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Influence of dietary black pepper and red pepper additives on the haematological and biochemical profiles of broiler chickens.

Kelvin Uhunoma AIKPITANYI[1] and James Atekha IMASUEN[2]

[1]Department of Animal Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria; Kelvin.aikpitanyi@aauekpoma.edu.ng

[2]Department of Animal Science, University of Benin, Benin City, Edo state, Nigeria; *james.imasuen@uniben.edu*

* Correspondence: *Kelvin.aikpitanyi@aauekpoma.edu.ng*

Abstract

This study investigates the impact of black pepper and red pepper on the blood and biochemical parameters of broiler chickens. Blood biochemical analysis is critical in monitoring poultry health and diagnosing diseases. Three hundred fifteen (315) broiler chicks were divided into seven treatment groups, each consisting of 45 birds. Each treatment group was replicated three times, with 15 birds per replicate, following a completely randomized design. Our findings show significant improvements in several key serum parameters amongst the treated groups. Serum protein levels, including total protein and albumin, were significantly higher in treated groups, with values reaching 3.43 g/dl and 2.00 g/dl, respectively, indicating improved protein utilization and liver function. The study also revealed significant reductions in white blood cell counts in most treated groups compared to the control, suggesting an enhanced immune response and reduced stress-induced leukocyte aggregation. For instance, the treated groups had white blood cell counts ranging from 25.87 $x10^3/\mu l$ to 61.13 $x10^3/\mu l$, significantly lower than the control group's count of 81.97 $x10^3/\mu l$. The atherogenic ratio of 3.56 was significantly higher in the control group compared to the treated groups, with Treatment 7 having the lowest ratio of 0.73. In conclusion, incorporating these additives and their combinations into broiler diets in this study significantly improved health indicators, boosted immune response markers, and optimized lipid profiles, leading to better overall health. These findings support the strategic use of these natural additives in broiler chicken nutrition, promising enhanced poultry health and healthier meat for consumers.

Keywords: broilers, black pepper, red pepper, haematology, serum biochemistry, phytogenic additives

1. Introduction

The poultry industry continuously seeks dietary interventions to enhance the health and productivity of broiler chickens. Among these interventions, natural feed additives, such as black pepper (Piper nigrum) and red pepper (Capsicum spp.), have garnered attention due to their bioactive compounds and potential health benefits. This study investigates the influence of dietary black pepper and red pepper additives on the blood profile characteristics of broiler chickens, providing insights into their physiological effects and potential applications in poultry nutrition.

Black pepper is renowned for its bioactive compound, piperine, which exhibits potent antioxidant and anti-inflammatory properties. Studies have shown that piperine can improve lipid profiles and antioxidant status in animal models by reducing markers of oxidative stress and inflammation (Khajuria *et al*., 2013). These findings suggest that piperine may positively impact blood parameters by mitigating oxidative damage and enhancing immune function.

Similarly, red pepper contains capsaicin, a compound known for its antioxidant and anti-inflammatory effects. Research on various animal species has demonstrated that capsaicin supplementation can modulate lipid metabolism, improve antioxidant capacity, and reduce inflammation (Omri *et al*., 2019). These properties imply that red pepper additives could potentially influence blood profiles by improving overall health and metabolic functions.

Because of these promising attributes, the specific effects of black pepper and red pepper on the blood profile characteristics of broiler chickens are being widely explored. Previous studies have primarily focused on poultry growth performance, meat quality, and immune responses (Bozkurt *et al*., 2014; Puvaca *et al*., 2015), leaving a gap in the understanding of their direct impact on blood biochemical parameters.

This study aims to fill this knowledge gap by examining the effects of dietary black pepper and red pepper additives on the blood profile of broiler chickens. By evaluating parameters such as total protein, albumin, cholesterol, triglycerides, glucose levels, and antioxidant status, this research seeks to elucidate the potential health benefits and physiological mechanisms associated with these natural feed additives. Understanding these effects could lead to developing more effective dietary strategies for enhancing the health and productivity of broiler chickens in the poultry industry. Conducting controlled studies such as this on broiler chickens would provide more accurate insights into the effects of pepper additives on their health and well-being.

2. Materials and Methods

2.1 Experimental Location

The research was conducted at the poultry unit of the Teaching and Research Farm, Faculty of Agriculture, University of Benin, Benin City, Edo State, Nigeria. The farm is situated at latitude 6°20'1.32" N and longitude 5°36'0.53" E, with a mean annual temperature of 34°C. The region experiences an average annual rainfall of 2000 mm and a relative humidity of 72.5% (Google Earth, 2016).

2.2 Preparations of test ingredients

The dried test ingredients for the research were sourced from the local market in Benin City. They were inspected to ensure uniform drying and the absence of rot. Subsequently, the red pepper was finely ground into powder and stored in airtight containers until incorporation into the experimental diets. The black pepper was ground in small batches, matching the proposed feed quantities to be formulated, to preserve its aromatic properties in the diets.

2.3 experimental diets

Broiler starter and finisher diets were formulated based on the recommendations from the National Research Council (1994) and Olomu (2010) to meet the nutritional requirements of the experimental birds. The chicks were initially fed a starter diet containing 23% crude protein and 3200 Kcal/kg ME for the first four weeks. Following this period, their diet was transitioned to a broiler finisher diet with 21% crude protein and 3000 Kcal/kg ME for an additional four weeks. The test ingredients, including black pepper, red pepper, and their combinations, were incorporated at varying inclusion levels as detailed in Tables 1 and 2.

Table 1 Composition of experimental broiler starter diets.

INGREDIENTS (%)	1	2	3	4	5	6	7
Maize	55.20	55.20	54.70	55.20	54.70	55.20	54.70
Soybean meal	28.00	28.00	28.00	28.00	28.00	28.00	28.00
Palm kernel cake	9.00	8.00	7.50	8.00	7.50	8.00	7.50
Fish meal	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Common salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin/mineral premix	0.50	0.30	0.30	0.30	0.30	0.30	0.30
Black pepper	-	1.00	1.50	-	-	0.50	0.75
Red pepper	-	-	-	1.00	1.50	0.50	0.75
Total	100	100	100	100	100	100	100
Crude protein (%)	23.10	23.04	23.00	23.04	23.00	23.04	23.00
Metabolizable energy (Kcal/Kg)	3240.04	3232.54	3214.94	3232.54	3214.94	3232.54	3214.94

Premix supplied per kilogram of feed: vit. A, 8,800 IU; vit. D3, 1,600 IU; vit. E, 12.8 mg; folic acid, 0.32 mg; pantothenic acid, 8 mg; biotin, 0.048 mg; niacin, 28 mg; vit. B6, 1.6 mg; riboflavin, 3.6 mg; thiamine, 0.96 mg; vit. B12, 12.8 µg; Vit. K3, 1.2 mg; copper, 9 mg; zinc, 60 mg; iodine, 1 mg; iron, 30 mg; manganese, 60 mg; selenium, 0.25 mg.

Table 2 Composition of experimental broiler finisher diet (%)

INGREDIENTS (%)	1	2	3	4	5	6	7
Maize	54.00	54.00	54.00	54.00	53.50	54.00	53.50
Soybean meal	20.80	20.80	20.80	20.80	20.80	20.80	20.80
Palm kernel cake	12.40	12.40	12.40	12.40	12.40	12.40	12.40
Wheat bran	7.00	6.00	5.50	6.00	5.50	6.00	5.50
Fish meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Bone meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Common salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin/Mineral premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Black pepper	-	1.00	1.50	-	-	0.50	0.75
Red pepper	-	-	-	1.00	1.50	0.50	0.75
Total	100	100	100	100	100	100	100
Crude protein (%)	20.62	20.56	20.52	20.56	20.52	20.56	20.52
Metabolizable energy (Kcal/Kg)	3088.15	3080.65	3063.05	3080.65	3063.05	3080.65	3063.05

Premix supplied per kilogram of feed: vit. A, 8,800 IU; vit. D3, 1,600 IU; vit. E, 12.8 mg; folic acid, 0.32 mg; pantothenic acid, 8 mg; biotin, 0.048 mg; niacin, 28 mg; vit. B6, 1.6 mg; riboflavin, 3.6 mg; thiamine, 0.96 mg; vit. B12, 12.8 µg; Vit. K3, 1.2 mg; copper, 9 mg; zinc, 60 mg; iodine, 1 mg; iron, 30 mg; manganese, 60 mg; selenium, 0.25 mg.

2.4 Experimental animals and design

A total of 315 day-old broiler chicks were procured from a reputable hatchery to ensure high-quality and disease-free birds for the study. The chicks were randomly assigned to seven experimental treatments, with each group comprising 45 birds. To reflect the proper experimental design and control, each treatment group was divided into three replicates, consisting of 15 birds per replicate. This study was conducted using a completely randomized design to ensure unbiased and statistically valid results.

2.5 Management of animals

Before the arrival of the birds, the brooding house and pens were meticulously cleaned and disinfected to ensure a sterile environment. All materials utilized throughout the study, including feeders, drinkers, and other equipment, were also thoroughly sanitized. The experimental birds were housed in a deep litter system. The brooding phase, crucial for early chick development, was conducted for the first two weeks.

Sufficient drinkers and feeders were provided during the brooding and rearing periods to prevent aggressive competition and ensure adequate access to feed and water. The birds were allowed free and continuous access to feed and water, ensuring they could consume as needed without restriction. Daily and routine management practices—including feeding, watering, litter management, medication, and vaccination, were diligently observed to maintain optimal health and growth conditions throughout the study.

2.6 Haematology and serum biochemistry studies

At the end of the experiment, six (6) birds per treatment (two per replicate) were tagged, isolated, and starved of feed overnight. Blood samples were collected from the jugular vein at slaughter from each bird into labeled sterile universal bottles containing ethylene diamine tetraacetic acid (EDTA), while serum samples were collected in heparin bottles without anticoagulant. Samples were also collected in other bottles containing fluoride oxalate and used in determining blood glucose levels.

Blood samples collected in EDTA-containing tubes were used to perform a full blood count (including red blood cells, haemoglobin, packed cell volume, platelets, lymphocytes, and white blood cells) using an automatic analyzer. The serum was separated from coagulated blood by centrifugation at 2500 rpm for 10 minutes and used to assess liver function, kidney function, antioxidant capacity, lipid profile, and blood serum metabolites.

2.7 Statistical analysis.

All data collected were analyzed using a one-way analysis of variance (ANOVA) with the general linear model procedure in Statistical Analysis System (SAS, 2012). Differences between treatment means were compared using Duncan's multiple range test, following the methodology of Steel and Torrie (1997). Statistical significance was set at $p < 0.05$.

3. Results

3.1 Antioxidant status of broiler chickens

Table 3 shows the antioxidant status parameters measured, their values, possible similarities, and significant differences of the experimental groups fed varying levels of black pepper, red pepper and their combinations. Among the parameters assessed, only Aspartate aminotransferase (AST) showed no significant difference ($P>0.05$) among treatments. Other parameters exhibited significant differences ($P<0.05$) between the control and treated groups.

Table 3 Assessment of the antioxidant status of broiler chickens fed black pepper, red pepper, and their combinations.

Parameters	T1	T2	T3	T4	T5	T6	T7	S.E.M.
Malondialdehyde (MDA) (mmol/ml)	67.80a	58.29b	45.14c	36.84c	41.66c	45.75c	44.34c	2.78*
Catalase (CAT)(mmol/ml)	191.27b	190.93b	209.17a	220.08a	208.15a	163.13c	172.83c	5.10*
Superoxide dismutase (SOD)(mmol/ml)	4.72b	5.09b	2.43cd	1.85de	1.74e	6.25a	4.52bc	0.19*
Glutathione peroxidase (GPx)(mmol/ml)	101.34c	95.76c	123.78ab	127.76a	116.74b	65.32d	102.63c	2.82*
Alkaline phosphatase (ALP)(U/L)	133.50a	115.75c	115.75c	132.25a	127.75ab	119.25bc	102.25d	3.01*
Alanine aminotransferase (ALT)(U/L)	15.75a	20.50a	10.00b	9.25b	9.00b	6.75b	5.75b	1.66*
Aspartate aminotransferase (AST)(U/L)	106.50	87.00	102.75	103.25	108.50	110.00	96.00	5.74NS

T = Treatment, SEM = standard error of mean, BP = black pepper, RP = red pepper, * = significant ($p<0.05$), NS = Not significant. a,b,c,d; Means in the same row with different superscript are significantly different ($p<0.05$).

T1 = Control, T2 = 1% BP, T3= 1.5% BP, T4 = 1% RP, T5 = 1.5% RP, T6 = 0.5% each of BP and RP, T7 = 0.75% each of BP and RP

The study demonstrated significant variations in oxidative stress markers and enzyme activities across the control and treated groups. Malondialdehyde (MDA) levels were highest in the control group (67.80 mmol/ml) and lowest in Treatment 4 (36.84 mmol/ml). Catalase (CAT) activity was significantly elevated in Treatments 3 (209.17 mmol/ml), 4 (220.08 mmol/ml), and 5 (208.15 mmol/ml), with the lowest values recorded in Treatments 6 (163.13 mmol/ml) and 7 (172.83 mmol/ml). The control (191.27 mmol/ml) was similar to Treatment 2 (1% BP).

Superoxide dismutase (SOD) activity was highest in Treatment 6 (6.25 mmol/ml), with the control group (4.72 mmol/ml) showing no significant difference from Treatments 2 (5.09 mmol/ml) and 7 (4.52 mmol/ml). The lowest SOD values were observed in Treatments 5 (1.74 mmol/ml) and 4 (1.85 mmol/ml). Glutathione peroxidase (GPx) activity was highest in Treatment 4 (127.76 mmol/ml), followed by Treatments 3 (123.78 mmol/ml) and 5 (116.74 mmol/ml), while Treatment 6 had the lowest value (65.32 mmol/ml). Alkaline phosphatase (ALP) was lowest in Treatment 7 (102.63 U/L) and highest in the control (133.50 U/L) and Treatment 4 (132.25 U/L). Alanine aminotransferase (ALT) values showed a decrease from Treatment 3 (10.00 U/L) to Treatment 7 (5.75 U/L), with no significant differences observed among these groups.

3.2 Serum Biochemistry Parameters

Among the assessed serum biochemistry parameters (Table 4), no significant differences (P>0.05) were found in total bilirubin, globulin, urea, sodium, and bicarbonate (HCO3). All other parameters showed significant differences (P<0.05), though no consistent pattern or trend was observed.

Table 4 Serum biochemistry of broiler chickens fed black pepper, red pepper and their combinations as additives

Parameters	T1	T2	T3	T4	T5	T6	T7	S.E.M.
Total bilirubin (mg/dl)	0.40	0.33	0.40	0.40	0.38	0.40	0.33	0.03NS
Conjugated bilirubin (mg/dl)	0.15b	0.15b	0.15b	0.20a	0.15b	0.23a	0.15b	0.02*
Total protein (g/dl)	2.73c	3.05abc	2.80bc	3.43a	2.60c	2.95abc	3.25ab	0.15*
Albumin (g/dl)	1.20c	1.50b	1.58b	1.88a	1.45bc	1.83a	2.00a	0.08*
Globulin (g/dl)	1.53	1.55	1.23	1.40	1.15	1.13	1.25	0.11NS
Urea (mg/dl)	10.50	9.50	10.50	10.50	11.75	10.50	12.25	0.82NS
Creatinine (mg/dl)	0.75a	0.73ab	0.68cd	0.55d	0.63d	0.70bc	0.48d	0.02*
Sodium (µmol/L)	133.25	134.00	137.75	139.25	130.50	136.00	136.00	1.77NS
Potassium (µmol/L)	5.13b	5.83a	4.23c	4.10c	3.13d	3.50d	4.10c	0.16*
HCO3 (µmol/L)	21.50	20.25	20.75	21.75	20.50	21.00	20.75	0.76NS
Chloride (µmol/L)	101.0c	101.7c	105.5ab	107.00a	101.75c	102.25c	103.5bc	0.83*
Glucose (mg/dl)	189.25bc	176.0d	184.5cd	197.75b	198.75ab	200.25ab	211.00a	3.87*

T = Treatment, SEM = standard error of mean, BP = black pepper, RP = red pepper, * = significant (p<0.05), NS = Not significant. a,b,c,d; Means in the same row with different superscript are significantly different (p<0.05).
T1 = Control, T2 = 1% BP, T3= 1.5% BP, T4 = 1% RP, T5 = 1.5% RP, T6 = 0.5% each of BP and RP, T7 = 0.75% each of BP and RP

Significant differences were observed in conjugated bilirubin levels between Treatments 4 (0.20 mg/dl) and 6 (0.23 mg/dl), which were higher than the other treatments, all of which had a value of 0.15 mg/dl. Total protein levels varied, with Treatment 4 showing the highest value (3.43 g/dl) and Treatment 5 the lowest (2.60 g/dl). Serum albumin was higher in all treated groups compared to the control, with Treatments 4 (1.88 g/dl), 6 (1.83 g/dl), and 7 (2.00 g/dl) showing significantly higher values. The control group recorded the lowest albumin value (1.20 g/dl), with no significant difference between it and Treatment 5 (1.45 g/dl).

Serum creatinine levels were highest in the control group (0.75 mg/dl), with treated groups ranging from 0.48 mg/dl in Treatment 7 to 0.73 mg/dl in Treatment 2. Serum potassium was highest in Treatment 2 (1% black pepper) at 5.83 µmol/L, while the lowest level (3.13 µmol/L) was observed in Treatment 4. Chloride levels were highest in Treatment 4 (107 µmol/L), statistically similar to Treatment 3 (105.50 µmol/L), and lowest in the control group (101 µmol/L), though not significantly different from most treated groups. Glucose levels increased progressively among the treated groups, ranging from 176 mg/dl in Treatment 2 to 211 mg/dl in Treatment 7, with the control group recording 189.25 mg/dl, showing no significant difference from Treatments 3, 4, 5, and 6.

3.3 Plasma lipid profile of broiler chickens

The plasma lipid profile of broiler chickens fed black pepper and red pepper additives are given in Table 5. Significant differences were observed in all assessed parameters except for triglyceride and very-low-density lipoprotein (VLDL) levels.

Table 5 Plasma lipid profile of broiler chickens fed black pepper, red pepper, and their combinations as additives.

Parameters	T1	T2	T3	T4	T5	T6	T7	S.E.M.
Total cholesterol (mg/dl)	209.25a	157.00bc	148.25c	161.00bc	189.25ab	128.50cd	103.00d	11.44*
Triglyceride (mg/dl)	278.25	139.75	139.00	183.75	139.75	186.25	103.75	35.06NS
HDL (mg/dl)	34.50c	54.75a	51.25ab	53.25ab	52.25ab	41.25bc	47.50bc	3.69*
LDL (mg/dl)	119.10a	74.30b	69.20b	71.00b	109.05a	50.00bc	34.75c	9.71*
VLDL mg/dl)	55.65	27.95	27.80	36.75	27.95	37.25	20.75	7.01NS
LDL/HDL ratio	3.56a	1.40bc	1.38bc	1.33bc	2.11b	1.21bc	0.73c	0.36*

TRT= Treatment, SEM = standard error of mean, BP = black pepper, RP = red pepper, * = significant ($p<0.05$), NS = Not significant a,b,c,d; means in the same row with different superscript are significantly different ($p<0.05$)

T1 = Control, T2 = 1% BP, T3= 1.5% BP, T4 = 1% RP, T5 = 1.5% RP, T6 = 0.5% each of BP and RP, T7 = 0.75% each of BP and RP

Total cholesterol was highest in the control group (209.25 mg/dl), with significant differences ($P<0.05$) compared to all treated groups except Treatment 5 (189.25 mg/dl). Cholesterol levels were notably lower in the treated groups, with Treatment 7 (0.75% black pepper and red pepper) showing the lowest value (103 mg/dl). High-density lipoprotein (HDL) levels were significantly higher ($P<0.05$) in the treated groups, ranging from 41.25 mg/dl in Treatment 6 to 54.75 mg/dl in Treatment 2, compared to the control, which had the lowest HDL (34.50 mg/dl). Conversely, low-density lipoprotein (LDL) was highest in the control (119.10 mg/dl) and lowest in Treatment 7 (34.75 mg/dl). The LDL/HDL ratio was significantly highest in the control (3.56), while Treatment 7 had the lowest ratio (0.73), with no treated group exceeding 2.11 recorded in Treatment 5.

3.4 Haematological profile of broiler chickens

The haematological profile of the experimental birds fed varying levels of black pepper, red pepper and their combinations as additives is shown in Table 6. Significant ($P>0.05$) differences were only observed in white blood cells, lymphocytes, monocytes and platelets.

Table 6 Haematological profile of broiler chickens fed black pepper, red pepper, and their combinations as additives.

Parameters	T1	T2	T3	T4	T5	T6	T7	S.E.M.
White blood cell (x103/µl)	81.97a	39.47bc	44.50bc	60.83ab	55.43b	25.87c	61.13ab	8.27*
Lymphocytes (x103/µl)	46.63a	29.40b	29.27b	51.73a	47.47a	22.30b	47.20a	4.57*
Monocytes (x103/µl)	8.10a	4.57b	1.73c	4.23b	5.50ab	1.47c	5.80ab	0.88*
Granulocytes (x103/µl)	7.20	5.53	3.47	4.80	5.83	2.00	8.13	6.86NS
Red blood cell	2.71	3.11	2.32	2.61	3.05	2.25	2.66	0.37NS
Haemoglobin	13.33	8.03	11.37	9.80	11.50	8.27	9.80	1.92NS
Packed cell volume (%)	34.27	24.47	26.83	27.53	33.70	27.43	28.90	4.68NS
MCV	113.77	116.63	115.30	108.13	110.70	107.77	109.40	3.16NS
MCH	42.77	38.33	47.27	37.57	37.63	36.50	37.10	3.98NS
MCHC	38.97	32.87	40.77	34.70	34.70	33.93	33.93	2.85NS
RDW	13.03	12.90	13.77	13.63	13.03	13.10	14.27	0.48NS
Platelet	38.67ab	30.67bc	28.33c	30.00bc	41.67a	33.00abc	36.67abc	2.72*
Mean platelet volume (MPV)	6.00	5.70	5.57	5.37	5.90	5.33	5.30	0.17NS
PDW	4.27	4.50	4.80	3.57	3.93	3.93	3.67	0.34NS

T= Treatment, SEM = standard error of mean, BP = black pepper, RP = red pepper, * = significant ($p<0.05$), NS = Not significant a,b,c,d; means in the same row with different superscript are significantly different ($p<0.05$)

T1 = Control, T2 = 1% BP, T3= 1.5% BP, T4 = 1% RP, T5 = 1.5% RP, T6 = 0.5% each of BP and RP, T7 = 0.75% each of BP and RP

For white blood cell (WBC) count, the control group recorded the highest value at 81.97 x10³/µl, which was significantly different ($P<0.05$) from all additive groups except Treatment 7 (61.13 x10³/µl). Treatment 6 (0.5% each of black pepper and red pepper) had the lowest WBC count at 25.87 x10³/µl.

Lymphocyte count was highest in Treatment 4 (51.73 x10³/µl), with no significant difference from the control (46.63 x10³/µl), Treatment 5 (47.47 x10³/µl), and Treatment 7 (47.20 x10³/µl). The lowest lymphocyte count was in Treatment 6, at 22.30 x10³/µl. The control group had the highest monocyte count (8.10 x10³/µl), which was statistically similar to Treatment 5 (5.50 x10³/µl) and Treatment 7 (5.80 x10³/µl). The lowest monocyte counts were observed in Treatments 3 (1.73 x10³/µl) and 6 (1.47 x10³/µl). Platelet count was lowest in Treatment 3 (1.5% black pepper) at 28.33 x10³/µl and highest in Treatment 5 (1.5% red pepper) at 41.67 x10³/µl. The control group, with a platelet count of 38.67 x10³/µl, shared a level of significance with several treated groups.

4. Discussion

4.1 Antioxidant status of broiler chickens

The liver, essential for numerous bodily functions, is vulnerable to both chemical and biological damage, often reflected in serum enzyme levels. Elevated serum enzymes, such as AST, ALT, and ALP, are commonly used as biomarkers for liver damage. Disruptions in liver function due to increased enzyme activity can compromise overall health and reduce productivity (Amstad *et al*., 1998). This study found that the control group had significantly higher levels of these enzymes, indicative of compromised liver function, whereas the treated groups showed improved liver health. These findings are consistent with reports that lower enzyme levels are associated with enhanced liver function (Fernandez *et al*., 1994), contrasting with studies that reported no significant differences in ALT and AST levels with similar additives (Adedoyin *et al*., 2019; Imasuen and Ijeh, 2017). Malondialdehyde (MDA) concentration, a key marker for oxidative stress and lipid peroxidation, was significantly higher in the control group. This aligns with previous findings suggesting that oxidative stress markers are elevated in the absence of antioxidant-rich additives (El-Shaieb *et al*., 2009). Capsaicin, a bioactive compound found in red pepper, has been documented to enhance antioxidant properties by reducing MDA levels (Abou-Elkhair *et al*., 2018). This effect was evident in the treated groups (Treatments 3-7), where MDA levels were considerably lower, in agreement with earlier studies showing that herbs and spices can increase serum SOD and GPx levels, thus lowering oxidative stress by decreasing MDA concentration (Hashemipour *et al.*, 2013).

Superoxide dismutase (SOD) activity, essential for neutralizing reactive oxygen species, was notably higher in Treatments 2 and 6, indicating that these additives helped reduce oxidative stress, a finding consistent with previous research (Yang *et al*., 2010; Xu *et al*., 2014). The challenge in interpreting SOD levels lies in distinguishing whether increased activity reflects an adaptive response to stress or an enhancement of the body's overall antioxidant capacity (Niki, 2014; Surai, 2015). The elevated SOD activity observed in Treatments 1, 2, 6, and 7 suggests improved antioxidant defense, while lower levels in Treatments 3, 4, and 5 may indicate an efficient antioxidant system requiring less enzyme production.

The activities of ALP, influenced by both liver and bone metabolism, showed no clear trend in this study, although ALP levels in all groups remained within the reference range for poultry (Oleforuh-Okoleh *et al*., 2015). Variations in ALP can be attributed to multiple factors, including biliary obstruction or enteric disease (Jenkins, 2000). However, the trend of lower ALP levels in treated groups supports previous findings that higher levels of dietary black pepper can reduce ALP concentrations, ultimately contributing to better overall health in broiler chickens, as evidenced by reduced mortality rates in treatment groups (Aikpitanyi and Imasuen, 2020).

4.2 Serum biochemistry of broiler chickens

Blood biochemical analysis is a key indicator for monitoring the health status of poultry and diagnosing potential diseases (Schmidt *et al*., 2007). While several studies on the effects of phytogenic additives report minimal or non-significant differences between treated and control groups (Oleforeh-Okoleh *et al*., 2015; Al-Kassie *et al*., 2012), our research demonstrated significant differences in multiple vital parameters. Serum proteins, predominantly synthesized in the liver, are critical for various physiological functions, including maintaining blood volume, buffering pH, transporting hormones and drugs, aiding coagulation, catalyzing biochemical reactions, regulating metabolism, and defending against pathogens (Melillo, 2013). The higher total protein levels in treated groups, particularly linked to elevated albumin concentrations, indicate improved dietary protein utilization (Rezende *et al*., 2017). Our study is consistent with these findings, revealing that increased albumin levels corresponded with higher total protein concentrations. The total protein levels in both control and treated groups (2.60–3.43 g/dl) fell within the acceptable range of 2.50–4.50 g/dl for broiler chickens (Harr *et al*., 2002), although these values were slightly lower than the 40–62 g/l range reported by Nanbol *et al*. (2016) for broilers in Nigeria.

Albumin, which plays a role in molecule transport and maintaining oncotic pressure, is also an indicator of protein utilization (Rezende *et al*., 2017). Our study found that the treated groups exhibited enhanced protein efficiency, with albumin values (1.45–2.00 g/dl) slightly exceeding the reference range of 1.08–1.61 g/dl provided by Ross *et al*. (1978). This increase in albumin may be attributed to the bioactive compounds present in the spices used in the treatments. In contrast to Adedoyin *et al*. (2019), who reported no significant change in glucose levels with the use of red pepper, our study observed elevated glucose levels across all treated groups, except in those receiving black pepper additives (Treatments 2 and 3). Similar findings were reported by Imasuen and Ijeh (2017), who observed increased glucose levels in birds fed black pepper and ginger, suggesting that homeostatic mechanisms regulate blood glucose based on factors like growth, feed intake, productivity, and environmental conditions (Platel and Srinivasan, 2004). The active components in black pepper may influence the adrenal gland, lowering ACTH secretion and causing stress, thereby raising blood glucose concentrations (Al-Kassie *et al*., 2012; Gao *et al*., 2013).

In contrast to Abou-Elkhair *et al*. (2014), who found that black pepper increased serum globulin levels, our study observed a numerical reduction in serum globulin among treated groups, although these reductions were not statistically significant compared to the control. This discrepancy could be due to the lower inclusion levels of additives in our study compared to theirs. Additionally, we found no significant differences (P>0.05) between control and treated groups for bicarbonate and chloride levels, both of which remained within the ranges reported by Nanbol *et al*. (2016) for broilers (bicarbonate: 7–13 mmol/l; chloride: 54–70 mmol/l). This stability further supports the minimal physiological disruption caused by the treatments.

4.3 Haematological profile of broiler chickens

A high white blood cell (WBC) count in peripheral blood is often indicative of stress, infection, trauma, toxicities, or neoplasms, while a low WBC count could suggest chronic inflammation, infections, or diminished bone marrow function (Doneley and Doneley, 2010; Clement *et al*., 2010). Capsaicin, the active component in red pepper, has been shown to inhibit sensory neurons, impacting the long-term release of substance P, a neuropeptide involved in modulating inflammation, stimulating enzyme release from lysosomes, promoting phagocytosis, and enhancing the activity of natural killer cells (Vicente *et al*., 2007). Galib *et al*. (2011) found that feeding broilers hot red pepper led to lower WBC counts during the finisher phase. This partially aligns with our findings, where WBC counts in Treatments 4 (60.83 x10^3/µl) and 7 (61.13 x10^3/µl) were statistically similar to the control (81.97 x10^3/µl), while the other treatments showed significantly lower values (25.87 x10^3/µl – 55.43 x10^3/µl). Anderson (2007) also noted that dietary Capsicum annuum could induce WBC aggregation after prolonged feeding, which may explain the reduced WBC counts in some of the treated groups. On the other hand, studies by Al-Kassie *et al*. (2012) and Imasuen and Ijeh (2017) reported no significant changes in WBC counts with black pepper additives..

Packed cell volume (PCV), representing the percentage of red blood cells (RBC) in the blood, fell within the normal range of 25-41% for broilers (Mitruka and Rawnsley, 1997) and was slightly below the 32-45% range reported by Nanbol *et al*. (2016). PCV is often used as an indirect measure of RBC count and can indicate conditions like anemia (Bashar *et al*., 2010). According to Reece (2009), hemoglobin, which makes up about one-third of RBCs, is a reliable indicator of red blood cell production (Sugiharto *et al*., 2011). Despite these observations, no significant differences were noted in PCV, RBC, or hemoglobin levels in our study. The PCV values, ranging from 24.47% to 34.27%, were consistent with the normal 22-35% range for broilers (Jain, 1993) and similar to the results reported by Imasuen and Ijeh (2017).

Red pepper contains phenolic compounds that can inhibit iron absorption (Siriporn *et al*., 2006). Galib *et al*. (2011) also reported a significant reduction in hemoglobin levels with dietary pepper supplementation, which could explain why chicks on the basal diet had the highest, though non-significant, levels of circulating erythrocytes and hemoglobin. This observation aligns with Al-Kassie's (2012) report, which found that hemoglobin levels were higher in control groups compared to treated birds.

Platelets play a critical role in primary hemostasis, adhering to damaged blood vessels, changing shape, and releasing proteins that attract additional platelets to form a plug that initiates coagulation. Phytogenic compounds can reduce platelet sensitivity to aggregating agents during the conversion of arachidonic acid to thromboxane (Muhammed and Lakshmi, 2007), suggesting that quality phytogenic additives may improve blood circulation by inhibiting excessive platelet aggregation. However, our study recorded variable platelet counts, except for the 1.5% red pepper treatment, which showed notable differences. These findings are consistent with Oleforeh-Okoleh *et al*.'s (2015) study, which reported reduced platelet counts in broilers treated with aqueous ginger extract.

4.4 lipid profile of broiler chickens

Puvaca *et al*. (2015) observed that control groups exhibited the highest levels of triglycerides (65.9 mg/dl), total cholesterol (97.2 mg/dl), and low-density lipoprotein (LDL) (36.7 mg/dl), with significant differences ($p < 0.05$), particularly in triglyceride concentration, which was as low as 14.4 mg/dl in their black pepper treatment. Similarly, Sayeed *et al*. (2016) found significantly higher levels of cholesterol (212.01 mg/dl), triglycerides (208.03 mg/dl), and LDL (146.54 mg/dl) in the control group compared to groups treated with black and red pepper. These findings align with our study, where significant differences ($p < 0.05$) were observed in cholesterol, high-density lipoprotein (HDL), LDL, and the LDL/HDL ratio. Although triglyceride levels were lower in the treated groups, they did not differ significantly from the control, which is consistent with Valiollahi *et al*. (2013), who explored the effects of ginger and black pepper.

Adedoyin *et al*. (2019) also reported similar trends in triglyceride, cholesterol, LDL, and HDL levels between broilers on a basal diet and those receiving various levels of red pepper. In contrast, Imasuen and Ijeh (2017) found significantly lower levels of triglycerides (40.33 mg/dl), cholesterol (125.70 mg/dl), and HDL (29.75 mg/dl) in broilers supplemented with black pepper, though they noted elevated LDL levels (70.08 mg/dl).

Other studies have also demonstrated that including garlic, black pepper, and hot red pepper in broiler diets (in amounts ranging from 0.25% to 1%) significantly reduces blood cholesterol and other related biochemical parameters (Alaa, 2010; Al-Kassie *et al*., 2012; Moradi *et al*., 2016). These reductions are often attributed to the inhibition of Acetyl-CoA synthase, an enzyme vital for fatty acid biosynthesis (Puvaca *et al*., 2015), or the suppression of hepatic lipogenic and cholesterogenic enzyme activities (Qureshi *et al*., 1983). Additionally, Case and Elson (1995) noted that a mere 5% inhibition of HMG-CoA reductase could lower serum cholesterol in poultry by up to 2%. Spices, herbs, and medicinal plants may also enhance enzymes involved in converting cholesterol to bile acids (Al-Kassie *et al*., 2012) or reduce hepatic lipogenic enzyme activity (Ciftci *et al*., 2010), contributing to lower cholesterol levels.

In this study, diets supplemented with combined black pepper and red pepper mixtures (Treatments 6 and 7) significantly decreased LDL levels compared to the control group. These findings are consistent with Moradi *et al*. (2016), who supported the use of these spices to improve HDL levels while reducing LDL. This was further corroborated by Akbarian *et al*. (2012) and Najafi and Taherpour (2014). The effects of these additives on lipid profiles may stem from their anti-peroxide properties, which protect LDL from oxidation, or from a reduced hepatic production of very-low-density lipoprotein (VLDL), the precursor to LDL in the blood (Kim *et al*., 2009). Given that high levels of LDL and VLDL can lead to atherosclerosis, reducing these parameters suggests that the inclusion of black pepper and red pepper in poultry diets could mitigate health risks associated with these blood components in both poultry and the consumers of poultry products.

5. Conclusion

In conclusion, this study provides compelling evidence that including phytogenic additives such as black pepper and red pepper in broiler diets positively influences various health and biochemical parameters, ultimately enhancing overall poultry health and production performance. The significant improvements in serum protein levels, such as total protein and albumin, point to enhanced protein utilization and improved liver function, which are crucial for optimal growth and metabolic efficiency. Additionally, the favourable modulation of blood glucose levels and immune response, reflected in reduced white blood cell counts, highlights the potential of these additives in promoting homeostasis and reducing stress in broilers.

Notably, the lipid profile analysis demonstrated substantial reductions in cholesterol, HDL, LDL, and the LDL/HDL ratio, reinforcing the role of black and red pepper additives in optimizing lipid metabolism and supporting cardiovascular health in broilers. These findings emphasize the potential of dietary spices in improving the overall well-being, physiological function, and resilience of broiler chickens, making them a valuable tool for enhancing animal welfare and sustainable poultry production.

Contribution of Authors

KUA., and JAI., were involved in the conceptualization and design of the study.
KUA., conducted the field experimentation.
KUA., and JAI.,contributed to data interpretation.
KUA., drafted the manuscript.
JAI., edited and approved the manuscript

Disclosure Statement

The authors declare that there is no conflict of interest.

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Ethical Approval

All experimental procedures followed the guidelines for the use of animals in research approved by the Boards of the Faculty of Agriculture and the Department of Animal Science, University of Benin, Benin City, Nigeria.

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