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Dietary Supplementation with Phytogenic Blend on Haematology, Serum Biochemistry, Carcass Traits and Meat Quality of Broiler Chickens

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ABSTRACT

A 42-day study was conducted with broiler to determine the blend effects of *Piper nigrum, Zingiber officinale, Azadirachta indica, Cymbopogon citratus and Citrus sinensis* on blood indices, carcass characteristics and meat quality. 240 day old chicks were assigned to 6 treatments with 5 replicates: basal diet (T1), basal diet plus oxytetracycline (T2), 5g (T3), 10g (T4), 15g (T5) and 20g (T6) PB respectively. Data on blood parameters, carcass and meat quality were obtained and analysed with ANOVA. Starter phase, packed cell volume (PCV), red blood cell (RBC), haemoglobin (Hb) and low density lipoprotein decreased (P <0.05) while alanine aminotransferase (ALT) and alkaline phosphatase increased as PB increased. Antibiotic group recorded higher (P <0.05) white blood cell (WBC), mean corpuscular haemoglobin concentration but lowest eosinophils . Mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were higher (P <0.05) in T1. Finisher phase, addition of PB decreased (P <0.05) PCV, RBC and Hb with the least value recorded for antibiotic group. Basophil increased (P <0.05) at higher level of PB while cholesterol, MCV and MCH reduced. Triglyceride and very low-density lipoprotein were least (P <0.05) in antibiotic group, others were similar. High-density lipoprotein and ALT were higher in 10g/kg group. Breast meat, spleen and kidney were higher (P <0.05) in the antibiotic group, others were similar. Percentage water absorption and refrigeration loss were higher (P <0.05) in 5g/kg and 10g/kg respectively. It was concluded that the PB did not improve carcass yield and meat quality however, the birds were healthy.

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INTRODUCTION

Public health concern on the risk of consuming poultry products raised with antibiotic growth promoter (AGP) and the potential development of antibiotic-resistant bacteria in animals and humans couple with the ban of antibiotics (Charis, 2000) in many countries has stimulated research into the use of botanical products known as phytogenic feed additives (PFAs). Phytogenic feed additives or botanicals, are substances of plant origin added to animal diets at recommended levels with the aim of improving animal performance. The choice of PFAs in poultry feeds is because they are generally recognized as safe by the Food and Drug Administration (FDA) of the United States (Diaz-Sanchez *et al.*, 2015).

The use of phytogenic feed additives like ginger, garlic, black pepper, clove, thyme, pepper fruit, orange peels, lemon grass and the likes as non-antibiotics in poultry production have received much attention globally. There are positive reports on the use of single application or combination of any of these PFAs on growth performance, gut morphology of broiler chickens. Fafiolu *et al*. (2020) reported that adding phytogenic feed additives containing lemon and orange peels promoted positive effect on villus height, improved red blood cell (RBC) production, enhanced the plasma protein reserves and lipid profile, and prevents muscle wastage. It was reported by Abou-Elkhair *et al*. (2014) that dietary supplementation with black pepper, coriander, or their combinations enhanced the performance and health status of broiler chickens. In the report of Karangiya *et al*. (2016) it is concluded that supplementation of garlic improves growth performance at 1% of broiler ration when compared with ginger only or the mixture of garlic and ginger. Fallah & Mirzaei (2016) reported that dietary inclusion of turmeric and thyme powders increase body weight, feed intake and improve FCR of broiler chickens at 42 days of age and decreased serum triglyceride, high density lipoprotein (HDL) and low-density lipoprotein (LDL) concentration, and enhance antibody titer production of broiler chickens at 18 and 28 days of age.

The novel about PFAs is the use of phytogenic blends as feed additive for likely positive interaction since they contain different phytochemicals with diverse biological functions.

For instance, Black pepper (*Piper nigrum*) is a flowering plant that it rich in glutathione peroxidase and glucose-6-phosphate dehydrogenase which a biological function of detoxifying hydroperoxides (Karthikeyan & Rani 2003). Al-Kassie *et al*. (2011) reported that black pepper improved health status, heat production of lipids and speed up energy metabolism in broiler chickens. Ginger have been reported to have strong stimulating effect on the immune and digestive systems in birds (Al-Shuwali *et al*., 2015). Addition of ginger power to broiler chicken diet was reported (Olefouh-Okoleh, 2014) to improved weight gain, feed efficiency, lowered mortality and increased liveability. *Azadirachta indica* (Neem) leaf contains some phytochemicals like azadiractin, nimbin, nimbindin, quercetin (Makeri *et al*., 2007) which performs certain biological functions such as antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic (Gayathri & Arun, 2021). Broiler chickens fed diets containing 1.5% (Makeri *et al.*, 2007) and 2.5 g/kg (Ansari *et al*., 2012) neem leaf meal recorded body weight gain (BWG) and feed conversion ratio (FCR) which was significantly similar to the control. Lemon grass (*Cymbopogon citratus*) contains antioxidant, flavonoids, phenolic chemicals, and terpenoids which could be responsible for its many biological actions, like anti-inflammatory, immune-modulatory, fungistatic and antisceptic properties (Alagawany *et al*., 2021). An improvement in growth performance and carcass characteristics of quails fed diets supplemented with lemon grass essential oil has been reported (Ayman *et al*., 2021). Sweet Orange (*Citrus sinensis*) peels (SOP) contain Flavonoid that can function as direct antioxidants and free radical scavengers, and have the capacity to modulate enzymatic activities and inhibit cell proliferation (Duthie & Crozier, 2000; Behera *et al*., 2019). Fafiolu *et al.* (2020) reported that *C. sinensis* has anti-inflammatory properties that can aid in digestion of fatty foods and reduce acidity and heartburn.

Therefore, it was hypothesized in this study that the blend of black pepper, ginger, sweet orange peels, neem leaf and lemon grass should be able to improve haematology, serum biochemistry, carcass traits and sensory evaluation of broiler chickens.

MATERIALS ANS METHODS

Study Area

The experiment was carried out at the Poultry Unit of the Directorate of University Farms (DUFARMS), Abeokuta, Ogun-State, Nigeria. The farm is located in the Tropical rainforest vegetation zone of South-Western Nigeria on latitude $7^0$10N and longitude $3^0$2E. The prevailing climate is tropical humid with an average annual rainfall of 1037mm and a mean ambient temperature of about 34.7^0C. Humidity of the experimental location is lowest (37- 85%) (Federal University of Abeokuta Meteorological Station).

Preparation of Phytogenic Blend

Dried ginger rhizomes (*Zingiber officinale*) and black pepper seed (*Piper nigrum*) was purchased from a local market in Abeokuta ogun state, Nigeria. The sweet orange peel (*Citrus sinensis*) was collected from the orange seller, fresh neem leaf (*Azadirachta indica*) and the lemon grass (*Cymbopogon citratus*) was obtained from the University Farm and air-dried for 7 days while the orange peel was sun-dried. The ingredients were milled separately into powder using kitchen blender. The blend was made by mixing 500g each of ginger, black pepper, sweet orange peel and 250g each of neem leaf and lemon grass in accordance with recommendation from past related studies.

Chemical Composition of Formulated Blend Dry matter and crude protein (CP) ($N \times 6.25$) of the PB sample were analyzed according to the methods of the Association of Official Analytical Chemists International (AOAC, 2000). Crude fat was determined by extraction with petroleum ether following acidification with 4 M hydrochloric acid as described by Wiseman *et al*. (1992). Gross energy was measured using an adiabatic bomb calorimeter (Rajdhani Scientific Instruments Company, India).

Experimental Diets, Design and Birds' Management

Basal diets (starter and finisher) were formulated according to the recommendation of NRC (1994). The composition of the basal diets is presented in Table1. The experiment was arranged in a completely randomised design.

The phytogenic blend was added to the diet as follows:

T1 = Negative Control (Basal diet only); T2 = Basal diet + tetracycline (positive Control)

T3 = Basal diet + 5g blend/kg; T4 = Basal diet + 10g blend/kg

T5 = Basal diet +15g blend/kg; T6= Basal diet + 20g blend/kg

A total of two hundred and forty (240) day old Cobb 500 broiler chicks were used for the study. Birds were allotted on weight equalization basis into 6 dietary treatments of 40 birds each, replicated 5 times. The temperature of the brooding house was monitored with the aid of a thermometer. The birds were managed intensively with feed and water given without restriction. Standard broiler chicken vaccination programme was strictly adhered to without the use of antibiotics and anticoccidial drugs.

Table 1: Composition of Basal Diets

Ingredients	Starter (day 0-21)	Finisher (day 22-42)
Maize	55.00	59.75
Soybean meal	36.60	32.00
Fish meal (72%)	2.50	2.00
Limestone	1.00	1.00
Bone meal	2.00	2.60
Wheat offal	1.75	1.60
Sodium chloride	0.25	0.25
Lysine	0.40	0.20
Methionine	0.25	0.25
*Broiler premix	0.25	0.25
Total (kg)	100.00	100.00
Analyzed nutrients		
ME (Kcal/kg)	2827.44	3030.11
CP%	26.60	24.58

Ingredients	Starter (day 0-21)	Finisher (day 22-42)
CF %	4.89	4.61
Fat %	4.26	4.73

*Composition of Premix per kg of Diet: Vitamin A, 12,500 I.U Vitamin E, 40mg; Vitamin K3 2mg; Vitamin B1, 3mg; Vitamin B2,5.5mg; Niacin, 55mg; Calcium Pantothenate,11.5mg; Vitamin B6, 5mg; Vitamin B12, 0.025mg; Chloride, 500; Folic Acid, 1mg; Biotin, 0.08mg, Manganese, 120; iron, 100mg; Zinc, 80mg, Copper 8.5mg; Iodine, 1.5mg; Cobalt, 0.3mg; Selenium, 0.12mg; Anti-Oxidant, 120mg

Data Collection

Blood Indices

At the end of the starter (day 21) and finisher (day 42) phases, 3 ml blood samples were collected from two birds per replicate through brachial vein puncture into tubes containing ethylene-diamine-tetra-acetate (EDTA) for haematological indices. Another 3 mL were collected into plain bottles without anti-coagulant which were centrifuged for 15 minutes at 503.1 × g to collect the serum for biochemistry indices. Haemoglobin concentration (Hb) was determined using the cyanmethaemoglobin method (Cannan, 1958). Haematocrit, red blood cell (RBC) and white blood cell (WBC) counts were determined using Wintrobe haematocrit tube according to the method of Schalm *et al*. (1975). Total serum protein, serum albumin, serum globulin (Varley *et al*., 1980) and serum uric acid concentration (Wootton, 1964) and serum creatinine (Bonsnes & Taussky, 1945) were measured according to standard procedures. Serum enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST) was measured using commercial Kits (Randox test Kits, Randox Laboratories, Antrim, UK). Cholesterol (Gordon & Amer, 1977) values were recorded while concentration of low density lipoprotein (LDL), high density lipoprotein (HDL) and triglycerides in the serum were recorded (Burtis & Ashwood, 1999).

Carcass and Organs Evaluation

At the end of finisher phase (day 42), two birds per replicate whose weight were close to the average weight of the group were selected and fasted for feed, for 12 hours (in order to empty the gut and prevent carcass contamination) for carcass evaluation. The final live weight of the birds was taken prior to slaughter. The birds were slaughtered by a ventral neck cut with a sharp knife to bleed. Following thorough bleeding, the birds were manually de-feathered by scalding in hot water as described by Hann & Spindler (2012). De-feathered birds were weighed, labelled and eviscerated. Each carcass was cut into their parts and parameters were calculated. Dressing percentage were calculated as:

$$\text{Dressing percentage} = \frac{\text{Dressed weight (kg)}}{\text{Live weight (kg)}} \times 100$$

Visceral organs were expressed as percentage of carcass weight.

Meat Quality and Organoleptic Attribute

Water Holding Capacity (WHC)

The water holding capacity of meat was determined by placing 1.0g of breast meat sample between two filter papers (9cm What man No 1) and then enclosed in between two 10.2 x 10.2cm flexi glasses and pressed between two jaws of a vice with approximately 35.60kg/cm3 for 1 minute. The areas of free water and pressed meat samples were measured using grid method while percent free water was calculated based on weight and moisture content (Suzuki *et al*., 1991).

Water holding capacity was calculated as:

$$\text{WHC} = \frac{100-(\text{Av}-\text{Am})\times 9.47}{\text{Wm}\times\text{Mc}} \times 100$$

Where: Aw = Area of water released from meat samples (cm2)

Am = Area of meat samples (cm2)

Wm = Weight of meat (g)

Mc = Moisture content of meat samples (%)

9.47 = Constant factor.

Cooking Loss

This was determined by collecting samples of known weight (50g) from the breast muscles from each carcass, hygienically wrapped in an air-tight polythene bag, labelled accordingly and immediately cooked in water bath to a temperature of 70°C for 15 minutes. Thereafter, the water released after cooking and cooling was separated and the weight of the cooked meat chops was taken to obtain the cooking loss.

Cooking loss (g) = Weight before cooking – Weight after cooking

$$\text{Cooking loss Percentage} = \frac{\text{Weight before cooking} - \text{Weight after cooking}}{\text{Weight before cooking}} \times 100$$

Chilling Loss

This was determined by measuring the weight of breast meat sample before chilling overnight and the weight after chilling at 4°C. The percent change in weight of meat is known as chilling loss.

$$\text{Chilling loss percentage} = \frac{\text{Weight before chilling} - \text{Weight after chilling}}{\text{Weight before chilling}} \times 100$$

Statistical Analysis

All generated data were subjected to Analysis of Variance (ANOVA) procedures appropriate for a completely randomized design (CRD) using SAS (2008) software package and the means of the parameters ($P < 0.05$) will be separated using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Results

Haematological Indices

At starter phase (Table 2), there was a linear decrease ($P < 0.05$) in packed cell volume (PCV) and haemoglobin (Hb) as the blend increased in the diets up to T5 with birds on the negative control (T1) recorded the highest value for these parameters. Red blood cell (RBC) deceased ($P < 0.05$) with increased level of PB with the least recorded for T2 (antibiotic group). Birds in T2 recorded higher ($P < 0.05$) white blood cell (WBC) concentration but with the least eosinophils (EOS) value. Birds in T6 recorded higher ($P < 0.05$) Lymphocytes (LYM) count but with the least heterophils (HET). Basophil (BAS) was least ($P < 0.05$) in T3 and similar for other treatment groups. Monocytes was significantly higher in T1 and least for T5. Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) were higher ($P < 0.05$) in T1, other treatments were similar. Significant higher ($P < 0.05$) Mean Corpuscular Haemoglobin Concentration (MCHC) was recorded for T2 and least for T1.

Result at the finisher phase (Table 3) revealed that PCV and Hb deceased as PB was supplemented in the diets with the least values ($P < 0.05$) recorded for T2 (antibiotic group). Red blood cell (RBC) was least ($P < 0.05$) for T2, other treatments were similar. Heterophils (HET) was similar among the treatments except T6 that recorded the least value ($P < 0.05$).

Lymphocytes (LYM) was significantly higher in T6 while other treatments were similar. Basophil (BAS) was higher (P <0.05) at T5 and T6 groups while other treatments were similar. Mean Corpuscular Volume (MCV) slightly reduced (P <0.05) with PB supplementation when compared with the two controls. Corpuscular Haemoglobin (MCH) decreased linearly (P <0.05) with PB supplementation when compared with the controls.

Table 2: Effect of Varying Levels of Phytogenic Blends Supplementation on Haematology of Starter Broiler Chickens

Parameters	Level of phytogenic blends								Orthogonal contrast (P-value)		
	T1	T2	T3	T4	T5	T6					
	0g/kg	Antibiotic	5g/kg	10g/kg	15g/kg	20g/kg	SEM	P-value	Linear	Quadratic	Cubic
PCV (%)	32.50^{a}	24.50^{c}	30.50^{ab}	28.50^{abc}	25.50^{c}	26.50^{bc}	0.686	0.000	0.002	0.332	0.042
Hb (g/dl)	10.60^{a}	8.45^{c}	10.25^{ab}	9.70^{abc}	8.50^{c}	9.00^{bc}	0.211	0.001	0.008	0.688	0.188
RBC ($\times 10^{12}$/L)	2.55^{ab}	2.20^{c}	2.85^{a}	2.55^{ab}	2.45^{bc}	2.35^{bc}	0.049	0.000	0.371	0.016	0.124
WBC ($\times 10^{9}$/L)	13.50^{ab}	15.05^{a}	11.40^{b}	12.80^{ab}	14.30^{a}	13.35^{ab}	0.320	0.007	0.748	0.149	0.890
HET. (%)	36.50^{a}	35.50^{a}	36.00^{a}	32.50^{ab}	38.00^{a}	28.00^{b}	0.891	0.003	0.009	0.097	0.041
LYM (%)	59.50^{b}	63.00^{ab}	62.00^{ab}	63.00^{ab}	58.00^{b}	69.50^{a}	1.041	0.012	0.043	0.202	0.007
EOS (%)	1.00^{b}	0.50^{b}	1.00^{b}	2.00^{a}	2.50^{a}	0.50^{b}	0.173	0.000	0.017	0.001	0.000
BAS (%)	1.00^{ab}	0.50^{ab}	0.00^{b}	1.50^{a}	1.50^{a}	1.50^{a}	0.170	0.020	0.022	0.158	0.031
MONO (%)	2.00^{a}	0.50^{b}	1.00^{ab}	1.00^{ab}	0.00^{b}	0.50^{b}	0.167	0.003	0.002	0.148	0.315
MCV (fl)	127.33^{a}	111.37^{b}	107.08^{b}	111.69^{b}	104.00^{b}	113.37^{b}	1.829	0.000	0.001	0.000	0.335
MCH (pg)	41.55^{a}	38.41^{ab}	36.00^{b}	38.02^{ab}	34.66^{b}	38.48^{ab}	0.575	0.002	0.008	0.003	0.832
MCHC (g/dl)	32.63^{c}	34.49^{a}	33.60^{b}	34.04^{ab}	33.31^{bc}	33.97^{ab}	0.142	0.000	0.042	0.008	0.000

^{abc}Mean on the Same Row having Different Superscripts are Significantly Different (P <0.05). PCV=Packed Cell Volume, Hb=Haemoglobin, RBC=Red Blood Cell, WBC=White Blood Cell, HET=Heterophils, LYM=Lymphocytes, -EOS=Eosinophils, BAS=Basophils, MONO=Monocytes, MCV=Mean Corpuscular Volume, MCH=Mean Corpuscular Haemoglobin, MCH=Mean Corpuscular Haemoglobin Concentration, SEM=Standard Error of Means.

Table 3: Effect of Varying Levels of Phytogenic Blends Supplementation on Haematology of Finisher Broiler Chickens

Parameters	Level of phytogenic blends								Orthogonal contrast (P-value)		
	T1	T2	T3	T4	T5	T6					
	0g/kg -ve control	Antibiotic +ve control	5g/kg	10g/kg	15g/kg	20g/kg	SEM	P-value	Linear	Quadratic	Cubic
PCV (%)	45.00^{a}	34.50^{b}	42.50^{ab}	37.50^{ab}	34.50^{b}	37.50^{ab}	1.082	0.006	0.018	0.208	0.514
Hb (g/dl)	15.00^{a}	11.45^{b}	13.90^{ab}	12.75^{ab}	11.55^{b}	12.60^{ab}	0.346	0.006	0.025	0.161	0.348
RBC ($\times 10^{12}$/L)	3.50^{a}	2.55^{b}	3.45^{a}	3.45^{a}	2.90^{ab}	3.32^{a}	0.087	0.000	0.695	0.720	0.043
WBC ($\times 10^{9}$/L)	14.35	15.55	14.95	14.45	13.50	14.10	0.270	0.354	0.162	0.468	0.099
HET. (%)	31.00^{ab}	35.50^{a}	36.00^{a}	36.50^{a}	36.00^{a}	28.00^{b}	0.838	0.002	0.283	0.000	0.291
LYM (%)	67.50^{ab}	63.00^{ab}	62.00^{ab}	60.50^{b}	61.00^{ab}	69.50^{a}	1.005	0.022	0.882	0.001	0.274
EOS (%)	0.50	0.50	1.00	1.50	1.50	0.50	0.180	0.293	0.338	0.089	0.132
BAS (%)	0.00^{b}	0.50^{ab}	0.00^{b}	0.50^{ab}	1.50^{a}	1.50^{a}	0.155	0.000	0.000	0.123	0.641
MONO (%)	1.00	0.50	1.00	1.00	0.00	0.50	0.155	0.349	0.216	0.773	0.844
MCV (fl)	128.01^{a}	135.31^{a}	127.74^{ab}	108.49^{b}	118.69^{ab}	118.08^{ab}	2.311	0.005	0.004	0.229	0.035
MCH (pg)	42.71^{ab}	44.89^{a}	40.49^{ab}	36.94^{b}	39.72^{ab}	39.79^{ab}	0.770	0.036	0.020	0.223	0.106
MCHC (g/dl)	33.40	33.18	32.71	34.06	33.43	33.61	0.147	0.159	0.270	0.665	0.192

^{abc}Mean on the Same Row Having Different Superscripts are Significantly Different (P<0.05). PCV=Packed Cell Volume, Hb=Haemoglobin, RBC=Red Blood Cell, WBC=White Blood Cell, HET=Heterophils, LYM=Lymphocytes, -EOS=Eosinophils, BAS=Basophils, MONO=Monocytes, MCV=Mean Corpuscular Volume, MCH=Mean Corpuscular Haemoglobin, MCH=Mean Corpuscular Haemoglobin Concentration, SEM=Standard Error of Means

Serum Biochemistry

The result of the starter phase (Table 4), indicated that there was a linear increase in Alanine amino transferase (ALT) as the level of PB increased in the diets with highest (P <0.05) value recorded for T6 and lowest for T1. Low-density lipoprotein (LDL) decreased (P <0.05) linearly as the level of PB increased in the diets with the lowest value recorded for T6. Aspartate amino transferase (AST) was significantly (P < 0.05) higher in T6 and least for T3. Alkaline phosphatase (ALP) values increased slightly as the level of PB increased in the diets. Other parameters were not significantly (P > 0.05) affected.

At the finisher phase (Table 5), creatinine was significantly higher (P <0.05) in T3 while other treatments were similar.

Serum cholesterol result is cubic and quadratic in nature, the values increased up to T4 and started decline with increased level of the PB. Triglyceride was least (P <0.05) in T2 while other treatments differ not significantly. Alanine amino transferase (ALT) and high-density lipoprotein (HDL) were significantly higher in T4, other treatments were similar. Very low-density lipoprotein (VLDL) is least (P <0.05) in T2 while other treatments differ not significantly.

Table 4: Effect of Varying Levels of Phytogenic Blends Supplementation on Serum Biochemistry of Starter Broiler Chickens

Parameters	Level of phytogenic blends								Orthogonal contrast (P-value)		
	T1	T2	T3	T4	T5	T6					
	0g/kg	Antibiotic	5g/kg	10g/kg	15g/kg	20g/kg	SEM	P-value	Linear	Quadratic	Cubic
Total Protein (g/dl)	6.05	6.05	5.90	5.25	5.90	5.25	0.14	0.30	0.08	0.99	0.94
Albumin (g/dl)	3.60	3.70	3.95	3.05	3.55	3.55	0.12	0.46	0.53	0.86	0.29
Globulin (g/dl)	2.45	2.35	1.95	2.20	2.40	1.70	0.19	0.87	0.43	0.90	0.45
Creatinine (mg/dl)	1.30	1.00	1.64	1.74	0.58	1.60	1.15	0.20	0.92	0.85	0.40
Urea (mg/dl)	12.15	10.45	11.30	11.00	10.50	11.65	0.21	0.11	0.49	0.05	0.79
Cholesterol (mg/dl)	237.00	246.70	224.25	212.70	198.80	205.45	6.26	0.19	0.02	0.89	0.26
Triglyceride (mg/dl)	178.65	175.70	171.60	208.30	172.05	208.35	7.60	0.52	0.28	0.7	0.92
AST (iu/l)	73.00^{bc}	85.00^{b}	70.50^{c}	85.50^{b}	78.00^{bc}	109.00^{a}	2.86	0.00	0.00	0.00	0.001
ALT (iu/l)	66.00^{b}	77.5^{b}	77.00^{b}	97.5^{ab}	81.50^{ab}	118.00^{a}	4.68	0.01	0.001	0.43	0.20
ALP (iu/l)	37.00^{b}	39.00^{ab}	39.00^{ab}	43.50^{ab}	50.00^{a}	34.50^{b}	1.45	0.01	0.29	0.03	0.01
HDL (mg/dl)	118.50	123.35	112.10	106.35	99.40	102.75	3.13	0.19	0.02	0.88	0.26
LDL (mg/dl)	82.75^{ab}	88.20^{a}	77.80^{ab}	64.70^{ab}	65.00^{ab}	61.10^{b}	2.90	0.01	0.00	0.94	0.16
VLDL (mg/dl)	35.75	35.15	34.30	41.65	34.45	41.65	1.52	0.52	0.29	0.70	0.92

^{abc}Mean on the Same Row Having Different Superscripts are Significantly Different (P<0.05). SEM=Standard Error of Means. AST=Aspartate Transaminase, ALT = Alanine Transaminase, ALP= Alanine Phosphatase, HDL=High Density Lipoprotein, LDL=Low Density Lipoprotein, VLDL=Very Low-Density Lipoprotein

Table 5: Effect of Varying Levels of Phytogenic Blends Supplementation on Serum Biochemistry of Finisher Broiler Chickens

Treatment	Level of phytogenic blends								Orthogonal contrast (P-value)		
	T1	T2	T3	T4	T5	T6					
Phytogenic (g/kg)	0g/kg	Antibiotic	5g/kg	10g/kg	15g/kg	20g/kg	SEM	P-value	Linear	Quadratic	Cubic
Total Protein (g/dl)	6.35	8.05	5.80	7.45	7.00	8.2	0.31	0.15	0.20	0.47	0.30
Albumin (g/dl)	4.80	4.55	4.50	4.80	4.40	5.10	0.77	0.08	0.35	0.04	0.56
Globulin (g/dl)	1.55	3.50	1.25	2.65	2.60	3.10	0.31	0.24	0.29	0.81	0.39
Creatinine (mg/dl)	2.27^{ab}	1.58^{b}	2.77^{a}	1.93^{ab}	1.57^{b}	2.38^{ab}	0.13	0.02	0.90	0.57	0.25
Urea (mg/dl)	13.05	15.05	14.70	14.35	13.40	12.55	0.44	0.55	0.41	0.12	0.49
Cholesterol (mg/dl)	196.40^{b}	189.00^{b}	217.60^{ab}	262.55^{a}	218.45^{ab}	193.10^{b}	6.71	0.003	0.25	0.00	0.02
Triglyceride (mg/dl)	254.95^{a}	139.85^{b}	260.65^{a}	268.90^{a}	177.05^{ab}	179.10^{ab}	13.16	0.002	0.18	0.21	0.04
AST (iu/l)	105.00	109.50	118.50	121.50	113.5	106.00	3.21	0.65	0.77	0.10	0.75
ALT (iu/l)	58.00^{b}	63.00^{b}	54.5^{b}	93.5^{a}	54.00^{b}	57.00^{b}	3.16	0.00	0.82	0.00	0.06
ALP (iu/l)	58.00	48.00	53.00	76.00	57.00	65.00	3.22	0.15	0.18	0.93	0.23
HDL (mg/dl)	98.15^{b}	94.5^{b}	108.85^{ab}	131.30^{a}	109.20^{ab}	96.55^{b}	3.35	0.003	0.25	0.00	0.02

Treatment	Level of phytogenic blends								Orthogonal contrast (P-value)		
	T1	T2	T3	T4	T5	T6					
Phytogenic (g/kg)	0g/kg	Antibiotic	5g/kg	10g/kg	15g/kg	20g/kg	SEM	P-value	Linear	Quadratic	Cubic
LDL (mg/dl)	47.20	66.55	56.70	77.50	73.80	60.75	3.71	0.17	0.13	0.09	0.56
VLDL (mg/dl)	51.00^{a}	28.00^{b}	52.10^{a}	53.75^{a}	35.40^{ab}	35.80^{ab}	2.63	0.002	0.18	0.21	0.04

^{ab}Mean on the Same Row Having Different Superscripts are Significantly Different (P<0.05). SEM=Standard Error of Means. AST=Aspartate Transaminase, ALT = Alanine Transaminase, ALP= Alanine Phosphatase, HDL=High Density Lipoprotein, LDL=Low Density Lipoprotein, VLDL=Very Low-Density Lipoprotein

Carcass Intestinal and Organs Traits

Table 6 shows that significant (P <0.05) higher breast meat was recorded for birds in T2 (antibiotic group), other treatments were similar. There was a slight decrease (P <0.05) in back percentage as the PB increased in the diets. Birds on antibiotic (T2) recorded significant (P <0.05) higher spleen and kidney while birds in T1 had significant higher liver. Birds in T5 recorded higher (P <0.05) jejunum while birds in T6 recorded significant (P <0.05) higher whole gizzard and abdominal fat. Other parameters did not differ significantly among treatment groups.

Meat Quality

The result of meat quality (Table 7). Percentage water absorption (PWA) was significantly (P <0.05) higher in T3 which did not differ significantly from T2 and T4. Birds in T5 recorded highest (P <0.05) percentage cooking loss (PCL) which did not differ significantly from birds in T2, T3 and T4. Percentage refrigeration loss (PRL) was highest (P <0.05) for birds in T4 and lowest for birds in T1 (negative control), other treatments were similar.

Table 6: Carcass and Cut Parts Characteristics (%) of Broiler Chickens Fed Diets Containing Varying Levels of Phytogenic Blends

Parameters	T1 (-ve) (0g/kg)	T2 (+ve) antibiotic	T3 (5g/kg)	T4 (10g/kg)	T5 (15g/kg)	T6 (20g/kg)	SEM	P-value
Live weight (g)	2017.50	2025.00	2200.00	2145.00	2000.00	2200.00	30.432	0.141
Eviscerated weight (g)	14920.75	1484.50	1620.50	1573.25	1488.00	1620.50	22.599	0.180
% Live Weight								
Head	2.33	2.68	2.78	2.29	1.90	1.93	0.118	0.144
Neck	3.11	3.37	3.39	3.44	3.55	3.33	0.090	0.865
Back	13.20^{ab}	15.92^{a}	11.98^{ab}	13.02^{ab}	10.26^{b}	10.78^{b}	0.524	0.009
Breast	20.95^{b}	26.89^{a}	19.45^{b}	22.37^{b}	16.70^{b}	18.0^{b}	0.848	0.001
Thighs	9.11	11.14	7.30	10.63	8.70	8.50	0.531	0.315
Drumsticks	9.20	10.10	9.21	10.70	8.44	0.30	0.280	0.241
Shanks	4.01	4.17	3.97	3.69	3.57	3.52	0.145	0.780
Wings	7.34	8.52	7.04	7.80	6.70	7.62	0.220	0.189

SEM: Standard Error of the Mean. ^{ab}Means with Different Superscript are Significantly Different (P<0.05)

Table 7: Meat Quality of Broiler Chicken Fed Diets Supplemented with Phytogenic Blends

Parameter	Level of phytogenic blends							
	T1	T2	T3	T4	T5	T6	SEM	P-value
Water absorption	0.27b	0.33ab	0.42a	0.30ab	0.21b	0.21b	0.0206	0.008
Percentage water absorption	8.78b	10.01ab	13.52a	9.19ab	6.80b	6.80b	0.6472	0.005
Cooking loss	23.07	25.73	31.12	28.94	27.36	27.36	1.0440	0.126
Percentage cooking loss	24.54b	26.70ab	26.07ab	30.43ab	32.83a	32.83a	0.9711	0.020
Refrigeration loss	0.41c	4.29ab	5.79a	1.77bc	2.50bc	2.50bc	0.5429	0.003
Percentage refrigeration loss	0.50c	4.28ab	5.16ab	7.81a	3.54ab	3.54ab	0.8379	0.050

SEM = Standard Error of Mean. ^{abc}Means with Different Superscript are Significantly Different (P<0.05)

Discussion

According to Purves *et al*. (2003) packed cell volume (PCV) is the percentage of red blood cells (RBC). The present result is in line with the findings of Adegoke *et al*. (2018) who reported similar values when they fed starter broiler chickens with diets containing turmeric powder and cayenne pepper. The values obtained falls within the normal range of 25–41% reported by Mitruka and Rawnsley (1997). It has been reported (Bashar *et al*., 2010) that PCV is the quickest indirect way of determining RBC in circulation and is often used to screen for anaemia. The site of RBC production is the long bone and its production depends on the amount of iron absorbed from ingested food. Haemoglobin is about one-third (Reece, 2009) of erythrocytes which generate increased haemoglobin (HB) concentration (Sugiharto *et al*., 2011). The values for these parameters (PCV, RBC and Hb) slightly declined as the level of the blend increased in the diet. Most phytogenic contains phenolic compound which according to (Siriporn *et al*., 2006) can inhibit iron absorption. This assertion was corroborated by Galib *et al.* (2011) who reported a significant decline in haemoglobin levels as consumption of dietary pepper in broiler chick diet increased.

This possibly explains why chicks fed diets containing higher inclusion level of the blend had lowest circulating erythrocytes and haemoglobin levels. The immunity value of the birds increased as the level of the blend increased in the diets which is similar to the control groups is an indication that the blend assisted the bird's immunity through stem cell production of the white blood cells (WBC). The mean cell haemoglobin concentration (MCHC) of 32.63-34.49 values reported in this study is similar to 26.38–33.04% by Azodo *et al.* (2021) and within the normal range of 25.3–32.5% reported by Mitruka and Rawnsley (1977). The normal physiological range for the MCHC reported in this study was a clear indication that the birds were not anaemic.

According to Upah *et al*. (2024) PCV and Hb are two parameters of high significance in determining health status of broiler chickens. The parameters were affected by levels of PB inclusion but both values were within the normal range (PCV = 35.9% - 41.0%; 25.20% - 45.20% and 11.60 - 13.68 g/dl; 10.02 - 15.1 g/dl) described for healthy birds by Hodgers, (1977) and Aeangwanich *et al*. (2004) respectively. The present results is an indication that the birds were healthy and the PB was not hazardous to the birds. Red blood cell is produced in the long bones of the body, and adequate production is dependent on the amount of iron absorbed from food digested. The red blood cell (RBC) increased slightly with the supplementation of PB in the diet when compared with the birds on diet with antibiotic. The basal group had the highest values for PCV, Hb and RBC, the reason for this may be because of phenol compound present in the PB. It has stated (Siriporn *et al.,* 2006) that this compound can inhibit iron absorption. This assertion has been corroborated by Galib *et al*. (2011) that there was significant decline in haemoglobin levels as consumption of dietary pepper in broiler chick diet increased at the third week experiment. The result of Adegoke *et al*. (2018) agreed with these findings. The value of MCV and MCH in this study is in tandem with normal physiological range of 89 - 203 fl and 39 - 61 pg reported by Aiello and Mays for MCV and MCH respectively. The present finding is similar to the values of 175.30 fl and 55.80 pg observed for MCV and MCH by Esiegwu and Obih (2019) who fed broiler chickens with graded levels of *Artenia urmania* powder. Upah *et al*. (2024) has reported similar results when finisher broiler chickens were fed graded levels of spurge weeds (*Euphorbia heterophylla*) leaf meal. Al-Nedawi, (2018) has opined that the variation in the values of MCV and MCH could be the consequence of the effect of age, sex, weight, type of diet, strain and climate on chickens, indicated that the observed significant difference in the values of MCV and MCH in the present study may not be deleterious to the birds as RBC, WBC, PVC, Hb and MCHC values were within normal physiological range. It has been stated (Upah *et al*., 2024) that the blood parameters with greater values could be a consequence of wide range of ambient temperature as previously reported by Nanbol *et al*. (2016). Increase in WBC differentials in the blood indicated enhanced immunological well-being of the body (Azodo *et al*., 2021). The lymphocyte (60.5-69.5) and heterophils (28-36.5) reported in this study are in the range of 45%-70%; 20%-50% respectively reported by Mitruka and Rawnsley (1977) which also agreed with the findings of Upah *et al*. (2024). Basophil significantly increased as the PB was supplemented in the diets is an indication that the PB may have stimulated immune response. This report did not support the findings of Upah *et al*. (2024) as they reported non-significant difference among the treatments.

Serum protein (SP), albumin and globulin reported in this study indicated that there was no treatment effect on these parameters this contradict the findings of Azodo *et al*. (2021) who reported significant higher SP and albumin for the basal group when broiler chicks were fed diet supplemented with turmeric and Negro pepper mixture. Adekoge *et al*. (2018) has earlier reported that broilers chicks fed the basal diet had significantly better serum protein, globulin and albumin than the other dietary groups. The discrepancies in these findings could be in the composition of the mixture, dosage or rate of supplementation and mode of application. There was a numerical linear reduction in cholesterol as PB was added into the diets. The activities of the liver enzymes; aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) in the blood are bio-indicators of liver function and damage (Yildirim *et al*., 2011). An increase in the concentration of liver enzymes has been linked to liver or muscle damage (Lumeij, 2008) resulting from the body's response to stress. Liver enzymes in the present study were significantly affected by the dietary supplementation of PB with birds on diets with 15 and 20g/kg of PB reported to have higher values for these parameters. Deviance to this finding, Adekoge *et al*. (2018) reported higher values for AST and ALT in the basal group. Additionally, Azodo *et al*. (2024) reported significant difference only for ALP. The increased in liver enzymes with higher doses of PB supplementation could not have been as a result of liver damage because mortality was across the treatments with lesser proportion recorded for these groups. Moreover, black pepper one of the component of the PB is rich in vitamins including vitamin B6 (pyridoxine) which is essential for the activities of AST. Harr, (2002) stated that ALT is still of poor diagnostic value in birds due to its existence in many tissues while AST is a very sensitive and non-specific bio-marker of liver disease in birds. The hypolipidemic effect observed in birds fed with dietary inclusions of PB could be attributed to the high fibre content of the blend. Fibre are known to decrease lipid absorption by adsorbing fatty acids, cholesterol and bile acids in the digestive tract, this prevent formation of micelles and cannot be absorbed in their bound form. The fibre-bound lipids are transported to the large intestine where they are excreted or degraded by colonic bacteria (Udeme *et al*., 2013). The observed decrease in the serum LDL-C level suggest a reduced risk in the development of atherosclerosis.

The results of serum biochemistry at the finisher phase is at divergence with the report of Azodo *et al*. (2021). In the present study cholesterol, triglyceride and HDL significantly increased with dietary PB supplementation while VLDL reduced. The cholesterol (193.10-262.55) reported in this study is higher than 52.00–148.00 mg dl–1 and 85.18-124.92 reported by Mitruka and Rawnsley, (1977) and Azodo *et al*. (2021) respectively. In the findings of Fallah and Mirzaei (2016), they discovered that supplementation of turmeric and thyme in the diets of broiler chickens ate day 42 reduced cholesterol, HDL and LDL. However, Adebiyi *et al*. (2020) reported increased HDL in rat fed diet supplemented with *Dennetia tripetala* (pepper fruit). The disparity in these findings could be as a result of diet and composition. When the energy need of birds is met, the excess is used to lay fat. Abdulkarim *et al*. (2013) opined that higher serum cholesterol may be the result of an increased lipid digestibility due to a higher secretion of bile and digestive enzymes. The hyperlipidemic effect observed in birds fed with dietary inclusions of PB did not result in liver damage because the liver enzymes were statistically similar across the treatment groups. Additionally, the slight mortality reported was evened out since it was not connected to a specific treatment. More so, it was the "good" cholesterol (HDL) that was increased while VLDL reduced significantly.

Carcass traits of broiler chickens could be improve by dietary supplementation of PFA (Jamroz *et al*. 2003; Mech *et al*. 2021). The observed significance decrease in breast and back with the supplementation of PB did not agree with the report of Petričević *et al*. (2021) when broiler chickens fed diet with (*Menthe piperita L*) peppermint. Fasasi *et al*. (2020) has equally reported that dietary supplementation of phytogenic blend had no effect on the carcass of broiler chickens. In addition, Tanimowo *et al*. (2026) reported no significant effect of diets supplemented with guava leaf meal of carcass yield. However, Oso *et al*. (2021) discovered that broiler chickens diets supplemented with blend of *A. lanata*, *C. dactylon, P. betle and P. nigrum* did not influence the breast meat but decrease the back. According to Oso *et al*. (2021), carcass traits are a reflection of growth response this may explain the reduction in the breast meat of birds fed PB when compared with those on antibiotic fed diet. Generally, the discrepancies in these findings could be as a result of constituents of the blends and rate of usage in the diets. Moreover, different phytogenic ingredients have different intensities and effectiveness in body of birds (Pooja *et al*., 2023).

Water-holding capacity (WHC) is one of the most important quality attributes of meat, in particular with regard to its processing suitability (Lipiński *et al*., 2019). The observed decrease in WHC at higher dosage of PB in this study align with the report of Lipiński *et al*. (2019) who fed broiler chickens with low energy diet supplemented with herbal feed additive. Other studies with phytogenic (Zdanowska-Sasiadek *et al*., 2019 and Purwanti *et al*., 2019) has revealed reduction in WHC of broiler chicken meat. However, the reports of Reis *et al*. (2018) and Puvača *et al*. (2014) indicated increased WHC of meat in broiler chickens receiving a feed additive based on essential oils and garlic powder respectively. Contrary to these findings, Küçükyilmaz *et al*. (2017) and (Salajegheh *et al*., 2018) found no significant differences in the WHC of meat of broiler chickens fed diets supplemented with lavender essential oil and lavender powder respectively. According to Barbera and Tassone. (2006) cooking loss percentage (CLP) is the most important physical characteristic (juiciness) as it directly affect the economic and palatability of processed meats. The present result indicated that CLP reduced significantly as PB was supplemented in the diets when compared with meat from birds fed basal diet only. This is consistence with the finding of Fakolade *et al*. (2016) who reported reduced CLP in meat of broiler chickens fed diets with honey supplementation. It may be that the PB contain dehydrating agent that makes the meat lost it moisture.

CONCLUSIONS

The findings of the present study revealed that the blend of *Piper nigrum, Zingiber officinale, Azadirachta indica, Cymbopogon citratus and Citrus sinensis* can be added in the diets of broiler chickens up to 20g/kg instead of pure antibiotic without negatively affecting their health, carcass and meat quality.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

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