Poultry Science Journal
ISSN: 2345-6604 (Print), 2345-6566 (Online) http://psj.gau.ac.ir
DOI: 10.22069/psj.2021.18370.1627

Influence of Dietary Supplementation of *Kigelia pinnata* and *Plukenetia conophora* Leaves on Cytokine Expression, Immunoglobulins, Blood Chemistry, Caecal Microbiota and Meat Quality in Broiler Chickens

Adeyemi KD, Adegoke MA, Mudashir MO, Owoyomi FM, Hamzat TO, Adeleke IA, Ibrahim SO & Abdulrahman A

Department of Animal Production, Faculty of Agriculture, University of Ilorin, PMB 1515 Ilorin, Nigeria

Poultry Science Journal 2021, 9(1): 27-39

Keywords
E. coli
Salmonella
Antioxidant
Immune status
Growth performance

Corresponding author
Kazeem D. Adeyemi
adeyemi.kd@unilorin.edu.ng

Article history
Received: September 16, 2020
Revised: December 18, 2020
Accepted: December 28, 2020

Abstract

This study examined the effect of dietary supplementation of *Kigelia pinnata* (KPL) and *Plukenetia conophora* (PCL) leaves in comparison with oxytetracycline (OXY) and butylated hydroxyanisole (BHA) on growth performance, selected blood biochemical parameters, caecal microbiota, splenic interleukins (IL), serum immunoglobulins (Ig), carcass traits, meat quality, and oxidative stability in broiler chickens. One day old Arbor Acres chicks (n=420) were randomly assigned to either basal diet only (BD); basal diet + 0.5 g/kg oxytetracycline + 0.12 g/kg BHA (OXYBHA); basal diet + 1 g/kg KPL (KPL-1); basal diet + 2 g/kg KPL (KPL-2); basal diet + 1 g/kg PCL (PCL-1); or basal diet + 2 g/kg PCL (PCL-2) for 42 d. Each dietary treatment had seven replicates with 10 chicks per replicate. Supplemented birds gained ($P < 0.05$) more weight and had a better feed conversion ratio compared with the BD birds. Hematological indices, IL-1β, and IL-6 did not differ among the treatments. BD birds had lower ($P < 0.05$) carcass weight and IL-10, and higher ($P < 0.05$) IgG, IgM, *Salmonella* spp., and *E. coli* counts than the supplemented birds. The KPL-2 birds had the least ($P < 0.05$) *E. coli* and *Salmonella* spp. counts and IgM among the supplemented birds. *Lactobacillus* spp. count was lower ($P < 0.05$) in OXYBHA birds compared with KPL and PCL birds. Carbonyl and malondialdehyde contents in the *Sartorius* muscle, and drip loss and carbonyl content in the *Pectoralis* muscle of the BD birds were higher ($P < 0.05$) than those of the supplemented birds. These results illustrate that the 2 g/kg KPL and 2 g/kg PCL could be used as an antioxidant and an antimicrobial in the diets of broiler chickens.

Introduction

One of the major concerns of contemporary animal agriculture is the shortcomings associated with the use of synthetic additives. The global estimated consumption of antibiotics in animal production and humans was between 10,000 to 20,000 tons (Manzetti and Ghisi, 2014) and it is projected to reach 105,596 (±3605) tons by 2030 (Van Boeckel *et al.,* 2015). The release of a large quantity of antibiotics encourages the cycle of bioaccumulation and biotransformation of antibiotics in the environment, which could have severe consequences on human and animal health (Sivagami *et al.,* 2020; Van *et al.,* 2020). In the hope of preserving human and animal health, there have been changes in legislation culminating in the ban or strict restrictions on the usage of antibiotics in animal feed (FDA, 2013; EFSA, 2016; NAFDAC, 2017). This scenario highlights the need to explore potential alternatives to antibiotics.

The chicken gut harbors numerous microbes whose abundance and diversity are amenable to changes in production factors and could have significant implications for production efficiency, health, and welfare of birds, environmental impact, and food safety (Kogut, 2019; Oviedo-Rondón, 2019; Kogut *et al*., 2020). Owing to the crucial role of the gastrointestinal tract in broiler production (Shang *et al.,* 2018; Kogut *et al*., 2020), the implications of

Please cite this article as Adeyemi KD, Adegoke MA, Mudashir MO, Owoyomi FM, Hamzat TO, Adeleke IA, Ibrahim SO & Abdulrahman A. 2021. Influence of Dietary Supplementation of *Kigelia pinnata* and *Plukenetia conophora* Leaves on Cytokine Expression, Immunoglobulins, Blood Chemistry, Caecal Microbiota and Meat Quality in Broiler Chickens. Poult. Sci. J. 9(1): 27-39.

probable changes in gut microbiota due to dietary supplements have been the focus of a plethora of studies (Dalal *et al.,* 2018; Oloruntola *et al.,* 2019; Vanessa *et al.,* 2019). The alterations in gut microbiota could stimulate an innate and adaptive immune response that could affect the health and production performance of broiler chickens (Oviedo-Rondón, 2019; Kogut *et al*., 2020).

Polyunsaturated fatty acids, which are abundant in broiler meat, are the major substrate for oxidative deteriorations (Tao, 2015; Adeyemi *et al*., 2020). Oxidative deterioration could have severe consequences on the nutritional quality, shelf life, and safety of meat (Iqbal *et al.,* 2015; Tao, 2015; Nakyinsige *et al*., 2016). The available scientific evidence illustrates that the dietary supplementation of antioxidants in livestock could prevent oxidative spoilage in meat (Iqbal *et al.,* 2015; Odhaib *et al.,* 2018b). Synthetic antioxidants are highly potent in maintaining the oxidative stability of foods (Pokorný, 2007; Adeyemi, 2021). However, recent toxicological evidence has imposed some cautions in their use (Yang *et al.,* 2018; Du *et al*., 2019). Moreover, the decline in the social acceptance of synthetic antioxidants has shifted research focus to alternative sources of antioxidants in livestock production.

In-feed use of medicinal plants is one of the possible alternatives for synthetic additives in animal husbandry (Hashemi and Davoodi, 2011). However, the available data on the potentials of medicinal plants as an alternative to antibiotics (Ansari *et al.,* 2013; Vanessa *et al.,* 2019; Oloruntola *et al.,* 2019) and synthetic antioxidants (Olorunsanya *et al*., 2012; Iqbal *et al.,* 2015; Adeyemi, 2021) are inconsistent. Thus, the elucidation of the antimicrobial and antioxidant potentials of medicinal plants requires, at least, some degree of coherent and systemic trials in different production systems.

Kigelia pinnata (Jacq.) DC and *Plukenetia conophora* (Mull. Arg) belong to the family *Bignoniaceae* and *Euphorbiaceae* respectively and both are found in the subtropical and tropical regions (Ajaiyeoba and Fadare, 2006; Gouda *et al.,* 2006). The phytochemical contents, and the ethnomedicinal, antimicrobial, and antioxidant properties of *K. pinnata* (Gouda *et al.,* 2006; Hussain *et al.,* 2016) and *P. conophora* (Ajaiyeoba and Fadare, 2006; Maduka *et al.,* 2018) have been documented. However, to date, scientific literature contains no information on the antimicrobial and antioxidant potential of *K. pinnata* and *P. conophora* leaves in broilers. This study aimed to examine the growth performance, immune indices, caecal microbiota, selected biochemical blood parameters, carcass traits, and meat quality in broiler chickens fed a diet supplemented with *K. pinnata* and *P. conophora* leaves in comparison with oxytetracycline, and butylated hydroxyanisole.

Materials and Methods

Animal welfare and ethics

The experimental procedures were approved (FERC/ASN/2018/062) by the Animal Care and Ethics Committee, University of Ilorin, Ilorin, Nigeria. All animal procedures were carried out following the animal welfare standards of the Department of Animal Production and Animal Husbandry Services, Federal Ministry of Agriculture and Rural Development, Nigeria.

Collection, processing, and phytochemical analysis of leaves

Fresh *K. pinnata* leaf (KPL) was harvested within the Ilorin metropolis, Kwara State, Nigeria. Fresh *P. conophora* leaf (PCL) was harvested at Osunjela, Osun State, Nigeria. The identity of the leaves was ascertained at the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. The leaves were air-dried at 35±2^{o}C for 4 days and milled (BLG 699, Binatone Limited, Hong Kong) into powder to pass through a 0.5 mm sieve. The leaf powders were packaged in polythene plastic bags, sealed, and kept at 34±3^{o}C until needed. Qualitative phytochemical screening was carried out according to the procedure described by Odebiyi and Sofowora (1978). Thereafter, the detected phytochemicals were quantified as follows. Total flavonoid was determined by the aluminum chloride method with quercetin as the standard (Talari *et al*., 2012). Results were expressed as mg quercetin equivalent (QE)/ 100 g dry weight (DW). Total polyphenol was determined by the Folin-Cicocalteau assay using gallic acid as the standard (Makkar *et al*., 2009). Results were expressed as mg gallic acid equivalent (GAE)/ 100 g DW. Coumarin content was determined as described by Yuying et al. (2005). Tannin content was determined by the Folin-Denis colorimetric method using tannic acid as the standard (Swain, 1979). Terpenoid and steroid were quantified as described by Makkar *et al*. (2009) and Oyekale *et al*. (2015). Alkaloid was quantified by distillation and titrimetric methods as described by Tolkachev *et al.* (1983). Anthocyanin content was quantified by the pH differential method described by Abu Bakar *et al*. (2009). The phytochemical composition of KPL and PCL is presented in Table 1.

Birds, Husbandry, and Experimental diets

One-day-old male Arbor Acres chicks (n=420) were obtained from a commercial hatchery. Upon arrival, the birds were weighed, and randomly distributed into 42-floor pens (1.45 m^2) consisting of wood shavings and sawdust (70:30) spread to a depth of 6 cm. The birds were vaccinated against infectious bursa disease on d 7 and 21, and Newcastle disease on d 14 and 28. Birds were allowed *ad libitum* access to water and feed during the trial. The birds were kept at 34^{o}C for

the first 7 days. Afterward, the temperature was reduced by 3°C per week until it reached 26°C. During the first week, 22 h of light was provided. Thereafter, the light hour was reduced to 17L:7D and remained constant until the end of the experiment. Birds were inspected daily and dead birds were removed following the recording of mortality (pen, date, and bodyweight).

The feeding program consisted of starter (1-21 d) and finisher (22-42 d) basal diets that were formulated according to the National Research Council (NRC, 1994) requirements. The pens were randomly assigned to either BD, basal diet only; OXYBHA, BD + 0.5 g/kg oxytetracycline + 0.12 g/kg butylated hydroxyanisole; KPL-1, BD + 1 g/kg *Kigelia pinnata* leaf; KPL-2, BD + 2 g/kg *Kigelia pinnata* leaf; PCL-1, BD + 1 g/kg *Plukenetia conophora* leaf; or PCL-2, BD + 2 g/kg *Plukenetia conophora* leaf. Each dietary group had seven replicates with 10 birds per replicate. The chemical composition of the basal diets was determined following the methods of the Association of Official Analytical Chemists (AOAC, 2000) and presented in Table 2. Feed was offered as mash (milled to pass through 2 mm-screen for starter diet and 4 mm-screen for finisher diet) and was prepared weekly. Each supplement was primarily mixed with a small quantity of its respective basal diet, then added to the remaining portion of the basal diet and mixed thoroughly.

Table 1. Phytochemical contents of *Kigelia pinnata* and *Plukenetia conophora* leaves

Phytochemical	*Kigelia pinnata* leaf	*Plukenetia conophora* leaf
Total polyphenol (mg GAE[1]/100 g DW[2])	48.45	54.34
Flavonoids (mg QE[3]/ 100 g DW)	38.40	33.70
Saponin (mg/ 100 g DW)	0.54	0.11
Alkaloids (mg/ 100 g DW)	0.90	0.56
Tannin (mg/ 100 g DW)	0.20	0.60
Phytate (mg/ 100 g DW)	1.33	-
Coumarin (mg/ 100 g DW)	-	0.84
Anthocyanine (mg/ 100 g DW)	-	0.05
Terpenoids (mg/ 100 g DW)	-	0.09
Steroids (mg/ 100 g DW)	0.36	-

[1]gallic acid equivalent. [2]dry weight. [3]quercetin equivalent.

Table 2. Ingredients and chemical composition of basal diet

Item	Starter	Finisher
Feed Ingredients (%)		
Maize	54.00	59.00
Soybean meal	31.00	22.00
Groundnut cake	5.00	10.00
Fish meal	5.00	3.00
Bone meal	2.25	2.25
Oyster shell	1.00	1.00
Dicalcium phosphate	1.00	2.00
DL-Methionine	0.15	0.15
L-Lysine HCL	0.10	0.10
Salt	0.25	0.25
Vitamin-mineral Premix[1]	0.25	0.25
Analyzed Composition		
Dry matter (%)	92.38	93.56
Ether extract (%)	5.21	5.22
Crude protein (%)	24.54	21.56
Crude fiber (%)	3.75	4.24
Ash (%)	3.55	3.74
Calculated analysis		
Metabolizable energy (kcal/kg)	3026.00	3200.00
Crude protein (%)	24.32	21.56
Calcium (%)	1.45	1.58
Phosphorus (%)	0.68	0.74
Methionine (%)	0.70	0.61
Lysine (%)	1.38	1.14

[1]Supplied per kg diet: Retinol 12000 IU; thiamine 1.43 mg; cholecalciferol 3500 IU; Niacin 40.17 mg; α-tocopherol 44.7 IU; riboflavin 3.44 mg; pantothenic acid 6.46 mg; pyridoxine 2.29 mg; biotin 0.05 mg; folic acid 0.56 mg; cyanocobalamin 0.05 mg; menadione 2.29 mg; Iron 120 mg; Zinc 120 mg; copper 15 mg; manganese 150 mg; cobalt 0.4 mg; selenium 0.3 mg; iodine 1.5 mg.

Growth performance
Feed intake (FI) and body weight of the birds per pen were measured weekly. Body weight gain (BWG), and feed conversion ratio (FCR) per pen were calculated. Mortality was recorded as and when it occurred. The FCR was adjusted for mortality.

Blood sampling and analysis
On day 41, blood samples were collected from randomly selected five birds per pen via brachial venipuncture into plain and EDTA bottles. Hematological parameters were determined with Sysmex-K 1000 (Sysmex Corporation, Kobe, Japan). Serum was obtained after centrifuging (3000 g, 10°C, 15 min) the blood samples in the plain bottles. Serum lipids were determined using an ELISA kit (ab65390, ABCAM, UK). Serum alanine transaminase (ALT) and aspartate transaminase (AST) were determined using Randox test kits (Randox Laboratories, WV, USA). Urea (DIUR-100), uric acid (DIUA-250), and creatinine (DICT-500) were determined using QuantiChrom™ Assay Kit (Bioassay Systems, Hayward, CA, USA). All assays were carried out according to the manufacturer's procedure. Total serum protein was assayed according to the method of Tietz (1995). Serum immunoglobulin (Ig) was determined with ELISA kits following the manufacturer's protocol. Serum IgG was assayed with Chicken IgG ELISA kit (Cat # MBS260043, MyBioSource, San Diego, CA 92195-3308, USA). Serum IgM was determined with chicken IgM ELISA kit (Cat # CSB-E11232Ch, CUSABIO Technology, Houston, TX 77054, USA).

Slaughter, and carcass analysis
On d 42, five birds per pen whose body weights were close to the mean weight of each replicate were deprived of feed overnight but had *ad libitum* access to water, and euthanized. After bleeding, scalding, plucking, and washing, the feet, head, and neck were removed and the carcasses were manually eviscerated.

The weight of carcass, carcass cuts, internal organs, and abdominal fat were measured. The weight of carcass cuts was expressed as a percentage of carcass weight, while the weight of internal organs and abdominal fat were expressed as a percentage of the live body weight of birds. The dressing percentage was calculated as follows:

$$Dressing\ \% = [Carcass\ weight \div Live\ weight] \times 100$$

Splenic cytokine expression
Spleen samples were aseptically excised from three birds per pen. A 100 mg of spleen was rinsed with phosphate buffer saline (PBS), homogenized in 1 mL of PBS, and stored overnight at -20°C. Thereafter, two freeze-thaw cycles were performed to break the cell membranes. The homogenates were centrifuged for 5 min at 5000 x g, at 4°C. The supernatant was removed and assayed immediately. Interleukins (IL) in the spleen samples were determined with ELISA kits according to the manufacturer's instructions. The IL-6 was determined using a chicken IL-6 ELISA kit (Cat # CSB-E08549Ch, CUSABIO Technology, Houston, TX 77054, USA). The IL-1β was determined using a chicken IL-1β ELISA kit (Cat # CSB-E11230C, CUSABIO Technology, Houston, TX 77054, USA). The IL-10 was determined using a chicken IL-10 ELISA kit (Cat # CSB-E12835C, CUSABIO Technology, Houston, TX 77054, USA).

Selected Caecal microbial population
Fresh caecal digesta was collected from three birds per pen. Digesta was sampled from the right and left caeca into sterile bijou bottles (Thermo Scientific™ Waltham, MA 02145, USA). Digesta (1 g) was introduced aseptically into a test tube containing 9 mL of PBS. The mixture was vortexed and dilution was made up to a ten-fold serial dilution. One mL of the mixture was removed from the test tube and introduced into Petri dishes and a sterile molten agar was introduced. *Escherichia coli* was cultured on eosin methylene blue agar (Merck-1.01347.0500, Merck KGaA, Darmstadt, Germany) and incubated at 37°C for 24 h, *Salmonella* spp. was counted on *Salmonella Shigella* agar (Merck-107667, Merck KGaA, Darmstadt, Germany) and incubated at 37°C for 48 h (Edwards and Hilderbrand, 1976). *Lactobacilli* spp. was cultured on Man Rogosa Sharpe agar (Merck-1.10660.500, Merck KGaA, Darmstadt, Germany) and incubated at 37°C for 48 h (Baurhoo *et al.,* 2007). All agars were prepared according to the manufacturer's instructions. Bacterial units were counted with a colony counter (Stuart®; Burlington, VT, USA). Bacterial counts were expressed as $\log_{10}$ colony-forming units (CFU) per gram of caecal digesta.

Meat quality analyses
Meat quality analyses were conducted on the breast (*Pectoralis*) and thigh (*Sartorius*) muscles.

Determination of muscle pH
The pH reading was measured on a meat sample using a handheld digital pH meter (MW102 pH meter, MILWAUKEE® instruments, Inc. NC, USA) fitted with pH (MA920B/1) and temperature (MA830R) probes. The pH meter was calibrated before taking readings by dipping the pH probe into a buffer solution of pH 7.0 followed by pH 4.0. About 5 g of the sample was homogenized with 25 ml of distilling water using an electric blender. The homogenate was transferred into a beaker and the pH was read. Triplicate pH readings were taken from each sample. The pH probe was rinsed with distilled water after each measurement.

Measurement of meat color
The meat samples were exposed to the air to bloom for 30 min before taking color readings. Measurements of meat color coordinates namely,

lightness (L*), redness (a*), and yellowness (b*) were made with a handheld colorimeter (WR-10, Shenzhen, China) following the International Commission on Illumination (CIE, 1976) L^* a^* b^* classification system with the D_{65} illuminant. Three color readings were performed on different points of each sample and the average was used for statistical analysis.

Determination of drip loss of meat samples

Muscle samples were weighed and the weight was recorded as initial weight (Wa). The weighed samples were placed in transparent vacuum bags, vacuum sealed, and stored in a refrigerator (HRF-200ALUX, PZ Cussons Holdings, Nigeria) at 5±1°C. After 1 d postmortem, the samples were removed from the vacuum bags, blotted dry, and weighed, and the weight was recorded as final weight (Wb). Drip loss was estimated using the formula below:

$$Drip\ loss\ (\%) = [(Wa - Wb) \div Wa] \times 100$$

Determination of cooking loss of meat samples

Muscle samples were weighed and the weight was recorded as initial weight (Wa). The samples were placed in vacuum bags, vacuum sealed, and cooked in a pre-heated water bath at 80°C until the internal temperature of the samples reached 78°C as monitored by a stabbing temperature probe, which was inserted into the center of the meat sample. The cooked meat samples were cooled with running tap water for 15 min, removed from the vacuum bags, blotted dry without squeezing, and reweighed (Wb). Cooking loss was calculated using the equation below:

$$Cooking\ loss\ (\%) = [(Wa - Wb) \div Wa] \times 100$$

Determination of meat oxidative stability

Lipid oxidation in meat was measured by thiobarbituric acid reactive substance (TBARS) assay based on the reaction between malondialdehyde (MDA) and thiobarbituric acid (TBA) (Sigma-Aldrich, St. Louis, MO, USA) in line with the protocol of Buege and Aust (1978). One gram of meat sample was mixed with 5 mL of 20% (v/v) trichloroacetic acid (TCA) (Sigma-Aldrich, St. Louis, MO, USA) and centrifuged at 3000 x g for 10 min. Then, 6 mL of 0.2 g/dL TBA was added to the supernatant. The mixture was heated in boiling water for 30 min. After cooling on ice, the resulting chromogen was extracted with 8 mL of n-butyl alcohol. The organic phase was separated by centrifugation at 3000 x g for 10 min and the absorbance was read at a wavelength of 530 nm on a spectrophotometer (Spectronic 21D, Milton Roy, 18974 PA, USA). The MDA solution which has been made freshly by the hydrolysis of 1,1,3,3-tetra methoxy propane (Sigma-Aldrich, St. Louis, MO, USA) was used as the standard. The TBARS value was expressed as mg MDA/kg meat.

Protein oxidation was measured by the quantification of carbonyl groups based on their reaction with 2, 4-dinitrophenylhydrazine (DNPH) (Sigma-Aldrich, St. Louis, MO, USA) to form hydrazones following the method of Levine *et al.* (1990). Briefly, 0.1 g of meat sample was incubated with 1.0 mL of 20 mM DNPH solution for 60 min. Proteins were precipitated by the addition of 20% (v/v) trichloroacetic acid (Sigma-Aldrich, St. Louis, MO, USA) and re-dissolved in DNPH. Thereafter, the proteins were precipitated from the solution using 20% (v/v) trichloroacetate; the protein pellet was washed thrice with ethanol and ethyl acetate and re-suspended in 1 mL of 6 M guanidine (Sigma-Aldrich, St. Louis, MO, USA). The absorbance was read at 370 nm on a spectrophotometer (Spectronic 21D, Milton Roy, 18974 PA, USA). Results were presented as µmol carbonyl/mg protein.

Statistical analysis

The experimental design was a completely randomized design with seven replicates. Data were checked for normality using the PROC UNIVARIATE procedure of SAS (SAS Institute Inc., Cary, NC, USA). Homogeneity of variance was tested with Levene's test. A pen of 10 birds was the experimental unit for growth performance. A replicate of five birds was the experimental unit for the blood chemistry, carcass traits, and meat quality. A replicate of three birds was the experimental unit for cytokine expression, serum immunoglobulin, and caecal microbiota. Data were subjected to the generalized linear model procedure of SAS. The statistical model is as follows:

$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$ where Y_{ij} is the j^{th} observation in treatment i, μ is the overall mean, τ_i is the fixed effect of treatment i, ε_{ij} is a random error with mean 0 and variance σ^2. The level of significance was set at $P < 0.05$. Means were separated using the Tukey HSD test.

Results

Feed intake and growth performance

The body weight was not different ($P > 0.05$) on days 1 and 21 among the treatments (Table 3). The supplemented birds had higher (P = 0.024) body weight than the BD birds on day 42. Dietary treatments had no significant effect on feed intake at the starter and finisher phases as well as the entire production cycle ($P > 0.05$). The supplemented birds presented greater BWG than the BD birds at the finisher phase (P = 0.034) and during the entire production cycle (P = 0.023). The FCR was not affected ($P > 0.05$) by diets at the starter and finisher phases. However, during the entire production cycle, supplemented birds had lower (P = 0.042) FCR compared with the BD birds. The percentage of mortality was not affected by dietary supplements (Table 3).

Table 3. Growth performance in broiler chickens fed the diet supplemented with *Kigelia pinnata* and *Plukenetia conophora* leaves

	Dietary treatments[1]							
Item	BD	OXYBHA	KPL-1	KLP-2	PCL-1	PCL-2	*SEM*	*P*-value
Body weight (g/bird)								
1	44.00	43.66	43.66	44.00	44.00	43.66	0.53	0.985
21	850.00	869.66	855.33	863.3	870	866.67	6.46	0.234
42	2333.70[b]	2533.40[a]	2553.30[a]	2498.70[a]	2501.60[a]	2546.70[a]	41.85	0.024
Body weight gain (g/bird/day)								
1-21	38.38	39.33	38.65	39.01	39.33	39.19	0.31	0.241
22-42	70.63[b]	79.22[a]	80.85[a]	77.85[a]	77.69[a]	80.00[a]	1.97	0.034
1-42	54.50[b]	59.28[a]	59.75[a]	58.44[a]	58.51[a]	59.59[a]	0.99	0.023
Feed intake (g/bird/day)								
1-21	65.00	65.00	65.00	64.66	64.40	64.67	0.67	0.970
22-42	150.67	150.3	152	147.33	150.00	151.67	1.44	0.317
1-42	107.83	107.67	108.50	106.00	107.61	108.17	0.76	0.314
Feed conversion ratio								
1-21	1.69	1.65	1.68	1.65	1.63	1.65	0.02	0.374
22-42	2.13	1.90	1.88	1.89	1.93	1.90	0.05	0.056
1-42	1.98[a]	1.82[b]	1.81[b]	1.81[b]	1.83[b]	1.81[b]	0.04	0.042
Mortality (%)	2.10	1.95	2.00	1.96	2.00	2.00	0.32	0.102

[ab] Means bearing different superscripts in a row are significantly different ($P < 0.05$) [1]BD, basal diet only; OXYBHA, basal diet + 0.5 g/kg oxytetracycline + 0.12 g/kg Butylated hydroxy anisole; KPL-1, BD + 1 g/kg *Kigelia pinnata* leaf; KPL-2, BD + 2 g/kg *Kigelia pinnata* leaf; PCL-1, BD + 1 g/kg *Plukenetia conophora* leaf; PCL-2, BD + 2 g/kg *Plukenetia conophora* leaf. *SEM*, standard error of mean.

Table 4. Carcass traits and organ weights in broiler chickens fed the diet supplemented with *Kigelia pinnata* and *Plukenetia conophora* leaves

	Dietary treatment[1]							
Carcass traits	BD	OXYBHA	KPL-1	KPL-2	PCL-1	PCL-2	*SEM*	*P* -value
Live weight (LW, g/bird)	2350.0[b]	2541.6[a]	2550.0[a]	2500.2[a]	2500.0[a]	2550.2[a]	40.11	0.019
Carcass weight (CW, g/bird)	1621.0[b]	1758.6[a]	1747.0[a]	1778.5[a]	1721.5[a]	1743.44[a]	40.21	0.040
Dressing %	68.98	69.21	68.51	71.14	68.86	68.37	2.15	0.545
Abdominal fat (% LW)	0.62	0.76	0.35	0.29	0.30	0.46	0.34	0.189
Carcass cut (% CW)								
Breast	29.51	28.52	29.46	29.65	28.45	29.86	1.62	0.347
Thigh	14.99	14.92	15.68	15.11	15.27	15.46	0.89	0.626
Drum stick	14.79	15.49	14.69	15.54	15.79	14.30	0.68	0.170
Wings	11.86	11.47	11.12	11.74	11.65	11.65	0.80	0.268
Back	21.79	22.55	22.07	21.87	22.18	21.69	0.91	0.564
Neck	2.06	2.03	2.11	2.27	2.39	2.28	0.14	0.911
Leg	1.96	1.85	2.00	2.26	2.05	1.85	0.17	0.105
Head	2.84	3.19	2.82	3.03	3.22	2.63	0.15	0.090
Organ weight (% LW)								
Heart	0.42	0.43	0.43	0.44	0.45	0.45	0.04	0.126
Crop	0.31	0.34	0.38	0.38	0.40	0.39	0.04	0.079
Proventriculus	0.44	0.47	0.43	0.43	0.45	0.48	0.05	0.314
Gizzard	1.95	1.96	1.79	1.84	1.94	1.94	0.09	0.447
Liver	1.90	1.90	1.84	1.84	1.94	1.87	0.13	0.505
Pancreas	0.25	0.24	0.22	0.22	0.24	0.24	0.03	0.529
Duodenum	1.30	1.31	1.28	1.27	1.28	1.34	0.16	0.200
Jejunum	0.87	0.90	0.87	0.87	1.00	1.07	0.37	0.065
Ileum	1.05	0.10	1.08	1.62	1.09	1.01	0.11	0.409
Caecum	0.48	0.50	0.51	0.49	0.48	0.48	0.12	0.417
Thymus	0.24	0.26	0.24	0.29	0.27	0.25	0.02	0.459
Spleen	0.12	0.12	0.11	0.11	0.12	0.10	0.01	0.124
Bursa of fabricious	0.10	0.12	0.11	0.12	0.11	0.10	0.02	0.123

[ab] Means bearing different superscripts in a row are significantly different ($P < 0.05$) [1]BD, basal diet only; OXYBHA, BD + 0.5 g/kg oxytetracycline + 0.12 g/kg butylated hydroxyanisole; KPL-1, BD + 1 g/kg *Kigelia pinnata* leaf; KPL-2, BD + 2 g/kg *Kigelia pinnata* leaf; PCL-1, BD + 1 g/kg *Plukenetia conophora* leaf; PCL-2, BD + 2 g/kg *Plukenetia conophora* leaf. *SEM*, standard error of mean.

Carcass traits

The BD birds had lower (P = 0.04) carcass weight compared with the supplemented birds (Table 4). The dressing percentage, abdominal fat pad, percentages of carcass cuts, and relative organ weights were not affected (P > 0.05) by the dietary treatments (Table 4).

Hematology and serum biochemistry

Hematological indices were not different (P > 0.05) among the dietary supplements (Table 5). The PCL and KPL birds had lower total serum cholesterol (P = 0.031) and LDL-cholesterol (P = 0.041) compared with the BD and OXYBHA birds. The PCL-2 birds had the least total serum cholesterol that was different from those of birds fed other diets. Experimental treatments did not affect (P > 0.05) serum total protein, creatinine, urea, uric acid, AST, and ALT concentrations in broiler chickens.

Table 5. Hematology and serum biochemical indices in broiler chickens fed the diet supplemented with *Kigelia pinnata* and *Plukenetia conophora* leaves

	Dietary treatments[1]							
Hematological indices	BD	OXYBHA	KPL-1	KPL-2	PCL-1	PCL-2	*SEM*	*P*- value
White blood cells (x 10^9/L)	13.22	14.72	11.53	14.20	12.80	14.43	2.56	0.192
Red blood cells (x 10^{12}/L)	2.30	2.48	2.30	2.35	2.56	2.56	0.25	0.101
Packed cell volume (%)	38.16	38.90	36.90	38.44	37.26	38.00	3.22	0.117
Hemoglobin (g/dL)	10.28	12.00	12.13	11.67	14.06	11.47	3.61	0.064
Lymphocyte (%)	80.15	84.11	85.13	80.93	83.33	83.00	5.93	0.942
Granulocyte (%)	12.10	14.00	9.16	9.83	9.06	10.36	4.53	0.105
Mean cell hemoglobin (pg)	34.38	35.86	35.60	35.94	36.14	40.12	2.64	0.140
Mean corpuscular volume (fL)	165.91	156.85	160.43	163.40	145.31	148.44	15.56	0.210
MCHC[2] (g/dL)	26.73	29.27	30.33	28.09	29.11	26.06	1.88	0.311
RDWSD[3] (%)	23.63	23.37	21.83	22.50	23.83	41.46	2.68	0.213
RDWCV[4] (%)	13.86	15.23	14.80	14.13	14.97	12.16	1.59	0.116
Platelets (x 10^3/L)	373.0	375.0	372.0	373.9	377.3	397.3	19.94	0.214
Mean platelet volume (fL)	9.13	9.23	9.70	10.67	9.43	9.57	0.55	0.309
Platelet distribution width (fL)	8.37	9.50	8.20	8.40	9.50	10.17	0.73	0.199
P-LCR[5] (%)	29.07	26.10	25.23	29.37	29.13	22.67	3.60	0.300
Plateletcrit (%)	0.34	0.34	0.36	0.39	0.35	0.37	0.04	0.156
Serum indices								
Total cholesterol (mg/dL)	266.54[a]	287.65[a]	207.28[b]	208.13[b]	196.00[b]	131.03[c]	31.23	0.031
Triglycerides (mg/dL)	92.00	98.24	105.26	109.45	89.06	89.40	7.91	0.282
LDL-cholesterol (mg/dL)	132.44[a]	138.11[a]	41.19[b]	65.43[b]	51.32[b]	33.37[b]	17.71	0.041
HDL-cholesterol (mg/dL)	115.70	130.00	131.00	121.33	124.26	113.20	29.68	0.270
Total protein (g/L)	11.52	12.80	13.43	13.96	12.24	13.77	2.82	0.088
Creatinine (µmol/L)	0.68	0.71	0.72	0.70	0.70	0.68	0.06	0.978
Uric acid (mg/dL)	4.78	4.67	4.82	4.66	4.35	4.90	0.22	0.356
Urea (mg/dL)	1.97	1.93	1.85	1.61	1.15	1.96	0.32	0.352
Aspartate transaminase (IU/L)	124.22	126.10	115.06	134.73	171.93	127.02	16.05	0.234
Alanine transaminase (IU/L)	46.00	45.85	46.01	43.96	44.60	44.86	0.86	0.593

[abc] Means bearing different superscripts in a row are significantly different (P < 0.05) [1]BD, basal diet only; OXYBHA, BD + 0.5 g/kg oxytetracycline + 0.12 g/kg butylated hydroxy anisole; KPL-1, BD + 1 g/kg *Kigelia pinnata* leaf; KPL-2, BD + 2 g/kg *Kigelia pinnata* leaf; PCL-1, BD + 1 g/kg *Plukenetia conophora* leaf; PCL-2, BD + 2 g/kg *Plukenetia conophora* leaf. [2]MCHC, mean cell hemoglobin concentration; [3]red blood cell distribution width standard deviation; [4]red blood cell distribution width coefficient of variation; [5]Plateletcrit-large cell ratio. *SEM*, standard error of mean.

Immune response and caecal bacterial population

The splenic interleukin-1β and interleukin 6 were not affected (P > *0.05*) by diets (Table 6). The splenic interleukin-10 was higher (P = 0.021) in the supplemented birds compared with the BD birds. The KPL birds had higher interleukin 10 than the PCL and OXYBHA birds. The BD birds had higher serum IgG (P = 0.031) and IgM (P = 0.013) compared with the supplemented birds. The KPL birds had lower IgM than the OXYBHA and PCL birds.

Lactobacilli spp. count in the OXYBHA birds was lower (P < 0.0001) than that of PCL and KPL birds (Table 6). The BD and KPL-1 birds had similar *Lactobacilli* spp. count. *Lactobacilli* spp. count in the PCL-2 birds did not differ from those of KPL-2 and PCL-1 birds but was higher than those of KPL-1 and BD birds. *Salmonella* spp. (P = 0.001) and *E. coli* (P < 0.0001) counts were higher in the BD birds compared with the supplemented birds. The KPL-2 birds had the least *Salmonella* spp. and *E. coli* counts compared with birds fed other diets. The *E coli* count in the PCL-1 and PCL-2 birds was lower than that of KPL-1 and OXYBHA birds.

Table 6. Immune response and caecal bacterial counts in broiler chickens fed the diet supplemented with *Kigelia pinnata* and *Plukenetia conophora* leaves

	Dietary treatments[1]							
Item	BD	OXYBHA	KPL-1	KPL-2	PCL-1	PCL-2	*SEM*	*P*-value
Splenic cytokines								
Interleukin-1β (pg/mL)	29.38	27.67	27.62	30.82	25.45	25.29	4.98	0.266
Interleukin-10 (pg/mL)	6.85^{c}	18.71^{b}	29.36^{a}	33.27^{a}	17.85^{b}	19.14^{b}	5.28	0.021
Interleukin-6 (pg/mL)	520.00	396.90	397.74	493.89	496.36	389.02	57.19	0.126
Serum Immunoglobulin								
Immunoglobulin G (µmol/L)	234.00^{a}	149.00^{b}	129.43^{b}	149.23^{b}	125.22^{b}	147.48^{b}	23.45	0.031
Immunoglobulin M (µmol/L)	109.00^{a}	45.00^{b}	25.90^{c}	20.31^{c}	49.89^{b}	51.16^{b}	13.52	0.013
Caecal microbiota (Log_{10} CFU[2]								
Lactobacilli spp.	3.42^{cd}	2.62^{d}	3.93^{bc}	4.60^{ab}	4.60^{ab}	5.13^{a}	0.20	<0.0001
Escherichia coli	4.13^{a}	2.63^{b}	2.54^{b}	0.13^{d}	1.69^{c}	1.28^{c}	0.17	<0.0001
Salmonella spp.	4.28^{a}	2.47^{b}	2.53^{b}	1.67^{c}	2.67^{b}	2.70^{b}	0.23	0.001

[abcd] Means bearing different superscripts in a row are significantly different ($P < 0.05$) [1]BD, basal diet only; OXYBHA, BD + 0.5 g/kg oxytetracycline + 0.12 g/kg butylated hydroxy anisole; KPL-1, BD + 1 g/kg *Kigelia pinnata* leaf; KPL-2, BD + 2 g/kg *Kigelia pinnata* leaf; PCL-1, BD + 1 g/kg *Plukenetia conophora* leaf; PCL-2, BD + 2 g/kg *Plukenetia conophora* leaf. [2]colony forming unit. *SEM*, standard error of mean.

Meat quality

In the *Sartorius* muscle, pH, cook loss, drip loss, lightness, redness, and yellowness did not differ ($P > 0.05$) among the diets (Table 7). The *Sartorius* muscle in the BD birds had higher ($P = 0.003$) carbonyl content and TBARS value than those of the supplemented birds. The *Sartorius* muscle of the PCL-1 and PCL-2 birds had the least ($P = 0.003$) TBARS value compared with those of birds fed other experimental diets. The least carbonyl content was recorded in the *Sartorius* and *Pectoralis* muscles of the PCL-2 birds. In the *Pectoralis* muscle, the BD birds had higher drip loss ($P = 0.016$) and carbonyl content than did birds fed other diets. Experimental diets had no effect ($P > 0.05$) on cook loss, pH, and color coordinates of *Pectoralis* muscle.

Table 7. Meat physicochemical properties assessed at 24 h *postmortem* in broiler chickens fed the diet supplemented with *Kigelia pinnata* and *Plukenetia conophora* leaves

		Dietary treatment[1]							
Muscle	Indices	BD	OXYBHA	KPL-1	KPL-2	PCL-1	PCL-2	*SEM*	*P*-value
Sartorius	Drip loss (%)	3.43	2.56	2.33	2.29	3.38	2.26	1.00	0.113
	Cook loss (%)	8.30	7.11	7.90	7.03	8.43	8.19	1.39	0.963
	pH	5.90	5.92	5.87	5.87	5.80	5.84	0.08	0.903
	Lightness	46.01	46.87	45.39	46.36	45.64	45.55	2.59	0.298
	Redness	6.79	7.57	7.21.	7.40	7.54	7.07	0.93	0.695
	Yellowness	9.49	7.31	8.29	9.34	8.38	8.24	0.80	0.348
	TBARS (mg MDA/kg)	0.25^{a}	0.14^{b}	0.13^{b}	0.11^{b}	0.07^{c}	0.07^{c}	0.03	0.003
	Carbonyl (µmol/kg protein)	1.27^{a}	0.70^{b}	0.80^{b}	0.72^{b}	0.75^{b}	0.53^{c}	0.12	0.003
Pectoralis	Drip loss (%)	6.31^{a}	3.51^{b}	3.86^{b}	3.81^{b}	3.55^{b}	3.76^{b}	1.37	0.016
	Cook loss (%)	11.62	10.26	10.40	10.65	10.54	11.05	2.07	0.693
	pH	5.83	5.87	5.87	5.82	5.94	5.95	0.06	0.601
	Lightness	46.17	43.49	41.72	44.88	46.25	42.95	2.00	0.532
	Redness	3.55	3.76	3.30	3.84	4.21	3.33	0.51	0.538
	Yellowness	9.02	9.60	8.62	8.61	9.32	7.81	1.51	0.237
	TBARS (mg MDA/kg)	0.12	0.09	0.09	0.08	0.10	0.09	0.03	0.312
	Carbonyl (µmol/kg protein)	0.83^{a}	0.63^{b}	0.63^{b}	0.58^{b}	0.61^{b}	0.40^{c}	0.12	0.025

[abc] Means bearing different superscripts in a row are significantly different ($P < 0.05$) [1]BD, basal diet only; OXYBHA, BD + 0.5 g/kg oxytetracycline + 0.12 g/kg butylated hydroxy anisole; KPL-1, BD + 1 g/kg *Kigelia pinnata* leaf; KPL-2, BD + 2 g/kg *Kigelia pinnata* leaf; PCL-1, BD + 1 g/kg *Plukenetia conophora* leaf; PCL-2, BD + 2 g/kg *Plukenetia conophora* leaf. *SEM*, standard error of mean.

Discussion

The improvement in BWG and feed efficiency in the supplemented birds may be ascribed to the changes in caecal bacteria populations, and immune indices induced by the oxytetracycline and the phytochemical contents of KPL and PCL. These observations concur

with the findings of Oloruntola *et al.* (2019), who found a significant improvement in BWG and feed efficiency in broiler chickens supplemented with 8-10 g/kg pawpaw seed and leaf blend. Furthermore, the supplementation of 1% *Withania somnifera* root powder enhanced feed efficiency and body weight gain in broiler chickens (Ansari *et al.,* 2013). The mortality rate was not affected by dietary treatments and was within the normal range for Arbor Acres broiler chickens. The improvement in carcass weight of the supplemented birds mirrored the improved BWG. Nonetheless, carcass yield, percentage of prime cuts, and relative organ weights did not differ among the treatments. Contrarily, the supplementation of 1% *Withania somnifera* root powder improved carcass yield and the weights of lymphoid organs in broilers (Ansari *et al.,* 2013).

Gut microbiota plays a crucial role in the health, production performance, and welfare of poultry (Kohl, 2012; Kogut *et al*., 2020). The reduction in *E. coli* and *Salmonella* spp. counts in the KPL and PCL birds, which was at par with that of oxytetracycline-supplemented birds, may reflect the phytochemical contents in KPL and PCL. Plant secondary metabolites can disrupt the cell membrane of pathogenic microbes and can promote the hydrophobicity of microbial species, which could affect the surface properties of microbial cells and influence the pathogenicity of the microbes (Vidanarachchi *et al.,* 2005; Hashemi and Davoodi, 2011). The increase in caecal *Lactobacillus* spp. counts in the KPL and PCL birds may indicate the selective antimicrobial effects of the additives and may be responsible for the reduction in the *E. coli* and *Salmonella* spp. counts. The shift in caecal microbiota towards the *Lactobacillus* spp. could promote the synthesis of lactate and short-chain fatty acids, which may create an acidic environment that could hinder the proliferation of pathogenic microbes (Vidanarachchi *et al.,* 2005; Hashemi and Davoodi, 2011). Our findings are consistent with those of Vanessa *et al.* (2018), who reported that the supplementation of neem oil reduced *Salmonella* spp. count and improved *Lactobacillus* spp. count in broiler chickens. Furthermore, the supplementation of *Emblica Officinalis* fruit powder reduced *E. coli* and improved *Lactobacillus* spp. counts in broiler chickens (Dalal *et al.,* 2018).

Cytokines are non-structural peptides that play intricate regulatory roles on immunity and inflammation (Wigley and Kaiser, 2003; Giansanti *et al.,* 2006). The expression of IL-1β and IL-6 exert pro-inflammatory effects, and the expression of IL-10, which exert anti-inflammatory effects in chickens (Giansanti *et al*., 2006; Kaiser and Stäheli, 2014) were examined in this study. Our results suggest that dietary supplements did not affect the expression of IL-6 and IL-1β in the spleen of broiler chickens. However, the expression of splenic IL-10 increased following the dietary supplementation of PCL, KPL, and OXYBHA in broiler chickens. This observation suggests the anti-inflammatory potential of the supplements. Similarly, the administration of *Calea uniflora* polyphenols enhanced the IL-10 in mice (da Rosa *et al.,* 2019).

The B cells synthesize immunoglobulins in response to oxidative stress, infection, or other immune stressors (Mast *et al.,* 2000). The supplementation of KPL and PCL down-regulated the expression of IgM and IgG as did the OXYBHA-supplemented diet. This finding may suggest that plant polyphenols and antibiotics may not stimulate the activation of B cells when oxidative stress, compromised health condition, or infection was absent. The reduction in IgM and IgG may probably be due to the lower *Salmonella* spp. and *E. coli* counts that may have scaled back the need for the production of antibodies. This observation is consistent with those of Su *et al.* (2016) who reported that the supplementation of 300 mg/kg Yucca extract reduced serum IgG and IgA in broilers. The result of splenic cytokine and serum immunoglobulin appears to be related to the caecal microbial profile. The KPL-2 birds had the least IgM concentration, *Salmonella,* and *E. coli* counts and the highest IL-10 concentration.

Blood indices are good indicators of the physiological, nutritional, and health status of livestock (Odhaib *et al.,* 2018a). The supplementation of PCL and KPL did not affect hematological indices in broiler chickens. Moreover, the hematological indices were within the normal range for healthy broiler chickens (Mitruka and Rausley, 1977). Similarly, the supplementation of Neem leaf extract (Nodu *et al.,* 2016) and Neem oil (Vanessa *et al.,* 2019) did not affect the blood indices in broiler chickens. There was a significant reduction in total serum cholesterol and LDL-cholesterol in birds fed the diet supplemented with PCL and KPL. This finding may be attributed to the phytochemical contents of the supplements. Plant secondary metabolites can reduce cholesterol by inhibiting the activity of 3-hydroxy-3-methyl glutaryl-CoA reductase, which is a crucial enzyme in the biosynthesis of cholesterol (Crowell, 1999). Furthermore, the improvement in caecal *Lactobacillus* spp. counts in the KPL and PCL birds may be responsible for the decrease in serum cholesterol. *Lactobacillus* spp. can bind cholesterol on cellular surfaces and convert intestinal cholesterol to coprostanol (Lye *et al.,* 2010). *Lactobacillus* spp. can de-conjugate bile salts and reduce pH, which is capable of hindering the absorption cycle of bile salts thereby increasing their fecal excretion. The liver accelerates bile synthesis from cholesterol to recover the intestinal-liver cycling of bile salts, thereby

reducing tissue and blood cholesterol levels (Ramasamy *et al*., 2010). Case in point, the PCL-2 birds had the least serum cholesterol and the highest *Lactobacilli* spp. count. The current observation is in tandem with that of Oloruntola *et al.* (2019), who observed a reduction in serum cholesterol in broiler chickens following the supplementation of pawpaw seed and leaf blend. No significant effects of the dietary supplements were found on serum total protein, AST, ALT, creatinine, urea, and uric acid concentrations. Serum total protein measures the amount of protein in the blood and may reflect dietary protein. The similarity in serum total protein may reflect the isonitrogenous nature of the dietary treatments. The AST and ALT are reliable indicators of hepatic health while creatinine, urea, and uric acid are good indicators of renal health and metabolism. Our observations may imply that the supplementation of PCL and KPL did not impair hepatic and renal health and metabolism. Similarly, the supplementation of pawpaw seed and leaf blend did not affect serum total protein, ALP, and creatinine in broiler chickens (Oloruntola *et al.,* 2019).

Dietary supplements did not influence the pH, color, and cook loss in the *Pectoralis* and *Sartorius* muscles in broiler chickens. The amount of muscle glycogen at slaughter and the rate of postmortem glycolysis have a profound influence on the muscle pH in animals (Salwani *et al*., 2016; Yusuf *et al.,* 2018). The similarity in muscle pH among the dietary treatments may be due to the homogenous dietary energy, and the management conditions employed in this study. The non-significant difference in muscle pH may account for the similarity in cook loss among the treatments. The lack of significant differences in muscle color coordinates (L* a* b*) may imply that the dietary supplements did not affect the concentration and the oxidative stability of myoglobin (Adeyemi *et al*., 2016). The influence of dietary supplements on drip loss, carbonyl content, and TBARS value was muscle-dependent. The supplementation of KPL and PCL lowered the carbonyl content and TBARS value of the *Sartorius* muscle and the carbonyl content of the *Pectoralis* muscle as did the BHA-supplemented diet. This observation may reflect the antioxidant properties of the polyphenols in KPL (Hussain *et al.,* 2016) and PCL (Maduka *et al.,* 2018). Polyphenols exhibit antioxidant properties by metal chelation, singlet oxygen quenching, lipoxygenase inhibition, and free radical scavenging (Bors *et al.,* 1996). The reduction in drip loss in the *Pectoralis* muscle of the supplemented birds may imply a reduction in the oxidative disruption of myofibrillar proteins typified by the lower carbonyl content. Amino acids in the muscle can undergo oxidative deterioration thereby generating carbonyl compounds that can affect the functionality of meat proteins (Serpen *et al.,* 2012).

Conclusion

The findings of this study suggest that the supplementation of KPL, PCL, and OXYBHA improved body weight gain at the finisher phase and during the entire production period and reduced feed conversion ratio during the entire production period in broiler chickens. Dietary supplementation of OXYBHA, KPL, and PLC improved splenic IL-10 and lowered caecal *E. coli,* and *Salmonella* spp. counts, and serum IgG and IgM in broiler chickens. Dietary supplementation of KPL and PCL lowered serum total cholesterol and LDL-cholesterol and enhanced caecal *Lactobacillus* spp. count in broiler chickens. Dietary supplements reduced carbonyl content and TBARS value in the *Sartorius* muscle, and drip loss and carbonyl content in the *Pectoralis* muscle of broiler chickens. Taken together, the KPL-2 exhibited higher antimicrobial and immunomodulatory effects than the PCL-2, while the PCL-2 exhibited greater antioxidant and cholesterol-lowering potential than the KPL-2. Thus, KPL-2 and PCL-2 could be considered as potential alternatives to synthetic additives in the diet of broiler chickens. Owing to the differences in antimicrobial, immunomodulatory, and antioxidant properties between KPL and PCL, future studies may consider a mix of KPL and PCL. The impact of KPL and PCL and their mix on diseased and/or oxidatively challenged broiler chickens should be investigated in future studies.

Conflict of interest

The authors declare that they have no competing interests.

ORCID

Kazeem D. Adeyemi
https://orcid.org/0000-0002-6719-2081
Mariam A. Adegoke
https://orcid.org/0000-0003-4491-9062
Mariam O. Mudashir
https://orcid.org/0000-0002-9906-0550
Funke M. Owoyomi
https://orcid.org/0000-0002-3631-8251
Toheeb O. Hamzat
https://orcid.org/0000-0002-5922-8056
Israel A. Adeleke
https://orcid.org/0000-0003-3579-3090
Sarafadeen O. Ibrahim
https://orcid.org/0000-0002-7400-1925
Abdulraheem Abdulrahman
https://orcid.org/0000-0002-5171-2513

References

Abu Bakar MF, Mohamed M, Rahmat A & Fry J. 2009. Phytochemicals and antioxidant activity of different parts of bambangan (*Mangifera pajang*) and tarap (*Artocarpus odoratissimus*). Food Chemistry, 113: 479-483. DOI: 10.1016/ j. food chem.2008.07.081

Adeyemi KD. 2021. Comparative effect of dietary *Morinda lucida* leaf and Butylated hydroxyanisole (BHA) on carcass traits, meat quality, and oxidative stability of broiler chickens. Journal of Food Science and Technology, DOI: 10.1007/ s13197-020-04916-2

Adeyemi KD, Abdulrahman A, Ibrahim SO, Zubair MF, Atolani O & Badmos AA. 2020. Dietary Supplementation of *Tetracarpidium conophorum* (African Walnut) seed enhances muscle n-3 fatty acids in broiler chickens. European Journal of Lipid Science and Technology, 122: 1900418. DOI: 10.1002/ejlt.201900418

Adeyemi KD, Shittu RM, Sabow AB, Ebrahimi M, Sazili AQ. 2016. Influence of diet and postmortem ageing on oxidative stability of lipids, myoglobin and myofibrillar proteins and quality attributes of gluteus medius muscle in goats. PloS one, 11: e0154603. DOI: 10.1371/journal. pone. 0154603

Ajaiyeoba EO & Fadare DA. 2006. Antimicrobial potential of extracts and fractions of the African walnut–Tetracarpidium conophorum. African Journal of Biotechnology, 5: 2322-2325.

Ansari J, Khan SH, Haq AU, Ahmad T & Abbass MI. 2013. Effect of Supplementation of *Withania somnifera* (Linn.) Dunal Roots on Growth Performance, Serum Biochemistry, Blood Hematology, and Immunity of Broiler Chicks. Journal of herbs, Spices and Medicinal Plants, 19: 144-158. DOI: 10.1080/10496475.2012.759169

Association of Official Analytical Chemists (AOAC) (2000) 'Official methods of analysis. Vol I.' 17th edn. (AOAC International Gaithersburg, MD).

Baurhoo B, Phillip L & Ruiz-Feria CA. 2007. Effects of purified lignin and mannan oligosaccharides on intestinal integrity and microbial populations in the ceca and litter of broiler chickens. Poultry Science, 86: 1070-1078. DOI: 10.1093/ps/ 86.6.1070

Bors W, Heller W, Michael C & Stettmaier K. 1996. Flavonoids and polyphenols: Chemistry and biology in: *Handbook of Antioxidants*, Cadenas E and Packer L (eds), New York, Marcel Dekker 409–66.

Buege JA & Aust SD. 1978. Microsomal lipid peroxidation. In Methods in enzymology. 52: 302-310. Academic Press, New York.

Commission International De I' Eclairage (CIE) (1976) 'Colorimetry.' 2nd edn. (CIE: Vienna, Switzerland).

Crowell PL. 1999. Prevention and therapy of cancer by dietary monoterpenes. Journal of Nutrition, 129: 775S-778S. DOI: 10.1093/jn/129.3.775S

da Rosa JS, Nascimento MVPDS, Parisotto EB, Lima TC, Santin JR, Biavatti MW, Zamoner A, Dalmarco EM & Fröde TS. 2019. Phenolic Compounds Isolated from Calea uniflora Less. Promote Anti-Inflammatory and Antioxidant Effects in Mice Neutrophils (Ex Vivo) and in Mice Pleurisy Model (In Vivo). Mediators of Inflammation, 1468502. DOI: 10.1155/2019/ 1468502

Dalal R, Ahlawat PK, Sonu V, Panwar VS, Tewatia BS & Sheoran N. 2018. Evaluation of Antimicrobial Effect of Emblica officinalis Fruit Powder on Intestinal Micro-biota in Broilers Chicken. International Journal of Current Microbiology and Applied Science, 7: 1432-1438. DOI: 10.20546/ijcmas.2018.704.162

Du B, Zhang Y, Lam JC, Pan S, Huang Y, Chen B, & Zeng L. 2019. Prevalence, biotransformation, and maternal transfer of synthetic phenolic antioxidants in pregnant women from South China. Environmental Science and Technology, 53:13959-13969. DOI: 10.1021/acs.est.9b04709

Edwards EA & Hilderbrand RL. 1976. Method for identifying Salmonella and Shigella directly from the primary isolation plate by coagglutination of protein A-containing staphylococci sensitized with specific antibody. Journal of Clinical Microbiology, 3: 339–343.

EFSA. 2016. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2014. European Food Safety Authority Journal, 4380.

FDA. 2013. Phasing out certain antibiotic use in farm animals. http://www.fda.gov/ For Consumers/ Consumer Updates /ucm378100.htm. 2013. Accessed June 2020.

Giansanti F, Giardi MF & Botti D. 2006. Avian cytokines-An overview. Current Pharmaceutical Design, 12: 3083-3099. DOI: 10.2174/ 138161206777947542

Gouda YG, Abdel-Baky AM, Mohamed KM, Darwish FM, Kasai R & Yamasaki K. 2006. Phenylpropanoid and phenylethanoid derivatives from *Kigelia pinnata* DC fruits. National Production Research, 20: 935-939. DOI: 10.1080/ 14786410500462702

Hashemi SR & Davoodi H. 2011. Herbal plants and their derivatives as growth and health promoters in animal nutrition. Veterinary Research Communications, 35: 169-180. DOI: 10.1007/ s11259-010-9458-2

Hussain T, Fatima I, Rafay M, Shabir S, Akram M & Bano S. 2016. Evaluation of antibacterial and antioxidant activity of leaves, fruit and bark of

Kigelia africana. Pakistan Journal of Botany, 48: 277-83.

Iqbal Z, Kamran Z, Sultan JI, Ali A, Ahmad S, Shahzad MI, Ahsan U, Ashraf S & Sohail MU. 2015. Replacement effect of vitamin E with grape polyphenols on antioxidant status, immune, and organs histopathological responses in broilers from 1-to 35-d age. Journal of Applied Poultry Research, 24: 127-134. DOI: 10.3382/japr/pfv009

Kaiser P & Stäheli P. 2014. Avian cytokines and chemokines. In Avian immunology. Academic Press. Pages, 189-204.

Kogut MH. 2019. The effect of microbiome modulation on the intestinal health of poultry. Animal Feed Science and Technology, 250: 32-40.

Kogut MH, Lee A & Santin E. 2020. Microbiome and pathogen interaction with the immune system. Poultry Science, 99: 1906-1913. DOI: 10.1016/ j.psj.2019.12.011

Kohl KD. 2012. Diversity and function of the avian gut microbiota. Journal of Comparative Physiology B, 182: 591-602. DOI: 10.1007/ s00360-012-0645-z

Levine RL, Garland D & Oliver CN. 1990. Determination of carbonyl content in oxidatively modified proteins. Method in Enzymology, 186: 464-478.

Lye HS, Rusul G & Liong MT. 2010. Removal of cholesterol by lactobacilli via incorporation and conversion tocoprostanol. Journal of Dairy Science, 93: 1383–1392. DOI: 10.3168/jds.2009-2574

Maduka HCC, Ugwu CE, Okpogba AN, Ogueche PN, Dike CC, Okonkwo CO & Nwanyanwu AC. 2018. Phytochemical analysis and antioxidant properties of the ethanolic extract from *Tetracarpidium conophorum* (African Walnut) and *Pterocarpus soyauxii* (oha) Leaf. Journal of Applied Life Science International, 18: 1-7. DOI: 10.9734/JALSI/2018/42439

Makkar HPS, Norsambuu T, Lkhavatsere S & Becker K. 2009. Plant Secondary metabolites in some medical plants of Mongolia used for enhancing animal health and production. Tropicultura 27: 159-167.

Manzetti S & Ghisi R. 2014. The environmental release and fate of antibiotics. Marine Pollution Bulletion 79: 7-15. DOI: 10.1016/j.marpolbul. 2014.01.005

Mast J, Buyse J & Goddeeris BM. 2000. Dietary L-carnitine supplementation increases antigen-specific immunoglobulin G production in broiler chickens. British Journal of Nutrition, 83: 161-166. DOI: 10.1017/S0007114500000209

Mitruka BM & Rawsley HM. 1977. Clinical Biochemistry and Haematological Reference Value in Normal Experimental Animal. Mason Publishing Company New York. pp. 35-50.

NAFDAC. 2017. Antimicrobial use and resistance in Nigeria. Situation analysis and recommendations. Federal Ministries of Agriculture and Rural Development, Environment and Health Report.

Nakyinsige K, Abdul Rahman NS, Salwani MS, Abd Hamid A, Adeyemi KD, Sakimin SZ & Sazili A. 2016. Effect of *Belimbing buluh* (Averrhoa bilimbi) juice extract on oxidative stability and microbiological quality of spent chicken meat. International Food Research Journal, 23: 2657-2680.

Nodu MB, Okpeku M, Akpoveta ZA & Iroegbu DO. 2016. Evaluation of azadirachta indica leave extract on hematology and biochemical profiles, organs weight and growth parameters of broiler chickens. Journal of New Science, 32: 5

NRC (National Research Council). 1994. Nutrient Requirements of Poultry. 9th ed. Washington: National Academy Press.

Odebiyi OO & Sofowora EA. 1978. Phytochemical screening of Nigerian medicinal plants II. Lloydia, 41: 234-246.

Odhaib, K.J, Adeyemi KD, Ahmed MA, Jahromi MF, Jusoh S, Samsudin AA, Alimon AR, Yakoob H & Sazili, AQ. 2018a. Influence of Nigella sativa seeds, Rosmarinus officinalis leaves and their combination on growth performance, immune response and rumen metabolism in Dorper lambs. Tropical Animal Health and Production, 50: 1011-1023. DOI: 10.1007/s11250-018-1525-7

Odhaib KJ, Adeyemi KD & Sazili AQ. 2018b. Carcass traits, fatty acid composition, gene expression, oxidative stability and quality attributes of different muscles in Dorper lambs fed Nigella sativa seeds, Rosmarinus officinalis leaves and their combination. Asian-Australasian Journal of Animal Science, 31:1345-1357. DOI: 10.5713/ajas.17.0468

Olorunsanya AO, Adeyemi KD & Babatunde IA. 2012. Effect of Bamboo (*Bambusa valgaris*) and Elephant grass (*Pennisetum purpureum*) leaf extracts on oxidative stability of cooked and raw b roiler meat. Journal of Agricultural Research and Development, 10: 1-10.

Oloruntola OD, Ayodele SO, Adeyeye SA, Jimoh AO, Oloruntola DA & Omoniyi IS. 2019. Pawpaw leaf and seed meals composite mix dietary supplementation: effects on broiler chicken's performance, caecum microflora and blood analysis. Agroforestry Systems, 94: 555-564. DOI: 10.1007/s10457-019-00424-1

Oviedo-Rondón EO. 2019. Holistic view of intestinal health in poultry. Animal Feed Science and Technology, 250: 1-8. DOI: 10.1016/j.anifeedsci. 2019.01.009

Oyekale KO, Odutayo OI, Esan EB, Ogunwemimo KO, Denton OA & Bolaji DT. 2015. Comparative studies on phytochemical and proximate

composition of four morphologically distinct segments of the conophor seedling (*Tetracarpidium conophorum* Hutch. & Dalziel). Brazilian Journal of Biological Science, 2: 91-100.

Pokorný J. 2007. Are natural antioxidants better–and safer–than synthetic antioxidants? European Journal of Lipid Science and Technology, 109: 629-642.

Ramasamy K, Abdullah N, Wong MC, Karuthan C & Ho YW. 2010. Bile salt deconjugation and cholesterol removal from media by *Lactobacillus* strains used as probiotics in chickens. Journal of the Science of Food and Agriculture, 90: 65-69. DOI: 10.1002/jsfa.3780

Salwani MS, Adeyemi KD, Sarah SA, Vejayan J, Zulkifli I & Sazili AQ. 2016. Skeletal muscle proteome and meat quality of broiler chickens subjected to gas stunning prior slaughter or slaughtered without stunning. CyTA Journal of Food, 14: 375-381. DOI: 10.1080/19476337. 2015.1112838

Serpen A, Gökmen V & Fogliano V. 2012. Total antioxidant capacities of raw and cooked meats. Meat Science, 90: 60-65. DOI: 10.1016/ j.meatsci. 2011.05.027

Shang Y, Kumar S, Oakley B & Kim WK. 2018. Chicken gut microbiota: importance and detection technology. Frontiers in Veterinary Science, 5: 254. DOI: 10.3389%2Ffvets.2018.00254

Sivagami K, Vignesh VJ, Srinivasan R, Divyapriya G & Nambi IM. 2020. Antibiotic usage, residues and resistance genes from food animals to human and environment: An Indian scenario. Journal of Environmental and Chemical Engineering, 8: 102221. DOI: 10.1016/j.jece.2018.02.029

Su JL, Shi BL, Zhang PF, Sun DS, Li TY & Yan SM. 2016. Effects of yucca extract on feed efficiency, immune and antioxidative functions in broilers. Brazilian Archive of Biology and Technology 59: e16150035. DOI: 10.1590/1678-4324-20161 50035

Swain T. 1979. Tannins and Lignins. In G.A Rosenthal and D.H. Janzen (eds.). Herbivores: Their interactions with plant secondary metabolites. Pp 657-682. Academic Press, New York.

Talari S, Rudroju S, Penchala S & Nanna Rama S. 2012 Quantification of total phenolics and Total Flavonoid contents in extracts of Oroxylum Indicum I. Kurz. Asian Journal of Pharmaceutical and Clinical Research, 5: 177-179

Tao L. 2015. Oxidation of polyunsaturated fatty acids and its impact on food quality and human health. Advances in Food Technology and Nutrition Science Open Journal, 1: 135-42. DOI: 10.17140/ AFTNSOJ-1-123

Tietz NW. 1995. Fundamentals of Clinical Chemistry. 3rd edn, WB Saunders Philladelphia, Pages, 878.

Tolkachev ON, Shemeryakin BV & Prolina NV. 1983. Isolation and purification of alkaloids. Chemistry of Natural Compounds, 19: 387-400.

Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, Teilant A & Laxminarayan R. 2015. Global trends in antimicrobial use in food animals. Proceedings of National Academy of Science, 112: 5649-5654. DOI: 10.1073/pnas.1503141112

Van TTH, Yidana Z, Smooker PM & Coloe PJ. 2020. Antibiotic use in food animals worldwide, with a focus on Africa: Pluses and minuses. Journal of Global Antimicrobial Research, 20: 170-177. DOI: 10.1016/j.jgar.2019.07.031

Vanessa MS, Raphaël KJ & Kissel NN. 2019. Blood and Gut Micriobiota Profiles of Broiler Chickens Fed on Diet Supplemented with Graded Levels of Neem (*Azadirachta indica*) Oil. Animal Veterinary Science 7: 78-82. DOI: 10.11648/ j.avs.20190703.12

Vidanarachchi JK, Mikkelsen LL, Sims I, Iji PA & Choct M. 2005. Phytobiotics: alternatives to antibiotic growth promoters in monogastric animal feeds. Recent Advances in Animal Nutrition in Australia, 15: 131-44.

Wigley P, & Kaiser P. 2003. Avian cytokines in health and diseases. Brazilian Journal of Poultry Science, 5: 1-14.

Yang X, Sun Z, Wang W, Zhou Q, Shi G, Wei F & Jiang G. 2018. Developmental toxicity of synthetic phenolic antioxidants to the early life stage of zebrafish. Science of Total Environment, 643: 559-568. DOI: 10.1016/j.scitotenv. 2018. 06.213

Yusuf AL, Adeyemi KD, Roselina K, Alimon AR, Goh YM, Samsudin AA & Sazili AQ. 2018. Dietary supplementation of different parts of Andrographis paniculata affects the fatty acids, lipid oxidation, microbiota, and quality attributes of longissimus muscle in goats. Food Research International, 111: 699-707. DOI: 10.1016/ j. foodres.2018.06.015

Yuying M, Shihong Z, Minru, Jia, Guihua J, Shengwu T, Zhengyou H, Rong L. 2005. Determination of total coumarin in Angelica dahurica by UV spectrophotometry. West China Journal of Pharmaceutical Sciences, 20: 159-160.