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PERFORMANCE OF BROILER CHICKEN FED DIETS SUPPLEMENTED WITH *IRVINGIA GABONENSIS* KERNEL POWDER AND *OCIMUM GRATISSIMUM* LEAF POWDER

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ABSTRACT

This study assessed the effects of *Irvingia gabonensis* kernel powder (IKP) and *Ocimum gratissimum* leaf powder (OLP) dietary supplementation on performance of broiler chicken. A basal diet divided into four portions, designated diet 1 (the control) and diets 2, 3 and 4 supplemented with 2.5g.kg^{-1} IKP, OLP and IKP+OLP composite mix 1:1 (IOCM), respectively. Two hundred and forty 1-day broiler chicks were randomly assigned to the four experimental diets (60 birds/diet; 10 birds per/replicate) using a Completely Randomised Design. At the finisher phase, the body weight gain and feed conversion ratio of the birds fed diets 2 and 4 were better ($P < 0.05$) than those fed the diets 1 and 3. During the overall period, the body weight gain of birds fed diet 4 was similar to those fed diet 2 but higher than those fed diets 1 and 3, while the feed conversion ratio of birds in diets 2 and 4 was better ($P < 0.05$) than those fed diets 1 and 3. The slaughtered and dressed weights of the birds fed diets 2 and 4 were significantly ($P < 0.05$) better than those birds fed the diets 1 and 3. The dressing percentage of the birds fed diets 2 and 4 were higher ($P < 0.05$) than those fed diet 1. White blood cells, granulocytes and lymphocytes counts were ($P < 0.05$) higher in birds fed diet 4 compared to those on other diets. Serum cholesterol concentration and meat lipid peroxidation activities were significantly ($P < 0.05$) lower in the birds fed diets 2, 3 and 4 compared to diet 1. The catalase concentration in the birds fed diets 3 and 4 were ($P < 0.05$) higher than those chickens fed diet 1, while the glutathione peroxidase concentration in the birds fed the diets 2 and 4 were ($P < 0.05$) higher than those fed diet 1. Glutathione concentration was higher ($P < 0.05$) in meat from birds fed diets 2, 3 and 4, compared to the birds fed diet 1. The meat cholesterol concentration recorded in the birds fed diet 4 was comparable ($P > 0.05$) to diets 2 and 3, but lower ($P < 0.05$) than diet 1. The IKP and IOCM supplementation improved the growth performance of the broiler chickens. The overall health status and meat quality were also improved by the phytogenic supplements in this study.

Key words: supplements; phytogenics; performance; anti-oxidative status; poultry

INTRODUCTION

The supplementation of diets with phytochemicals or phytogenic supplements in poultry nutrition and production has attracted considerable attention to enhancing the performance, carcass traits, health status and potentially reduce the negative effect of anti-oxidative stress since the past couple of years (Valenzuela-Grijalva *et al.*, 2017; Oloruntola *et al.*, 2018a). This is primarily because of an increased number of consumers, who are conscious of the quality and the type of their food and increased awareness of the numerous health risks associated with the use of synthetic chemicals in animal production (Gonzalez

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and Angeles, 2017). Besides, the increasing global adoption of prohibiting law against the use of antibiotic growth promoters (OJEU, 2003) further catalyses the increased hunger for searching the alternatives such as secondary plant metabolites, otherwise known as phytochemicals (Valenzuela-Grijalva *et al.*, 2017; Oloruntola *et al.*, 2019). These phytochemicals aside being considered a suitable replacement for antibiotic growth promoters are also used as other steroidal compounds (testosterone and progesterone) being used to improve the growth of animals (Gonzalez-Rios *et al.*, 2016).

Phytogenic feed supplements are a large group of compounds having diversified chemical bioavailability and structure (Surai, 2014). These phytogenic bioactive compounds in the plants vary, depending on some factors, such as the specific part of the plant, the harvest season, production techniques or methods and geographical location (Ganguly, 2013). Phytochemicals have some biological properties (antioxidant, anti-stress, antimicrobial and immunomodulatory) that prompt their consideration for use as growth promoters in livestock production (Hashemi and Davoodi, 2010).

The use of various types of phytochemicals such as extracts or parts of red pepper, lemon, clove, black cumin seed, artemisia leaf among others in broiler chicken's production produced positive results (Valenzuela-Grijalva *et al.*, 2017), while a few studies reported no significant effects (Barreto *et al.*, 2008; Goliomytis *et al.*, 2014).

Irvingia gabonensis trees produce mango-like fruits. The fruit is 4 – 7 cm long, and its kernel and pulp are edible by animals and man. The kernel is rich in oil, fat and protein and is being considered the most valuable component of the fruit (Mgbemena *et al.*, 2019). Previous phytochemical screenings of *Irvingia gabonensis* kernel reveals the presence of biologically active compounds such as tannins, alkaloids, terpenoids, steroids, saponins and glycosides, which are known for aiding antimicrobial activities (Igbinosa *et al.*, 2009; Lillehoj *et al.*, 2018; Mgbemena *et al.*, 2019).

Ocimum gratissimum is a perennial herb, which grows up to 1-2 m and having an erect stem. The *Ocimum gratissimum* plant is used in traditional medicine in India and Africa for treatment of cases, such as headache and influenza, fever, gonorrhoea, inflammation of the ears, throat or eyes, skin diseases, stomach pain and diarrhoea (Rabelo *et al.*, 2003; Adebolu and Salau, 2005; Kabir *et al.*, 2005). Prabhu *et al.*, (2002) reported the antimicrobial, antifungal, ovicidal, leishmanicidal and anti-diarrhoeal activities of *O. gratissimum* extracts.

It was observed that relatively low work was done to assess the effects of the use of *I. gabonensis* kernel and *O. gratissimum* leaf powders in broiler production compared to other medicinal plants. Also, since there could be positive effects resulting from the interactions of the various bioactive compounds in these botanicals (Brenes and Roura 2010; Oloruntola *et al.*, 2018a), this feeding trial was conducted to assess the consequences of dietary supplementation of *I. gabonensis* kernel powder, *O. gratissimum* leaf powder and their combinations on the performance, carcass traits, health status, meat analysis and antioxidant status of broiler chickens.

MATERIAL AND METHODS

Ethical approval, phytogens gathering and processing

This experiment was carried out according to the specifications and guidelines of animal and animal protocol approved by the Research and Ethics Committee of the Department of Animal Science, Adekunle Ajasin University, Akungba-Akoko, Nigeria. The pericarp, mesocarp and the endocarp of freshly plucked ripe fruits of *I. gabonensis* were removed with sharp stainless knives to expose the kernel. After that, the kernels were chopped with a stainless knife into smaller pieces, spread on a clean tarpaulin, air-dried for 21 days and ground to about 70 µm to produce *I. gabonensis* kernel powder (IKP). Freshly plucked leaves of *Ocimum gratissimum* were also chopped into smaller pieces with sharp stainless knives, spread lightly on a tarpaulin to air-dry for 14 days and milled to the particulate size of 70 µm to produce *O. gratissimum* leaf powder (OLP). Equal portions (1:1) of IKP and OLP were mixed to form *I. gabonensis* and *O. gratissimum* leaf powder composite mix (IOCM). After that, the IKP, OLP, and IOCM were analysed for saponin (Brunner, 1984), flavonoids (Bohm and Kocipal-Abyazan, 1994), phenol (Ignat *et al.*, 2013), terpenoids (Sofowora 1993) and 2,2-diphenyl-1-picrylhydrazy hydrate (Gyamfi *et al.*, 1999).

Diets, housing and experimental design

A broiler chickens' basal diet each was prepared for the starter phase (0 to 28 days) and finisher phase (29-56 days) to meet the requirements of the birds (NRC, 1994). At each of the phases, the basal diet was divided into four equal portions and designated to Diets 1 to 4. Diet 1 was the control, while the diets 2, 3 and 4 were supplemented with 2.5 g of IKP, OLP and IOCM.kg^{-1}, respectively. The experiment was performed at the Avian Unit of the Teaching and Research Farm, Adekunle Ajasin University, Akungba-Akoko, Nigeria.

Two hundred and forty (240) 1-day old Cobb 500 broiler chicks with an average initial body weight of 44.99 ± 0.90 g were randomly assigned to four experimental diets (60 birds per diet; 10 birds per replicate) using a completely randomised design (CRD). The floor of the experimental pen (200 x 100 cm) used for housing each replicate was covered with wood shaving while the temperature of the experimental house was maintained at 31 ± 2 °C for the first week and gradually being reduced by 2 °C after each consecutive week until the experimental house temperature was 26 ± 2 °C. The lighting duration was 23 hours per day, while the feed for the birds was provided *ad libitum* throughout the experiment.

Growth performance

The experimental birds' body weight (BW) and feed intake (FI) were determined and recorded on a 7-day interval. The average body weight gain (BWG) was calculated as the differences between the initial weights and final weights of the birds while their feed conversion ratio (FCR) was estimated as the ratio of feed consumed to weight gain.

Slaughtering procedures, collection of blood samples and carcass analysis

On day 56 of the experiment, 18 birds randomly selected from each dietary treatment (3 birds/replicate) were tagged, weighed, stunned and sacrificed by cutting the two jugular veins in the neck region with a stainless-steel knife. Blood was allowed to flow into a plain blood sample bottle for serum biochemicals and enzymes (creatinine, aspartate aminotransferase, alanine aminotransaminase, and cholesterol); antioxidant enzymes (catalase, superoxide dismutase and, glutathione peroxidase) and also into EDTA bottle for haematological studies. The blood sample in each of the plain bottles was spun and its serum decanted into another plain bottle and frozen at -20 °C before analysis. The haematological indices were determined within 2 hours post-collection as described by Shastry (1983). The concentrations of serum enzymes were determined on a Reflectron ®Plus 8C79 (Roche Diagnostic, GombH Mannheim, Germany), using kits. The serum catalase, superoxide and glutathione peroxidase were determined as described by Aebi (1974), Misra and Fridovich, (1972) and Rotruck *et al.*, (1973), respectively.

The selected slaughtered experimental birds were de-feathered, dressed and weighed. After that, the dressed percentage was estimated as a percentage of the slaughtered weight. The internal organs (liver, heart, lung, pancreas, gall bladder, gizzard and proventriculus, and the spleen) were carefully excised, wiped clean with tissue paper and weighed with a sensitive scale. The relative internal organ weight was expressed as a percentage of the bird's slaughtered weight. About 100 g of the meat was excised from the breast meat for determination of the level of the meat cholesterol (Allain *et al.*, 1974), lipid peroxidation (Bostoglou *et al.*, 1994), catalase activity (Hadwan and Khabt, 2018) and glutathione peroxidase activity (Cichoski *et al.*, 2012).

The contents of the caeca from the experimental birds (1 bird/replicate) were collected for bacterial population's analysis by serial dilution. Agar plates were aseptically prepared a day before the caecal content collection. The plates were streaked on the experimental site to determine the bacteria's growth. The aerobic bacteria were cultured in the nutrient agar, lactic acid-producing bacteria were cultured on Man Rogosa agar, while the coliforms and intestinal negative lactose bacteria were cultured in the MacConkey agar (Dibaji *et al.*, 2014; Seidavi and Simoes 2015).

Analysis of data

The model: $Txy = \mu + \alpha x + \beta xy$, was used in this experiment, where Txy = any of the response variables; x = the overall mean; αx = effect of the *xth* treatment (T = diets 1, 2, 3 and 4); and βxy = random error due to experimentation. All the data were subjected to one-way ANOVA using SPSS version 20. The differences among the treatment means were determined ($P < 0.05$) by Duncan multiple range test of SPSS.

RESULTS

The contents of saponin (34.74 mg.g^{-1} vs. 26.08 mg.g^{-1}), flavonoid (3.19 mg.g^{-1} vs. 0.26 mg.g^{-1}), phenol (19.61 mg.g^{-1} vs. 14.97 mg.g^{-1}) and terpenoid (94.18 mg.g^{-1} vs. 89.42 mg.g^{-1}) in IKP- versus OLP are presented in Figure 1. The concentrations of the phytochemicals determined were higher in IKP- compared to OLP. Figure 2 shows the antioxidant property (2,2-diphenyl-1-picrylhydrazyl hydrate) of IKP- (28.10 %) and OKM- (35.50 %).

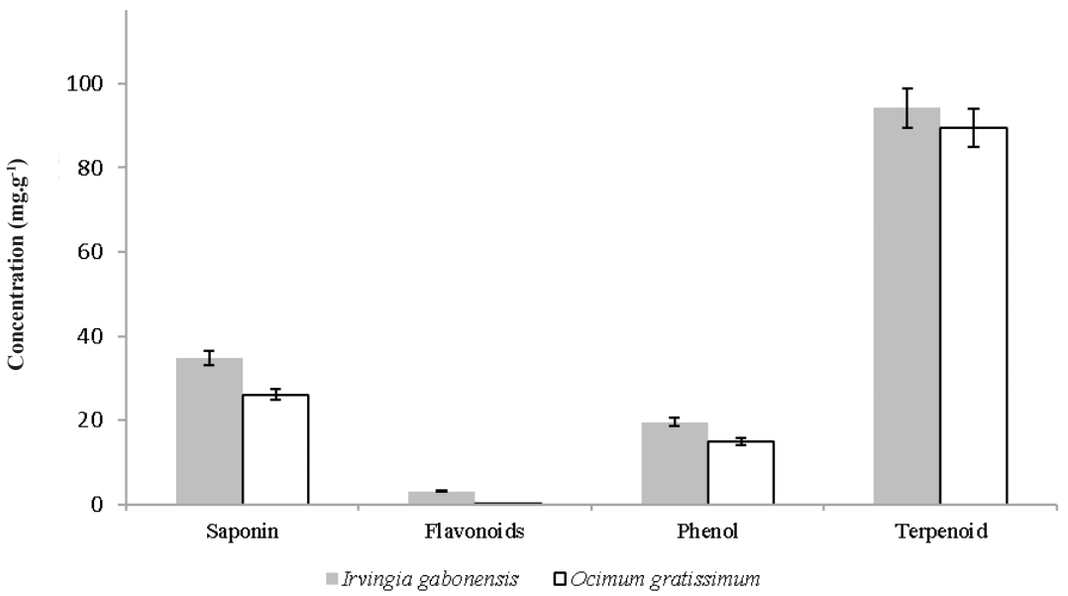

Figure 1. Phytochemical compositions of *I. gabonensis* kernel and *O. gratissimum* leaf powders

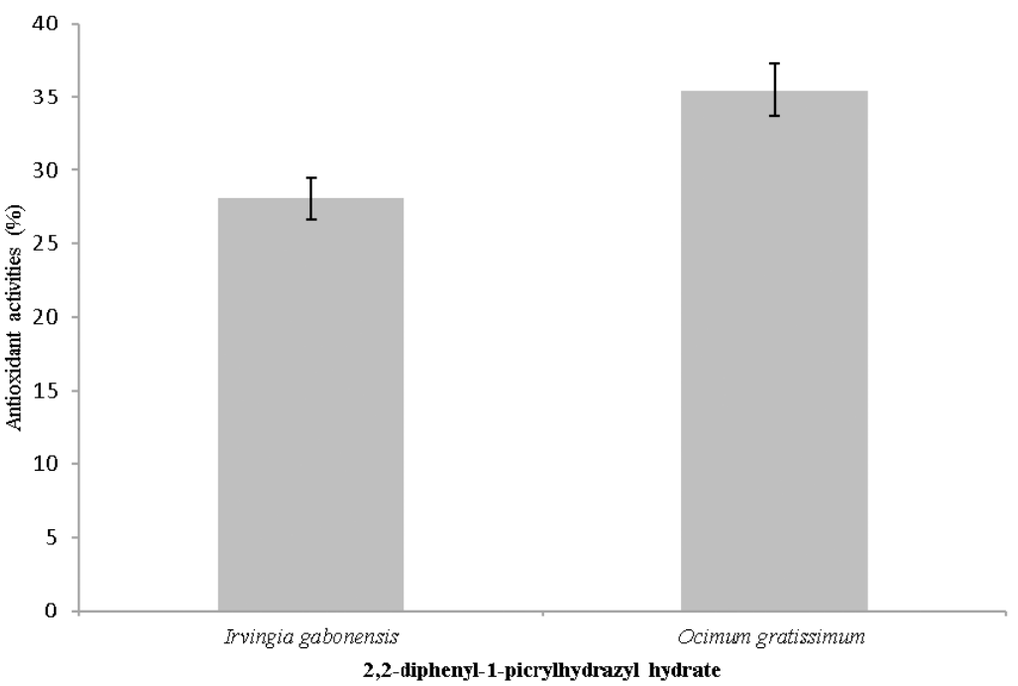

Figure 2. Antioxidant activities of *I. gabonensis* kernel and *O. gratissimum* leaf powders

Table 1. Composition of the experimental diets

Ingredients	Starter diet	Finisher diet
Maize	52.35	59.35
Maize bran	7.00	0.00
Rice bran	0.00	6.00
Soybean meal	30.00	24.00
Fish meal	3.00	3.00
Soy oil	3.00	3.00
Bone meal	3.00	3.00
Limestone	0.50	0.50
Lysine	0.25	0.25
Methionine	0.30	0.30
Salt	0.30	0.30
Premix	0.30	0.30
Nutrient composition (%)		
*Crude protein	22.18	20.03
Metabolizable energy (Kcal.kg^{-1})	3018.89	3108.10
Lysine	1.36	1.24
Methionine	0.68	0.66
Calcium	1.01	0.99
Available phosphorus	0.70	0.73

*Analysed composition

The effects of the phytogenic supplementations on the body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) during the starter phase (1-28 day), finisher phase (29-56 day) and overall (1-56 day) are presented in Table 2. During the finisher phase, the BWG and FCR of the birds fed IKP and composite mix of IKP and IOCM supplemented diets (Diets 2 and 4) were similar ($P > 0.05$), but significantly ($P < 0.05$) better than those birds fed the control diet and OLP-supplemented diet. For the overall period of the experiment, the BWG of the birds fed diet 4 was similar to those fed diet 2, but significantly ($P < 0.05$) higher than those fed the diets 1 and 3. The FCR of the birds fed diets 2 and 4 were similar ($P > 0.05$) to those fed diet 3, but better ($P < 0.05$) than those fed diet 1. The slaughtered and dressed weights of the birds fed IKP- and IOCM-supplemented diets were higher ($P < 0.05$) compared to the control diet and OLP-supplemented diet (Table 3). Similarly, the dressing percentage of the birds fed diets 2 and 4 were similar ($P > 0.05$) to those fed diet 3, but higher ($P < 0.05$) than those fed the control diet.

The significant dietary treatment effects were not recorded for the haematological indices except for the white blood cell (WBC), granulocyte and lymphocyte counts that were significantly ($P < 0.05$) higher in birds fed diet 4 compared to those fed the other diets (Table 4). Table 5 shows the effects of the phytogenic supplements on the serum metabolites

Table 2. Effects of the phyto-supplements on the performance characteristics of broiler chickens

Parameters	Diet 1 Control	Diet 2 0.25 % IKP	Diet 3 0.25 % OLP	Diet 4 0.25 % IOCM	SEM	*P*-value
Starter phase (1 to 28 day)						
IBW (g/bird)	43.09	47.52	45.42	43.93	0.90	0.35
BW G (g/bird)	990.10	1052.60	1040.01	1156.22	34.86	0.44
FI (g/bird)	1407.46	1307.15	1406.66	1412.30	35.91	0.74
FCR	1.44	1.27	1.35	1.22	0.06	0.63
Finisher phase (29 to 56 day)						
BWG (g/bird)	1824.30^{b}	2161.11^{a}	1687.26^{b}	2218.98^{a}	79.85	0.01
FI (g/bird)	3561.04	3238.67	3250.77	3532.37	77.07	0.30
FCR	1.95^{a}	1.51^{b}	1.93^{a}	1.59^{b}	0.07	0.01
Overall (1 to 56 day)						
BWG (g/bird)	2314.40^{b}	3213.71^{ab}	2727.27^{b}	3374.20^{a}	103.65	0.04
FI (g/bird)	4968.51	4545.83	4657.43	4944.68	101.17	0.40
FCR	1.77^{a}	1.42^{b}	1.71^{ab}	1.46^{b}	0.05	0.05

Means within a row with different letters are significantly different ($P < 0.05$); IKP: Irvingia kernel powder; OLP: Ocimum leaf powder; IOCM: Irvingia and Ocimum composite mix (1:1); SEM Standard error of the mean.

Table 3. Effects of phyto-additives on carcass and relative internal organ weights (% slaughtered weight) of broiler chickens

Parameters	Diet 1 Control	Diet 2 0.25 % IKP	Diet 3 0.25 % OLP	Diet 4 0.25 % IOCM	SEM	*P*-value
Slaughtered weight (g/bird)	2618.66^{b}	3254.67^{a}	2570.65^{b}	3436.01^{a}	115.34	0.01
Dressed weight (g/bird)	2016.65^{b}	2630.34^{a}	2118.02^{b}	2795.65^{a}	107.52	0.01
Dressing percentage (%)	79.95^{b}	80.78^{a}	79.34^{ab}	81.31^{a}	0.66	0.04
Liver	1.62	1.46	1.61	1.33	0.05	0.19
Heart	0.41	0.34	0.34	0.35	0.01	0.46
Lung	0.47	0.42	0.44	0.40	0.22	0.82
Pancreas	0.16	0.11	0.16	0.13	0.01	0.67
Gall bladder	0.13	0.09	0.16	0.10	0.01	0.13
Gizzard and proventriculus	2.37	1.96	2.09	1.94	0.09	0.42
Spleen	0.13	0.11	0.10	0.08	0.01	0.50

Means within a row with different letters are significantly different ($P < 0.05$); IKP: Irvingia kernel powder; OLP: Ocimum leaf powder; IOCM: Irvingia and Ocimum composite mix (1:1); SEM Standard error of the mean.

Table 4. Effects of phyto-additives on haematological indices of broiler chickens

Parameters	Diet 1 Control	Diet 2 0.25 % IKP	Diet 3 0.25 % OLP	Diet 4 0.25 % IOCM	SEM	*P*-value
Packed cell volume (%)	33.66	33.50	33.50	37.90	0.81	0.06
Red blood cells (x10^{12}.l^{-1})	3.00	2.90	2.30	3.00	0.18	0.53
Haemoglobin conc. (g.dl^{-1})	11.46	11.16	11.16	12.55	0.23	0.10
Mean cell haemoglobin conc. (g.dl^{-1})	35.19	33.72	33.23	33.37	0.32	0.10
Mean cell volume (fl)	110.53	123.48	149.61	133.90	8.01	0.40
Mean cell haemoglobin (pg)	39.02	41.16	49.87	40.36	2.75	0.55
White blood cells (x10^{9}.l^{-1})	3.90^{b}	2.60^{c}	3.40^{bc}	7.23^{a}	0.55	0.00
Granulocytes (x10^{9}.l^{-1})	0.74^{c}	0.51^{c}	1.67^{b}	2.97^{a}	0.30	0.00
Lymphocytes (x10^{9}.l^{-1})	3.10^{b}	2.05^{c}	1.64^{c}	4.17^{a}	0.30	0.00
Monocytes (x10^{9}.l^{-1})	0.04	0.03	0.08	0.06	0.01	0.57

Means within a row with different letters are significantly different ($P < 0.05$); IKP: Irvingia kernel powder; OLP: Ocimum leaf powder; IOCM: Irvingia and Ocimum composite mix (1:1); SEM Standard error of the mean.

and enzymes of the broiler chickens. The serum creatinine, aspartate aminotransferase and alanine aminotransferase were not affected ($P > 0.05$) by the dietary treatment, while the serum cholesterol concentrations were significantly ($P < 0.05$) lower in birds fed diets 2, 3 and 4 compared to those fed the diet 1. The serum catalase concentrations in the birds fed diets 3, and 4 were significantly ($P < 0.05$) higher than in those chickens fed the control diet, while the highest serum glutathione peroxidase concentration recorded in the birds fed diets 2 and 4 was comparable ($P > 0.05$) to those fed the diet 3 but significantly ($P < 0.05$) higher than those fed the diet 1.

The effects of phytogenic supplements on the lipid peroxidation, antioxidant enzymes and cholesterol of the meat were shown in Table 6. The lipid peroxidation activities were significantly ($P < 0.05$) reduced in diets 2, 3 and 4, so that the least lipid peroxidation was recorded in meat from the birds fed diet 4. The catalase activity was not affected ($P > 0.05$) by dietary treatment. However, the glutathione concentration was higher ($P < 0.05$)

Table 5. Effects of phyto-additives on serum metabolites and serum antioxidant enzymes of broiler chickens

Parameters	Diet 1 Control	Diet 2 0.25 % IKP	Diet 3 0.25 % OLP	Diet 4 0.25 % IOCM	SEM	*P*-value
Serum metabolites						
Creatinine (μmol.L^{-1})	43.48	47.33	29.00	35.16	6.83	0.82
Aspartate aminotransferase (IU.L^{-1})	75.82	108.66	89.00	83.56	5.98	0.26
Alanine aminotransferase (IU.L^{-1})	28.67	30.05	31.96	28.84	1.28	0.83
Cholesterol (μmol.L^{-1})	6.68^{a}	3.07^{b}	3.15^{b}	3.13^{b}	0.55	0.02
Serum antioxidant enzymes						
Catalase (mM.ml.min^{-1})	5.90^{c}	9.09^{bc}	17.40^{a}	12.40^{ab}	1.51	0.01
Superoxide dismutase (%)	75.38	75.64	74.46	61.93	3.99	0.62
Glutathione peroxidase (μg.g^{-1})	77.68^{b}	133.16^{a}	119.56^{ab}	145.36^{a}	9.76	0.04

Means within a row with different letters are significantly different ($P < 0.05$); IKP: Irvingia kernel powder; OLP: Ocimum leaf powder; IOCM: Irvingia and Ocimum composite mix (1:1); SEM Standard error of the mean.

Table 6. Effects of phyto-additives on the quality of broiler chicken meat

Parameters	Diet 1 Control	Diet 2 0.25 % IKP	Diet 3 0.25 % OLP	Diet 4 0.25 % IOCM	SEM	*P*-value
Lipid oxidation (mg MDA.100 g^{-1})	13.72^{a}	7.94^{b}	7.39^{bc}	3.43^{c}	1.24	0.00
Catalase (U.ml^{-1})	1.93	2.05	1.38	2.87	0.28	0.35
Glutathione peroxidase (mg.ml^{-1})	149.83^{b}	253.50^{a}	227.59^{a}	247.08^{a}	15.11	0.02
Cholesterol (mg.dl^{-1})	218.75^{a}	138.12^{ab}	106.25^{ab}	43.75^{b}	23.72	0.03

Means within a row with different letters are significantly different ($P < 0.05$); IKP: Irvingia kernel powder; OLP: Ocimum leaf powder; IOCM: Irvingia and Ocimum composite mix (1:1); SEM Standard error of the mean.

Table 7. Effects of the phyto-supplements on intestinal microbiology (log10 CFU.g^{-1}) of broiler chickens

Parameters	Diet 1 Control	Diet 2 0.25 % IKP	Diet 3 0.25 % OLP	Diet 4 0.25 % IOCM	SEM	*P*-value
Aerobic bacteria	5.05	4.98	5.35	5.05	0.14	0.84
Lactic acid-producing bacteria	5.80	5.79	5.38	5.53	0.07	0.13
Coliform bacteria	5.13	4.75	4.73	4.51	0.15	0.64
Intestinal negative bacteria	5.05	4.68	4.81	5.43	0.14	0.28

Means within a row with different letters are significantly different ($P < 0.05$); IKP: Irvingia kernel powder; OLP: Ocimum leaf powder; IOCM: Irvingia and Ocimum composite mix (1:1); SEM Standard error of the mean.

in meat from birds fed diets 2, 3 and 4, compared to the meat from the birds fed the diet 1. The least meat cholesterol concentration recorded in the meat of the birds fed the diet 4 was comparable ($P > 0.05$) to those fed diets 2 and 3, but significantly ($P < 0.05$) lower than those fed the diet 1. The phytosupplements did not cause significant difference ($P > 0.05$) in the aerobic bacteria, lactic-acid producing bacteria, coliform bacteria and intestinal negative bacteria populations in the chickens' intestine (Table 7).

DISCUSSION

In this study, the presence of secondary bioactive compounds such as saponin, flavonoids, phenol and terpenoid and the antioxidant activities of IKP and OLP suggests that these phytogenics, when incorporated into the diets of the broiler chickens, could generate beneficial effects on their performance and health status.

The variability of the birds' response to the dietary treatment between the starter and finisher phases of the birds suggests that the age and another factors, such as differences in the nutritional requirements and management practices between the two phases, may influence the growth response of these birds to dietary phyto-supplementation. This is in agreement with Oloruntola *et al.* (2018a), who recorded the effects of phyto-supplements on the growth performance of the broiler chicken at the finisher phase but not at the starter phase. According to Valenzuela-Grijalva *et al.* (2017), most of the *in vivo* studies evaluating the effects of dietary phyto-supplementation on the growth performance of broiler chickens were positive. In this study, the observed improved BWG and FCR of the experimental birds fed IKP- and IOCM-supplemented diets during the finisher phase (29-56 day) and overall (1-56 day) could be due to the activities of the constituents of these phytogenics. The biological activities (antimicrobial, antioxidant and flavour enhancer) of phytochemicals were reported (Negi, 2012; Valenzuela-Grijalva *et al.*, 2017) and could have contributed to the improved BWG and FCR recorded in these groups of birds. Besides, phytochemicals were reported to exert anabolic effects and modulate the animals' metabolism to influence the increase of the muscle tissue (Devi *et al.*, 2015; Gonzalez-Rios *et al.*, 2016). In particular, the flavonoid, one of the phytoconstituents of IKP and OLP is known for its active role in the suppression of reactive oxygen species (ROS) formation, scavenging for ROS and protection and upward regulation of antioxidant defences (Halliwell and Gutteridge, 1998; Mishra *et al.*, 2013). Flavonoid is also known as an established hepatoprotective, anticancer, anti-inflammatory and antiviral agent (Kumar and Pandey, 2013). Therefore, the antioxidant activities of the phytogens used in this study may also contribute to the enhanced weight gain and feed conversion ratio recorded in the birds fed IKP- and IOCM-supplemented diets in this study.

Lillehoj *et al.* (2018) reported that a combination of multiple phytogenics exerted synergistic effects to ameliorate the adverse consequences of intestinal infection. This could be responsible for the relatively superior growth performance observed in the birds fed with IOCM-supplemented diet in this study. Since the phyto-supplementation did not exert any effect on the feed intake in the experimental birds across the various dietary treatments in this study, the enhanced growth performance recorded in the birds fed IKP- and IOCM-supplemented diets may be due to the activities of the phytoconstituents of IKP and the synergistic effects IOCM to stimulate the functions of the intestinal tract to improve the digestive secretion, nutrient digestion, absorption and metabolism. Phytochemicals exhibit biological activities, such as a decrease of pathogenic load, increase of digestive secretions, development of antioxidant and anti-inflammatory activities in the intestinal lumen and improved intestinal morphology, which may result in improved nutrient utilisation and enhanced growth (Dhama *et al.*, 2014).

The improved slaughtered weight dressed weight, and dressed weight percentage recorded in the birds fed the IKP- and IOCM-supplemented diets in this study agreed with the earlier reports that supplementation of the broiler chickens diet with phytochemicals such as thyme, lemon balm, essential oil blend and cinnamon improved the carcass weight and dressed percentage (Kanduri *et al.*, 2013; Valenzuela-Grijalva *et al.*, 2017). This suggests that the phytogenic supplements used in this study have phytoconstituents or bioactive compounds (e.g., hydroxycinnamic acid derivatives of the phenylalanine) that can modulate animal metabolism in a similar pattern with β-adrenergic agonist compound (Gorewit, 1983; Valenzuela-Grijalva *et al.*, 2017). These plant-based compounds have a similar structure with the catecholamines (the natural animal hormones). They could interact with β-adrenergic receptor agonists to modulate animal metabolism by increasing lipolysis and protein synthesis and by decreasing lipogenesis (Dominguez-Vira *et al.*, 2009). The decrease or increase in the relative weights of the internal organs of the animals has been reported as a possible

response of their internal organs to toxins in their diets (Ayodele *et al.*, 2016). The similarity in the growth response of these animals' internal organs to the phytogenic supplementation in this study suggests the support of the supplements to the normal functioning of the birds' internal organs.

There is a relationship existed between nutritional deficiency and changes in the blood constituents. The haematological indices are among the known good indicators of the physiological status of the animals (Khan and Zafar, 2005). In this study, the stable erythrogram constituents' values recorded in the birds across the various dietary treatments indicate that the dietary phyto-supplements did not have a harmful interference on the haematopoiesis in the experimental birds. The variations recorded in the white blood cell values and their differentials across the dietary treatments in this study may indicate the immunomodulatory effects of the phytogenic supplements in the experimental birds, as reported by Oloruntola *et al.* (2016). The white blood cells play a significant role in the fighting against an infection. Therefore, the rise in the white blood cell and the differential counts (granulocytes and lymphocytes) of the birds fed the IOCM-supplemented diets in this study suggests that the IOCM (i.e. the composite mix of IKP and OLP) could trigger a complex but beneficial immunomodulatory response in the birds. This is supported by the earlier report of Hang and Lee (2018). The phytochemicals such as flavonoids, carotenoids and vitamin C have been shown to possess immune-stimulatory properties by improving the activities of lymphocytes, monocytes, macrophages, the immunoglobulin response and NK cells (Frankic *et al.*, 2009; Alipour *et al.*, 2015).

The similar creatinine concentration in the birds fed phytogenic-supplemented diets and the control suggest that the supplements used in this study did not pose any threat to the renal functions of the birds (Peters and Susan, 1991). The aspartate aminotransferase and alanine aminotransferase levels are commonly used to detect hepatic cell infraction and inflammation (Oloruntola *et al.*, 2018a, b). The stable aspartate aminotransferase and alanine aminotransferase concentrations negate the occurrence of the liver and biliary system disease, skeletal muscle disease, myocardial disease and non-specific tissue injury in the experimental birds as a result of the phytogenic supplementation tested in this study (Peter and Susan, 1991). Excess of serum cholesterol concentration promotes cholesterol accumulation on the artery walls creating plaques that lead to the narrowing of the arteries lumen and reduction of the rate of blood flow to the heart (Oloruntola *et al.*, 2018a, c). Therefore, the observed reduced serum cholesterol concentration recorded in the birds fed the phytogenic-supplemented diets in this study is of health benefit because abnormally high serum cholesterol concentration has an association with arteriosclerosis and sudden death syndrome in broiler chickens (Kawada *et al.*, 1994; Olkowski *et al.*, 2007) what also suggests IKP, OLP and IOCM possessed hypo-cholesterol properties.

The observed reduced cholesterol level, as a result of phytogenic supplementation in this study, may be due to the activities of secondary metabolites (e.g. saponin) in IKP, OLP and IOCM, that promoted the reduction of the gut uptake of cholesterol through the intra-luminal physicochemical interaction (Oloruntola *et al.*, 2018a). Also, phytochemicals or phytogens inhibit 3-hydroxy-3methylglutarycoenzyme A (HMG Co-A) reductase; and the consequence of this inhibition may be pleiotropic, because mevalonate, the product of HMG Co-A reductase reaction is the precursor for cholesterol (Bellosta *et al.*, 2000; Vaughan *et al.*, 2000). Enzymatic activities, such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx), provide protection against oxidative stress (Young and Woodside, 2001). The relatively higher catalase and glutathione peroxidase activities recorded in the birds fed the IKP-, OLP- and IOCM- supplemented diets in this study further unveiled the antioxidant and anti-stress properties of these phytogenics (Hashemi and Davoodi, 2010). The higher antioxidant activities recorded in the birds fed the IKP-, OLP- and IOCM-supplemented diets in this work may be due to the polyphenolic contents (flavonoids or phenolic acids) of the phytogens (Goyal and Brahma, 2014). According to Dhama *et al.* (2015), the plants' active ingredients generate strong antioxidant effects that scavenge the radicals or increase the CAT, SOD and GPx activities. The antioxidant activities of these phytogenics may also have contributed to the superior performance recorded in those birds fed

the phytogenic-supplemented diets, compared to those fed the control diet in this study. According to Biswas *et al.* (2011), antioxidants play a significant role in the performance of poultry.

Oxidative process during the shelf-life of meat can depreciate its nutritional and sensory values (Kumar *et al.*, 2015). Therefore, strategies that can promote the avoidance of the oxidation of lipids and proteins will contribute to the extension of the useful life/shelf life of meat (Velasco and Williams, 2011). This is because the progression of lipid oxidation promotes the loss of physiological function, membrane property alteration, enzyme inactivation, denaturation and rupture, causing cellular component leakage (Bekhit *et al.*, 2013). Currently, the dietary inclusion of natural antioxidants during animal production is being proposed (Brewer, 2011) to forestall the deposition of antioxidants in the meat during the life of the animals and subsequently enhancing the health status of the animals and the shelf life of the meat by enhancing the oxidative status ante-mortem (Descalzo and Sancho, 2008).

The reduced lipid peroxidation activities in the meat of the birds fed the IKP-, OLP- and IOCM-supplemented diets in this study further support the fact that dietary inclusion of phytogenics in the animals' diet during production could reduce the lipid peroxidation activities and subsequent improvement of the meat shelf life (Descalzo and Sancho, 2008, Valenzuela-Grijalva *et al.*, 2017). Peroxide (H_2O_2) can diffuse within the cell and produce noticeable damages to the cells and the muscle systems of living organisms (Bekhit *et al.*, 2013). Therefore, the availability of catalase, thioredoxin peroxidase and glutathione peroxidase is essential in eliminating H_2O_2 (Marchi *et al.*, 2012). The higher glutathione peroxidase concentration recorded in the meat from birds fed the IKP-, OLP- and IOCM-supplemented diets in this study unveiled the possibility of these phytogenic supplements to modify the antioxidant enzymes in the muscular system and subsequent increase of the meat shelf life (Bekhit *et al.*, 2013). Presently, the type of cholesterol and fatty acids are of health importance to the consumers because of the existing relationship between the consumption of high cholesterol and saturated fat and an increased possibility of acquiring diseases such as high blood pressure, cancer, heart disease and obesity (Walker *et al.*, 2005). The reduced meat cholesterol recorded in birds fed IKP-, OLP- and IOCM-supplemented diets compared to those fed the control diet in this study may have an association with the low blood serum recorded in the same group of birds. There is a need to research further if there is a relationship exists between the blood and meat cholesterol concentrations. However, the reduced meat cholesterol level recorded in these birds, is of health benefit because of the enormous health complication associated with the ingestion of high cholesterol meat.

The gut of broiler chickens is densely colonised by the community of microorganisms that is intimately linked to the general health and development of the host (Oakley *et al.*, 2014). The avian caeca housed microorganism that functions in the breaking down of indigestible fibre substance and maintenance of the health status of the birds (Zulkifli *et al.*, 2009; Oakley *et al.*, 2014). For instance, lactic acid bacteria (probiotic microorganism), are usually associated with the maintenance and enhanced gut health and productivity because of their activities in reducing enteric diseases (Noohi *et al.*, 2014). Also, controlled adhesion of antimicrobial phytogens and subsequent modulation of the gut microflora promotes the maintenance of the intestinal epithelium integrity, reduced toxin production and increased nutrient availability for absorption (Dhama *et al.*, 2014). The observed similarity in the intestinal microbial population of the broiler chickens in this study implies the phytogenic supplementations used, which are able to maintain the healthy and stable intestinal microflora. A stable increase of non-pathogenic gut bacteria was reported as a necessary condition for inhibition of the proliferation of pathogens, improved growth performance and production, reduced morbidity and mortality (Gou *et al.*, 2004; Oloruntola *et al.*, 2020).

CONCLUSION

The IKP and OLP have phytochemicals of health benefits and possess antioxidant properties. The IKP and IOCM at 0.25 % dietary supplementation improved the BWG, FCR, slaughtered weight and dressed weight of broiler chickens at the finisher

phase. The phytogenic supplementation also caused immunomodulatory and hypocholesterolemic effects on the broiler chickens. The IKP, OLP and IOCM at 0.25 % dietary supplementation reduced the lipid peroxidation, increased the glutathione concentration and reduced the cholesterol level of the breast meat of the broiler chickens.

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