

Phytogenic blend as feed additive in the nutrition broiler chickens: Growth performance, apparent digestibility, gut morphology and caeca microbiota

Adeyeye, E. A^{1*}, Adeyeye, O. A^{2,3}, Idowu, O. P. A², Kareem, D. U^{1,5}, Amos, A. T¹, Orbugh, A. T¹, Lala, A. O^{1,4} and Oso, A. O¹

¹Department of Animal Nutrition, Federal University of Agriculture, P.M.B. 2240 Abeokuta, Nigeria

²Agricultural Media Resources and Extension Centre (AMREC), Federal University of Agriculture, P.M.B. 2240 Abeokuta, Nigeria

³Department of Agricultural Extension and Rural Development, Federal University of Agriculture, P.M.B. 2240 Abeokuta, Nigeria

⁴Institute of Food Security, Environmental Resources and Agricultural Research (IFSERAR), Federal University of Agriculture, P.M.B. 2240 Abeokuta, Nigeria

⁵Faculty of Animal Health and Veterinary Medicine, The University of Sydney, Sydney Australia

⁶Department of Animal Production and Health, Federal University of Agriculture, P.M.B. 2240 Abeokuta, Nigeria



*Corresponding email: adeyeyee@funaab.edu.ng, +2348067331986

Abstract

Public health awareness on the risk of consuming poultry products with residual antibiotics has geared nutritionist and researchers to seek alternative in phytogenic feed additives. The effects of dietary supplementation with phytogenic blend (PB) of *Piper nigrum*, *Zingiber officinale*, *Azadirachta indica*, *Cymbopogon citratus* and *Citrus sinensis* on growth performance, nutrient digestibility, gut morphology and caeca microbiota were investigated in a 42-day broiler chicken feeding trial. Two hundred and forty day old Cobb 500 broiler chicks were distributed to six treatments (5 replicates of 8 birds each): basal diet (T1), basal diet supplemented with 1g/5kg oxytetracycline (T2), 5, 10, 15 and 20g PB/kg of feed (T3, T4, T5 and T6), respectively. At the starter phase, broiler chicks fed with T2 and T6 had reduced ($p < 0.05$) final live weight and weight gain when compared with the control group. Birds fed with T3, T4 and T5 had similar final live weight and weight gain with control group. Feed conversion ratio improved ($p < 0.05$) in birds fed with T1, T2, T3 and T4 when compared to those fed with T4 and T5. Growth response was not affected by dietary supplementation with phytogenic blend (PB) at the finisher phase. Dietary supplementation of PB had no effect ($p > 0.05$) on all nutrient digestibility. No growth of *Staphylococcus aureus* was observed in the caeca of birds with T2, T3 and T4. *Klebsiella pneumoniae* count was least in birds fed with T2 and T6. Broilers fed with T6 had the least ($p < 0.05$) *Proteus vulgaris* count and highest ($p < 0.05$) *Escherichia coli* count while those fed with T4 recorded the highest ($p < 0.05$) *Enterobacter aerogenes*. Duodenal villi height, mucosa thickness and villus/crypt ratio of birds in T4 were ($p < 0.05$) higher. Cryptal depth was significantly higher in T1 and T6. Ileum villi height and mucosa thickness were ($p < 0.05$) higher for T4 and T5 with higher cryptal depth for T3. Jejunum villi height, mucosa thickness and cryptal height of birds in T2 were significantly higher. Villus/crypt ratio was similar across the treatments with highest ratio recorded for T3. In conclusion, the diets of broiler chicken can be supplemented with phytogenic blend at 10g/kg to modify their gut for nutrients absorption and without deleterious effect on growth performance.

Key words: Digestibility, gut, microbiota, oxytetracycline, performance, phytogenic blend

Mélange phytogénique utilisé comme additif alimentaire dans l'alimentation des poulets de chair : performances de croissance, digestibilité apparente, morphologie intestinale et microbiote du cæcum



Résumé

La prise de conscience, en matière de santé publique, des risques liés à la consommation de produits avicoles contenant des résidus d'antibiotiques a incité les nutritionnistes et les chercheurs à rechercher des alternatives sous forme d'additifs alimentaires phytogéniques. Les effets d'une supplémentation alimentaire à base d'un mélange phytogénique (PB) composé de Piper nigrum, Zingiber officinale, Azadirachta indica, Cymbopogon citratus et Citrus sinensis sur les performances de croissance, la digestibilité des nutriments, la morphologie intestinale et le microbiote du cæcum ont été étudiés dans le cadre d'un essai d'alimentation de 42 jours mené sur des poulets de chair. Deux cent quarante poussins de chair Cobb 500 âgés d'un jour ont été répartis en six traitements (5 réplicats de 8 oiseaux chacun) : régime de base (T1), régime de base complété par 1 g/5 kg d'oxytétracycline (T2), 5, 10, 15 et 20 g de PB/kg d'aliment (T3, T4, T5 et T6), respectivement. Au cours de la phase de démarrage, les poulets de chair nourris avec les régimes T2 et T6 ont présenté un poids vif final et un gain de poids réduits ($p < 0,05$) par rapport au groupe témoin. Les volailles nourries avec les régimes T3, T4 et T5 ont présenté un poids vif final et un gain de poids similaires à ceux du groupe témoin. L'indice de conversion alimentaire s'est amélioré ($p < 0,05$) chez les volailles nourries avec les régimes T1, T2, T3 et T4 par rapport à celles nourries avec les régimes T4 et T5. La réponse de croissance n'a pas été affectée par la supplémentation alimentaire en mélange phytogénique (PB) pendant la phase de finition. La supplémentation alimentaire en PB n'a eu aucun effet ($p > 0,05$) sur la digestibilité de l'ensemble des nutriments. Aucune prolifération de Staphylococcus aureus n'a été observée dans le cæcum des volailles nourries avec les régimes T2, T3 et T4. Le nombre de Klebsiella pneumoniae était le plus faible chez les volailles nourries avec les régimes T2 et T6. Les poulets de chair nourris avec le régime T6 présentaient la plus faible densité ($p < 0,05$) de Proteus vulgaris et la plus forte densité ($p < 0,05$) d'Escherichia coli, tandis que ceux nourris avec le régime T4 présentaient la plus forte densité ($p < 0,05$) d'Enterobacter aerogenes. La hauteur des villosités duodénales, l'épaisseur de la muqueuse et le rapport villosités/cryptes des volailles du groupe T4 étaient ($p < 0,05$) plus élevés. La profondeur des cryptes était significativement plus élevée dans les groupes T1 et T6. La hauteur des villosités iléales et l'épaisseur de la muqueuse étaient ($p < 0,05$) plus élevées pour les groupes T4 et T5, tandis que la profondeur des cryptes était plus élevée pour le groupe T3. La hauteur des villosités du jéjunum, l'épaisseur de la muqueuse et la hauteur des cryptes des poulets du groupe T2 étaient significativement plus élevées. Le rapport villosités/cryptes était similaire entre les traitements, le rapport le plus élevé ayant été enregistré pour le groupe T3. En conclusion, l'alimentation des poulets de chair peut être complétée par un mélange phytogénique à raison de 10 g/kg afin de modifier leur intestin pour favoriser l'absorption des nutriments, sans effet néfaste sur les performances de croissance.

Mots-clés : digestibilité, intestin, microbiote, oxytétracycline, performances, mélange phytogénique

Introduction

Public health concern on the risk of consuming poultry products raised with antibiotic growth promoter (AGP) and the potential development of bacteria resistant to synthetic antibiotics in human beings and animals and couple with the ban on you use synthetic antibiotics as growth promoter (Charis 2000) in many countries has

stimulated research into the use of botanical products known as phytogenic feed additives (PFAs). Phytogenic feed additives are plant based substances included in livestock diets at specific dosage with the purpose of ameliorating their performance. The choice of PFAs in poultry feeds is because they are generally recognized as safe by the Food and

Drug Administration (FDA) of the United States (Diaz-Sanchez *et al.*, 2015).

The use of PFAs like ginger, garlic, black pepper, clove, thyme, pepper fruit, orange peels, lemon grass and the likes as non-antibiotics in poultry production have received much attention globally. There are positive reports on the use of single application or combination of any of these PFAs on growth performance, gut morphology of broiler chickens. Fafiolu *et al.* (2020) reported that adding phytogenic feed additives containing lemon and orange peels promoted positive effect on villus height, improved red blood cell (RBC) production, enhanced the plasma protein reserves and lipid profile, and prevents muscle wastage. It was reported by Abou-Elkhair *et al.* (2014) that dietary supplementation with black pepper, coriander, or their combinations enhanced the performance and health status of broiler chickens. In the report of Karangiya *et al.* (2016) it was recommended that addition of garlic at 1% improves growth performance of broiler chickens when compared with ginger only or the mixture of garlic and ginger. Fallah and Mirzaei (2016) reported that addition of turmeric and thyme powders to broiler chickens diets increase body weight, feed intake and improve FCR at 42 days of age and decreased serum triglyceride, high density lipoprotein (HDL) and low-density lipoprotein concentration, and enhance antibody titer production of birds at 18 and 28 days of age. The novel about PFAs is the use of phytogenic blends as feed additive for likely positive interaction since they contain different phytochemicals with diverse biological functions. *Piper nigrum* (black pepper) is a flowering plant rich in enzymes that help cells to counterbalance oxidative stress (Karthikeyan and Rani 2003). Al-Kassie *et al.* (2011) reported that black pepper improved health status, heat production of lipids and speed up energy metabolism in broiler chickens. Ginger have been reported to have strong stimulating effect on the immune and digestive systems in birds (Al-

Shuwali *et al.*, 2015). Addition of ginger power to broiler chicken diet was reported (Olefouh-Okoleh *et al.*, 2014) to improved growth performance. *Azadirachta indica* (Neem) leaf contains some phytochemicals like azadiractin, nimbin, nimbindin, quercetin (Makeri *et al.*, 2007) which performs certain biological functions such as antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic (Gayathri and Arun 2021). Neem leaf meal added at 1.5% (Makeri *et al.*, 2007) and 2.5 g/kg (Ansari *et al.*, 2012) in broiler chickens diets recorded similar body weight gain (BWG) and feed conversion ratio (FCR) to the control. Lemon grass (*Cymbopogon citratus*) contains antioxidant, flavonoids, phenolic chemicals, and terpenoids which could be responsible for its many biological actions, like anti-inflammatory, immune-modulatory, fungistatic and antiseptic properties (Alagawany *et al.*, 2021). Lemon grass essential oil was reported (Ayman *et al.*, 2021) to enhanced growth performance and carcass traits of quails. Sweet orange (*Citrus sinensis*) peels (SOP) contain flavonoids that can function as direct antioxidants and free radical scavengers, and have the capacity to modulate enzymatic activities and inhibit cell proliferation (Duthie and Crozier 2000; Behera *et al.*, 2019). The findings of Fafiolu *et al.* (2020) revealed that *C. sinensis* has anti-inflammatory properties that can aid in digestion of fatty foods and reduce acidity and heartburn.

Therefore, it was hypothesized in this study that the blend of black pepper, ginger, sweet orange peels, neem leaf and lemon grass should be able to improve growth performance, nutrient digestibility, gut morphology and caeca microbiota of broiler chickens.

Materials and Methods

Study area

The trial was conducted at the Poultry Unit of the Directorate of University Farms (DUFARMS), Abeokuta, Ogun-State, Nigeria. The farm lies in the Tropical rainforest vegetation zone, South-

Western Nigeria on latitude 7°10'N and longitude 3°2'E. The prevailing climate is tropical humid with an average annual rainfall of 1037mm and a mean ambient temperature of about 34.7°C. Humidity of the experimental location is lowest (37- 85%) (Federal University of Abeokuta Meteorological Station).

Preparation of Phytogetic Blend

Dried ginger rhizomes (*Zingiber officinale*) and black pepper seed (*Piper nigrum*) were procured from a local vendor in Abeokuta Ogun State, Nigeria. The sweet orange peel (*Citrus sinensis*) was collected from the orange seller, fresh neem leaf (*Azadirachta indica*) and the lemon grass (*Cymbopogon citratus*) was obtained from the University Farm and air-dried for 7 days while the orange peel was sun-dried. Kitchen blender was used to mill the ingredients separately into powder. The blend was made by mixing 500g of ginger, 500g of black pepper, 500g of sweet orange peel and 250g of neem leaf and 250g of lemon grass in accordance with recommendation from past related studies (Ansari *et al.*, 2012; Olefouh-Okleh *et al.*, 2014; Abou-Elkhair *et al.*, 2014; Fafiolu *et al.*, 2020 and Ayman *et al.*, 2021) to form a single blend (Ginger + black pepper + sweet orange peel + neem leaf + lemon grass-2kg). The method of AOAC (2000) was used to analyze for chemical composition of formulated blend, dry matter and crude protein (CP) ($N \times 6.25$). Crude fat was determined by extraction with petroleum ether following acidification with 4 M hydrochloric acid as described by Wiseman *et al.* (1992). Gross energy was measured using an adiabatic bomb calorimeter (Rajdhani Scientific instruments Company, India).

Quantification of Bioactive Compounds in the blend

The phenolic acids, flavonoids, phytol, thymol, eugenol, and piperine content of PB were determined by the methods described previously with modifications (Hajimehdipoor *et al.*, 2010; Adosraku *et al.*, 2013; Xiao *et al.*, 2013; Inam *et*

al., 2014). An Acquity UPLC system coupled with TQD-MS/MS (Waters) with electrospray ionization source was used for the determination of phenolic acids and flavonoids. The chromatography system was equipped with a degasser, quaternary pump, auto injector, and a diode array detector. MassLynx software was used for integrating the data. An aqueous phase of 0.1% formic acid in water and organic phase of 0.2% formic acid in methanol was used as mobile phase. Separation was achieved using a UPLC BEH- C18 column (2.1×50 mm, Waters) having 1.7 µm guard column; temperature was set at 25°C. The flow rate was 0.15 mL/min. The metabolites eluted were pumped directly without any split into the TQD-MS/MS system, which was optimized for the analysis of phenolic acids and flavonoids. Phenolic acids and flavonoid standards were procured commercially (Sigma Chemical Co.). An Acquity Ultra Performance Liquid Chromatography system coupled with TQD-MS/MS (Waters, USA) with Mass Lynx software was employed for the quantification of phytol, thymol, eugenol, and piperine content of PB. The mobile phases for phytol and eugenol were methanol-acetonitrile and methanol-acetonitrile-water, respectively. For thymol and piperine, an acetonitrile– water–acetic acid mixture was used. Flow rate was 0.15 mL/min. Phytol, thymol, eugenol, and piperine standards were procured commercially (Sigma Chemical Co.).

Ethical approval

Animal Ethics Committee guidelines of the Federal University of Agriculture, Abeokuta (FUNAAB 2014) was strictly adhered to throughout the duration of the experiment.

Experimental diets, design and birds' management

Basal diets (starter and finisher) were formulated according to the recommendation of NRC (1994). Table 1 shows the gross composition of the experimental diets. Completely randomized design was used as a statistical design for the

experiment. The phytogenic blend was added to the diet as follows:

T1 = Negative Control (Basal diet only); T2 =

Basal diet + tetracycline (positive Control)

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

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

A total of 240 1- day old Cobb 500 chicks was used for the study. Birds were allotted on weight equalization basis into 6 dietary treatments of 40 birds each, replicated 5 times. The birds were reared and managed according to the guidelines of Ethics Committee for animal use in researches of the Federal University of Agriculture (FUNAAB, 2014), Abeokuta, Nigeria. Birds in

each replicate were housed (open sided deep litter) in separate pens with similar environmental conditions in all the pens throughout the experimental period. Each pen had a dimension of 1.1 × 1.0 m and the birds were given *ad libitum* access to water and feed with lightning program of 16 hours of light: 8 hours of dark throughout the trial period. The temperature of the brooding house was monitored with the aid of a thermometer. Standard broiler chicken vaccination programme was strictly adhered to without administration of anti-coccidial and antibiotic. Two birds in each replicate were used for nutrient digestibility. Also two birds per replicate were used for gut morphology sample collection as well as carcass characteristics.

Table 1: Composition of basal diets

Ingredients	Starter (day 0-21)	Finisher (day 22-42)
Maize	55.00	59.75
Soybean meal	36.60	32.00
Fish meal (72%)	2.50	2.00
Limestone	1.00	1.00
Bone meal	2.00	2.60
Wheat offal	1.75	1.60
Common salt	0.25	0.25
Lysine	0.40	0.20
Methionine	0.25	0.25
*Broiler premix	0.25	0.25
Total (kg)	100.00	100.00
Analyzed nutrients		
ME (Kcal/kg)	2827.44	3030.11
CP%	23.05	20.10
CF %	4.89	4.61
Fat %	4.26	4.73

*Composition of Premix per kg of diet: vitamin A, 12,500 I.U vitamin E, 40mg; vitamin K3 2mg; vitamin B1, 3mg; vitamin B2,5.5mg; niacin, 55mg; calcium pantothenate,11.5mg; vitamin B6, 5mg; vitamin B12, 0.025mg; chloride, 500; folic acid, 1mg; biotin, 0.08mg, manganese, 120; iron, 100mg; zinc, 80mg, copper 8.5mg; iodine, 1.5mg; cobalt, 0.3mg; selenium, 0.12mg; Anti- oxidant, 120mg

Data collection

Determination of growth performance

Initial body weight (g/bird) was obtained before the commencement of the experiment. Weekly live body weight was measured. Average daily weight gain per bird was calculated as total final

body weight minus total initial body weight divided by the number of birds in a replicate. The value was divided further by seven to obtain the average daily weight gain per bird/day. Daily feed intake (g) was obtained as the difference between quantity of feed given the previous day

and the left over. The figure was divided by the number of birds in a replicate. Feed conversion ratio (FCR) was calculated as the ratio of daily feed intake and daily weight gain.

Mortality is the total number of broiler chickens that died during the study. It was expressed in percentage (%) as; Mortality (%) = $\frac{\text{Number of dead broilers}}{\text{Total number of broilers}} \times 100$

broilers

Nutrient digestibility

Apparent digestibility of dry matter, crude fibre, crude protein, ether extract, and ash of the diets was measured at the end of the starter and finisher phases according to the method of (Bourdillon *et al.*, 1990) which is total collection of excreta. Two birds per group were randomly picked and housed individually in an already prepared cages for a 3-day acclimation period, during which water and experimental diets were given without restriction. The birds were fasted for a day to empty their GIT. The birds were given a known quantity of feed above their daily intake and excreta were collected daily for three consecutive days by means of clean trays placed under the cages. The collected samples were oven dried at 80 °C, milled, and stored in a cool dry place for subsequent analyses.

The method of AOAC, (2000) was used to carry out the proximate analysis of the respective samples (faeces and diets).

Gut morphology

At 42nd day of the experiment, the two birds per replicate whose weights were closest to the average weight, and which was slaughtered for the carcass characteristic was used. The gastro intestinal tract (GIT) was removed and separated into intestinal segments. The duodenum was taken distally from the gizzard to the end of the pancreatic loop to merckel diverticulum, the ileum distally from merckel diverticulum to the ileco-caecal junction. A sample portion (0.5cm in length) was taken at the medium part of each of the three intestinal segments (duodenum, jejunum

and ileum) for gut morphometric analysis. The sample was kept for 24hrs in acetic acid/ethanol (25/75, v/v) followed by dehydration in a bath of ethanol/water (50/50, v/v) and then in distilled water. Thereafter, the samples were stained with feulgen reaction, hydrolysed in HCL at 60°C for 6 minutes rinsed three times with distilled water and stained with Schiff reagent for 30 minutes. The sample was rinsed in distilled water and stored in acid/water (45/45, v/v) at 40°C until further analysis. Intestinal villi with their crypts were individually separated under a dissecting microscope as described by (Goodlad *et al.*, 1991). Villi width and length were determined according to the technique outlined by (Hampson 1986).

Caeca microbiota

Two birds per replicate whose weights were closest to the average weight were slaughtered for the carcass characteristic were used. The caeca contents were collected separately from each of the birds into a sterile bottle under ice condition. Fresh samples of about 1g was placed in sterile bottles for bacterial analysis.

In the laboratory, the samples were plated on selected culture media, the plates were incubated anaerobically at 37°C for 48 hours. The following selective culture media was used: MRS (de Man, Rogosa and Sharpe) agar (Oxoid CM 361) for *Lactobacillus sp.* And KFS (Kenner Formula for *Streptococcus*) agar (Oxid CM 701) for *Streptococcus sp.* By spread-plate technique (Szermer-Olearnik *et al.*, 2014). Counting of total bacteria was carried out using colony counter (Stuart Scientific) and was weighed in terms of colony-forming units (cfu) per gram digesta.

Statistical analysis

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

Results

Table 2. Proximate composition, metabolizable energy of the phytogenic blend

Compositions	Quantity
Proximate composition (%)	
Dry matter	90.75
Ash	6.55
Crude protein	11.69
Ether extract	4.66
Crude fibre	13.24
Neutral detergent fibre	46.56
Acid detergent fibre	24.38
Acid detergent lignin	5.02
Metabolizable energy (MJ/Kg)	9.33

Table 3. Phytochemical composition of the phytogenic blend

Compositions	Quantity
Phytochemicals	
Octanol	3.385
Dodecane	5.668
Decanol	5.874
Teradecene	8.008
Caryophyllene	8.432
1-methyl-4	9.021
Cyclohexa-1,3-diene	9.055
Benzene, 1-4-methyl	9.176
1,3-cyclohexadiene	9.279
α -farnesene	9.319
β -bisabolene	9.484
Cyclohexene	10.091
Cyclohexadecane	10.171
Caryophyllene oxide copaene	10.749
Butan-2-one, 4	10.823
2-butanone	11.951
1-octadecene	11.951
2,4-decadienamide, N-isobutyl	13.284
n-hexadecanoic acid	13.461
Hexadecenoic acid, ethyl ester	13.650
9,12-octadecadienoic acid-oleic acid	14.852
Linoleic acid ethyl 5-ester	14.966
-9-octadecenoic acid	15.006
Octadecanoic acid, ethyl ester	15.189
4-hydroxy-3-methoxyphenyl	15.527
1-(4-hydroxy-3-methoxyphenyl) dec-4	16.093
Phenol	16.460
Gingerol	17.060
Propylene glycol	18.136
Benzoic acid, 3-amino, 3-methylbutyl ester	18.336
Ethenone, 1-(1-hydroxy-2,6	18.554
(Piperidum-1-yl) pat-2en-1-one	20.293

Growth performance

Starter phase table 4, birds on basal diet only without antibiotic, 5g, 10g and 15g has significantly ($p < 0.05$) had higher final weight and weight gain while birds on 20g of PB recorded least values for these parameters. Birds on 5g PB recorded significant higher ($p < 0.05$) feed intake while the least values were recorded for birds in the control groups. Feed conversion ratio (FCR) were similar ($p < 0.05$) for birds in the control groups, 5g and 10g PB while birds on

20g PB recorded the worst FCR. Percentage mortality did not differ among treatment groups. Final weight (FW), weight gain (WG), feed intake (FI) and feed conversion ratio (FCR) were not significantly ($p > 0.05$) different among treatment groups at the finisher phase (Table 4).

Nutrient digestibility

The results of nutrient digestibility table 5, shows that none of the parameters were significant among treatments at both phases.

Table 4: Growth performance of starter and finisher broiler chickens fed diets with varying levels of phytogenic blends

Parameter	T1 (-ve control)	T2 +ve control	T3 5g/kg	T4 10g/kg	T5 15g/kg	T6 20g/kg	SEM	P-value
Starter phase (Day 0-21)								
Initial weight(g/bird)	33.25	33.25	33.25	33.25	33.25	33.25	0.52	1.00
Final weight (g/bird)	560.00 ^a	511.50 ^{bc}	555.25 ^{ab}	537.50 ^{ab}	530.00 ^{ab}	482.50 ^c	6.66	0.00
Total weight gain (g/bird)	526.00 ^a	478.50 ^{bc}	521.75 ^{ab}	504.00 ^{ab}	496.50 ^{ab}	449.00 ^c	6.66	0.00
Feed intake (g/bird)	1007.00 ^c	1003.00 ^c	1092.00 ^a	1050.75 ^b	1073.25 ^{ab}	1053.75 ^b	7.07	0.00
Feed conversion ratio	1.91 ^c	2.10 ^{bc}	2.09 ^{bc}	2.08 ^{bc}	2.17 ^{ab}	2.35 ^a	0.32	0.00
% Mortality	5.00	5.00	2.50	5.00	2.50	2.50	1.01	0.93
Finisher phase (Day 22-42)								
Final weight (g/bird)	2017.50	2010.00	2100.00	2075.00	1950.00	1975.00	43.64	0.92
Total weight gain (g/bird)	1457.50	1571.80	1550.00	1537.50	1412.50	1492.50	43.30	0.95
Feed intake(g/bird)	3850.00	4203.00	3957.00	3987.00	3505.00	3801.30	158.12	0.77
Feed conversion ratio	2.64	2.67	2.54	2.59	2.48	2.55	0.11	0.84
% Mortality	11.11	5.56	5.56	5.56	5.56	11.11	1.28	0.43

^{abc} Mean in the same row with different superscript are different significantly (P< 0.05), SEM = Standard Error of mean

Table 5. Nutrient digestibility of broiler chickens fed diets supplemented with phytogenic blend

Parameters (%)	T1	T2	T3	T4	T5	T6	SEM	P value	Linear	Quadratic	Cubic
Starter phase (day 0-21)											
Dry matter	81.88	80.76	83.92	79.42	81.32	81.23	1.29	0.967	0.837	0.998	0.819
Ash	90.50	84.74	90.65	87.65	89.55	88.77	0.95	0.520	0.890	0.689	0.353
Crude protein	80.58	81.31	76.96	75.59	77.06	81.49	1.60	0.859	0.790	0.295	0.492
Crude fibre	86.63	79.87	83.00	83.84	82.46	81.91	1.14	0.710	0.549	0.633	0.266
Ether extract	86.20	84.47	88.35	84.81	89.04	87.68	0.95	0.694	0.398	0.885	0.754
Finisher phase (day 22-42)											
Dry matter	73.54	79.80	69.45	74.24	77.51	60.41	2.24	0.148	0.125	0.194	0.322
Ash	78.20	82.94	79.85	78.25	80.54	71.60	1.71	0.565	0.260	0.248	0.867
Crude protein	66.60	71.17	69.21	64.72	72.94	60.24	2.78	0.841	0.618	0.504	0.792
Crude fibre	76.06	80.32	63.37	70.76	79.75	61.52	2.37	0.056	0.125	0.852	0.158
Ether extract	77.41	78.50	69.84	76.91	75.95	64.06	2.19	0.373	0.145	0.491	0.291

T1= 0g/kg of phytogenic blend (negative control); T2= antibiotic (Positive control); T3= 5g/kg of phytogenic blend; T4=10g/kg of phytogenic blend;
T5= 15g/kg of phytogenic blend; T6= 20g/kg of phytogenic blend

Gut morphology

Table 6, the villus height, mucosa thickness and villus/crypt ratio of duodenum were higher ($p < 0.05$) in birds on 20g PB which did not significantly differ from those on 5g PB except for villus/crypt ratio. The least values for these parameters were recorded for birds in the antibiotic group except for mucosal thickness which was least for birds on 20g PB. Cryptal depth was significantly higher in the negative control group and the group fed diet containing 20g PB while the least value was recorded for birds on 10g and 15g PB. Villi height and mucosa thickness of ileum were significantly ($p < 0.05$) higher for birds on 10g and 15g PB. Other treatments differ not significantly for villi height while the least mucosa thickness was recorded for the control groups and birds on diet with 20g PB. Higher ($p < 0.05$) Cryptal depth was reported for birds on 5g PB while birds in the control groups, 10g and 20g PB recorded the least value. Villus/crypt ratio was least for birds on diet with 5g PB while other treatments were similar. Jejunum villi height, mucosa thickness and cryptal height of birds antibiotic were significantly ($p < 0.05$) higher. Other treatments differ not significantly for villi height. The least

cryptal depth was recorded for birds fed diet containing 5g PB while birds on 20g PB recorded the least value for mucosa thickness. Villus/crypt ratio was significantly higher in T3 and other treatments were significantly similar.

Caeca microbiota

Table 7, total bacteria count show no significant ($p > 0.05$) difference among the treatment groups. Birds on basal diet without antibiotic and birds on 15g PB recorded higher ($p < 0.05$) values for *Staphylococcus aureus* while birds on antibiotic, 5g and 10g PB reported the least significant values. *klebsiella pneumoneae* count was significantly ($p < 0.05$) similar for birds on basal diet without antibiotic, birds on 5g and those on 15g PB, the least value for this parameter was recorded for antibiotic group and birds fed diet containing 20g PB. Birds on 5g PB recorded significant ($p < 0.05$) higher *Proteus vulgaris* and *Pseudomonas aeruginosa* while birds on 20g PB recorded the least values for these parameters. *Enterobacter aerogenes* was significantly higher in birds fed diet with 10g PB while other treatments were similar. Birds on 20g PB recorded higher ($p < 0.05$) value for *Escherichia coli*, other treatments differ not significantly.

Table 6. Gut morphology of finisher broiler chickens fed diets supplemented with phytogenic blend											
Parameters	T1	T2	T3	T4	T5	T6	SEM	P value	Linear	Quadratic	Cubic
Duodenum											
Villi height (mm)	524.75 ^{bc}	411.60 ^c	652.48 ^{ab}	786.98 ^a	373.75 ^c	483.60 ^{bc}	32.73	0.00	0.57	0.00	0.37
Cryptal depth (mm)	372.63 ^a	277.48 ^{ab}	207.38 ^{bc}	145.50 ^c	172.08 ^c	314.90 ^a	18.56	0.00	0.00	0.00	0.03
Mucosal thickness (mm)	777.15 ^{bc}	786.2 ^{5b}	908.35 ^a	893.33 ^a	569.48 ^d	731.38 ^c	23.78	0.00	0.00	0.00	0.00
Villius/crypt ratio	1.43 ^c	1.47 ^c	3.23 ^b	5.40 ^a	2.18 ^{bc}	1.55 ^c	0.32	0.00	0.08	0.00	0.01
Ileum											
Villi height (mm)	300.80 ^b	303.30 ^b	354.55 ^b	547.30 ^a	684.30 ^a	282.85 ^b	33.74	0.00	0.00	0.00	0.00
Cryptal depth (mm)	84.88 ^c	84.88 ^c	310.18 ^a	119.28 ^c	183.18 ^b	83.63 ^c	17.79	0.00	0.39	0.00	0.71
Mucosal thickness (mm)	368.25 ^c	368.25 ^c	643.53 ^b	731.15 ^{ab}	822.38 ^a	400.68 ^c	39.59	0.00	0.00	0.00	0.00
Villus/crypt ratio	3.55 ^a	3.62 ^a	1.10 ^b	4.60 ^a	3.75 ^a	3.45 ^a	0.25	0.00	0.23	0.12	0.00
Jejunum											
Villi height (mm)	360.20 ^b	552.73 ^a	320.35 ^b	260.83 ^b	261.58 ^b	249.73 ^b	24.39	0.00	0.00	0.75	0.00
Cryptal depth (mm)	111.60 ^{bc}	187.30 ^a	64.68 ^d	93.18 ^c	95.18 ^c	124.78 ^b	8.25	0.00	0.00	0.00	0.00
Mucosal thickness (mm)	474.90 ^b	711.55 ^a	423.85 ^{bc}	375.15 ^{bc}	375.15 ^{bc}	327.18 ^c	27.89	0.00	0.00	0.25	0.00
Villus/crypt ratio	3.20 ^b	2.95 ^b	5.00 ^a	2.75 ^b	2.73 ^b	2.00 ^b	0.23	0.00	0.01	0.00	0.31

^{abcd}Means on the same row with different superscripts are significantly different when $p < 0.05$. SEM= standard error of mean.

T1= 0g/kg of phytogenic blend (negative control); T2= antibiotic (Positive control); T3= 5g/kg of phytogenic blend;

T4=10g/kg of phytogenic blend; T5= 15g/kg of phytogenic blend; T6= 20g/kg of phytogenic blend

Table 7. Caeca microbiota of finisher broiler chickens fed diets containing varying levels of phytogenic blends

Parameters ($\times 10^6$ cfu/mL)	Levels of Phytogenic blends						SEM	P-value
	T1	T2	T3	T4	T5	T6		
Total bacterial count	5.17	4.93	5.23	5.04	5.08	5.26	0.04	0.06
<i>Staphylococcus aureus</i>	4.93 ^a	0.00 ^c	0.00 ^c	0.00 ^c	4.99 ^a	4.68 ^b	0.51	0.00
<i>Klebsiella pneumoneae</i>	4.90 ^{ab}	4.57 ^c	4.97 ^a	4.79 ^b	4.82 ^{ab}	4.59 ^c	0.03	0.00
<i>Proteus vulgaris</i>	4.63 ^{bc}	4.68 ^{ab}	4.78 ^a	4.41 ^d	4.52 ^{cd}	0.00 ^c	0.36	0.00
<i>Pseudomonas aeruginosa</i>	0.00 ^b	0.00 ^b	4.41 ^a	0.00 ^b	0.00 ^b	0.00 ^b	0.34	0.00
<i>Enterobacter aerogenes</i>	0.00 ^b	0.00 ^b	0.00 ^b	4.57 ^a	0.00 ^b	0.00 ^b	0.36	0.00
<i>Escherichia coli</i>	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b	4.90 ^a	0.38	0.00

^{abcde} Means on the same row having different superscripts are significantly different when $p < 0.05$. SEM= standard error of mean.

T1= 0g/kg (negative control); T2= 0g/kg (Positive control + antibiotic); T3= 5g/kg of phytogenic blends; T4=10g/kg of phytogenic blends; T5= 15g/kg of phytogenic blends; T6= 20g/kg of phytogenic blends

Discussion

At the starter phase, significant higher final weight, weight gain and better feed conversion ratio recorded for birds fed basal diet only was not expected. This finding contradicts the report of Oso *et al.* (2019) which stated that body weight gain (BWG) and feed conversion ratio (FCR) improved with addition of 1% phytogenic blend in the diet of broiler chickens. Also, Syed *et al.* (2021) reported improved BWG with starter broiler chickens fed with an Encapsulated Blend of a Phytogenic Feed Additive. The reasons for the results of this present study could be due to drug failure on the part of the oxytetracycline (OTC) which occasion from drug-ion complex formation between the drug and bivalent ions in the feed (Chopra and Roberts, 2001). Maddaleno *et al.* (2021) opined that intestinal absorption of oxytetracycline might be altered if it administered along with feed, substances that increase stomach pH or contain bivalent or trivalent cation which may trigger the development of chelating complexes. It has been reported (Schmitt and Schneider 2000, Jin *et al.*, 2007) that OTC bind divalent ions and form non-absorbable chelates affecting drug absorption from gastrointestinal tract (GIT). The birds had unrestricted access to feed and water, Ziółkowski *et al.* (2016) suggested a negative correlation between divalent ion concentration in water and bioavailability of orally administered OTC. On the other hand, the non-significance difference among the treatments with phytogenic blend (PB) up to 15g/kg and those fed basal diet only could be as a result of toxicity in the birds caused by the phytogenic blend which delayed growth.

The changes in intestinal morphology with supplementation of phytogenic feed additives (PFA) is an indication on possible benefits of the blend to the gastrointestinal tract. In this study, T4 supplemented with Phytogenic blends (PB) had similar duodenal villi height with the T3 group, but recorded longer jejunum villi height in T2 (positive control + antibiotics) than the other

group. The trend noticed in the jejunum is vital since majority of absorption of digested products (from fat, starch, and protein) is to a large extent completed in the jejunum (Oso *et al.*, 2019). Increased villi height of the jejunum with broilers fed diet supplemented with T2 (positive control + antibiotics) is an indication of improved absorptive surface of the available nutrients (Iji *et al.*, 2001). Reduced jejunum crypt depth obtained in birds fed with T6 is indicative of decreased cellular turnover and improved intestinal health. Cellular turnover is an energy consuming process that uses resources that might otherwise be utilized toward growth; hence, shallower crypts have been linked to improved performance (Markovi *et al.*, 2009). Increased villi height/crypt depth ratio obtained for groups fed diet containing phytogenic blend (PB) suggested that the small intestine digestive capacity was enhanced. Montagne *et al.* (2003) reported that villus height/crypt depth ratio is a useful criterion for estimating the digestive capacity of the small intestine. The results of this present study on crypt depth/villus height ratio are in agreement with previous reports on broilers fed essential oils containing thymol and eugenol (Mohammadi *et al.*, 2014; Du *et al.*, 2016). Furthermore, Oso *et al.* (2019) and Salihu *et al.* (2020) reported increased villi height/crypt ratio when broiler chickens were fed diets containing Phytogenic blends. In overall the trend of results in this study is in tandem with previous reports that addition of PFA enhanced intestinal morphology (Geyra *et al.*, 2001).

The increase in *Staphylococcus aureus* population recorded in the cecum content of birds with increasing dietary supplementation of PB beyond 10g/kg of feed suggests improved gut ecology and immune-stimulation initiated by antibacterial properties exhibited in PB. However, its presence is generally considered undesirable in poultry farming because it can cause various diseases and have negative effects on chicken production. To prevent the negative

impact of *Staphylococcus aureus* on broiler chicken health and production, biosecurity measures, proper sanitation, and vaccination programs are often implemented in commercial poultry farming operations. Phytogenic feed additives have been reported to cause a shift in gut microbiota (Jamroz *et al.* 2005; Mountzouris *et al.*, 2011) by decreasing the production of growth depressing microbial metabolites (Jamroz *et al.* 2003), selection of healthier microbial groups (Castillo *et al.*, 2006), enhancement of the growth of beneficial bacteria (Tiihonen *et al.*, 2010), enhancement of feed break down (Kamel 1999), an increasing nutrient availability to the host (Anderson *et al.*, 1999). *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Pseudomonas aeruginosa* are bacteria that can inhabit the cecum of broiler chickens. Each of these bacteria can play various roles, some beneficial and some potentially harmful, in the chicken's digestive system. Bacteria like *Klebsiella pneumoniae* and *Proteus vulgaris* can aid in the digestion of complex carbohydrates and proteins that are hard for chickens to break down on their own. They help ferment these substances, making them more easily absorbed by the chickens. Some bacteria in the cecum can synthesize certain vitamins, such as B vitamins. This can be beneficial for the chicken's overall health as it provides essential nutrients. By occupying space and competing for nutrients in the cecum, these bacteria can help prevent the growth of harmful pathogens. This is known as competitive exclusion and is beneficial for the chicken's gut health. On the other hand, if these bacteria overgrow or if the chicken's immune system is compromised, they can potentially become pathogenic. *Pseudomonas aeruginosa*, in particular, is known for its pathogenicity and can cause infections if it enters the bloodstream or other tissues. The absence of this bacteria in the cecum of birds fed diets with higher levels of PB is an indication that the PB contains substances that can reduce or eliminate harmful bacteria in the gut. It's essential to note

that the balance of these bacteria in the cecum is crucial for the overall health of the broiler chicken. An imbalance or overgrowth of harmful bacteria can lead to digestive disorders and other health problems. Proper nutrition, hygiene, and management practices are important in maintaining a healthy gut microbiome in broiler chickens. The increase in *Escherichia coli* population recorded in the caecum content of birds with 20g/kg can have both positive and negative implications. *E. coli* can aid in the absorption of nutrients in the cecum. It can break down complex carbohydrates and help the chicken extract more energy from its feed. Like other bacteria in the cecum, *E. coli* contributes to the fermentation of undigested food particles, making them more digestible for the chicken. *E. coli*, when present in healthy numbers, can compete with and help exclude harmful pathogens. Phytogenic feed additives have been reported to decrease harmful bacteria by depressing the formation of wrong metabolites (Oso *et al.*, 2019). It occupies space and competes for resources, reducing the risk of pathogenic bacterial overgrowth. Some strains of *E. coli* can synthesize vitamins, such as certain B vitamins, which can be beneficial for the chicken's overall health. Generally, the concentrations of the examined bacteria in the broiler caeca digesta were in range with the respective ones according to the reviewed by (Rehman *et al.*, 2007). Beneficial effects of phytogenic on gut flora composition have been reported by other studies (Giannenas *et al.*, 2003; Mitsch *et al.*, 2004; Jamroz *et al.*, 2005). In addition, it was shown that administration of a PFA, similar to the one used in this work, helped control *Clostridium perfringens* and *Necrotic enteritis* in broilers (McReynolds *et al.*, 2009).

Conclusion

The blend of black pepper, ginger, sweet orange peels, neem leaf and lemon grass can be included at 10g/kg in the diets of broiler chickens to modulate the caeca activity and without

deleterious effect on growth performance. This blend is recommended for use in substitute for antibiotic in broiler chicken production since it did not hamper the growth of the birds.

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