

Effect of dietary supplementation of black pepper (*Piper guineense*) leaf and seed meals on growth performance and blood characteristics of the finisher broiler chickens

Effiong, O. O., Udoekong, E. C and Ochagu, S. I.

Animal Science Department, University of Calabar, PMB 1115, Calabar, Nigeria.

Corresponding Author: Email: Okokoneffiong48@gmail.com Phone Number: +234 8036602048

Target Audience: Feed millers, poultry farmers and food technologist

Abstract

The study was designed to evaluate the performance of broiler chickens fed diets supplemented with *Piper guineense* leaf (PLM) and seed (PSM) meals. The harvested samples were air dried, blended and their proximate composition determined. Seven diets were formulated, with the treatment diets supplemented with the PSM and PLM at 0.2, 0.4 and 0.6 %, respectively. One hundred and eighty nine, 28 days old broiler chicks were divided into seven (7) groups and each assigned to one of the seven diets and fed for 28 day of the experiment. The experimental design used was the randomized complete block design. The crude protein values of 15.86% and 10.16% were recorded in the PLM and PSM, respectively. Dietary inclusion of PSM improved the total weight gain, with the highest value of 72.08 g/bird observed in the 0.6% PSM diet group. Birds on a control diet recorded the highest average daily feed intake of 138.50g/bird, while those on a 0.4% PLM diet had the lowest average daily feed intake of 122.86 g. Dietary inclusion of PSM and PLM improved the feed conversion ratio of chickens relative to those on control group. Supplementation of diets with PSM and PLM did not influence ($P > 0.05$) the dressing percentage of the chickens, but the relative weights of neck and wing were influenced ($P < 0.05$) at 0.6% of PLM. The concentrations of haemoglobin, mean corpuscular haemoglobin, packed cell volume, and white blood cells in the chickens fed PSM and PLM diets were significantly higher ($P < 0.05$) than in those fed the control diet. The results on the biochemical indices showed significant ($P < 0.05$) differences in cholesterol, globulin, glucose and total protein levels between birds on a control diet and those fed treatment diets. It was therefore concluded that dietary supplementation with PSM improved the performance of finisher broiler chickens relative to the PLM. Broiler finisher diets may be supplemented with 0.6% of PSM for optimum performance.

Keywords: Black pepper seed, black pepper leaf, proximate composition, growth performance

Description of Problem

Medicinal herbs, aromatic plants, and spices (phytogenic feed additives) have been used successfully in animal feed for the improvement of animal welfare after the ban of antibiotics due to the emergence of microbe resistance. According to (1), the withdrawal of antibiotics as growth promoters has led to low performance, high feed conversion ratio, and a rise in the

incidence of certain animal diseases. (2) stated that growth promoters have the capacity to maintain the feed hygiene and also beneficially effect gastrointestinal microbiota by moderating the growth of pathogens. They are rich in oils such as curcumin, thymol, piperin, and eugenol, with capacity to reduce *Clostridium perfringens* concentrations in both the gastro-intestinal tract and dropping of broiler chickens at

growth phase (3). (4 and 5) stated that essential oils in their powder forms are usually used alone or in combination as feed additives in commercial broiler feed production. Garlic has been traditionally used for a variety of reasons, most of which have been approved scientifically: anti-atherosclerosis, anti-microbial, hypolipidemic, anti-thrombosis, anti-hypertension, and anti-diabetes (6). The active ingredients in garlic include diallyl sulfide, *s*-alkyl cysteine, ajoene, and allicine, (7). According to Alder and Holub (1997) and (5), allicine has the potential to reduce low density lipoprotein cholesterol in serum and tissues, hence has been used in the treatment of cardiovascular diseases (8). (9) recorded an improved growth rate, a good feed conversion ratio, and a decreased mortality rate when broiler chickens were fed diets supplemented with garlic powder. Garlic was also found to manifest hypocholesterolemic effects when fed to chickens through inhibition of trihydroxy-trimethylglutaryl coenzyme A reductase, cholesterol-7 α hydroxylase, and the synthesis of fatty acids. According to (10), black pepper has been found to improve feed digestibility. Pepper efficiency compounds consist of capsaicin, capsinin, and capsantine, some of which allay rheumatic aches. Black pepper is rich in glutathione peroxidase and glucose-6-phosphate dehydrogenase, and it has been shown that piperine can dramatically increase absorption of selenium, vitamin B complex, β carotene, and curcumin, as well as other nutrients (11). (12) affirmed that piperine enhances the thermogenesis of lipids and accelerates energy metabolism in the body and also increases serotonin and β -endorphin production in the brain.

The aim research evaluate the growth parameters of finisher broiler chickens fed diets containing different levels of black seed

and leaf meals, the carcass characteristics of finisher broilers on diets with black pepper seed and leaf meals and the hematology and blood biochemical indices of finisher broiler birds fed diets with black pepper seed and leaf meals.

Materials and methods

Site of the experiment

The proximate analysis of the test samples was carried out at the Animal Science Department Laboratory, University of Calabar, while the feeding trial was conducted at the Poultry Unit of the Teaching and Research Farm of the above department.

Collection and processing of the research materials

Black pepper seed and leaves were gathered from the home garden at Akamkpa Local Government Area of Cross River State. They were air-dried to a moisture content of 12% by spreading thinly on a concrete floor, blended using an electric blender, and stored on a well-labeled screw-capped plastic container.

Proximate analysis

The proximate composition of the test samples were determined by using the methods outlined by (13). The nitrogen free extract (NFE) was determined by a difference. In other words, % NFE = 100% (% ether extract +% crude protein +% ash% crude fibre).

Experimental diets

Seven broiler finisher diets were formulated during the feeding trial. The control diet had none of the test samples. Diets 2, 3 and 4 were supplemented with the PSM at 0.2, 0.4, and 0.6 %, and diets 5, 6, and 7, supplemented with PLM at 0.2%, 0.4%, and 0.6%, respectively.

Table 1: Gross composition of finisher broiler diet

Ingredients	Different levels of <i>P. guineense</i> seed and leaf meals						
	Control	0.2 PLM	0.4 PLM	0.6 PLM	0.2 PSM	0.4 PSM	0.6 PSM
Yellow corn	60.10	59.90	59.70	59.50	59.90	59.70	59.50
Soybean meal	24.50	24.50	24.50	24.50	24.50	24.50	24.50
Fish meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Palm kernel cake	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Wheat offal	6.00	6.00	6.00	6.00	6.00	6.00	6.00
<i>P. guineense</i> meal	–	0.20	0.40	0.60	0.20	0.40	0.60
Dicalcium phos.	2.55	2.55	2.55	2.55	2.55	2.55	2.55
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Lysine	0.10	0.10	0.10	0.10	0.10	0.10	0.10
*Vit./min. premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Proximate analysis:							
% Crude protein	19.99	19.97	19.95	19.93	19.97	19.95	19.97
Met. Energy (KcalME/kg)	3002	30017	3015	3013	3016	3014	3014

*Vitamin/mineral premix contains the following / kg: vit. A- 4000000IU; vit. D₃- 800,000IU; vitamin E- 2500IU vit. K- 1000mg; thiamine,-750mg; riboflavin-2,000mg; pyridoxine B₆ -750mg; antioxidant- 62.5g; niacin -750mg vit. B₁₂- 5mg; pantothenic acids- 2500mg; folic acid- 250mg; biotin-10mg; choline 100g- mn- 40g; zn -25g; fe- 10g; cu- 2.5g; Iodine -1.2g; Sel- 100mg; Co- 100mg.

PLM: = *Piper guineense* leaf meal

PSM: = *Piper guineense* seed meal

Research birds and experimental design

One hundred and eighty nine, four-week-old Hubbard broiler chicks were used for this study. The birds were scaled with electronic weighing balance and thereafter shared to seven (7) groups of 27 chicks. Each group was assigned one of the seven diets and replicated thrice with nine chicks per replicate. The completely randomized design (CRD) was used and the feeding trial lasted for 28 days.

Data collection

The feed intake was monitored on daily basis, while the weight gain was taken on the weekly basis. The feed conversion ratio was computed using the average daily feed intake and weight gain.

Blood collection

Three days prior to the termination of

the growth experiment, three chickens were selected from each treatment and blood collected for analysis. Two milligrams of blood were collected by puncturing the prominent wing vein with a 5 millimeter scalp vein needle and syringe into two separate sterile plastic bottles. The bottle containing Ethylene diamine tetra acetic acid (EDTA) as an anti-coagulant was used to collect blood for a haematological assay, while the other bottle without EDTA was used to collect blood for the serum biochemical indices.

Haematological and serum biochemical indices

White blood cell counts were carried out using the micro-hematocrit method (14). Packed cell volume (PCV) was determined using the Wintrob's micro-hematocrit method (15). The cyanomethaemoglobin method (16) was used to determine

hemoglobin concentration, while an improved Neubauer haemocytometer (17) was used to estimate red cells. Mean corpuscular volume (MCV), mean haemoglobin concentration (MHC) and mean corpuscular haemoglobin concentration (MCHC) were computed according to the method of (17). Spectrophotometry method was used to determine total protein, cholesterol, and globulin using the, while method described by (18) was used in determining glucose concentration.

Carcass and internal organs evaluation

At the end of the growth experiment nine chickens were selected randomly from each treatment, starved of feed for 12 hours, scaled and kill by cutting the jugular vein. They were thoroughly bled and scalded in water of about 69°C for 5 minutes and their plumage manually removed. The carcasses were weighed individually to get the weight of carcass, open through the vent to remove internal organs, and weighed again to establish the dressed weights. The dressed carcasses were thereafter cut into different parts for the determination of their relative

weights. The internal organs weights were taken using a sensitive weighing balance and expressed as percentages of live weight, while the intestinal length taken using the measuring tape.

Statistical analysis

Data collected were subjected to standard ANOVA procedures using the software package SPSS 11.0 for Windows.

Results and Discussion

Table 2 shows the result of the proximate analysis of *P. guineense* leaf and seed meals. The crude protein value of 15.86% recorded for *P. guineense* leaf meal (PLM) was higher than 10.16% observed in the *P. guineense* seed meal (PSM). The crude fat extract, fibre and ash contents of the PLM were 2.52%, 4.43% and 2.24% as against 1.35%, 6.30% and 3.60% recorded for PSM. The availability of crude protein, lipids, and carbohydrates in black pepper seed and leaf meal showed that they have the capacity to supply nutrients when used in monogastric animal feed formulation.

Table 2: Composition of *P. guineense* leaf and seed meal

Constituent (%)	Leaf meal	Seed meal
Crude protein	15.86±0.04*	10.16±0.66
Crude fibre	4.43±0.03	6.30±0.07*
Ether extract	2.52±0.04*	1.35±0.02
Ash	2.24±0.02	3.60±0.09*
Nitrogen Free Extract	58.64±0.47	61.18±0.12

Values are means of triplicate determination, *Significant means ($p < 0.05$)

Table 3 gives the results of the growth characteristics of broiler chickens on diets supplemented with PSM and PLM at the finisher phase. The final weight gain, mean daily weight gain, mean daily feed intake, and the feed conversion ratio showed significant ($P < 0.05$) differences among the treatment groups. The diet with no treatment samples had the least total weight gain of

1572.12 g/bird, while birds on fed diet with 0.6% PSM recorded the highest total weight gain of 2019.23 g/bird. Similarly, birds on 0.6% PSM record the highest mean daily weight gain corresponding to 72.08 g/bird, followed by those on a 0.2% PSM diet, whose value (64.62 g/bird) was similar ($P > 0.05$) to those on 0.4% PSM, 0.2% PLM, and 0.4% PLM diets, respectively. The mean

daily feed intake was highest (138.50g/bird) in group consuming control diet and least (122.86 g) among birds consuming diet with 0.4% PLM. The addition of PSM and PLM in the feed of finishers' broiler chickens led to the improvement of their feed conversion ratio (FCR) compared to those on diet without the treatment sample. Chickens fed 0.6% PSM diet had the best FCR values among the treatment diets. The improvement in the FCR values for birds on PSM and PLM diets means that they contain substances whose action leads to the improvement of feed utilization. (19) observed that plant proteolytic enzymes found in spices have the capacity to enhance the digestion of feed protein. PLM and PSM could also serve as pancreatic lipase enhancers as in ginger (20), with the capacity to promote the intestinal lipase, sucrose, disaccharide, and maltase activity. (20). PSM and PLM also have relevant minerals and vitamins which can promote nutrient digestibility, and in turn improve feed utilization and feed conversion efficiency. Ascorbic is known for its important role in gut micro flora improvement and in enhancing the absorption of nutrient (21). According to (22) several spices including

ginger, onions, curcuma, and cayenne have the capacity to aid the production of bile acids in the liver and their excretion in the bile, which in turn aid the digestion and absorption of fats. Herbs and spices extracts have the potential of increasing feed digestion rate and reduce the transit time of feed in the digestive track (23). (24) observed that animals on herbs and spices supplemented diets can express greater resistance when exposed to varying stress conditions with greater capacity to absorb essential nutrients, required for the optimum growth performance. (25) discovered that piperin can also positively influence lipid homogenesis and increase the flow of digestive juice. (26) observed that piperine and curcumine are present in *P. guineense* leaf and seed meal and can facilitate the release of amylase, lipase, and proteases, which are capable of enhancing digestion as well as reduce the rate at which feed pass through gastro intestinal tract. (27) recorded a reduction in mean feed intake with an improvement in the feed conversion efficiency of broiler chickens fed a diet with different levels of garlic powder, at 0.3% inclusion level, compared to the rest of the treatment groups.

Table 3: Growth characteristics of broiler chickens on diets supplemented with black pepper leaf and seed meals.

Parameters	Control	0.2%PLM	0.4%PLM	0.6%PLM	0.2%PSM	0.4%PSM	0.6%PSM	±SEM
Initial weight(g/bird)	796.67	785.86	796.67	793.87	795.60	796.67	797.64	1.13
Final weight (g/bird)	23677.78 ^e	2554.92 ^c	243.21 ^e	2516.15 ^d	2605.94 ^b	2592.02 ^b	2815.86 ^a	11.59
Total weight gain(g/bird)	1572.12 ^g	1769.06 ^d	1667.01 ^f	1722.28 ^e	1810.34 ^b	1795.40 ^c	2019.23 ^a	2.56
Mean daily weight gain(g/bird)	56.11 ^c	63.18 ^{bc}	59.54 ^{bc}	61.51 ^{bc}	64.62 ^b	64.12 ^b	72.08 ^a	2.35
Mean daily feed intake(g/bird)	138.50 ^a	133.04 ^{ab}	122.86 ^d	125.18 ^{bcd}	127.08 ^{bc}	123.33 ^{cd}	130.95 ^{abc}	2.41
Feed conversion ratio	2.47 ^a	2.11 ^b	2.06 ^{bc}	2.04 ^{bc}	1.98 ^c	1.92 ^{cd}	1.82 ^d	0.002

^{a,b,c,d,e} means on the same row with different superscripts are significantly different (P<0.05)

PLM - *Piper guineense* leaf meal; **PSM** - *Piper guineense* seed meal; **SEM** - Standard error of means

The carcass and internal organs evaluation revealed that the dressing percentage of the chickens were not affected ($P>0.05$) by the treatment, but the relative weights of the back, neck and wings were significantly influenced ($P<0.05$), with the addition of PLM and PGM to the feed. Chickens fed the control feed gave a higher ($P<0.05$) relative back weight (16.03%) relative to those on PLM and PGM diets. The back weight of 15.46% observed for birds on a 0.6% PSM diet was similar to that of the chickens on control feed. The absence of significant differences in the relative weights of the internal organs (liver, heart, gizzard, and lungs) observed in broiler chickens fed control feed and those whose feeds were supplemented with the PLM and PGM are supported by the observation made by (28).

The authors stated that utilization of herbs in broiler nutrition had no significant influence on the organs. The use of phytobiotics in poultry feed production has been reported influence the digestive system positively by increasing the enzymes production and aiding the utilization of digestive products through the improved liver function (29). According to (30), irregularities observed in internal organ weights result from the organs' increased metabolic rate in an attempt to convert toxic substances to non-toxic metabolites. Therefore, the absence of these effects on organs is an implication that both the PLM and PGM were devoid of appreciable toxin that would have impaired the functionality of these all-important organs.

Table 4: Carcass characteristics of broiler chickens fed diets containing *Piper guineense* leaf and seed meals

Parameters	Control	0.2%PLM	0.4%PLM	0.6%PLM	0.2%PSM	0.4%PSM	0.6%PSM	SEM
Live weight (g)	2400.00	2266.67	2100.00	2133.33	2216.67	2166.67	2266.67	129.50
Dress weight (g)	2283.33	2170.00	1940.00	1933.33	2050.00	2076.67	2133.33	98.30
Dressing percentage	72.10	74.10	69.40	71.50	74.30	74.40	74.30	2.94
Carcass characteristics (% live weight)								
Back	16.03 ^a	14.40 ^{abc}	13.57 ^{bcd}	12.15 ^d	12.84 ^{cd}	13.95 ^{bcd}	15.46 ^{ab}	0.64
Breast	26.54	25.30	22.82	25.74	26.18	25.71	24.60	2.01
Thigh	22.97	22.61	20.93	20.82	22.25	22.61	22.05	1.48
Wings	8.07 ^{ab}	7.77 ^b	7.89 ^{ab}	9.61 ^a	7.62 ^b	7.98 ^{ab}	8.17 ^{ab}	0.52
Neck	5.07 ^{ab}	4.94 ^{ab}	4.35 ^b	5.52 ^a	4.46 ^b	4.28 ^b	4.52 ^b	0.24
Head	1.97	2.07	2.19	2.04	2.03	2.00	1.20	0.14
Shank	3.23	3.13	3.54	3.03	2.79	2.91	2.78	0.26
Internal organs characteristics (% live weight)								
Heart	0.39	0.38	0.39	0.38	0.38	0.38	0.40	0.03
Liver	1.78	1.65	2.00	1.91	1.83	1.85	1.81	0.20
Gizzard	2.14	2.12	2.44	2.68	2.14	2.51	2.68	0.17
Lungs	0.54	0.48	0.60	0.53	0.52	0.54	0.60	0.06

Means of the same row with different superscripts are significantly different ($P<0.05$)

PLM - *P. guineense* leaf meal; **PSM** - *P. guineense* seed meal; **SEM** - Standard error of mean

The haemoglobin (Hb) and mean corpuscular (MCH) of broiler chickens on the PSM and PLM diets (Table 5) were

higher ($P < 0.05$) than those of the chickens in the control diet. Broiler chickens on 0.6% PLM got the highest Hb (10.80 g/dl), MCH

(156.50%), PCV (37.40%) and WBC (1247.80 $\times 10^9/\text{mm}^3$) concentrations, followed by broiler chickens fed 0.6% PSM feed with Hb, PCV and WBC values corresponding to 8.20 g/dl, 29.30% and 1236.50 ($\times 10^9/\text{mm}^3$), respectively. However, haemoglobin values between birds fed 0.4% PGLM and those fed 0.6% PGSM were statistically similar. An increase in the chickens' blood PCV and Hb concentration fed PSM and PLM diets indicate an improved oxygen carrying capacity of the cells, which translates to a better nutrient availability to the chickens, thereby affecting their well-being (31). The increase in total white blood count seen in this research indicates a healthy immune response. Hematological indices according to (32) is a good indicator of the physiological status of the animals. The blood chemistry parameters indicate significant ($P < 0.05$) differences in cholesterol, globulin, glucose and total protein levels among the treatment groups. Chickens on 0.6% PSM had the least cholesterol concentration, with the value corresponding to 1.90 mg/dl, while chickens

consuming control diet had the highest value corresponding to 2.30mg/dl. Different studies have indicated that the supplementation of garlic and ginger in broiler chickens' diets have caused the reduction in the cholesterol level of blood. (33) discovered that extract from ginger had an anti-hypercholesterolemia effect in cholesterol. The findings of this research corresponded with the reports of the above authors. Broiler chickens on 0.6% PLM diet recorded the highest globulin concentration (2.690 mg/dl), while those on 0.2% PSM diet recorded the least concentration, with a value corresponding to 1.93 mg/dl. Globulin in the blood of broiler chickens was observed to increase as the dietary inclusion of PSM and PLM increased. Broiler chickens on a 0.6% PSM diet recorded the highest blood glucose and total protein concentrations, corresponding to 11.50g/l and 3.50g/l, respectively. The glucose concentration of 10.30 g/l was recorded among chickens fed control diet while those on 0.2% PSM recorded the least total blood protein value of 2.40 g/l.

Table 5 : Hematological and biochemical indices of broiler fed Piper guineense leaf and seed meals

Treatments	Control	0.2%PLM	0.4%PLM	0.6%PLM	0.2%PSM	0.4%PSM	0.6%PSM	SEM
Hematological indices (mg/dl)								
Haemoglobin (Hb)	7.00 ^{cd}	7.20 ^c	8.30 ^b	10.80 ^a	6.73 ^d	6.80 ^d	8.20 ^b	0.9
Mean corpuscular	139.80 ^e	143.40 ^d	150.80 ^b	156.50 ^a	143.60 ^d	139.23 ^f	147.50 ^c	0.14
Packed cell volume	26.40 ^f	26.73 ^e	28.20 ^d	37.40 ^a	23.93 ^g	28.90 ^c	29.30 ^b	0.06
White blood cell counts	1201.40 ^f	1209.70 ^e	1227.00 ^c	1247.80 ^a	1211.50 ^d	1211.50 ^d	1236.50 ^b	0.09
Serum biochemistry (g/l)								
Albumin	1.23	1.17	1.23	1.00	1.23	1.00	1.10	0.19
Cholesterol	2.30 ^a	2.20 ^a	2.20 ^a	2.20 ^a	2.29 ^a	2.00 ^a	1.90 ^b	0.04
Globulin	2.10 ^b	1.93 ^{bc}	2.00 ^{bc}	2.60 ^a	1.50 ^d	1.80 ^c	2.40 ^a	0.07
Glucose	10.30 ^f	10.50 ^e	10.80 ^d	10.90 ^c	11.50 ^a	11.30 ^b	11.50 ^a	0.03
Total protein	3.00 ^b	2.90 ^{bc}	2.70 ^{de}	2.60 ^e	2.40 ^f	2.80 ^{cd}	3.50 ^a	0.05

a, b, ...f means of the same row with different superscripts are significantly different ($P < 0.05$)

- 0.2%PLM - 0.2% *P. guineense* leaf meal
- 0.4%PLM - 0.4% *P. guineense* leaf meal
- 0.6%PLM - 0.4% *P. guineense* leaf meal
- 0.2%PSM - 0.2% *P. guineense* seed meal
- 0.4%PSM - 0.4% *P. guineense* seed meal
- 0.6%PSM - 0.6% *P. guineense* seed meal
- SEM - Standard error of means

Conclusion and Applications

1. It was therefore concluded that dietary inclusion with PSM improved the performance of finisher broiler chickens relative to the PLM.
2. Broiler chicken diets may be supplemented with 0.6% of PSM for optimum performance at the finishers' stage.

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