CHEMICAL COMPOSITION AND GROWTH PROMOTING EFFECT OF *PIPER GUINEENSE* LEAF AND SEED MEALS ON BROILER CHICKS AT STARTER PHASE

EFFIONG, Okokon Okon and OCHAGU, Simeon Iyai
Department of Animal Science, Faculty of Agriculture, University of Calabar, PMB 1115, Calabar, Nigeria.

Corresponding Author: Effiong, O. O. Department of Animal Science, Faculty of Agriculture, University of Calabar, PMB 1115, Calabar, Nigeria **Email:** okokoneffiong48@gmail.com **Phone:** +234 8037313657

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ABSTRACT

The study was designed to compare the chemical composition of Piper guineense leaf and seed meals, and their effect on the performance of broilers at starter phase. The P. guineense leaves and seeds were processed and their chemical composition determined. Seven (7) broiler starter diets were formulated, with the control without the test samples, while diets 2, 3 and 4 were formulated to include 0.2, 0.4 and 0.6 % of P. guineense seed meal, respectively. P. guineense leaf meal was included at 0.2, 0.4 and 0.6 % respectively in diets 4, 5 and 6. Two hundred and ten day-old broiler chicks were divided into seven groups and each group assigned to one of the experimental diets in a randomized complete block design and data generated were analyzed using SPSS 17.0. P. guineense leaf meal was superior (p<0.05) in crude protein (15.87 %) and ether extract (2.52 %), relative to the seed meal with higher crude fibre (6.30 %) and ash (3.60 %) contents. P. guineense seed meal contained lower phytochemical compounds compared to the leaf meal. Broiler chicks fed P. guineense seed and leaf meals supplemented diets were superior (p<0.05) to those fed on control diet in their mean daily weight gain and feed conversion ratio. Within the treatment groups, birds on 0.4 % P. guineense seed meal diet recorded the best performance. It was concluded that broiler diets could be supplemented with P. guineense seed meal at 0.4% during the starter phase.

Keywords: *Piper guineense* leaf, *Piper guineense* seed, Mineral composition, phytochemical composition, Broiler performance

INTRODUCTION

One of the major challenges facing poultry industry in Nigeria is how to improve the performance of birds to enhance higher profit margin, while reducing cost of production, especially feeding cost. A lot of research and production strategies have been employed, including the use of antibiotics to achieve improved growth (Kehinde *et al*., 2010; Fakhim *et al*., 2013; Oleforuh-Okoleh *et al*., 2015). The use of antibiotics in poultry industry as feed additives and treatment of diseases for many years have caused microbiological and clinical evidences of resistant bacteria that might be zoonotic resulting in infections that are more difficult to treat. Due to the growing concern of this health implication, the use of antibiotics has been banned in many countries. Consequently, there is an increasing interest in finding alternatives to antibiotics in poultry production.

Herbs, spices and plant extracts have been considered as alternatives for the health and nutrition of the poultry birds (Manan *et al*., 2012). They have been reported as good appetite and digestion stimulants, enhancing physiological function, promoting good health and also improving body performance (Frankic *et al.,* 2009). Puvaca *et al*. (2013) also opined that phyto-additives in animal nutrition have attracted attention for their potential role as alternatives to antibiotic growth promoters. Windisch *et al*. (2008) reported that herbs and spices enhanced the resistance of animals exposed to different stress conditions and also increased the absorption of essential nutrients, thereby promoting the growth of the animals. Some leaf meal/extracts which have been used as growth promoters in broiler chicken feeds include *Moringa olerifera* (Portugaliza and Fernandez*,* 2012), lemon grass leaf (Mmereole, 2010), pawpaw leaf (Heuze and Tran, 2015), bitter leaf, ginger and garlic (Oleforuh-Okoleh, *et al*., 2015). Another spice which may be of importance is the leaf and seeds of *P. guineense.*

Piper guineense has been noted for its multiple economic purposes, such as culinary preservative, cosmetic, medicinal as well as insecticidal usage (Dalziel, 1999). Research has confirmed that it enhances good health and it is nutritionally safe for humans (Scott *et al*., 2004). The genus *Piper* is made up of about 1,050 species of tropical shrubs and small trees, most of which are important as spices, flavoring agents and medicine (Mabberley, 2008). Members with economic importance include *P. nigrum* (black pepper) (Ravidran, 2000), *P. betel* (Betal pepper) (Satyal and Setzer, 2012), *P. metlysticum* (narcotic pepper) (Shulgin, 1973) and *P. longum* (long pepper) (Zaveri *et al.,* 2010). The essential oils of numerous piper species have been analyzed and examined for their biological properties (Moura Do Carmo *et al.,* 2012)*. P. guineense* is a flowering plants belonging to the family Piperaceae. It bears small drupe fruits of about five millimeters in diameter and red when fully mature. The seeds are otherwise called peppercorn when dried and is used as spice and seasoning (Dutta and Dutta 1997) as well as preservative. The plant has been reported to originate from India and had been rated the world most important spice. It has been cultivated most importantly for its fruit. This is usually dried and it is one of the most common spices in European cuisine and has been reported since antiquity for both its flavor and medicinal purposes (McGee, 2007). The seed of *P. guineense* has been used for the production of white and green pepper (Dalby, 2002). Several studies on the medicinal applications including anticonvulsant effect (Abila *et al.* 1993), non-sedating anti-convulsant activity (Ashorobi and Akintoye, 1999) and the antimicrobial activity (Oyedeji *et al*., 2005) of *P. guineense* have been investigated. *P. guineense* seeds have been used externally as counter-irritant or as a stimulating ointment, internally as stomachic (reduce flatulence). The leaves have been used to treat wounds, intestinal diseases and rheumatism (Sumathykutty *et al*., 1999), while the twigs are excellent in the treatment of cough and bronchitis (Oliver-Bever, 1986). The essential oils of *P. guineense* from Cameroon (Tchoumbougnang *et al.,* 2009) and from Nigeria (Oyedeji *et al*., 2005) have been examined and several chemo-types are apparent.

There is paucity information on the utilization of *P. guineense* leaf and seed meals as additive in broiler chickens diet hence the need for this research. This study was designed to: (i) compare the chemical composition of *P. guin*eense leaf and seed meals and (ii) evaluate the growth performance of broiler chicks fed diets containing graded levels of *P. guineense* seed and leaf meals at starter phase.

MATERIALS AND METHODS

Experimental Site: The proximate, minerals and phytochemical analyses of the *P. guineense* seed and leaf meals were carried out at the Department of Animal Science Laboratory, University of Calabar, while the feeding trial was conducted at the Poultry Unit of the Teaching and Research Farm, Department of Animal Science University of Calabar, Calabar, Nigeria.

Sources and Processing of *P. guineese* Seed and Leaf: *P. guineense* seed and leaf were obtained from farmers at Odukpani and Akpabuyo Local Government Areas of Cross

River State. The seeds and leaves were separated gently from the twig, air-dried to a moisture content of 12 % by spreading thinly on a concrete floor, blended to powder using a Kenitone Millennium Quality Electric Blender and stored individually in a screw capped plastic container, prior to chemical analyses and feed formulation.

Chemical Analyses of the Test Samples

Proximate composition: The *P. guineense* seeds and leaves were analyzed for their percentage crude protein (N x 6.25), crude fibre, ether extract and total ash using the methods described by AOAC (2010).

Phytochemical screening: Concentrations of some important phytochemicals present in the *P. guineense* seeds and leaves meals were determined. Phytic acid content was determined by the method described by Wheeler and Ferrel (1971), using two grams of each dry samples. Quantitative estimation of tannins was carried out using the modified vanillin-HCl method of Price *et al.* (1987), while oxalates was determined by the method outlined by Dye (1956). Cyanogenic glycoside was estimated by determining the amount of hydrogen cyanide released on hydrolysis (AOAC, 1990).

Mineral composition determination: Atomic absorption spectrophotometer (Unicam-1929, Cambridge, England) was used for the determination of calcium, magnesium, iron and copper concentrations, while sodium was determined using of flame photometer. The molybdovanadate method (AOAC 1990) was used for the determination of total phosphorus.

Experimental Diets: Seven diets (Table 1), were formulated at starter phase of the experiment with the control diet formulated without the test samples. Diets 2, 3 and 4 were formulated to include 0.2, 0.4 and 0.6 g/kg of *P. guineense* seed meal, respectively while *P. guineense* leaf meal was included at the rate of 0.2. 0.4 and 0.6 g/kg, respectively to diets 4, 5 and 6.

Experimental Animals and Design: Two hundred and ten (210) day-old broiler chicks were purchased from CHI Hatchery Limited, Ibadan, Nigeria for the experiment. The chicks were weighed and grouped according to their body weight into seven groups of thirty (30) chicks. Each group was further divided into three replicates of ten chicks, to ensure that the overall mean weights and weight ranges were similar across the groups. Each group was randomly assigned to one of the seven experimental diets in a completely randomized design (CRD).

Diets were fed as mash throughout the experiment, which lasted for 28 days and water was offered to the birds at free choice. Feed intake per bird on replicate basis was recorded daily by subtracting the left over feed from the quantity offered after 24 hours post feeding and divided by the numbers of birds per replicate. The weights of birds were taken on weekly basis and the average weekly weight gain determined by subtracting the weight of a particular week from the previous week. Average weight gain per bird for each replicate was determined by dividing the total weight gain by the number of birds in the replicate. The average weekly feed intake and weight gain were used to calculate the feed conversion ratio.

Statistical Analysis: Data generated from the experiment were subjected to analysis of variance (ANOVA) using SPSS 17.0 while the significant means were separated using the same statistical software.

RESULTS AND DISCUSSION

Proximate Composition of *Piper guineense* Leaf and Seed Meals: The results of the proximate composition of *P. guineense* leaf and seed meals are presented in Table 2. The percentage crude protein value of 15.87 % observed in the *P. guineense* leaf meal was significantly ($p<0.05$) higher than 10.17 % recorded in the seed meal. Similarly, variations in the ether extract, crude fibre and ash composition between the leaf and seed meals of *P. guineense* were significant ($p<0.05$).

Table 1: Composition of *Piper guineense* leaf and seed meals supplemented diets fed to broiler chicks at the starter phase

Ingredients	Control	0.2 % PGLM	0.4 % PGLM	0.6 % PGLM	0.2 % PGSM	0.4 % PGSM	0.6 % PGSM
Maize	53.70	53.50	53.30	53.10	53.50	53.30	53.10
Soybean meal	34.90	34.90	34.90	34.90	34.90	34.90	34.90
Fish meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Palm kernel cake	2.00	2.00	2.00	2.00	2.00	2.00	2.00
P. guineense	-	0.20	0.40	0.60	0.20	0.40	0.60
Wheat offal	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Bone meal	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Lysine	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin/ mineral 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
Chemical analysis:							
crude protein	22.571	22.611	22.593	22.575	22.611	22.259	22.575
Metabolizable energy (Kcal ME/Kg)	2838.76	2831.90	2825.04	2818.17	2831.9	2825.04	2818.17

*PGLM = Piper guineense leaf meal, PGSM = Piper guineense seed meal, *mineral/ vitamin premix containing the following per kg. Vitamin A, 5,50000 IU ; vitamin D3 1,0000 IU; vitamin 5,10,000 IU; vitamin 2,500 mg; pyridoxine 1375; antioxidant, 62.5 g; Niacin, 13.750 mg; vitamin B 12 7.5 mg; pathogenic acid 3750 mg; biotin, 10 mg; chlorine chloride 200 g; manganese 40 g; zinc 25; Iron 10 g; copper 2.50 g; iodine, 0.6 g; selenium 200 mg; cobalt 200 mg*

Table 2: Proximate composition of *Piper guineense* leaf and seed meal

Constituents (%)	Leaf meal	Seed meal
Crude protein	15.87 ± 0.04*	10.17 ± 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)*

From the results, crude fibre and ash values were higher in *P. guineense* seed meal (6.30 and 3.60 % respectively) than in the leaf meal, while the leaf meal was superior to seed meal in ether extract content. The ether extract of the *P. guineense* leaf and seed meals were 2.52 and 1.35 % respectively. The nitrogen free extract of 61.81 % recorded in *P. guineense* seed meal was not statistically different (p>0.05) from 58.04 % contained in the leaf meal. The presence of protein, fat and energy in *P. guineense* leaf and seed meals indicated their potentials in supplying nutrient, when used in compounding feed for animals. The percentage crude protein, ether extract, ash and total fibre values, corresponding to 4.94, 23.65, 21.29 and 38.76, respectively reported by Zou *et al*. (2015) for hot pepper were higher than the values obtained in this experiment. Oladunjoye *et al*. (2010) reported a crude protein range of 4.31 – 4.85 % for bread fruit meal, while the crude fibre, fat, ash and starch, ranging from 5.00 – 5.38, 2.56 – 2.90 and 58.38 – 69.20 % respectively reported by these authors were similar to the result of this experiment. The crude protein values (22.23, 27.31 and 25. 08 %), ash (7.96, 8.93 and 6.63 %) and ether extract (16.41, 12.40 and 12.29 %) reported by Aye and Adegun (2013) for *Moringa, Leucaena* and *Gliricidia* leaf meals respectively were also higher than the values observed in the leaf and seed meals of *P. guineense* of this research. The nitrogen free extract range (34.64 – 40.28 %) observed by Aye and Adegun (2013) was however lower than 58.64 % for *P. guineense* leaf meal and 61.18 % for *P. guineense* seed meal reported in this study. As one of food components, the dietary fibre is beneficial for animal health. A number of studies reported by Elleuch *et al*. (2011) showed that the dietary fibre could prevent certain diseases such as colon cancer, obesity, cardiovascular issues as well as determine the rate at which the feed passes through the digestive tract. The high nitrogen free extract (58.64 and 61.18 %, respectively) noted in the leaf and seed meals of *P. guineense* suggests the availability of substantial energy in the meals.

Mineral composition of *Piper guineense* leaf and seed meals: The mineral compositions of *P. guineense* leaf and seed meals are presented in Table 3.

Table 3: Mineral composition of *Piper guineense* leaf and seed meal (mg/g)

Minerals	Leaf	Seed
Sodium	11.70 ± 0.01	11.40 ± 0.008
Potassium	48.00 ± 0.009	47.60 ± 0.04
Calcium	25.90 ± 0.10	56.00 ± 0.00*
Magnesium	27.90 ± 0.09	27.60 ± 0.30
Iron	1813.50 ± 0.73	1810.00 ± 0.93
Phosphorus	38.70 ± 0.09	38.60 ± 0.44
Manganese	510.00 ± 1.88	475.00 ± 2.36

*Values are means of triplicate determinations, *Significant means (P<0.05)*

The results showed that phosphorus was the most abundant mineral in the *P. guineense* seed and leaf meals (1813.50 ± 0.73 and 1810.00 ± 0.02 mg/100 g respectively) followed by manganese (510.00 ± 1.88 mg/100 g) for the leaf meal and 475.00 ± 2.36 mg/100 g for the seed meal. Sodium had the least concentration in the two samples, with 11.70 ± 0.01 mg/100 g observed in the leaf meal and 11.40 ± 0.008 mg/100 g in the seed meal. The concentration of minerals between the two samples showed no statistical difference (p>0.05), except for calcium, where 56.00 ± 0.00 mg/100 g observed in *P. guineense* seed meal was significantly higher than 25.90 ± 0.1 mg/100 g in *P. guineense* leaf meal. Miele *et al*. (2015) noted that diets high in potassium have the capacity to lower blood pressure and consequently the risk of mortality due to cardiovascular diseases. Calcium is one of the most important components in bone synthesis, while iron is associated with the production of blood cells (Sousa *et al*., 2014). Sodium have also been reported to enhance the regulation of plasma volume, acid balance and promotes nerves and muscle contraction. Sodium concentration of 12.38 mg/100 g reported by Zou *et al.* (2015) for hot pepper seed meal was similar to the values recorded in this experiment for *P. guineense* seed and leaf meals, while values for potassium (654.12 mg/100 mg), calcium (174.71 mg/100 mg) and magnesium (237.59 mg/100 mg) were higher than 25.90 ± 0.1 mg/100 mg and 56.00 ± 0.00 mg/100 mg for *P. guineense* seed and leaf meals, respectively. Concentrations of iron (1813.50 ± mg/100 mg and 1810.00 ± 0.73 mg/100 mg) and manganese (510.00 ± 1.88 mg/100 mg and 475.00 ± 2.36 mg/100 mg) for *P. `guineense* seed and leaf meals were higher than values of 7.49 mg/100 g for manganese and 2.16 mg/100 g for iron, reported by Zou *et al.* (2015) for hot pepper seed meal.

Phytochemical composition of *Piper guineense* leaf and seed meals: Results of the phytochemical contents of the *P. guineense* leaf and seed meals are presented in Table 4.

Table 4: Phytochemical composition of *Piper guineense* leaf and seed meals (%)

Parameters	Leaf	Seed
Tannins	3.30 ± 0.09	3.00 ± 0.001
Flavonoids	0.20 ± 0.09	0.20 ± 0.005
Saponins	13.40 ± 0.14	13.00 ± 0.19
Alkaloids	33.50 ± 0.12	33.00 ± 0.02
Hydrogen cyanides	15.00 ± 0.009	15.00 ± 0.02

Values are means of triplicate determinations

The results revealed the presence of tannins, flavonoids, saponins, alkaloids and hydrogen cyanides in both the leaf and seed meals of *P. guineense*. Variation in the concentration of these compounds between the two samples was however, not significantly different (p>0.05). The concentrations of tannins, flavonoids, saponins, alkaloids and hydrogen cyanides were 3.30 ± 0.03, 0.2 ± 0.09, 13.40 ± 0.14, 33.50 ± 0.12 and 15.000. ± 0.009 mg/100 g respectively for the leaf meal and 3.00 ± 0.001, 0.2 ± 0.005, 13.00 ± 0.19, 33.00 ± 0.02 and 15.00 ± 0.02 mg/100 g respectively for the seed meal, were within the safety limits recommended for monogastric animals (Attia-Ismail, 2015).

Study has shown that phenolic substances possess strong anti-inflammatory and anti-oxidative properties and considerable anti-carcinogenic and anti-mutagenic activities (Rice-Evans *et al.,* 1995; Cook and and Samman, 1996; Kumar *et al*., 2013; Brglez Mojzer *et al*., 2016). Shirin Adel and Prakash (2010) observed the presence of polyphenols, tannins and flavonoids in the ginger root meal.

Performance of broiler chicks fed *Piper guineense* leaf and seed meal diets at starter phase: The results of dietary inclusion of graded levels of *P. guineense* leaf and seed meals on the growth performance of broiler chicks at starter phase are presented in Table 5.

Table 5: Performance of broiler fed diets containing *Piper guineense* leaf and seed meals at the starter phase

Parameters	Control	0.2 % PGLM	0.4 % PGLM	0.6 % PGLM	0.2 % PGSM	0.4 % PGSM	0.6 % PGSM
Initial weight (g/bird)	33.33 ± 0.00	33.33 ± 0.00	33.33 ± 0.00	33.42 ± 0.01	34.35 ± 0.01	34.76 ± 0.01	33.82 ± 0.01
Final weight (g/bird)	819.66 ± 9.22[a]	1099.33 ± 70.39[d]	1042.35± 19.90[b]	818.55 ± 7.20[a]	1107.68 ± 47.37[e]	1143.09 ± 23.57[f]	1078.82 ± 9.81[c]
Total weight gain (g/bird)	786.33 ± 8.55[a]	1066.00 ± 23.00[d]	1009.02± 4.24[b]	785.23 ± 5.20[a]	1073.33 ± 9.96[d]	1108.33 ± 29.07[e]	1045.00 ± 15.56[c]
Av. daily weight gain (g/bird)	28.10 ± 0.57[a]	39.26 ± 0.94[b]	36.04 ± 0.97[b]	29.22 ± 0.98[a]	38.33 ± 1.94[b]	39.58 ± 1.37[b]	37.32 ± 1.51[b]
Av. daily feed intake (g/bird)	57.86 ± 1.46[c]	53.69 ± 1.73[bc]	52.44 ± 1.36[bc]	51.59 ± 1.73[b]	57.20 ± 2.16[c]	48.86 ± 1.83[a]	52.86 ± 0.85[ab]
Feed conversion ratio	2.06 ± 0.04[e]	1.37 ± 0.02[b]	1.45 ± 0.01[bc]	1.76 ± 0.01[d]	1.49 ± 0.09[c]	1.23 ± 0.01[a]	1.42 ± 0.01[bc]

means on the same row with different superscripts are significantly different (p<0.05), PGLM - Piper guineense leaf meal, PGSM - Piper guineense seed meal

Broiler chicks fed diet with 0.4 % *P. guineense* seed meal had significantly (p<0.05) higher final weight, total weight gain and average daily weight gain relative to control group with the final weight, total weight gain and average daily weight gain, corresponding to 819.66 ± 9.22, 786.33 ± 8.55 and 28.1 ± 0.52 g per bird respectively. The result showed that the dietary inclusion of *P. guineense* leaf and seed meals had significant impact on the weight of broilers. At 0.6 % inclusion level, *P. guineense* leaf meal did not positively influence the total and the average weekly weight gain.

The average daily feed intake of 57.86 ± 1.46 g observed for bird fed control diet was higher (p<0.05) than the value for birds fed treatment diets, except for those on diet with 0.2 % *P. guineense* seed meal whose value (57.20 ± 2.16 g) was similar to that of the control group. Birds on 0.6 % *P. guineense* seed meal recorded the least average daily feed intake of 48.86 ± 1.83 g. Dietary inclusion of *P. guineense* leaf and seed meals significantly improved (p<0.05) feed conversion ratio, with the best value (1.23 ± 0.01) recorded on birds fed 0.6 % *P. guineense* seed meal. Broiler chicks fed control diet had the most inferior feed conversion ratio of 2.06 ± 0.04. The increase in the final weight, total weight gain and average daily weight gain per bird relative to control group, suggested the presence of growth promoting substances in the *P. guineense* leaf and seed meals. Essential oil from *P. guineense* leaf and seed has been reported to promote digestive process in birds and other farm animals (Cabuk *et al*., 2003). According to Platel and Srinivasan (2000), piperin and cucurmin found in *P. guineense* can also influence the activity of pancreatic digestive enzymes, such as amylase, protease and lipases, thereby enhancing the digestive system. Furthermore, piperin and cucurmin promote digestion and also reduces the time feed passes through the digestive track. The use of herbs and spices as phytobiotics, with growth promoting properties have been reported to increase feed stability and enhanced gastro intestinal ecosystem through interference with the growth of pathogenic organisms. Report by Windisch *et al*. (2008) revealed that animal fed diets supplemented with herbs and spices are usually more resistant when exposed to different stress conditions and also with higher absorption of essential nutrients, thereby enhancing the growth performance of the animals.

Reduction in the feed intake, recorded among birds fed 0.6 percent *P. guineense* seed meal agreed with the findings of Jugl-Chizzola *et al*. (2006) who revealed that weaned pigs recorded significant lower feed intake when fed diet containing thyme compared to those fed control diet. Fadlalla *et al*. (2013) noted a decrease in feed intake, with improvement in feed conversion ratio of broiler chickens fed diet containing graded levels of garlic powder at 0.3 % level of inclusion, relative to other dietary treatment groups. The present findings also affirmed the works of Oleforuh-Okoleh *et al*. (2015) who reported a better performance of birds fed aqueous extract of ginger and garlic diets relative to those fed control diet. Saeid and Al-Nasry (2010) reported a higher body weight gain and feed conversion ratio for broiler birds fed coriander plant supplemented diet, which is similar to the finding of this research. Report by Güler *et al*. (2005) also revealed that supplementation of Japanese quail diet with *Coriander sativum* at 0.2 % increased their body weight and feed conversion ratio compared to those fed control diet.

Conclusion: From the results of this study, it was therefore concluded that dietary inclusion of *P. guineense* leaf and seed meals had no negative influence on the performance of broiler birds at starter phase. Broiler diets can be supplemented with *P. guineense* seed meal at 0.4 % during brooding phase for optimum performance.

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