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Effect of *Phyllanthus Amarus* supplemented diet on Egg production and Egg quality traits of Japanese Quail

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The effects of *Phyllanthus amarus* supplemented diet on Japanese quail egg production and egg quality traits were studied in a five (5) week trial period. A total of 48 female Japanese quail birds were randomly allotted into 4 dietary treatments: A- {Basal diet + zero antibiotic with 0% *Phyllanthus amarus* (Negative Control)}, B- {Basal diet + Antibiotic with 0% *Phyllanthus amarus* (Positive Control)}, C- (Basal diet +0.2% *Phyllanthus amarus* with zero antibiotic), D- (Basal diet +0.4% *Phyllanthus amarus* with zero antibiotics). The basal diet was formulated to contain 2,800Kcal metabolizable energy and 23% Crude Protein. Data were collected on Egg production traits and Egg quality traits. Data obtained were subjected to analysis of variance using the model suitable for completely randomized design. Although there were numerical increase in Hen-day production and Egg number with supplementation at 0.4% *Phyllanthus amarus*. Also for the egg quality traits, egg weight egg length and albumen weight revealed significant ($P<0.05$) differences and was highest at 0.4% *Phyllanthus amarus* supplementation. Yolk ratio was significantly ($P<0.05$) improved when supplemented with 0.2% *Phyllanthus amarus* and comparable with the Control diet. It was concluded that *Phyllanthus amarus* supplementation improved egg quality traits and production traits in Japanese quail.

Keywords: Japanese quails, egg production, antibiotics, egg quality traits, *Phyllanthus amarus*

INTRODUCTION

The importance of poultry production to the socio-economic development of any country cannot be over emphasized as a result of its ability to meet the people's dietary demand for animal protein in the form of meat and eggs. The potential for further growth is obvious in view of the value of eggs and poultry meat as basic healthy foods in human diet (Daghir, 2001).

It has been observed in poultry breeding that the quails were benefited as much as hens both for their meat and eggs, therefore, commercial quail breeding have become widespread (Altinel *et al.*, 1996). Corticulture is an important activity that is currently highly relevant in agricultural sector but despite the increasing production, much is still unknown about

Japanese quail nutrition. The egg is considered a functional food, as it is a source of protein, vitamins and lipids, such as phospholipids and polyunsaturated fatty acids (Meluzzi *et al.*, 2000; Anton *et al.*, 2006). Quail eggs have advantages compared to other poultry eggs; they are rich in minerals (Ca, Fe, Mg, P, K, Na, Zn, Cu, Mn and Se), vitamins (A, B1, B3, B12 and E) and pantothenic acid (Al-Daraji, *et al.*, 2010). On the other hand quail egg yolks have a higher cholesterol content (as much as 746 mg/100 g) (Aviati *et al.*, 2014) than chicken (352 mg/100 g) and duck (734 mg/100 g) eggs (Bagchi, 2012).

Antibiotic growth promoters (ADP) have undoubtedly improved animal performance and health status. About 70% of the use of ADP's has facilitated the efficient treatment and prevention of some threatening diseases in poultry birds either by killing or limiting the growth of bacteria or harmful microorganisms. However, the profound dependence on antibiotics in animal production

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has resulted in bacterial resistance to many modern antibiotics used for life-threatening diseases in humans (Hughes and Heritage, 2007).

Long term use of antibiotics may cause resistance and leave residues in the meat and egg product that may be harmful to the consumer. Edible tissues and egg products containing veterinary drug residues jeopardizes human health, including direct toxic effects, allergic reactions and increased bacterial resistance to common antibiotics (Botsoglou and Fletouris, 2001; Donoghue, 2005; Companyo *et al.*, 2009). Therefore, there is a demand for alternatives to antibiotics in an increasingly health conscious market.

As an alternative, herbal extracts and plants are now being investigated for use due to their natural antimicrobial properties. The importance of feed additives especially of plant origin has greatly increased in recent years and a number of studies have reported the beneficial effect of herbals or their active components on digestion process and better performance in birds (Al-Kassie, 2009; Hashemi and Davoodi, 2011). Also herbals have received increased attention as possible antibiotic growth promoter replacements (Denli *et al.*, 2004, Faghani *et al.*, 2014).

Biological trials of herbal formulation used as growth promoters have established beyond doubt the improved overall performance with respect to weight gain, feed efficiency, lowered mortality and also as therapeutic against liver damage due to feed contaminants like aflatoxins, toxicity caused by chemical drugs and in improving digestion (Ramoppa *et al.* 1975; Rao and Reddy, 1986; Devegowda *et al.*, 1990, Devegowda *et al.*, 1996).

In Nigeria and many other African countries, rural and urban communities have continued to use or consult local herbs/medicine-men for remedies to a variety of diseases. The entry of such herbs into the feed resource base of poultry farmers could significantly enhance poultry production. For example, in southwestern Nigeria, where most of the country's poultry farms are located, utilization of local herbs could help reduce cost of treating diseases in poultry.

Phyllanthus amarus which is one of the species belonging to a large family, Euphorbiaceae, is found in the tropical and sub-tropical region worldwide (Joseph and Raj, 2011). *Phyllanthus amarus* is said to possess claims of medicinal values which include hepatoprotective, anti-diabetic, anti-hypertensive, analgesic, anti-inflammatory and anti-microbial properties which are essential to enable efficient production in poultry (Adeneye *et al.*, 2006; Okiki *et al.*, 2015). These medicinal values are attributed to the chemical compounds including alkaloids, flavonoids, lignans and phenols which have been reportedly isolated from this plant (Adeneye *et al.*, 2006). The use of *Phyllanthus amarus* as a feed additive to replace antibiotics and natural phytochemicals have been reported. Its effect as an

antibacterial and antioxidant on immunity and body resistance, and have also been documented.

However, the potentials of this plant as a feed additive on egg production and egg quality traits of Japanese quail are yet to be documented. This experiment therefore investigates the effect of *Phyllanthus amarus* leaf meal on the egg laying performance and egg quality of Japanese quail layers.

However, the recent ban of antibiotics in animal production in Nigeria has led to the search for alternative sources to improve healthy consumption of protein in man. The medicinal properties of *Phyllanthus amarus* have been well documented, however its effect on egg production and egg quality traits of Japanese quail have not been extensively explored and are yet to be established, hence this study was carried out to evaluate the egg production and egg quality traits of Japanese quail hens fed *Phyllanthus amarus* supplemented diet.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted at Teaching and Research Farm of the Faculty of Agriculture, University of Ilorin, located at Latitude 8°30'N and Longitude 4°33'E with an average annual temperature of 26.5°C, an average rainfall of 107mm and a 51.1% average relative humidity. The experiment lasted for five (5) weeks.

Experimental Birds and Their Management

A total number of 48 six (6) weeks old female Japanese quail birds were randomly selected from the existing flock of birds. The birds were assigned using completely randomized design (CRD) into 4 treatment levels designated A, B, C and D. Treatment A was the basal diet with no *Phyllanthus amarus* and antibiotic (Control), Treatment B was the basal diet with antibiotic and no *Phyllanthus amarus*, Treatment C was the basal diet with 0.2% *Phyllanthus amarus* and no antibiotics, Treatment D was the basal diet with 0.4% *Phyllanthus amarus* and no antibiotics.

A total of twelve (12) birds were allocated per treatment and divided in three replicates. The birds were given multivitamins as provided as anti-stress with no vaccination, nor antibiotics throughout the experimental period. Feed and water was given ad-libitum throughout the experimental period.

The birds were reared in a multiple bird cage with a capacity ranging from 2 to 10 birds following a thorough cleaning and disinfection of the poultry house and equipment with a commercial disinfectant to ensure a clean and hygienic environment.

Experimental Treatment and Preparation

The fresh plant of *Phyllanthus amarus* were collected and air dried for a minimum period of six (6) days at room temperature to reduce the moisture content of the leaves while maintaining its green color and to achieve a relatively constant weight. The leaves were then grounded into fine powder using an electric blender and

stored properly prior to the commencement of the experiment to ensure that the quality was maintained. Proximate analysis of the samples were determined and known commercial antibiotic (Oxytetracycline) used in this study and gotten from a reputable feed concentrate and premixes company. The basal diet is shown in table 1

Table 1: Composition of Basal Diet

Feed ingredients	Percentage (%)
Maize	50.00
Wheat offal	13.00
Soya bean meal	20.00
Full fat soya bean	10.00
Fish meal (72%)	3.00
Oyster shell	1.00
Bone meal	2.00
DL- Methionine	0.25
Lysine	0.25
Salt	0.25
Broiler Premix	0.25
Total	100
Analyzed composition %	
Dry Matter	90.20
Crude Protein	22.20
Crude fat	6.00
Crude fiber	19.50
Total ash	11.32
Nitrogen free extract	31.18
Metabolizable energy (KCal)	2800

Data Collection

The following data were collected during the experimental period.

Egg production trait data includes: Egg Number, Hen Day Production (HDP), Age at First Egg (AFE), Body Weight at First Egg (BWFE) while *data on internal and external egg quality traits* which includes: Weekly Egg Weight (g): Shape Index: Albumen Height: Yolk Index: The height of the yolk (mm): Haugh Unit and The yolk index parameters were collected according to (Card *et al.*, (1979); Doyon *et al.*, (1986) and Anderson *et al.*, 2004).

Statistical Analysis

Data obtained were subjected to a Complete Randomized Design (CRD) analysis of variance (ANOVA) according to SPSS (2008). Significant differences among

the means were determined using the Duncan Multiple Range Test.

RESULTS AND DISCUSSION

Phytochemical screening of *Phyllanthus amarus* and proximate analysis

The results of phytochemical (quantitative and qualitative) screening of *Phyllanthus amarus* and proximate composition of diets are presented in Table 2 and 3. Table 2 shows that tannin, phenolics, steroids, flavonoids, coumarins, glycosides, alkaloids and fixed oil were present.

There were no traces of saponin, phlobatanin, anthocyanin, amino acids. Table 3 shows the proximate composition of the diets.

Table 2: Phytochemical screening (Quality and Quantity) of *Phyllanthus amarus*

Phytochemicals	Quality	Quantity (mg/kg)
Saponin	-	
Tannin	+	9.7322
Phlobatanin	-	
Phenolics	+	31.156
Steroids	+	135.8497
Flavonoids	+	316.9744
Coumarins	+	45.4082
Anthocyanin	-	
Amino acid	-	
Terpenoid	+	22.84082
Glycosides	+	7.8012
Triterpenes	-	
Alkaloids	+	41.9333
Fixed oils	+	

+ :detected and - :non detected

Table 3: Proximate composition of experimental diets

Parameters	Treatment A	Treatment B	Treatment C	Treatment D
Dry Matter (%)	90.2	89.58	89.94	90.1
Moisture Content (%)	9.8	10.42	10.06	9.9
Crude Protein (%)	22.2	20.8	23.6	21.2
Ether Extract (%)	6	5.5	6.5	5.5
Crude Fiber (%)	19.5	18	17.7	18.4

Treatment A = (No *P. amarus* and no Antibiotics); Treatment B = (0% *P. amarus* with antibiotics) : Treatment C = (0.2% *P. amarus*) and Treatment D = (0.4% *P. amarus*)

Effects of *Phyllanthus amarus* Supplemented Diet on Egg Production of Japanese quail

The relative effects of the plant *Phyllanthus amarus* on the egg production of Japanese Quails are shown in Table 4. The Hen-day production of the birds was not significantly different ($P>0.05$). The highest mean output of (43.20) was recorded at 0.4% *Phyllanthus amarus* while the lowest mean output (8.49) was recorded at 0% *Phyllanthus amarus*. The Age at first Egg among the birds showed no significant difference ($P>0.05$). Age at first egg of birds fed 0.4% *Phyllanthus amarus* was the highest with a mean output of (47.00) followed by birds fed 0% *Phyllanthus amarus* and antibiotic with a mean

output of (46.67) followed by birds fed 0.2% *Phyllanthus amarus* with a mean output of (50.67) while birds in negative control had the lowest mean of (50.67). The Egg number among the birds was not significantly different ($P>0.05$).

However, a significantly higher mean output of (3.02) was recorded at 0.4% *Phyllanthus amarus* compared to the negative control which had the lowest mean output of (0.67). There was no significant difference ($P>0.05$) in the BWFE of the birds fed with the various dietary treatments. BWFE of the birds was the highest at 0.4% *Phyllanthus amarus* with mean output of (1.4519) and the lowest at 0% *Phyllanthus amarus* with antibiotic with a mean output of (1.3742).

Table 4: Effects of *Phyllanthus amarus* Supplemented Diet on Egg Production Traits of Japanese Quail

Parameters	Treatment A	Treatment B	Treatment C	Treatment D	P- value	SEM
A FE (days)	50.67	46.67	46.33	47.00	0.533	1.165
Egg Number	0.67	1.17	1.33	3.02	0.188	0.591
BWFE (g)	144.05	137.42	145.70	145.19	0.828	2.271
HDP	8.49	15.81	17.73	43.20	0.186	8.733

A FE =Age at first egg, BWFE = Body Weight at First egg, HDP = Hen Day Production: Treatment A = (No *P. amarus* and no Antibiotics); Treatment B = (0% *P. amarus* with antibiotics) : Treatment C = (0.2% *P. amarus*) and Treatment D = (0.4% *P. amarus*)

Effects of *Phyllanthus amarus* Supplemented Diet on Egg Quality Traits of Japanese quail

The effects of *Phyllanthus amarus* supplemented diet on the external qualities of Japanese quail eggs are shown in Table (5). There was a significant difference ($P < 0.05$) in egg weight. Birds fed 0.4% *Phyllanthus amarus* laid the largest eggs with an average egg weight of (10.1193), followed by eggs from birds fed 0% *Phyllanthus amarus* with antibiotic having an average egg weight of (9.1792) followed by eggs from negative control having an average egg weight of (8.73430) while birds fed 0.2% *Phyllanthus* had the smallest eggs with an average egg weight of (7.8800). There was a significance difference in the egg length ($P < 0.05$) with 0.4% *Phyllanthus amarus* supplementation having the highest mean output (2.5850), followed by a mean output of (2.4983) at 0% *Phyllanthus amarus* with antibiotic supplementation followed by negative control with a mean output of (2.3629) while 0.2% *Phyllanthus amarus* Supplementation had the lowest mean output of (2.2650).

Egg width showed no significant difference ($P > 0.05$) among the treatments with eggs from 0.4% *Phyllanthus amarus* having the highest mean output (1.807) and eggs from birds fed 0.2% *Phyllanthus amarus* having the lowest mean output of (1.7120). There were no significant differences ($P > 0.05$) recorded among the different treatments for egg shell weight, egg shape index and egg shell ratio. The highest shell weight was recorded at 0.4% *Phyllanthus amarus* supplementation with a mean output of (0.6843) while the least shell weight of mean output (0.4990) was recorded at 0.2% *Phyllanthus amarus* supplementation. Egg shape index showed no significant differences ($P > 0.05$) among the various treatments. Highest mean output of (75.8325) was recorded at 2% *Phyllanthus amarus* supplementation and lowest mean output of (71.3982) at 0.4% *Phyllanthus amarus* supplementation, Egg shell ratio also showed no significant differences ($P > 0.05$) among the treatments. Negative control had the highest mean output of (7.0463) and the lowest mean output of (6.3516) was recorded at 0.2% *Phyllanthus amarus*.

Table 5: Effects of *Phyllanthus amarus* Supplemented diet External Egg Quality Traits of Japanese Quail

Parameters	Treatment A	Treatment B	Treatment C	Treatment D	P- value	SEM
Egg weight (g)	8.73 ^{ab}	9.18 ^{bc}	7.88 ^a	10.12 ^c	0.002	0.427
Egg length	2.36 ^{ab}	2.50 ^{bc}	2.27 ^a	2.59 ^c	0.003	0.158
Egg width (cm)	1.75 ^a	1.81 ^{ab}	1.71 ^a	1.84 ^b	0.084	0.064
Shell thickness (mm)	0.25	0.29	0.23	0.35	0.501	0.064
Shell Weight (g)	0.62	0.62	0.50	0.68	0.144	0.087
Egg Shape	74.44	72.77	75.83	71.40	0.282	2.171
Shell Ratio	7.05	6.82	6.35	6.87	0.860	0.301

Means with no common superscripts letter within a row differ significantly ($P < 0.05$), Treatment A = (No *P. amarus* and no Antibiotics): Treatment B = (0% *P. amarus* with antibiotics) : Treatment C = (0.2% *P. amarus*) and Treatment D = (0.4% *P. amarus*).

Table 6: Effect of *Phyllanthus amarus* supplemented diet on the internal egg quality traits of Japanese Quail

Parameters	Treatment A	Treatment B	Treatment C	Treatment D	P- value	SEM
Albumen weight (g)	4.76 ^{ab}	4.61 ^{ab}	3.95 ^a	5.52 ^c	0.011	0.741
Yolk Weight (g)	2.81	3.23	2.96	3.07	0.493	0.178
Albumen Height (mm)	4.30	3.61	3.53	4.09	0.271	0.381
Albumen Ratio	54.55	50.44	48.58	54.36	0.513	3.160
Yolk Ratio	32.17 ^{ab}	34.97 ^{ab}	39.24 ^b	30.43 ^a	0.046	4.253
Haugh Unit (%)	90.01	86.27	87.08	88.23	0.511	1.609

Means with no common superscripts letter within a row differ significantly ($P < 0.05$), Treatment A = (No *P. amarus* and no Antibiotics): Treatment B = (0% *P. amarus* with antibiotics) : Treatment C = (0.2% *P. amarus*) and Treatment D = (0.4% *P. amarus*).

Effects of *Phyllanthus amarus* supplemented diet on the internal egg quality traits

The effects of *Phyllanthus amarus* supplemented diet on the internal egg quality traits are shown in Table 6. The result showed that albumen weight was significantly different ($P < 0.05$). It was significantly higher ($P < 0.05$) at 0.4% *Phyllanthus amarus* with a mean output of (5.5179)

and was the lowest at 0.2% *Phyllanthus amarus* with a mean output of (3.9500). Yolk weight showed no Significant differences ($P > 0.05$) among the treatments, 0% *Phullanthus amarus* with antibiotic supplementation had the highest mean output (3.2292) and negative control had the lowest mean output (2.8171).

There were no significant differences ($P > 0.05$) in albumen height, the highest mean output (4.2971) was

recorded in negative control while the lowest mean output of (3.5300) was recorded at 0.2% *Phyllanthus amarus*. The result showed no significant differences ($P>0.05$) in albumen ratio with the negative control having the highest mean output (54.5501) and the lowest mean output of (48.5816) at 0.2% *Phyllanthus amarus* supplementation. Yolk ratio differed significantly ($P<0.05$) with a record of the highest mean output of (39.2410) at 0.2% *Phyllanthus amarus* and the lowest mean output of (30.4254) at 0.4% *Phyllanthus amarus*.

Haugh Unit showed no significant differences ($P>0.05$) with the negative control having the highest mean output (90.0126) followed by a mean output of (38.2284) at 0.4% *Phyllanthus amarus* followed by a mean output of (87.0770) at 0.2% *Phyllanthus amarus* and 0% *Phyllanthus amarus* With antibiotic having the lowest mean output of (86.2653).

DISCUSSION

Phyllanthus amarus supplemented diet didn't significantly affect egg production parameters, such as hen-day production, age at first egg, body weight at first egg and egg number of Japanese quail hens as there were no significant differences, this is in accordance to Hammershoj and Steinfeldt (2012) study on the effect of feeding the following phytobiotics to layers; kale (*Brassica oleracea ssp. acephala*), thyme (*Thymus vulgaris*) and basil (*Ocimum basilicum*) as a forage material who reported no significant difference in laying rate between treatment groups. Also Gharaghani *et al.*, (2015) who studied effects of fennel in productive performance showed no significant difference. Although there was an improvement in the Egg number and Hen-day production of Japanese quail fed 0.4% *Phyllanthus amarus* supplemented diet compared to birds fed basal diet only, AFE and BWFE weren't improved. Very limited reports from literature had evaluated the effect of *Phyllanthus amarus* supplementation on layer performance.

Due to the scarcity of available reports on the effects of *Phyllanthus amarus* on egg production and egg quality traits of Japanese quail, comparison was done with other herbal supplements. The statistical analysis of our data clearly suggested that *Phyllanthus amarus* supplemented diet affected some egg external quality traits and interior egg quality such as egg weight, egg length, albumen weight and yolk ratio with no sign of interaction. In contrast, Olobatoke and Mulugeta (2011) observed no significance in dietary garlic supplementation in egg quality and Yildiz *et al.*, (2006) also showed no effects of *Jerusalem artichoke* containing high amounts of flavonoid on the egg quality parameters in laying hens.

This study revealed that egg weight of birds improved when fed 0.4% *Phyllanthus amarus* with an average egg weight of 10.12g. This is in line with Bonomi *et al.* (1976)

study on the phytochemical effect of the photobiotic Propolis (10, 20, 30 mg/kg diet, which significantly increased egg weight, and in contrast to a study carried out by Asrat *et al.*, (2018) on effects of supplementation of different levels of garlic (*Allium sativum*) on white leghorn chicken. Egg weight is strongly determined genetically and influenced by the age of hens and production intensity (Washburn, 1990). The lack of information about the nutrient use from *Phyllanthus amarus* administration and/or the digestion and absorption abilities makes the increase in egg components weights less well understood and opens the door for further studies. There were no significant effects on egg width, egg shape, shell thickness, shell ratio and shell weight.

Egg shell thickness is an important trait for hatchability, safer eggs and increased shelf life. Egg shell thickness should be between 0.33mm and 0.35mm while a few eggs with Shell thickness less than 0.27mm will hatch as reported (Khan, *et al.*, 2004). From the results in this present study, there was no significant difference in the shell thickness as well as shell weight, similar to Lim *et al.* (2006) study on garlic powder. This study showed a shell thickness of range 0.23- 0.3 5mm, 0.2% *Phyllanthus amarus* having the lowest shell thickness of 0.2250mm. But an improved shell thickness and shell weight was shown at 0.4% *Phyllanthus amarus* having the highest shell thickness of 0.3543mm.

Results obtained from this study showed that *Phyllanthus amarus* supplemented diet had a significant effect on yolk weight ratio to egg weight ($P = 0.046$). Usually, the higher egg weight is linked with the higher albumen content and respectively lower yolk content (Suk and Park, 2001), however birds fed 0.4% *Phyllanthus amarus* had the largest eggs in weight but had the lowest egg yolk ratio while highest yolk weight ratio was at 0.2% *Phyllanthus amarus* as shown in Table 4 and 5.

The yolk index is a measure of the standing-up quality of the yolk. Previous reports have demonstrated that egg yolk can be enriched in antioxidant compounds or nutrients. There was an improvement in the albumen weight of eggs of birds fed 0.4% *Phyllanthus amarus* in contrast to Asrat *et al.*, (2018) study on effects of supplementation of different levels of garlic (*Allium sativum*) which showed no significant improvement in albumen weight. Similar to Asrat *et al.*, (2018) study on garlic inclusion levels in white leghorn layers diet, there was no significant effect on the yolk weight in this present study. In contrast to Mottaghitalab and Taraz (2002) study of dietary garlic supplemented diet on laying hens, this study showed no significant improvement in the albumen height.

The Haugh unit and albumen ratio of Japanese quail was not significantly different among the treatments (Table 5), similar to Yalcin *et al.* (2006) who reported that the supplementation of garlic powder had no effect on albumen ratio and egg Haugh unit values when laying

hens were fed 5 and 10 g/kg garlic powder for 22 weeks. TSS, (1999) reported that it is generally accepted that the higher the Haugh unit value the better the egg equality, studies have shown in the U.K that there is consumer resistance to purchase eggs with Haugh unit below 60, in this present study the Haugh unit showed in this study is between the range of 86.27% -90.0%. Haugh unit was the highest in the negative control diet having 90.01%.

CONCLUSION AND RECOMMENDATION

From the result, *Phyllanthus amarus* supplementation significantly increased egg weight, egg length, egg number and albumen weight at 0.4%. It is therefore concluded that *Phyllanthus amarus* can be supplemented in Japanese quail diet.

It is recommended that *Phyllanthus amarus* can be used at 0.4% as a replacement for antibiotics in quail diet.

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