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Comparative evaluation of black pepper (*Piper nigrum*) meal and oxytetracycline on growth performance, nutrient digestibility and microbial counts of growing Japanese quails (*Coturnix coturnix japonica*)

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Target Audience*: Nutritionists and Researchers*

Abstract

A 4-week feeding trial was conducted on comparative evaluation of black pepper meal and Oxytetracycline on the growth performance, nutrient digestibility and microbial counts of growing Japanese quails. A total of 210 growing Japanese quails, aged 2 weeks were used for the experiment. The birds were randomly assigned to the diets in a completely randomized design; such that the diets had 0, 250, 500, 750g/kg black pepper meal and Oxytetracycline at manufacturer's recommendation. Each diet had 42 birds, replicated three times with each replicate having 14 birds. Results obtained showed that the body weight gain, and feed intake were significantly ($p<0.05$) increased in quail chicks fed 750g black pepper compared to those fed 0g/kg black pepper meal and Oxytetracycline while the best feed conversion ratio was obtained for chicks fed 750g/kg black pepper. The nutrient digestibility was significantly ($p<0.05$) influenced by dietary supplement of black pepper at 750g except for crude fibre that was not significantly ($p>0.05$) influenced. The microbial count was significantly ($p<0.05$) decreased for the harmful bacteria while the beneficial bacteria were significantly ($p<0.05$) increased in the quails fed 750g black pepper compared to those fed the control and Oxytetracycline diets. It was concluded that addition of black pepper meal could significantly improve performance and the general health status of growing Japanese quails.

Keywords: *black pepper, oxytetracycline, performance, microbial, Japanese quail, digestibility*

Description of Problem

Antibiotics have been widely used in animal production for decades to treat and improve animal health, growth rate, and feed conversion ratio (1; 2). The danger of the continuous emergence of new antibiotic resistance mechanisms in different microorganisms prompted the European Commission to ban antibiotics as growth enhancers in animal feed. However, the ignorance of the hazards related to synthetic antibiotic therapy in food producing animals has escalated the emergence of antimicrobial resistance (3) and residues found in livestock products could be harmful for consumer's health (4). Due to these, there has been an increasing interest in the use of natural plants or non-synthetic alternatives such as herbs, spices and essential oils as additives in animal diets (5). Phytogenic compounds are the groups of feed additives that have been reported to possess a potential for growth enhancement of livestock species due to presence of a number of pharmacologically active substances. The beneficial effects of these natural plants or plant products resides

in the presence of active substances such as phenols, tannins, alkaloids, flavonoids, terpenoids, eugenol and sulphur compounds (6). These secondary metabolites when incorporated in animal feed can increase feed digestibility (7; 8), improve growth performances, activate digestive enzymes and stimulate immune function through increased gut secretions, anti-oxidative and anti-inflammatory actions (6).

Black pepper (*Piper nigrum*) called the King of spices, is one of the pungent or aromatic substances of vegetable origin used as food adjuncts and was used as stimulant of gastric secretion, raises the flow of digestive juice (9), and develops thermogenesis of lipid (10). Black pepper contains piperine, essential oils, piperettine, piperidine and chavicine (11) which has been shown to improve poor digestion, enhance health due to their anti-inflammatory, antioxidant and disease fighting qualities. Oxytetracycline on the other hand, is a highly active broad-spectrum antibiotic used in the poultry industry that works by inhibiting bacterial protein synthesis by preventing the attachment of aminoacyl-tRNA with the bacterial ribosome to the ribosomal acceptor (A) site, and is commonly administered to chickens through feed and drinking water to treat several diseases, such as chronic respiratory disease, infectious coryza, and fowl cholera.

Recently, rearing of quail has gained importance in the poultry industry. The consumption of quail eggs and meat has significantly increased and there is scope for increasing healthy quail products in the market. Quail meat has extremely low skin fat and low cholesterol value. It is rich in micronutrients and a wide range of vitamins including the B complex, E and K and folate. There is need to find more efficient alternatives or combinations of different alternatives for maintaining health and improving performance of poultry and other livestock species. Hence, the purpose of this study was to evaluate the effect of black pepper (*Piper nigrum L*) in feed in comparison with Oxytetracycline (antibiotics) on growth performance, nutrient digestibility and microbial counts of growing Japanese quails (*Coturnix japonica*).

Materials and Methods

Experimental Site

The experiment was conducted at the Poultry Unit, Kabba College of Agriculture, Division of Agricultural Colleges, Ahmadu Bello University, Zaria, Kaduna State. Kabba is located in the Southern Guinea Savannah Ecological Zone of Nigeria on the Latitude $7^{o}53'$ N, Longitude 6 $^{o}02$ E (12) with an average rainfall of about 1500mm per annum and an average temperature range from $18^{o}C$ - $32^{o}C$. It is 427m above sea level (13).

Source of test materials and experimental birds

Black pepper was purchased from a local market in Kabba, Kogi State. The dry black pepper was ground before being incorporated into the experimental diets. The oxytetracycline was purchased from a reputable pharmaceutical store, while a total of 210 (Two hundred and ten) growing Japanese quails (*Coturnix japonica*) were obtained from a reputable farm in Ibadan, Oyo State, Nigeria.

Experimental Diets

Five experimental diets were formulated for the experiment

Control - contained no black pepper or Oxytetracycline

Treatment 1 –250g Black pepper

Treatment 2 –500g Black pepper

Treatment 3 –750g Black pepper

Treatment 4—Basal diet with

oxytetracycline as feed additives (200g dosage was used as recommended by the manufacturer). The diets were formulated to contain 25% crude protein.

Design and Management of Experimental Birds

A total of 210 (Two hundred and ten) growing Japanese quails at 2 weeks of age with average initial body weight ranging from 52.03- 52.08g were used for the experiment. The quails were randomly assigned to five dietary treatments with forty-two (42) quails per each treatment, replicated three times with fourteen (14) quails each in a completely randomized design The diets were formulated to meet the nutrient requirements of Japanese quail chicks during growth period according to the National Research Council requirements (14). Though quail is known to be resistant to most viral diseases of poultry, a good hygiene, cleanliness and biosecurity measures were ensured throughout the experimental period.

Data collection

Growth performance

The birds were weighed at the beginning of the experiment and their average initial weights were taken. The chicks were subsequently weighed every week to determine the weight gained. Feed supplied and the left over were also weighed daily to determine the daily feed intake. Average final weight, daily weight gain, average daily weight gain, daily feed intake, average daily feed intake, feed to gain ratio and percentage mortality were computed. The cost/kg of feed and cost of feed /kg (₦) gains were computed for each dietary treatment. Feed intake and body weight gain were taken weekly. Mathematically:

Feed intake (g) = feed given (g) – left over feed (g) on daily basis.

Body weight=

$$\frac{\text{Total weight of quails (replicate) (g)}}{\text{Number of quails (replicate)}}$$

Weight gain= weight of the current week - weight of preceded week (g).

Feed conversion ratio (FCR) was calculated on the basis of unit of feed consumed to unit of body weight gain.

$$\textbf{FCR} = \frac{\text{Feed consumed (g)}}{\text{Weight gain (g)}}$$

Mortality Rate: The percentage mortality was estimated for the first 6 weeks on weekly basis. This was estimated using the formula:

$$\textbf{Mortality rate} = \frac{\text{Number of dead quail over the week x } 100}{\text{Number of quails at the beginning of the week } 1}$$

Digestibility trial

Digestibility trial was carried out at the last week of the growing phase (5 weeks of age). Seven quail birds from each replicate were taken to a clean, separate and dis-infected metabolic cage with polythene bag attached to the beneath of the cage. They were allowed 5 days for acclimatization before the commencement of the study. A known weight of feed was fed to the birds by 8:00am daily during the digestibility trial. Faeces were collected daily for a period of seven days. The dried feacal samples were weighed and ground after which feacal samples along with the sample of the feed given were taken to the Biochemical Laboratory, Department of Animal Science, Faculty of Agriculture, Ahmadu Bello University, Zaria, to determine the proximate composition according to the methods described by AOAC (15). Nutrient retention was determined for crude protein, crude fibre, ether extract, ash, and nitrogen free extract.

$$\text{Nutrient Retention} = \frac{\text{Nutrient intake – Nutrient output}}{\text{Nutrient intake}} \times 100$$

Microbial Isolation

At the end of growing phase, bacterial cell species and numbers in the ileum and caecum were determined using three quails per replicate that is nine quails per treatment. This was carried out at the Department of Veterinary Microbiology, University of Ilorin, Ilorin. The total bacteria load and isolation of *Salmonella, Staphylococcus and Escherichia coli* from the samples were done using different selective media for isolation of bacteria groups and characterization based on sugars fermentation using Microbat 12E kit and conventional biochemical methods. The media used were Eosin Methylene Blue Agar and Salmonella Agar (16).

Chemical analysis

The proximate composition of the test ingredient (Black pepper meal) and experimental diets were determined using the procedures of the Association of Analytical Chemist (15).

Statistical Analysis

Data generated were statistically analyzed using the general linear model procedure of statistical analysis system (17). Significant different between treatments means were separated using the Duncan multiple range Test (18). The procedure for the general linear model used is shown as $\mathbf{Y_{ij} = \mu + M_i + e_{ij}}$

Where,

$\mathbf{Y_{ij}}$= the j^{th} observation in the i^{th} levels of black pepper meal or Oxytetracycline.

$\boldsymbol{\mu}$= overall mean

$\mathbf{M_i}$= fixed effect of black pepper meal levels (0,250g, 500g and 750g) or Oxytetracycline.

$\mathbf{e_{ij}}$= random error (All error terms were assumed to be random, normally distributed and independent with expectation equal to zero).

Results and Discussion

Proximate Composition of Black Pepper Meal

The results of the proximate composition of Black pepper meal is presented in Table 1. The percentage dry matter content of the seeds supports the result of moisture content. The seed of black pepper contain high fibre and ash compared to the result reported by (19). Fibre is known to increase the bulk of diet content and also enhance the frequent release of bowel content (20). This has shown positive impact in human health since it can reduce certain conditions such as constipation. However, the frequent removal of bowel content can cause indigestibility in animal such as human. Adequate caution should be taken in consumption of food materials that are very high in fibre since indigestibility will cause loss of nutrients and utilization of the undigested food materials. The percentage ash obtained showed that there will be a corresponding high mineral content. Carbohydrates and fat/lipid are known to be good sources of high energy generation compound (20, 21). The human body depends on carbohydrates and lipid/fat largely to drive its regular activities. The values obtained for lipid and carbohydrates content of black pepper in this study showed it could be a better source of carbohydrates and lipids.

Effects of black pepper meal and Oxytetracycline on growth performance of Japanese quail chicks

The results of the growth performance of Japanese quails fed graded levels of black pepper meal (BPM) and Oxytetracycline is presented in Table 2. There was significant ($p < 0.05$) improvement in final weight, weight gain, average daily weight gain (ADWG), total feed intake (TFI), average daily feed intake (ADFI), feed conversion ratio (FCR) and mortality rate across the

treatment. There was significant increase in body weight of quails fed with 750g black pepper meal (184.43g/b) when compared to control (164.90g/b) and oxytetracycline (172.25g/b) which might be due to the antioxidant property of black pepper and increased digestibility property of black pepper included in the quail diets, which was similar to the findings of (22) and (23) who observed that phytogenic feed additives like ginger, black pepper, garlic et.c increased body weight when included in the diet up to 2% level in the diet. This was contrary to the findings of (24); (25) and (26). Feed intake revealed significant ($p< 0.05$) difference in quails fed diet containing 750g BPM (418.42g/b) compared to the quails fed the control (400.00g/b) and oxytetracycline (409.91g/b). The significant ($p> 0.05$) decrease in FCR recorded was best for quails fed with 500g, 750g black pepper meal and oxytetracycline (3.44, 3.45 and 3.47) respectively and is supported by the findings of (27) and (28) who reported that phytogenics feed additives increase significantly growth performance in poultry compared to the control and oxytetracycline group. Active ingredient (*Piperine*) found in black pepper causing an increase in the absorption of nutrients, enhance digestion, and greater efficiency in the utilization of feed, resulting in enhanced growth (29). (30) who found that herbal plant affects weight gain and showed improvement in the health of birds, in addition to other performance traits, feed conversion ratio and feed intake. Similar results were recorded by (31) and (32), who reported that phytogenic feed additives had a positive effect on growth performance of broiler and this might be due to the effects of the flavor of the phytogenics on the chicks' appetite and improved secretions of proteolytic enzymes, lipase enzymes and amylase (33) and enhance antioxidants and control hyperlipidemia (34). There was no significant ($p>0.05$) difference between quails fed with graded levels of BPM and oxytetracycline (1.01%) respectively on percentage mortality and are numerically similar compared to the control group. (35), however reported significant reduction in mortality rate in broilers fed with phytogenic feed additives.

Apparent nutrient digestibility of quail chicks containing graded levels of black pepper meal and oxytetracycline

The effect of feeding level of BPM and oxytetracycline on the apparent nutrient digestibility of Japanese quails is presented in Table 4. All the parameters measured were significantly ($p < 0.05$) affected by dietary treatments except for crude fibre. The percentage crude protein (CP), ether extracts (EE), nitrogen free extracts (NFE) and ash retention increased with increase in BPM inclusion, although crude fibre was not significantly different. Dry matter reduces as the inclusion level of black pepper increases while crude protein, ether extracts, nitrogen free extracts and ash digestibility were higher in quails fed 750g BPM ($p<0.05$) with values 85.64%,63.44%, 71.76% and 76.84% respectively. The result that were significantly higher in digestibility of dry matter, crude protein, ash observed in birds fed 750g BPM are confirmed with the report of (36) who indicates that the plant extracts at certain levels improved digestibility. (37) and (38) also reported that spices and essential oils could be used to aid digestion in monogastric animals. (39) attributed better nutrient digestion to antimicrobial property of the essential oil in black pepper. (40) linked it to the ability of black pepper to induce saliva secretion, hydrochloric acid and mucus production. (33) attributed that the pungent properties black pepper stimulated digestive enzymes activities. The inability of the black pepper to improve

digestibility of fibre could have resulted to the insignificant reduction of fecal moisture at levels higher than 0.25%. There was significant ($p < 0.05$) difference in the nitrogen free extract across the dietary treatment. Quails fed 750g BPM had the highest value of NFE (71.76%) compared to the control and oxytetracycline group. This result contradicts the report of (41) who reported poor nitrogen free extract digestibility and energy utilization exhibited at 1.0% spice. This present study disagreed with (42) who reported that high doses of active principles in spices could lead to poor digestibility at higher level.

Effects of black pepper meal and oxytetracycline on microbial counts of Japanese quail chicks

The caecal microbiota of growing Japanese quail is presented in Table 5. Quails fed diets supplemented with 750g BPM exhibited lower population of *Clostridium, E. coli, Salmonella* and *Pseudomonas spp* colonization (2.97, 1.01, 0.00 and 0.00 $x10^3$ CFU/g**)**) respectively than those in the control and Oxytetracycline groups while a significant increase was observed for quails fed with 750g BPM for *Bacillus* and *Lactobacillus spp* concentration (8.82 and 14.23 $x10^3$ CFU/g) respectively. The treatments affected the microbial composition of the intestine, where levels of different additives showed an improvement in the quantity and type of the tested bacterial species, for example, increased *Bacillus* and *Lactobacillus* count and decreased *Clostridium, E. coli, Salmonella* and *Pseudomonas spp* count. In this study, the present results are consistent with those reported by (43) who used 250 mg/kg diet of phytogenic feed additive from anise and thyme and found that there was an decrease in the intestinal microbiota (*Clostridium perfringens, Lactobacillus, Escherichia coli*). These results were inconsistent with those of (3) who indicated that *Lactobacillus* count in the small intestine was not affected ($P < 0.05$) by thyme supplemented at 15 or 20 g/kg diet of broilers. This result is in accordance with (44) who reported an inclusion of thyme essential oil in the diet of Japanese quail significantly ($P < 0.05$) increased *Lactobacillus* and decreased *E. coli* in the ileum, this can result can be due to active compounds of thyme that have antibacterial properties. Therefore, it can cause a distortion of the membrane's physical structure (45; 46) and can eliminate pathogenic bacteria in the intestine (47).

Table 1: **Proximate composition of Black Pepper Meal**

Proximate composition	Content (%)
Moisture content	10.03
Dry matter	89.97
Crude protein	2.20
Crude fibre	13.00
Ether extracts	4.20
Ash retention	5.40
Nitrogen free extract	64.29

Table 2: Composition of experimental diets for Japanese quail chicks containing Black Pepper Meal and Oxytetracycline (2 - 6 weeks)

	Levels of Black pepper meal (g)				
Ingredients	**0**	**250**	**500**	**750**	**Oxy**
Maize	51.70	51.45	51.20	50.95	51.70
Soybean cake	30.00	30.00	30.00	30.00	30.00
Ground Nut Cake	15.00	15.00	15.00	15.00	15.00
Bone meal	0.70	0.70	0.70	0.70	0.70
Limestone	2.00	2.00	2.00	2.00	2.00
Black Pepper Meal	0.00	0.25	0.50	0.75	0.00
Common salt	0.15	0.15	0.15	0.15	0.15
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10
Vitamin Premix[A]	0.25	0.25	0.25	0.25	0.25
Total (%)	100	100	100	100	100
Calculated Analysis					
ME Kcal\kg	2844	2838	2833	2828	2844
Crude Protein (%)	25.00	25.00	25.00	25.00	25.00
Crude fibre (%)	4.52	4.56	4.59	4.62	4.52
Ether extracts (%)	4.87	4.88	4.88	4.88	4.87
Calcium (%)	1.00	1.00	1.00	1.00	1.00
Phosphorus (%)	0.23	0.23	0.23	0.23	0.23
Meth + cys (%)	0.75	0.75	0.75	0.75	0.75
Lysine (%)	1.27	1.27	1.27	1.27	1.27
Feed Cost #/Kg	130.03	136.40	139.70	142.90	130.03

[A]Vitamin-mineral premix provides per kg of diet; Vit. A, 10,000,000 IU; Vit. D3, 2,000,000 IU; Vit. E 40,000mg; Vit. K3, 2000mg, Vit B1, 1500mg; Vit. B2, 5,000mg, Vit.B6, 4,000mg; Vit. B12, 20mg; Niacin, 40,000mg; Folic acid, 1,000mg; Biotin, 100mg; Choline Chloride, 30,000mg; Cobalt, 300mg; Copper, 80,000mg; Iodine, 800 mg; Iron, 40,000mg; Manganese, 80,000 mg; Selenium, 200 mg; Zinc, 60,000mg; ME = Metabolizable Energy, Meth + cys, =Methionine + cysteine, Oxy= Oxytetracycline.

Table 3: Effects of Black Pepper and Oxytetracycline on Growth performance of Japanese quails

	Levels of Black Pepper (g)					
PARAMETERS	**0**	**250**	**500**	**750**	**Oxy**	**SEM**
Initial weight(g/bird)	52.03	52.06	52.06	52.03	52.06	0.03
Final weight (g/bird)	164.90^{e}	170.75^{d}	174.43^{b}	184.43^{a}	172.25^{c}	1.20
Weight gain(g/bird)	100.71^{e}	101.73^{d}	119.43^{b}	121.23^{a}	118.20^{c}	1.20
ADWG (g/bird/day)	7.19^{e}	7.27^{d}	8.53^{b}	8.66^{a}	8.44^{c}	0.02
Feed Intake (g/bird)	400.00^{e}	401.21^{d}	411.09^{b}	418.42^{a}	409.91^{c}	0.51
ADFI(g/bird/day)	28.57	28.66	29.36	29.89	29.28	3.22
Feed Conversion Ratio	3.97^{a}	3.94^{b}	3.44^{c}	3.45^{c}	3.47^{c}	0.04
Mortality (%)	2.02^{a}	1.01^{b}	1.01^{b}	1.01^{b}	1.01^{b}	1.03

[abcde]: Means with different superscripts on the same row are significantly different (P<0.05), SEM = Standard Error of Mean, Oxy= Oxytetracycline.

Table 4: Effects of Black pepper and Oxytetracycline on Nutrient digestibility of Japanese quail chicks

	Levels of Black Pepper (g)					
PARAMETERS	**0**	**250**	**500**	**750**	**Oxy**	**SEM**
Dry matter	75.67[a]	73.13[b]	64.73[c]	55.13[e]	57.06[d]	0.40
Crude protein	76.13[c]	75.77[d]	80.62[b]	85.64[a]	80.67[b]	0.03
Crude fibre	67.61	67.88	67.76	68.25	67.82	0.12
Ether extract	43.31[d]	62.80[b]	60.59[c]	63.44[a]	60.71[c]	1.53
NFE	64.00[b]	65.63[b]	70.19[a]	71.76[a]	71.68[a]	0.39
Ash retention	64.71[d]	71.10[c]	76.18[b]	76.84[a]	76.02[b]	0.14

[abcd]: Means with different superscripts on the same row are significantly different (P<0.05), SEM = Standard Error of Mean, Oxy= Oxytetracycline. NFE= Nitrogen free extract.

Table 5: Ceacal microbial count of Japanese quail chicks fed graded levels of Black pepper and Oxytetracycline

	Levels of black pepper (g)					
Bacteria (x10^3 CFU/g)	**0**	**250**	**500**	**750**	**Oxy**	**SEM**
Bacilli spp	4.31[d]	5.41[c]	6.10[b]	8.82[a]	7.12[a]	1.16
Lactobacilli spp	4.19[d]	10.64[c]	12.37[b]	14.23[a]	12.22[b]	0.11
Clostridium spp	4.05[a]	3.44[b]	3.11[c]	2.97[d]	3.03	0.42
Escherichia spp	5.08[a]	3.00[b]	1.01[c]	1.01[c]	1.10[c]	0.53
Salmonella spp	3.12[a]	1.07[b]	1.01[c]	0.00[d]	1.01[c]	0.17
Pseudomonas spp	2.08[a]	0.00[b]	0.00[b]	0.00[b]	0.00[b]	0.14

abcd: Means with different superscripts on the same row are significantly different (P<0.05), SEM = Standard Error of Mean, Oxy= Oxytetracycline.

Conclusion and Applications

1. The dietary supplementation of black pepper meal at (750 g/kg) can enhance the growth performance, increase crude protein, ash and nitrogen free extracts and also, decrease gram negative intestinal pathogens compared to that of the control and oxytetracycline.
2. Black pepper has some compounds that enhance digestion and absorption of some nutrients in the diets, thus improving the health status of growing Japanese quails.

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