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ORIGINAL RESEARCH ARTICLE

Effect of supplemental copper on growth, haematology and reproductive performance of japanese quails

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ABSTRACT

The study was carried out to evaluate the effect of supplemental copper (0, 200, 300, 400 mg/100 kg diet) on growth, haematology, laying performance and egg quality of Japanese quails. A total of 360 birds randomly allocated to four dietary treatments with 30 birds per replicate in a completely randomized design were used for the growth trial for a period of four weeks. The laying phase comprised of 132 six-week old Japanese quails randomly allocated to four treatments with three replicates of 11 birds each in a completely randomized design for four weeks. The result of the growth phase showed no significant (P>0.05) difference in feed consumption, final weight, weight gain and feed conversion ratio, weight of first egg, age at peak lay, number of eggs and mortality. Significant (P<0.05) difference was observed in age at first egg, age at 10% lay and age at 50% lay There was also no significant (P>0.05) difference in packed cell volume (PCV), haemoglobin (Hb), red blood cells (RBC), white blood cell (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). The result of the laying phase showed no significant (P>0.05) difference in final weight, weight gain, daily feed intake, feed conversion ratio, egg number, average egg weight, egg mass, hen-housed production and hen-day production. However, yolk diameter significantly (P<0.05) increased with the inclusion of dietary copper. Supplemental copper (0, 200, 300, 400 mg/100 kg diet) did not improve growth, haematological indices, laying performance and egg quality of Japanese quails.

Key words: quails, copper, growth, haematology, egg

INTRODUCTION

The shortage of animal protein intake among the ever increasing human population of the third world countries has long been recognized and remains one of the greatest issues of concern today (Omoikhoje *et al*., 2008). The need has arisen to develop the poultry industry since it is the fastest means of providing the needed animal protein for the growing population. One way of increasing protein supply is to diversify poultry production as well as increase the production of other micro livestock species with short generation interval. The optimum performance of livestock depends largely on the quality and quantity of their dietary nutrients. Quality feed should be fed to livestock to meet the requirement of the animal for effective growth. Nutrients required for optimum growth include minerals, even though they are required in minute quantity. Trace mineral premixes should be formulated and supplemented to poultry feeds separate from the vitamin premix due to potential vitamin oxidation by the trace minerals (Scheideler, 2008).

Copper can be routinely added as supplement in swine and poultry diets at concentrations above requirement of the animal because phamarmacological concentrations of copper act as a growth stimulant in these species, thereby improving growth performance, including increase in body weight gain and feed intake (Lien *et al*., 2004). Copper between 125 and 250 mg/kg feeding stuff is also effective in promoting growth of broiler chicken (Skrivan *et al*., 2000). Copper (at 125-250 mg/kg feeding stuff) have been shown to enhance the laying performance of hens and reduce egg cholesterol (Pesti and Bakalli, 1998). Copper is required for normal red blood cell formation by allowing iron absorption from small intestine and release of iron in tissue into the blood plasma. However, Njike *et al*. (1987); Oyewola and Longe (1999) and Babangida and Ubosi, (2006) have reported that nutrient requirements established under temperate conditions may not be entirely satisfactory in the tropical environment characterized by high ambient temperature and low quality feedstuffs. Little work has been carried out on the effect of supplemental copper in quails found in the tropics. This study was carried out to determine the effect of supplemental copper in growing Japanese quail birds in the tropics. This study was carried out to evaluate the effect of supplemental copper on growth, haematology, laying performance and egg quality characteristics of Japanese quails.

Table 1: Composition of experimental diets (2-6weeks)

	Levels of supplemental copper (mg/kg)			
Ingredient (%)	0	200	300	400
Maize	53.50	53.50	53.50	53.50
Groundnut cake	21.34	21.34	21.34	21.34
Soya bean cake	22.00	22.00	22.00	22.00
Bone meal	0.98	0.98	0.98	0.98
Limestone	1.00	1.0	1.0	1.0
Common salt	0.35	0.35	0.35	0.35
Methionine	0.18	0.18	0.18	0.18
Lysine	0.30	0.30	0.30	0.30
Vitamin premix	0.35	0.35	0.35	0.35
Copper (mg/kg)	0.00	200	300	400
Total	**100**	**100**	**100**	**100**
Calculated nutrients				
ME Kcals/kg DM	2948	2948	2948	2948
Crude protein (%)	24.10	24.10	24.10	24.10
Crude fibre (%)	4.86	4.86	4.86	4.86
Ether extract (%)	3.69	3.69	3.69	3.69
Calcium (%)	0.83	0.83	0.83	0.83
Phosphorus (%)	0.58	0.58	0.58	0.58
Lysine (%)	1.40	1.40	1.40	1.40
Methionine (%)	0.52	0.52	0.52	0.52
Copper (mg/kg)	132	332	432	532

Biomix vitamin premix: Vitamin A 8000000 iu, Vitamin D_3 1500000 iu, Vitamin E 7000mg, Vitamin K_3 1500 mg, Vitamin B_1 2000 mg, Vitamin B_2 2500 mg, Niacin 15000 mg, Pantothenic acid 5500 mg, Vitamin B_6 2000 mg, Vitamin B_{12} 10 mg, Folic acid 500 mg, Biotin 250 mg, Choline chloride 175000 mg, Cobalt 200 mg, Copper 3000 mg, Iodine 1000 mg, Iron 21000 mg, Manganese 4000 mg, Selenium 200 mg, Zinc 31000 mg, Antioxidant 1250 mg.

MATERIALS AND METHODS

Experimental Site

The experiment was carried out at the Poultry Unit of the Department of Animal Science Research and Teaching Farm, Ahmadu Bello University Zaria. It is located within the Northern Guinea Savannah zone of Nigeria: latitude 11° 12'N and longitude 7° 33'E at an altitude of 610 meters above sea level (Ovimaps, 2012). The climate is characterized by a well defined dry and wet seasons. The climate is relatively dry with a mean annual rainfall of 700-1400mm.

Experimental Design

The design of the experiment was completely randomized design (CRD). Three hundred and sixty two old birds were randomly allocated into four treatments of 30 birds per replicate. The experiment lasted for four weeks. The broilers were weighed at the beginning of the experiment and weekly thereafter. Four experimental diets were formulated with copper at 0, 200, 300 and 400 mg/kg diet respectively (Table 2). Feed and water was provided at 7.00 am daily. Values for feed consumption, weight gain, feed to gain ratio and mortality were calculated.

A total of 132 six-week old laying Japanese quails (*Coturnix coturnix japonica*) were used for the laying phase. They were randomly allotted to four treatment groups of 33 birds each with three replicates of 11 birds. Four experimental diets formulated were supplemented with Copper (II) chloride dihydrate at 0, 200, 300 and 400 mg/100 kg diet (Table 2). All routine management practices and vaccinations were duly followed. Eggs were picked in the morning and evening and weighed with a digital scale. At the laying phase parameters monitored included initial body weight, feed intake, hen day production, hen-house production, body weight gain, final body weight, egg number, average egg weight, egg mass, total feed consumed, daily feed consumed and feed conversion ratio (FCR). Egg quality traits were assessed using three eggs per replicate. The eggs were sampled at random once a week. Each egg was assessed separately for internal and external egg quality traits. For external quality traits, data on egg weight (g), egg height (mm), egg diameter (mm), shell weight (g) and shell thickness (mm) were collected. Individual egg weight was measured using a sensitive electronic balance (Mettler PM 400 model) while egg length and breadth were measured using a Vernier calliper. Shell thickness was measured for individual dry egg shells to the nearest 0.01mm using a micrometer screw gauge. The interior egg quality traits were measured in terms of Haugh unit. The Haugh unit was calculated from egg weight and albumen height (Haugh, 1937).

Haugh unit $=100 Log10\ (H + 7.57 - 1.7\ W^{0.37})$

Where H = Albumen height (mm)

Yolk quality was evaluated through the yolk index: $YI = h/D$, where h is the yolk height and D – the yolk diameter (Romanoff, 1956).

W = Weight of the egg (g), 7.57-1.7 are constants.

Chemical analysis

The compounded diets were analyzed for their proximate compositions (AOAC, 2000) in the Biochemical laboratory of the Department of Animal Science, Ahmadu Bello University Zaria.

An egg separator was used to separate the yolk from the albumen. The height of the albumen was measured at its widest expanse and midway between the yolk edge and the external edge of the thick albumen using a tripod micrometer (Adeyemo and Longe, 2008).Yolk height was measured at the highest point using a tripod micrometer. The yolk and albumen were placed in separate Petri dishes, which had initially been weighed. The differences in the weight of each Petri dish after and before the introduction of the yolk and albumen were taken as the weights of the yolk and albumen respectively. The weights were taken with the aid of Mettler analytical balance (Mettler PM 400 model).

Evaluation of Haematological Parameters

Blood samples were collected from three birds in each replicate when the birds were six weeks old. Blood samples were transferred to test tubes containing EDTA. Haematological parameters measured were red blood cell count (RBC), haemoglobin (Hb) content, white blood cell count (WBC), packed cell volume (PCV) while mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin volume (MCHC) were determined using the following formulas; MCH=(Hb*10)/RBC , MCV=(WBC÷RBC)×10, and MCHC= (Hb÷ PCV). Uncoagulated blood samples were used for determination of haemoglobin (Hb) concentration and packed cell volume (PCV) using cyanmethaemoglobin and Wintrobe microhaematocrit methods respectively (Coles, 1986). The total red blood cell count (RBC) was determined using improved Neuberhaemocytometer. Total WBC count was carried out using haemocytometer, while differential WBC count was determined by preparing blood smear stained with Wrights stain.

Statistical Analysis.

Data collected was subjected to ANOVA using the SAS (2002) general linear model procedure and treatment means were compared for significance using Duncan multiple range test.

Table 2: Composition of experimental diets (6-10 weeks)

	Levels of supplemental copper			
Ingredients (kg)	0	200	300	400
Maize	60.00	60.00	60.00	60.00
Groundnut cake	11.25	11.25	11.25	11.25
Soya bean cake	21.00	21.00	21.00	21.00
Bone meal	4.20	4.20	4.20	4.20
Limestone	3.10	3.10	3.10	3.10
Common salt	0.15	0.15	0.15	0.15
Methionine	0.15	0.15	0.15	0.15
Lysine	0.10	0.10	0.10	0.10
Vitamin premix	0.05	0.05	0.05	0.05
Copper (mg/kg)	0.00	200	300	300
Total	100	100	100	100
Calculated nutrients				
ME Kcals/kg DM	2889.83	2889.83	2889.83	2889.83
Crude protein (%)	19.70	19.05	19.05	19.05
Crude Fibre (%)	3.14	3.14	3.14	3.14
Ether extract (%)	4.17	4.17	4.17	4.17
Calcium (%)	2.77	2.77	2.77	2.77
Phosphorus (%)	0.95	0.95	0.95	0.95
Lysine (%)	1.01	1.01	1.01	1.01
Methionine (%)	0.45	0.45	0.45	0.45
Copper (mg/kg)	1200	1400	1500	1600

Biomix vitamin premix: Vitamin A 8000000 iu, Vitamin D_3 1500000 iu, Vitamin E 7000mg, Vitamin K_3 1500 mg, Vitamin B_1 2000 mg, Vitamin B_2 2500 mg, Niacin 15000 mg, Pantothenic acid 5500 mg, Vitamin B_6 2000 mg, Vitamin B_{12} 10 mg, Folic acid 500 mg, Biotin 250 mg, Choline chloride 175000 mg, Cobalt 200 mg, Copper 3000 mg, Iodine 1000 mg, Iron 21000 mg, Manganese 4000 mg, Selenium 200 mg, Zinc 31000 mg, Antioxidant 1250 mg.

Table 3: Growth and early laying performance of quails fed graded levels of supplemented copper.

	Levels of supplemental copper (mg/kg)					
Parameters	0	200	300	400	SEM	P value
Initial weight(g)	75.55	76.63	76.09	75.53	0.703	0.669
Feed consumed(g/bird)	405.17	388.33	390.0	383.10	10.12	0.072
Final weight(g)	133.08	131.33	130.84	132.48	3.67	0.969
Weight gain(g/bird)	59.09	54.70	54.75	56.95	3.60	0.928
Feed/gain ratio	7.0	8.0	7.3	7.0	0.63	0.768
Weight at 1st egg	7.2	7.1	7.06	6.73	0.59	0.665
Age at 1st egg (Days)	41.33^{a}	44.0^{b}	43.33^{b}	43.33^{b}	1.39	0.027
Age at 10% lay (Days)	42.66^{a}	45.0^{b}	44.33^{b}	45.66^{b}	1.23	0.021
Age at 50% lay (Days)	46.33^{a}	48.33^{b}	48.33^{b}	48.0^{b}	0.94	0.004
Age at peak lay (Days)	57.66	56.33	54.66	55.33	4.88	0.939
Number of eggs	10.66	9.33	10.0	11.66	1.09	0.561
Mortality (%)	3.3	3.3	3.3	2.2	1.73	0.802

^{ab} means within rows with different superscript are significantly different ($p<0.05$), SEM=Standard error of means.

RESULTS AND DISCUSSION

Effect of Supplemental Copper on Growth and Early Laying Performance of Quails.

Table 3 shows growth and early laying performance of quails fed diets supplemented with copper at 0, 200, 300 and 400 mg/100 kg diet. No significant (p>0.05) difference was observed in final weight, feed consumption, weight gain, and feed to gain ratio. This agrees with the report of Abaza *et al.* (2009) who observed no significant difference in body weight and weight gain when copper was fed at 100 and 200 mg/kg to quail birds. Zahedi *et al*. (2014) also observed no significant difference in body weight, weight gain, and feed conversion ratio when copper was fed at 50, 100, 150 and 200 mg/kg to quail birds. Pang and Applegate (2007) also observed that copper supplementation at 250 mg/kg diet had no effect on the body weight gain, feed intake and feed to gain ratio of broiler birds. Banks *et al.* (2004) also observed that supplementation of 250 mg copper/kg had no significant effect on broiler body weight gain and feed conversion efficiency. However, Jenkins *et al.* (1970) reported an increase in growth and feed intake of birds fed 250 mg copper/kg. Pesti and Bakalli, (1996) also reported an improved growth and feed conversion efficiency of broilers at inclusion level of 125 or 250 mg Cu/kg for poultry. Adu and Egbunike (2010) also observed an increase in final live weight, daily feed intake, daily weight gain and feed conversion ratio in rabbits fed diets supplemented with dietary copper levels until 200ppm. The lack of growth-enhancing effect of supplemental Cu in the present study may be related to the environmental temperature. High temperature has a negative effect on poultry such as decrease in body weight, feed consumption and feed efficiency.

There was no significant (p>0.05) difference in weight at first egg, age at peak lay, number of eggs and mortality. This disagrees with the report of Abaza *et al.* (2009) that dietary copper supplementation at 100 and 200 mg/100 kg diet increased number of eggs laid by quails. Pesti and Bakalli (1998) also observed an increase in egg production at 250 mg/kg supplemental copper fed to poultry. Delfani *et al.* (2013) reported an increase in egg production and egg weight in quail birds when copper was supplemented at 250 mg/kg. A significant (p<0.05) difference was observed for age at first egg, age at 10% lay and age at 50% lay. The birds in the control group started laying earlier than birds fed supplemental copper. The response of birds to higher levels of Cu supplementation is variable and in some studies, growth- suppressive effects of using higher levels of have even been reported (Karimi *et al.,* 2011).

Table 4 shows the effect of supplemental copper (0, 200, 300, 400 mg/ 100 kg diet) on the haematological indices of quails. There was no significant (P>0.05) difference across the treatments for PCV. The values obtained (28.22 to 32.45%) fell below the normal range of 37 to 69% for poultry birds (Campbell, 1988). Ayub *et al.* (2012) also reported a PCV range of 31.45 to 35.57 % for Japanese quails. There was no significant (P>0.05) difference across the treatments for haemoglobin. The values obtained (9.22 to 10.79g/dl) fell below the normal range of 12 to 15.52g/dl (Campbell, 1988). There was no significant (p>0.05) difference across the treatment for RBC. The values obtained (4.89 to 5.36×10^6/µl) fell within the normal range of 3.8 to 5.5×10^6/µl (Campbell, 1988). There was no significant (p>0.05) difference for WBC. The values obtained (4.19 to 5.41×10^3/µl) fell within the normal range of 4.1 to 10.9×10^3/µl (Campbell, 1988) indicating that the birds were healthy. No significant (p>0.05) difference was observed for MCV across the treatments. The values obtained (60.17 to 60.58fl) fell below the normal range of 78 to 101fl (Campbell, 1988). There was also no significant (p<0.05) difference for MCH and MCHC across the treatment.

Table 4: Effect of supplemented copper on the haematological indices of Quails.

	Levels of supplemental copper (mg/kg)					
Parameters	0	200	300	400	SEM	P value
PCV (%)	28.22	29.44	27.78	32.45	1.95	0.422
Hb (g/dl)	9.36	9.78	9.22	10.79	0.66	0.413
RBC ($\times 10^6$/µl)	4.69	4.89	4.59	5.36	0.32	0.452
WBC ($\times 10^3$/µl)	4.72	4.78	5.41	4.19	0.52	0.243
MCV (fl)	60.24	60.17	60.43	60.58	0.11	0.283
MCH (g/dl)	19.87	20.00	19.75	19.83	0.22	0.874
MCHC (%)	32.99	33.22	32.68	32.62	0.39	0.646

PCV=packed cell volume, Hb=haemoglobin, TP=total protein, RBC=red blood cell, WBC=white blood cell, MCV=mean corpuscular volume, MCH=mean corpuscular haemoglobin, MCHC=mean corpuscular haemoglobin count

Table 5 shows there was no significant (P>0.05) difference in egg production, hen-day production, egg weight, feed consumed (g/hen/day), feed conversion ratio (FCR), and egg mass. The inclusion of copper chloride (0, 200, 300, 400 mg/ 100 kg diet) had no effect on laying performance of Japanese quail. This is in accordance with the work of Banks *et al.* (2004) that egg production and egg weight were not significantly affected by diets containing Cu up to 250 ppm from copper chloride. EL-Huseiny *et al.* (2009) also reported that different dietary copper levels (0, 300, 400, 500 mg/kg) had no influence on egg production, egg weight, feed intake, body weight gain, egg-day production and feed conversion ratio in laying chickens.

Table 6 shows that copper had no significant (P>0.05) effect on most of the egg quality traits measured but had a significant (P<0.05) effect on yolk diameter. Yolk diameter significantly (P<0.05) increased with the inclusion of copper. This does not agree with the report of EL-Huseiny *et al.* (2009) that copper had no effect on yolk diameter. The decrease in yolk is important to consumers because of total cholesterol content in the yolk. A decrease in the relative amount of yolk would decrease the total cholesterol content of the egg (Schideler *et al.*, 1996)

CONCLUSION

Supplemental copper (0, 200, 300, 400 mg/100kg diet) did not improve growth, haematology, laying performance and egg quality of Japanese quails.

Table 5: Laying performance of female Japanese quail fed varying levels of dietary copper (6-10 weeks)

	Levels of supplemental copper (mg)					
Parameters	0	200	300	400	SEM	P value
Initial Wt (g/bird)	145.5^{a}	143.9^{a}	144.0^{a}	145.5^{a}	6.20	0.997
Final Wt (g/bird)	163.4^{a}	164.9^{a}	158.8^{a}	159.4^{a}	2.710	0.474
Wt change (g/bird)	17.90^{a}	21.0^{a}	14.8^{a}	13.9^{a}	4.26	0.74
Egg number (production)	177.3^{a}	150.0^{a}	157.7^{a}	151.7^{a}	15.0	0.68
Ave. egg wt (g)	7.65^{a}	7.10^{a}	7.21^{a}	7.05^{a}	0.41	0.790
Egg mass (g/bird)	1365^{a}	1093^{a}	1137^{a}	1087^{a}	159.9	0.689
Total feed con. (g/bird)	824.3^{a}	822.0^{a}	816.7^{a}	780.3^{a}	22.77	0.625
Daily feed con. (g/bird/day)	29.4^{a}	28.91^{a}	29.17^{a}	27.9^{a}	0.744	0.611
FCR	621.6^{a}	851.3^{a}	718.1^{a}	781.3^{a}	0.123	0.712
g feed/dozen eggs	56.70^{a}	69.53^{a}	2.14^{a}	63.49^{a}	6.481	0.695
Hen House production (%)	57.6^{a}	48.7^{a}	51.0^{a}	49.24^{a}	4.882	0.680
Hen day production (%)	57.57^{a}	50.40^{a}	51.2^{a}	49.24^{a}	5.045	0.750
Mortality (%)	0.00^{a}	3.03^{a}	0.00^{a}	0.00^{a}	1.31	0.441

^{a,b,c,d} : means with different superscript on the row are significantly different (p< 0.05)

Table 6: Egg quality characteristics of Japanese quail fed varied level of dietary copper (6-10 weeks)

	Levels of supplemental copper (mg)					
Parameters	0	200	300	400	SEM	P value
External Quality						
Egg Wt (g)	9.01	9.05	8.67	8.99	0.1873	0.137
Egg Height (mm)	28.83	29.30	28.58	28.75	0.5120	0.488
Egg Diameter (mm)	25.04	25.07	24.86	25.27	0.2292	0.229
Shell Weight (g)	1.07	1.08	1.01	1.08	0.0328	0.119
Shell Thickness (mm)	1.34	1.29	1.31	1.39	0.0453	0.101
Internal Quality						
Albumen Height (mm)	2.28	2.25	2.18	2.27	0.0969	0.735
Albumen Weight (g)	5.16	4.79	4.86	4.93	0.1676	0.142
Yolk Height (mm)	2.34	2.39	2.22	2.37	0.0949	0.228
Yolk Diameter (mm)	1.98^{b}	2.03^{a}	2.05^{a}	2.04^{a}	0.0160	0.001
Yolk Weight (g)	2.46	2.39	2.25	2.21	0.1177	0.103
Yolk Index	0.48	0.49	0.49	0.49	0.0069	0.850
Haugh Unit	86.88	86.69	85.69	86.81	0.7828	0.349

$^{a\,b}$Means with different superscript on the same row are significantly different ($p<0.05$)

RECOMMENDATION

From the findings of this study, supplemental copper (200-400 mg/100 kg diet) is not required for improved growth, haematological indices and early laying performance of quail birds.

CONFLICT OF INTEREST

I certify that this is an original research work conducted by the authors.

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