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The Effects of Super-Dosing Copper Supplementation on Productive and Reproductive Performance of Breeder Quail Fed a Diet with Reduced Non-Phytate Phosphorus Level

Research Article

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ABSTRACT

A total of 132 ten-week-old breeder quails were used in a 2 × 2 factorial experiment arranged in a completely randomized design, consisting of 4 treatments and 6 replicates. Each replicate comprised either 4 females and 2 males (4 replicates) or 3 females and 2 males (2 replicates). The study was conducted over a 28-week period to evaluate the effects of dietary copper supplementation (0 or 250 mg/kg) and non-phytate phosphorus (NPP) levels (0.13% or 0.35%) on performance, reproductive performance, egg quality traits, and selected blood metabolites. The results of this study showed that adding 250 mg/kg of copper from copper sulfate improved body weight gain, egg production (P<0.01), egg mass (P<0.01), egg weight, feed conversion ratio, albumen and yolk ratios (P<0.05). The hatchability percentage of total set eggs and fertile eggs increased (P<0.01) with the dietary supplementation of 250 mg/kg copper. Additionally, excess copper reduced (P<0.05) yolk cholesterol (P<0.01), serum cholesterol, and low-density lipoprotein concentrations, while increasing serum high-density lipoprotein (P<0.05) and copper (P<0.01) concentrations. In conclusion, supplementing the diet of breeder quails with 250 mg/kg of copper enhanced productive and reproductive performance without any interaction with the NPP level in the diet. Additionally, reducing the NPP to 0.13% did not have any negative impact on the production rate of breeder quails.

KEY WORDS breeder quails, egg quality traits, non-phytate phosphorus, productive performance, reproductive performance, super-dosing copper.

INTRODUCTION

Copper is an essential trace mineral that serves as a cofactor in numerous enzymatic systems in the body, including cytochrome oxidase, lysyl oxidase, ceruloplasmin, superoxide dismutase, tyrosinase, hydroxyphenylpyruvate hydroxylase, and dopamine beta-hydroxylase (Ali, 2018). Although the precise copper requirements for quail species are not fully established, the National Research Council (NRC, 1994) recommends a dietary level of 5 mg/kg for both breeder and laying Japanese quails. In many countries, elevated copper levels have been utilized as a growth promoter in broiler diets in recent years (Nguyen *et al.* 2020; Sharif *et al.* 2021). However, the primary use of excess copper in the diets of laying hens has been as a strategy to reduce egg cholesterol levels (Puertas and Vázquez, 2019; Elnesr *et al.* 2024). Supplementing diets with excess copper has been shown to improve nutrient absorption in the digestive system and alter the microbial population, thereby enhancing productive performance (El Kazaz and Hafez, 2020; Yausheva, 2021; AL-Ruwad *et al.* 2024; Gül *et al.* 2024). Commercial organic sources of copper, such as copper proteinate and copper lysinate, as well as inorganic copper salts like copper sulfate, copper chloride, and copper oxide, are commonly used for supplementation, each with varying bioavailability (Akbari Moghaddam Kakhki *et al.* 2024). Copper sulfate pentahydrate is more widely used than other copper sources due to its easier availability and lower cost.

Phosphorus is a vital mineral required for growth and the development of the skeletal system, playing a crucial role in various metabolic pathways, including acid-base balance (Goldenberg and Fernandez, 1966; Shi et *al.* 2024). The majority of phosphorus in plant-based feed ingredients is present as phytate, which has low bioavailability; only about one-third of the total phosphorus in plant sources is accessible to poultry (Shi *et al.* 2024). The National Research Council (NRC, 1994) recommends a non-phytate phosphorus (NPP) level of 0.35% for both laying and breeder Japanese quails. Given phosphorus's importance in maintaining optimal production and eggshell quality, as well as the adverse environmental effects of excess phosphorus excretion through manure, it is crucial to manage accessible phosphorus levels in the diet to ensure optimal production performance.

The concentration of specific minerals in the diet can affect the hydrolysis of phytate in feed by forming complexes with phytate molecules, which in turn reduces the bioavailability of phosphorus (Pang and Applegate, 2006). Copper significantly reduced the efficacy of phytase as the pH approached neutrality, with greater inhibition observed at higher copper concentrations (Pang and Applegate, 2006). Copper sulfate readily dissociates in the acidic environment of the proventriculus, enabling copper to interact with phytate and form copper-phytate complexes (Ren *et al.* 2021). Supplementing up to 250 mg/kg of copper from copper sulfate source in broiler diets did not affect phytase efficacy but reduced apparent phosphorus retention by 11-15 percentage points (Banks *et al.* 2004a). Supplementation with 250 mg Cu/kg of copper from copper citrate similarly reduced apparent phosphorus retention (Banks *et al.* 2004b).

Considering the economic and environmental importance of reducing dietary phosphorus levels while meeting the birds' phosphorus requirements, failing to consider factors that reduce phosphorus bioavailability in the diet can make it more difficult to accurately determine the birds' phosphorus needs, which could result in reduced performance. The potential interactions between excess copper levels and the bioavailability of non-phytate phosphorus in the diet motivated this study, which aimed to investigate the effects of excess copper supplementation and reduced NPP levels on the production, reproductive performance, and egg quality traits of breeder Japanese quails.

MATERIALS AND METHODS

Birds, diets and management

A total of 132 quails (10 weeks old) were used in a 2×2 factorial experiment within a completely randomized design with 4 treatments and 6 replicates (4 replicates consisting of 4 females and 2 males; 2 replicates consisting of 3 females and 2 males) to evaluate the effects of different levels of copper (0 and 250 mg/kg) and NPP (0.13% and 0.35%) on performance, hatchability, egg quality traits, and some blood metabolites of breeder quails. The experimental treatments were :1) control group (required copper and NPP levels), 2) 250 mg/kg copper supplementation with the required level of NPP (0.35%), 3) required copper level with reduced NPP (0.13%), and 4) 250 mg/kg copper supplementation with reduced NPP (0.13%). According to NRC (1994), Japanese quails require 5 mg/kg copper at early, growing, and laying stages, which is typically met through the basal diet (Table 1). Excess copper levels of 100-300 mg/kg, known as pharmacological and therapeutic levels, were used in this study (Feng *et al.* 2020). Throughout the 28-week experimental period, quails had free access to water and feed. Lighting was provided for 16 hours of light and 8 hours of darkness during the laying period. The experimental diets were formulated according to the NRC (1994) recommendations for Japanese quails. The copper source used was copper sulfate pentahydrate (Merck, molar mass: 249.684 g/mol).

Measurements

Body weight and feed intake were measured weekly. Eggs were collected and weighed daily, and production performance parameters including egg production, egg weight, egg mass, body weight gain, feed intake, and feed conversion ratio were calculated for the entire experimental period (10-38 weeks).

To evaluate reproductive performance traits, eggs produced during the 36 and 37-week of age were collected and incubated for 18 days in an incubator. The incubation process was carried out using automatic incubators with a relative humidity of 60 %, a temperature of 37.6 °C, and egg turning every 2 hours. On the 15th day of incubation, egg turning was halted, and the eggs were transferred to the hatchers, where the temperature was maintained at 37.6 °C and relative humidity at 70% until hatching. The reproductive performance traits assessed in this study included hatchability of set eggs (the number of hatched chicks relative to the total number of eggs placed in the incubator; Elibol *et al.* 2002), hatchability of fertile eggs (the number of hatched chicks relative to the number of fertile eggs; Elibol *et al.* 2002).

Table 1 Composition of experimental diets and calculated and analyzed composition (as-fed basis)

Ingredients	Unit	Rearing period		Laying period			
			Copper (mg/kg)	0	0	250	250
			NPP[1] (%)	0.35	0.13	0.35	0.13
Corn	%	55.04		54.11	54.11	54.11	54.11
Soybean Meal	%	43.73		34.85	34.55	34.85	34.55
Oil	%	0.08		3.33	3.33	3.33	3.33
Dicalcium phosphate	%	0.75		1.18	0.00	1.18	0.00
Oyster Powder	%	1.23		5.51	6.31	5.51	6.31
Sodium chloride	%	0.36		0.34	0.34	0.34	0.34
Dl-Methionine	%	0.05		0.17	0.17	0.17	0.17
Sand	%	0.00		0.00	0.69	0.00	0.69
Vitamin Premix[2]	%	0.25		0.25	0.25	0.25	0.25
Mineral Premix[3]	%	0.25		0.25	0.25	0.25	0.25
Calculated composition[4] (%)							
ME	Kcal/kg	2900		2900	2900	2900	2900
CP	%	24		20	20	20	20
Calcium	%	0.8		2.50	2.50	2.50	2.50
AP	%	0.30		0.35	0.13	0.35	0.13
M+C	%	0.75		0.85	0.85	0.85	0.85
Sodium	%	0.15		0.16	0.16	0.16	0.16
Chlorine	%	0.20		0.15	0.15	0.15	0.15
Potassium	%	0.40		0.87	0.86	0.87	0.86
Analyzed composition							
Copper	mg/kg			9.26	9.22	9.26	9.22

[1] NPP: non-phytate phosphorous.

[2] Each kilogram of the saral vitamin premix contained the following: vitamin A: 3600000 IU; vitamin D3: 800000 IU; vitamin E: 7200 IU; vitamin B1: 720 mg; vitamin B2: 2640 mg; Pantothenic acid: 4000 mg; Nicotinic acid: 12000 mg; vitamin B6: 1200 mg; Folate: 400 mg; vitamin B12: 6 mg; vitamin K3: 800 mg; Biotin: 40 mg; Choline chloride: 100000 mg and Antioxidants: 40000 mg.

[3] Each kilogram of the saral mineral premix contained the following: Manganese: 40000 mg; Iron: 20000 mg; Copper: 4000 mg; Iodine: 400 mg; Selenium: 80 mg; Zinc: 33880 mg and choline chloride: 100000 mg.

[4] ME: meatbolizable energy; AP: available phosphorous and M + C: methionine + cysteine.

To determine fertility, all eggs that were unhatched on days 18-19 were cracked open, and infertile eggs were separated from those containing dead embryos, after which the number of fertile eggs and the number of fertile but unhatched eggs were calculated (Elibol *et al.* 2002).

For measuring the cholesterol content of quail egg yolk during the 30-31-week period, the Folch *et al* (1957) method was used for yolk extraction, and the Zak (1977) method was employed to determine the cholesterol levels. Egg quality parameters, including eggshell ash, yolk, albumen, eggshell ratios, and yolk color, were assessed by collecting all eggs twice weekly for each replicate. These egg quality traits were evaluated during the weekly intervals of 10-15, 16-21, 22-27, and 28-31 weeks. To determine the weights of the egg component, each egg yolk was individually weighed using a laboratory balance with 0.01 g precision. The shell weight was measured following washing and drying for 48 hours at 60 °C. The calculation of albumen weight was also based on EW – (yolk weight+shell weight). The yolk color score was evaluated using DSM Fan Color Scale ranging from light yellow (score 1) to reddish-orange (score 15).

Blood samples were collected in heparin tubes at the end of the experiment (at 266 days of age) from two birds per replicate (one male and one female). A portion of the blood samples was centrifuged at 3500 × g for 10 minutes at 4 °C to obtain plasma. The resulting heparinized plasma samples were collected and stored at -20 °C for subsequent analysis. Glucose, total protein, cholesterol, triglycerides, high-density lipoprotein (HDL), copper, calcium, and phosphorous were analyzed using commercial enzymatic kits (Pars Azmoon Kits, Pars Azmoon Inc., Tehran, Iran) with a plasma autoanalyzer (Theauto-Analyzer-Biochemical Analyzer-BT3000 TARGA-Made in Italy). The calculation of low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) in poultry plasma is typically done using specific formulas. LDL is calculated using the formula:

LDL= Total Cholesterol - HDL - (Triglycerides / 5).

Where:
Total cholesterol, HDL, and triglycerides: measured in the plasma.
VLDL: calculated by dividing the triglyceride level by 5.

Copper concentration in the experimental diets was also measured using atomic absorption spectrophotometry (Phoenix-986, Germany). The remaining blood samples were immediately analyzed to determine hematocrit and red blood cell count.

Statistical analysis

The data obtained were statistically analyzed using the GLM procedure of SAS software in a 2 × 2 factorial experiment with a completely randomized design, consisting of 4 treatments and 6 replications. The statistical model applied in this study was as follows:

$$Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + e_{ijk}$$

Where:
Y_{ijk}: value of the trait.
μ: population mean.
A_i: effect of copper level.
B_j: effect of NPP (non-phytate phosphorus) level.
AB_{ij}: interaction effect of copper and NPP levels in the diet.
e_{ijk}: error term or unknown factors influencing each observation.

Mean comparisons for each trait were carried out using Duncan's test at the 5% significance level. For blood parameters, the gender effect was also analyzed and incorporated into the model.

RESULTS AND DISCUSSION

The effects of excess copper levels, NPP, and their interactions on body weight gain, feed intake, egg production, egg weight, egg mass, and feed conversion ratio in Japanese quail breeders are summarized in Table 2. Supplementation of the diet with excess copper at 250 mg/kg over the entire period (from 10 to 38 weeks) resulted in significant improvements in body weight gain, egg production (P< 0.01), egg mass (P<0.01), egg weight, and feed conversion ratio (P<0.05). However, excess copper supplementation did not significantly affect feed intake during the entire experimental period (P>0.05). None of the performance parameters throughout the experimental period were influenced by NPP levels or the interaction between excess copper and NPP (P>0.05).

Based on the data in Table 3, supplementing with 250 mg/kg of excess copper significantly reduced yolk cholesterol during the 28-31-week period (P<0.01). The inclusion of excess copper in the diet also led to a significant increase (P<0.01) in both the hatchability of set eggs and the hatchability of apparently fertile eggs. However, hatchability was not influenced (P>0.05) by the levels of NPP or the interaction between excess copper and NPP.

The results of the current study indicate that egg shell percentage and shell ash were not significantly affected (P>0.05) by excess copper supplementation, NPP levels, or their interactions across the 10-15, 16-21, 22-27, and 28-31 week periods (Table 4). However, supplementing the diet with 250 mg/kg of excess copper led to a significant (P<0.01) increase in yolk color score during the 16-21 and 22-27 week periods. Conversely, a reduction in NPP levels during the 22-27-week period resulted in a significant (P<0.01) decrease in yolk color intensity.

Egg weight significantly increased with excess copper supplementation during the 10-15 (P<0.01) and 22-27 (P<0.05) week periods (Table 5). Additionally, supplementing the diet with 250 mg/kg copper resulted in a significant (P<0.05) increase in the percentage of albumen during the 10-15, 22-27, and 28-31 week periods, as well as in the percentage of yolk during the 10-15, 16-21, and 22-27 week periods (Table 5). However, none of the internal egg quality traits were affected (P>0.05) by the level of NPP or the interaction between NPP and excess copper (Table 5).

According to the results in Table 6, supplementation with 250 mg/kg of excess copper significantly (P<0.05) reduced cholesterol and low-density lipoproteins (LDL) while increasing high-density lipoproteins (HDL) in the plasma of breeder quails at 266 days of age. However, plasma triglycerides, very low-density lipoproteins (VLDL), glucose, and total protein levels remained unchanged (P>0.05) across all experimental treatments at 266 days of age. Moreover, neither sex nor the interactions between sex, excess copper supplementation, and NPP levels in the diet had a significant (P>0.05) effect on any of the traits studied.

As shown in Table 7, dietary supplementation with 250 mg/kg of excess copper significantly increased plasma copper levels at 266 days of age (P<0.01). However, calcium, phosphorus, red blood cell count, and hematocrit levels were not influenced by excess copper supplementation, non-phytate phosphorus levels, sex, or their interactions (P>0.05).

The NRC (1994) recommended copper levels for various poultry species range between 5-8 mg/kg. For example, the copper requirement for broiler strains such as Ross 308 is set at 16 mg/kg (Aviagen, 2022), for Cobb 500 at 15 mg/kg (Cobb-vantress, 2012), and for Hy-Line W-80 layers, it is recommended at 20 mg/kg during the rearing period and 10 mg/kg during the production period (Hy-Line International, 2023). Poultry diets typically contain copper at levels of 8-15 mg/kg, which is usually sufficient to meet all or a significant portion of the bird's copper requirements through the basal diet. However, the practice of copper superdosing has gained attention due to its positive effects on performance, cost-effectiveness, and accessibility (Feng *et al.* 2020).

Table 2 The effects of varying levels of excess copper (ECu) and dietary non-phytate phosphorus (NPP) on the production performance of breeder quails between 10 and 38 weeks of age

	Bodyweight (g)	Feed intake (g)	Egg weight (g)	Egg mass (g)	Egg production (%)	FCR[1]
ECu (mg/kg)						
0	245.80[b]	178.50	12.32[b]	11.00[b]	78.30[b]	2.53[a]
250	249.40[a]	178.40	12.36[a]	11.15[a]	89.90[a]	2.51[b]
SEM[2]	1.185	0.386	0.013	0.026	2.09	0.006
NPP (%)						
0.35	247.20	178.50	12.33	11.08	84.70	2.53
0.13	248.00	178.50	12.35	11.08	83.50	2.52
SEM	1.185	0.386	0.013	0.026	2.09	0.006
ECu × NPP						
0 × 0.35	245.30	178.60	12.31	10.99	79.00	2.54
250 × 0.35	249.10	178.30	12.35	11.16	90.40	2.52
0 × 0.13	246.30	178.40	12.32	11.01	77.70	2.53
250 × 0.13	249.80	178.50	12.37	11.15	89.40	2.51
SEM	1.676	0.546	0.019	0.037	2.95	0.008
P-value						
ECu	0.012	NS	0.025	< 0.001	0.002	0.013
NPP	NS[3]	NS	NS	NS	NS	NS
ECu × NPP	NS	NS	NS	NS	NS	NS

[1] FCR: feed conversion ratio
[2] SEM: standard error of the means.
[3] NS: non-significant.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 3 The effects of varying levels of excess copper (ECu) and dietary non-phytate phosphorus (NPP) on egg yolk cholesterol (at 36 weeks) and reproductive performance (at 36-37 Weeks) in breeder quails

	Yolk cholesterol (mg/g)	Hatchability of set eggs (%)	Hatchability of fertile eggs (%)
ECu (mg/kg)			
0	11.80[a]	66.70[b]	78.30[b]
250	11.47[b]	77.50[a]	89.90[a]
SEM[1]	0.086	1.71	2.09
NPP (%)			
0.35	11.63	77.50	84.70
0.13	11.63	71.70	83.50
SEM	0.086	1.71	2.09
ECu × NPP			
0 × 0.35	11.90	67.10	79.00
250 × 0.35	11.36	77.90	90.40
0 × 0.13	11.70	66.30	77.70
250 × 0.13	11.57	77.10	89.40
SEM	0.121	2.41	2.95
P-value			
ECu	0.009	0.001	0.001
NPP	NS[2]	NS	NS
ECu × NPP	NS	NS	NS

[1] SEM: standard error of the means.
[2] NS: non-significant.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 4 The effects of varying levels of excess copper (ECu) and dietary non-phytate phosphorus (NPP) on egg quality traits in breeder quails across the age periods of 10–15, 16–21, 22–27, and 28–31 weeks

	Shell weight (%)				Shell ash (%)				Yolk color score			
	10-15	16-21	22-27	28-31	10-15	16-21	22-27	28-31	10-15	16-21	22-27	28-31
ECu (mg/kg)												
0	8.60	8.40	8.50	8.50	54.00	51.90	51.30	51.60	4.60	4.70[b]	4.60[b]	4.70
250	8.40	8.40	8.40	8.50	53.50	51.40	50.80	52.50	4.60	4.90[a]	4.80[a]	4.70
SEM[1]	0.138	0.061	0.071	0.069	0.829	0.428	0.591	0.625	0.077	0.067	0.063	0.082
NPP (%)												
0.35	8.60	8.30	8.50	8.50	55.00	52.20	50.90	51.80	4.50	4.90	4.80[a]	4.70
0.13	8.50	8.40	8.40	8.50	52.50	51.10	51.10	52.30	4.70	4.70	4.60[b]	4.70
SEM	0.138	0.061	0.071	0.069	0.829	0.428	0.591	0.625	0.077	0.067	0.063	0.082
ECu × NPP												
0 × 0.35	8.60	8.40	8.50	8.90	55.30	52.30	50.70	51.40	4.50	4.80	4.70	4.70
250 × 0.35	8.60	8.30	8.60	8.60	54.60	52.10	51.20	52.10	4.70	4.90	5.00	4.80
0 × 0.13	8.60	8.40	8.50	8.60	52.70	51.40	51.80	51.80	4.80	4.50	4.70	4.80
250 × 0.13	8.30	8.40	8.30	8.50	52.30	50.80	50.50	52.90	4.70	4.90	4.70	4.70
SEM	0.196	0.086	0.101	0.098	0.117	0.605	0.823	0.884	0.109	0.095	0.089	0.116
P-value												
ECu	NS[2]	NS	NS	NS	NS	NS	NS	NS	NS	0.0079	0.0037	NS
NPP	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
ECu × NPP	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1] SEM: standard error of the means.
[2] NS: non-significant.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 5 The effects of varying levels of excess copper (ECu) and dietary non-phytate phosphorus (NPP) on egg quality traits in breeder quails across the age periods of 10–15, 16–21, 22–27, and 28–31 weeks

	Egg weight (g)				Albumen (%)				Yolk (%)			
	10-15	16-21	22-27	28-31	10-15	16-21	22-27	28-31	10-15	16-21	22-27	28-31
ECu (mg/kg)												
0	11.80[b]	12.60	12.00[b]	12.0	53.90[b]	54.30	55.20[b]	54.20[b]	31.90[b]	32.00[b]	31.50[b]	30.60
250	12.20[a]	12.60	12.30[a]	12.2	55.30[a]	54.80	55.90[a]	55.40[a]	23.90[a]	32.90[a]	32.20[a]	31.20
SEM[1]	0.083	0.079	0.089	0.113	0.470	0.240	0.210	0.380	0.270	0.310	0.191	0.350
NPP (%)												
0.35	12.00	12.60	12.20	12.10	54.10	54.60	55.70	54.50	32.40	32.60	31.80	31.20
0.13	12.10	12.60	12.10	12.10	55.10	54.50	55.40	54.90	32.40	32.30	31.90	30.50
SEM	0.083	0.081	0.089	0.113	0.470	0.240	0.210	0.380	0.290	0.310	0.176	0.35
ECu × NPP												
0 × 0.35	11.70	12.50	12.10	12.00	53.50	54.30	55.50	53.80	32.30	32.40	31.50	31.10
250 × 0.35	12.30	12.70	12.30	12.20	54.80	55.00	56.00	55.60	32.60	32.90	32.20	31.40
0 × 0.13	12.00	12.60	12.00	11.90	54.30	54.40	55.00	54.70	31.50	31.60	31.50	29.90
250 × 0.13	12.20	12.60	12.20	12.30	55.80	54.50	55.90	55.20	33.20	32.90	32.30	31.20
SEM	0.118	0.112	0.126	0.160	0.680	0.350	0.300	0.540	0.39	0.44	0.270	0.500
P-value												
ECu	0.001	NS	0.048	NS	0.045	NS	0.021	0.044	0.035	0.011	0.011	NS
NPP	NS[2]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
ECu × NPP	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1] SEM: standard error of the means.
[2] NS: non-significant.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 6 The effects of varying levels of excess copper (ECu) and dietary non-phytate phosphorus (NPP) on plasma lipids, glucose and total protein of breeder quails at 266 days of age

	Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLD (mg/dL)	Glucose (mg/dL)	Total protein (g/dL)
ECu (mg/kg)							
0	230.60[a]	146.20	123.80[b]	77.10[a]	29.30	175.40	4.50
250	223.50[b]	146.60	126.70[a]	69.10[b]	28.50	186.90	4.30
SEM[1]	1.90	1.40	0.900	2.22	0.279	4.18	0.143
NPP (%)							
0.35	227.40	143.90	126.20	72.60	28.80	180.50	4.40
0.13	226.70	145.30	124.20	74.40	29.10	181.80	4.50
SEM	1.90	1.40	0.900	2.24	0.280	4.18	0.143
Sex							
Female	229.20	144.70	126.00	74.80	28.90	182.80	4.50
Male	225.10	144.50	124.40	72.00	28.90	179.50	4.40
SEM	1.91	1.40	0.900	2.23	0.280	4.18	0.143
ECu × NPP							
0 × 0.35	230.70	145.00	125.30	75.60	29.00	172.60	4.50
250 × 0.35	224.00	142.60	127.00	69.10	28.50	188.30	4.30
0 × 0.13	230.40	147.40	122.30	78.50	29.50	178.10	4.60
250 × 0.13	222.90	142.70	126.40	69.00	28.50	185.40	4.40
SEM	2.69	1.98	1.27	3.15	0.395	5.91	0.201
P-value							
ECu	0.019	NS	0.026	0.022	NS	NS	NS
NPP	NS[2]	NS	NS	NS	NS	NS	NS
ECu × NPP	NS	NS	NS	NS	NS	NS	NS
Sex	NS	NS	NS	NS	NS	NS	NS

[1] SEM: standard error of the means.
[2] NS: non-significant.
[3] HDL: high density lipoprotein; LDL: low density lipoprotein and VLDL: very low density lipoprotein.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 7 The effects of varying levels of excess copper (ECu) and dietary non-phytate phosphorus (NPP) on plasma copper, calcium, phosphorus and blood red blood cell (RBC) count and hematocrit of breeder quails at 266 days of age

	Copper (µg/mL)	Calcium (mg/dL)	Phosphorus (mg/dL)	RBC count ($10^{\times 4}$/µL)	Hematocrit (%)
ECu (mg/kg)					
0	0.16[b]	10.60	4.04	4.44	42.90
250	0.20[a]	10.20	4.00	4.42	43.40
SEM[1]	0.007	0.350	0.099	0.025	0.470
NPP (%)					
0.35	0.18	10.10	4.15	4.43	43.60
0.13	0.18	10.60	3.90	4.42	42.70
SEM	0.007	0.350	0.098	0.025	0.470
Sex					
Female	0.17	10.70	4.04	4.43	42.90
Male	0.18	10.00	4.00	4.42	43.40
SEM	0.007	0.35	0.099	0.025	0.470
ECu × NPP					
0 × 0.35	0.16	10.40	4.08	4.41	43.30
250 × 0.35	0.19	9.80	4.23	4.46	43.90
0 × 0.13	0.16	10.80	4.01	4.47	42.50
250 × 0.13	0.21	10.50	3.79	4.37	42.90
SEM	0.009	0.490	0.139	0.036	0. 660
P-value					
ECu	0.002	NS	NS	NS	NS
NPP	NS[2]	NS	NS	NS	NS
ECu × NPP	NS	NS	NS	NS	NS
Sex	NS	NS	NS	NS	NS

[1] SEM: standard error of the means.
[2] NS: non-significant.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Various studies have demonstrated performance improvements, such as enhanced weight gain and improved feed conversion ratios in broilers, with copper supplementation levels ranging between 100-300 mg/kg (Pesti and Bakalli, 1996; Arias and Koutsos, 2006; Payvastegan *et al.* 2017; Nguyen *et al.* 2020; Deo *et al.* 2023; Haldar *et al.* 2023). In line with the results of current study, Almansour (2006) also reported that adding 250 and 500 mg/kg of excess copper from copper sulfate improved body weight gain in Japanese quail.

In Japanese quail, Abaza *et al.* (2009) observed that adding excess copper from copper sulfate at levels of 100 and 200 mg/kg increased egg production and egg mass while decreasing the feed conversion ratio. In laying hens, improved egg production percentages were observed with supplementation of 250 mg/kg of copper from a sulfate source (Pekel and Alp, 2011). Similarly, a feed conversion ratio improvement was reported when 300 mg/kg of copper from a sulfate source was added to the diet (Kim *et al.* 2016). Additionally, Zhou *et al.* (2021) noted both linear and quadratic increases in egg production percentages in laying hens supplemented with copper from a lysinate-glutamate source at levels of 0, 15, 75, 150, and 200 mg/kg. At lower supplementation levels, Berwanger *et al.* (2018) observed that broiler breeder hens experienced increases in egg production percentages and egg weights when given 9.38, 12.92, 16.83, and 20.19 mg/kg of copper from copper sulfate. Thus, despite potential environmental issues, copper superdosing is regarded as both effective and cost-efficient (Feng *et al.* 2020).

A suggested mechanism for the growth-promoting effects of excessive dietary copper supplementation is its ability to alter gut microbiota, which is linked to copper's antibacterial properties (Pang *et al.* 2009; EL- Kazaz and Hafez, 2020). This change reduces the bird's susceptibility to pathogenic bacteria, decreases the permeability of intestinal lymphocytes, and ultimately increases nutrient absorption (Pang *et al.* 2009; EL-Kazaz and Hafez, 2020). Excess copper levels in poultry diets can positively alter gut microbiota by reducing pathogen populations, increasing nutrient availability—particularly proteins—and thereby improving performance, including body weight gain (Yausheva, 2021). The growth-promoting effects of excess copper supplementation may also be attributed to improved nutrient absorption, which could result from copper's beneficial impact on small intestine morphology—such as increased goblet cell numbers, elongated villi, and reduced crypt depth—as well as enhanced activity of digestive enzymes like protease, lipase, and amylase in the intestinal contents (Attia *et al.* 2012; Chen *et al.* 2023; AL-Ruwad *et al.* 2024).

In line with the findings of the present study, Amoah *et al.* (2012) observed in an experiment with laying Japanese quail that feed intake, egg production, feed conversion ratio, and egg mass were unaffected by varying levels of NPP (0, 0.25%, and 0.35%). Similarly, Bello *et al.* (2020) reported no impact on production traits in laying hens when the NPP requirement was reduced to 0.2%. While the NRC (1994) recommends an NPP level of 0.35% for breeder and laying quail, the present study demonstrated that lowering the NPP level from 0.35% to 0.13% had no effect on egg production, egg weight, egg mass, feed intake, feed conversion ratio, or body weight. These findings suggest that reducing the NPP requirement does not compromise the productive performance of Japanese breeder quail.

Copper is an essential element in poultry physiology, particularly during growth and embryonic development (Gou *et al.* 2021). Consistent with the findings of the present study, Abaza *et al.* (2009) demonstrated in an experiment with Japanese breeder quail that supplementing diets with excess copper from copper sulfate at levels of 100 and 200 mg/kg improved the hatchability of fertile eggs. Similarly, Attia *et al.* (2011) reported an increase in hatchability percentages in breeder hens supplemented with 10, 60, and 120 mg/kg of copper from sulfate and lysinate sources. Copper sulfate has also been shown to reduce susceptibility to infections caused by Gram-positive and Gram-negative bacteria as well as fungi (Tavakkoli *et al.* 2015). In the same study, Tavakkoli *et al.* (2015) found that *in ovo* copper sulfate injection mitigated the effects of fungal contamination during incubation without negatively impacting embryo quality or development. Pathogens can adversely affect the reproductive system either reversibly or irreversibly (Hassan *et al.* 2020). Moreover, copper plays a significant role in enhancing the body's antioxidant system, strengthening the immune system, and boosting digestive enzyme activity (Sharif *et al.* 2021; AL-Ruwad *et al.* 2024). Therefore, excess copper supplementation may potentially enhance hatchability and fertility in Japanese quail by improving nutrient digestibility, reducing infections, and supporting immune and antioxidant systems.

In agreement with the present study, Kimiaeitalab *et al.* (2013) showed that supplementing 150, 200, 250, and 300 mg/kg of copper from copper sulfate reduced total cholesterol levels in yolk and serum. In another study, Jegede *et al.* (2015) found that supplementing 50, 100, and 150 mg/kg of proteinates and copper sulfate in laying hens significantly reduced blood and yolk cholesterol. Additionally, a decrease in yolk cholesterol levels in laying hens was observed in the study by Noori *et al.* (2023) with supplementation of 50 and 100 mg/kg of copper-methionine, and in the study by Baghban-Kanani *et al.* (2023) using 75 and 150 mg/kg of copper nanoparticles and bioplex copper. Studies indicate that excess dietary copper affects lipid metabolism (Chowdhury *et al.* 2002; Pesti and Bakalli, 1996).

Cholesterol biosynthesis is regulated by the rate-limiting step involving the conversion of beta-hydroxy-beta-methylglutaryl-CoA (HMG-CoA) to mevalonate, a reaction catalyzed by HMG-CoA reductase (Mondal *et al.* 2007). Since this enzyme relies on glutathione thiol groups for its activity, higher dietary copper levels, and the resulting increase in liver copper concentration, reduce the ratio of reduced to oxidized glutathione, thereby influencing cholesterol synthesis. As a result, the conversion of HMG-CoA to mevalonate is inhibited, disrupting the carbon flow in the cholesterol biosynthetic pathway and subsequently reducing cholesterol synthesis (Jegede *et al.* 2015; Cufadar *et al.* 2022; AL-Ruwad *et al.* 2024). The liver enzyme 7-alpha-hydroxylase is the initiator and rate-limiting enzyme in the biosynthesis of bile acids from cholesterol (Chiang and Ferrell, 2020). According to research, adding copper to the diet is associated with a decrease in the activity of the cholesterol 7-alpha-hydroxylase enzyme, indicating that the activity of this enzyme is regulated based on substrate availability (Konjufca *et al.* 1997).

In line with the observed increase in yolk ratio following excess copper supplementation in the present study, Moradi *et al.* (2011) also observed that supplementing 125 and 250 mg/kg of copper from copper sulfate increased yolk ratio in laying hens. Similarly, Berwanger *et al.* (2018) reported an increase in yolk ratio with the supplementation of 9.38 mg/kg of copper from copper sulfate in broiler breeder hens. The increase in both yolk and albumen percentage can be attributed to the effective role of copper in improving nutrient absorption and utilization efficiency, which leads to the production of more yolk and albumen, ultimately enhancing yolk and albumen ratios. Furthermore, in the present study, the yolk color score also increased with excess copper supplementation, which is consistent with the results of Gou *et al.* (2021) in broiler breeder hens (at a level of 13.5 mg/kg) and Cufadar *et al.* (2022) in Japanese breeder quails (at a level of 225 mg/kg). Additionally, following a reduction in the NPP level in the diet, yolk color score decreased. Given the role of phosphorus in fat metabolism (Li *et al.* 2016), it is likely that the reduction in NPP levels affected the digestion and absorption of fats, as well as fat-soluble vitamins. Carotenoids can act as provitamin A (Shastak and Pelletier, 2024).

Consistent with the findings of this study, Mondal *et al.* (2007) reported that excess copper supplementation at levels of 200 and 400 mg/kg from copper sulfate in broiler diets did not affect calcium, phosphorus, glucose, albumin, total protein, or globulin levels, but significantly increased plasma copper concentration. Attia *et al.* (2011), in an experiment with excess copper supplementation at levels of 10, 60, and 120 mg/kg from copper sulfate and proteinate sources, also reported that serum total protein, albumin, and globulin concentrations were unaffected by the treatments, while serum copper concentration significantly increased. Similarly, Noori *et al.* (2023) reported an increase in serum copper concentration in laying hens after excess copper supplementation, with no observed effect on serum calcium and phosphorus concentrations. Plasma and liver copper concentrations are indicative of dietary copper levels, with higher dietary copper leading to increased copper content in both the liver and plasma (Gou et *al.* 2021).

Regarding hematological traits, consistent with the findings of this study, Mondal *et al.* (2007) reported that excess copper supplementation did not affect hematocrit percentage or red blood cell count in broiler chicks. Similarly, Payvastegan *et al.* (2012) found no effect on hemoglobin concentration, red blood cell count, or hematocrit percentage in broiler chicks supplemented with 125 and 250 mg/kg of copper in the diet. Since copper plays an indirect role in enhancing iron absorption and hemoglobin synthesis, copper supplementation is expected to increase the capacity for iron transport via ferritin and transferrin only when the diet is deficient in copper (McDowell, 1992).

CONCLUSION

The results of this study showed that supplementing 250 mg/kg of excess copper from copper sulfate enhanced productive and reproductive performance in Japanese breeder quail. Furthermore, lowering the NPP level to 0.13% did not negatively impact egg production or hatchability in Japanese breeder quail.

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