

## Journal of the Hellenic Veterinary Medical Society

Vol 73, No 3 (2022)



### The Effect of Organic Copper Levels in Breeding Japanese Quail (*Coturnix coturnix Japonica*) Diets on Performance, Egg Quality, Incubation Parameters and Blood Parameters

*Y Cufadar, B Curabay, SA Gökmen, Y Bahtiyarca, B Sevim*

doi: [10.12681/jhvms.28129](https://doi.org/10.12681/jhvms.28129)

Copyright © 2022, Yusuf Cufadar, Barışcan Curabay, Seyit Ahmet Gökmen, Yılmaz Bahtiyarca, Behlül Sevim



This work is licensed under a [Creative Commons Attribution-NonCommercial 4.0](https://creativecommons.org/licenses/by-nc/4.0/).

#### To cite this article:

Cufadar, Y., Curabay, B., Gökmen, S., Bahtiyarca, Y., & Sevim, B. (2022). The Effect of Organic Copper Levels in Breeding Japanese Quail (*Coturnix coturnix Japonica*) Diets on Performance, Egg Quality, Incubation Parameters and Blood Parameters. *Journal of the Hellenic Veterinary Medical Society*, 73(3), 4619–4626. <https://doi.org/10.12681/jhvms.28129>

## The Effect of Organic Copper Levels in Breeding Japanese Quail (*Coturnix coturnix Japonica*) Diets on Performance, Egg Quality, Blood and Incubation Parameters

Y. Cufadar<sup>1</sup>, B. Curabay<sup>1\*</sup>, S. A. Gökmen<sup>1</sup>, Y. Bahtiyarca<sup>1</sup>, B. Sevim<sup>2</sup>

<sup>1</sup>Selcuk University, Faculty of Agriculture, Department of Animal Science, Konya, Turkey

<sup>2</sup>Aksaray University, Eski Vocational School, Department of Veterinary, Aksaray, Turkey

**ABSTRACT:** This study was conducted to determine the effect of different levels of organic copper (organic Cu) supplementation on performance, egg quality, serum parameters and incubation characteristics in Japanese quail diets. 120 Japanese breeder quails of 7 weeks old were used in the study and lasted 84 days. Four diets containing different levels of organic copper (0, 75, 150 and 225 mg/kg) were used in the study. The study was a random design consisting of 20 subgroups with 5 replicates, and 4 females and 2 males were used in each subgroup. As a result of this study, final body weight and body weight gain in Japanese quails was significantly lower in the 75 and 150 mg/kg organic copper groups than in the groups containing 0 and 225 mg/kg copper ( $p < 0.01$ ;  $p < 0.05$ ). Egg production, egg weight, egg mass, feed intake and feed conversion ratio were not affected by dietary copper levels ( $p > 0.05$ ). Eggshell thickness and eggshell ratio were significantly higher in the groups containing 150 and 225 mg/kg organic copper than the control and 75 mg/kg organic copper groups ( $p < 0.05$ ). Serum cholesterol level was significantly lower in 75 and 150 mg/kg copper groups compared to other groups ( $p < 0.05$ ). Serum albumin, globulin and total protein levels were significantly lower in the 75 and 150 mg/kg copper groups compared to the control group ( $p < 0.01$ ). Serum albumin and total protein concentrations were significantly higher in the 75 and 150 mg/kg copper groups than the other groups ( $p < 0.01$ ). The highest serum calcium (Ca) level was in the group containing 225 mg/kg copper ( $p < 0.01$ ). In the study, incubation parameters were not significantly affected by different copper levels in the diet. The results of the study showed that adding 150 mg/kg organic Cu to quail diets can be effective in improving eggshell quality and lowering serum cholesterol levels.

**Keywords:** Breeding quail; Egg quality; Incubation parameters; Organic copper; Performance

*Corresponding Author:*

B. Curabay, Selcuk University, Faculty of Agriculture, Department of Animal Science, Konya, Turkey  
E-mail address: bcurabay@hotmail.com

*Date of initial submission:* 04-10-2021  
*Date of acceptance:* 03-12-2021

## INTRODUCTION

Copper (Cu) is an important mineral in poultry nutrition and acts as a cofactor in many enzyme systems, including cytochrome oxidase, lysyl oxidase, ceruloplasmin, dopamine beta-hydroxylase, and superoxide dismutase (Fouad et al., 2016; Hill and Shannon, 2019). Most of the mineral sources added to the diets are derived from inorganic compounds such as sulfates, oxides and carbonates (Bao et al., 2007). Organic micro mineral sources can be an alternative to inorganic sources. Organic minerals remain electrically neutral at acidic gastric pH and are protected from chemical reactions with other molecules in the intestinal lumen, optimizing their absorption and increasing their bioavailability relative to inorganic sources. (Swiątkiewicz et al., 2014). The National Research Council recommends the addition of 5 mg/kg Cu to breeder quail diets (NRC, 1994). Although the Cu needs of the poultry are stated at low levels, it can be found in the diet at the levels of 100-250 mg/kg for growth stimulators and therapeutic purposes (Guclu et al., 2008). Copper is often used commercially at much higher pharmacological levels (100 to 300 mg/kg) due to its growth-promoting properties (Azimi Youvalari et al., 2017). However, since excessive Cu accumulation in the body causes toxicity, the maximum tolerable dietary Cu level for poultry has been determined as 300 mg/kg (NRC, 1994). In addition, excess Cu (more than 300 mg/kg) can inhibit the growth of broilers (Persia et al., 2004) and it cannot be ruled out that excessive Cu supplementation causes environmental pollution.

Reports on the efficacy of Cu used at pharmacological levels as an alternative to antibiotic growth promoters in poultry diets are inconsistent (Nguyen et al., 2020). Some researchers have suggested that feeding 250 mg/kg Cu initially has negative consequences on poultry performance, and moderate Cu (125 mg/kg) has beneficial effects on performance in the market age (Karimi et al., 2011). Adding high levels of Cu (200-300 mg/kg) to the diet of laying hens reduced egg weight and feed intake, but increased eggshell strength (Kaya et al., 2018). The addition of Cu to the diet increased eggshell thickness in laying hens (Olgun and Aygün, 2017) and Japanese quail (Olgun et al., 2020). According to studies on the relationship between high levels of dietary Cu (100-300 mg/kg) and broiler performance, Cu has a significant inhibitory effect on serum cholesterol content and can significantly increase the survival rate of chickens, which is especially important in breeding (Arias and Koutsos,

2006; Berwanger et al., 2018). The process of transporting Cu trace elements in the blood occurs through the binding of Cu to a unique transport protein, ceruloplasmin, and through albumin, transcuprein, and Cu-amino acid complexes (Megahed et al., 2013). Copper is effective on the lipid metabolism of poultry (Jegade et al., 2012) and lowered plasma cholesterol in growing poultry (Jegade et al., 2015).

Copper deficiency negatively affects embryo development with resulting gross structural and biochemical abnormalities (Roychoudhury et al., 2016). Because of the tight involvement of Cu in collagen synthesis, an adequate supply of this mineral is also essential for embryo bone development and, therefore, for the independent feed seeking immediately after hatching. (Berwanger et al., 2018). Adding Cu to the chicken diet reduced the clostridia population in the poultry digestive tract. In general, the coliform bacteria population was affected by the addition of 200 mg/kg Cu to the diet, while the lactobacillus population was stimulated with 150 mg/kg Cu (EFSA, 2016).

The effects of high levels of copper supplementation in poultry diets are still controversial. For this reason, the main aim of this study is to eliminate the lack of resources with the effects of high-levels Cu addition to the diets of laying breeder quails.

## MATERIALS AND METHODS

### Animals and diets

A total of 120 Japanese quails (*Coturnix coturnix Japonica*) at the age of 7 weeks were randomly distributed among four dietary treatment groups and has lasted 12 weeks. The initial body weights of the quails were between 210 g and 218 g. Each dietary treatment was replicated in five cages, each containing six quails (4 females/2 males). The control group hens fed a basal diet. Experimental diets were prepared from the basal diet by adding different levels of Cu: 0, 75, 150, or 225 mg/kg diet. Organic Cu (Glycinoplex-Cu) was used as Cu source. All experimental diets, except the Cu level, were prepared to the levels recommended by NRC (1994) for quail breeding (Table 1). During the experiment, feed and water were provided ad-libitum and lighting was provided for 16 hours/day.

### Performance parameters

Livability was determined by logging all the deaths in all subgroups throughout the experiment and this was taken into consideration when measuring the

**Table 1.** The ingredients and calculated nutrient composition of the basal diet

Ingredients	%	Calculated nutrients	
Corn	47.40	Metabolizable Energy, kcal/kg	2905
Soybean Meal, 46 % crude protein	31.00	Crude Protein %	20.06
Sunflower Seed Meal, 35% crude protein	8.00	Calcium %	2.54
Vegetable Oil, 8800 kcal ME/ kg	5.80	Available phosphorus %	0.45
Limestone	5.30	Methionine %	0.42
Dicalcium Phosphate	1.75	Lysine %	1.03
Salt	0.30	Copper mg/kg*	8.18
Vitamin Premix <sup>1</sup>	0.25		
Mineral Premix <sup>2</sup>	0.10		
DL-Methionine	0.10		
<b>Total</b>	<b>100</b>		

<sup>1</sup>Vitamin pre-mix in 1 kg of the ration: Vitamin A, 8,800 IU; Vitamin D3, 2,200 IU; Vitamin E, 11 mg; Nicotinic acid, 44 mg; Cal-D-Pan, 8.8 mg; Riboflavin, 4.4 mg; Thiamine, 2.5 mg; Vitamin B12, 6.6 mg; Folic acid, 1 mg; D-Biotin, 0.11 mg; Choline: 220 mg.

<sup>2</sup>Mineral premix in 1 kg of the ration: Manganese, 80 mg; Iron, 60 mg; Zinc, 60 mg; Cobalt, 0.20 mg; Iodine, 1 mg; Selenium, 0.15 mg.

\* Analyzed value.

performance values for each group. The body weight of the quails was determined per group by weighing at the beginning and the end of the experiment and body weight gain has been calculated from these data. Eggs were collected and recorded daily and egg production was calculated to these data. Egg weight was recorded by weighing the eggs collected during the last two days of each period. Egg mass was calculated for each period by multiplying the egg production percentage with average egg weight and dividing it into 100. Feed intake was calculated by weighing the amount of feed given to the quails during the period. The feed conversion ratio was calculated by dividing the average feed intake for each period by the egg mass in the same period.

### Egg quality parameters

Measurements of egg quality parameters were carried out on a total of 200 eggs, 10 eggs collected from each subgroup in the last 3 days of each 28 days. Egg shape index was calculated by the formula (Egg Width (mm) / Egg Length (mm)) x 100; egg specific gravity was determined by using the Archimedes' principle and the formula defined by Wells (1968), and egg surface area was calculated by using the formula developed by Carter (1975). Eggshell breaking strength (kg) was determined by using an eggshell breaking instrument (Egg Force Reader) and shell strength per unit area has been calculated by dividing eggshell breaking strength by the eggshell surface area. Eggshell weight (with membrane) was determined by weighing them on a scale. Eggshell ratio (%) was calculated by using the following formula: (shell weight (g) / egg

weight (g)) x 100; shell index = (shell weight (g) / egg surface area (cm<sup>2</sup>)) x 100. Shell thickness (mm) was measured by using a micrometer. Eggshell density was calculated by using the following formula: Eggshell density (g/cm<sup>3</sup>) = eggshell weight (g) / (shell surface area (cm<sup>2</sup>) x eggshell thickness (cm)). Egg albumen index (%) was defined by using the following formulas: (Egg albumen height, mm / (average of the length and width of egg albumen (mm))) x 100; Haugh unit = 100 x Log(egg albumen height + 7.57 - egg albumen<sup>0.37</sup>). Egg yolk index (%) was calculated by using the following formula: (height of egg yolk, mm / width of egg yolk, mm) x 100, and the Roche scale has been used to determine the egg yolk color.

### Blood serum parameters

At the end of the experiment, 3 ml of blood was taken from a total of 40 quail, 2 females from each subgroup. The collected blood samples were centrifuged for 10 minutes (3000 rpm) and their serums were separated. Serum biochemical parameters were determined using an autoanalyzer.

### Incubation parameters

The determination of incubation parameters was carried out on all eggs collected for 3 consecutive days in the middle of the last period of the experiment. Chicks hatched at the end of the 18-day incubation period were weighed and their weight was determined. Later, the eggs that did not hatch were broken and the fertilized and unfertilized eggs were determined and the period of embryonic death in the fertilized eggs was determined. The fertility rate was

calculated by dividing the number of fertilized eggs by the total number of incubated eggs. Hatchability was calculated by dividing the number of live chicks hatched by the total number of eggs hatched. The hatchability of fertile eggs was calculated by dividing the number of fertilized eggs incubated by the number of live chicks hatched. The determination of embryo deaths was made according to the method determined by Aygun et. al (2012).

### Statistical analysis

Data were then subjected to one-way ANOVA analysis in a completely randomized design using the General Linear Model procedure of MINITAB (Minitab Inc. USA, release 17.1), and the differences between means of the groups were determined by

Duncan's multiple comparison test.

## RESULTS

### Performance parameters

Adding 0, 75, 150 and 225 mg/kg organic Cu to the breeding quail diets had a statistically significant effect on the end of the experiment body weight and body weight change ( $p < 0.01$ ;  $p < 0.05$ ). Adding 75 and 150 mg/kg organic Cu to the diets had significantly reduced the end of experiment body weight and body weight change parameters when compared to containing 0 (control) and 225 mg/kg organic Cu groups. Among other performance parameters, egg production, egg weight, egg mass, feed intake and feed conversion ratio werenot significantly affected by dietary different Cu levels (Table 2).

**Table 2.** The effect of dietary different levels of organic copper on the performance parameters of breeding Japanese quails

Parameters	Dietary Supplemental Organic Cu Levels (mg/kg)				SEM	P-Value
	0	75	150	225		
Initial body weight (g/quail)	210.17	215.17	214.93	218.13	3.648	0.504
Final body weight (g/quail)	251.26 <sup>a</sup>	231.43 <sup>b</sup>	227.83 <sup>b</sup>	249.31 <sup>a</sup>	5.592	0.016
Body weight gain (g/quail)	41.09 <sup>A</sup>	16.27 <sup>B</sup>	12.90 <sup>B</sup>	31.17 <sup>A</sup>	4.129	0.001
Liveability (%)	96.67	100.0	96.67	96.67	2.887	0.801
Egg production (%)	88.07	88.81	86.98	86.81	1.963	0.873
Egg weight (g)	12.46	12.59	12.61	12.07	0.238	0.369
Egg mass (g/quail/day)	11.00	11.21	10.98	10.50	0.372	0.588
Feed intake (g/quail/day)	28.31	27.25	26.83	27.27	0.351	0.051
Feed conversion ratio (Feed intake/egg mass)	2.57	2.43	2.44	2.60	0.083	0.338

SEM: Standard error mean.

<sup>A,B</sup>The differences indicated by different letters on the same row are statistically significant ( $p < 0.01$ ).

<sup>a,b</sup>The differences indicated by different letters on the same row are statistically significant ( $p < 0.05$ ).

**Table 3.** The effect of dietary different levels of organic copper on egg quality characteristics of breeding Japanese quails

Parameters	Dietary Supplemental Organic Cu Levels (mg/kg)				SEM	P-Value
	0	75	150	225		
Eggshell breaking strength (kg)	1,39	1.43	1.53	1.55	0.055	0.143
Eggshell thickness (mm)	0.202 <sup>b</sup>	0.204 <sup>b</sup>	0.217 <sup>a</sup>	0.217 <sup>a</sup>	0.004	0.030
Eggshell ratio (%)	7.69 <sup>b</sup>	7.74 <sup>b</sup>	8.20 <sup>a</sup>	8.32 <sup>a</sup>	0.15	0.017
Eggshell index (mg/cm <sup>2</sup> )	39.56 <sup>b</sup>	39.99 <sup>ab</sup>	42.34 <sup>a</sup>	42.46 <sup>a</sup>	0.828	0.044
Eggshell density (g/cm <sup>3</sup> )	1.963	1.956	1.956	1.958	0.013	0.974
Eggshell strength per unit surface area (g/cm <sup>2</sup> )	57.51	58.57	62.68	65.60	2.505	0.124
Egg shape Index (%)	76.03	77.16	75.90	77.40	0.555	0.169
Egg surface area (cm <sup>2</sup> )	24.19	24.37	24.43	23.63	0.555	0.169
Specific gravity (g/cm <sup>3</sup> )	1.070	1.071	1.073	1.074	0.001	0.068
Haugh unit (%)	89.08	89.52	89.15	90.53	0.433	0.108
Albumen index (%)	10.48	10.74	10.55	11.37	0.236	0.063
Egg yolk index (cm <sup>2</sup> )	47.44	47.75	47.97	49.04	0.453	0.110
Egg yolk color	4.24 <sup>b</sup>	4.29 <sup>b</sup>	4.44 <sup>ab</sup>	4.65 <sup>a</sup>	0.089	0.019

SEM: Standard error mean.

<sup>a,b</sup>The differences indicated by different letters on the same row are statistically significant ( $p < 0.05$ ).

**Table 4.** The effect of dietary different levels of organic copper on the serum parameters of breeding Japanese quails

Parameters	Dietary Supplemental Organic Cu Levels (mg/kg)				SEM	P-Value
	0	75	150	225		
Triglyceride (mg/dl)	779.5	676.8	671.3	680.6	135.61	0.550
Cholesterol (mg/dl)	159.4 <sup>A</sup>	149.6 <sup>AB</sup>	131.6 <sup>BC</sup>	129.2 <sup>C</sup>	10.27	0.001
Albumen (mg/dl)	1.33 <sup>A</sup>	1.14 <sup>B</sup>	1.11 <sup>B</sup>	1.39 <sup>A</sup>	0.055	0.000
Globulin (mg/dl)	2.36 <sup>A</sup>	1.86 <sup>B</sup>	1.92 <sup>B</sup>	2.46 <sup>A</sup>	0.201	0.000
Total protein (mg/dl)	3.69 <sup>A</sup>	3.00 <sup>B</sup>	2.97 <sup>B</sup>	4.05 <sup>A</sup>	0.344	0.000
Uric acid (mg/dl)	2.90	2.30	2.47	2.71	0.600	0.432
Calcium (mg/dl)	20.89 <sup>B</sup>	19.32 <sup>C</sup>	19.50 <sup>C</sup>	25.22 <sup>A</sup>	0.710	0.000
Phosphorus (mg/dl)	5.81	5.92	5.85	6.28	1.032	0.883

SEM: Standard error mean.

<sup>A,B</sup>: The differences indicated by different letters on the same line are statistically significant ( $p < 0.01$ ).**Table 5.** The effect of dietary different levels of organic copper on the incubation parameters of breeding Japanese quails

Parameters	Dietary Supplemental Organic Cu Levels (mg/kg)				SEM	P-Value
	0	75	150	225		
Fertility (%)	98.33	96.25	95.83	96.39	2.733	0.918
Hatchability (% of fertile eggs)	98.33	89.97	91.34	98.09	2.573	0.066
Hatchability (% of setting eggs)	96.67	86.97	87.77	94.65	4.294	0.314
Chick's weight (g)	8.91	8.65	8.72	8.62	0.168	0.617
Embryonic mortality (%)						
Early stage	0.01	4.73	3.16	0.92	2.013	0.365
Mid stage	0.01	2.66	0.01	1.01	0.736	0.069
Late stage	1.67	0.01	0.92	0.01	0.948	0.555

SEM: Standard error mean.

### Egg quality parameters

The effect of supplementation of different levels of Cu to diets on egg quality parameters has been given in Table 3. According to the findings obtained, although the addition of organic Cu to the diets had statistically significant effects on eggshell thickness, eggshell ratio, eggshell index and egg yolk color ( $p < 0.05$ ), but no statistically significant was observed effect on other quality characteristics of eggs ( $p > 0.05$ ). The addition of organic Cu to Japanese quail breeding diets increased the eggshell thickness and eggshell ratio values in the groups containing 150 and 225 mg/kg Cu compared to the control and 75 mg/kg level Cu groups. While the eggshell index was higher in the groups containing 150 and 225 mg/kg Cu compared to the control group, it was similar to the group containing 75 mg/kg Cu. The egg yolk color of the group containing 225 mg/kg organic Cu to the diet was higher than the control and 75 mg/kg organic Cu added groups but was similar to the group with 150 mg/kg Cu added ( $p < 0.05$ ).

### Serum parameters

The effect of diets containing different levels of organic Cu on serum triglyceride, phosphorus and uric acid concentrations was insignificant ( $p > 0.01$ ). Serum cholesterol concentration was significantly affected by the addition of Cu to the diet and was lower in the 0 and 75 mg/kg Cu added groups than in the groups with 225 mg/kg Cu added. Serum total protein and globulin levels were significantly higher in the control and 225 mg/kg Cu added groups than 75 and 150 mg/kg Cu added groups ( $p < 0.01$ ). Serum Ca concentration was significantly higher in the control and groups with 225 mg/kg Cu added than 75 and 150 mg/kg Cu, but the highest in the group with 225 mg/kg Cu added ( $p < 0.01$ ).

### Incubation parameters

The effects of adding different levels (0, 75, 150 and 225 mg/kg) of organic Cu to breeding Japanese quail diets on incubation parameters are given in Table 5. The addition of different levels of organic Cu (0, 75, 150, 225 mg/kg) to the diets did not cause any

statistical differences in the incubation parameters when compared to the control group ( $p>0.05$ ). Despite not observing any statistical differences between parameters, there was a relative reduction in fertility and late stage death when compared to the control group ( $p>0.05$ ).

## DISCUSSION

### Performance Parameters

The addition of Cu to the diet at 75 and 150 mg/kg levels decreased the final body weight and body weight gain, but this decrease was not at the level of 225 mg/kg in this study. Different dietary Cu levels did not cause a change in feed intake, egg production and feed conversion ratio in this study. The corn-soybean diet in this study could provide sufficient Cu to meet Cu requirements for maintenance and production, and supplementation of extra Cu did not affect egg production, egg weight and feed efficiency or feed intake. These results were supported by the findings of the researchers who found that 100 mg/kg Cu-protein supplement did not affect egg production, egg weight, and feed efficiency or feed intake (Kara et al., 2021). It was stated that performance parameters were not affected in studies where Cu added to the diet was lower than 300 mg/kg (Olgun et al., 2020; El-Husseiny et al., 2018; Azimi Youvalari et al., 2017; Jegede et al., 2012). In addition, it was reported that the addition of Cu to the diet at levels of 300 mg/kg and above caused a decrease in feed intake (Kim et al., 2016; Kaya et al., 2018). In the current study, performance characteristics such as egg production and feed intake were not affected by the addition of Cu to the diet, so it was concluded that the Cu level in the basal diet (8.18 mg/kg) in quails was sufficient for production performance.

### Egg quality parameters

It is well known that Cu has an effect on the calcite crystal formation in the eggshell and regulates the crystal holographic structure of the eggshell. The current study results showed that the addition of high levels of Cu (150 and 225 mg/kg) to the diet improved eggshell thickness and eggshell ratio, but did not improve eggshell breaking strength. Although the membrane thickness does not regulate the amount of mineral deposition in the other eggshell layers, it appears to be important for the structural organization. Copper most likely plays an important role in the thickening of the eggshell membrane, and, therefore, it is expected to affect eggshell breaking resistance (Berwanger

et al., 2018). El Shafei et al. (2012) reported that the addition of 125 and 250 mg/kg Cu to Japanese quail diets significantly increased eggshell thickness and eggshell weight compared to the control group. In another study with similar results to the present study, Kaya et al. (2018) reported that adding 200, 250 and 300 mg/kg Cu to laying hen diets had a positive effect on eggshell strength, but there was a non-statistically significant improvement in eggshell thickness. On the contrary, the addition of organic Cu (Cu-lysine) at the level of 250 mg/kg to the laying hens diet caused a significant decrease in eggshell thickness compared to the control group (Pekel and Alp, 2011). Olgun et al. (2020) reported that while the eggshell thickness was higher with the diet containing 20 mg/kg organic Cu than the control group, contrary to the results of the present study, the eggshell breaking strength and eggshell ratio were lower than the control group.

In the present study, egg albumen index, yolk index, egg specific gravity and Haugh unit were not significantly affected by the addition of Cu to the diet. Similar results were reported by Kaya et al. (2018), and in this study, the effect of Cu addition to the diet on the Haugh unit, yolk index and albumen index was insignificant in laying hens. The positive effect of Cu-Protein did not affect the Haugh unit, yolk index and yolk color score, eggshell thickness and egg-specific gravity (Kara et al., 2021). The results supporting the present study were also reported by Attia et al. (2011). Trindade-Neto et al. (2019) resulted that adding different levels of Cu to laying hen diets (from 18 to 111 mg/kg) did not have a significant effect on yolk height, albumen height, specific gravity, egg surface area and Haugh unit. In another study performed in laying hens (Pekel and Alp, 2011), they were reported that diets containing 250 mg/kg inorganic and organic Cu sources did not cause a significant difference in specific gravity.

The addition of 150 and 250 mg/kg Cu to the diet significantly increased the color of the egg yolk in this study. Previous study results reported that the addition of Cu to the diet did not cause any change in egg yolk color in laying birds (El Shafei et al., 2012; Fouad et al., 2016; Kaya et al., 2018; Kara et al., 2021). When dietary lipids produce peroxide, yolk pigmentation may be adversely affected due to the oxidation of carotenoids (Faitarone et al., 2016). Copper also protects cells from free radical damage, as it is a component of superoxide dismutase and increases iron transport as part of ceruloplasmin (Kara et al., 2021).

Therefore, by adding high-level Cu to the diet, it can prevent oxidation, thus protecting carotenoids and contributing to the egg yolk color.

### Serum parameters

The present results showed that the addition of high levels of Cu to the diet reduced serum cholesterol, but did not cause a change in serum triglyceride content. It may be due to the fact that the addition of organic Cu to the diet alters lipid metabolism and lowers cholesterol. Jegede et al. (2015) stated that adding 150 mg/kg Cu-protein to the laying hen diet reduced serum cholesterol concentration. Olgun et al. (2013) reported that 75 and 300 mg/kg Cu levels in laying hens decreased serum HDL values compared to control and 150 mg/kg levels. El-Shafei et al. (2012) reported that serum cholesterol values decreased with the addition of Cu to the diet. Contrary to these results, it was reported that the effect of diets supplemented with 200, 250 and 300 mg/kg Cu on serum cholesterol concentrations in laying hens was insignificant (Kaya et al., 2018). Since this study was performed in laying hens, this may be the possible reason for the inconsistency with the current study results. Fouad et al. (2016) reported that the addition of Cu at increased levels up to 28.6 mg/kg to the diet in laying ducks did not cause a significant change in serum cholesterol content, but the dietary Cu levels in this study were much lower than in the current study.

In the present study, serum triglyceride content was not affected by different dietary Cu levels. A similar result was found in Attia et al. (2011). According to Kaya et al. (2018) reported that the effect of diets supplemented with 200, 250 and 300 mg/kg Cu on serum cholesterol concentrations in laying hens was insignificant, but the serum triglyceride concentration was higher in the groups containing 250 and 300 mg/kg Cu compared to the control group. In the current study, serum total protein, albumin and globulin contents were decreased in groups containing 150 and 225 mg/kg Cu compared to the control group. In a study

that reported a contrary result, higher serum protein and globulin content were observed in chickens fed 120 mg/kg Cu than in the control group (Elsherif et al., 2019).

### Incubation parameters

In the present study, the addition of Cu to the diet did not cause any difference in fertility, hatchability, chick weight and embryonic mortality as incubation parameters. Attia et al. (2011) the dietary Cu level did not significantly affect fertility, hatchability and embryonic mortality. In another study conducted on incubation parameters in quails 100 and 200 mg/kg Cu have been added to the diet and it has been reported that there was no significant effect on fertility and hatchability (Abaza et al., 2009). El-Shafei et al. (2012) conducted a study on quails and reported that adding 125 mg/kg and 250 mg/kg Cu to the diet significantly reduced fertility but had no impact on the hatchability. Contents of Cu in eggs are dependent on the dietary supply to breeder hens (Kim et al., 2016) and, therefore, deficiencies or excesses affect egg quality and subsequent performance of the progeny. Deficiencies observed with the low Cu contents in the diets fed resulted in decreases in yolk Cu concentration (Berwanger et al., 2018).

### CONCLUSIONS

As a result, the addition of high levels of organic Cu to breeder quail diets did not cause a significant improvement in egg production parameters and reproductive characteristics. However, the addition of organic Cu to the diet at levels of 150 mg/kg and above resulted in a decrease in serum cholesterol concentration, while an increase in eggshell thickness was obtained. These results concluded that there is no need to add high levels of Cu to the diets of breeding quails, but it is effective in reducing serum cholesterol levels.

### CONFLICT OF INTEREST

None declared by the authors.

## REFERENCES

- Abaza IM, Ezzat W, Shoeib MS El-Zaiat AA, Hassan II (2009) Effects of copper sulfate on productive, reproductive performance and blood constituents of laying Japanese quail fed optimal and sub-optimal protein. *Int J Poult Sci* 8: 80-89.
- Arias VJ, Koutsos EA (2006) Effects of copper source and level on intestinal physiology and growth of broiler chickens. *Poult Sci* 85:999-1007.
- Attia YA, Abdallah AA, Zeweil HS, Bovera F, Tag El-Din AA, Araft MA (2011) Effect of inorganic or organic copper additions on reproductive performance, lipid metabolism and morphology of organs of dual-purpose breeding hens. *Arch Geflügelk* 75: 169-178.
- Aygun A, Sert D, Copur G (2012) Effects of propolis on eggshell microbial activity, hatchability, and chick performance in Japanese quail (*Coturnix coturnix Japonica*) eggs. *Poult Sci* 91: 1018-1025.
- Azimi Youvalari S, Farhoomand P, Baghban Kanani P, Hosseintabar-Ghasemabad B (2017) Effects of copper sulfate and arginine supplements on performance and carcass traits in broiler chickens fed with canola meal based diet. *Iran J Appl Anim Sci* 7(4): 647-654.
- Bao YM, Choct M, Iji PA, Bruerton K (2007) Effect of organically complexed copper, iron, manganese, and zinc on broiler performance, mineral excretion, and accumulation in tissues. *J Appl Poult Res* 16: 448-455.
- Berwanger E, Vieira SL, Angel CR, Kindlein L, Mayer AN, Ebbing MA, Lopes M (2018) Copper requirements of broiler breeder hens. *Poult Sci* 97:2785-2797.
- Carter TC (1975) The hen's egg: A rapid method for routine estimation of flock means shell thickness. *Br Poult Sci* 16: 131-143.
- EFSA (2016) Revision of the currently authorised maximum copper content in complete feed. *EFSA J* 14:4563.
- El-Shafei AA, Hassan MSH, Al-Gamal MA, El-Sayed OA (2012) Influence of fenugreek and copper sulfate levels in the diet on physiological and productive performance of laying Japanese quail. *Egypt Poult Sci J* 32: 909-930.
- Elsharif H, Fouad A, Nassar S, Wahba F, Elsabagh M, El-Iraqi K (2019) Effect of dietary copper sulphate on laying hen performance, egg quality, and oxidative stress in hot climate conditions. *Europ Poult Sci* 83.
- Faitarone ABG, Garcia EA, Roça RO, Andrade EN, Vercese F, Pelicia K (2016) Yolk color and lipid oxidation of the eggs of commercial white layers fed diets supplemented with vegetable oils. *Braz J Poult Sci* 18(1): 9-16.
- Fouad AM, Li Y, Chen W, Ruan D, Wang S, Xie WG, Lin YC, Zheng CT (2016) Effects of dietary copper supplementation on laying performance, egg quality and plasma cholesterol fractions in laying ducks. *Pak J Nutr* 15: 878-882.
- Guclu BK, Kara K, Beyaz L, Uyanik F, Eren M, Atasever A (2008) Influence of dietary copper proteinate on performance, selected biochemical parameters, lipid peroxidation, liver, and egg copper content in laying hens. *Bio Trac Elem Res* 125: 160-169.
- Hill GM, Shannon MC (2019) Copper and zinc nutritional issues for agricultural animal production. *Bio Trac Elem Res* 188: 148-159.
- Jegade AV, Oduguwa OO, Oso AO, Fafiolu AO, Idowu OMO, Nollet L (2012) Growth performance, blood characteristics and plasma lipids of growing pullet fed dietary concentrations of organic and inorganic copper sources. *Livestock Sci* 145: 298-302.
- Jegade AV, Oso AO, Fafiolu AO, Sobayo RA, Idowu OMO, Oduguwa OO (2015) Effect of dietary copper on performance, serum and egg yolk cholesterol and copper residues in yolk of laying chickens. *Slovak J Anim Sci* 48(1): 29-36.
- Kara K, Guclu BK, Senturk M, Eren M, Baytok E (2021) Effects of catechin and copper or their combination in diet on productive performance, egg quality, egg shelf-life, plasma 8-OHdG concentrations and oxidative status in laying quail (*Coturnix coturnix japonica*). *J Appl Anim Res* 49(1): 97-103.
- Karimi A, Sadeghi GH, Vaziry A (2011) The effect of copper in excess of the requirement during the starter period on subsequent performance of broiler chicks. *J Appl Poult Res* 20: 203-209.
- Kaya H, Kaya A, Macit M, Celebi S, Kaynar O (2018) Effects of dietary copper supplementation on performance, egg quality parameters, yolk cholesterol and fatty acid profiles in laying hens. *Indian J Anim Res* 52: 1623-1627.
- Kim JW, Kim JH, Shin JE, Kil DK (2016) Relative bioavailability of copper in tribasic copper chloride to copper in copper sulfate for laying hens based on egg yolk and feather copper concentrations. *Poult Sci* 95: 1591-1597.
- Megahed MA, Hassanin KMA, Youssef IMI, Elfgi ABA, Amin KA (2013) Alterations in plasma lipids, glutathione and homocysteine in relation to dietary copper in rats. *J Invest Bio* 1: 1-7.
- Nguyen HTT, Morgan N, Roberts JR, Swick RA, Toghiani M (2020) Copper hydroxychloride is more efficacious than copper sulfate in improving broiler chicken's growth performance, both at nutritional and growth-promoting levels. *Poult Sci* 99:6964-6973.
- NRC (1994) National Research Council, Nutrient Requirements of Poultry. 9th Rev. Ed., National Academy Press, Washington DC: pp 11-19.
- Olgun O, Aygun A (2017) Effect of copper supplementation on performance, eggshell quality and heterophil: lymphocyte ratio in aged laying hens housed at different stocking densities. *Anim Nutr Feed Technol* 17(1): 25-33.
- Olgun O, Yildiz A, Senturk ET (2020) The effect of supplementation of organic copper to commercial quail diets on performance, egg quality and haematological parameters. *Turkish J Agr Food Sci Tech* 8: 1517-1521.
- Olgun O, Yazgan O, Cufadar Y (2013) Effect of supplementation of different boron and copper levels to layer diets on performance, egg yolk and plasma cholesterol. *J TraEle Med Biol* 27: 132-136.
- Pekel AY, Alp M (2011) Effects of different dietary copper sources on laying hen performance and egg yolk cholesterol. *J Appl Poult Res* 20: 506-513.
- Persia ME, Baker DH, Parsons CM (2004) Tolerance for excess basic zinc chloride and basic copper chloride in chicks. *Br Poult Sci* 45: 672-676.
- Roychoudhury S, Nath S, Massanyi P, Stawarz R, Kacaniova M, Kolesarova A (2016) Copper-induced changes in reproductive functions: In vivo and in vitro effects. *Physiol Res* 65: 11-22.
- Świątkiewicz S, Arczewska-Włosek A, Jozefiak D (2014). The efficacy of organic minerals in poultry nutrition: review and implications of recent studies. *World's Poult Sci J* 70: 475-486.
- Trindade Neto MA, Saccomani APO, TseMLP, Dadalt JC (2019) Effect of dietary chelated copper and methionine and cysteine on performance, egg quality, and nutrient balance in brown laying hens from 20 to 49 wk old. *Can J Anim Sci* 99: 283-299.
- Wells RG (1968) Egg quality Characteristics in Egg Quality: A Study of the Hens Egg. Eds: Carter, T. C., Oliver and Boyd, Edinburgh: pp 214-225.