The Antibacterial effects and chicks quality response of copper nanoparticles of Japanese quail in hatching

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Abstract

An experiment was conducted to determine the effects of nano-copper at different levels (0, 14, 16 and 18 ppm named respectively as G1, G2, G3 and G4) as antibacterial against *E. coli* bacteria. Each group of 100 eggs was injected with *E. coli* bacteria (1 ml × 10^{-7}) and was exposed to 38°C in the hatcher. Significant increase was observed in G1 for the *E. coli* and total count bacteria. There was significant improvement for G5 in abnormal chicks with significant improvement for G2, G3, and G4 in addled eggs, live pipped chicks and dead pipped chicks. Significant improvement was also observed for nano copper groups in activity, general situation, appearance and feather condition, case of yolk retracted inside the abdomen, navel case and residual yolk membrane. There was a significant decrease in cholesterol, glucose, and triglyceride for the G2, G3, and G4 groups and a significant increase for the G2 group in GSH. At the same time, a significant decrease in MDA in the G2 and G3 groups and a significant increase in AST enzyme for the G1 group were observed.

Keywords: Antibacterial, nanotechnology, copper, hatching, Japanese quail

INTRODUCTION

The problem of high temperatures in hatcheries is considered as one of the dangerous problems that affect embryonic growth and development, especially when this coincides with the proliferation of microorganisms, especially harmful (AL-Saeedi *et al*., 2023a). Bird reproductive organs are home to bacteria that can contaminate egg yolks, egg whites and shell membranes. In bird breeding facilities, eggs may also be contaminated by bacteria via bird droppings or litter. In these situations, the bacteria will infiltrate the eggshell and shell membranes and when it reaches the embryo, it either causes pathological damage or malformations or it causes the embryo to die (Sharif *et al*., 2021; Ibrahim *et al*., 2022a,b). *E. coli* causes various illnesses, including cellulitis, omphalitis, coligranuloma, swollen head syndrome, septicemia by *E. coli* and peritonitis. As a result, the bacteria kills chicks during their early breeding, which can result in up to 25% of economic losses (Mroczek-Sosnowska *et al*., 2015). Heat stress also causes a negative balance between the amount of heat generated by the animal and the amount of heat dissipated in its environment. This affects the raising of broilers in particular due to the deterioration of their physiological, immune and production parameters (Al-Khafaji and Al- Jebory, 2018; Al-Jebory *et al*., 2023a; Al-Jebory *et al*., 2023b; Al-Jebory *et al*., 2023c). Nanoparticles of copper are considered a significant part of many antioxidant enzymes. Adding copper nanoparticles to broiler feed can improve antioxidant status, as it has been proven that copper nanoparticles in broiler feed improved Superoxide Dismutase (SOD) activity and reduced the level of malondialdehyde (Ognik *et al*., 2017), and copper nanoparticles have been used in several ways in feeding broilers. They have given positive results on growth performance (Mroczek-Sosnowska *et al*., 2016), improved physiological traits (Scott *et al*., 2018), and immune status (Ognik *et al*., 2017). Moreover, it works as an antibacterial agent (DeAlba-Montero *et al*., 2017). Goel *et al*., (2013) explained that copper nanoparticles improve the immune response of birds in addition to having many immune regulatory functions such as regulating the process of formation of white and red blood cells, their differentiation and maturation activity, macrophages, and modulate the immune response to disease resulting from inflammation, and stimulate immune responses. The current study aims to study the effect of injecting hatching eggs with nano-copper on the characteristics of hatched chicks and some physiological traits and pathological and thermal challenges in the hatchery.

MATERIALS AND METHODS

The first experiment, where the eggs were injected, was carried out at the Jaflawi poultry company hatchery in the Mahaweel region of Babil Governorate from February 3 to February 15, 2020. Four hundred eggs were used to incubate Japanese quail eggs (average weight of 11 g). 30 chicks from each group were selected, with each group being split into three duplicates, each containing ten chicks.

The egg injection solution was made using distilled water and nano-copper from Nano-sany Corporation. The particles were 10-20 nanometers in size, and distilled water was used to dilute them to the necessary concentrations. A 1 g sample of *E. coli* bacteria was mixed using 9 ml of sterile peptone water to get a dilution of 10^{-1}. Further, gradual dilutions were made by transferring 1 ml of it to several test tubes containing 9 ml of sterile peptone water until the dilution reached 10^{-7} (Son and Taylor, 2021). Eggs were injected at 15 days of age into the amniotic sac using an automatic syringe. The hole was closed with paraffin wax, the eggs were flagged, and all groups were exposed to 38°C in the hatcher.

The first group (G1) was injected with 0.1 ml/egg of a bacterial solution (1 ml x 10^{-7}) as a control group. The second group (G2) was injected with 0.1 ml/egg of copper nano solution (14 ppm with *E. coli* bacteria solution 1 ml x 10^{-7}). The Third group (G3) was injected with 0.1 ml/egg of copper nano solution (16 ppm with *E. coli* bacteria solution 1 ml x 10^{-7}) and the fourth group (G4) injected with 0.1 ml/egg of copper nano solution (18 ppm with *E. coli* bacteria solution 1 ml x 10^{-7}).

The chicks' hatched traits, hatching traits, and the phenotypic chick's traits were measured according to Tona *et al*., (2003); Al-Saeedi *et al*., (2022) and Al-Jaryan *et al*., (2023).

RESULTS AND DISCUSSION

Bacterial contamination

The bacterial contamination in nano-copper injection groups was lower than the control group (Table 1). There was a significant decrease (P<0.05) in *E. coli* bacteria, total bacteria, compared with the control group.

Table 1: Antibacterial effects of copper nanoparticles of Japanese quail in hatching (means ± standard error)

Groups	*E. coli*	Total bacteria
G1	6.18 ± 0.30 a	9.76 ± 0.41 a
G2	4.32 ± 0.80 c	6.59 ± 0.27 c
G3	5.10 ± 1.25 b	7.37 ± 0.51 b
G4	3.68 ± 1.76 d	6.11 ± 1.00 c
Significant	*	*

Hatching traits

The effect of the study appears on the physical traits of the chicks in table 2. We noted a significant increase (P<0.05) in abnormal chicks in G1, G2, and G3 groups compared with the G4 group, meanwhile no significant difference in chick's length, wing's length and leg length. There was a significant improvement (P<0.05) in addled eggs for G2, G3, and G4 groups, and there was a significant increase (P<0.05) in live pipped chicks and dead pipped chicks for G2 and G4, respectively (Table 3).

Chicks phenotypic traits

In chicks, phenotypic traits in table 4 showed a significant improvement (P<0.05) for nano copper groups in activity, general situation, appearance, feather condition, case of yolk retracted inside the abdomen, navel case and residual yolk membrane, as the superiority of groups G3 and G4 compared with the rest of the groups. Meanwhile no significant difference between groups in eye's appearance, Leg appearance and residual yolk.

The improvement in the productive qualities of copper treatments may be due to copper's immunological role through its ability to destroy pathogenic bacteria *in vitro* and *in vivo* (Xia *et al*., 2004; AL-Saeedi *et al*., 2023a). Copper also increases the production of growth hormone (Ibrahim *et al*., 2022a). Thus, it increases the metabolism of amino acids and proteins. Copper also acts as an antioxidant, as it can bind to free radicals and get rid of them by giving it two electrons. Copper is also

Table 2: effect of copper nanoparticles in chicks hatching traits of Japanese quail (means ± standard error)

Traits	Groups				Significant
	G1	G2	G3	G4	
Abnormal chicks %	5.00 ± 1.00 b	6.00 ± 2.10 a	5.00 ± 1.50 b	4.00 ± 1.65 c	*
Chick's length (cm)	7.00 ± 0.20	7.21 ± 0.13	7.10 ± 0.17	7.00 ± 0.75	N.S
Wing's length (cm)	1.02 ± 0.02	1.06 ± 0.08	1.00 ± 0.01	1.00 ± 0.03	NS
Leg length (cm)	1.55 ± 1.00	1.40 ± 0.23	1.50 ± 0.30	1.50 ± 0.41	NS

Table 3: effect of copper nanoparticles in chicks hatching traits of Japanese quail (means ± standard error)

Traits	Groups				Significant
	G1	G2	G3	G4	
Addled eggs %	41.7 ± 2.00 a	35.2 ± 2.45 b	33.5 ± 3.01 b	33.2 ± 2.75 b	*
Live pipped chicks %	8.00 ± 1.00 b	10.00 ± 1.12 a	7.00 ± 1.25 bc	6.50 ± 0.90 c	*
Dead pipped chicks %	5.00 ± 0.50 c	6.00 ± 0.75 b	4.00 ± 0.23 c	7.50 ± 0.80 a	*

Table 4: effect of copper nanoparticles in chick phenotypic traits of Japanese quail (means ± standard error)

Traits	Groups				Significant
	G1	G2	G3	G4	
Activity	4.32 ± 1.00 b	5.01 ± 1.05 a	4.96 ± 1.25 b	5.24 ± 0.75 a	*
General situation	75.5 ± 3.10 b	70.2 ± 4.50 c	80.0 ± 2.25 a	78.5 ± 75 ab	*
Appearance and feather condition	6.50 ± 0.01 c	7.00 ± 0.05 b	8.00 ± 0.14 a	7.50 ± 0.03 b	*
Case of yolk retracted inside the abdomen	7.00 ± 0.65 c	8.00 ± 0.13 b	9.00 ± 0.24 a	9.25 ± 0.30 a	*
Eye's appearance	10.0 ± 1.00	10.5 ± 2.04	10.0 ± 0.60	10.2 ± 1.25	NS
Leg appearance	10.0 ± 0.45	10.0 ± 0.85	10.5 ± 1.55	10.5 ± 1.00	NS
Navel case	8.00 ± 0.30 c	9.50 ± 0.50 b	10.0 ± 0.25 a	10.0 ± 0.13 a	*
Residual yolk membrane	7.50 ± 0.17 c	12.5 ± 1.00 a	9.00 ± 0.14 b	10.0 ± 0.98 ab	*
Residual yolk	12.0 ±2 .10	12.5 ± 1.25	13.1 ± 1.08	12.0 ± 1.40	NS

involved in the formation of ceruloplasmin, superoxide and catalase, as these compounds are characterized by their antioxidant activity (El-Basuini *et al.*, 2016). Adding copper nanoparticles as an alternative to antibiotics can have similar benefits on the growth performance of broilers (Ibrahim *et al.*, 2022). Copper nanoparticles are considered to enhance the growth performance of broilers to a greater extent because their doses are lower and also because of their size, which can improve digestive system absorption (Mohamed *et al.*, 2022). All of these properties can reduce the burden of thermal and disease stress to which the embryos were exposed in the hatchery, the effect of which was demonstrated in the chicks hatched from the eggs of the first treatment. The deterioration of the characteristics of hatching chicks in the first treatment may be caused by *E. coli* bacteria, which causes deterioration in the growth of the embryos and inflammation of the omphalitis, and yolk sac infection, as well as infections of the digestive gut, and incomplete development of the embryos (AL-Saeedi *et al.*, 2023a).

Biochemical traits

The effect of copper nanoparticles on some biochemical traits at hatching, showed a significant decrease (P<0.05) in cholesterol, glucose and triglyceride for G2, G3 and G4 groups compared to G1 group (Table 5). In GSH, the G2 group was significantly higher (P<0.05) than the G3, and G4 groups, at the same time the G1 group showed a significant increase (P<0.05) in MDA and AST, while no significant difference in ALT.

The significant increase in the concentration of cholesterol and triglycerides in the blood for group G1 chicks may be due to exposure to a disease challenge and the high temperature of the hatchery (AL-Saeedi *et al.*, 2023a). The reason for this increase may be a decrease in the rate of secretion of the thyroid hormones thyroxine, triiodide in T3, and thyroxine in T4 during exposure of fetuses to high temperatures, as the decrease in the activity of the thyroid glands generally leads to an increase in the level of cholesterol in the blood through a decrease in both the rate of cholesterol formation and the rate of its excretion in bile (AL-Saeedi *et al.*, 2023 b) and the decrease in cholesterol. The triglycerides in groups G2, G3, and G4 may be due to the role of copper nanoparticles, as they may lead to decreased cholesterol accumulation in tissues by reducing cholesterol synthesis or higher cholesterol degradation in the liver (Kim *et al.*, 1992; Bakalli and Pesti, 1995). The decrease in cholesterol concentration in the groups compared to the control group may be due to a deterioration in the process of cholesterol synthesis or the manufacture of low-density lipoprotein particles and low-density lipoprotein particles in the liver cells, which are the primary site of cholesterol synthesis and are then excreted into the circulation (Olotede, 2005). Exposure to high temperatures causes an increase in the levels of the hormones epinephrine, norepinephrine and the glucocorticoid hormone in the first stage, which leads to an increase in glucose levels in the blood through the process of glycogen lysis in the second stage. Heat stress leads to an increase in the hormone corticosterone in the birds' blood plasma, which leads to an increase in the level of glucose, as a result of its formation from protein sources (Stryer, 2000) which explains the high level of glucose in the control treatment, while its decrease in the two treatments with nano-copper may be due to its antioxidant role, which reduced the severity of heat stress (Abdullah *et al.*, 2022; AL-Saeedi *et al.*, 2023a). Exposure to diseases and high temperatures cause an imbalance in the production of free radicals (ROS), the activity of glutathione peroxidase and antioxidant enzymes (SOD) in broilers (De Vega *et al.*, 2016), where the production and activation of glutathione peroxidase is the mechanism of the cellular antioxidant defense system that increases upon the production of radicals. Free radicals, which increase with increasing oxidative stress to protect tissues from damage (Altan *et al.*, 2003), and the improvement in the level of this enzyme in groups G3 and G4 may be due to the role of copper in stimulating the activity of the antioxidant system, as copper works as an antioxidant by removing free radicals and preventing peroxidation. Lipids and nano-copper are considered part of the active sites of many enzymes (Johnson *et al.*, 1992), as they participate with zinc in the activity of superoxide dismutase (Cu-Zn-SOD) (Gaetke and Chow, 2003). These enzymes play an important role in antioxidant defense, connective tissue formation and mitochondrial respiration (Angelova *et al.*, 2011; Maltais *et al.*, 2013; Palumaa, 2013). The improvement in antioxidant activity explains the decrease in MDA level in the nano-copper injection groups. As for the AST enzyme, we note that its level is high in the serum of control group G1 birds, which may be due to the high corticosterone hormone due to heat stress, which affects several liver enzymes, including AST and ALT, leading to an increase in their levels in the blood (Richard and Preston, 2006), and this is a particular stimulus for that

Table 5: effect of copper nanoparticles in some biochemical traits at the hatching of Japanese quail (means ± standard error)

Groups	G1	G2	G3	G4	Significant
Cholesterol	174.2 ± 2.13 a	162.8 ± 6.56 b	146.9 ± 0.21 c	137.9 ± 1.94 cd	*
Glucose	225.2 ± 1.22 a	187.8 ± 1.43 b	183.7 ± 1.16 b	159.7 ± 0.53 c	*
Triglyceride	146.6 ± 0.65 a	128.1 ± 1.89 b	116.8 ± 1.03 c	114.5 ± 0.83 c	*
GSH	187.6 ± 1.34 ab	191.0 ± 1.06 a	170.8 ± 0.55 b	171.1 ± 1.55 b	*
MDA	8.26 ± 0.27 a	6.23 ± 0.17 b	6.32 ± 0.06 b	7.04 ± 0.07 ab	*
AST	46.9 ± 0.11 a	43.5 ± 0.03 ab	40.1 ± 0.07 b	44.1 ± 0.04 ab	*
ALT	3.22 ± 0.21	3.45 ± 0.23	3.14 ± 0.08	3.55 ± 0.10	NS

increase. The activity of these enzymes may be attributed to the particular requirements for generating glucose from non-carbohydrate sources such as amino acids, as these enzymes stimulate the transfer of the amino group from alpha-type amino acids to keto acids. Keto acids are an essential source of energy that is part of the carbs cycle to produce energy in mitochondria (Stryer, 2000), while the low level of the AST enzyme in group G3 may be caused by copper, which is due to its antioxidant role (Mohamed *et al*., 2022).

CONCLUSION

Supplementation of nano-copper (16 and 18 ppm) was able to enhance the chick's quality, hatching and physical traits of embryo growth under challenging conditions by bacterial contamination and height temperature of hatcher. Injecting nano-copper in hatching eggs, or sparing on eggs or sterilized hatcher would reduce *E. coli* infection.

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