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Effect of in Ova Injection with Nano-copper in Productive Performance of Japanese Quail Exposed to Pathological and Environmental Challenges

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ABSTRACT

To evaluate the effects of nano-copper on pathogenic bacteria in vivo, 400 fertilized eggs of quail were used; divided into four groups (T_1, T_2, T_3 and T_4) each group of 100 eggs was injected with *E. coli* bacteria (1 m × 10^{-7}). Further, groups T_2, T_3 and T_4 were injected with copper nanoparticles at concentrations of 14, 16 and 18 ppm, respectively. Each group was exposed to 38 ^{0}C in hatcher to receive 30 chicks from the hatched eggs. Significant increase was observed in T_1 for the percentage of embryo mortality and total mortality, in group T_4 for the rate of weight gain and feed conversion efficiency, and in group T_3 for the percentage of hatching, chick weight at hatching and total live body weight. The rate at which feed was consumed was also significantly higher in T_4.

Key words: *E. coli*, Japanese quail, injection, hatching eggs, nano-copper

INTRODUCTION

Bacteria can live in the reproductive organs of birds, causing contamination of egg yolk, egg white and shell membranes. Eggs can also be exposed to bacterial contamination when they come into contact with bird droppings or litter in bird breeding halls. In both cases, the bacteria will penetrate the egg shell and shell membranes. It reaches the fetus and leads to the death of the fetus or the occurrence of pathological injury or deformities (Sharif *et al.*, 2021; Ibrahim *et al.*, 2022 a, b). The bacteria causes many diseases such as peritonitis, omphalitis, coligranuloma, swollen head syndrome, septicemia caused by *E. coli*, cellulitis, and more and thus causes the death of chicks during the first ages, causing economic losses up to 25% due to the death of chicks during the first week (Mroczek-Sosnowska *et al.*, 2015). The most common type of bacteria that contaminates hatching eggs is *E. coli* and one of the modern means to reduce the severity of contamination or disease infections is the use of water copper nanoparticles which are characterized by their small size of particles, which enable them to penetrate the membranes of bacterial cells (Rossetto *et al.*, 2014). Copper nanoparticles directly affect the bacterial cell membrane, causing its rupture and thus the death of bacteria. Copper is with the rare mineral important for growth and metabolism inside the body (Swiatkiewicz *et al.*, 2014). Scott *et al.* (2017) showed that injecting nano-copper into chicken embryos boosted final body weight, weight gain rate, feed conversion efficiency, as well as its function in enhancing body muscle mass and metabolic rate. It participates in the processes of phosphorylation oxidation in mitochondria and boosts the generation of thyroxine and growth hormones, iron metabolism and its resistance to free radicals (Ibrahim *et al.*, 2022b) with multiple immune roles (Valli and Balakrishnan, 2016). Based on the aforementioned information, the goal of this study was to determine the impact of injecting copper nanoparticles into hatching eggs of Japanese quail exposed to an *E. coli* pathological challenge in order to demonstrate how this affects bacterial infection of the embryos and enhances performance of the subsequent growth of the chicks that have been hatching.

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MATERIALS AND METHODS

The first experiment, in which the eggs were injected, took place at the Jaflawi poultry company hatchery in the Mahaweel region of Babil Governorate from February 3 to 15, 2020. The second experiment, in which the eggs from the first experiment's hatched, chicks were raised, took place at a poultry farm in Babil Governorate from February 3 to April 12, 2020.

Four hundred eggs were used for the incubation of Japanese quail eggs, with an average weight of 11±1 g. Thirty chicks from each group were selected and raised for 42 days, with each group being split into three duplicates, each containing 10 chicks.

The egg injection solution was made using distilled water and nano-copper from the American Company Nano-sany Corporation. The particles were 10-20 nanometers in size, and distilled water was used to dilute them to the necessary concentrations.

One g of *E.coli* bacteria sample was mixed using 9 ml of sterile peptone water to get a dilution of 10^{-1}. Further, gradual dilutions were made from it 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 and the eggs were flagged and all groups exposed to 38 ^{0}C in hatcher.

The first group (T_1) was injected with 0.1 ml/egg of a bacterial solution (1 ml x 10^{-7}) as a control group. The second group (T_2) was injected with 0.1 ml/egg of copper nano solution (14 ppm with *E. coli* bacteria solution 1 ml x 10^{-7}). Third group (T_3) was injected with 0.1 ml/egg of copper nano solution (16 ppm with *E. coli* bacteria solution 1 ml x 10^{-7}). Fourth group (T_4) was injected with 0.1 ml/egg of copper nano solution (18 ppm with *E. coli* bacteria solution 1 ml x 10^{-7}). The chicks were fed on the standard diet (Table 1).

The hatching %, weight at hatching (g) and the embryonic mortality % was calculated. The average of live bodyweight and weekly weight gain rate (g/m) was calculated as feed consumption and feed conversion ratio. The mortality was computed based on data collected from the beginning of the study until the end of the fifth week as.

Table 1. The percentages of diet components and their chemical analysis

Ingredients	Starter (%)	Finisher (%)
Yellow corn	30	40
Wheat	28.25	24
Soybean meal (48% protein)	31.75	24.8
Protein concentrate*	5	5
Sunflower oil	2.9	4.4
Limestone	0.9	0.6
DCP calcium diphosphate	0.7	0.9
Salt	0.3	0.1
Mix vitamins and minerals	0.2	0.2
Total	100	100
Crude protein (%)	23	20
Metabolic energy (kilo calories/kg feed)	3027	3195.3
Lysine (%)	1.2	1.1
Methaionine (%)	0.49	0.46
Cystine (%)	0.36	0.32
Methaionine+cysteine (%)	0.85	0.76
Available phosphorus (%)	0.45	0.49
C/P (%)	131.61	159.77

*BROCON-5 SPECIAL W protein concentrate.

Total mortality rate (%) = 100 x [(The number of mortality birds during the experiment)/(The total number of birds)]. The data were analyzed statistically using Analysis of Variance.

RESULTS AND DISCUSSION

A significant difference was observed between groups T_3 and T_4 and groups T_1 and T_2 in terms of the rate at which chicks hatched, as well as the superiority of group T_2 over group T_1. There was no discernible difference between groups T_3 and T_4 on the one hand and groups T_2 and T_4 on the other (Table 2). Group T_2 surpassed group T_1 in both performance and effectiveness. Groups T_3 and T_4 considerably outperformed groups T_1 and T_2, and the group in terms of the

Table 2. Effect of in ova injection with nano-copper in productive performance of Japanese quail exposed to pathological and environmental challenges in hatching (%), weight at hatching g/chicks) and embryonic mortality (%)

Groups	Mean±stander error		
	Hatching (%)	Weight at hatching (g/chick)	Mortality (%)
T_1	58.25±1.54c	6.55±0.50c	41.75±0.17a
T_2	64.75±1.03b	6.80±0.04b	35.25±0.33b
T_3	66.50±1.35a	7.10±0.07a	33.50±0.11c
T_4	66.75±1.72a	6.85±0.30ab	33.25±0.20c
Significance	**	**	**

*Different letters indicate significance.

percentage of fetal fatalities. There was no discernible change between T_3 and T_4 therapies as compared to T_2 and T_1, respectively.

It was also used as an alternative to antibiotics that affected chicken performance by affecting the metabolism of nutrients as it affected the immune system, leading to metabolic changes known as immune stress. Copper affects the function of living organisms as the mechanism of action of copper molecules by affecting pathogenic microorganisms, which had a direct impact on animal growth performance (Valli and Balakrishnan, 2016) usually the animals were placed in large groups to produce high-density food and were quickly reared for slaughter, and this significantly improved the resistance of the embryos to high temperature in the hatchery.

The improvement in hatching rate, embryo mortality and hatched chick weight in T_4 and T_3 groups may be due to the injection of nano-copper, as Mroczek-Sosnowska *et al.*, (2015, 2016) indicated that nano-copper helped in the development of embryos' blood vessels. The injection of nano-copper into chicken embryos improved the body weight and feed conversion factor compared to the control group. Also, nano-copper reduced the harmful action of *E. coli* bacteria, as it had the ability to reduce harmful bacteria associated with potentially fatal microbial infections that returned to the fetus. Due to the small size of the particles and their high surface area, which allowed them to bond closely with the membranes of bacterial cells, where the copper nanoparticles directly affected the bacterial cell membrane, causing its rupture and thus the death of bacteria (Upadhaya *et al.*, 2016). Cu nanoparticles showed more toxicity on *E. coli* and *Lactobacillus brevis* compared to normal size Cu nanoparticles, where Cu nanoparticles caused bacteria degradation. Rossetto *et al.* (2014) attributed the reason for the effect of copper nanoparticles on bacteria due to their small size and ability to penetrate the bacteria membrane, causing damage (Harikumar and Anisha, 2016). Also, the development in hatching traits may be due to the injection of silver nanoparticles into the amniotic fluid. The intake by the embryo at the stage of closing the navel to reduce the stress that the embryo was exposed to in the last third of hatching, resulted by the high temperature of the embryo and the hatcher, as well as the process of inverting the eggs when they were transferred from the incubator to the hatcher, which led to the development of hatching characteristics as a result of the properties of the antioxidant copper, Arafat *et al.* (2019) noted that injecting different sources of copper (Nanocopper, Cu acetate) at the age of 10-day old chicken embryos improved the weight of the hatching chicks as Nano Cu stimulated formation of red blood cells and hemoglobin (Amaral *et al.*, 2018) and promoted the growth of embryos through its participation in many metabolic processes and raised the level of arginine in the liver. Copper increased the production and formation of thyroid hormones in T_3 and T_4, which had an effective role in increasing the growth of bird embryos by raising metabolism and improving oxygen consumption. As for the high percentage of embryos' deaths in the T_1 control group, this may be due to the injection of eggs with *E. coli* bacteria, which caused a pathological infection of the embryos and their inability to hatch due to omphalitis and Yolk sac infection. Claudia *et al.* (2019) indicated that bacterial contamination with *E. coli* bacteria in chicken embryos was concentrated in the ileum and caecum. The researcher used injecting eggs with probiotics as a group to reduce the infection of *E. coli* bacteria.

Table 3 displays the impact on live body weight during 1-6 weeks of a bird's life after injecting nano-copper with *E. coli* bacteria into hatching eggs. The superiority of group T_4 over the other groups and the superiority of groups T_1 and T_2 over group T_3 were both observed during the first week. In the second, third and fifth weeks, group T_3 was superior to groups T_1 and T_2 and groups T_2 and T_4 were superior to groups T_1. Groups T_2 and T_4 exhibited superiority in the fourth week compared to groups T_1 and T_3. In the last week of the experiment, the data revealed significant superiority for group T_1.

Table 4 shows the impact of injecting *E. coli* bacteria into hatching eggs on the rate of weight gain for 1 - 6 weeks. It was discovered that group T_4 significantly outperformed groups T_1, T_2 and T_3 during the first week. During the second week, group T_3 significantly outperformed the other groups under study, while group T_2 outperformed groups T_3 and T_4, respectively. Groups T_3 and T_4 considerably outperformed T_1 in the third week. The fourth

Table 3. Effect of in ova injection with nano-copper in productive performance of Japanese quail exposed to pathological and environmental challenges in the live body weight (g/chick)

Groups	Mean±stander error					
	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
T_1	30.19±0.72b	61.21±0.32c	92.67±1.80c	131.18±0.39c	169.03±2.13c	190.92±2.00c
T_2	30.50±0.13b	64.75±0.10b	96.13±0.74b	138.24±0.80a	172.17±2.10b	193.27±2.02b
T_3	29.90±0.25c	66.80±0.90a	102.00±2.00a	135.09±1.00b	175.93±2.25a	198.70±2.11a
T_4	32.20±0.50a	65.00±0.75ab	100.35±1.50ab	137.80±0.55a	173.20±2.17ab	197.13±2.25a
Significant	*	*	*	*	*	*

*Different letters indicate significance.

Table 4. Effect of in ova injection with nano-copper in productive performance of Japanese quail exposed to pathological and environmental challenges in weight gain (g/chick)

Groups	Mean±stander error						
	1 week	2 week	3 week	4 week	5 week	6 week	Total
T_1	23.09±1.09b	31.02±0.36c	31.46±1.52b	38.51±3.10b	37.85±2.15b	21.89±1.11c	183.82±1.01c
T_2	23.70±1.67b	34.25±0.47b	31.38±1.21b	42.11±2.00a	33.39±1.75d	21.10±1.98c	186.47±1.22b
T_3	23.35±1.71b	36.90±1.03a	35.20±1.37a	33.09±2.33c	40.84±1.40a	22.77±1.19b	192.15±1.51a
T_4	25.35±1.49a	32.80±0.94	35.35±1.20a	37.45±2.41b	35.40±1.07c	23.93±1.20a	190.28±1.85ab
Significant	*	*	*	**	*	*	*

*Different letters indicate significance.

week of the trial saw a highly significant superiority for the T_2 over the other groups that had been investigated and the control group.

Table 5 shows the impact of injecting *E. coli* bacteria into hatching eggs on the rate of feed consumption during 1 - 6 weeks. For the first, second and fourth weeks, it was discovered that there were no significant differences between groups. However, in the third week, a significant superiority was detected between the groups T_3 and T_4 and the groups T_1 and T_2. In the fifth week, the groups T_1 and T_4 considerably exceeded the groups T_2 and T_3 whereas in the sixth week, the group T_3 significantly outperformed the group T_1. Thus, the group had a highly significant impact on the rate of total feed consumed.

The impact of infusing nano-copper with *E. coli* bacteria into hatching eggs on the feed conversion factor for weeks 1-6 is shown in Table 6. T_4 group showed a significant improvement compared to the other groups during the first week, while T_3 group showed a substantial improvement in second, third and fifth weeks compared to the other groups. In fourth week, group T_2 improved compared to groups T_3 and T_4. The group T_4 improved compared to group T_3. In the sixth week, groups T_3 and T_4 improved significantly compared to the rest of the studied groups. Similarly, for total feed conversion ratio, groups T_3 and T_4 improved compared to the rest of the study groups.

Table 7 indicates the mortality rate during the breeding period (1-6 weeks), and it was found that there was a significant increase ($P<0.05$) in the mortality rate in the control group T_1 compared to the rest of the studied groups.

Table 5. Effect of in ova injection with nano-copper in productive performance of Japanese quail exposed to pathological and environmental challenges in feed consumed (g/chick)

Groups	Mean±stander error						
	First week	Second week	Third week	Fourth week	Fifth week	Sixth week	Total
T_1	25.00±1.00	90.16±0.80	115.63±0.17b	139.27±1.10	151.19±0.50a	154.67±1.90b	650.92±1.15b
T_2	25.35±0.95	89.80±1.00	113.51±0.25b	140.00±1.00	147.13±0.41b	155.25±1.30ab	645.69±1.83c
T_3	25.10±1.18	89.00±0.75	119.20±0.23a	138.95±1.30	148.70±0.87b	156.50±0.95a	652.35±1.79ab
T_4	25.47±1.25	90.31±0.60	120.13±0.35a	139.41±1.50	150.50±0.59a	155.18±1.25ab	655.53±1.03a
Significant	NS	NS	*	NS	*	*	**

*Different letters indicate significance.

Table 6. Effect of in ova injection with nano-copper in productive performance of Japanese quail exposed to pathological and environmental challenges in feed conversion ratio (kg feed/kg meat/chick)

Groups	Mean±stander error						
	1 week	2 week	3 week	4 week	5 week	6 week	Total
T_1	1.082±0.179a	2.906±0.110a	3.675±0.117a	3.616±0.120bc	3.994±0.312b	7.065±0.259b	3.541±0.124a
T_2	1.069±0.126c	2.621±0.169c	3.617±0.201b	3.324±0.210c	4.336±0.255a	7.357±0.281a	3.462±0.117b
T_3	1.074±0.330b	2.411±0.132d	3.386±0.141d	4.199±0.183a	3.641±0.301c	6.873±0.217c	3.395±0.105c
T_4	1.004±0.171d	2.753±0.107b	3.403±0.137c	3.722±0.192b	4.251±0.337ab	6.484±0.251d	3.445±0.193b
Significant	*	*	*	**	**	*	*

*Different letters indicate significance.

Table 7. Effect of in ova injection with nano-copper in productive performance of Japanese quail exposed to pathological and environmental challenges in mortality (%)

Groups	Mean±stander error
T_1	18.00±2.91a
T_2	2.50±3.00c
T_3	5.75±2.18b
T_4	4.25±2.51b
Significant	*

*Different letters indicate significance.

The improvement in the growth performance of nano-copper injections, as copper participated in many metabolic activities, as it contributed to energy metabolism and helped to increase the metabolic rate by stimulating the thyroid gland to secrete thyroxine. It also regulated its absorption into the blood by controlling calcium levels, and thyroxine was necessary in protein and energy metabolism, as it worked to increase muscle growth and build proteins in the body and enhanced the functions of growth hormone (Al-Behadili, 2015) as it worked to increase the production of growth hormone (Ibrahim *et al.*, 2022a). It increased the metabolism of amino acids and proteins. Copper also acted as an antioxidant as it had the ability to bind with free radicals and get rid of them by giving it two electrons (El-Basuini *et al.*, 2016). Copper was also included in the formation of ceruloplasmin, superoxide dismutase and catalase, where these compounds were characterized by their antioxidant activity. The low percentage of chick mortality in groups T_2, T_3 and T_4 may be caused by the injection of nano-copper, which inhibited the growth of bacteria due to its harmful effect on them. As for the high mortality in group T_1 it may be infection with *E. coli* bacteria, which caused this bacteria infecting many diseases such as peritonitis, omphalitis, coligranuloma, swollen head syndrome, colisepticaemia, and cellulitis and thus causing the death of chicks (Swiatkiewicz *et al.*, 2014).

CONCLUSION

It was concluded from the study that nano-copper had the ability to inhibit the ability of *E. coli* bacteria to disease in embryos and after hatching as well as the role of nano-copper in the early feeding of embryos through the reduced effect of environmental stress in hatchery and farm

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