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EFFECT OF INJECTION IN HATCHING EGGS WITH DIFFERENT CONCENTRATIONS OF NANO-SILVER AT 17.5 DAYS AGE IN SOME HATCHING TRAITS AND BLOOD PARAMETERS FOR BROILER CHICKENS (ROSS 308)

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Abstract

This study was conducted in Al-Anwar Hatchery Company for Poultry by injecting hatching eggs at the age of 17.5 days from the embryos with the silver nanoparticle. The hatched chicks were raised from the injected eggs in the poultry field belonging to the Department of Animal Production, College of Agriculture, Al-Qasim green University for the period from 6/8/2017 to 9/9/2017. After that, the laboratory works were then conducted, where 168 chicks of unsexed broiler chickens (Ross 308) were used, with one day age, which raised in pens with dimensions of (1 x 1.5 m). The chicks were randomly divided into 7 treatments, each treatment included 3 replicates, each replicate containing 8 chicks, nano-silver was used by injecting it into the eggs with concentrations of (0, 4, 6, 8, 10, 12,14 ppm) for the treatments of (T1, T2, T3, T4, T5, T6, T7), respectively. The chicks were raised in the field for 35 days. The study showed the following results. There was a significant superiority ($P < 0.05$) in the percentage of hatching for the T7 and T6 treatment, which recorded the lowest percentage of embryonic mortalities and it occurred a significant superiority for the T6 treatment in weight at hatching. There was significant excelling ($P < 0.05$) in the number of white blood cells for the treatments T7 and T1. As for the percentage of Neutrophils cells, the T1 treatment was excelled compared to the rest of the treatments, the T5, T3, and T2 treatments have also significantly excelled. As for the H / L ratio, The control treatment T1 was significantly excelled compared to the rest of the treatments.

***Keyword*:** Nano-silver, broiler, injection

Introduction

Nanotechnology is considered a modern technology and has many applications that include manufacturing the particles in the nanometer range. The unique properties of nanoparticles are due to their small size as well as their chemical composition and surface structure. The characteristic properties and physical changes for various materials in the nanometer have given rise to the development of the properties of industrial products, resulting in a real and significant increase in industrial and medical applications (Ahmedi *et al.*, 2013). Ajobiewe *et al.* (2014) indicated that silver metal is a natural antimicrobial agent (killing harmful bacteria, yeast, fungi, and viruses), it has not yet had a negative effect on human tissue, silver is also anti-inflammatory, reducing pain and promoting natural healing. Silver is widely used in medicine, which reduces pain and promotes natural healing. Silver is widely used in medicine, and to protect us from food poisoning, new applications are being developed daily and include many areas in the field of dentistry and medicine. Studies have shown that the new silver liquid may save your life in case of an H5-N1 bird flu pandemic. It is safe to say that silver is used almost every day by every man, woman or child in the civilized world (fung and Bowen, 1996). The sources indicated that the injection of nano-silver has led to increasing the immunity of the hatched eggs (Saki and Salary, 2015; Subrat *et al.*, 2015). Lane *et al.* (2012) noted that the injection of hatched eggs with silver nanoparticle enhanced the absorption of yolk in embryos and stimulated growth and embryonic development, where nano-silver is considered an antimicrobial against a wide spectrum of bacteria (Feng *et al.*, 2000; Lansdown, 2004). Al-Jubouri *et al.* (2018) indicated that injection of hatching eggs with silver nanoparticle at concentrations of (18, 16, 14, 12 ppm) improved the percentage of hatching and the weight at hatching and reduced the percentage of embryonic mortalities and increased the length and width of the villus. Therefore, the current study aims to know the effect of injecting hatching eggs at the age of 17.5 days from embryonic age with different levels of nano-silver in some hatching traits and blood Parameters for Broiler Chickens (Ross 308) and determine the best level of injection.

Materials and Methods

The saline solution (NaCl) was used in the preparation of egg injection solutions. The nano-silver material was obtained from Nanosany company, with size (20 nm) and morphological (spherical) form. A168 chicks of unsexed broiler chickens (Ross 308) were used, with one day age, which raised in pens with dimensions of (1 x 1.5 m). The chicks were randomly divided into 6 treatments, with 3 replicates, each replicate containing 8 chicks, nano-silver was injected into the eggs with concentrations of (0, 4, 6, 8, 10, 12, 14 ppm) for the treatments of (T1, T2, T3, T4, T5, T6, T7), respectively, where hatching eggs are injected at 17.5 days of embryo age, where the injection was with rate of (0.25 ml/egg).

Feeding diets

The chicks were fed on the initiator diet (23% protein content and the amount of metabolic energy is 3027 kcal/kg feed) from one day age until the third week of the birds' age. It was then replaced by the growth diet (20% protein content and the amount of metabolic energy is 3195.3 kcal/kg feed) until the end of the fifth week. Feed and water were provided freely and the used diet is as shown in Table (1)

Table 1: shows the percentage of the used diet ingredients in the study and their chemical composition.

Feed materials	Initiator diet 1-21 day	Growth diet 22-35 day
yellow corn	30	40
wheat	28.25	24
Soybeans meal (48% protein)	31.75	24.8
concentrated Proteins*	5	5
Sunflower oil	2.9	4.4
limestone	0.9	0.6
Calcium diphosphate DCP	0.7	0.9
salt	0.3	0.1
A mixture of vitamins and minerals	0.2	0.2
Total	100	100
Crude protein (%)	23	20
The calculated metabolic energy (kcal / kg feed)	3027	3195.3
Lysine (%)	1.2	1.1
Methionine (%)	0.49	0.46
Cysteine (%)	0.36	0.32
Methionine + Cysteine (%)	0.85	0.76
Available phosphorus (%)	0.45	0.49
percentage of Energy: Protein C / P%	131.61	159.77

* Concentrated Protein (BROCON-5 SPECIAL W): Chinese origin, containing 40% raw protein, 3.5% fat, 1% fiber, 6% calcium, 3% available phosphorus, 3.25% lysine, 3.90% methionine + cysteine , 2.2% sodium, 2,100 kcal / kg metabolic energy, 20000 IU vitamin A, 40000 IU vitamin D3, 500 mg vitamin E, 30 mg vitamin K3, 15 mg vitamin B1 + B2, 150 mg B3, 20 mg B6,300 mg B12, 10 mg folic acid, 100 µg biotin, 1 mg iron, 100 mg copper, 1.2 mg manganese, 800 mg zinc, 15 mg iodine, 2 mg selenium, 6 mg cobalt, 900 mg anti-oxidant (BHT).

** the chemical analysis for the diet according to (NRC, 1994).

The studied traits

(i) The percentage of Hatching

It was calculated for the treatments according to the following equation (Khattab *et al.*, 1992):

$$\text{The percentage of Hatching} = \frac{\text{Number of hatched chicks}}{\text{Number of enriched eggs}} \times 100\%$$

(ii) Weight at hatching (g).

The hatched chicks were weighed for each treatment in the hatchery, where the box is weighed empty and then weighing it with the chick and then the empty weight of the box is extracted from the weight of the box with the chick. This represents the weight of the chick after hatching.

(iii) The percentage of embryonic mortalities

It was calculated as follows:

$$\text{The percentage of embryonic mortalities} = \frac{\text{Number of mortality chikcs}}{\text{Number of enriched eggs}} \times 100\%$$

(iv) Blood parameters

The Packed cell volume (PCV), Red blood cells count, and white blood cells count were calculated by taking blood samples in tubes containing a coagulation inhibitor, which included 1 male bird and 1 female from each replicate at age of 7 days from the femoral vein. The Packed cell volume (PCV) was calculated According to the method of (Archer, 1965), the white and Red blood cells count were calculated According to the method of (Pierson, 2000). Heterophil (H) and lymphocytes (L) were counted by taking blood smears on glass slides and dyed with Wright-Giemsa according to (Shen and Patterson, 1983). They were then examined under a microscope as reported in (Campbell, 1988). The H/L ratio was extracted by dividing the total number of heterophils on total lymphocytes.

(v) Statistical analysis

The Statistical Analysis System (SAS, 2012) was used in data analysis to study the effect of different treatments in the studied traits according to completely randomized design (CRD). The significant differences between the averages were compared with the Duncan's New Multiple (Duncun, 1955) test.

Mathematical Model:

$$Yij = \mu + Ti + eij$$

Results and Discussion

The percentage of Hatching (%), the percentage of embryonic mortalities (%) and weight at hatching (g)

Table (2) shows the effect of injection of hatching eggs with the silver nanoparticles for the broiler chicks on the percentage of Hatching (%), the percentage of embryonic mortalities (%) and weight at hatching (g). It was showed a significant excelling ($P<0.05$) for the treatments (T4, T6, T7) was observed in the percentage of hatching, As for the weight at hatching (g), The T6 treatment was excelled followed by T7 treatment compared to the rest of the treatments. The T4 treatment has excelled on the treatments (T3, T3, T2, T1). The T3 treatment was excelled on the treatment (T5, T2, T1), while did not show a significant difference between the treatments of (T5, T2, T1), As for the percentage of embryonic mortalities, the two treatments (T3, T1) were excelled compared to the other treatments. There were no significant differences between the treatments (T7, T6, T5, T4, T2). The superiority of hatching and the weight at hatching may be due to the fact that nano-silver injection has led to increasing metabolic rate in the embryos and improving the growth in the hatched chicks. It may also lead to increase the efficiency of utilization from the fat found in the yolk for the production of energy for the embryo and increase transport Oxygen on the surface level (Lane Pineda *et al.*, 2012; Sato *et al.*, 2006; Druyan, 2010; Saki and Salary,

2015). The injection of eggs hatching with silver nanoparticles led to improving the growth of the hatched chicks by improving their immune system due to the antibacterial properties of silver nanoparticles, as evidenced by the superiority of the percentage of hatching for the treatments (T7, T6), which gave the highest percentage of hatching and this result did not agree with (Subrat *et al.*, 2015) who indicated that nano-silver did not significantly affect the average weight of the chicks body when hatching but improved its immunity, where It was found that the injection of nano-silver with the amino acid (Cysteine) led to increasing the immune level of the embryo due to the nano-silver properties against the germs and the role that Cysteine played in the production of antibodies (Grimble, 2006), which is agree with (Sirimongkolkasem, 2007) that Nano-silver increases the immunity of embryos. This is similar to (Grodzik and Sawosz, 2006) found that nano-silver injection with concentration of (10 ppm) at age of (5, 11, 17) from the age of embryos which did not affect in the growth of embryos and the weight of the chick at hatching, The reason is that nano-silver is associated with the Glycoprotein in the bacterial cell membrane causing damage it (Elechiguerra *et al.*, 2005) without damage to animal cells, In a study conducted by Iwona *et al.* (2014), they found that nano-silver injection of 50 ppm did not improve embryos weight, liver weight, and spleen weight, but it improved the heart weight of embryo relative to the total weight of embryo and improved the level of connective tissue and collagen in the embryo, The development of hatching traits may also be due to the fact that injection of nano-silver in amniotic fluid and ingestion it by the embryo at age (19 days) has reduced the stress experienced by the embryo in the last third of hatching, resulting from high temperatures of embryo and hatcher, The eggs were rotated when it transferred from the incubator to the hatchery, leading to the development of hatching traits.

Table 2: Effect of injection of hatching eggs with silver nanoparticle in the percentage of Hatching (%), the percentage of embryonic mortalities (%) and weight at hatching (g) for the broiler chicken (Ross 308).

Average ± standard error			Treatments
Percentage of embryonic mortalities (%)	**Weight at hatching (g)**	**Percentage of Hatching (%)**	
0.46 ± 15a	0.08 ± 41.81e	2.79 ± 85b	T1
0.25 ± 8b	0.03± 40.88e	3.64 ± 92ab	T2
0.62 ± 12a	0.03± 42.22d	2.96 ± 88b	T3
0.08 ± 4b	0.09± 42.66c	4.15 ± 96a	T4
0.25 ± 8b	0.01± 40.75e	2.93 ± 92ab	T5
0.00 ± 0b	0.10± 43.55a	0.00 ± 100a	T6
0.08 ± 4b	0.03± 43.12b	4.31 ± 96a	T7
*	**	*	significant

The averages with different letters within the same column vary significantly among them at the level of *(P <0.05) and ** (P <0.01). The T7, T6, T5, T4, T3, T2, treatments are injections with concentrations of (14, 12, 10, 8, 6, 4) from silver nanoparticle solution, respectively. T1: The control treatment without injection.

Table 3: Effect of injection of hatching eggs with silver nanoparticle on some blood parameters for broiler chicken (Ross 308).

Average ± standard error						Treatments
H/L ratio %	**Lymphocyte %**	**Heterophil %**	**WBC X 10^3 Cell / mm^3 blood**	**RBC X 10^6 Cell / mm^3 blood**	**PCV%**	
0.40 0.01± a	67.50 0.50± ab	0.50± 27.00a	33.50 0.50 ± a	1190.000.02 ±	1.00± 27.00	T1
0.25 0.01± c	69.00 1.00± a	0.50± 17.50c	28.00 0.10 ± bc	1395.000.75±	1.00± 27.00	T2
0.25 0.01± c	69.50 1.00±a	0.50± 18.00c	25.50 0.55 ± c	1180.000.11 ±	0.00± 28.00	T3
0.38 0.03± b	65.00 0.50± c	0.50± 25.00ab	30.50 0.75 ± ab	1350.0000.2 ±	2.00± 28.00	T4
0.23 0.04± c	70.00 1.00± a	1.50± 16.50c	31.00 0.15 ± ab	1545.000.50 ±	2.00± 28.00	T5
0.33± 0.07 b	66.00 0.50±b	22.00 1.00± b	28.50 3.0 ± bc	1195.001.00±	0.00± 26.00	T6
0.29 0.04± bc	67.00 1.00± c	20.00 1.00± b	32.50 0.50 ± a	1320.000.03 ±	0.50± 28.50	T7
**	*	**	**	NS	NS	significant

The averages with different letters within the same column vary significantly among them at the level of *(P <0.05) and ** (P <0.01). The T7, T6, T5, T4, T3, T2, treatments are injections with concentrations of (14, 12, 10, 8, 6, 4) from silver nanoparticle solution, respectively. T1: The control treatment without injection.

Blood traits

Table (3) shows the effect of injection of hatching eggs with the silver nanoparticles for the broiler chicks on blood traits at the age of 35 days from birds age. It did not show a significant difference between the treatments in the Packed cell volume (PCV) and Red blood cells count. As for white blood cells count, The treatments of (T7, T1) were significantly excelled (P <0.01) compared to the other treatments. The results of the statistical analysis showed no significant differences between the treatments (T5, T4) and the treatments (T6, T3, T2). As for the percentage of Heterophile cells, the T1 treatment was significantly (P <0.01) excelled on the other treatments. The T7 and T6 treatments were also excelled on the T5, T3, and T2 treatments. There was no significant difference between T4 treatment and the treatments of (T7, T6, T1). As for The percentage of Lymphocytosis cells, The results of the statistical analysis showed a significant excelling (P <0.05) for the treatments (T5, T3, and T2) compared to the rest of the treatments. The T6 treatment was excelled on the two treatments of (T7, T4). There was no significant difference

between treatment T1 and the treatments (T6, T5, T3, T2). In the H / L ratio, the T1 treatment was significantly ($P < 0.01$) excelled compared to the rest of the treatments. The two treatments (T6, T4) were excelled on the treatments (T5, T3, T2). There was no significant difference between the treatment T7 and the treatments (T6, T5, T4, T3, T2). The increase of the red blood cells counts in the silver injection treatments compared to the control treatment may be due to that the nano-silver has increased the immunity of the raised birds, thus reflected positively on blood traits as mentioned by (Ognik *et al.*, 2016; Al-Jubouri, 2018) that nano-silver stimulated the Phagocytosis process in chickens, which explains the rise of white blood cells counts in the T7 treatment, as well as the high H / L ratio in the control treatment T1 caused by the exposure of birds to a state of oxidative stress and that Nano-silver has alleviated stress in injection treatments.

Conclusions

It has been demonstrated that the injection of silver nanoparticles to chicken's embryo influences the morphology and functioning of embryonic development, as well as the parameters of immune and redox status. This effect varies depending on the dose and size of the used silver nanoparticle, so there is a need for further investigation in order to assess the suitability of nano-silver in poultry.

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