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IMPACT OF *IN- OVO* INJECTION WITH SELENIUM NANOPARTICLES AND OR NICOTINAMIDE ON SOME POST-HATCH TRAITS OF BROILER CHICKS

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ABSTRACT: This work aimed to study the effects of selenium nanoparticles and nicotinamide *in-ovo* injection on hatchability and physiological traits of broiler chicks at hatch day. A total of 500 eggs from Arbor Acres breeder hens were weighed and distributed into five treatments with four replicates for each. At day 18 of incubation, eggs of the first group were non-injected and served as control, while the 2nd group was injected with normal saline at 0.9 % (saline group). But, the 3rd group was injected with selenium nanoparticles (SeNPs) (20 µg SeNPs/ egg) the 4th group was injected with nicotinamide (NAM) (300 mM NAM/ egg) and the 5th group was injected with their mixture (20 µg SeNPs plus 300 mM NAM/ egg). The results showed that chicks of saline and mixture *in-ovo* injection groups had higher significant weights at hatch compared to all other studied groups. In contrast, eggs of the *in-ovo* NAM injected group had higher hatch of fertile egg percentage values with insignificant differences with both control and mixture injected groups. The chicks of NAM and mixture *in-ovo* injected groups had significantly highest WBC values compared to all other studied treatments. The chicks of SeNPs, NAM, and mixture *in-ovo* injected groups have significantly higher low-density lipoprotein compared to control and saline groups. This suggested that the *in-ovo* injection with NAM and the mixture groups used in the present study had a considerably positive effect on hatchability percentage, chick weight, relative chick weight, white blood cell count, and blood glucose level and the activity of total antioxidant capacity (TAC). In conclusion, injection of eggs *in-ovo* of broiler chicks with 300 mM nicotinamide/ egg and mixture between 20 µg SeNPs plus 300 mM NAM/ egg enhanced hatchability and some physiological traits for chicks.

Keywords: *In-ovo* injection, broiler, SeNPs, NAM, hatchability, blood parameters.

INTRODUCTION

The global poultry sector is characterized by faster growth in consumption and trade than any other major agricultural sector (FAO, 2008). In addition to genetic selection, the broiler chick meat industry has used various tools to increase the productivity of these birds to produce more meat such as feeding regimens, early feeding and the *in-ovo* feeding (Hassan et al., 2022). One of these tools is the *in-ovo* injection/ feeding of nutrients to improve the biological characteristics of these chicks (Ferket and Reynolds, 2021).

In-ovo feeding (IOF) technology is used safely to introduce external nutrients, for instance, amino acids, carbohydrates, vitamins, minerals, hormones, minerals, etc., into developing embryos (Zhai et al., 2011a, b). In addition, it may be used to get around growth restrictions in the early stages of embryogenesis and post-hatch development of chickens, which can advance the development of the growing embryo before and after the hatch (Uni and Ferket, 2003). Moreover, this technology could be used to benefit important physiological and biochemical parameters (Malheiros et al., 2012). Interestingly, IOF has an epigenetic impact by activating genes involved in critical metabolic pathways and activities in tissues and organs, which in turn impacts performance later (Li et al., 2016).

Selenium is a critical nutrient for both animals and humans since it plays an important role in thyroid gland metabolism, cell growth, and antioxidant activity and is the most important element for the immune system (Kim and Mahan, 2003). Also, selenium is an essential microelement that serves as a biological natural antioxidant to protect cellular membranes from oxidative damage, and promotes the growth and health of birds (Khan et al., 2016). Nanomaterials have small size of particles that are capable of accessing organ parts that aren't typically exposed to bulk materials (Nel et al., 2006). It has become a significant aspect of daily life in the twenty-first century and has an increasing number of publications (Gupta and Xie, 2018). The SeNPs is a better than other forms of this microelement, this example of applied nanotechnology in the field of dietary supplements demonstrates advantages and unique qualities, including stronger surface activity, higher solubility, mobility, high cellular absorption, and exceptional bioavailability, then it can be a more effective way would eliminate the drawbacks of selenium obtained in conventional forms (Wang et al., 2007; Zhang et al., 2008).

Nicotinamide (NAM) is a form of vitamin B3 that is present in food and utilized as a medicine, dietary supplement and pellagra deficiency is prevented and treated (WHO, 2009). It is the amide form of niacin, and both are precursors to nicotinamide adenine dinucleotide (NAD+), a coenzyme that participates in a number of cellular activities, such as DNA repair and energy metabolism. Furthermore, it can be transformed into nicotinamide mononucleotide, NMN (Maiese et al., 2009). Sirtuin and poly (ADP-ribose) polymerase, which control protein deacetylation and DNA repair, but not niacin, are also inhibited by nicotinamide (Avalos et al., 2005). Additionally, NAM can up-regulate the expression of antioxidant genes and enhance stress resistance (Tran et al., 2016).

Therefore, in this study, we hypothesize that the *in-ovo* feeding of SeNPs or/and

NAM to the developing broiler embryo may cause an increase in hatchability percentage and improve the physiological indicators of chicks at hatch.

MATERIALS AND METHODS

Ethics Statement

The study was conducted at the Poultry Research Center, Faculty of Agriculture, Alexandria University and Livestock Research Department, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications, New Borg El-Arab city, Alexandria, Egypt. The directive 2010/63/EU of the European Parliament and of the Council of September 22, 2010, on the protection of animals and birds employed for research purposes, was complied with, the authors certify.

The *in-ovo* injection solutions

Selenium nanoparticles (SeNPs) were prepared as inorganic material by bottom-down method at the City of Scientific Research and Technological Applications laboratory. The selenium bulk material was grinded to nanoscale using the ball milling mechanical method via Gear-Drive 0.4L Planetary Ball Mill. The speed of the gridding was recorded at 400 rpm for 90 min. Moreover, the material:ball ratio was 1:20, respectively. The fabricated selenium nanoparticles were further characterized to identify their characteristics. The data of the Transmission Electron Microscopy (TEM) demonstrated that the average particle size of the synthesized selenium nanoparticles was 54 nm.

Nicotinamide (C6H6N2O) was obtained from Rival Company for animal health, New Damietta, Egypt. Imported from Lason's factory for nutrients, in India. It is a white crystalline powder, freely soluble in water and ethanol. Then, an appropriate weight, from each material was melted in the saline solution. Just prior to the injection, the injection fluid was produced and gradually warmed to the incubation temperature.

Experimental design

Eggs were collected from *Arbor Acres* breeder hens (42 weeks old) obtained from a commercial flock (Atmida Company for Hatchers, 72 km Alexandria, Cairo desert rood, Egypt). Eggs were incubated under optimal conditions at the Poultry Research Center, Faculty of Agriculture, Alexandria University using an automatic incubator (Model S380, PTO Incubation System Co., Alexandria, Egypt). For the first 18 days of incubation, eggs were placed in an electric forced draught incubator with 37.8 ^{o}C and 53 % relative humidity. Following the injection procedure, the eggs were automatically rotated 24 times per day at angles of 45 degrees before being transferred to the hatcher in covered trays for the last three days of incubation at 37.5 ^{o}C and 70 % relative humidity. All of the eggs were incubated during the incubation period in accordance with customary practices.

A total number of eggs (N=500) were weighed and divided into five treatments, each treatment contained four replicates, with 25 eggs each with nearly equal weight. Eggs of the first group were non-injected and served as control (negative control), while th2nd group was injected with normal saline at 0.9 % (positive control). But, the 3rd group was injected with SeNPs by 20 µg of/ egg, the 4th group was injected with NAM by 300 mM/ egg, and finally, the 5th group was injected with their mixture (20 µg SeNPs plus 300 mM NAM/ egg).

In-ovo injection procedures

At18-day of the incubation, the eggs candled, and those with evidence of living embryos were used (unfertilized eggs were replaced with the same average weight). All the eggs were taken out of the incubator for nearly 20 min/ tray to equalize the conditions for the injection process for all treatments. A mini grinder had to be used to make a proper hole in the broadside of the eggshell. Then, using 21 needle-gauge at 18 days of incubation, all eggs from the 2nd to the 5th groups were injected from the top of the egg with *in-ovo* injection solutions (0.5 ml/ egg) into the amniotic fluid. The site of injection was sanitized with ethanol 70 % and sealed by using the wax gun after injection as described by Hassan et al. (2018). Throughout the incubation period, all eggs were incubated according to the common routine procedures.

Hatchability traits

On the day of hatch, hatchlings chicks were weighed (g), and chick yield (%), fertility percentage (FP), hatchability of set eggs (HOS), and hatchability of fertile eggs (HOF) were calculated, as follows: Chick yield % = chick weight at hatch/ egg weight × 100, FP % = the number of fertile eggs/ the number of set eggs × 100, HOS % = the number of hatched chicks/ the number of set eggs × 100, and HOF % = the number of hatched chicks/ the number of fertile eggs × 100.

Hematological and biochemical parameters

Four blood samples (about 1 ml) from chicks of each treatment (a sample per replicate) were collected at slaughter time on the day of the hatch. The sample was retained with heparin in tubes and divided into two parts, the first part was to estimate the complete blood count test, and the second part was separated by centrifuging the blood samples at 4000 rpm for 15 min to obtain plasma, then storing them at -20°C until the biochemical analysis. According to Feldman et al. (2000), the red blood cell count (RBC 106/mm3), white blood cells count (WBC 103/mm3), and platelets (PLT, 103/µl) were determined. Hemoglobin (Hb, g/dl) concentration and packed cell volume (PCV, %) percentage were measured according to Provan et al. (2004).

Total protein was measured using special kits delivered from sentinel CH Milano, Italy by means of a spectrophotometer (Beckman DU-530, Germany) according to the guidelines of Armstrong and Carr (1965). Albumin was determined using special kits delivered from sentinel CH Milano, Italy according to the method of Doumas et al. (1971). Globulin level was calculated by the difference between total protein and albumin since the fibrinogen usually comprises a negligible fraction (Sturkie, 1986). Total lipids, triglyceride, and cholesterol levels were measured by using special kits by means of a spectrophotometer according to the recommendation of Fossati and Prencipe (1982). High-density lipoprotein (HDL) was determined using the colorimetric method by commercial kits obtained from Reactivos GPL, Barcelona, Spain. Low-density lipoprotein (LDL) was calculated when a lipid panel was performed as LDL (mg/ dl) = Cholesterol – HDL cholesterol – (Triglycerides/ 5) guidelines of Raya et al. (2014). Glucose concentration (mg/dl) was measured by the method of Trinder (1969). The enzyme-linked immune-sorbent assay (ELISA) technique was applied to assess levels of Immunoglobulin-G (IgG) and Immunoglobulin-M (IgM) were also measured (Micini et al., 1965) using commercial kits obtained from Biosystems S.A. Costa Brava, Barcelona, Spain. The activity of total antioxidant capacity (TAC) was measured (Koracevic et al., 2001) using commercial kits obtained from Bio Diagnostics, Giza, Egypt.

Statistical Analysis

Data were subjected to analysis of variance using the General Linear Model (GLM) procedure of the statistical analysis system of the SPSS software program package (SPSS, 2016). Data were analyzed by a one-way method using the following model. $Yij = \mu + Ti + eij$ Where; Yijk = The observation value of the statistical measured، μ= The general overall means، Ti = The effect of treatment groups (i=1,2,3,4 and 5) and eij = The experimental standard error. Before analyses, all percentages were first transformed to arcsine being analyzed to approximate normal distribution before ANOVA. Also, significant differences among means were determined by Duncan's multiple-range test (Duncan, 1955).

RESULTS AND DISCUSSION

Chick and hatchability traits

The results of **Table** 1 showed significant differences among all studied groups except for egg weight. However, the results of chick weight and yield indicated significant differences among treatments. The chicks of saline and mixture *in-ovo* injection groups have higher significant weights (42.95 and 43.08g, respectively) compared to all other studied groups. Also, the chicks of saline and mixture *in-ovo* injection groups have higher significant values 69.09 and 69.36%, respectively compared to the corresponding values of the control group and NAM *in-ovo* injected groups which were 67.51 and 67.32%, respectively.

The muscle development of farm animals is influenced by maternal factors, while poultry species have no such influence after laying their eggs (Greene et al., 2019; Gauvin et al., 2020). Muscle growth is mostly determined during embryogenesis and the ultimate number of muscle fibers is attained in prenatal and early post-hatch periods (Velleman, 2007). Within the first two weeks after hatching, chickens go through their quickest developmental stage (Oliveira et al., 2015). According to numerous studies, the nutrients in the egg are insufficient to enable maximum growth (Zielinska et al., 2011), As a result, researchers are looking into *in-ovo* feeding as a way to provide more nutrients. *In-ovo* injection of different nutrients provides directly to the growing fetus for development (Hassan et al., 2018; Jha et al., 2019), and helps to overcome any constraint of inadequate egg nutrition (Selim et al., 2012).

The present results are in acceptance of the results of Abd El-Fatah et al. (2018) showed that Arbor Acres broiler chick hatch body weight of *in-ovo* injection with SeNPs (0, 10, and 20 μg/ egg) at day 16 of incubation has significant differences (40.63, 39.56, and 40.00 g, respectively). However, the insignificant differences in hatch body weight as a result of *in-ovo* injection with SeNPs were found by El-Deep et al. (2020) and Ibrahim et al. (2020) at different concentrations, locations, date of injection and broiler strains (Habbard Star-Bro, Inshas and Hubbard broiler chick, respectively).

In explanation, more interaction is made possible by nanoparticles' enhanced surface area in biological better bioavailability and functionality are produced by the interface, their prolonged gut retention, and the efficient transport of functional chemicals to target locations

(Chen et al., 2006), improve the uptake (Feng et al., 2009). Both nutritional transporters and active compounds aid in nano-nutrition to provide bioactive ingredients for the sustenance of embryos (Sawosz et al., 2012). Finally, nanoparticles can be better absorbed by animals, thus reducing the excretion of minerals which makes environmental protection (Matuszewski et al., 2020).

On the other hand, the current results showed that *in-ovo* injection of NAM has insignificant differences associated with the control group in respect of chick weight in Table 1, which are in line with the findings of Davis et al. (2018) showed that Cobb 500 chick weight at hatching day was not affected by treatment of *in-ovo* injection (0 or 2.5 mM nicotinamide riboside, NR) or location (injected into yolk or albumen) at day 10 of incubation. However, this increases the pectoralis major muscle (PM) development of the embryo especially when NR is injected into the yolk. Gonzalez and Jackson (2020) studied the *in-ovo* injection of NR in different locations (albumen or yolk sac) or levels (0 or 250 mM) of Cobb 500 eggs on day 10 of the incubation period and found that body weight at hatching has insignificant differences among treatments ranged between 42.96 and 44.02 g. Xu et al. (2021) found that Cobb 500 chicks from eggs injected at day 10 of incubation into the yolk sac with 1 mol NR had significantly greater fiber density than other treatments (250 mmol, 500 mmol) than control chicks, but did not differ from each other. In a review article, Vincenzo Tufarelli et al. (2022) showed that improving one-day weight was achieved by the *in-ovo* injection of 100 g of B-group vitamins (thiamine or riboflavin) into broiler eggs. At last, the *in-ovo* injection of SeNPs plus NAM has significant enhancements on chick weight and yield compared to chicks of the control group without affecting chick quality.

The results of Table 1 showed that the eggs of the *in-ovo* 20 µg SeNPs injected group have significantly lowest fertility and HOS values (87.74 and 84.00%, respectively) compared to all other studied groups. On the other hand, the eggs of the 300 mM NAM *in-ovo* injected group had higher HOF values with insignificant differences with both control and mixture injected groups (97.73, 96.78, and 96.79%, respectively), however, it has significant differences compared to the values of both saline and 20 µg SeNPs *in-ovo* injected groups (95.79 and 95.74%, respectively).

The present results found that *in-ovo* injection of SeNPs has insignificant differences in HOF values compared to control and saline groups in Table 1, similarly, recent studies reported that the *in-ovo* injection of metal. At day 18 of incubation, NPs such as silver, zinc, copper, and selenium had no impact on embryo growth or hatchability rates (Patra and Lalhriatpuii, 2019). Also, the hatchability percentages have insignificant differences among control, deionized water and Vitamin E-Se *in-ovo* injected groups of Ross 308 breeder eggs (88.89, 86.67 and 88.89%, respectively), as found by Abdul-Majeed and Abdul-Rahman (2022). In contrast, the hatchability percentages of Habbard Star-Bro broiler eggs (Mohammad et al., 2019) and Inshas broiler chicken strain (El-Deep et al., 2020) were significantly higher as affected by *in-ovo* SeNPs injection. In addition, *in-ovo* Se injection at day 10 of incubation into the yolk decreases the hatchability percentage of broiler chicks (Macalintal, 2012).

On the other hand, the present results showed that NAM has a big impact on HOF compared to saline groups with insignificant differences compared to the control group in Table 1. Vincenzo Tufarelli et al. (2022) indicated that the *in-ovo* injection of B-group vitamins (thiamine and riboflavin) at a concentration of 100 µg into broiler eggs boosted hatchability percentages, according to the results of many trials, which were reviewed. However, the *in-ovo* injection of choline or folic acid did not affect the hatchability rate. Momeneh and Torki (2018) concluded that *in-ovo* nurturing of vitamin B12 (1000 µl) into broiler eggs improved the hatchability percentage (70.83%) in comparison to the control group (58.33%). However, Teymouri et al. (2019) found that the hatchability of Ross 308 eggs *in-ovo* injected with vitamin B12 (20 and 40 µg) on days 13 and 15 of incubation and 20 µg on days 15 of incubation showed a significant reduction, but not with 40 µg on 15 d of the incubation.

In broiler breeder strains, hatching performance traits (average egg size, chick weight, quality and yields, moisture loss, fertility, hatchability of fertile eggs) are affected by production phases/ age, egg weight, storage period before incubation, incubation conditions (Ishaq et al., 2018). The differences among *in-ovo* studies may be due to either the genetic and breeder hen age, injection technique, the compound that is injected into the egg, concentrations, date of injection, a combination of both technique and compound, egg size, or conditions of incubation. In the present study, the *in-ovo* injection of NAM was more effective/positive on the hatchability of Arbor Acres breeder eggs compared to the SeNPs injection.

Hematological parameters

The results of Table 2 showed insignificant differences among studied treatments in regard to red blood cell (RBC, 2.14 to 2.60 106/ mm3), hemoglobin (Hb, 10.77 to 11.97 g/dL), and packed blood volume (PCV, 29.75 to 32.57%). However, it was highly significant for the white blood cell (WBC) and platelets (PLT) values. The chicks of NAM and mixture *in-ovo* injected groups have significantly highest WBC values compared to all other studied treatments. The chicks of control, saline, SeNPs and NAM *in-ovo* injected groups have significantly higher PLT values (12.67, 12.33, 13.33 and 14.00 103 /µl, respectively) compared to the value of the mixture *in-ovo* injected group (10.33 103 /µl).

Livestock with good blood composition are likely to show good performance (Isaac et al., 2013). Moreover, blood act as a pathological reflector of the status of exposed animals to different conditions (Olafedehan et al., 2010). Blood characteristics are used to determine various statuses of the body and stress due to environmental, nutritional, and/or pathological factors (Ashour et al., 2014), as well as the diagnosis and monitoring of diseases (Zhong et al., 2020). The physiological condition of newly hatched chicks during the hatch may be influenced by the environment the embryo experiences during incubation or by the hatching process itself (Molenaar et al., 2011). Bojarski et al. (2021) reported that RBC and Hb levels of one-day Ross 308 old chicks in the control group averaged 2.8 106 µL and 166.7 g/L, respectively. According to Chineke et al. (2006) posited that high PCV reading indicated either an increase in the number of RBCs or a reduction in circulating plasma volume. Furthermore, Isaac et al. (2013) reported that PCV is engaged in the movement of ingested nutrients and oxygen. A higher PCV indicates improved transportation, which causes a rise in both primary and secondary polycythemias.

In a comparative study with Ross, Cobb, Arian, and Arbor-Acres broiler strains, Talebi et al. (2005) showed the age of birds' effect of age on erythrocytic and leukocytic parameters. In general, animals with high WBC counts were capable of generating antibodies and had a high degree of resistance to diseases (Soetan et al., 2013) and improved ability to adapt to regional environmental and disease-prone situations (Isaac et al., 2013). The *in-ovo* injection effect of vitamin plus selenium into fertilized eggs of Ross 308 was studied by Abdul-Majeed and Abdul-Rahman (2022), who found a significantly increasing and decrease in Hb and PCV values of hatchling chicks respectively as compared with the control.

On the other hand, chicken thrombocytes/ platelets are nucleated blood leukocytes and represent the most abundant white blood cell types in chicken blood (Chang and Hamilton, 1979). Moreover, avian thrombocytes have been shown to play a major role in hemostasis like mammalian platelets by aggregating to form a hemostatic plug (Hodges, 1979). Platelets release a vast array of bioactive molecules (Elzey et al., 2005). According to the results in Table 2 and the literature, the birds of all studied treatments, especially that *in-ovo* injected with the NAM injected group, have good health conditions.

Blood biochemical parameters

Protein profile

The results in Table 3 showed insignificant differences among studied treatments in regard to all three traits; total protein, (TP, 3.60 to 4.43 g/dL), albumin, (Alb, 1.83 to 2.03 g/dL), and globulin, (Glo,1.71to 2.60 g/dL). The TP protein blood change in its levels depends on many external and internal factors and results from the physiological role of blood proteins. The results of earlier studies on protein profile changes in bird blood in relation to age (Anna Piotrowska et al., 2011). Albumin, one of the main serum proteins, serves as the most favorable source of amino acids for the synthesis of tissue proteins in the period of quick somatic growth of birds (Filipovic et al., 2007).

The obtained values of the blood protein profile were within the wide range of physiological values specified for growing broiler chickens (Anna Piotrowska et al., 2011). Bojarski et al. (2021) found that TP and Alb levels of one-day-old Ross308 chicks in the control group averaged 17.3 and 8.0 g/L, respectively. The effect of *in-ovo* injection of vitamin plus selenium into fertilized eggs of Ross 308 was studied by Abdul-Majeed and Abdul-Rahman (2022), which showed a significant increase in serum globulin and a significant decrease in albumin concentrations of hatchling chicks as compared with control. The effect of B12 *in-ovo* injected into Ross 308 broiler eggs through the blunt end on days 13 and 15 of the incubation period was studied by Teymouri et al. (2019) and showed insignificant differences among chicks at day one post-hatch of all studied groups in respect of TP (3.22 – 3.45 g/dl) and Alb (3.22 – 3.45 g/dl) values. The obtained results may be due to the good *in-ovo* injection practice/nutrients in the present study which reflect enhancements in metabolic profile, and health condition, which reflect good production patterns in rapidly growing Arbor Acres chickens during the fattening period.

Lipids profile

The results in Table 4 showed highly significant differences among studied treatments in all studied lipids profile traits. The chicks of the NAM *in-ovo* injected group have significantly the highest total lipids (TL) value (321.00 mg/dL) compared to all other studied treatments. The chicks of NAM and mixture *in-ovo* injected groups have significantly higher cholesterol (Cho) values (215.00 and 217.33 mg/dL, respectively) compared to all other studied treatments. The chicks of the control group have significantly higher triglycerides (TG) value (130.33 mg/dL) compared to all other studied treatments. The chicks of saline and mixture *in-ovo* injected groups have significantly lower High-density lipoprotein (HDL) values (69.00 and 72.33 mg/dL, respectively) compared to all other studied treatments. The chicks of SeNPs, NAM, and mixture *in-ovo* injected groups have significantly higher low-density lipoprotein (LDL) values (103.00, 101.33, and 106.33 mg/dL, respectively) compared to the corresponding values of control and saline groups (92.33 and 86.00, respectively).

One of the main effects of nicotinic acid is associated with reduced lipids (low-density lipoproteins, fatty acids and cholesterol) (Kamanna et al., 2013). The effect of B12 *in-ovo* injected into Ross 308 broiler eggs on d13 and d15 of the incubation period was studied by Teymouri et al. (2019) and showed insignificant differences among chicks at day one post hatch of all studied groups in respect of TG (84.65 – 87.42 mg/dl), HDL (63.14 – 66.31 mg/dl) and LDL (161.35 – 169.25 mg/dl) values. The effect of *in-ovo* injection of vitamin E plus selenium into fertilized eggs of Ross 308 was studied by Abdul-Majeed and Abdul-Rahman (2022), who reported a significant decrease in cholesterol and triglycerides levels of hatchling chicks as compared with control.

Glucose, immunoglobulins and total antioxidant capacity

The results in Table 5 showed highly significant differences among studied treatments in regard to glucose level, the chicks of saline and SeNPs *in-ovo* injected groups have significantly lower values (185.00 and 186.00 mg/dL, respectively) compared to the corresponding values of control, NAM, and mixture groups (188.33, 192.00 and 208.33 mg/dL, respectively). The immunoglobulin G (IgG, 32.40 to 35.83 mg/dL) and immunoglobulin M (IgM, 15.08 to 17.67 mg/dL) have insignificant differences among treatments. On the other hand, the differences were highly significant for the total antioxidant capacity (TAC) value, the chicks of SeNPs, NAM, and mixture *in-ovo* injected groups have significantly higher values compared to the corresponding values of control and saline groups (3.88, 4.83, 5.39, 1.63, and 2.39 nmol/L, respectively).

The results of the present study indicated that the *in-ovo* injection of SeNPs plus NAM enhanced glucose level, which is one of the energy sources in the chicken body. Klasing et al. (2002) explicated that glucose with triglycerides is the primary metabolites that have a direct impact on the body's ability to sustain its energy supply and carry out its physiological and biochemical processes. The blood glucose level in broilers is often greater than that of mammals, ranging from 180 to 250 mg/dL. (Hazelwood, 2000). The *in-ovo* administration effect of chromium in air cells of Ross 308 broiler breeder eggs was studied by Bojarski et al. (2021), who found that blood glucose levels of chicks at hatch day in the control group averaged 229.0 mg/dL.

The *in-ovo* injection effect of vitamin E plus selenium into fertilized eggs of Ross 308 was studied by Abdul-Majeed and Abdul-Rahman (2022) and showed a significant decrease blood glucose level of hatchling chicks as compared with the control. The effect of B12 *in-ovo* injected into Ross 308 broiler eggs through the blunt end on days 13 and 15 of the incubation period was studied by Teymouri et al. (2019) and showed significant differences among chicks at day one post-hatch of all studied groups in respect of blood glucose level ranged between 172.35 – 207.75 mg/dl.

Jenkins (2008) hypothesized that the difference among studies in blood glucose levels may be due to the variations in some factors such as stress, blood collection methods, and housing conditions.

Natural antibodies (NAb), IgM, IgG, or IgA, are defined as antibodies found in healthy individuals who have not undergone vaccination or intentional antigenic stimulation (Baumgarth et al., 2005). It can recognize all tested species, even poultry (Chou et al., 2008). It has been suggested that they can operate as a first line of defense against infection by directly neutralizing bacteria or viruses or by enhancing certain immune responses (Ochsenbein and Zinkernagel, 2000). Individuals' changing NAb profiles throughout time have been linked to or suggestive of a variety of physiological states and pathogenic infections (Nagele et al., 2013).

Silver, zinc, copper, and selenium metal nanoparticles do not impair embryo development, and they improve the immune system (Patra and Lalhriatpuii, 2019). Also, the *in-ovo* injection effect of vitamin E plus selenium into fertilized eggs of Ross 308 was studied by Abdul-Majeed and Abdul-Rahman (2022), who concluded that vitamin E and selenium have enhanced some immunological aspects of broiler chicks.

Assessment of the plasma oxidative state for several animal species could reflect the health conditions of animals. In farm animals, oxidative stress is involved in a number of pathological conditions, including those associated with animal production, reproduction and welfare (Pastorelli et al., 2010) and is associated with the specific and non-specific response of the immune system (Hildeman, 2004). Moreover, Meineri et al. (2017) found that oxidative stress measurements can serve as indicators for spotting illegal hormonal therapies, which have an impact on the safety and quality of animal products for human use. Shokraneh et al. (2020) reported that the injection of Nano-Se and Nano-ZnO into Cobb 500 eggs improved antioxidant activity and decreased oxidative stress, which had a significant impact on reducing the detrimental effects of high-temperature incubation and heat stress.

The available research shows that nicotinamide is an effective source of niacin when offered to animals (Ivers and Veum, 2012). The expression of antioxidant genes can be increased by nicotinamide (John et al., 2012) and improve stress resistance ability (Tran et al., 2016). Protein breakdown and skeletal muscle atrophy can result from oxidative stress over activating the ubiquitin-proteasome system (Abrigo et al., 2018). According to previous findings in the literature and the present results of the TAC antioxidant parameter, the SeNPs, NAM and SeNPs plus NAM *in-ovo* injected chicks, have better oxidative stability, compared to control and saline groups. Moreover, these injections do have not any effect on studied natural antibodies.

CONCLUSION

It can be indicated that the *in-ovo* injection with nicotinamide and the mixture (selenium nanoparticles + nicotinamide) used in the present study was the best in terms of hatchability percentage, chick weight, relative chick weight, white blood cell count, glucose level, and antioxidant status. Accordingly, it could be recommended the use the *in-ovo* injection with a mixture of (SeNPs + NAM) into broiler breeder eggs at day 18 of incubation to obtain good results in hatching performance without any adverse effects on chick quality, and hematological and biochemical characteristics.

Table (1): Mean and pooled standard error (SEM) of egg and chick weights, hatchability traits of *Arbor Acres* breeder eggs *in-ovo* injected by different levels of Selenium Nano particles, Nicotinamide, or their mixture.

Treatments	Egg weight (g)	Chick weight (g)	Chick yield (%)	Fertility (%)	Hatchability (%)	
					HOS	HOF
Control	62.60	42.24^{c}	67.51^{b}	92.99^{a}	90.00^{a}	96.78^{ab}
Saline	62.20	42.95^{a}	69.09^{a}	92.91^{a}	89.00^{a}	95.79^{b}
SeNPs 20µg	62.65	42.58^{b}	68.01^{ab}	87.74^{b}	84.00^{b}	95.74^{b}
NAM 300mM	62.65	42.15^{c}	67.32^{b}	92.09^{a}	90.00^{a}	97.73^{a}
SeNPs plus NAM	62.15	43.08^{a}	69.36^{a}	92.98^{a}	90.00^{a}	96.79^{ab}
SEM	0.39	0.12	0.33	0.70	0.83	0.47
P-value	0.921	0.001	0.021	0.043	0.052	0.031

Means having different superscripts at the same column has significant differences ($P \leq 0.05$)
SeNPs: Selenium Nano particles; NAM, nicotinamide; HOS, hatchability of set eggs; HOF, hatchability based on fertile eggs; SEM, pooled standard error; *P*-value, probability value.

Table (2): Mean and pooled standard error (SEM) of hematological parameters at hatching day of *in-ovo* injected *Arbor Acres* chicks by different levels of Selenium Nano particles, Nicotinamide or their mixture.

Treatments	**Hematological parameters**				
	RBC (10^6/ mm^3)	**Hb (g/dl)**	**PCV (%)**	**WBC ($10^{3/}mm^3$)**	**PLT ($10^{3/}$ µl)**
Control	2.60	11.60	29.91	11.33^b	12.67^a
Saline	2.38	11.97	32.57	11.67^b	12.33^a
SeNPs (20µg)	2.52	11.90	31.69	11.33^b	13.33^a
NAM (300mM)	2.14	10.77	30.00	13.90^a	14.00^a
SeNPs plus NAM	2.25	11.13	29.75	13.60^a	10.33^b
SEM	0.08	0.30	7.08	3.52	0.83
P-value	0.374	0.721	0.695	0.005	0.003

Means having different superscript letters in the same column are significantly different ($P \leq 0.05$).
SeNPs, Selenium Nano particles; NAM, nicotinamide; RBC, red blood cell; Hb, hemoglobin; PCV, packed cell volume; WBC, weight blood cells; PLT, platelets; SEM, pooled standard error; *P*-value, probability value.

Table (3): Mean and pooled standard error (SEM) of some blood protein profile at hatching day of *in-ovo* injected *Arbor Acres* broiler chicks by Selenium Nano particles, Nicotinamide or their mixture.

Treatments	**Blood protein profile**		
	TP (g/dL)	**Albumin (g/dL)**	**Globulin (g/dL)**
Control	3.60	1.87	1.73
Saline	4.43	1.83	2.60
SeNPs (20µg)	4.02	2.03	1.99
NAM (300mM)	3.92	1.93	2.00
SeNPs plus NAM	3.68	1.97	1.71
SEM	0.20	0.04	0.18
P-value	0.776	0.665	0.618

Means having different superscript letters in the same column are significantly different ($P \leq 0.05$).
SeNPs, Selenium Nano particles; NAM, nicotinamide; TP, total protein; SEM, pooled standard error; *P*-value, probability value.

Table (4): Mean and pooled standard error (SEM) of blood lipids profile at hatching day of *in-ovo* injected *Arbor Acres* broiler chicks by Selenium Nano particles, Nicotinamide, and/or their mixture.

Treatments	**Blood lipids profile**				
	TL (mg/dL)	**Cho (mg/dL)**	**TG (mg/dL)**	**HDL (mg/dL)**	**LDL (mg/dL)**
Control	277.33^{bc}	200.67^{b}	130.33^{a}	78.67^{a}	92.33^{b}
Saline	258.67^{d}	171.00^{c}	107.33^{d}	69.00^{c}	86.00^{b}
SeNPs (20µg)	269.33^{cd}	206.33^{b}	115.33^{c}	77.67^{ab}	103.00^{a}
NAM (300mM)	321.00^{a}	215.00^{a}	112.33^{cd}	79.33^{a}	101.33^{a}
SeNPs plus NAM	286.33^{b}	217.33^{a}	122.67^{b}	72.33^{c}	106.33^{a}
SEM	4.40	3.31	1.67	1.11	1.82
P-value	0.001	0.001	0.001	0.003	0.001

Means having different superscript letters in the same column are significantly different (P≤0.05). SeNPs, Selenium Nano particles; NAM, nicotinamide; TL, total lipids; Cho, cholesterol; TG, triglycerides; HDL, high density lipoprotein; LDL, low density lipoprotein; SEM, pooled standard error; *P*-value, probability value.

Table (5): Mean and pooled standard error (SEM) of blood biochemical parameters at hatching day of *in-ovo* injected *Arbor Acres* broiler chicks by Selenium Nano particles, Nicotinamide, or their mixture.

Treatments	**Blood biochemical parameters**			
	Glucose (mg/dL)	**IgG (mg/dL)**	**IgM (mg/dL)**	**TAC (nmol/L)**
Control	188.3^{ab}	32.40	17.33	1.63^{c}
Saline	185.0^{c}	33.57	15.08	2.39^{c}
SeNPs (20µg)	186.0^{c}	35.83	16.78	3.88^{b}
NAM (300mM)	192.0^{b}	33.00	17.67	4.83^{ab}
SeNPs plus NAM	208.3^{a}	33.63	16.17	5.39^{a}
SEM	1.76	0.50	0.35	0.40
P-value	0.001	0.268	0.106	0.0001

Means having different superscript letters in the same column are significantly different (*P*≤0.05) SeNPs, Selenium Nano particles; NAM, nicotinamide; IgG, immunoglobulin G; IgM, immunoglobulin M; TAC, total antioxidants capacity; SEM, pooled standard error; *P*-value, probability value.

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الملخص العربي

تأثير حقن البيض بالنانو سيلينيوم والنيكوتيناميد على صفات الفقس والصفات الفسيولوجية لكتاكيت التسمين عند الفقس

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[1] قسم إنتاج الدواجن ، كلية الزراعة ، جامعة الإسكندرية ، الإسكندرية ، مصر.

[2] قسم بحوث الحيوانات المزرعية، معهد بحوث زراعة الأراضي القاحلة ، مدينة الابحاث العلمية والتطبيقات التكنولوجية، برج العرب الجديدة ، مصر.

أجريت الدراسة في المزرعة البحثية الخاصة بوحدة بحوث الدواجن التابعة لقسم انتاج الدواجن – كلية الزراعة – جامعة الاسكندرية، بغرض دراسة تأثير حقن بيض التفريخ بمحلول يحتوي علي جزيئات النانو سيلينيوم وكذلك النيكوتيناميد او مخلوط منهما معا علي صفات الفقس وبعض الصفات الفسيولوجية لكتاكيت التسمين عند الفقس. تم استخدام عدد 500 بيضه وتقسيمها الي خمس مجموعات وكل مجموعه بها 4 مكررات و في اليوم الثامن عشر من التحضين تم معامله المجموعات بالحقن بحجم 0.5 ميلليلتر لكل بيضه؛ بقيت المجموعة الاولي بدون حقن (كنترول) وتم حقن المجموعه الثانيه بمحلول الملح 0.9% و الثالثه بمحلول بتركيز 20 ميكروجرام من النانوسلينيوم و المجموعه الرابعه بمحلول النيكوتيناميد بتركيز 300 ميلليمول و المجموعه الخامسه تم حقنها بخليط من كلا التركيزين (20 ميكروجرام نانو سلينيوم و 300 ملليمول نيكوتيناميد). أظهرت النتائج وجود فروق معنويه للوزن عند الفقس بالنسبه للكتاكيت التي تم حقنها بالمحلول الملحي وخليط التركيز من (نيكوتيناميد و نانوسلينيوم) مقارنه بباقي المجموعات بينما وجدت اعلي قيمه لنسبه الفقس في المجموعه التي تم حقنها بالنيكوتيناميد مع عدم وجود فروق معنويه لكل من مجموعه الكنترول و مجموعه الخليط. واوضحت النتائج عدم وجود فروق معنويه للصفات محل الدراسه في معظم وزن الاعضاء الداخليه المتوقعه للكتاكيت. اظهرت النتائج وجود فروق معنويه في عدد كرات الدم البيضاء في كتاكيت المجموعه التي تم حقنها بالخليط مقارنه بباقي المعاملات محل الدراسه؛ كما وجدت فروق معنويه في كلا من المجموعه التي تم حقنها بالنانوسليينيوم ومحلول الخليط في الليبوبروتين منخفض الكثافه مقارنه بمجموعه الكنترول و المجموعه التي تم حقنها بالمحلول الملحي. وفي النهاية يمكن ان نستخلص من هذة الدراسة انه يتضح ان حقن البيض بكل من النيكوتيناميد و محلول الخليط (نيكوتيناميد + نانوسلينيوم) محل الدراسه لها نتائج جيده في كل من نسبه الفقس ووزن الكتكوت عند الفقس، كرات الدم البيضاء و مستوي الجلوكوز و كذلك مضادات الاكسده ومعظم الصفات محل الدراسه.

الكلمات الدالة: حقن بيض التفريخ، النانو سيلينيوم، النيكوتيناميد، نسبة الفقس، صفات الدم