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EFFECT OF NANO- SELENIUM SUPPLEMENTATION AND *IN-OVO* INJECTION ON GROWTH PERFORMANCE, HEMATO-BIOCHEMICAL PARAMETERS AND IMMUNOLOGICAL RESPONSES OF POST-HATCH CHICKS REARED UNDER HOT CONDITIONS

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ABSTRACT: This work aimed to study the effects of Nano- selenium (Nano-Se) supplementation and *in- ovo* injection on the growth performance and physiological responses of post-hatch chicks reared under hot conditions. A total number of 60 laying hens and 8 cocks Doki-4 strain (74 weeks old and average body weight of female and male 1663.75±34.9 g and 2383.33±147.0, respectively) were randomly divide into two equal groups (30 hens and 4 cocks of each). The first group considered as parents of treatment 1 (Tr1) and treatment 2 (Tr2). The second group considered as parents of treatment 3 (Tr3) and treatment 4 (Tr4). After four weeks of start experiment, 300 fertile eggs collected from two groups (150 eggs / group), the fertile eggs and post-hatch chicks treated as following; Tr1, parents and post-hatch chicks fed on basal diet plus *in- ovo* injection with 0.5 ml saline solution /egg (control treatment). Tr2, parents and post-hatch chicks fed on basal diet plus *in- ovo* injection with 0.5 ml saline solution (containing 0.2 µg Nano –Se) /egg. Tr3, parents fed with basal diet supplemented with 0.3 mg Nano –Se /kg feed and post-hatch chicks fed on basal diet plus *in- ovo* injection with 0.5 ml saline solution (containing 0.2 µg Nano –Se)/ egg. Tr4, parents and post-hatch chicks fed on basal diet supplemented with 0.3 mg Nano –Se /kg feed plus *in- ovo* injection with 0.5 ml saline solution (containing 0.2 µg Nano –Se) / egg. The results showed that, hemoglobin and mean corpuscular volume significantly ($P<0.05$) increased in Tr2 and Tr3 in compared to Tr1 (control). Heterophils / lymphocyte ratio and hatchability % significantly ($P<0.05$) improved in Tr3 and Tr4 in compared to Tr1. Immunoglobulin M significantly ($P<0.05$) increased in Tr2 and Tr3 in compared to Tr1 and Tr4, rectal temperature significantly ($P<0.05$) decreased in Tr4 in compared with Tr1, from one day to 6 weeks of age from hatch, Tr3 and Tr4 show better performance in body weight gain, feed intake and feed conversion when compared with Tr1 and Tr2. In conclusion, supplementation the diets of parents and post-hatch chicks with 0.3 mg Nano-Se / kg feed plus *in-ovo* injection with 0.2 µg Nano-Se /egg enhance growth performance, hematological parameters and immunological responses of post-hatch chicks reared under hot conditions.

Key words: Blood -Chicks- Growth - *In-ovo* injection -Nano-Selenium.

INTRODUCTION

Poultry production becomes one of the most important industries that contribute to the economy of countries. Therefore, it must be taken care of all the obstacles related to reduce its production. In Egypt, Chickens subjected to heat stress when the outdoor ambient temperature reached 45 °C and the summer months show a higher heat waves frequency of temperatures above 35 °C (Faisal et al., 2008 and Morsy et al., 2018). High environmental temperature caused detrimental effects on the physiological responses of poultry through altered blood chemistry, decreasing productive performance and increased mortality rate (Barrett et al., 2019; Attia et al. 2016; Kilic and Simsek, 2013; Song et al., 2012; Khan et al., 2011 and Oguntunji and Alabi 2010). Recently, micro (trace) mineral elements supplementations are used to alleviate the harmful effects of heat stress (Habibian et al., 2014; Perai et al., 2015 and Elfiky et al., 2021).

Selenium (Se) is a necessary micro mineral for poultry nutrition and low Se status leads to reduce growth performance, immune responses, exudative diathesis, nutritional muscular dystrophy, antioxidant status and lipid peroxidation (Cantor et al., 1982 and Yang et al., 2016). Se deficiency with heat stress caused oxidative stress which resulted from the disturb balance between antioxidant defense system and production of free radicals. The induced-oxidative stress led to damage wide variety of biomolecules including lipids, proteins and DNA, resulting in damage of tissues and organs failure (Fischer et al., 2008 and Liu et al., 2014). Additionally, synthesizing new selenoproteins such as Glutathione Peroxidase (GPxs) may increase under hot conditions, which may impair a low Se deficiency or may increase Se requirement (Lyons et al., 2007).

In poultry diets, Se supplements typically come in mineral or organic forms. Despite the fact that organic selenium has a greater potential for uptake, mineral selenium and vitamin E have been shown to have a more beneficial effect on poultry performance (Perez et al., 2010 and Elfiky et al., 2021). Some researchers believe that feeding chickens with organic or mineral Se has no effect on their immune systems (Biswas et al., 2006), numerous studies have demonstrated that a novel source known as Nano-Se performs better in poultry diets. (Shirsat et al., 2016 and Elfiky et al., 2021).

Nano-elemental Se (Nano-Se) has received more attention, due to its high bioavailability, high catalytic efficiency, strong adsorbing ability and low toxicity when compared to selenite in chickens diets (Wang et al., 2009), Nano-Se is deeply involved in many biological functions like growth, general health status, immunity, antioxidant status, production and reproductive performance (Saad et.al., 2009; Pappas and Zoidis, 2012 and Elfiky et al., 2021). These effects of Se made it very necessary to be included in poultry feed. The role of supplemental Se in enhancing the fertility of laying hens, embryonic development and hatched chicks % was proved in many reports (Edens, 2002 and Davtyan et al., 2006). Selenium is a structural component of the enzyme system GPxs, which protects cellular components from oxidative stress by acting as an antioxidant (Larsen et al., 1997).

El-Deep et al. (2016) found that broilers' diets supplemented with Nano-Se at 0.3 mg/kg could improve growth performance by enhancing anti-oxidative or immune properties when raised at high temperatures, similar trend was observed by Safdari-Rostamabad et al., (2017) who found that supplement Nano-Se in broilers diet improved growth performance, the health of internal organs, the immune response and the morphology of the jejunum by reducing heat stress-induced by oxidative stress. Moreover, Hassan (2018) showed that no harmful effect of Nano-Se on the embryo, which can be used to boost broiler performance after hatching in semi-arid environments when injected with 15 parts per million.

Therefore, this work aimed to study the effects of Nano-Se supplementation and *in- ovo* injection on the growth performance and physiological responses of post-hatch chicks reared under hot conditions.

MATERIALS AND METHODS

The present study conducted in the Al-Azzab integrated poultry project, located at El-Fayoum desert that belongs to El-Fayoum governorate, Egypt. The experiment started on June up to September 2021.

Experimental design

A total number of 60 laying hens and 8 cocks Doki-4 strain (74 weeks old and average body weight of female and male 1663.75±34.9 and 2383.33±147.0 g, respectively) were randomly divide into two equal groups (30 hens and 4 cocks of each). The first group considered as parents of Tr1 and Tr2, the second group considered as parents of Tr3 and Tr4. After four weeks of start experiment, 300 fertile eggs collected from two groups (150 eggs / group) with average weight 46.32±0.59 g and 47.09±0.59 g for first and second group, respectively, and kept in storage cool room (16-17 ^{0}C) until setting in the hatchery, the fertile eggs and post-hatch chicks treated as following; Tr1, parents and post-hatch chicks fed on basal diet plus *in- ovo* injection with 0.5 ml saline solution /egg (control treatment). Tr2, parents and post-hatch chicks fed on basal diet plus *in- ovo* injection with 0.5 ml saline solution (containing 0.2 µg Nano –Se) /egg. Tr3, parents fed with basal diet supplemented with 0.3 mg Nano –Se /kg feed and post-hatch chicks fed on basal diet plus *in- ovo* injection with 0.5 ml saline solution (containing 0.2 µg Nano –Se)/ egg. Tr4, parents and post-hatch chicks fed on basal diet supplemented with 0.3 mg Nano –Se /kg feed plus *in- ovo* injection with 0.5 ml saline solution (containing 0.2 µg Nano –Se) / egg.

The parents stock of chickens reared on floor and kept under the same hot conditions for 6 weeks. The basal diet of parents stock containing crud protein 16% and metabolizable energy 2700 kcal/kg feed according to NRC (1994).

The post-hatch chicks from the four treatments separately housed in galvanized wire cage batteries for 6 weeks. Chicks fed on basal diet (the starter diet from 0-4 weeks of age (19 % crude protein and metabolizable energy 2950 Kcal/Kg diet) and the grower diet from 5-6 weeks (17.5% crude protein and metabolizable energy 3000 Kcal/Kg diet) according to NRC (1994). The hatched chicks from the four treatments brooded and kept under the same hot conditions.

Animal ethics

This study was conducted according to the guidelines for care and use of

laboratory animals by Beni-suef University (BSU-IACUC). Approval number (022-359).

Egg collection, treatment and injection procedures

All procedures done in the hatchery (reform hatchery model, 84) of Al-Azzab integrated poultry project. All eggs in all treatments were incubated under standard conditions (T = 37.8°C, RH = 55-60%) from 1 to 18 days of incubation and during the last three days of incubation (T = 36.8°C, RH = 60-65%). On 16th day of incubation, infertile eggs and eggs containing dead embryos removed from all treatments by candling.

At 17th day of incubation (400 h), eggs putted out the incubator, during *in-ovo* injection; the wide ends of the eggs disinfected with 96% ethanol. The fertile eggs were candled to determine the injection sites and a small hole in the wide end of the egg was made with a 21G needle, and the injections were performed at an approximate depth of 25 mm from the eggshell into amniotic fluid. After injection, the holes sealed with sterile wax, and then the eggs returned to the incubator according to standard hatchery practices (Shokraneh et al., 2020).

Preparation of Nano form of selenium and their characterization

Selenium nanoparticles (Se-NPs) were prepared by mechanochemical activation of purchased Selenium in anatase phase with purity 99.99%. In mechanochemical activation process, the Se-NPs powder was milled in a ball mill for 24 hours at 950 rounds per minute. The weight ratio of the sample to the balls was 1: 50 and the balls were made of ceramic alumina.

The obtained Se-NPs fine powder was then characterized using X-Ray Diffraction (XRD) (Fig. 1). Characterization of Se-NPs is important to understand and control nano particles synthesis and applications. Characterization of Se-NPs shows that Se-NPs are prepared in semi-crystalline anatase phase with average crystallite size of 32.1 nm. Se-NPs source are the central laboratory of faculty of post graduate studies and advanced sciences-Beni-seuf university-Egypt.

Ambient temperature and relative humidity

Table (1) indicated monthly indoor climatic conditions recorded during the experimental period using electronic digital thermo-hygrometer (Model 303, China). The relationship between ambient temperature (AT) and relative humidity (RH) were termed as temperature-humidity index (THI) and calculated according to Marai et al. (2001).

$$THI = db°C - [(0.31 - 0.031 \times RH) \times (db°C - 14.4)]$$

Where, db°C = dry bulb temperature in centigrade and RH = relative humidity/100. The THI values were classified as absence of heat stress (<27.8), moderate heat stress (27.8-28.8), severe heat stress (28.9-29.9) and very severe heat stress (>30.0).

Sampling, measurements and analysis

Hatchability percentage calculated at the end of incubation period based on the number of fertile eggs. On the end of the experiment of post-hatch chicks (42 days from hatching), individual blood samples of about 5 ml, from randomly 60 birds (15 birds / treatment) collected from wing vein into six mL heparinized tubes. Plasma was removed by using centrifugation at 3000 r.p.m for 15 minutes and stored at –20 ^{0} C until analysis.

Total proteins, albumin, cholesterol, triglycerides and creatinine were analyzed

using commercial kits (Spectrum Company, Egypt). Globulin concentration calculated according to the formula: globulin = (total protein - albumin) Alanine aminotransferases (ALT) and aspartate aminotransferase (AST) were analyzed using commercial kits (Diamond Diagnostic, Cairo, Egypt),. Determination of red blood cells (RBC's), white blood cells (WBC,s) and their differential counts, hemoglobin (Hb), hematocrit % (Ht %), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) were determined by blood using complete blood counter (CBC,Model, HA-Vet. Clinding, Belgium). Heterophils/Lymphocytes ratio (H/L ratio) calculated according to (Gross and Siegel, 1983).

Subclasses of immunoglobulin levels (IgY and IgM) in plasma were determined by turbidimetry with commercial kits (Sanwei Biotech Firm, Weifang, China). Biweekly live body weight measured and recorded by digital balance. Weight gain, feed intake and feed conversion calculated from one day - 2 weeks of age, 2-4 weeks, 4-6 weeks and one day-6 weeks of age.

Rectal temperature (RT) measured by inserting clinical thermometer 2-3 cm in cloca for one minute. Data were analyzed by the least square analysis of variance using the General Linear Model Procedure (SAS, 2004) according to following model:

$Y_{ij}=\mu + Tr_i + e_{ij}$

Where, Y_{ij} = observations, μ = overall mean, Tr_i = effect of ith treatment (i: 1-4), e_{ij} = experimental error. Duncan's New Multiple Range Test (Duncan, 1955) separated differences among treatment means.

RESULTS AND DISCUSSIONS

Hematological parameters

There were insignificant differences in red blood cells counts (RBC's), white blood cells counts (WBC's) and mean corpuscular hemoglobin concentration (MCHC) as affected by treatment. Additionally, there were significant ($P<0.05$) differences among treatments in hemoglobin (Hb), hematocrit (Ht, %), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and heterophils / lymphocyte ratio (H/L ratio), however, Hb concentration significantly increased in Tr2 and Tr3 in compared to Tr1 (control), on the same trend, MCV significantly increased in Tr2, Tr3 and Tr4 when compare with Tr1, meanwhile, H/L ratio significantly improved in Tr3 and Tr4 when compared with Tr1. In addition, Ht % significantly increased in Tr2 when compared with Tr1 (Table 2). From these results, we note that using Nano-Se in Tr2, Tr3 and Tr4 might alleviate adverse effects of heat stress. Where, heat stress impaired these parameters in control treatment (Tr1). These outcomes may be attributable to the use of Nano-Se, a form of elemental selenium that is less toxic and better absorbed when given to chickens under oxidative stress and the Nano-Se reduced oxidative stress in the hens' bodies and Se made the avian immune system better by making immune-competent cells more likely to respond to an antigen challenge **(**Qu et al., 2017). These results agreement with Bealish et al. (2018) who showed that Silver Montazah chicks received diet containing 0.25 mg Nano-Se/kg diet recorded the highest significant value of PCV%, heterophils% and lymphocytes %

and Hb compared with other treatments. El-Sheikh et al. (2010) reported the similar trend in Bandarah strain. It was also observed that both cellular and humoral immunity were significantly increased in chicks fed on diet containing 0.3 ppm Nano-Se after 8 weeks of post-feeding (Mohapatra et al., 2014). On the other hand, Mohamed et al., (2016) found that supplemented Nano-Se to the diet resulted in a significant increase in Hb concentration in local Sinai chickens strain when exposed to heat stress. Alagawany et al. (2021) showed that Hb were increased (linear, P < 0.05) by the addition of Nano-Se at 0.4 and 0.6 g/kg in quails diet. Fuxiang et al. (2008) showed that H/L ratio significantly decreased when Nano-Se supplemented to broilers diets. Additionally, Hassan (2018) demonstrate that under semi-arid conditions, When compared to the control group, the effect of *in-ovo* injection of broiler eggs with Nano-Se resulted in an significantly increased in RBC counts of approximately 24.39 %, HGB of approximately 18.14 % and Ht of approximately 13.41%, while, a significantly decreased in MCHC of approximately 4.59 %.

Bio-chemical parameters

There were insignificant (P>0.05) differences among treatments in all bio-chemical parameters as showed in Table (3). These results agree with Alagawany et al. (2021) who found that TP, albumin and AST were not affected (P > 0.05) by dietary supplementation of Nano-Se in quails diet for four weeks (1-5 weeks of age) during growth periods. In the same trend, Qu et al. (2017) found that there were insignificant differences between the control treatment and Nano-Se treatment (0.5 mg /kg diet) in TP, albumin, AST, ALT and creatinine when laying hens diet supplemented with Nano-Se for four consecutive weeks. Additionally, Alian et al. (2020) found that there were no statistical differences in the serum ALT, AST, TP, albumin and creatinine level between control and treatment which supplemented with 0.3 mg Nano-Se / kg feed in broilers diet. As in agreement with the current study, Visha et al. (2020) found that there were insignificant (differences between control group and treatment group (diet supplemented with 0.3 mg Nano –Se / kg feed of broiler chicken) in triglycerides level.

Immunological responses

Figure 2 showed that effect of Nano- Se supplementation and *in-ovo* injection on Immunoglobulin M **(**IgM) and Immunoglobulin Y **(**IgY) of post-hatch chicks under hot conditions. There were insignificantly (P>0.05) increased in IgY level in Tr2, Tr3 and Tr4 when compared with Tr1 (control), while, IgM showed significantly increased in Tr2 and Tr3 when compared with Tr1 and Tr4. On the other hand, there were insignificant (P>0.05) differences between Tr1 and Tr4 in IgM level. These results may be attributable to Nano-Se's essential biological function of increasing T helper cells and boosting cytokine secretion **(**Shabani et al., 2019). These results agree with Cai et al. (2012) who found that no differences were found in IgY and IgM levels across all treatments on day 21, but increased IgM level was found in group of broiler chickens supplemented with 0.3 mg Nano-Se / kg feed from 1-45 day of age. In the same trend, El-Deep et al. (2016) found that under the high ambient temperature condition, feeding a diet containing Nano-Se increased the relative weights of immune organs. These results were attributed to the possibility that

feeding a diet containing Nano-Se might have an immune-stimulatory effect on chickens and alleviating the negative effects of high ambient temperature.

Rectal temperature

Rectal temperature significantly (P<0.05) decreased in Tr4 when compared with Tr1 (control), while, There were insignificant (P>0.05) differences between Tr1, Tr2 and Tr3 or between Tr2, Tr3 and Tr4 (Fig. 3). These results may be due to that in heat stressed birds using Nano-Se can improve antioxidant status through decrease of malondialdehyde (MDA) and mediating GSH-Px levels (Senthil Kumaran et al., 2015). Similarly, during chromium toxicity, Nano-Se prevents cell damage and regulates altered levels of antioxidant enzymes like catalase, superoxide dismutase, glutathione, and MDA (Hassanin et al., 2013). These results disagreement with Mohamed et al. (2016) who found that dietary supplementation of Nano-Se in Sinai chickens during growth period under heat stress had no significant effect on rectal temperature.

Hatchability %

Figure (4) showed the effect of supplementation of Doki-4 parent's diet and *in-ovo* injection with Nano-Se in hatchability %. Hatchability % was significantly increased in Tr4 and Tr3 when compared with Tr1 (control). On the other hand, there were insignificant differences between Tr1 and Tr2 or among Tr2, Tr3 and Tr4. These results agreement with Rizk et al. (2017) who indicated that heat stress severely reduced the hatchability % of the eggs of Sinai hens, whereas hatchability % was improved by dietary selenium sources (Nano-Se at 0.3 ppm) supplementation during laying period under heat stress conditions, without any adverse effect on the vitality of hens. In the same side, Bealish et al. (2018) found that the highest percentages of the hatchability percentages of all eggs of all treatments that fed on diet containing of 0.25 mg Nano-Se/kg feed than the other treatments in parents of Silver Montazah chickens. Additionally, Abbas et al. (2020) found that using Nano-Se *in-ovo* injection at different levels can improve hatchability percentages under normal conditions in quail performance. However, Sallam et al. (2019) found that hatchability % significantly decreased in eggs of Japanese quail when *in-ovo* injection with Nano-Se (2.5 ppb / egg) compared with control group.

Growth performance

Effect of Nano-Selenium (Nano-Se) supplementation and *in-ovo* injection on body weight (BW) of post-hatch chicks under hot conditions is presented in Table 4. There were insignificant differences among treatments in BW at one day and two weeks of age. At four weeks of age, BW was significantly (P<0.05) increased in Tr3 when compared with Tr1 and Tr2. While, there were insignificant differences among Tr1, Tr2 and Tr4 or between Tr3 and Tr4. At six weeks of age, body weight was significantly (P<0.05) increased in Tr3 when compared with Tr1 and Tr2. While, there were insignificant differences between Tr1 and Tr2 or between Tr3 and Tr4.

Effect of Nano-Se supplementation and *in-ovo* injection on feed intake (FI), body weight gain (BWG) and feed conversion (FCR) of post-hatch chicks under hot conditions is presented in Table (5). From one day - two weeks of age, there were insignificant differences among treatments in BWG and FCR, while, FI

significantly (P<0.05) decreased in Tr3 and Tr4 when compared with Tr1 and Tr2. From 2-4 weeks of age there were insignificant differences among treatments in FI and FCR, while, BWG significantly (P<0.05) increased in Tr3 when compared with Tr1 and Tr2. additionally, there were insignificant differences among Tr1, Tr2 and Tr4. From 4-6 weeks of age, chicks in Tr4 showed better performance in BWG and FCR when compared with other treatments, while, FI significantly (P<0.05) increased in Tr2, Tr3 and Tr4 when compared with Tr1 (control). However, from one day - 6 weeks of age, Tr3 and Tr4 showed better performance in BWG, FI and FCR when compared with Tr1 and Tr2.

These outcomes may be attributable to the increased utilization of Nano-Se, which is associated with the distinctive properties of the Nano form, such as high cellular uptake, improved bioavailability, increased solubility, and increased surface activity. Se plays a role in regulating a number of enzymatic systems that hinder energy metabolism and the breakdown of apurinic and apyrimidinic base essential fatty acids (Zhang et al., 2007). These results in the present study agreement with Alagawany et al. (2021) who reported that the best growth performance parameters recorded in the group fed 0.4 g Nano-Se /kg feed. They found that fed quails with diets containing Nano-Se had significantly higher BW, FCR and BWG during the completely experimental period (1-5 weeks of age). FI was decrease in Nano-Se groups compared with that in the control group. Similar results was reported by El-Deep et al. (2016) they found that FCR was remarkably improved (P＜0.05) when broiler diets were supplemented with Nano-Se under hot conditions. Alian et al. (2020) showed that BW and BWG were significant improved by supplementation of broiler chickens diet with 0.3 mg Nano-Se/ kg feed compared with control group. Zhou and Wang (2011) found that final body weight was significant improved by feeding diet supplemented with 0.10, 0.30, and 0.50 mg Nano-Se /kg feed as compared to the control after 90 days of feeding.

In addition, Mohamed et al. (2016) found that under heat stress, FI was significantly decrease as a result of supplementing Nano-Se to the diet of local Sinai chickens strain during the periods of 12-16 and 8-16 wks of age. While, FCR was significantly improve by supplementing Nano-Se in Sinai chick's diets as compared with control diet.

CONCLUSION

From these results, we can concluded that, supplementation the diets of parents and post-hatch chicks with 0.3 mg Nano Se / kg feed plus *in-ovo* injection with 0.2 µg Nano Se /egg enhance growth performance, hematological parameters and immunological responses of post-hatch chicks reared under hot conditions.

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Table (1): Indoor ambient temperature, average relative humidity and temperature-humidity index during experimental period.

Months	AMAT (^{0}C)	ARH %	THI
June	33.2	36.0	29.5
July	36.4	25.8	32.5
August	39.0	25.5	32.4
September	33.9	47.0	30.7

AMAT =average maximum ambient temperature; ARH % = average relative humidity; THI= temperature-humidity index

Table (2): Effect of Nano-Se supplementation and *in-ovo* injection on hematological parameters of post-hatch chicks reared under hot conditions.

Traits	Treatments				±SE
	Tr1	Tr2	Tr3	Tr4	
RBC (× 10^6)	1.33	1.55	1.50	1.40	0.04
Hb (g/dl)	5.36^b	6.96^a	7.23^a	6.30^{ab}	0.24
Ht (%)	15.76^{bc}	18.63^a	18.20^{ba}	15.25^c	0.51
MCV (fl)	119.48^a	120.21^a	119.50^a	110.23^b	1.46
MCH (pg)	36.98^b	46.51^a	47.28^a	45.41^a	1.10
MCHC (%)	38.18	37.95	40.23	41.13	0.24
WBC (10^3/mm^3)	35.71	36.26	39.03	31.85	1.28
H/L ratio	1.05^a	0.85^{ab}	0.69^b	0.63^b	0.06

RBC's =red blood cells; Hb=hemoglobin; Ht= hematocrit %; MCV= mean corpuscular volume; MCH= mean corpuscular hemoglobin; MCHC= mean corpuscular hemoglobin concentration, WBC's= white blood cells H/L ratio= heterophils / lymphocytes ratio

Tr1, parents and post-hatch chicks fed on basal diet plus in- ovo injection with 0.5 ml saline solution /egg (control treatment). Tr2, parents and post-hatch chicks fed on basal diet plus in- ovo injection with 0.5 ml saline solution (containing 0.2 µg Nano –Se) /egg. Tr3, parents fed with basal diet supplemented with 0.3 mg Nano –Se /kg feed and post-hatch chicks fed on basal diet plus in- ovo injection with 0.5 ml saline solution (containing 0.2 µg Nano –Se)/ egg. Tr4, parents and post-hatch chicks fed on basal diet supplemented with 0.3 mg Nano –Se /kg feed plus in- ovo injection with 0.5 ml saline solution (containing 0.2 µg Nano –Se)/egg.

^{a,b,c} Means bearing different superscripts within the same row are significantly different ($P<0.05$).

Table (3): Effect of Nano-Se supplementation and *in-ovo* injection on bio-chemical parameters of post-hatch chicks reared under hot conditions.

Traits	Treatments				±SE
	Tr1	Tr2	Tr3	Tr4	
Total Protein (g/dl)	3.78	4.31	3.95	3.68	0.17
Albumin (g/dl)	1.16	1.61	1.60	1.36	0.09
Globulin (g/dl)	2.61	2.70	2.35	2.31	0.13
Creatinine (mg/dl)	0.46	0.41	0.40	0.38	0.02
ALT (IU/L)	16.83	16.33	26.00	21.83	2.16
AST(IU/L)	169.33	172.33	210.67	210.67	10.39
Triglycerides (mg/dl)	110.50	78.00	78.17	83.83	6.53

ALT= Alanine aminotransferase AST= Aspartate transferase

Tr1, parents and post-hatch chicks fed on basal diet plus in- ovo injection with 0.5 ml saline solution /egg (control treatment). Tr2, parents and post-hatch chicks fed on basal diet plus in- ovo injection with 0.5 ml saline solution (containing 0.2 µg Nano –Se) /egg. Tr3, parents fed with basal diet supplemented with 0.3 mg Nano –Se /kg feed and post-hatch chicks fed on basal diet plus in- ovo injection with 0.5 ml saline solution (containing 0.2 µg Nano –Se)/ egg. Tr4, parents and post-hatch chicks fed on basal diet supplemented with 0.3 mg Nano –Se /kg feed plus in- ovo injection with 0.5 ml saline solution (containing 0.2 µg Nano –Se)/egg.

Table (4): Effect of Nano-Se supplementation and *in-ovo* injection on body weight of post-hatch chicks reared under hot conditions.

Body weight	Treatments				±SE
	Tr1	Tr2	Tr3	Tr4	
One day	30.86	29.66	31.28	29.11	0.48
2 weeks	95.73	96.00	96.00	92.55	1.52
4 weeks	276.00[b]	279.00[b]	318.71[a]	288.44[ab]	6.24
6 weeks	600.00[c]	627.67[bc]	672.29[a]	657.78[ab]	7.49

Tr1, parents and post-hatch chicks fed on basal diet plus in- ovo injection with 0.5 ml saline solution /egg (control treatment). Tr2, parents and post-hatch chicks fed on basal diet plus in- ovo injection with 0.5 ml saline solution (containing 0.2 µg Nano –Se) /egg. Tr3, parents fed with basal diet supplemented with 0.3 mg Nano –Se /kg feed and post-hatch chicks fed on basal diet plus in- ovo injection with 0.5 ml saline solution (containing 0.2 µg Nano –Se)/ egg. Tr4, parents and post-hatch chicks fed on basal diet supplemented with 0.3 mg Nano –Se /kg feed plus in- ovo injection with 0.5 ml saline solution (containing 0.2 µg Nano –Se)/egg.

[a,b,c] Means bearing different superscripts within the same row are significantly different ($P<0.05$).

Table (5): Effect of Nano-Se supplementation and *in-ovo* injection on body weight gain, feed intake and feed conversion of post-hatch chicks reared under hot conditions.

Age	Traits	Treatments				±SE
		Tr1	Tr2	Tr3	Tr4	
One day – 2 wks	BWG (g)	64.86	66.33	64.71	63.44	1.63
	FI (g)	236.06 [a]	233.66 [a]	222.71 [b]	218.22 [b]	1.78
	FC	3.74	3.56	3.58	3.54	0.10
2- 4 wks	BWG (g)	180.27 [b]	183.00 [b]	222.71 [a]	195.89 [ab]	6.48
	FI (g)	652.80	665.33	659.71	658.44	2.73
	FC	3.96	3.75	3.08	3.37	0.18
4- 6 wks	BWG (g)	324.00 [c]	348.66 [b]	353.57 [b]	369.33 [a]	3.67
	FI (g)	904.53 [b]	932.44 [a]	934.71 [a]	930.11 [a]	2.48
	FC	2.79 [b]	2.67 [b]	2.66 [b]	2.51 [a]	0.02
One day- 6 wks	BWG (g)	569.13 [c]	598.00 [bc]	641.00 [a]	628.67 [ab]	7.58
	FI (g)	1793.40 [c]	1831.44 [ab]	1817.14 [ab]	1806.77 [bc]	3.98
	FC	3.16 [a]	3.07 [ab]	2.85 [c]	2.87 [bc]	0.03

BWG=body weight gain, FI=feed intake, FC=feed conversion

Tr1, parents and post-hatch chicks fed on basal diet plus in- ovo injection with 0.5 ml saline solution /egg (control treatment). Tr2, parents and post-hatch chicks fed on basal diet plus in- ovo injection with 0.5 ml saline solution (containing 0.2 µg Nano –Se) /egg. Tr3, parents fed with basal diet supplemented with 0.3 mg Nano –Se /kg feed and post-hatch chicks fed on basal diet plus in- ovo injection with 0.5 ml saline solution (containing 0.2 µg Nano –Se)/ egg. Tr4, parents and post-hatch chicks fed on basal diet supplemented with 0.3 mg Nano –Se /kg feed plus in- ovo injection with 0.5 ml saline solution (containing 0.2 µg Nano –Se)/egg.

[a,b,c] Means bearing different superscripts within the same row are significantly different (P<0.05).

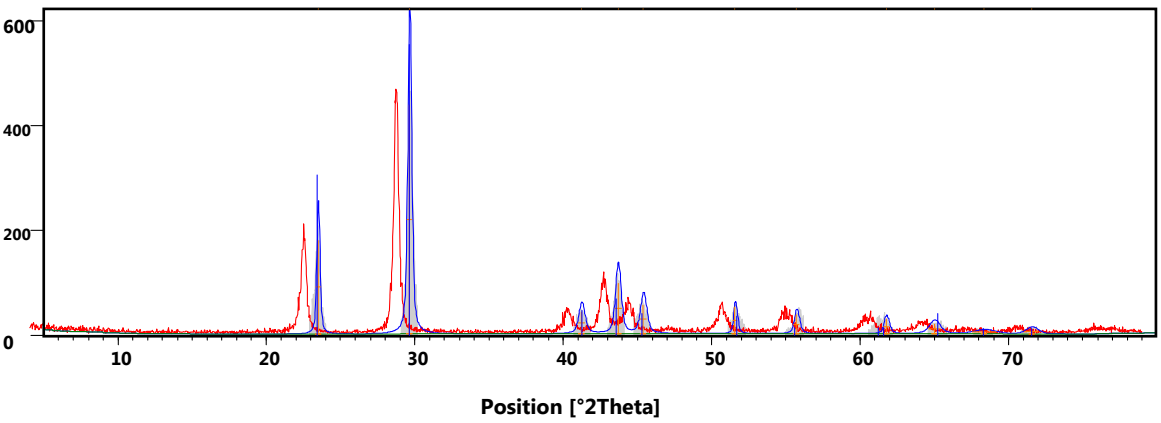

Fig. (1): X-Ray Diffraction crystalline phase of synthesized selenium nanoparticles

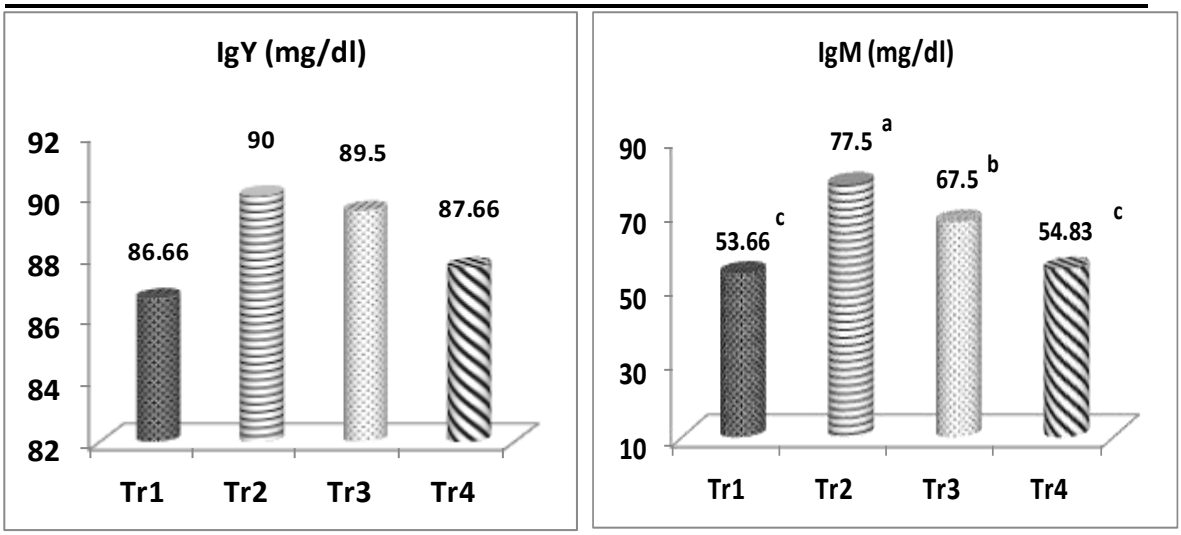

Fig. (2): Effect of Nano-Se supplementation and *in-ovo* injection on IgM and IgY of post-hatch chicks reared under hot conditions

IgM= Immunoglobulin M ; IgY= Immunoglobulin Y

Tr1, parents and post-hatch chicks fed on basal diet plus in- ovo injection with 0.5 ml saline solution /egg (control treatment). Tr2, parents and post-hatch chicks fed on basal diet plus in- ovo injection with 0.5 ml saline solution (containing 0.2 µg Nano –Se) /egg. Tr3, parents fed with basal diet supplemented with 0.3 mg Nano –Se /kg feed and post-hatch chicks fed on basal diet plus in- ovo injection with 0.5 ml saline solution (containing 0.2 µg Nano –Se)/ egg. Tr4, parents and post-hatch chicks fed on basal diet supplemented with 0.3 mg Nano –Se /kg feed plus in- ovo injection with 0.5 ml saline solution (containing 0.2 µg Nano –Se)/egg.

a,b,c Means bearing different superscripts are significantly different (P<0.05).

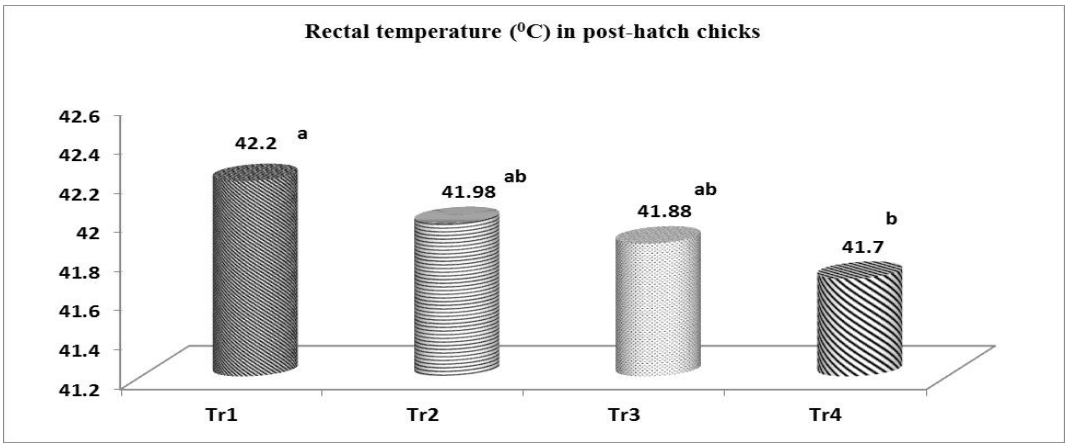

Fig. (3): Effect of Nano-Se supplementation and *in-ovo* injection on rectal temperature of post-hatch chicks reared under hot conditions

Tr1, parents and post-hatch chicks fed on basal diet plus in- ovo injection with 0.5 ml saline solution /egg (control treatment). Tr2, parents and post-hatch chicks fed on basal diet plus in- ovo injection with 0.5 ml saline solution (containing 0.2 µg Nano –Se) /egg. Tr3, parents fed with basal diet supplemented with 0.3 mg Nano –Se /kg feed and post-hatch chicks fed on basal diet plus in- ovo injection with 0.5 ml saline solution (containing 0.2 µg Nano –Se)/ egg. Tr4, parents and post-hatch chicks fed on basal diet supplemented with 0.3 mg Nano –Se /kg feed plus in- ovo injection with 0.5 ml saline solution (containing 0.2 µg Nano –Se)/egg.

[a,b] Means bearing different superscripts are significantly different (P<0.05).

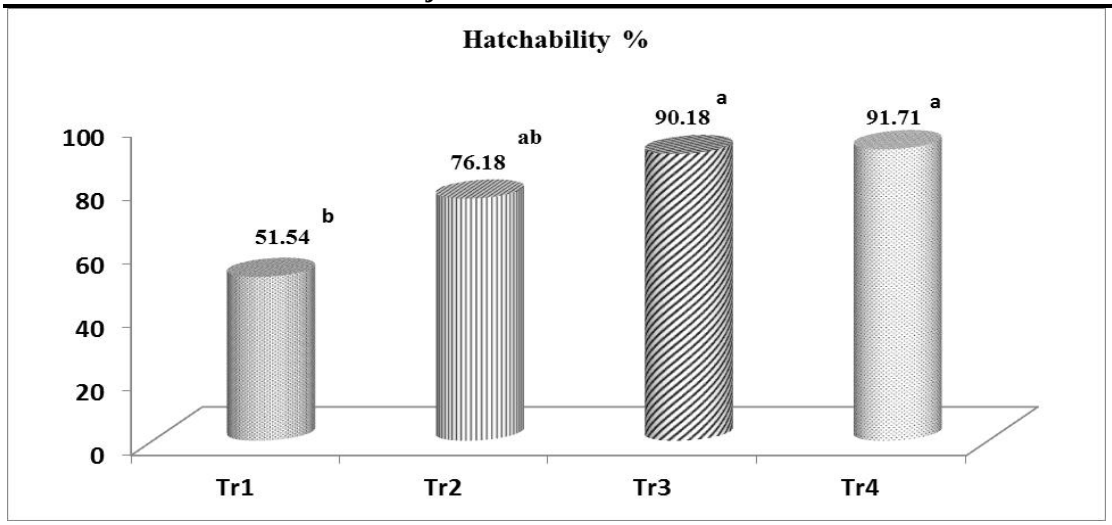

Fig. (4): Effect of Nano-Se supplementation and *in-ovo* injection on hatchability percentage

Tr1, parents and post-hatch chicks fed on basal diet plus in- ovo injection with 0.5 ml saline solution /egg (control treatment). Tr2, parents and post-hatch chicks fed on basal diet plus in- ovo injection with 0.5 ml saline solution (containing 0.2 µg Nano –Se) /egg. Tr3, parents fed with basal diet supplemented with 0.3 mg Nano –Se /kg feed and post-hatch chicks fed on basal diet plus in- ovo injection with 0.5 ml saline solution (containing 0.2 µg Nano –Se)/ egg. Tr4, parents and post-hatch chicks fed on basal diet supplemented with 0.3 mg Nano –Se /kg feed plus in- ovo injection with 0.5 ml saline solution (containing 0.2 µg Nano –Se)/egg.

[a,b] Means bearing different superscripts are significantly different ($P<0.05$).

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المخلص العربى

تأثير إضافة النانوسيلينوم وحقن البويضة على أداء النمو و صفات الدم والإستجابات المناعية للكتاكيت الناتجة بعد الفقس المرباة تحت الظروف الحارة

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يهدف هذا العمل الى دراسة إضافة النانوسيلينوم وحقن البويضة على أداء النمو والإستجابات الفسيولوجية للكتاكيت الناتجة بعد الفقس المرباة تحت الظروف الحارة.

في هذه التجربة تم استخدام ستون دجاجة بياضة وثمانية ديوك من سلالة دقى 4 (عمر أربعة وسبعون اسبوع بمتوسط وزن للإناث والذكور 1663.75 ± 34.9 ، 2383.33±147 على التوالى) وتم تقسيمهم عشوائيا إلى مجموعتين (ثلاثون دجاجة واربعة ديوك / مجموعة). المجموعة الاولى اعتبرت اباء المعاملة الاولى والثانية. المجموعة الثانية اعتبرت اباء المعاملة الثالثة والرابعة. بعد اربعة اسابيع من التجربة . تم تجميع 300 بيضة مخصبة من المجموعتبن (150 بيضة / مجموعة). البيض المخصب والكتاكيت الناتجة بعد الفقس عُملت كلاتى ، المعاملة الاولى ، الاباء والكتاكيت الناتجة بعد الفقس غذيت على العليقة الاساسية وتم حقن البويضة بنصف مللى من المحلول الملحى / بيضة (معاملة التحكم). المعاملة الثانية، الاباء والكتاكيت الناتجة بعد الفقس غذيت على العليقة الاساسية مع حقن البويضة بنصف مللى من المحلول الملحى (يحتوى على 0.2 ميكرو جرام من النانوسيلينوم) / بيضة. المعاملة الثالثة ، غذيت الاباء بعليقة اساسية مع امدادها ب 0.3 مللى جرام نانوسيلينوم / كجم علف والكتاكيت الناتجة غذيت على عليقة اساسية مع حقن البويضة بنصف مللى من المحلول الملحى (يحتوى على 0.2 ميكرو جرام من النانوسيلينوم) / بيضة. المعاملة الرابعة، الاباء والكتاكيت الناتجة بعد الفقس غذيت على عليقة اساسية مع امدادها ب 0.3 مللى جرام نانوسيلينوم / كجم علف مع حقن البويضة بنصف مللى من المحلول الملحى (يحتوى على 0.2 ميكرو جرام من النانوسيلينوم) / بيضة.

أظهرت النتائج زيادة معنوية فى الهيموجلوبين ومتوسط حجم كرات الدم الحمراء فى المعاملة الثانية والثالثة مقارنة بالمعاملة الاولى (معاملة التحكم)، نسبة الهيتروفيل الى الليمفوسيت ونسبة الفقس زادت معنويا فى المعاملة الثالثة والرابعة مقارنة مع المعاملة الاولى، زادت الاجسام المضادة من النوع IgM فى المعاملة الثانية والثالثة مقارنة بالمعاملة الرابعة والاولى ، إنخفضت درجة حرارة المستقيم فى المعاملة الرابعة مقارنة بالمعاملة الاولى ، ابدت المعاملة الثالثة والرابعة أداء أفضل فى وزن الجسم المكتسب والغذاء الماكول ونسبة التحويل الغذائى مقارنة بالمعاملة الاولى والثانية خلال الفترة من عمر يوم وحتى عمر 6 أسابيع من العمرمن الفقس.

يمكننا التوصية بأن إمداد علائق الاباء والكتاكيت الناتجة بعد الفقس بـ 0.3 مللى جرام نانوسيلينوم / كجم علف بالإضافة الى حقن البويضة بـ 0.2 ميكروجرام نانوسيلينوم / بيضة يحسن أداء النمو و صفات الدم والإستجابات المناعية للكتاكيت الناتجة بعد الفقس المرباة تحت الظروف الحارة.