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Effect of In-Ovo Organic Zinc Injected and High Temperature During the Late Stage of Chicken Eggs Incubation on Post Hatch Performance

Hanaa M. Khalil[1], Faika G. El-Wediny[1], Sabria B. Abou-El-Soud[2] and Yahya Eid [2]

[1] *Department of Poultry Breeding Research, Animal Production Research Institute, Agriculture Research Center, Egypt.*

[2] *Department of Poultry Production, faculty of agriculture, Kafrelsheikh University, 33516 Kafr Elsheikh, Egypt.*

THIS study aimed to investigate the effect of in ovo injection of organic zinc and eggs subjected to high temperature (39.8°C) during the late stage of incubation on post-hatch performance and immune response of local Egyptian strain (Bandarah) chicks. A total of 400 fertile eggs were weighed and randomly distributed to 8 groups with 5 replicates 10 eggs each. On the 14th day of incubation, eggs were injected asT1: Eggs not injected (control), T2: eggs injected with 1ml saline solution, T3: eggs injected with 1ml saline solution containing 50µg Zn-Met, T4: eggs injected with a saline solution containing 100µg Zn-Met. Treatments from T1 to T4 incubated at normal temperature of 37.8°c and 60%RH, treatments from T5 toT8 injected similar doses as described and subjected to high temperature of 39.8°c when eggs are transferred from setter to hatcher machine for 3 hours at 18, 19, and 20 days of incubation. Results showed that in ovo injection with 50 and 100 µg Zn-Met/egg and subjected to high temperature during late incubation could improve hatching, chicks' weight, antioxidant activities, and increased WBC's and lymphocyte. Group injected with 100µg Zn-Met/egg was more effective in improving high-density lipoprotein, low-density lipoprotein, triglycerides, cholesterol, glucose, zinc, and T_3.

Keywords: *In ovo* injection, organic zinc, hatchability, immune response, biochemical parameters.

Introduction

Egg yolk is the main compartment storage of nutrients for embryos, most of the zinc is exhausted by the late embryonic stage [1]. *In ovo* injection of nano, minerals could help embryos at the late stage of embryogenesis with a supplementary amount of nutrients. Nutrients are transferred into the amniotic fluid and then absorbed by the developing embryo [2]. Antioxidants play an important role in cells protecting from reactive oxygen species by reducing free radicals, zinc acts as one of these antioxidants. In tropical and semi tropical regions, raising broiler out of their thermal comfort zone can cause economic loss in the poultry industry. In poultry that exposed to elevated temperature showed desperation on the immune responses, body weight and feed efficiency [3] while plasma corticosterone and heterophil/ lymphocyte ratio are improved [4]. The increased temperature during incubation causes stress before hatching and it affects the development of embryonic organs. *In ovo* injection of zinc could improve the performance of chicks by enhancing the immune system, improving the antibody synthesis [5], and performance of non-specific immunity systems for instance neutrophils and natural killer cells [6]. This experiment was designed to study the effect of in ovo injection of zinc-methionine and subjected to high temperature during the late incubation on the hatchability, post-hatch-performance, and immune response of Bandarah chicks.

*Corresponding author: Yahya Z. Eid, E-mail: yahya.eid@agr.kfs.edu.eg, Tel.: 00201111819340
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Material and Methods

The present study was conducted at the El-Sabahia poultry Research station in Alexandria belonging to Animal Production Research Institute. Six hundred hatching eggs produced from Bandarah hens at 48 wks were collected at the same day. All eggs were individually numbered and weighed (the average egg weight was 49±1) prior to the beginning of the incubation. Eggs incubated in Egyptian made incubator model S100 at 37.8°C and 60% RH during setting phase of incubation. For starting our experiment four hundred fertile eggs were weighed and randomly distributed to 8 groups with 5 replicates. On the 14th day of incubation the injected was performed included.T1: eggs not injected (control), T2: eggs injected with 1ml saline solution (possitive control), T3: eggs injected with 1ml saline solution containing 50µg Zn-Met, T4: eggs injected with a saline solution containing 100µg Zn-Met. The pH of the solution was 7.0. Treatments from T1 to T4 incubated at normal temperature of 37.8°c and 60%RH, treatments fromT5 to T8 injected as the similar doses as described and subjected to high temperature of 39.8°c and 66%RH, when eggs are transferred from setter to hatcher machine for 3 hours daily at 18, 19 and 20 days of incubation.

In ovo injection technique

On the 14th day of incubation, eggs were injected according to the previous doses through the amniotic route using a hypodermic needle (25 mm long) and the pinpoint hole was sealed using wax.

Zinc methionine complex (zinpro®180) zinc concentration 18% was used in this experiment.

Characteristics investigated: Hatching chicks were individually weighed at the initial of the experiment, and then they were weighed for each 4 weeks in each replicate.

Five chicks at one day old from each replicate were slaughtered. Blood samples were taken into heparinized tubes for measuring white blood cells (WBC's) counted using hemocytometer using a light microscope at 100x magnification after diluted the blood samples 20 times with diluting fluid (1% acetic solution with little of Leishman's stain) before counting according to Hepler [7] Plasma was obtained by centrifugation of the remaining blood at 3000 rpm for 20 minutes, and kept at -20°c until used for biochemical analysis. Plasma glucose concentration was determined by the method of Trinder [8] using commercial kits (Diamond Diagnostics). Plasma low density lipoprotein LDL and high-density lipoprotein HDL were determined according to Wieland and Seidel and Lopes-Virella *et al.* [9,10] respectively. Total antioxidant capacity (TAC) and malondialdehyde (MDA) were determined according to Erel and Ricard *et al.* [11,12] respectively.

Plasma total cholesterol determined according to the method of Stein [13], triglycerides, glutathione (GSH-Px), Superoxide dismutase (SOD) activities and copper-zinc superoxide dismutase (Cu-Zn-SOD), mineral (Zn) were determined using commercial kits. Triiodothyronine (T_3) was measured by using radioimmunoassay (RIA) kits according to the method described by Hollander and Shenkman [14].

The statistical analysis was performed using 2-way analysis by general linear model procedure of SAS version 9.4 (SAS Institute lnc., Cary, North Carolina, USA).

The statistical model used in this study was: $Y_{ijk}= \mu+A_i+ G_j +AG_{ij} +e_{ijk}$

Where: Y_{ijk}= an observation from the kth bird, µ =General mean, A_i=the effect of injection, G_j= the effect of temperature, AG_{ij}= the effect of interaction between injection and temperature, and e_{ijk}= random error. The statistical significance of the effects was assessed at a p-value of 0.05.

Results and Discussion

Table (1) showed the effect of in ovo injection of Zn-Met and subjected to high temperature (HT) in late incubation and their interaction on hatching%, chick's body weight and hatch weight as a percentage of egg weight. Regardless of HT hatchability%, chick weights at 0day, 4wks, 8wks , and hatch weight as a percentage of egg weight were higher ($p<0.05$) *in ovo* injection of Zn-Met groups than in control groups. However, non-hatched eggs were decreased ($p<0.05$) *in ovo* injection of Zn-Met levels than in control groups. Concerning the effect of HT in incubation on hatchability% and chick weights at 0day, 4wks, 8wks and hatch weight% of egg weight were lower ($p<0.05$) under HT than the group under normal (NT). However, non-hatched eggs were increased ($p<0.05$) under HT. Hatchability, chick's weight at 0day, 4wks, 8wks and hatch weight % /egg weigh were significantly affected by an interaction between in ovo injection Zn-Met levels and HT and NT hatchability, chicks weight from 0day to 8 wks and hatch weight % /egg weight were higher ($p<0.05$) in ovo injection of Zn-Met levels group under HT or NT compared to control groups (T1 andT2). Moreover, the group of Zn-Met at 100µg/egg recorded the highest chicks weight at 4wks, 8wks and hatch weight % /egg weight under HT and NT. Followed that the group of 50µg/egg. Control (T2) recorded the highest value ($p<0.05$) concerning non-hatched eggs, compared to the other groups. Results are in agreement with, Biria *et al.* [15] who found nano ZnO *in ovo* injection increased

the hatchability of eggs and decreased the early embryo mortality rate in broilers. High incubation temperature could negatively affect hatchability and post-hatch growth performance [16]. Wilson [17] noted that each 1g of increase in BW at hatch leads to 8 to 13g of increase in BW at marketing. Tako *et al.* [18] concluded that Zn-Met *in ovo* injected improved villus surface at hatching and improved weight gain in broilers. Hassan [19] found *in ovo* injection nano-zinc improved the hatching weight of chicks, and weight gain, of broiler chicks under heat stress.

In addition, Hee *et al.* [20] noted that *in ovo* injection of Zn (200 mg/egg) at 18 embryonic days, increased body weight. Kouassi *et al.* [21] reported that development of the *in ovo* technology allowing for the delivery of bioactive substances into chicken embryos during their development represents a way to accommodate the perinatal period, late embryo development, and post- hatch growth. Also, Sogunle *et al.* [22] found that in ovo injection of Zn at 80µg.egg^{-1} and/or Cu at 16µg.egg^{-1} enhanced growth in broiler chickens. Effects of *in ovo* injection of zinc methionine and subjected to high temperature (HT) in incubation and their interaction on the immune response of post-hatch chicks are shown in Table(2). Regardless of HT, WBC's count, and lymphocyte% were increased ($p<0.05$) by injection Zn-Met groups compared with two control groups. While heterophil and H/L ratios were lower ($p<0.05$) than in the control groups. Regarding the effect of HT, it was shown that WBC's count, and lymphocyte were significantly lower ($p<0.05$) than normal temperature (NT) while heterophil and H/L ratio increased.

The interaction effect between Zn-Met levels and HT noted that the highest value for WBC's, and lymphocytes was observed for 50 and100µg Zn-Met/egg either with HT or NT. While injected Zn-Met levels with HT and NT had the lowest value for heterophil and H/L ratio compared with control groups (T1and T2). Results agreement with, Biria *et al.* [15]. Sogunle *et al.* [22] found that *in ovo* injection of Zn at 80µg.egg^{-1} and/or Cu at 16µg.egg^{-1} enhanced immune response of blood serum in broiler chickens. They found nano ZnO in ovo injection increased immune responses of broilers. Zinc has a well-known role in the development of the immune system of the chicks [23] and dietary Zn supplementation improve the antibody synthesis [5] and performance of non-specific immunity system for instance neutrophils and natural killer cells [6] in broilers. Shokraneh *et al.* [24] reported that injection of Nano-ZnO had alleviated the negative effects of high-temperature incubation. In contrast, Jose *et al.* [25] found *in ovo* administration of different forms of Zn did not positive response on the immune status of birds post-hatch. Table (3) summarized the effect of *in ovo* injection of Zn-Met and subjected to HT in incubation and their interaction on antioxidant activity (TAC), GSH-Px, (SOD), (Cu-Zn-SOD) and (MDA) in post-hatch chicks. Regardless of HT the activities of TAC, GSH-Px, SOD and CuZnSOD were enhanced ($p<0.05$) in ovo injection of Zn-Met levels than control groups. However, MDA was reduced for Zn-Met levels than in control groups.

Concerning the effect of HT in incubation, the activities of GSH-Px, SOD, CuZnSOD, and MDA were decreased except TAC increased ($p<0.05$) compared with the groups subjected to normal temperature (NT). Concerning the interaction between ovo injection Zn-Met levels and HT in incubation, the antioxidant was significantly affected, our results recorded that the activity of the antioxidant *in ovo* injection under HT and NT increased ($p<0.05$) than those control exposed to HT. However, MDA was lower in Zn-Met levels under HT than in control groups. *In ovo* Zn injection with 0, 50,150, 200µg Zn/egg, up-regulated the metallothionein (MT) mRNA expression levels in the embryonic liver at E20, and Cu-Zn-SOD activities. While MDA did not affect. Shokraneh *et al.* [24] recorded that *in ovo* injection of nano-Zinc oxide and high eggshell temperature during late incubation increased ($p<0.05$) activity of GSH-Px and SOD at high EST. In our experiment injection of Zn-Met had a significant role in alleviating the negative effects of high temperature during incubation by increased antioxidant activity and reduced oxidative stress. Also, Xiao *et al.* [26] observed high incubation temperature is a stressor for embryos of all ages. So during high eggshell temperature, ROS generation and oxidative damage were increased and reduced antioxidant activity. Table (4) summarized the effect of *in ovo* injection of Zn-Met and HT during late incubation and their interaction on (LDL), HDL, triglycerides, cholesterol, glucose, Zn, and triiodothyronine (T_3). Regardless of HT in incubation, LDL, triglycerides, and cholesterol were decreased ($p<0.05$) by *in ovo* injection of 50 or 100µg Zn-Met/egg compared with control groups (T1 andT2), however. HDL, glucose, and T_3 were higher ($p<0.05$) in Zn-Met 100µg/egg than in other treatment groups. *In ovo* injection, 50 or 100 µg/egg plasma zinc concentration increased ($p<0.05$) in other groups. Irrespective of *in ovo* injection of Zn-Met levels, HDL was not affected by HT. However, LDL, triglycerides, and cholesterol were affected by HT and increased ($p<0.05$) in NT groups. While, glucose, zinc, and T_3 were reduced ($p<0.05$) by HT than NT. Results indicated that the interaction between *in ovo* injection of Zn-Met levels with HT and NT lowered ($p<0.05$) both LDL, triglycerides,

cholesterol, and glucose than in other control groups. While, HDL, zinc, and T_3 were higher ($p<0.05$) under HT with *in ovo* injection of Zn-Met levels and Zn-Met with NT than in control groups (T1and T2). Results, Willemsen *et al.*, and Willemsen *et al.* [27, 28] found that high incubation temperature increased plasma levels of triglyceride and cholesterol. Sarica *et al.* [29] reported that the high levels of stress hormones stimulate lipolysis, which explains the increasing concentration of cholesterol and triglyceride under high EST and decreased plasma concentration of total protein is due to the catabolism of proteins to free amino acid for using as gluconeogenic substrates. Ayo *et al.* [30] noted that changes in incubation temperature and subsequently hormonal alterations led to changes in some metabolic responses.

On the other hand, Biria *et al.* [15] noted that injection of 50, 75, and 100 ppm nano-ZnO in broilers. Causes increasing cholesterol, LDL, and HDL [31]. Found that high temperature (38.8^{o}c) during d 10 to 18 of incubation increased T_3 in the broiler.

The results of Shokraneh *et al.* [24] are in agreement with our results they found that ovo injection with NaCl solution containing 500µg Nano-ZnO and incubated at high EST significantly decreased the levels of triglyceride, cholesterol, glucose, and T_3 at high EST. Thyroid hormones were increased and reached a peak during external piping. So, T_3 was beneficial during hatching and supplied more energy. While high incubation temperature caused decreased T_3 hormone and non-hatch due to decreased T_3 hormone [32].

Conclusion

It could be recommended that in ovo injection zinc methionine at 50 or 100µg/egg could alleviate the negative effects of high temperature during late incubation by increasing antioxidant activity, improving hatchability, chicks' performance, and immune response of hatched chicks.

Conflicts of interest

"There are no conflicts to declare".

TABLE 1. Effect of in ovo injection of zinc methionine and subjected to high temperature in late incubation and their interaction on hatching traits and chicks weights hatch represented by mean ± SE

Mean effect		Hatchability from fertile eggs%	Non-hatched from fertile eggs%	Hatch weight% / egg weight	Chick weight at 0 day (g)	Body weight at 4wks (g)	Body weight 8wks(g)
Inject							
Non injected control(T_1)		74.79 ± 4.1^{c}	8.54 ± 2.4^{b}	69.51±1.0^{c}	35.02± 0.53^{c}	179.75±3.34^{c}	657.25 ±4.5^{c}
Injected with saline control(T_2)		70.07 ± 3.7^{d}	17.65 ± 2.31^{a}	68.5 ± 0.94^{d}	34.52 ± 0.47^{c}	173.23 ±4.02^{c}	650.15 ± 5.45^{c}
Inject 50µg Zn-Met(T_3)		91.99 ± 0.43^{b}	6.53 ± 0.21^{c}	79.5 ± 0.9^{b}	40.07 ± 0.5^{b}	305.93 ±1.91^{b}	829.67±2.15^{b}
Inject100µg Zn-Met(T_4)		93.89 ± 0.68^{a}	5.12 ± 0.33^{d}	81.84 ± 0.49^{a}	41.23 ± 0.23^{a}	315.45 ±0.36^{a}	843.20 ± 0.9^{a}
P value		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Temperature							
Normal temperature		87.39 ± 0.33^{a}	9.06 ± 0.45^{b}	75.54 ± 0.03^{a}	38.58 ± 0.13^{a}	247.98 ±2.51^{a}	751.21 ±1.04^{a}
High temperature		77.89 ±1.36^{b}	9.87 ± 0.54^{a}	74.2 ± 0.56^{b}	36.84±0.35^{b}	239.20±2.54^{b}	738.93 ±3.4^{b}
P value		0.0001	0.0001	0.0006	0.0026	0.0001	0.0001
Interaction between injecting and temperature							
Non injected control(T_1)	Normal	83.77±0.54^{b}	10.31 ±0.33^{c}	71.75±0.01^{c}	36.14±0.31^{c}	186.55 ±0.84^{c}	666.95±1.1^{c}
	High	65.82±1.31^{d}	6.76±0.33^{d}	67.28 ±0.01^{d}	33.89 ±0.28^{b}	172.95±2.97^{d}	647.55±3.20^{c}
Injected with saline control(T_2)	Normal	79.31±0.13^{c}	15.29±0.33^{b}	70.58 ±0.54^{c}	35.55 ±0.03^{c}	182.35 ±0.09^{c}	662.35 ±0.09^{c}
	High	60.82±1.31^{e}	20.01 ±0.57^{a}	66.50 ±0.01^{d}	33.50 ±0.28^{d}	164.10 ±0.69^{e}	637.95 ±0.03^{d}
Inject 50µg Zn-Met(T_3)	Normal	92.05 ±0.71^{a}	6.10 ±0^{d}	81.67 ±0.03^{a}	41.13 ±0.92^{a}	306.90±3.03^{b}	830.45 ±3.32^{b}
	High	91.94±0.65^{ab}	6.95 ±0.33^{d}	77.42 ±0.65^{b}	39.00^{b} ±0.02	304.95^{b}± 2.82	828.90± 3.4^{b}
Inject100µg Zn-Met(T_4)	Normal	94.45 ±1.31^{a}	4.55 ± 0.58^{e}	82.41 ±0.93^{a}	41.50 ± 0.12^{a}	316.10 ±0.5^{a}	845.10 ±1.03^{a}
	High	93.32 ±0.38^{a}	5.68 ±0.33^{e}	81.29±0.17^{a}	40.95 ±0.43^{a}	314.80 ±0.12^{a}	841.30 ±0.17^{a}
P value		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

$^{a,b,\cdots}$Means within a column within each factor not sharing similar superscripts are significantly different , P-value=probability level

TABLE 2. Effect of in ovo injection of zinc methionine and subjected to high temperature in late incubation and their interaction on immune response in post-hatch chicks represented by mean ± SE .

Mean effect		WBC's 10^3mm^3	Lymphocyte%	Heterophil%	H/L ratio
Inject					
Non injected control(T_1)		21.53 ±0.19^{b}	34.08 ±0.5^{b}	23.28 ±0.48^{a}	68.45 ±1.9^{b}
Inject with saline, control(T_2)		21.18 ±0.43^{b}	32.48 ±0.5^{b}	23.94 ±0.47^{a}	73.83 ±1.8^{a}
Inject 50µg Zn-Met(T_3)		25.27 ±0.16^{a}	41.24 ±0.1^{a}	20.14 ±0.28^{b}	49.19 ±1.7^{c}
Inject100µg Zn-Met(T_4)		24.87 ±0.2^{a}	42.99 ±0.72^{a}	19.78 ±0.26^{b}	46.17 ±1.2^{c}
P value		0.0001	0.0001	0.0001	0.0001
Temperature					
Normal temperature		23.72 ±0.35^{a}	38.76 ±0.9^{a}	21.35 ±0.49^{b}	56.46 ±1.96^{b}
High temperature		22.71 ±0.19^{b}	36.64 ±0.71^{b}	22.21 ±0.51^{a}	62.37 ±1.76^{a}
P value		0.0001	0.0040	0.0317	0.0001
Interaction between injecting and temperature					
Non injected control(T_1)	Normal	21.73 ±0.34^{b}	35.12 ±0.44^{c}	22.7 ±0.46^{a}	64.54 ±1.94^{b}
	High	21.33 ±0.17^{b}	32.93 ±0.27^{c}	23.85 ±0.81^{a}	72.37 ±2.03^{a}
Inject with saline, control(T_2)	Normal	22.34 ±0.34^{b}	33.73 ±0.31^{c}	23.57 ±0.79^{a}	69.81 ±1.7^{b}
	High	20.02 ±0.19^{b}	31.23 ±0.45^{d}	24.30 ±0.54^{a}	77.85 ±1.69^{a}
Inject 50µg Zn-Met(T_3)	Normal	25.61 ±0.18^{a}	42.26 ±1.58^{a}	19.70 ±0.34^{b}	46.93 ±2.3^{d}
	High	24.93 ± 0.16^{a}	40.23 ± 1.54^{b}	20.57 ± 0.38^{b}	51.45 ± 2.21^{c}
Inject100µg Zn-Met(T_4)	Normal	25.18 ±0.23^{a}	43.83 ±1.28^{a}	19.43 ±0.4^{b}	44.56 ±1.9^{d}
	High	24.56 ±0.27^{a}	42.16 ±0.56^{a}	20.12 ±0.29^{b}	47.78 ±1.12^{d}
P value		0.0019	0.0050	0.0038	0.0051

$^{a,b,\cdots}$Means within a column within each factor not sharing similar superscripts are significantly different, P-value=probability level, WBC's=white blood cells.

TABLE 3. Effect of in ovo injection of zinc methionine and subjected to high temperature in late incubation and their interaction on antioxidant activity in post-hatch chicks represented by mean ± SE .

Mean effect		TAC (mg/dl)	GSH-Px (Umol/l)	SOD (µ/ml)	CuZnSOD (µ/ml)	MDA (U/l)
Inject						
Non injected control(T_1)		394.17±1.05 ^{b}	823.49 ±1.18 ^{b}	153.58 ±0.52 ^{d}	162.08 ±0.69 ^{c}	11.80 ±0.29 ^{a}
Inject with saline control(T_2)		375.61 ±3.6 ^{c}	823.89±0.99 ^{b}	155.34±0.46 ^{c}	162.73 ±0.84 ^{c}	12.22±0.21 ^{a}
Inject 50µg Zn-Met(T_3)		447.14 ±1.5 ^{a}	958.09 ±2.7 ^{a}	172.86±0.95 ^{b}	184.36±2.11 ^{b}	10.29±0.02 ^{b}
Inject 100µg Zn-Met(T_4)		448.34 ±1.7 ^{a}	961.10 ±3.2 ^{a}	174.95±0.81 ^{a}	187.95 ±.46 ^{a}	10.08 ± 0.16 ^{b}
P value		0.0001	0.0001	0.0001	0.0001	0.0001
Temperature						
Normal temperature		406.6 ±1.65 ^{b}	894.23 ± 1.09 ^{a}	165.98 ±0.28 ^{a}	177.73 ±0.34 ^{a}	11.33 ±0.21 ^{a}
High temperature		426.13 ±0.84 ^{a}	889.06 ±1.06 ^{b}	162.33 ±0.5 ^{b}	170.83 ±0.62 ^{b}	10.86 ±.33 ^{b}
P value		0.0001	0.0001	0.0001	0.0001	0.0053
Interaction between injecting and temperature						
Control(T_1)	Normal	425.52±3.06 ^{b}	826.21±1.6 ^{c}	152.17 ±0.25 ^{c}	160.17 ±0.51 ^{d}	11.03 ±0.2 ^{a}
	High	362.83±0.9 ^{d}	820.77±0.25 ^{d}	154.99 ±0.42 ^{c}	163.99±0.18 ^{d}	12.57 ±0.96 ^{a}
Inject with saline(T_2)	Normal	392.19 ±1.92 ^{c}	826.67±0.70 ^{c}	154.40±0.35 ^{c}	161.40±0.28 ^{d}	12.19±0.45 ^{a}
	High	359.02 ±0.08 ^{d}	821.11 ±0.21 ^{d}	156.04 ±0.72 ^{c}	164.04±1.51 ^{d}	12.24 ±0.01 ^{a}
Inject 50µg Zn-Met(T_3)	Normal	442.71±0.84 ^{b}	950.42 ±1.56 ^{ab}	170.06±0.1 ^{ab}	178.06 ±0.18 ^{c}	10.30±0.04 ^{b}
	High	451.56 ±0.57 ^{a}	965.78 ±0.29 ^{a}	175.59 ± 0.34 ^{a}	190.65 ± 0.34 ^{a}	10.82±0.03 ^{b}
Inject 100µg Zn-Met(T_4)	Normal	444.09 ±0.8 ^{a}	952.93 ±0.49 ^{a}	172.66 ±0.42 ^{a}	183.66±0.38 ^{b}	9.92±0.15 ^{c}
	High	452.59 ±1.81 ^{a}	969.27 ±3.5 ^{a}	177.24 ±0.43 ^{a}	192.24 ±0.43 ^{a}	10.23±0.3 ^{b}
P value		0.0001	0.0001	0.0002	0.0001	0.0041

$^{a,b,\ldots}$Means within a column within each factor not sharing similar superscripts are significantly different, P-value=probability level, TAC=total antioxidant capacity, GSH=glutathione-peroxidase, SOD=superoxide dismutase, CuZnSOD=copper zinc superoxide dismutase. MDA=Malonaldehyed

TABLE 4. Effect of in ovo injection of zinc methionine and subjected to high temperature in late incubation and their interaction on some biochemical parameters in post-hatch chicks represented by mean ± SE .

Mean effect		LDL (mg/dl)	HDL (mg/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)	Glucose (mg/dl)	Zinc (mg/dl)	T_3 (nmol/l)
Inject								
Non injected control(T_1)		96.41 ±0.54 ^{a}	35.74±0.34 ^{c}	96.79±0.57 ^{a}	202.79±1.56 ^{a}	161.73±1.58 ^{d}	70.88±0.54 ^{b}	1.42±0.02 ^{c}
Inject with saline control(T_2)		95.94±0.18 ^{a}	35.95±0.38 ^{c}	97.77±0.14 ^{a}	202.28±1.5 ^{a}	163.16±1.31 ^{c}	70.68±0.42 ^{b}	1.46±0.07 ^{c}
Inject 50µg Zn-Met(T_3)		88.93±0.16 ^{b}	50.43±0.44 ^{b}	66.61±0.54 ^{b}	169.61±0.54 ^{b}	183.13 +0.95 ^{b}	83.65±0.20 ^{a}	1.94±0.01 ^{b}
Inject100µg Zn-Met(T_4)		85.63±0.69 ^{c}	51.93±0.7 ^{a}	62.80±0.98 ^{c}	164.80±1.3 ^{c}	189.78±1.46 ^{a}	84.21±0.15 ^{a}	2.00±0.02 ^{a}
P value		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Temperature								
Normal temperature		91.169±0.3 ^{b}	43.59±0.5	79.59±0.37 ^{b}	181.34±0.37 ^{b}	178.54±0.23 ^{a}	77.88±0.36 ^{a}	1.78±0.1 ^{a}
High temperature		92.29±0.6 ^{a}	43.42±0.25	82.4±0.24 ^{a}	188.41±0.25 ^{a}	170.36±0.34 ^{b}	76.82±0.25 ^{b}	1.63±0.05 ^{b}
P value		0.0051	NS	0.0001	0.0001	0.0001	0.0001	0.0001
Interaction between inject and temperature								
Non injected control (T_1)	Normal	95.26±0.34 ^{a}	34.93±0.25 ^{c}	95.15±0.23 ^{a}	198.15±0.23 ^{b}	167.15±0.08 ^{c}	72.18±0.61 ^{b}	1.46±0.24 ^{c}
	High	97.56±0.74 ^{a}	36.54±0.36 ^{c}	98.45±0.23 ^{a}	207.45±0.23 ^{a}	156.3±0.20 ^{d}	69.58±0.33 ^{c}	1.38±0.14 ^{c}
Inject with saline control (T_2)	Normal	96.22±0.22 ^{a}	36.34±0.65 ^{c}	97.69±0.29 ^{a}	197.69±0.29 ^{b}	167.07±0.19 ^{c}	71.75±0.17 ^{b}	1.64±0.03 ^{b}
	High	95.67±0.23 ^{a}	35.26±0.08 ^{c}	97.86±0.03 ^{a}	206.86±0.03 ^{a}	159.25 ±0.29 ^{d}	69.61±0.44 ^{c}	1.27 ±0.05 ^{d}
Inject 50µg Zn-Met(T_3)	Normal	88.89±0.26 ^{b}	49.23±0.25 ^{b}	65.62±0.75 ^{b}	168.62±0.76 ^{c}	185.81± 0.46 ^{b}	83.42 ±0.39 ^{a}	1.96±0.01 ^{a}
	High	88.96±0.23 ^{b}	51.63±0.27 ^{a}	67.59±0.52 ^{b}	170.00±0.52 ^{c}	180.43±0.43 ^{b}	83.87±0.08 ^{a}	1.92±0.01 ^{a}
Inject100µg Zn-Met(T_4)	Normal	84.31±0.22 ^{c}	53.59±0.79 ^{a}	59.89±0.20 ^{c}	160.89±0.20 ^{d}	194.11±0.20 ^{a}	84.19±0.28 ^{a}	2.04±0.02 ^{a}
	High	86.96±1.13 ^{c}	50.26±0.29 ^{a}	65.71±0.19 ^{b}	168.71±0.19 ^{c}	185.5±0.44 ^{b}	84.24±0.16 ^{a}	1.96±0.01 ^{a}
P value		0.0086	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

$^{a,b,\ldots}$Means within a column within each factor not sharing similar superscripts are significantly different, P-value=probability level, NS=No significantly, LDL=Low density lipoprotein, HDL=High density lipoprotein, T_3= triiodothyronine

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تأثير الحقن داخل البيضة بالزنك العضوي وارتفاع درجة الحرارة خلال المرحلة المتأخرة من حضانة بيض الدجاج على الأداء بعد الفقس

هناء خليل[١] ، فايقة الودينى[١] و صبرية أبو السعود[٢] و يحيى عيد[٢]

[١] قسم أبحاث تربية الدواجن - معهد بحوث الانتاج الحيوانى - مركز البحوث الزراعية - مصر.

[٢] قسم إنتاج الدواجن - كلية الزراعة - جامعة كفر الشيخ - مصر.

هدفت هذه الدراسة إلى تقصي تأثير الحقن داخل البيضة بالزنك العضوي وتعريض البيض لدرجة حرارة عالية (٣٩,٨ درجة مئوية) خلال المرحلة المتأخرة من الحضانة على الأداء بعد الفقس والاستجابة المناعية لفراخ السلالة المصرية المحلية (البندرة). تم وزن ما مجموعه ٤٠٠ بيضة خصبة وتوزيعها عشوائياً إلى ٨ مجموعات مع ٥ مكررات من ١٠ بيضات لكل منها. في اليوم ١٤ من الحضانة، تم حقن البيض كالتالي: T1: بيض غير محقون (مجموعة ضابطة)، T2: بيض محقون بـ ١ مل محلول ملحي، T3: بيض محقون بـ ١ مل محلول ملحي يحتوي على ٥٠ ميكروجرام من ميثيونين الزنك، T4: بيض محقون بمحلول ملحي يحتوي على ١٠٠ ميكروجرام من ميثيونين الزنك. تمت حضانة المعاملات من T1 إلى T4 عند درجة حرارة طبيعية ٣٧,٨ درجة مئوية ورطوبة نسبية ٦٠%، المعاملات من T5 إلى T8 حُقنت بجرعات مماثلة كما وصف وتعرضت لدرجة حرارة عالية ٣٩,٨ درجة مئوية عند نقل البيض من جهاز الحضانة إلى جهاز الفقس لمدة ٣ ساعات في الأيام ١٨ و١٩ و٢٠ من الحضانة. أظهرت النتائج أن الحقن داخل البيضة بـ ٥٠ و١٠٠ ميكروجرام من ميثيونين الزنك/بيضة وتعريضها لدرجة حرارة عالية خلال المرحلة المتأخرة من الحضانة يمكن أن يحسن من الفقس ووزن الفراخ والأنشطة المضادة للأكسدة، ويزيد من عدد كريات الدم البيضاء واللمفاويات. كانت المجموعة المحقونة بـ ١٠٠ ميكروجرام من ميثيونين الزنك/بيضة أكثر فاعلية في تحسين البروتين الدهني عالي الكثافة والبروتين الدهني منخفض الكثافة والأحماض الدهنية الثلاثية والكوليسترول والجلوكوز والزنك وهرمون T_3.

الكلمات الدالة: الحقن داخل البيضة ، الزنك العضوي ، نسبة الفقس ، المناعة.