

***Original Article***

# **Progress Phenotypic Traits of Hatched Chicks and Growth Indicators of Broiler Chicks Fed Embryonically with Zinc Methionine**

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## **Abstract**

Nutritionists focused on introducing minerals as additives to poultry feed to achieve good bioavailability and increase absorption of these additives. Therefore, the current study aimed to demonstrate the effect of injecting hatching eggs with zinc methionine on the characteristics of hatched chicks and growth index. The hatching eggs injection technique was applied to inject the hatching eggs with zinc-methionine to study the traits of hatched chicks and the growth index of broilers where 300 eggs were used from the broiler breeder, and the eggs were divided into four treatments; each treatment of 75 eggs injected with zinc-methionine and at concentrations (0, 60, 80, 100 ppm) for the treatments T1, T2, T3, T4 respectively, and the studied traits were taken when the chicks hatched. The recorded data in this study showed that there was no significant effect on inactivity, general situation, case of yolk retracted inside the abdomen, abnormal checks, wing length and leg length, and significant improvement ( $P \leq 0.05$ ) to T4 treatment in appearance and feather condition, Eye's appearance and Leg appearance meanwhile significant improvement ( $P \leq 0.05$ ) to T3 treatment in naval case, residual yolk membrane, residual yolk and checks length, significant improvement of all Zn-methionine injection treatments in hatch window and total incubation period, significant improvement ( $P \leq 0.05$ ) to T2, T4 treatments in growth hormone level in hatching.

**Keywords:** Checks phenotype, Injection, Zinc, Methionine

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## **1. Introduction**

Nutritionists focused on introducing minerals as additives to poultry feed to achieve good bioavailability and increase absorption of these additives (1). One of the materials for which this technology was introduced is zinc, and this preference for choosing zinc in the field of nanotechnology is due to its role in cell proliferation, increasing growth, improving fertility, immunity, and gene expression, as well as being an essential part in the activity of many enzymes, and it directly participates in metabolic pathways and is one of the components Essential for cellular defence against

oxidative stress as an essential part of the enzyme superoxide dismutase (2), zinc positively affects the utilization of food through its participation in the metabolism of carbohydrates, proteins, and fats (3). The essential amino acids for the growth and development of embryos, where Zaki and Dakhel (4) found that the injection of hatching eggs with zinc methionine improved the productive characteristics of the hatched chicks, the hatching rate, the weight of the hatched chicks, and the embryo mortality rate. The antioxidant status of the birds also improved during the study period. Also, the delay of the chicks in the

hatchery until they are transferred to the breeding houses is a severe problem and until the counting and sorting process ends for a day or two without water and feed, which exposes the chicks to weight loss and low immunity. The technology of early feeding of eggs with various nutrient solutions may limit that; it is considered a source for the development of the hatched chicks (5-7).

Therefore, the current research aimed to demonstrate the effect of injecting hatching eggs with zinc methionine on the characteristics of hatched chicks and growth index.

## 2. Materials and Methods

This experiment was conducted in the hatchery of Jaflawi Poultry Company in the Mahaweel area / Babil Governorate from 5/2/2020 to 25/2/2020. The eggs were obtained from the Jaflawi Poultry Company hatchery (imported from the Turkish company EGE-TAV AS). All the hatching eggs were weighed individually with a digital scale type SF-400 Electronic Kitchen scale (broiler mothers type (ROSS 308), and an average weight of  $53 \text{ g} \pm 1$  was recorded. All the dirty or malformed eggs were excluded from this study.

### 2.1. Solutions Injecting

Distilled water was used to prepare the egg injection solution, and Zn-methionine was obtained from the Zinpro USA company.

The treatment was as follows: 300 eggs were randomly divided into 4 equal groups ( $n=75$ ). T1: without injection was considered the control group, and T2, T3, and T4 were injected with 60,80,100 ppm Zn-methionine/0.3 ml, respectively.

### 2.2. Studied Treats

#### 2.2.1. The Incubation Period, Hatching, and Hatching Window

The hatching machines were checked periodically in the middle of the 19th day of incubation and at the beginning of hatching for the treatments. The duration of incubation and hatching for each treatment was calculated by summing the number of hours from the beginning of the embryonic development at the time

the eggs gained heat in the hatching machine until the beginning of the hatching process (5% of the total fertilized eggs), As for the hatching window, it was calculated based on the number of hours that the chicks take to complete and according to the method in which the hatching process took place from the beginning of the first click of the first chick until the exit of the last chick for each treatment sequentially reported by Tona, Kempes (8) and Nielsen, Juul-Madsen (9), as the hatched chicks were calculated Every two hours, which was carried out inside the hatching machine, taking into account the closing of the door as a result of the impact of the chicks in the hatching process by low temperatures.

#### 2.2.2. Phenotypical Characteristics of Hatched Chicks

Fifteen chicks were taken from each treatment after hatching and randomly to assess the general and specific condition of the phenotypic characteristics of the hatched chicks, which included activity, effectiveness, external appearance, fluff condition, the condition of the yolk sac withdrawn into the abdomen, the appearance of the eyes, the condition of the leg, the condition of the umbilicus and the remaining yolk membrane With placing points for evaluation for each of the phenotypic traits, as reported by Tona, Bamelis (10).

#### 2.2.3. Physical Characteristics of Hatched Chicks

After hatching, 15 chicks were taken from each treatment (5 chicks for each replicate), and the length of the chick was measured by extending it on a graduated ruler from the beginning of the beak to the end of the middle finger (third) of the legs without the nail (the nail implant) (11). The wing length was measured by brushing it on a measuring ruler and calculating it from the shoulder bone (flap bone) to the second finger of the wing (12). The length of the shin bone was measured from the beginning of the hock joint and ending with the palm of the leg using the electronic vernier (11). As for the inertia of the tension movement, the chicks (5 chicks/repeated) were turned on their back, and the time needed to return to their

normal position was calculated to calculate their general activity (13).

**2.2.4. Growth Hormone**

Blood collection from hatched chicks and after isolating the plasma in a centrifuge, growth hormone was measured using a kit ELISA.

**2.3. Statistical Analysis**

The data were analyzed using a completely random design (CRD) to study the effect of the studied factors on the different traits. Significant differences between the averages were compared using Duncan's (14) Multiple-Range Test. SAS ; Cary (15) was used in the statistical analysis according to the following mathematical model:

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

where

$Y_{ij}$ : the value of viewing  $j$  to treatment  $i$ .

$\mu$ : general average for the trait.

$T_i$ : effect of treatment  $I$  (the study included the effect of four treatments).

$e_{ij}$ : a random error that is normally distributed with an average of zero and a variation of  $\sigma^2e$

**3. Results**

The results (Table 1) indicated that there were no

significant differences in the effectiveness of chicks, the general phenotypic condition, and the case of the yolk withdrawn to the abdomen, and a significant superiority ( $P \leq 0.05$ ) of the T4 treatment appeared in the case of chicks, the appearance of feathers, the condition of eyes and the appearance of the leg compared to the rest of the treatments. ( $P \leq 0.05$ ) for the treatment T3 in the case of the umbilicus and the improvement of the treatments, T2 and T3 in the case of the yolk sac compared to the rest of the treatments.

As for the physical specifications of the hatched chicks (Table 2), it was found that there were no significant differences between the treatments in the disabled chicks, wing length and stem length, and a significant improvement ( $P \leq 0.05$ ) for all zinc methionine injection treatments in chick length, hatching window and total incubation period compared to the control treatment.

As for the growth indicators (Table 3), it was shown that there was a significant increase ( $P \leq 0.05$ ) for T2 and T4 treatments in the concentration of growth hormone compared with the control treatment, and this explains the apparent role of zinc methionine injection in supporting the role of growth hormone inside the hatched chicks' body.

**Table 1.** Effect of in ova injection of Zn- methionine on the phenotypic characteristics of hatched chicks (mean±standard error)

Treats	Treatments				Significant
	T1	T2	T3	T4	
Activity	6.16±0.75	6.10±0.80	5.97±0.70	6.31±0.55	N. S
General situation	91.00±1.80	91.08±1.75	90.60±2.00	91.13±1.50	N. S
Appearance and feather condition	9.00±0.50 <sup>b</sup>	8.95±1.15 <sup>b</sup>	9.11±1.05 <sup>b</sup>	10.13±1.00 <sup>a</sup>	*
case of yolk retracted inside the abdomen	10.35±0.75	9.90±0.80	10.00±1.00	10.10±0.75	N.S.
Eye's appearance	11.65±2.06 <sup>c</sup>	11.50±1.70 <sup>c</sup>	12.25±1.53 <sup>b</sup>	13.98±1.25 <sup>a</sup>	*
Leg appearance	13.43±0.96 <sup>b</sup>	13.20±0.35 <sup>b</sup>	13.00±0.18 <sup>b</sup>	14.15±0.65 <sup>a</sup>	*
navel case	11.27±0.47 <sup>b</sup>	11.17±0.53 <sup>b</sup>	12.21±0.33 <sup>a</sup>	11.35±0.88 <sup>b</sup>	*
residual yolk membrane	9.95±1.20 <sup>b</sup>	11.13±1.00 <sup>a</sup>	10.88±0.87 <sup>a</sup>	9.68±0.98 <sup>b</sup>	*
residual yolk	14.39±0.50 <sup>a</sup>	14.70±0.64 <sup>a</sup>	14.03±0.15 <sup>a</sup>	13.36±0.50 <sup>b</sup>	*

Means with different letters indicate a significant difference in probability level \* ( $P \leq 0.05$ ), N. S: insignificant. T1: 0 and T2, T3, T4 injection with 60, 80 and 100 ppm Zn- methionine solution, respectively

**Table 2.** Effect of in ova injection of Zn- methionine on some checks hatching characters, hatching window, and total incubation period (mean±standard error)

Treats	Treatments				Significant
	T1	T2	T3	T4	
Abnormal checks %	2.00±1.15	0.00±0.00	0.00±0.00	1.00±3.10	N. S
Check's length (cm)	16.27±2.20 <sup>c</sup>	16.88±1.52 <sup>b</sup>	17.11±1.85 <sup>a</sup>	16.92±1.18 <sup>b</sup>	*
Wing's length (cm)	3.60±0.13	3.67±0.28	3.65±1.00	3.62±0.67	N. S
leg length (cm)	2.10±0.19	2.44±0.45	2.13±0.50	2.23±0.55	N. S
Hatching window (h)	20.55±1.13 <sup>a</sup>	19.63±1.35 <sup>b</sup>	19.27±0.90 <sup>b</sup>	19.12±1.63 <sup>b</sup>	*
The total incubation period (h)	501.16±2.40 <sup>a</sup>	489.50±3.12 <sup>b</sup>	492.95±2.95 <sup>b</sup>	495.36±2.61 <sup>b</sup>	*

Means with different letters indicate a significant difference in probability level \* ( $P \leq 0.05$ ), N. S: insignificant. T1: 0 and T2, T3, T4 injection with 60, 80 and 100 ppm Zn- methionine solution, respectively

**Table 3.** Effect of in ova injection of Zn- methionine on growth hormone when hatching (mean±standard error)

Treatments	Growth hormone pg/ml
T1	2070.13±4.30 <sup>b</sup>
T2	2153.81±3.70 <sup>a</sup>
T3	2098.65±4.18 <sup>ab</sup>
T4	2164.37±5.14 <sup>a</sup>
Significant	*

Means with different letters indicate a significant difference in probability level \* ( $P \leq 0.05$ ), N. S: insignificant. T1: 0 and T2, T3, and T4 injection with 60, 80, and 100 ppm Zn-methionine solution, respectively

#### 4. Discussion

The development of the characteristics of the hatched chicks at hatching for zinc methionine injection treatments may be because zinc can replace zinc salts as a promoter of health and growth due to its high reactivity and bioavailability (16), or the reason may be due to zinc stimulating the growth of the fetal alimentary canal and increasing the effectiveness of the villi for optimal absorption of nutrients, i.e., an increase in the rate of metabolism and absorption in the embryos, thus increasing the body mass (17). This explains the improvement in the physical characteristics of the chicks, as zinc is a vital element for the growth of birds, the development of bones and feathers, and the regulation of the absorption of nutrients for the embryos (18), and its interaction with other elements positively affects the increase in weight at hatching and the increase in hatchability (19). Zinc also participates in the metabolism of carbohydrates, fats, and proteins, thus increasing the utilization of the food available to the fetus inside the egg (3, 4).

Alternatively, the reason may be that zinc is a cofactor in more than 300 critical enzymes in metabolic pathways, so its deficiency negatively affects protein and carbohydrate metabolism within the bird's body, and this explains its role in promoting growth hormone action in birds (20). Alternatively, because of zinc's association with enzymes that maintain the integrity of cells that participate in the body's immune response and then improve the characteristics of the hatched chicks and their productive and physiological performance during the breeding period (21), and the reason for the superiority may be due to the role of zinc in regulating protein metabolism in the body protects it from breakdown by preventing the oxidative process in the cell membranes, that is, it is one of the requirements for the normal synthesis of protein (22), as well as zinc increases the synthesis of metallothionein, a cysteine-rich protein that functions As an antioxidant, it can inhibit free radicals, as well as zinc, is part of the enzyme (superoxide dismutase) that has an antioxidant role (23). The reason may also be due to the role of zinc

in maintaining the synthesis of proteins that enter into the formation of hormones, including growth hormone (G.H.) and insulin, and this role contributes to maintaining the normal growth and development of the body, as these hormones regulate glucose uptake and regulate cellular processes (22). The reason may also be due to the role that zinc plays in improving the health of birds by increasing the effectiveness of the metabolic rate in the body as a result of the increase in the level of thyroxine (T4) when zinc is added, as the researcher Morley, Gordon (24) found that a decrease in zinc in the blood serum is accompanied by a decrease in the levels of the thyroid stimulating hormone (TSH), which is secreted from the pituitary gland, and then a decrease in the thyroid hormones Thyroxine and Triiodothyronine, which shows that zinc has a role in contributing to the building of body tissues by increasing the level of thyroid hormones, or the improvement in the characteristics of the hatched chicks and their physical characteristics in zinc-methionine injection treatments may be due to the role of methionine. In addition, methionine enters into the methylation reaction of DNA. Brosnan and Brosnan (25) explained that methionine forms the amino acid cysteine inside the body and improves the immune status of birds. Methionine also enters the metabolic processes inside the body, where it works to balance other amino acids such as cysteine and participates in the synthesis of betaine, choline, vitamin B12, and folic acid metabolism (26); methionine is also an important source of sulfur inside the body and an influential donor of methyl groups that contribute to the formation of many vital compounds inside the body such as creatine and choline inside the body (27), that Amino acids in general and methionine, in particular, is essential for immunity and the formation of antibodies inside the body, especially when there is a pathological challenge, as indicated by Liu, Lu (28) that zinc-methionine is used as a guide to the bioavailability of zinc in live animals, where the rate of zinc absorption increases when it binds with acids amino or proteins

(29). Methionine is necessary for protein synthesis processes. Methionine also enters the methylation reaction of DNA. Brosnan and Brosnan (25) explained that methionine is the amino acid cysteine inside the body and improves the immune status of birds. Others, such as cysteine, show evidence of zinc methionine's role in supporting the growth and development of the characteristics of hatched chicks and supporting the action of growth hormone.

The injection of hatching eggs with zinc methionine gave a great stimulus to support the growth and development of the embryos, thus developing the characteristics of the hatched chicks and enhancing the action of the growth hormone. Also, the injection of eggs at the age of 17.5 days from the age of the embryos and into the amniotic sac caused the embryos to swallow zinc methionine with the amniotic sac into the abdominal cavity after age 19 days of embryonic growth and development, and thus will have a significant role in the growth and development of the intestine, and thus will be reflected on the subsequent performance of the hatched chicks.

#### **Authors' Contribution**

Study concept and design: H. H. A.

Acquisition of data: M. K. I. A.

Analysis and interpretation of data: H. H. A.

Drafting of the manuscript: M. K. I. A.

Critical revision of the manuscript for important intellectual content: M. H. A.

Statistical analysis: M. K. I. A.

Administrative, technical, and material support: H. H. A.

#### **Ethics**

The study design was approved by the ethics committee of the Al-Qasim Green University, Al Qasim, Iraq.

#### **Conflict of Interest**

The authors declare that they have no conflict of interest.

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