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EFFECT OF *IN OVO* INJECTION OF ZINC METHONINE ON HATCHING TRAITS, PRODUCTION PERFORMANCE AND IMMUNITY RESPONSE OF BROILER CHICKENS

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Abstract

This study included two experiments, in order to investigate the effect of *In ovo* injecting of different levels of Zinc Methonine on Hatching Traits, Production Performance and immunity response of Broiler Chickens and it was conducted in Almanar private hatchery- Diayla governorate and poultry farm, Animal production Department, College of Agriculture, University of Diyala during 21/10 - 29/11/2017.

In first experiment, 720 fertilized eggs were used from breeders of the broiler (Ross 308), distributed into six treatments, 120 egg per treatment by three replicates per treatment (40 eggs/rep.), at the 18th day of incubation when the eggs are transferred from the setter to the hatcher, they were injected into the amniotic sac according to the experimental treatments T_1 (Negative control) hatching eggs without injection, T_2 (Positive control) hatching eggs injected 0.2 ml/egg distilled water only and T_5, T_4, T_3, T_6 hatching eggs were injected by 0.2 mL/egg of different concentrations of Zinc Methonine solutions 15, 20, 25, 30 mg/100 ml achieves 54, 72, 90, 108 µg/egg respectively. The results showed a significant improvement in the, body weight, body length, relative weight of the yolk sac and humoral immunity in chicks of Zinc Methonine treatments compared with both control treatments (T_1, T_2) and The second experiment: In this experiment, 255 broiler chicks Ross308 were used, their took from the chicks in the first experiment of the best two treatments in the results that's means the fifth and sixth treatments (T_4, T_5) as well as the treatment of control (T_1) form randomly and an equal number of 75 chicks of each treatment, they were raised in standard conditions up to the age of 35 days. The results showed an improvement in live weight, weight gain mortality, production index and humoral immunity response against both diseases of Infectious Bursal (IBD), Newcastle (ND) and Infectious Bronchitis (IB) of broiler in both treatment which injected of Zinc Methonine (T_2, T_3) compared to birds of control treatment.

Key words*: In ovo* Injection, Zinc Methonine, Broiler, Hatching Traits, Production Performance, immunity response.

Introduction

Because of the increased metabolic rate of the embryos of today, the embryonic nutrient reserves are insufficient and might be depleted in the prenatal period (Yair *et al.,* 2013). Such nutritional insufficiencies may induce long-lasting adverse consequences on progeny performance (Petry and Hales 2000). Therefore, intervention strategies involving pre-hatch nutrient supplementation have been developed to reduce nutritional restrictions (Oliveira *et al.,* 2015). *In ovo* injection technology provides a practical means to safely introduce external nutrients into developing embryos (Foye *et al.,* 2007; Kadam *et al.*, 2008; Bello *et al.,* 2014). Feeding the embryo before hatch by *in ovo* administration of external feed components was reported to cause a positive effect on hatchability, development of the digestive tract, body weight and nutritional status of the hatchling (Uni and Ferket 2004).

Zinc (Zn) is an essential trace element required for development and growth of broiler chickens. This essential element has a key part in the structure and maintenance of the skeleton and acts as cofactor in many metabolic processes that necessary for hormone secretion *i.e.* growth and insulin hormones (Tsai *et al.,* 2016) DNA synthesis, gene expression and cellular division, enhanced the immune status. Also, zinc is vital free radical's scavenger of the in-antioxidant defense system (Parashuramulu *et al.,* 2015; Ibrahim *et al.,* 2017) Zinc

deficiency also results in poor hatchability and abnormal embryonic development in hens and It has been demonstrated that defects in DNA maintenance methylation in the embryo were associated with disruption of embryonic implantation and development (Yin *et al.,* 2012). Zinc is typically added to diets for poultry in a supplemental form, usually as inorganic feed grade like zinc sulfate, oxide, or one of the organic forms complexed to amino acids, proteins, and carbohydrates like zinc methonine. Which is the most bioavailable (Salim *et al.,* 2008) and because the Zn is exhausted by the late embryonic stage (Yair and Uni 2011) the aim of study was designed to determine the effect of *In ovo* injection of different levels of Zinc Methonine at 18th days of incubation on Hatching Traits, Production Performance and immunity response of broiler chickens (Ross 308).

Materials and Methods

Experimental Design and Treatments

The hatchery experiment was carried out in Petersime hatchery, 800 hatching eggs were used from one herd of broiler breeders (Ross 308) at the age of 46 weeks, a stored for two days and a weight average of 61 ± 1g/egg, incubation of eggs in the Setter at 99.8ºF and relative humidity 60%, on the 18th day of the incubation, before the transfer of eggs from the Setter to the hatcher, candled eggs were scanned for detection of fertilized eggs, and used 720 fertilized eggs divided into six treatments by three replicates per treatment (40 eggs/ rep.) post *In ovo* injection according experiment treatments :

- T_1 (Negative control) hatching eggs without injection
- T_2 (Positive control) hatching eggs injected 0.2 ml/egg distilled water only.
- T_5, T_4, T_3, T_6 hatching eggs were injected by 0.2 mL/ egg of different concentrations of Zinc Methonine solutions 15, 20, 25, 30 mg/100 ml achieves 54, 72, 90, 108 µg/egg respectively.

The incubation of eggs in the hatcher was repeated at 98.6°F and a relative humidity 65% until hatching. The injection solution was prepared using Zinc Methonine Glacial from USA-Zinpro Eden prairie chemicals company with sterilized distilled water, In the process of injecting eggs automatic syringe (Self-Refilling Syringe) processed by Socorex Swiss with a volume of 1 ml, a length of 1 inch and measuring Gauge22, the blunt end of each egg was disinfected by cotton immersed in ethanol, which was followed by the process of injecting each egg with a solution treatment by the automatic medical syringe to connecting the needle in the aminion fluid level surrounding the embryo post the piercing of the shell with the drill and closed the injection hole post completion by the paraffin wax (Zhai *et al.,* 2008), then put the eggs in plastic boxes and returned the process of incubation in the hatcher until the date of hatching.

Hatching Traits

When hatching, taking the live weight of the newly chicks using a Sensitive Balance to two decimal order of type HD-KE1200, and calculate the length of the body chicks using the measurement ruler and Calculated the relative weight of the remaining yolk sac according to the equation referred to by Seifi *et al.,* (2015) and the hatchability according to Desha *et al.,* (2015).

Production Performance

The farm experiment was conducted to monitor the effect of *In ovo* injection of Zinc Methoninepost-hatching on productive performance of broiler chickens, used 225 resulting chicks from the best two treatments for the first experiment, which were both treatment of the eggs treated with Zinc Methonine 72, 90µg/egg respectively, as well as resulting chicks from control treatment, which were treatment the eggs without injections which transferred to the raising farm after dividing they into three replicates per treatment (25 chick/rep.) as follows: first treatment T1 chicks from hatching eggs without injection, second treatment T_2 chicks produced from *In ovo* injected of Zinc Methonine 72µg/egg, third treatment T_3 chicks produced from *In ovo* injected of Zinc Methonine 90 µg/ egg. The chicks were raised in standard conditions until 35 days According to Ross Broiler Management Manual Guide, Aviagen (2014), and fed on diets containing chemical analysis as in table (1).

Table 1: Chemical analysis of the diets used in the experiment.

Structure	Starter 1-10 d.	Grower 11-24 d.	Finisher 25-35 d.
Crude protein (%)	22.50	20	18
ME (kcal / kg feed	3069	3185	3250
Ratio of energy to	1:136.4	1:159.25	1:180.56
protein(C:P)			
Crude fat (%)	4.45	5.61	5.95
Lysine (%)	1.33	1.21	1.21
Methionine (%)	0.64	0.56	0.50
Calcium (%)	0.98	0.95	0.90
Available phosphorus (%)	0.45	0.43	0.41

The birds and the feed intake were weighed weekly, as well the weight gain, mortality and feed conversion ratio, according to Diarra *et al.,* (2014). Production index was calculated according to the equation indicated by Koreleski *et al.,* (2010).

Immunity response

Blood samples four birds of each treatment of both sexes were collected at slaughter directly from the jugular Vein. Serum antibody titres against Newcastle disease (ND), Infectious Bronchitis (IB) and Infectious Bursal (IBD) were determined by commercial ELISA kits (Synbiotics Laboratories, USA), according to manufacturer's instructions.

Statistical Analyses

The experimental design for this experiment was Completely Randomized Design (CRD) each treatment with three replicates and the significance of differences between means detected by Duncan's Multiple Ranges test (Duncan, 1955) and the analysis of variance performed by used SPSS programe (SPSS, 2011).

Results and Discussion

Table 2 shows the result of effect of *In ovo* injecting of Zinc Methonine at 18th days on body weight (BW) and body Length (BL) of hatched chicks, it's noted from the table a significant effect ($P \leq 0.05$) on the BW hatched chicks in all injected treatment of Zinc Methonine compared to the control treatment of without injected Where it reached body weight 41.52, 42.84 and 42.66 g in T_4, T_5 and T_6 Respectively Compared with 39.26 and 40.81 in T_1 and T_2 treatments The current study also noted that the body length of the hatched chicks was significantly higher ($P \leq 0.01$) in the T_4, T_5 and T_6 Zinc Methonine injections compared to T_1 and T_2 and the Relative weight of yolk sac was 15.52 and 14.01 % and significantly decreased ($P \leq 0.05$) to 12.50, 11.18 and 9.19 in treatments of zinc Methonine injections T_3, T_4 and T_6 Respectively.

Table 2: Effect of *in ovo* Injection of Zinc Methonine on Hatching Traits (Mean ± SE).

Treatment	BW hatched chicks (g)	BL hatched chicks (cm)	Relative weight of yolk sac (%)
T_1	39.26 ± 0.88^{b}	17.71 ± 0.06^{c}	15.52 ± 1.22^{a}
T_2	40.81 ± 0.89^{b}	18.07 ± 0.10^{bc}	14.01 ± 2.11^{ab}
T_3	40.91 ± 0.32^{ab}	18.63 ± 0.16^{b}	12.50 ± 0.10^{b}
T_4	41.25 ± 0.79^{a}	18.75 ± 0.21^{a}	11.18 ± 0.51^{bc}
T_5	42.84 ± 0.52^{a}	19.00 ± 0.11^{a}	14.04 ± 0.23^{ab}
T_6	42.66 ± 0.17^{a}	18.72 ± 0.33^{a}	9.19 ± 0.98^{c}
Sig.	*	**	*

- T_1 (Negative control) Hatching eggs without injection, T_2 (Positive control) eggs hatching injected 0.2 ml / egg distilled water only and T_5, T_4, T_3, T_6 hatching eggs were injected by 0.2 mL/egg of different concentrations of Zinc Methonine solutions 15, 20 , 25 , 30 mg/100 ml achieves 54, 72, 90, 108 µg/egg respectively.
- The different letters within a single column indicate that there are significant differences between the treatments.
- *Mean significant effects found of treatment at $P \leq 0.05$ in variance analysis table.
- ** Mean significant effects found of treatment at the probability level $P \leq 0.01$ in the variance analysis table.

Table 3 shows an improvement in humoral immunity response against both diseases of Infectious Bursal (IBD), Newcastle (ND) and Infectious Bronchitis (IB) in chicks treatments of acid injection compared to both control treatments, as the titter of antibodies against Newcastle Disease was significantly increased ($P \leq 0.01$) in the Zinc Methonine injection treatments compared to T_1 and T_2, titter of antibodies against Infectious Bronchitis and Infectious Bursal was also significantly increased ($P \leq 0.01$) in all Zinc Methonine injection treatments compared with control treatments.

Table 4 shows that the improvement in chicks' characteristics which hatched from hatching eggs injected with Zinc Methonine at 18th days of incubation significantly affected the production characteristics of broilers at marketing, as the superiority of treatment of Zinc Methonine T3 significantly ($P \leq 0.05$) in the live body weight compared with the control treatment T_1 Where it reached 2173.00 g in control treatment T_1 Compared with 2301.66 g in T_3 and treatment T_2 was not significantly different from treatments T_1 and T_2 also the T_3 of Zinc Methonine injection treatments were significantly ($P \leq 0.05$) higher in the weight gain compared with the control treatment T_1 and its was reached 2263.15 g in T_3 compared with 2136.73 g in the control treatment T1 and 2199.74 g in T_2 The values of the production guide were 395.91 and 423.83 in T_1 and T_2 compared with 447.83 in treatment of Zinc Methonine injection T_3. injection of hatching eggs with zinc methionine did not significantly affect on Feed intake, Feed conversion ratio and Mortality (table 3).

Results of immune response, presented in Table 5 show that different levels of Zinc Methonine injection at 18th days of incubation had a positive effect on humoral immunity at 7 and 35 days as measured by antibody titres against ND, IB and IBD when compared with the control treatment T_1 . The highest scores of antibody titres against ND, IB and IBD were attained by broilers injection 72 and 90 µg Zn/egg (T_2 and T_3) compared with control treatment T_1.

The positive results were recorded in the treatments of Zinc Methonine injection compared with control treatments (T_1 and T_2) In body weight and Length of hatched chicks because the Zinc is an essential micro-mineral that is involved in several metabolic routes that are fundamental for growing and living (Ezzati *et al.,*

Table 3: Effect of *in ovo* Injection of Zinc Methonine on immunity response of chicks(Mean ± SE).

Treatment	IBD	ND	IB
T_1	16906.00 ± 420.30^{c}	6473.33 ± 159.98^{c}	6627.00 ± 158.65^{c}
T_2	16815.33± 205.34^{bc}	6607.66 ± 639.21^{c}	6757.33 ± 77.47^{bc}
T_3	16287.33 ± 355.57^{ab}	7098.00 ± 951.62^{b}	7190.33 ± 387.87^{bc}
T_4	18009.33± 357.80^{a}	8855.33 ± 311.08^{a}	9125.00 ± 238.24^{a}
T_5	18471.33 ± 144.03^{a}	8643.66 ± 286.09^{a}	9091.00 ± 419.95^{a}
T_6	17571.33 ± 236.71^{a}	7694.66 ± 109.10^{ab}	7820.33 ± 597.78^{b}
Sig.	**	**	**

- T_1 (Negative control) Hatching eggs without injection, T_2 (Positive control) eggs hatching injected 0.2 ml/egg distilled water only and T_5, T_4, T_3, T_6 hatching eggs were injected by 0.2 mL/egg of different concentrations of Zinc Methonine solutions 15, 20, 25, 30 mg/100 ml achieves 54, 72, 90, 108 µg/egg respectively.
- The different letters within a single column indicate that there are significant differences between the treatments.
- ** Mean significant effects found of treatment at the probability level $P \leq 0.01$ in the variance analysis table.

Table 4 : Effect of *in ovo* Injection of Zinc Methonine on production performance of broiler chickens at age 35 days (Mean ± SE).

Characteristics / Treatment	T_1	T_2	T_3	Sig.
Live body weight (g)	2173.00 ±8.38^{b}	2237.33±3.78^{ab}	2301.66±43.81^{a}	*
Weight gain (g/bird)	2136.73±7.56^{b}	2199.74±4.13^{ab}	2263.15±43.54^{a}	*
Feed intake (g/bird)	3322.66±35.31	3318.33±112.58	3320.66±15.02	NS
Feed conversion ratio	1.55±0.01	1.50±0.04	1.46±0.02	NS
Mortality (%)	1.00 ± 0.57	0.33 ± 0.33	0.33 ± 0.33	NS
Production index	395.91±5.54^{b}	423.83 ± 1.79^{ab}	447.83 ± 16.47^{a}	*

- T_1 (Negative control) Hatching eggs without injection, T_2 and T_3 eggs hatching eggs were injected by 0.2 mL/egg of different concentrations of Zinc Methonine solutions 20 and 25 mg/100 ml achieves 72 and 90 µg/egg respectively
- The different letters within a single column indicate that there are significant differences between the treatments .
- * Mean significant effects found of treatment at the probability level $P \leq 0.01$ in the variance analysis table.
- NSMean non a significant effects found of treatment in the variance analysis table.

2013) because of its role in building body tissues and stimulating the building of proteins, muscle and synthesis of metalloenzyme (Batal *et al.,* 2003) and improve the health of birds and increase the level of Insulin like growth factor (IGF-I) and Somatorinin Which stimulates the growth hormone, leading to increased cell division and growth (Ma and Yamaguchi, 2001; Bozalioglu *et al.,* 2005). The increased length of the chicks is due to the important zinc role in the construction of the skeleton Zinc participates in important regulatory pathways for bone and cartilage formation, such as collagen synthesis and hydroxyapatite crystallization (Oliveira *et al.*, 2015) Lourens *et al.,* (2006) also confirmed that there is a strong correlation between the length of the newly hatched chick with its zero yolk mass and the negative association between the remaining yolk and its yolk-free mass. Also the consumption of yolks at an early time (table 2) gives better results to the broiler chicks because the remaining yolk contains all the nutrients of fat, amino acids, vitamins and minerals needed by the embryo for its growth and development (Meijerhof, 2009).

The improvement in the production characteristics of the both birds treatments of Zinc Methonine injection (table 4) may be a reflection of the superiority of the characteristics of chicks at hatching, which was ideals by improved all of their weight, length, internal organs and immunity, which were great benefit to birds by increasing their ability to digestion, absorption and disease resistance. This is consistent with Tona *et al.,* (2003) who conclude that the quality of a one-day-old broiler is a good indicator of the future production performance of broilers. Willemsen *et al.,* (2008) further confirm that the improved weight and length of broiler chickens during hatching are positively reflected on production performance when marketing.

The present study demonstrated that Zinc Methonine injection positively affected the antibody titre against ND, IB and IBD in broiler chickens (Table 3 and 5). Bartlett and Smith (2003) reported that dietary Zn supplementation improved lymphoid organ weights, primary and secondary antibody responses, phagocytic ability of macrophages, total IgM and IgG antibody titres in male broilers also Zinc is essential for thymulin, a thymic hormone that regulates T lymphocyte maturation so the birds provided diets supplemented with a more available zinc source (Zinc Methonine) might have induced thymulin activity, and therefore promoted immune responses through increased maturation of T-lymphocytes and activation of B-lymphocytes by T-helper cells (Ezzati

Table 5 : Effect of *in ovo* Injection of Zinc Methonine on immunity response of Broiler Chickens at age 7 and 35 days (Mean ± SE).

Treatment	T_1	T_2	T_3	Sig.
7 days				
IBD	5413.66 ± 394.86^b	7374.00 ± 241.57^a	8160.66 ± 373.25^a	**
ND	1491.33 ± 110.56^b	2653.00 ± 421.11^b	4241.33 ± 569.47^a	*
IB	1525.33 ± 130.27^b	2534.33 ± 104.21^a	2774.33 ± 101.20^a	**
35 days				
IBD	7130.66 ± 158.26^b	15666.00 ± 1268.50^a	13627.00 ± 833.68^a	**
ND	1564.66 ± 86.85^b	2342.66 ± 173.58^a	2875.00 ± 325.15^a	*
IB	4222.33 ± 229.49^c	16130.00 ± 155.30^b	18325.00 ± 482.09^a	**

- T_1 (Negative control) Hatching eggs without injection, T_2 and T_3 eggs hatching eggs were injected by 0.2 mL/egg of different concentrations of Zinc Methonine solutions 20 and 25 mg/100 ml achieves 72 and 90 µg/egg respectively.
- The different letters within a single column indicate that there are significant differences between the treatments.
- * Mean significant effects found of treatment at the probability level P≤ 0.01 in the variance analysis table.
- ** Mean significant effects found of treatment at the probability level P≤ 0.01 in the variance analysis table.

et al.,, 2013 ;Chitithot *et al.,*, 2014).

Conclusion

Our results indicated that Zinc Methonine injection could improve Hatching Traits, Production Performance like Live body weight, Weight gain and production index but it has not any effects on feed intake, feed conversion ratio and mortality also the results showed the antibodies obtained in different levels of Zinc Methonine injection had a positive effect on humoral immunity in chicks and broiler at age 7 and 35 days.

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