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The effects of *in ovo* injection of L-threonine in broiler breeder eggs on characters of hatching and growth performance broiler chickens

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ABSTRACT

The experiment was conducted to investigate the effects of in ovo injection of L-threonine on characters of hatching and growth performance broiler chickens. On the 8th day of incubation, 1008 fertile eggs, on based completely randomized design were divided into seven treatments with four replicates per treatment and 36 eggs per replicate. Experimental groups were following: 1) control (without injection), 2) injected with 0.5 ml deionized water (sham group), 3) injected with 15 mg L-threonine in 0.5 ml deionized water, 4) injected with 20 mg L-threonine in 0.5 ml deionized water, 5) injected with 25 mg L-threonine in 0.5 ml deionized water, 6) injected with 30 mg L-threonine in 0.5 ml deionized water and 7) injected with 35 mg L-threonine in 0.5 ml deionized water. At day 8 of incubation 0.5 ml on in ovo solution were injected into albumen. Upon hatch, chicks were weighed and transferred to experimental house and reared for 21 days. Results showed, in ovo injection had significantly lower percent hatching than control group. Also, Chicks weight from eggs injected with 30 and 35 mg L-threonine had significantly higher body weight as compared to sham and control group (P<0.01). The effect of injection of L-threonine wasn't significantly on chick's body weight grain on 21 days. The results of the study indicate that, effect of different levels of L-threonine injection had highest influence on improving the weight of newly-hatched chickens.

Key words: *In ovo* injection, L-threonine, hatching, performance, broiler.

INTRODUCTION

The essential amino acid, L-threonine (Thr), is used in important metabolic processes such as protein synthesis and uric acid formation. Threonine is the third most limiting amino acid, especially in a low crude protein diet [1, 2]. Poultry cannot synthesize threonine making it a nutritionally essential amino acid. Poultry can utilize only L-Threonine [2], making it metabolically expensive. Threonine has also been shown to hinder methionine influx and stimulate lysine influx into the epithelial cells of the intestinal lumen [3].

All nutrients needed for embryogenesis are provided by the hen by the time the fertile egg is laid [4]. If nutritional deficiencies occur during the formation of the egg, it can have significant repercussions on the developing embryo. Hen diets are composed mainly of corn and soy, which contain low levels of L- threonine. Therefore, eggs contain little or no L- threonine [5]. Moreover, A novel method of supplementing the *in ovo* (IO) nutriture of oviparous species, described as *in ovo* feeding (IOF) within the US Patent (6592878) of Uni and Ferket [6], was demonstrated to be an effective way to administer exogenous nutrient to support the development of the embryos and neonates in broiler [7]. *In ovo* feeding of supplemental nutrients may help to overcome the constraint of limited egg nutrients [8]. In such situations, exogenous supplementation of L-threonine could prove advantageous [6] and could in turn be used by the chick during hatching. In overall, the organogeneses of important segments of the chicken embryo are occurred at first week of incubation, in this regard, gastro-intestinal organogenesis includes foregut, mid gut and hindgut differantions was reported at 4-7 days of incubation. In other side formation of most important organs includes ovary, ileum, femur, pancreas, gastrocnemius muscle; duodenum and etc are done at day-9 of incubation. in the past studies on "*in ovo* feeding", almost all of the works were conducted at the days of 17-21, late-embryonic or pre-hatch stages [9, 10, 12, 13, 14], but in the present study, we evaluated the effects of *in ovo* feeding in early-embryonic life of chicken as the unique point of present study, and in general, the *in ovo* injection of nutrient supplementation in early embryonic life, like maternal nutrient supplements that can be useful during the whole embryonic life of the bird (to hatching). Nutrient *in ovo* injection may provide poultry companies with an alternative method to increasing weight of newly-hatched chicken and growth performance [15]. Therefore, in this study, L-threonine was injected into the albumen of broiler breeder eggs on d 8 of incubation to determine hatching traits and performance of broiler chickens. The objective of this research is to evaluate the effects of *in ovo* injection of L-threonine in broiler breeder eggs on characters of hatching and growth performance broiler chickens

MATERIALS AND METHODS

Incubation and in ovo injection

1008 fertile eggs were obtained from (Ross-308) broiler breeder strain at 30 weeks of age. All eggs were collected from the same breeder flock and weighed on a balance with 0.1 g precision and eggs with a weight of 60 ± 1 g were incubated at 37.8 °C and %63 RH. On the 7^{th} day of incubation, the eggs were candled, and the infertile ones or those containing only dead embryos were removed. At 8 d of incubation, fertile eggs based on a completely randomized design were divided into7 treatments with 4 replicates per treatment and 36 eggs per replicate. The *in ovo* injection solutions were the following: 1) control (without injection), 2) injected with 0.5 ml deionized water (sham group), 3) injected with 15 mg L-threonine in 0.5 ml deionized water, 4) injected with 20 mg L-threonine in 0.5 ml deionized water, 5) injected with 25 mg L-threonine in 0.5 ml deionized water, 6) injected with 30 mg L-threonine in 0.5 ml deionized water and 7) injected with 35 mg L-threonine in 0.5 ml deionized water. Then, each egg was candled to identify the location of the injection. A hole was then punched using a 22-gauge needle and 0.5 mL of (*in ovo* injection) solution injected into the albumen using a 22-gauge needle to a depth of about 13 mm. The injection whole area was disinfected with an ethyl alcohol-laden swab, sealed with cellophane tape, and transferred to hatching baskets. Control eggs were removed from the incubator together with the treated groups, and kept in the same environment. The group of eggs designated as sham-injected controls were injected with 0.5 mL of deionized water. Deionized water injections were included as sham controls primarily to rule out a possible negative response caused by the stress of injection and handling. Pure L-threonine was supplied from Merck® Co (anhydrous ≥ 98%).

Birds

After hatching, Chicks were transferred to experimental house and reared for 21 days with the same ration according to standard broiler ration (National Research Council, 1994) (Table 1) [16]. Each treatment group and chick was identified by the neck tag and recorded. All chicks and treatments were randomly assigned to 1 of 28 pens. Each pen was bedded with soft pine wood shavings and equipped with automatic drinkers, and manual self-feeders. Food and water were available ad-libitum. All animal experimentation was conducted in accordance with the regulations of Islamic Azad University, Animal Ethics Committee.

Data Collection

Upon hatch, the hatchability and weight of newly-hatched chickens were measured. The Weight of newly-hatched chickens was determined by weighing all chicks hatched one by one. Hatchability was calculated by considering the ratio of chickens hatched to the live chicken's embryo after the treatment and expressed as a percentage of fertilized eggs. In each pen, bird body weight was recorded on d 0 and 21 post hatch. Then, mean body weight gain was calculated for each pen (replicate) between 0 and 21d. Then, body weight gain was calculated and expressed as grams per bird.

Statistical Analysis

Results were analyzed by ANOVA using the GLM procedure of SAS software (SAS institute, 2001) [17]. Differences between treatments were compared by the Duncan's multiple range tests following ANOVA, and values were considered statistically different at ($P < 0.05$) [18]. When data were percentages they were transformed by arc sin square root.

RESULTS AND DISCUSSION

Results showed, i*n ovo* injection had significantly lower percent hatchability than control group. Probably the decreasing rate of hatching was because of the injection into the albumin. Another reason by allergic cavity that is under the air sac had been causing the respiration of developing embryo to stop and die. Previous studies on *in ovo* administration of hormones such as corticosteroids at embryonic day-7 resulted in 35% decline of hatchability [19]. Any of reviewed relative studies with *in ovo* manipulation, including the present study, especially in early embryonic life weren't successful in terms of hatchability [20, 21, 22]. Also, in the present study, the *in ovo* injection of L-threonine into fertile chicken eggs at 8 d of incubation did not significant effect on hatchability sham group (injected with 0.5 mL of deionized water) than other injected groups. According to the past studies and our present observations, it seems that any *in ovo* injection at early embryonic life can harmful for internal environment susceptibility and would have negative effect on hatching; this effect is largely independent from injected L-threonine effect. Also, Ohta et al. [23] showed the effect of Amino Acid administration *in ovo* on chicken hatchability may be related to injection site *in ovo*. Chicks weight from eggs injected with 30 and 35 mg L-threonine had significantly higher body weight as compared to sham and control group ($P<0.01$). AL-Murrani [24] and Ohta et al. [15] found that injection of amino acids into the air cell of fertile chicken eggs during the first week of incubation increased amino acid contents of embryo, yolk albumen, and allantoic and amnionic fluids on d 19 of incubation and elevated embryonic body weight (BW). These experiments demonstrate the benefits of adding external nutrients to hatching eggs and clearly illustrate the limitations of avian species, which, unlike mammals, do not have a continuous energy supply from a maternal source to support embryonic and neonatal growth.

The effect of injection of L-threonine wasn't significantly on chick's body weight grain on 21 days. Previous studies have indicated that hatching weight is a major predictor of marketing weight in chickens. Wilson [25] reported that each 1 g of increase in BW at hatch leads to 8 to 13 g of increase in BW at marketing. Although this correlation between hatch weight and market weight may differ among strains, the influence of hatch weight on market weight is apparently increasing as broiler breeding companies continue to select for ever increasing growth rate [25, 26, 27]. Uni et al. [9] stated that a 2-g difference in BW at hatch due to *in ovo* feeding resulted in 50 to 60 g of increase in BW at d 25. But, in this study, the effect of injection of L-threonine wasn't significantly on chick's body weight grain on 21 days.

Table1. Ingredient percentages and calculated analysis of broiler diet

Item	Diet	
	Starter	Grower
Ingredient (%)	0 to 10 d	11 to 21 d
Corn	60.36	65.44
Soybean meal(44% CP)	34.12	28.62
Vegetable fat	1.23	1.74
Dicalcium phosphate	1.83	1.8
Oyster sell-ground	1.22	1.19
Salt	0.35	0.3
Sodium bicarbonate	0.11	0.07
Vitamin premix[1]	0.25	0.25
Mineral premix[2]	0.25	0.25
DL-Met	0.17	0.18
L-Lys	0.11	0.16
Calculated analysis		
ME (kcal/kg)	2894	2987
CP (%)	20.3	18.3
Ca (%)	1	0.96
Available P (%)	0.50	0.48
Met (%)	0.46	0.44
Met + Cys (%)	0.89	0.84
Lys (%)	1.20	1.10

[1]Vitamin premix provided the following per kilogram of diet: vitamin A, 11,013 IU; vitamin D3, 3,525 IU; vitamin E, 33 IU; vitamin K, 2.75 mg; riboflavin, 7.7 mg; pantothenic acid, 17.6 mg; niacin, 55.1 mg; choline, 478 mg; vitamin B12, 0.028 mg; pyridoxine, 5.0 mg; thiamine, 2.2 mg; folic acid, 1.1 mg; biotin, 0.22 mg.
[2]Trace mineral premix provided the following per kilogram of diet: manganese, 64 mg; zinc, 75 mg; iron, 40 mg; copper, 10 mg; iodine, 1.85 mg; and selenium, 0.3 mg.

Table 2. The effect of *in ovo* injection of L-threonine on hatchability, weight of newly-hatched chickens and body weight gain in 0-21 day of age of broiler chickens

Treatments	Hatchability (%)	Weight of newly-hatched chickens (g)	body weight gain (0-21) day of age (g)
Control	83.25[a]	41.34[bc]	564.75
*Sham	74.75[b]	41.36[bc]	572.25
15 mg (Thr)*	76.25[b]	41.55[ab]	560.50
20 mg (Thr)	72.50[b]	41.21[c]	552.25
25 mg (Thr)	72.25[b]	41.62[ab]	551.00
30 mg (Thr)	75.75[b]	41.87[a]	571.00
35 mg (Thr)	76.25[b]	41.76[a]	568.75
P-Value	0.0009	0.0014	0.38
SEM	1.36	0.10	8.16

Different letters (a, b, c, d or e) show significant difference.
**Sham=injected with 0.5 mL of deionized water, Thr = Threonine*

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