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Effect of *In ovo*Injection of Various Nutrients on the Hatchability, Mortality Ratio and Weight of the Broiler Chickens

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Abstract: *The main goal of this paper is to study the effect of injectingthe eggs embryos with certain levels of nutrient solutions (vitamin E 1.5 mg / egg, the amino acid lysine 1.5 mg / egg, the amino acid methionine 1.5 mg egg and a mixture of vitamin E and lysine and methionine 1.5 mg / egg) in the proportion of mortality of embryonic and hatchability and initial weight 1 g / day. Results wereindicated a significant decrease (P <0.05) in the percentage of embryonic mortality in the treatmentinjection with methionine and the treatment of the mixture of vitamin E, lysine and methionine. Though arithmetic improvements in the percentage of hatching and in the initial weight of the hatched chicks (1g/day) were achieved in case of vitamin E injection treatment, theseimprovements weren't getinto the significance (P <0.05) in the percentage of hatching ratio, and in theinitial weight of hatched chicks. Moreover, arithmetic improvements weren't get the significance (P <0.05) in case of vitamin E, lysine, and methionine mixture injection treatment. We conclude from this study, the in ovo possibility of injecting eggs embryoswith 1.5 mg / egg of vitamin E solution to improve hatchability and initial weight, methionine solution and lysine - methionine – vitamin E solution to reduce the proportion of embryonic mortality.*

Keywords: *Nutrients, In-ovo injection, Mortality, Weight, Broiler Chickens*

I. Introduction

Avian embryogenesis is an external process (Foye, 2005). Chicken embryos are dependent on the nutrients withinthe egg, which provide the energy and building blocks required for the metabolic needs of the growingembryo during the normal 21-day incubation (Foyeet al., 2006).One unique way of introducing nutrients to the incubating embryo is through in ovoinjection (Macalintal, 2012). Studies have shown that in ovoinjection of nutrients, such as carbohydrate, proteins, vitamins and amino acids as well as vaccines, can enhance growth anddevelopment of the embryo, improve energy status, promote early gut development and improve immune status, as well as alleviate the stress of hatching (Takoet al, 2005; Gore and Qureshi, 1987; Ohtaet al., 2001; Johnston et al., 1997; Gaafaret al.;, 2013). Thus, any embryonic growth and development inside the fertilized egg is taken place under the effect of the concentration and the type of nutrients that involved in the constitution of the fertilized egg (Al-Murraniet al., 1982). However, the injection of fertilized eggs with nutrients plays an important role in replacing any deficiency in the synthesis of food materials involved in the formation of eggs that is taken place as a result for the possible maternal malnutrition (Selimet al., 2012). Moreover, it was mentioned that in ovo injection with nutrients might be useful and much more efficient than the normal nutrition (Bhanjaet al., 2007). Add to that, in ovoinjection with nutrients reduces post-hatching mortalities and diseases as long as enhances the immune responses (Gore and Qureshi, 1987). Also, early development by enhancing intestinal function and development is enhanced too (Takoet al., 2004).

The in ovo nutrient administration could be considered as an alternative method to improve hatchability and duckling weights since either vitamin E or ascorbic acid resulted in significantly ($P < 0.05$) higher body weights at hatch, final body weights, and feed intakes than the un-injected control group (Selimet al., 2012). Vitamin E is regarded as the major lipid soluble antioxidant vitamin, which plays in turn on preventing the oxidation of phospholipids that involved in the construction of brain cells (Suraiet al.,1999). The use of antioxidants, especially vitamin E, has been proven to reduce harmful peroxidation of lipids and cholesterols in animal models (Singh, et al., 2005). Therefore, developed and improved nutritional status afforded by in ovo feeding subsequently improved hatchability percentage, hatching weight, growth performance and immune responses (Al-Zuhairyet al., 2013; Bakyarajet al., 2012; Selimet al., 2012).Moreover, the injection of hatching eggs with vitamin E leads to an enrichment of eggs contents of this vitamin, thus, it's possible to be exploited for chicks' development and growth (Cherianet al., 1997;Suraiet al., 1999). The in ovo injected vitamin E plays a significant role in the increasing of fatty acids oxidation since the day 14 of hatching (Cherianet al., 1997).

It was referred that methionine has an active role in enhancing body weight and growth and a fundamental role in the formation of choline that considered as one of B complex compounds (Coskunet al., 2014). Add to that, the embryonic development might be enhanced after the injection of some amino acids in ovo (Al-Murraniet al., 1982).

It was suggested that DL-methionine may use as an in ovo feed additive for obtain heavier chick (Coskunet al., 2014). Moreover, the in ovo injection of amino acids reduces the danger of some diseases through blocking the deficiency in the concentration of these amino acids in hatching eggs in such away it leads into acceleration of immune response (Khalid et al., 2013). Therefore, this study was designed to investigate the effects of administration of a mixture of amino acids and vitamins ((vitamin E); (Lys); (Met); (vitamin E + Lys + Met)) at a late stage of incubation on hatchability traits and performance of hatching chicks.

II. Materials And Methods

2.1 Eggs pre-injection incubation: Standard size fertile broilers eggs (n= 200) were collected from a commercial breeders chicks from a maternal flock of only one strain (Ross) that fed on adequate nutritional diet. Eggs were distributed into five groups of 40 eggs each. The eggs were incubated under optimal conditions (at 37 to 37.5ºC and 70% relative humidity) at the Department of Animal Resources at Al-Qasim Green University.

2.2 Eggs injection: The groups of the chosen fertile eggs were distributed into five groups. Each treatment that contained 40 eggs was randomly distributed on two repeats. Each egg was injected with 0.1ml (1.5%) nutrient saline as the following; T_1 control treatment (without injection), T_2 injection treatment with vitamin E (1.5 mg/egg), T_3 injection treatment with lysine (1.5 mg/egg), T_4 injection treatment with methionine (1.5 mg/egg), and T_5 injection treatment with the mixture of vitamin E, lysine, and methionine (1.5 mg/egg). The right eggs for hatching were chosen with average weight 62 g/bird (± 1g). Next Day, the package was introduced into the hatcher. The candling was performed at 7 days after hatching depending on each individual treatment. Before injecting the desired solution, the surface of eggs was disenfected by 70% ethanol. In their broad end, the eggs were pierced by a fine needle (gauge 23) of the commercially available 1ml insulin syringe.

2.3 Eggs post-injection incubation: After performing injection, the egg hole was sealedwith a sterile paraffin wax immediately after injection. The injected eggs were returnedinto the incubator. After hatching, the candling was repeated again after 24 and 18 days to identify the mortalities ratio in these ages. After the last test, the eggs were transported into the hatcher to complete hatching. After 21 days after hatching, the hatched and non-hatched eggs ratios were calculated. The initial body weight of the hatched chicks of one day age was calculated as well.

2.4 Statistical Analysis: The obtained data were represented by the completely randomized design (CRD) to analyze the effect of different treatments on the studied characters. Analysis of variance (ANOVA) was used to test the significance of the difference between different treatments and statistical differences were established using a Duncan's Multiple Range Test (Duncan, 1955) at the level of $P < 0.05$. Also, the significant differences were compared among the averages were represented by using SAS program (2001) in the statistical analysis according into the following equation;

$$Y_{ijk} = M + T_i + e_{ijk}$$

III. Results And Discussion

The Different nutrients supplements that in ovo administrated in the present study had shown an increased rate of hatchability and initial body weight. It was noticed from table (1) the presence of a significant reduction of the embryonic mortality at 14 days after hatching at significant level ($P < 0.05$) for the mixture injection treatment with the nutrient solutions (T_5) that notified (0.0%). On the other hand, an important reduction for methionine injection (T_4) treatment was occurred but without reaching the level of significance($P < 0.05$). In T4 treatment the ratio of embryonic mortalities was estimated to be 3.33% compared with T1 control treatment and T_2 and T_3 injection treatments, in which any significant differences among them weren't observed. However, the differences among T_1, T_2, and T_3 treatments were only 6.66%. Add to that, any significant difference between T_5 and T_4 injection treatment wasn't observed as well.

Concerning the effect of the nutrient solutions in the embryonic mortalities ratio after 18 days of hatching, it was noticed that there is a significant reduction ($P < 0.05$) in T5 injection treatment since it reached 0.0%. Though it was an important reduction, it wasn't reached the level of significance concerning T_2 injection treatment, which reached 7.17% compared with T_1 control treatment and other injection treatments (T_3 and T_4), in which any significant differences weren't observed. These results were come in accordance with what it was come out some papers (Selimet al., 2012). Since vitamin E participated in turn in the preventing of the oxidation phospholipids that involved in the growth and development of brain cells and other variable embryonic tissues properly (Dror and Bartov, 1982; Bhanjaet al., 2007). Moreover, this vitamin acts as a reducer for free radicals that cause deleterious damages for unsaturated fatty acids that existing in the embryonic cellular membranes (Cherian and Sim, 1992 and 1997).

The effect of injection of nutrient solutions into the eggs didn't hatched was clarified by the reduction of the injection treatment with methionine (T_4) compared with the control treatment (T_1) and other treatments (T_2, T_3, and T_4). But these reductions were only mathematical and weren't getting the level of significance ($P < 0.05$). These results, however, were come in accordance several observations that reported a significant reduction in the embryonic mortalities in injection treatments with lysine and methionine, in which the reason behind the reduction of mortal embryos might be attributed into the important roles played by these two amino acids in the reduction of glycolysis (Uni and Ferkit, 2003, Peebles et al., 2006). Consequently, the body energy resources will be conserved inside the body and the energy level will be elevated through the formation glucose from non-carbohydrate sources, such as amino acids (Sunny et al., 2007).

Table (1): the effect of injecting eggs with nutrient solutions on the ratio of mortality

Feature \ Treatment	T_1	T_2	T_3	T_4	T_5
Embryonic mortalities ratio (14 days after hatching)	6.66 a ±2.67	6.66 a ± 2.67	6.66 a ±2.67	3.33 ab ± 1.69	0.0 b ± 0.70
Embryonic mortalities ratio (18 days after hatching)	10.71 a ±3.30	7.17 a ±2.76	10.71 a ± 3.30	14.28 a ± 3.72	0.0 b ±0.70
Eggs didn't hatched ratio	8.01 a ±2.91	3.84 b ± 1.78	8.33 a ± 2.42	11.68 a ± 3.47	9.99 a ± 3.19

- Different letter within the same column indicate to the presence of significant differences among treatments within the probability levels ($P < 0.05$)

It was noticed from table (2) the presence of an important superiority in the hatching ratio for the injection treatment with vitamin E (T_2), and the injection treatment with methionine (T_5) compared with the control treatment (T_1) and the remaining injection treatments (T_3 and T_4). But, this superiority was only mathematical, and wasn't getting the level of significance ($P < 0.05$). These results were come in accordance with the results obtained by Selim and his colleagues (2012). Vitamin E plays on the increasing of fatty acids oxidation that begins at the day 14 after hatching (Chereian and Sim, 1992 and 1997). The situation was the same for the initial weight at one day age, in which it was noticed that superiority of the injection treatment with vitamin E (T_2), since it reached 44 g compared with the control treatment (T_1) and other injection treatments (T_3, T_4, and T_5). But this superiority was mathematical and wasn't getting the level of significance ($P < 0.05$). The in ovo injection of vitamin E plays on the increasing of the vitamin E egg's content, and consequently it will be exploited in the development and growth in addition to the ability of chick to utilize of this vitamin after hatching (Cherianet al., 1997; Suraiet al., 1999).

Table (2): the effect of injecting eggs with nutrient solutions on the hatching ratio and initial weight for hatched chicks (g/1 day).

Feature \ Treatment	T_1	T_2	T_3	T_4	T_5
Hatching ratio	83.33 a ±9.15	89.99 a ±9.53	83.33 a ± 9.13	76.66 a ± 8.78	89.99 a ± 9.51
Initial body weight 1g/day	42.50 a ±4.25	44.0 a ± 4.40	42.50 a ± 4.23	42.50 a ± 4.28	43.50 a ± 4.35

- Different letter within the same column indicate to the presence of significant differences among treatments within the probability levels (**$P < 0.05$**)

IV. Conclusion

It may be concluded that early supplementation of nutrients through inovoinjection such as amino acids and vitamin mixture can beregarded as a possible method to improvehatchability, body weight at hatch, marketing weights of broilers.Further investigations are needed to highlight theeffect of in-ovo injection of amino acids mixture onthehumoral, cell mediated immunity, anddevelopment of digestive organs in broilers.

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