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The effect of in ovo injection of hatching eggs with omega-3 oil on some productive traits of broilers

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Abstract:
This study was conducted to investigate the effect of in ovo injection of hatching eggs with Omega 3 Oil at 18 days old, in the amniotic sac in the air sac, on some productive characteristics of broilers ROSS 308. The eggs were injected into the Al-Bazz hatchery in the holy governorate of Karbala, as for raising hatched chicks from injected eggs, it was conducted in the poultry hall in the research station of the Department of Animal Production, College of Agriculture and Marshes, Thi-Qar University, from 12/1/2020 to 25/1/2021. A total of 450 eggs from ROSS 308 broiler breeder were used in this experiment, distributed into five treatments as follows: T1; The first treatment (negative control): hatching eggs left without injection. T2; The second treatment (positive control): hatching eggs injected with sunflower oil. And another treatment T3, T4 and T5 were injected hatching eggs with 0.1, 0.2 and ml of omega-3 oil respectively. 300 chicks from the hatched chicks were distributed in cages with dimensions of 1 x 1.5 m for each cage, at a rate of 60 chicks for each treatment, with three replicates of the treatment (20 chicks for each replicate). The results of the study indicated that there was a significant ($P < 0.05$) improvement in the studied productive traits (body weight, weight gain, feed intake, feed conversion factor).

Keywords: in ovo injection, hatching eggs, omega-3, productive traits, broilers.

I. INTRODUCTION

The poultry industry occupies a prominent position among the food industries, it is considered an important pillar of the economy of a number of countries in the world, its importance to meet the needs of the population, it provides the consumer with two important sources that cannot be easily replaced, namely meat and eggs (Al-Fayyad *et al*., 2011). These two products contain important and necessary nutrients for the human body, whether in the form of proteins, calories or fats, which the human body cannot do without (Baidi *et al*., 2013). Poultry meat also has good production specifications, it is characterized by its high efficiency in speed of growth and weight gain and high efficiency in converting feed (Al-Gharawi, 2012). These specifications increased their nutritional requirements, therefore, researchers have conducted many studies in order to advance this industry, and access to the international standards of the market, the hatching eggs produced from the flocks of broiler breeder, one of the best ways in the development of the poultry industry is through the production of chicks, then provide white meat. (Al-Zujaji and Khalil, 1982). Food additives such as sugar solutions and vitamins are added to the breeders' diet, only 25-30% were transmitted to the fetus, as for the greater part, it goes to the body of the bird (Abdul-Latif and Al-Jaf, 2011). The quality of the hatched chicks depends on the feeding of

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the parents and the specifications and characteristics of the hatching eggs and the components of the hatching process, therefore, the injection technique was devised (Al-Azzawi, 2018). The in ovo injection process is a useful mechanism for the development of embryos, protein and energy are derived from the yolk (Salmanzadeh, 2021). The importance of this method becomes clearer, it is believed that birds cannot reach food until after 36-48 hours, which may subsequently affect body weight and muscle development (Noy and Uni, 2009). In the last twenty years, improved feeding, management and genetic selection of poultry flocks, but in commercial hatcheries, the hatchability of broiler eggs was not elevated (Schaal and Cherian, 2007). Egg injection technique may improve hatchability and chick weight health, and growth performance through early feeding of the developing fetus (Bakyaraj *et al*., 2011). Injecting the hatching eggs with nutrients before hatching helps the chicks to overcome the stress of hatching, and to obtain their nutritional requirements immediately after hatching, Because it may wait a day or two in the hatchery before reaching the hall, injection is an early feeding of fetuses in the last period of fetal development, it is performed by injecting nutrient solutions into the amniotic sac surrounding the fetuses, to reduce the stress that the chicks are exposed to, whether it is health or nutritional, producing good quality chicks with a high capacity for productive performance (Selim *et al*., 2012). Omega-3 fatty acids possess anti-inflammatory or less inflammatory properties by reducing the release of pro-inflammatory cytokines eicosanoids (Stulnig, 2003). Cytokines produced by white blood cells act as body regulators by exerting various effects on lymphocytes and other immune cells in response to infection and injury, administration of high-quality fatty acids into eggs may be beneficial for improving energy production during embryonic development and hatching (Singh et al., 2005). In addition to the increase in body weight in the marketing age, in terms of human health, omega-3 fatty acids are essential to play an important role in preventing coronary heart disease, atherosclerosis, high blood pressure and inflammation, as well as boosting immunity (El-Yamany *et al*., 2008). It also lowers cholesterol and triglycerides in the blood and improves response to insulin, it was useful for the health of the fetus's brain, visual development and blood pressure regulation, and poultry meat can also acquire these characteristics (Al-Khalisi, 2011). The current study, which includes injecting the hatching eggs with different levels of omega-3 fatty acid, aims to determine the effect omega-3 fatty acid on some productive traits of broilers.

II. MATERIALS AND METHODS

This experiment was conducted in Al-Bazz hatchery in the holy governorate of Karbala for the period from 12/1/2020 to 21/12/2020. The hatchlings were reared in the poultry field belonging to the Agricultural Research and Experiment Station, College of Agriculture and Marshlands, Thi-Qar University from 12/22/2020 to 01/25/2021. A total of 450 hatching eggs were used from the fields of Ross 308 broiler breeders, eggs were taken from one incubator and stored for two days. The hatchable eggs were selected on the basis that they are approximately homogeneous in weight, the average weight of an egg was 53.5 g, exclude cracked, dirty, cracked eggs, small, large, elongated, and deformed eggs. Incubating eggs in a Belgian-made Petersime incubator, after regulating the temperature of the incubator 37.8 °C and the relative humidity of 70%, the eggs were turned automatically until the end of the eighteenth day of incubation, then the eggs were transferred to the incubator in flat-base trays, with a temperature set of 37.0 °C and a relative humidity of 80%, the eggs were not turned during the last three days of the hatching period. On the 18th day of the embryo's life before transferring

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the eggs from the incubators to the hatchers, the eggs were scanned and the amniotic sac was identified in the air space, use a pointed drill for the purpose of piercing the egg shell, while avoiding the occurrence of fracture or cracking of the crust, and taking care not to damage the blood vessels of the fetus as much as possible, the crust is pierced with a circular motion of the piercing, then injected the nutrients used in the experiment using a syringe, inserted the syringe needle (Gauge22), inside the egg, penetrating the egg shell and membranous shell into the amniotic sac, according to the method indicated (Versteeg, 1985). The eggs were injected using a sterile (1 ml) insulin syringe, measuring 22 Gauge, it is completely inserted for the purpose of ensuring that it reaches the amniotic sac surrounding the fetus, after sterilizing the piercing area with a cotton dipped in alcohol, after completing the injection process, sterilize the puncture site with alcohol, close it with nail polish and return the eggs to the hatching machine, after completing the injection process, transfer the injected eggs to the hatching trays, mark the eggs with special signs of transactions until the time of hatching. After hatching, a total of 300 one-day-old Ross 308 chicks, which hatched from the five treatments in the first experiment, were randomly distributed to five experimental treatments with 60 chicks for each treatment and for three replicates (20 chicks/ replicate). The hatched chicks were reared in cages of dimensions (1.5 m in length and 1) m in width, each cage contains 20 broiler chicks (each cage represents one replicate per treatment). Heat was saved by using gas incubators and air extractors, while monitoring the temperature by using a mercury thermometer from one day old to (35) days old. Feed and water were provided *ad libitum*, and used the continuous lighting system 23 hours a day throughout the experiment period, with one hour of darkness given. The birds were fed two types of diets, starter feed from 1 to 21 days old, and the final diet is from the age of 22 days until the end of the experiment at the age of 35 days, and Table (1) shows the chemical composition of the diets.

Table (1) The chemical composition of the diets used in the experiment and their chemical analysis during the initiator and finite periods.

Item	Starter diet (1-21 day)	Finisher (22-35 day)
Maize	43.5	47.4
Wheat	17	17
Soybean meal (44%)	28	26
Protein concentrate	10	6
Plant oil	0.5	2.6
Limestone	0.7	0.7
Salt	0.3	0.3
Total	100	100
Chemical analysis		
Crude protein (%)	22.44	20.31
Metabolize energy (kilo calorie/ kg diet)	2966.5	3154
C/P ratio	132.16	155.24
Crude fiber (%)	4.06	3.91
Calcium (%)	1.20	0.85

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Available phosphorus (%)	0.71	0.59
Methionine (%)	0.55	0.41
Lysine (%)	1.31	1.11
Methionine+ Cysteine (%)	1.00	0.83

*The chemical composition of the feed materials included in the diets was calculated according to the recommendations of the NRC (1994).

* The protein concentrate used is animal (Al-Wafi), of Dutch origin, imported from Al-Muwafaq Company. It contains 40% crude protein, 5% crude fat, 2% crude fiber, 6.5% calcium, 4% phosphorous. Available 3.85% lysine, 3.70% methionine, 4% Methionine + cysteine, 2.3% sodium, 2100 kg calories / kg represented energy and contains a mixture of vitamins and rare minerals to meet the needs of the bird. Phytase enzyme 15000 units/kg concentrate, 5000 mg/kg choline chloride concentrate.

Studied traits:

Average weekly live body weight

Chicks were weighed at the age of one day, and the birds were weighed weekly for each replicate of the experiment using an electronic scale during the first three weeks, then a scale with a 50 kg capacity was used for the last two weeks of the experiment and the following equation was applied to find out the weight of the bird within one replicate.

$$Live\ body\ weight\ (g) = \frac{\text{Sum of birds' weights in replicate}}{\text{Total number of birds in the replicate}}$$

Weekly weight gain rate

The daily weight gain was calculated from calculating the weight gain achieved weekly, as mentioned by Al-Zubaidi (1986) according to the following equation:

Weight gain (g/bird) = live body weight at the end of the period - weight of the live body at the beginning of the period

Feed intake

The weekly feed consumption for each bird was calculated from calculating the amount of feed consumed for each repeater weekly, by weighing the remaining amount of feed at the end of the period for each repeater and subtracting it from the total amount provided during the period according to the equation provided by Al-Zubaidi (1986): The amount of weekly consumed feed (g) = the feed provided at the beginning of the period - the remaining feed at the end of the period.

feed conversion factor

The daily food conversion factor was calculated according to the following equation, as mentioned by Al-Zubaidi (1986):

$$Feed\ conversion\ efficiency = \frac{\text{The average amount of feed intake per week (g)}}{\text{Average weekly weight gain (g)}}$$

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Statistical analysis

The complete random design (CRD) was used to study the effect of different treatments on the studied traits, and the significant differences between the means were compared with Duncan (1955) polynomial test under the significance level of 0.05, and the SPSS (2012) program was used in the statistical analysis.

III. RESULTS AND DISCUSSION

The effect of in ovo injection of hatching eggs with omega-3 on the average weekly live body weight (g) of broilers:

Table (2) shows the effect of in ovo injecting of hatching eggs with omega-3 oil on the average weekly body weight of broilers. At the first week of chick life, there was a significant difference (P<0.05) for treatment T5 compared to treatment T3, which was significantly superior (P<0.05) compared to treatments T1 and T2. At the third week of bird life, it was also noticed that treatment T5 was significantly superior (P<0.05) compared to treatments T3 and T4 that were significantly superior (P<0.05) compared to treatment T2, which was significantly superior (P<0.05) at the expense of the control treatment. At the ages of 2, 4 and 5 weeks, treatment T5 was significantly superior (P<0.05) compared to treatment T4, which was significantly superior (P<0.05) compared to treatment T3 that was significantly superior (P<0.05) at the expense of treatment T2, which was significantly superior (P<0.05) compared to the control treatment. The final average weight was 2037.00, 2110.00, 2241.67, 2303.00 and 2395.33 g for treatments T1, T2, T3, T4 and T5 respectively.

Table (2) The effect of in ovo injecting of hatching eggs with omega-3 oil on the average weekly body weight (g) of broilers ± standard error.

Treatments	Age (Week)				
	1	2	3	4	5
T_1	1.20±163.67 c	1.45±375.33 e	4.67±763.33 d	3.17±1330.33 e	2.30± 2037.00 e
T_2	3.48±165.67 c	1.76±384.66 d	13.73±787.67 c	4.04±1383.00 d	4.04±2110.00 d
T_3	2.30±176.00 b	2.88± 414.00 c	2.60± 834.33 b	3.48± 1480.67 c	2.33± 2241.67 c
T_4	2.60±180.33 ab	3.17± 425.33 b	2.88±858.00 b	2.30± 151400 b	4.61± 2303.00 b
T_5	2.90±184.06 a	4.04± 439.00 a	3.17±885.33 a	3.75± 1572.33 a	4.33± 2395.33 a
Sig.	N.S	*	*	*	*

T1: negative control without injection. **T2:** Positive control by in ovo injection of hatching eggs with sunflower oil. **T3:** in ovo injection of hatching eggs with 0.1 ml of omega-3 oil. **T4:** in ovo injection of hatching eggs with 0.2 ml of omega-3 oil. **T5:** in ovo injection the hatching eggs with 0.3ml of omega-3 oil.

* The vertically different letters indicate that there are significant differences between the means at the 0.05 level of significance. N.S Non-significant.

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The effect of in ovo injection of hatching eggs with omega-3 on the rate of weekly weight gain (g) for broilers:

Table (3) shows the effect of in ovo injecting of hatching eggs with omega-3 oil on the rate of weekly weight gain for broilers. It was noticed that there was a significant improvement (P<0.05) for treatment T5 compared to treatment T3, which is significantly superior (P<0.05) compared to treatments T1 and T2 at the first week of life of the birds. At the third week, treatments T3, T4 and T5 significantly outperformed (P<0.05) compared to treatments T1 and T2. As for the second, fourth and fifth weeks and the total weight gain, the results showed that treatment T5 was significantly superior (P<0.05) compared to treatment T4, which was significantly superior (P<0.05) compared to treatment T3 that was significantly superior (P<0.05) at the expense of treatment T2, which was significantly superior. P<0.05) compared to the control treatment. The total weight gain was 1994.00, 2067.00, 2198.67, 2260.00 and 2352.33 g for treatments T1, T2, T3, T4 and T5, respectively.

Table (3) The effect of in ovo injection hatching eggs with omega-3 oil on the average weekly weight gain (g) for broilers ± standard error.

Treatments	Age (Week)					Total
	1	2	3	4	5	
T_1	1.20±120.67 c	2.60±211.67 e	5.56±388.00 b	7.00±567.00 e	0.88±706.67 e	2.30±1994.00 e
T_2	3.48±122.67 c	1.73±219.00 d	12.09±403.00 b	9.76±595.33 d	0.52±727.00 d	4.04±2067.00 d
T_3	2.30±133.00 b	0.58±238.00 c	0.33±429.33 a	0.88±637.33 c	1.15±761.00 c	2.33±2198.67 c
T_4	2.60±137.33 ab	0.58±245.00 b	0.33±432.67 a	0.58±656.00 b	2.30±789.00 b	4.61±2260.00 b
T_5	2.90±141.06 a	1.20±254.33 a	0.88±446.33 a	0.58±687.00 a	0.57±823.00 a	4.33±2352.33 a
Sig.	*	*	*	*	*	*

T1: negative control without injection. **T2:** Positive control by in ovo injection of hatching eggs with sunflower oil. **T3:** in ovo injection of hatching eggs with 0.1 ml of omega-3 oil. **T4:** in ovo injection of hatching eggs with 0.2 ml of omega-3 oil. **T5:** in ovo injection the hatching eggs with 0.3ml of omega-3 oil.

* The vertically different letters indicate that there are significant differences between the means at the 0.05 level of significance.

The effect of in ovo injection of hatching eggs with omega-3 on the weekly feed intake rate (g) for broilers:

Table (4) indicates the effect of in ovo injecting of hatching eggs with omega-3 oil on the rate of weekly feed intake for broilers. The table shows a significant increase (P<0.05) in the rate of weekly feed intake at the first week of bird life in treatment T5 compared to treatment T3, which showed a significant increase (P<0.05) compared to the positive and negative control treatments. At the third week of bird life, a significant increase (P<0.05) is observed in treatments T3, T4

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and T5 compared to the positive and negative control treatments. As for weeks 2, 4, 5 and total feed intake, the same table indicates that treatment T5 led to a significant increase (P<0.05) in the rate of feed consumption compared to treatment T4 that was significantly higher (P<0.05) at the expense of treatment T3, which led the results to Significant increase (P<0.05) compared to treatment T2, which was significantly superior to treatment T1. The total feed intake was 3063.67, 3149.00, 3297.00, 3359.00 and 3456.67 g for treatments T1, T2, T3, T4 and T5 respectively.

Table (4) The effect of in ovo injection hatching eggs with omega-3 oil on the average weekly feed intake rate (g) for broilers ± standard error.

Treatments	Age (Week)					Total
	1	2	3	4	5	
T_1	2.30±161.00 c	2.33±315.33 e	4.50±596.00 c	3.52±879.67 e	2.72±1111.67 e	0.88±3063.67 e
T_2	2.02±162.33 c	0.88±324.67 d	3.17±613.67 b	4.93±920.00 d	2.33±1128.33 d	6.11±3149.00 d
T_3	0.88±175.33 b	2.72±347.33 c	2.33±635.67 a	2.18±969.33 c	2.96±1169.33 c	5.29±3297.00 c
T_4	3.51±179.00 ab	0.88±354.33 b	1.52±636.00 a	2.18±989.33 b	7.88±1200.33 b	1.52±3359.00 b
T_5	0.88±183.67 a	1.20±362.67 a	4.48±646.67 a	1.73±1020.00 a	3.75±1243.67 a	10.39±3456.67 a
Sig.	*	*	*	*	*	*

T1: negative control without injection. **T2:** Positive control by in ovo injection of hatching eggs with sunflower oil. **T3:** in ovo injection of hatching eggs with 0.1 ml of omega-3 oil. **T4:** in ovo injection of hatching eggs with 0.2 ml of omega-3 oil. **T5:** in ovo injection the hatching eggs with 0.3ml of omega-3 oil.

* The vertically different letters indicate that there are significant differences between the means at the 0.05 level of significance.

The effect of in ovo injection of hatching eggs with omega-3 on the feed conversion factor (g diet/g weight gain) for broilers:

Table (5) shows the effect of injecting hatching eggs with different levels of omega-3 on the feed conversion factor of broilers. It is noticed that there are no significant differences between all the treatments for the characteristic of the feed conversion factor at the first and third week of life of the birds. At the second and fourth week of bird life, a significant improvement (P<0.05) appeared in the feed conversion factor for treatment T5 compared to treatments T1 and T2, and there was also a significant improvement (P<0.05) for treatment T5 compared to treatments T1, T2 and T3. As for the total feed conversion factor, the results showed a significant improvement in treatment T5 compared to treatments T3 and T4, which showed a significant improvement (P<0.05) compared to the negative and positive control treatments. The total feed conversion factor was 1.53, 1.52, 1.49, 1.48 and 1.46 g diet/g weight gain for treatments T1, T2, T3, T4 and T5, respectively.

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Table (5) The effect of in ovo injection hatching eggs with omega-3 oil on the average weekly food conversion factor (g diet/ g weight gain) for broilers ± standard error.

Treatments	Age (Week)					Total
	1	2	3	4	5	
T_1	0.007±1.33	0.018±1.49 c	0.028±1.53	0.024±1.55 b	0.004±1.57 d	0.001±1.53 c
T_2	0.20±1.32	0.014±1.48 bc	0.052±1.52	0.017±1.54 b	0.003±1.55 cd	0.005±1.52 c
T_3	0.016±1.31	0.008±1.45 abc	0.006±1.48	0.003±1.52 ab	0.002±1.53 bc	0.001±1.49 b
T_4	0.016±1.30	0.005±1.44 ab	0.003±1.47	0.004±1.50 ab	0.013±1.52 ab	0.003±1.48 b
T_5	0.032±1.29	0.005±1.42 a	0.008±1.44	0.003±1.48 a	0.005±1.51 a	0.006±1.46 a
Sig.	N.S	*	N.S	*	*	*

T1: negative control without injection. **T2:** Positive control by in ovo injection of hatching eggs with sunflower oil. **T3:** in ovo injection of hatching eggs with 0.1 ml of omega-3 oil. **T4:** in ovo injection of hatching eggs with 0.2 ml of omega-3 oil. **T5:** in ovo injection the hatching eggs with 0.3ml of omega-3 oil.

* The vertically different letters indicate that there are significant differences between the means at the 0.05 level of significance. N.S Non-significant.

The results indicate that in ovo injection of the hatching eggs with omega-3 oil has improved the productive performance of the hatched broiler broilers, represented by body weight, weight gain, feed conversion factor. Abdul Wahed (2017) explained that in ovo injection of hatching eggs with fish oil significantly increased the average body weight compared to the control treatment. Al-Zuhairy and Taher (2014) also noted that the average live body weight of broilers showed a significant superiority when adding flaxseed oil to the feed. That this increase may be due to the presence of essential oils in flaxseed, because flaxseeds rich in omega-3 lead to the activation of bile, which increases the digestion of fats in the intestines and increases the effectiveness of the digestive system in digesting and absorbing nutrients in the gut, it may be due to the improvement in the vitality of the hatched chicks, which increases the rate of their weight in later ages. It may be attributed to the metabolism of adipose hormones (Caston *et al*., 1994). Al-Khalisi (2011) indicated that omega-3 has a positive role in raising the body weight rate, where it works to convert carbohydrates in the feed of vegetable origin into glucose. It is transmitted by insulin that binds to unsaturated fatty acids and converts them to triglycerides in adipose tissue, which increases body weight and thus improves the productive performance of broilers.

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