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# Effect of *in Ovo* Injection of Vitamins E and D3 on Hatchability and Embryos Development of Poultry

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**Abstract**
This study was conducted in the field of animal production of Agriculture college / University of Karbala on 7 February 2022 until 30 February 2022 to find out the effect of injecting broiler chicken eggs with vitamins E and D3 in some productive qualities of broiler chicks, where in the first experiment 480 eggs were used and distributed to eight transactions at 60 eggs per treatment, where eggs were injected with vitamins on the seventh day of incubation in the air sac of eggs. The study showed a significant increase ($P \leq 0.01$) in the hatching rate of eggs enriched for vitamin E injections for the third treatment and vitamin D3 for the sixth treatment compared to negative control treatment and the fifth treatment vitamin E injection and the seventh treatment vitamin D3 injection, while We did not find significant differences between the rest of the trial transactions, and for the average and late fetal deaths of vitamin injections during the intermediate stages of fetal development, there was a significant decrease ($P \leq 0.05$) in the proportion of average fetal fatalities of the sixth treatment. Vitamin D3 injections (0.01%) compared to negative and positive control factors A significant decrease ($P \leq 0.05$) was found in the percentage of fetal decimations delayed for the eighth treatment vitamin D3 injection (0.11%) compared to the treatment of 4th, 5th and 7th, while accounting differences were found with negative and positive control factors.

**Keywords:** Egg injection, Vitamin E, Vitamin D3, Hatching

## Introduction

The process of injecting bird embryos is known as the processing of the fetus with external nutrients by injecting eggs before hatching, which improves hatching ratios and productive qualities by modifying the anatomical composition of the gut (Unio Ferkat, 2004), which leads to a higher hatching rate and high weights for the body of chicks compared to hatching chicks of uninjuled eggs, thus making the important start for the production of chicks up to the weight of marketing faster and reducing the factor of conversion (Zhava and Ferkat, and reduces the factor of conversion (Zhava and Ferkat ;2005). The process of injecting hatching eggs includes the introduction of different nutrients into hatching eggs, whether they are amino acids such as argenin amino acid (Al-Daraji et al., 2012), vitamin E and vitamin D3 (Ibrahim et al., 2012; Bello et al., 2013, or injection of vitamin pyrodoxin (B6) (Elkholy, 2013). Vitamin E is essential for the embryonic growth of birds, as many studies have confirmed its importance in improving hatching, as some symptoms of vitamin E deficiency appear on the 7th and 15th day of incubation period, which include blood spots in the eye and muscle decay, and fetal death may sometimes occur on the fourth day of incubation (Lin, 2011). Vitamin D3 is also a fat-dissolved vitamin and the effective form of vitamin D3 is [1.25 (OH)2D3] 1α,25-dihydroxy cholecalciferol D3 as chicken needs it through its main role in regulating the level of calcium and phosphorus in the blood and the formation of skeleton,

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beak, claws and eggshell (De-Boland and Norman, 1990). For his role in the manufacture of calcium binding protein (CaBP) Calcium Binding Protein (Chatterjee, 2001), which participates in the effective transfer of calcium through bowel membranes and possibly through uterine membranes (Norman, 1985). Vitamin D3 helps improve body cell growth and increase chick weight immediately after hatching (Gonzales et al., 2013). This study deals with the effect of injecting different concentrations of vitamin E and vitamin D3 in the first week of incubation period on the rate of hatching and fetal mortality rates during the growth period of the chicken embryo.

**Materials and Methods**

**Eggs used in the experiment:**

In this study, 480 eggs were used to hatch the mothers of broiler chicks (Ross 308), the mothers of the meat chicks were 40 weeks old and the eggs used four days and the experiment was carried out in the hatching of the Faculty of Agriculture / University of Karbala in Karbala province. For the period from 7 February 2022 to 30 February 2022, the choice of hatching eggs was taken into account in terms of homogeneity in weight and free from defects in shape, calcification and dirt.

**Source of vitamins used in the study:**

The fat dissolved vitamins (E and D3) used in the experiment were obtained from one of the offices specializing in veterinary equipment and supplies in Baghdad/Sink (Orok Office) and sourced from the Swiss company DSM Nutritional Products, Inc., which is a pure oil vitamin that dissolves with fat packed in a 10ml glass bottle and held in a plastic container tightly to prevent exposure to light and protect it from damage.

- **-**Vitamin E is a pure, colorless, very sticky oil vitamin that dissolves with 99% fat concentration (999 mg/ml), packed with a small 10 ml glass bottle.

--Vitamin D3 is a pure oily vitamin that dissolves in light yellow fat with a concentration of 4,000,000 IU/ml, packed with a small 10 ml glass bottle .

**How to prepare vitamin concentrations**

To prepare the concentration of injected vitamins for current study about an hour before the injection process and the method of preparation was as follows::

Vitamin E: Three concentrations have been used and include as follows: -

-First concentration: 2,248 ml oily vitamin, which has a concentration of 99% (999 mg/ml) was added 5 ml of corn oil, each 100 microliter tow gives 15 IU of vitamin per egg.

- Second Concentration: 1.57 ml oily vitamin, which has a concentration of 99% (999 mg/ml) was added a 3.5 ml bank with corn oil, each 100 microliter tow giving 20 IU of vitamin per egg.

-Third Concentration: 1.12 ml oily vitamin, which has a concentration of 99% (999 mg/ml) was added 2.5 ml of corn oil, each 100 microliter withdrawal giving 25 IU per egg.

Using the following equations:

$Mg \times 0.67 = 15$ IU

$Mg = 22.48$ mg

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22.48 mg ⟹ (15 IU)
C1 ×0.05 = 22.48 mg
C1=449.6 mg / ml
For the first concentration vitamin E
5 ml oil × 449.6 = V × 999
Vitamin E V = 2.248 ml
For the second concentration of vitamin E
3.5 × 449.6 = V × 999
Vitamin E V = 1.57 ml
For the third concentration of vitamin E
2.5 × 449.6 = V × 999
Vitamin E V = 1.12 ml

**Vitamin D3: Use in this study three concentrations as follows:**

--The first concentration: 10 microliter of vitamin D3 oil has been added to 20 ml corn oil is added to oil ,each 100 microliter tow gives 100 IU of vitamin per egg. (Sixth treatment)

--The second concentration: 15 microliter of vitamin D3 oil has been added to 30 ml oil, with each cloud 100 microliter gives 150 international vitamin units per egg (seventh treatment),

--Third concentration: 20 microliter of vitamin D3 oil has been added to 40 ml of corn oil with each cloud 100 microliter gives 200 international vitamin units per egg (Eighth treatment) .

The following equation:
20× = 2000 V × 4000000 First concentration of vitamin D3 -
Vitamin D3 V= 10 microliters
30× = 2000 V ×4000000 The second concentration of Vitamin D3 -
Vitamin D3 V= 15 microliters
40× = 2000 V × 4000000 The third concentration of Vitamin D3 -
Vitamin D3 V= 20 microliters

**Egg injections:**

The egg injection was performed in the hatch of the Agriculture college / University of Karbala / Karbala province and the egg was injected at a week's age after the eggs were inserted into the hatchery of the egg and the injection of eggs was performed at a concentration of 70% and then the vitamins used in the experiment were injected by syringe with a capacity of 1 ml measurement (Gauge26) and allocated to each egg injected only once and the needle was inserted into inside the air sac, and when the injection is completed, the hole is closed by pure medical wax. The lap temperature was 37.7°C, relative humidity was 65% and the eggs were flipped 24 times a day and automatically from the first day until the 14th day of the incubation period, then the eggs were transferred to the hatching part of the hatchery and the temperature was 37° and the relative humidity was 85% and the eggs were not flipped in this part where the egg laying lasted three days until the end of the hatching period.

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**Experience treatment:**

-Eight treatment were used in this experiment, each of which contained 60 hatching eggs and was distributed over three repeaters per repeater containing 20 eggs, including the follows:

-The first treatment (T1): Negative control treatment includes eggs not injected with vitamin or oil as negative control treatment.

-Treatment 2 (T2): Positive control treatment includes eggs injected with 100 microliters with corn oil as positive control treatment.

-Treatment 3 (T3) : Eggs injected with 100 microliters include vitamin E per egg (15 IU/egg) vitamin E treatment.

-Treatment 4 (T4): Eggs injected with 100 microliters include vitamin E per egg (20 IU/egg) vitamin E treatment.

-Treatment 5 (5 T): Eggs injected with 100 microliters include vitamin E per egg (25 IU/egg) vitamin E treatment.

-Treatment 6 (6 T): Eggs injected with 100 microliters include vitamin D3 per egg (100 IU/egg) vitamin D3 treatment.

-Treatment 7 (7 T): Includes eggs with 100 Microliter vitamin D3 per egg (150 IU/egg) vitamin D3 treatment.

-Treatment 8 (8 T): Eggs injected with 100 microliters include vitamin D3 per egg (200 IU/egg) vitamin D3 treatment.

**Thoughtful qualities:**

**Percentage hatching:**

The percentage of hatching per treatment was calculated according( **Pedroso** et al ,2006 ) and according to the following equation:

Percentage of hatching of fertilized eggs=$\frac{Number\ of\ hatched\ chicks}{Number\ of\ fertilized\ eggs} \times 100$

Measuring the proportion of fetal deaths:

As reported by Naji and others (2007) and measured using the following equation

$\frac{Number\ of\ fetal\ deaths\ (mortal\ embryos)}{Number\ of\ fertilized\ eggs} \times 100(\%)$ =Percentage of fetal death

**Statistical analysis**

Use complete random design (CRD) to study the effect of different treatments on the qualities studied, and compared the significant differences between the averages of the Duncan test (1955) multi-border below a significant level of 0.05. The SAS program (2012) was used in statistical analysis according to the following mathematical model:

Yij =μ +Ti + eij

**Result and Discussion**

Table (1) shows the effect of injecting eggs with fat-dissolved vitamins E and D3 in the percentage of hatching from fertilized eggs, where note a significant improvement ($P \leq 0.01$)

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in the hatching rate in the third treatment vitamin E injection 0.84% and the sixth treatment vitamin D injection 3 (0.85%) compared to the first two negative control factors 0.64%, while we find a significant decrease ($P \leq 0.01$) in the hatching rate of the seventh treatment vitamin D3 injection if it amounts to (0.51%) compared to injection transactions while we did not find significant differences between Other injection treatments.

The significant improvement in hatching in vitamin E and D3 injections may be due to the fact that nutrient injections can cross the inner membrane of the egg to reach the developing fetus and improve its growth (Ghobadi and Hemati,2015), and the importance of vitamin E necessary for the development of the vascular system of the fetus Vascular Sysem by increasing the circulation of the concentration of vitamin E (α-Tocopherols) which is associated with the modification of fetal development by increasing blood flow and processing the nutrients of the fetus (Theresa et al , 2006), increasing energy production that increases fetal growth (Schaal, 2008). In addition to its work as an antioxidant, it interferes with preventing protein damage or decay, leading to better digestion and utilization of nutrients (Ciftci et al., 2005). It also has a role in preserving nitric oxide (NO) from the harmful effects of free radicals, which helps stimulate cellular metabolism (Leornzoni and Ruiz-Feria, 2006). Nitric oxide also helps increase muscle growth, regulate blood flow and differentiate muscle cells (Stamler and Meissner, 2001)

This result is consistent with that of Salary et al. (2014) and with al-Shamery and AL-Shuhaib (2015) who found a significant improvement in hatching when injecting the eggs of the mothers of chicken with vitamin E. It is consistent with a result in a study that obtained al-Musawi (2011) a signficant increase in the rate of hatching when injecting hatching eggs to Japanese quail birds with concentrations of vitamin E/egg on the seventh day of embryonic development compared to the negative and positive control factors as well as found a rise Morally in the weight of the hatched chick, the average weight of the hatched chick for the study transactions was 9.34, 9.43, 9.44, 9.44 and 9.47 grams, respectively.

. Selim et al. (2012) found when hatching eggs were injected to ecumenical duck mothers at the age of 12 days of incubation with vitamin E with a concentration of 10 mg dissolved with water, a significant improvement in hatching, as well as a significant improvement in the weight of hatched chicks at the age of one day for injection treatment compared to control .

The outcome of Salary et al. (2014) agrees on a significant improvement in hatching when hatching eggs are injected with vitamin E. Al-Shamery and AL-Shuhaib (2015) agree when injecting the eggs of mothers of chicken chicks with vitamin E concentration 1.5 mg/egg at the broad end of the egg and injection dose 0.1 ml/ egg and then enter the eggs into hatchery moral improvement in hatching rate and also found a significant improvement in the weight rate of hatchery for injection treatment compared to the control treatment. These results were not consistent with gore and Qureshi(1997), which found a significant decrease in hatching when injecting meat chick eggs with vitamin E (10 IU/egg) at the age of 14 days of incubation, the reason for the disagreement may be due to the difference in the injection date.

. For the significant improvement in the rate of hatching and the weight of the hatched chick in the treatment of vitamin D3 injections due to its role in stimulating cell reproduction and differentiation through special receptors of vitamin D3, through which it is associated with the target genes in the DNA molecule and leads to increased synthesis of the body protein

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(Ross et al., 1992). Vitamin D3 has an important role to play in the development and increase of blood cell numbers (Yetgin and 2004, Yalç needn). Vitamin D3 (1.25 (OHD3) also increases the production of Nitric oxide NO (Andrukhova et al., 2014). Vitamin D3 also has an important role in improving the tissue development of the formation of micro intestines by increasing the length of the 12th and fasting hgulations in the small intestine, in a cycle that helps improve the growth of body cells and improve the efficiency of the food conversion of birds (Chou et al. (2009), Bello et al. (2013 ,(

This finding is consistent with Ibrahim et al. (2012) When 180 IU vitamin D3 was injected into the air chamber of the hatching eggs of a week-old ostrich bird during the incubation period, a significant improvement in the hatching rate of fertilized eggs compared to the hatching rate of negative control treatment (without injection) and positive (injection).

These results were not consistent with that of Gonzales et al. (2013) and bello et al. (2013) who, when injecting the eggs of mothers of chicken chicks with vitamin 3D, found no significant improvement in hatching and hatching chick weight.

**Table 1 Effect of Vitamin E and D3 injections in local chicken eggs on hatching**

| Treatment | Standard error± averages |
|---|---|
| | Hatching rate |
| Treatment 1 Control | $^{C}$0.00± 0.64 |
| Treatment 2 corn oil injections | AB0.02 ±0.79 |
| Treatment 3 vitamin E1 injections | $^{A}$0.02 ±0.84 |
| Treatment 4 vitamin E2 injections | $^{BC}$0.06 ± 0.69 |
| Treatment 5 vitamin E3 injections | $^{C}$0.09 ±0.67 |
| Treatment 6 vitamin D1 injections | $^{A}$0.07 ±0.85 |
| Treatment 7 vitamin D2 injections | $^{D}$0.07 ± 0.51 |
| Treatment 8 vitamin D3 injections | $^{ABC}$0.02 ±0.75 |
| L S D | 0.1156 |
| A significant level | 0.01 |

Different letters within a single column mean significant differences between transactions (P<0.01)

The first treatment (T1): Negative control treatment includes eggs not injected with vitamin or oil as negative control treatment. Treatment II (T2): Positive control treatment includes eggs injected with 100 microliters with corn oil as positive control treatment. Treatment 3: Eggs injected with 100 microliters include vitamin E per egg (15 IU/egg) vitamin E treatment. 20 IU/Egg Treatment Vitamin E.V .5 T: Eggs injected with 100 microliters include vitamin E per egg (25 IU/egg) vitamin E treatment. Eggs injected with 100 microliters include vitamin D3 per egg (100 IU/egg) vitamin D3.Treatment VII (7 T): Eggs injected with 100 microliters include vitamin D3 per egg (150 IU/egg) vitamin D3 treatment (8 T): Eggs with 100 microliters include vitamin D3 per egg (200 IU/IU).

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**The percentage of mortal embryos during the various stages of embryonic development:**

Table (2) for medium and late fetal deaths of vitamin injections during fetal development stages, The average rate of fetal death decreased morally (0.05 (P≤ for the sixth treatment vitamin D3 injection (0.02%) compared to negative and positive control and the seventh and eighth treatment (0.17. and 0.13, 0.14 and 0.13%), respectively, but we found no significant differences between the rest of the trial transactions, In terms of delayed fetal fatalities, a significant decrease (0.05 (P≤ for the sixth treatment was found to be vitamin D3 injections (0.08%) compared to the fourth, fifth and seventh transactions (0.19, 0.24 and 0.24%), respectively, while there were mathematical differences with negative and positive control (0.14 and 0.13%), respectively.

The low rate of moderate and late fetal death of vitamin injections during the fetal development stages of injected eggs may be due to the action of vitamin E mainly antioxidant against harmful free radicals on cellular membranes and the prevention of fat oxidation that has a role in growth processes (Ryu et al,.2005). This increases fat digestion (Brenes et al., 2008). This leads to increased use of fat to produce energy that improves fetal development and reduces fetal death (Schaal, 2008).

. The result of Al-Shamery and AL-Shuhaib (2015) found when the eggs of mothers of meat chicks were injected with vitamin E concentration of 1.5 mg/egg at the broad end of the egg a decrease in the proportion of premature fetal death after 14 days and late deaths after 18 days. For vitamin D3 injection treatment, this may be due to the fact that vitamin D3 converts metabolism into active metabolism, as vitamin 1.25 (OH) 2D3 D3 is the active hormonal form of vitamin cellular receptors that adjusts the rate of reproduction of targeted genes from key genes responsible for the biological response that regulates cell growth and reproduction (Adriana et al.), 2005). It also plays a role in supporting growth by stimulating cell proliferation and differentiation in the stages of growth and also increasing the activity of the cells of the bowel mucosa as well as vitamin D3 is associated with vitamin receptors found in the DNA molecule, which stimulates both ribose acid mRNA and protein synthesis.

. (Binderman and Weisman, 1983). This finding is consistent with Ibrahim et al. (2012) when 180 IU vitamin D3 was injected into the air chamber of the ostrich bird one week old during the incubation period, he found no fetal death in the treatment of vitamin D3 injections after injection, with the rate of fetal death after injection of vitamin D3 (0%) compared to the treatment of control and treatment of oil injections, in which the rate of post-injection fatalities (30 and 50%). Fatemi and his colleagues (2020) disagreed when eggs were injected with vitamin D3 on the eighth day of incubation, a significant difference with the treatment of control.

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**. Table (2) The effect of vitamin E and D3 injections in local chicken eggs on the proportion of fetal fatalities (%) during the different stages of embryonic development (average ± standard error)**

| Treatment | | )Standard error± averages( Percentage of fetal deaths during hatching period | . |
|---|---|---|---|
| | First week | Second week | Third week |
| Treatment 1 Control | 0.02 ±0.10 | $^{AB}$0.00±0.017 | $^{BC}$0.00±0.14 |
| Treatment 2 corn oil injections | 0.12 ± 0.11 | $^{AB}$0.02 ±0.013 | $^{BC}$0.11±14 .0 |
| Treatment 3 vitamin E1 injections | 0.02 ± 0.11 | $^{BC}$0.04±0.06 | $^{BC}$0.12 ±0.13 |
| Treatment 4 vitamin E2 injections | 0.02 ± 0.10 | $^{ABC}$0.05 ±0.09 | $^{AB}$0.15 ±0.19 |
| Treatment 5 vitamin E3 injections | 0.07 ± 0.10 | $^{BC}$0.03± 0.06 | $^{A}$0.12 ±0.24 |
| Treatment 6 vitamin D1 injections | 0.04 ± 0.11 | $^{C}$0.05±0.02 | $^{C}$0.10 ±0.08 |
| Treatment 7 vitamin D2 injections | 0.04 ± 0.11 | $^{B}$0.04± 0.14 | $^{B}$0.12 ±0.23 |
| Treatment 8 vitamin D3 injections | 0.02 ± 0.10 | $^{B}$0.05±0.13 | $^{BC}$0.17 ±0.11 |
| L S D | **N.S** | 0.0692 | 0.0697 |
| A significant level | N.S | 0.02 | 0.01 |

Different letters within a single column mean moral differences between transactions (P<0.01)

The first treatment (T1): Negative control treatment includes eggs not injected with vitamin or oil as negative control treatment. Treatment II (T2): Positive control treatment includes eggs injected with 100 microliters with corn oil as positive control treatment. Treatment 3: Eggs injected with 100 microliters include vitamin E per egg (15 IU/egg) vitamin E treatment. 20 IU/Egg Treatment Vitamin E.V .5 T: Eggs injected with 100 microliters include vitamin E per egg (25 IU/egg) vitamin E treatment. Eggs injected with 100 microliters include vitamin D3 per egg (100 IU/egg) vitamin D3.Treatment VII (7 T): Eggs injected with 100 microliters include vitamin D3 per egg (150 IU/egg) vitamin D3 treatment (8 T): Eggs with 100 microliters include vitamin D3 per egg (200 IU/IU).

## Conclusion

In the summary of the study we find that the process of injecting eggs with vitamin E and D3 in the air sac of chicken eggs is a significant decrease in the proportion of fetal death during

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fetal development stages and a significant improvement in the hatching rate of vitamin E treatment when injecting 15 IU/egg and vitamin D3 treatment when injecting 100 IU/egg.

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