IMPACT OF VITAMIN E AND SELENIUM TREATMENT IN-ovo AND AFTER HATCHING OF BROILER
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ABSTRACT
This study was aimed to investigate the effect of in-ovo injection with vitamin E and selenium during the incubation and post-hatching period. 360 fertilized eggs of Ross 308 broiler breeders were incubated, then distributed on 10th day into three groups (120 eggs/group). 1st group: uninjected eggs, 2nd group: eggs were injected with 0.1 ml deionized water/egg in the chorioallantoic cavity, and 3rd group: eggs were injected with 0.1 ml/egg of Introvit-E-Selen in chorioallantoic cavity. After hatching, 270 chicks were randomly distributed into six groups, and reared until 42 days aged as follows: birds in 1st, 3rd and 5th groups drinks tap water only (free from any addition), while birds of other groups, were reared on drinking water supplemented with 12.5 mg vitamin E and 500 µg sodium selenite/liter water. Results showed a significant increase in hemoglobin, lymphocytes% and serum globulin, and a significant decrease in packed cell volume%, heterophils%, heterophils/lymphocytes ratio, glucose, cholesterol, triglycerides and albumin concentration as compared with control. In conclusion, vitamin E and selenium have enhanced some immunological aspects and reduced stress, as well as a number of hematological parameters of broiler chicks, also, the continuity of Vit.E-Selenium addition led to continuous improvement of physiological parameters, and when it stopped, the values of those parameters were retracted.

Keywords: Egg injection, sodium selenite, inorganic selenium, stress.

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INTRODUCTION
The technique of the in-ovo injection is one of the early feeding techniques for the avian embryos (25) for healthy chicks with high production capacity by improving the physiological, immunological and productive chicks performance (20, 39, 52). Now the in-ovo injection techniques is widely applied with various elements such as carbohydrates, proteins, amino acids and vitamins (1, 8, 10, 27), as well as vaccines (31) and antioxidants (32, 37), as research has shown that early chicken embryos feeding will enhance their growth before and after hatching (30, 6), by improving their physiological, biochemical, immunological state and the antioxidant status (47) that related to embryonic development (52). Dietary supplementation (as antioxidants) is an essential factor in poultry nutrition, some of them are added to the diet such as Vit. E, carotenes and selenium (3), others can be produced in the bird's body such as Vit. C and CoQ (48). The dietary supplementation could be given starting from the egg, by eggs feeding (In-Ovo Injection) with substances that enhance the first defense line against oxidative stress, or by enhancing the Co-factors that enhance the first enzymatic defense line such as Se, Zn, and Mn, which are important for the enzymatic activity such as SOD and GSH-Px (44). Vitamin E is one of the essential vitamins for human and animals (46), due to its inability to produce it (13, 33), and it has an important role in various biological and physiological processes, including its antioxidant activity (41) which enhance the antioxidant status in the body (48), as it increases the immunological response as it stimulates the immune system (2, 28, 36) and it increases the immunoglobulin in broilers, turkeys and ducks (40), so it protects the body from stress (26). Vitamin E as known is an antioxidant that is found mainly in the cell membranes, and it has an important role in scavenging the free oxygen radicals (36), and prevents cell membrane damage (43), by reducing or preventing the lipid peroxidation that resulting from oxidative stress (22). Also, it significantly improves the hatchability rate of fertilized eggs and the feed conversion rate of hatched chicks as well as their weight (40). As for selenium, it is an antioxidant (48), and one of the essential nutrients for the growing and development of humans and animals, especially poultry (19). It was discovered by the Swedish chemical scientist Berzelius in 1817 as an essential component of cellular function (12), and for many metabolic processes (45), so it is usually added to the rations (18). Selenium has an important role in maintaining the sparing effect of Vit. E (43), and such an effect of selenium was also reported by Tufarelli et al. (51) who stated that the selenium maintains the level of Vit. E in the eggs. Therefore, the objective of this study is to evaluate the effect of vitamin E and selenium during embryonic development in-ovo and post-hatching, and then follow-up the effect of the continuity or discontinuity (stoppage) of the treatment during rearing the chicks until 42 days age, on the physiological and productive performance of broilers.

MATERIALS AND METHODS
Three hundred sixty fertilized eggs of Ross 308 broiler breeders were randomly distributed into 3 groups (120 eggs/group with 4 replicates: 30 eggs/replicate), then eggs were incubated in a Turkish incubator (Cimuka 800 eggs), at 37.7°C and 75% relative humidity, and the eggs were turned every 4 hours up to 18 days aged. On the 10th day of the incubation, the eggs were candled to verify the embryonic growth and to perform in-ovo injection in the chooroallantoic cavity by automatic medical syringe (gauge 30), the injection site puncture closed by nail polish, the eggs returns immediately into the incubator until hatching. The treatment was as follows:

1st group (negative control): 120 fertilized eggs that were non injected with any substance.
2nd group (positive control): 120 fertilized eggs were injected with 0.1 ml deionized water/egg.
3rd group (Vit. E-Se group): 120 fertilized eggs were injected with 0.1 ml/ egg of Introvit-E-Selen* (5 mg vitamin E and 50 µg sodium selenite/egg).

*(Introvit-E-Selen manufactured by Interchemie / Holland, each ml contains 50 mg vitamin E and 0.5 mg sodium selenite).

On the day of hatching, the hatched chicks were weighed, and the hatchability% and embryonic mortality% were calculated, then
six of hatched chicks were slaughtered from each group and the blood samples were collected from it directly in two types of tubes: 1st tube with anticoagulant (EDTA) for determining the hemoglobin concentration (Hb), packed cell volume (PCV%), and differential leukocyte count (DLC), and the 2nd tube without anticoagulant to collect the serum which preserved at (-20 °C) until the biochemical tests performing to estimating the blood glucose concentration, total cholesterol, triglycerides, total protein, albumin, globulin by using Biosystems kits (Spanish made). The remaining hatched chicks (270 chicks) were randomly distributed into 6 groups, each group is 45 chicks with 3 replicates, each replicate 15 chicks, and reared until 42 days age. The feed and water were allowed ad libitum, and the treatments were as follows:

T1: Chicks came from negative control group, reared on standard ration and tap water.

T2: Chicks came from negative control group, reared on standard ration and tap water supplemented with Vitamin E-S Forte** (12.5 mg vitamin E and 500 µg sodium selenite/liter water).

T3: Chicks came from positive control group, reared on standard ration and tap water.

T4: Chicks came from positive control group, reared on standard ration and tap water supplemented with Vitamin E-S Forte** (12.5 mg vitamin E and 500 µg sodium selenite/liter water).

** (Vitamin E-S Forte manufactured by Vapco Co., Jordan, each gm contains 50 mg Vitamin E and 2 mg Sodium Selenite).

On the 42 days of age, the birds were weighed, then six of birds were slaughtered from each group, and the blood samples were taken as in the first stage of this study for the hematological and biochemical tests.

### Statistical Analysis

All data were analyzed by Statistical Analysis Statics program (7), and were analyzed using one-way analysis of variance. The differences between the means were determined with Duncan's Multiple Range test at (P≤0.05) (16).

### RESULTS AND DISCUSSION

From Table 1 we note that the injection of Introvit-E-Selen in eggs had no negative effects on the growth and embryonic development, and they had no effect on the hatchability%, embryonic mortality% and hatched chicks' weight in the T3 group (Vit. E-Se group) compared with the control groups (T1 and T2).

### Table 1. Mean (±SE) effect of Introvit-E-Selen in-ovo injection on hatchability, embryonic mortality and hatched chicks' weight.

| Treatments          | Hatchability (%) | Parameters Embryonic mortality (%) | Hatched chicks' weight (gm) |
|---------------------|------------------|-----------------------------------|-----------------------------|
| T1                  | 88.89±1.57 a     | 11.11±1.57 a                      | 43.52±0.52 a                |
| T2                  | 86.67±2.72 a     | 13.33±2.72 a                      | 42.78±0.24 a                |
| T3                  | 88.89±1.57 a     | 11.11±1.57 a                      | 42.73±0.17 a                |

Different letters in each column mean significant differences at (P≤0.05).

T1: Negative control group (non-injected eggs).

T2: Positive control group (eggs injected with 0.1 ml deionized water/egg).

T3: Vit. E-Se group (eggs injected with 0.1 ml Introvit-E-Selen/egg).

Results were in consistent with Goel et al. (21) who recorded that in-ovo injection had no passive effect on hatchability. Also, agreed with Babacanoğlu et al. (9) when they reported that the hatchability% and embryonic mortality% of broilers were not affected by vitamin E injection. While our results were not in agreement with Salary et al. (36) who found that vitamin E increased hatchability compared with the un.injected group, but we agreed with him when he said that weight of the hatched chick was not affected. This may be due to the that vitamin E and selenium have antioxidant properties that helped to preserve the embryonic internal environment despite the hatching stress (49). Results in Table 2 showed that the supplementation of vitamin E-S (injection or/and addition) did not affect the body weight gain, feed consumption, feed conversion ratio and final body weight of broilers at 42 days aged. Our results agreed with Ibrahim et al. (24) when he said that the
selenium did not lead to a significant increase in the feed consumption compared with the control group, on the other hand, results not agreed with his results when he records that the selenium led to an increase in the body weight of the Hubbard broilers. Also, results agreed with Deniz (14) and Downs et al. (15) when they record that the body weight and feed conversion ratio were not affected while disagreeing with the findings of the researchers El-Said and Tag-El-Din (17) when they mentioned that the ovo injection with selenium (SeNPs / 20 ppb or 40 ppb) had an effect on body weight and weight gain compared with the control group.

Table 2. Mean (±SE) effect of vitamin E-S forte supplementation on body weight, weight gain, feed consumptions, and feed conversion ratio of broilers for 1-42 days

| Treatments | Hatched chicks' weight (gm) | Body weight gain (gm) | Feed consumption (gm) | Feed conversion ratio (gm) | Final body weight (At 42 day age) (gm) |
|------------|-----------------------------|-----------------------|-----------------------|---------------------------|----------------------------------------|
| T1         | 43.18 ±0.19 a               | 2877.32 ±50.52 a      | 5353.09 ±18.65 a      | 1.86 ±0.04 a              | 2920.50 ±50.36 a                      |
| T2         | 42.94 ±0.21 a               | 2960.64 ±55.00 a      | 5314.35 ±24.52 a      | 1.80 ±0.03 a              | 3003.58 ±54.84 a                      |
| T3         | 42.82 ±0.32 a               | 2894.12 ±46.53 a      | 5305.81 ±52.82 a      | 1.83 ±0.04 a              | 2936.93 ±46.68 a                      |
| T4         | 42.47 ±0.51 a               | 2885.80 ±44.55 a      | 5234.56 ±73.42 a      | 1.81 ±0.01 a              | 2928.27 ±44.58 a                      |
| T5         | 42.27 ±0.38 a               | 2953.46 ±35.64 a      | 5243.06 ±73.32 a      | 1.78 ±0.03 a              | 2995.73 ±35.95 a                      |
| T6         | 42.12 ±0.22 a               | 2841.45 ±33.21 a      | 5250.71 ±27.49 a      | 1.85 ±0.02 a              | 2883.57 ±33.27 a                      |

Different letters in each column mean significant differences at (P≤0.05).

T1: Chicks came from negative control group, reared on standard ration and tap water.
T2: Chicks came from negative control group, reared on standard ration and tap water supplemented with Vitamin E-S Forte (12.5 mg vitamin E and 500 µg sodium selenite/liter water).
T3: Chicks came from positive control group, reared on standard ration and tap water.
T4: Chicks came from positive control group, reared on standard ration and tap water supplemented with Vitamin E-S Forte (12.5 mg vitamin E and 500 µg sodium selenite/liter water).
T5: Chicks came from Vit. E-Se group, reared on standard ration and tap water.
T6: Chicks came from Vit. E-Se group, reared on standard ration and tap water supplemented with Vitamin E-S Forte (12.5 mg vitamin E and 500 µg sodium selenite/liter water).

Data in Table 3 declare that the ovo-injection of Vit. E-Se and/or their addition was significantly affect Hb and PCV% values. It was observed that the hemoglobin concentration was significantly higher in group 6, while the percentage of PCV was significantly lower in group 6 and 2 compared with the other groups. This is in agreement with Tayeb and Qader (50), Ali and Al-Hassani (5) and Al-Hassani et al., (4) who said that the addition of selenium and Vit. E lead to significant changes in the hematological parameters of broilers. Also, it agreed with Raza et al. (34), while the results of the current study was not in agreement with Ibrahim et al. (24) who said that the selenium injection did not lead to significant changes in the hematological parameters of broilers. Also, it not agreed with Boostani et al. (11) who confirmed a non significant differences in the values of Hb and PCV.

Table 3. Mean (±SE) effect of vitamin E-S forte supplementation on Hb and PCV% of broiler at 42 days aged

| Treatments | Hb g/dl | PCV % |
|------------|---------|-------|
| T1         | 11.90 ±0.29 bc | 31.00 ±0.45 a |
| T2         | 12.28 ±0.14 ab  | 29.20 ±0.58 b  |
| T3         | 12.06 ±0.11 bc  | 29.20 ±0.58 ab |
| T4         | 11.54 ±0.14 c   | 30.40 ±0.68 a  |
| T5         | 11.85 ±0.33 bc  | 30.60 ±0.51 a  |
| T6         | 12.89 ±0.24 a   | 29.40 ±0.51 b  |

Different letters in each column mean significant differences at (P≤0.05).
T1-T6: as in Table 2.
Results in Tables 4 and 5 showed that the in-ovo injection of Vit. E-Se and/or their addition causes a significant increase in the lymphocyte% and a significant decrease in heterophils and H/L ratio. These results are in agreement with what Raza et al. (34) found, also, the results agreed with Selim et al. (38) when they said that administration of selenium will increase the lymphocyte counts and decreased the H/L ratio in broilers while disagreeing with Habibian et al. (23) when they reported that heterophils, lymphocytes and the H/L ratio were not affected by Se treatments. The results may be due to the antioxidant capacity of vitamin E and selenium which are consider the major antioxidants in avian species (42), which enhance also the antioxidant status of eggs (43), or because of the protective effect of vitamin E against oxidative stress by enhancing the antioxidant status and protecting the cell from the oxidation, especially the phospholipids of the cell membrane from the reactive oxygen species (37).

Table 4. Mean (±SE) effect of Introvit-E-Selen in-ovo injection on differential leukocyte count of broiler chicks on the day of hatching.

| Treatments | Lymphocytes (%) | Heterophils (%) | Monocytes (%) | Eosinophils (%) | Basophils (%) | H/L Ratio |
|------------|-----------------|-----------------|--------------|----------------|--------------|-----------|
| T1         | 68.00 ±0.58 b   | 26.67 ±0.33 a   | 2.33 ±0.67 a | 1.67 ±0.33 a   | 1.33 ±0.33 a | 0.39 ±0.01 a|
| T2         | 67.00 ±0.58 b   | 27.00 ±0.58 a   | 2.67 ±0.33 a | 2.00 ±0.58 a   | 1.33 ±0.33 a | 0.40 ±0.01 a|
| T3         | 71.33 ±0.88 a   | 22.67 ±1.20 b   | 2.33 ±0.33 a | 1.67 ±0.33 a   | 2.00 ±0.00 a | 0.32 ±0.02 b|

- Different letters in each column mean significant differences at (P≤0.05).
- T1-T3 : as in Table 1

Table 5. Mean (±SE) effect of vitamin E-S forte supplementation on differential leukocyte count of broiler at 42 days aged

| Treatments | Lymphocytes (%) | Heterophils (%) | Monocytes (%) | Eosinophils (%) | Basophils (%) | H/L Ratio |
|------------|-----------------|-----------------|--------------|----------------|--------------|-----------|
| T1         | 76.60 ±1.21 b   | 17.60 ±1.08 a   | 1.80 ±0.37 a | 2.20 ±0.37 a   | 1.80 ±0.20 a | 0.23 ±0.02 a|
| T2         | 79.60 ±0.51 ab  | 15.40 ±0.51 ab  | 1.80 ±0.37 a | 1.80 ±0.37 a   | 1.40 ±0.25 a | 0.19 ±0.01 ab|
| T3         | 78.40 ±1.12 b   | 17.20 ±0.97 a   | 1.20 ±0.20 a | 1.20 ±0.20 a   | 2.00 ±0.32 a | 0.22 ±0.02 a|
| T4         | 79.80 ±1.11 ab  | 15.80 ±0.86 ab  | 1.80 ±0.58 a | 1.20 ±0.20 a   | 1.40 ±0.40 a | 0.20 ±0.01 ab|
| T5         | 78.20 ±1.24 b   | 17.60 ±1.03 a   | 1.80 ±0.49 a | 1.20 ±0.37 a   | 1.20 ±0.20 a | 0.23 ±0.02 a|
| T6         | 82.20 ±1.43 a   | 13.00 ±1.76 b   | 1.40 ±0.24 a | 1.60 ±0.24 a   | 1.80 ±0.20 a | 0.16 ±0.02 b|

- Different letters in each column mean significant differences at (P≤0.05).
- T1-T6 : as in Table 2

It can be seen from the data of Table 6, that the continued addition of selenium led to decreasing in glucose and cholesterol in the blood until it reached the significant level in group T2, T4 and T6, while the triglycerides levels decreased and reached the significant level in group T6 compared with group T1. Our results agreed with the researchers El-Said and Tag-El-Din (17) when they inject the eggs with selenium and led to a decrease in glucose, cholesterol and triglycerides. Also, in agreement with Sahin et al. (35) when they gave the vitamin E and led to a significant decreasing in blood glucose, cholesterol and triglycerides. While our study did not agree with Yang et al. (53) which found that cholesterol and triglycerides were not significantly affected by the addition of selenium. The total protein was not significantly affected by adding vitamin E-S forte, this is agreed with Ibrahim et al. (24) which who stated that the addition of selenium had no significant effect on the total protein level, while they record the serum albumin level decreased and the serum globulin level increased. However, our study is not agreed with Mohamed et al. (29) when they said there was an increase in total protein, and the albumin was not affected.
Table 6. Mean (±SE) effect of vitamin E-S forte supplementation on some blood parameters of broiler at 42 days aged

| Treatments | Parameters | Lymphocytes % | Heterophils % | Monocytes % | Eosinophils % | Basophils % | H/L Ratio |
|------------|------------|---------------|---------------|-------------|---------------|-------------|-----------|
| T1         |            | 76.60 ±1.21 b | 17.60 ±1.08 a | 1.80 ±0.37 a | 2.20 ±0.37 a  | 1.80 ±0.20 a | 0.23 ±0.02 a |
| T2         |            | 79.60 ±0.51 ab| 15.40 ±0.51 ab| 1.80 ±0.37 a | 1.80 ±0.37 a  | 1.40 ±0.25 a | 0.19 ±0.01 ab |
| T3         |            | 78.40 ±1.12 b | 17.20 ±0.97 a | 1.20 ±0.20 a | 1.20 ±0.20 a  | 2.00 ±0.32 a | 0.22 ±0.02 a |
| T4         |            | 79.80 ±1.11 ab| 15.80 ±0.86 ab| 1.80 ±0.58 a | 1.20 ±0.20 a  | 1.40 ±0.40 a | 0.20 ±0.01 ab |
| T5         |            | 78.20 ±1.24 b | 17.60 ±1.03 a | 1.80 ±0.49 a | 1.20 ±0.37 a  | 1.20 ±0.20 a | 0.23 ±0.02 a |
| T6         |            | 82.20 ±1.43 a | 13.00 ±1.76 b | 1.40 ±0.24 a | 1.60 ±0.24 a  | 1.80 ±0.20 a | 0.16 ±0.02 b |

- Different letters in each column mean significant differences at (P<0.05).
- T1-T6: as in Table 2

CONCLUSION
We found that selenium and vitamin E at doses given in our research (ovo injection or adding to drinking water) did not lead to negative effects in physiological and productive performance during embryonic development and after hatching, no contrary it leads to significant improvement in the stress index (H/L), a significant decrease in the serum glucose and lipid profile, and an improvement in the body immunity status, and thus these doses may be recommended as in ovo injection or added to drinking water to improve the physiological, immunological status and productive efficiency of hatched chicks.

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CONFLICT OF INTEREST
The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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