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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تأثير المعاملة بفيتامين E والسيلينيوم أثناء حضانة البيض وبعد الفقس لفروج اللحم

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المستخلص

هدفت هذه الدراسة إلى معرفة تأثير حقن البيض بفيتامين هـ والسيلينيوم أثناء فترة الحضانة وبعد الفقس. حُضِنَت 360 بيضة مخصبة من أمهات فروج اللحم سلالة روس 308، ثم وزع البيض في اليوم العاشر من الحضانة في ثلاث مجموعات (120 بيضة/ مجموعة). المجموعة الأولى: بيض غير محقون، المجموعة الثانية: بيض محقون بـ 0,1 مل ماء منزوع الأيونات/ بيضة في التجويف المشيمي اللقانقي، المجموعة الثالثة: بيض محقون بـ 0,1 مل/بيضة من Introvit–E–Selen في التجويف المشيمي اللقانقي. بعد الفقس، وزع عشوائياً 270 فرخاً في ست مجموعات، وربيت الأفراخ حتى عمر 42 يوماً كالآتي: الطيور في المجموعة الأولى والثالثة والخامسة أعطيت ماء شرب اعتيادي (خالياً من أية إضافة)، بينما الطيور في المجموعات الأخرى أعطيت ماء شرب مضافاً إليه 12,5 ملغم فيتامين هـ و 500 مايكروغرام من سيلينيت الصوديوم/ لتر ماء. تبين من النتائج أن هنالك زيادة معنوية في تركيز الهيموكلوبين والخلايا اللمفاوية والكلوبيولين في الدم، وانخفاض معنوي في حجم خلايا الدم المرصوصة% ونسبة الخلايا المتغايرة ونسبة الخلايا المتغايرة/ الخلايا اللمفاوية وتركيز الكلوكوز والكولسترول والكليسيريدات الثلاثية والألبومين مقارنة مع مجموعة السيطرة. نستنتج من ذلك، أن فيتامين هـ والسيلينيوم قد عززا من بعض الجوانب المناعية وخفضا من حالة الإجهاد وعدد من المعايير الدموية لفروج اللحم، وأن استمرارية إعطاء فيتامين هـ والسيلينيوم أدى إلى التحسن المستمر للمعايير الفسلجية، وعندما توقف إعطاؤهما تراجعت قيم تلك المعايير.

الكلمات المفتاحية: حقن البيض، سيلينيت الصوديوم، السيلينيوم غير العضوي، الإجهاد.

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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 chorioallantoic 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:

T_1: Chicks came from negative control group, reared on standard ration and tap water.

T_2: 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).

T_3: Chicks came from positive control group, reared on standard ration and tap water.

T_4: 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).

T_5: Chicks came from Vit. E-Se group, reared on standard ration and tap water.

T_6: 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).

**(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 ($P \leq 0.05$) (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 T_3 group (Vit. E-Se group) compared with the control groups (T_1 and T_2).

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

Treatments	Parameters Hatchability (%)	Embryonic mortality (%)	Hatched chicks' weight (gm)
T_1	88.89 ±1.57 a	11.11 ±1.57 a	43.52 ±0.52 a
T_2	86.67 ±2.72 a	13.33 ±2.72 a	42.78 ±0.24 a
T_3	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).

T_1: Negative control group (non-injected eggs).

T_2: Positive control group (eggs injected with 0.1 ml deionized water/egg).

T_3: 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 uninjected 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	Parameters				
	Hatched chicks' weight (gm)	Body weight gain (gm)	Feed consumption (gm)	Feed conversion ratio (gm)	Final body weight (At 42 day age) (gm)
T_1	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
T_2	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
T_3	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
T_4	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
T_5	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
T_6	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).

T_1: Chicks came from negative control group, reared on standard ration and tap water.

T_2: 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).

T_3: Chicks came from positive control group, reared on standard ration and tap water.

T_4: 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).

T_5: Chicks came from Vit. E-Se group, reared on standard ration and tap water.

T_6: 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	Parameters	
	Hb g/dl	PCV %
T_1	11.90 ±0.29 bc	31.00 ±0.45 a
T_2	12.28 ±0.14 ab	29.20 ±0.58 b
T_3	12.06 ±0.11 bc	29.20 ±0.58 ab
T_4	11.54 ±0.14 c	30.40 ±0.68 a
T_5	11.85 ±0.33 bc	30.60 ±0.51 a
T_6	12.89 ±0.24 a	29.40 ±0.51 b

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

T_1-T_6 : 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	Parameters					
	Lymphocytes %	Heterophils %	Monocytes %	Eosinophils %	Basophils %	H/L Ratio
T_1	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
T_2	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
T_3	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).
- T_1-T_3 : 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	Parameters					
	Lymphocytes %	Heterophils %	Monocytes %	Eosinophils %	Basophils %	H/L Ratio
T_1	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
T_2	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
T_3	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
T_4	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
T_5	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
T_6	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).
- T_1-T_6 : 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 T_2, T_4 and T_6, while the triglycerides levels decreased and reached the significant level in group T_6 compared with group T_1. 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
T_1	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
T_2	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
T_3	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
T_4	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
T_5	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
T_6	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).

- T_1-T_6 : 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.

REFERENCES

1. Abdul-Lateif, K. M. and M. A. Al-Shamari. 2014. The effect of *in ovo* injection with different concentrations of vitamin a on some blood traits of broiler chicken. Iraqi Journal of Agricultural Science, 45(4) (Special Issue),376-383. https://www.iasj.net/iasj/download/3c5533a802da0985

2. Abdulwahid, M. T., A. H. Zahid, and M. J. Kadhum. 2016. Effect of vitamin e and cod liver oil supplement with bivalent oil based vaccine of newcastle disease and infectious bronchitis disease on immune response of the broilers. Iraqi Journal of Agricultural Science, 47(3),892-899.

3. Al-Hammed, S. A. M., M. H. Abdul-Abass, and S. K. Ahmed. 2015. Effect of adding different levels of Vit. E and rosemary leaves to the diet on broiler performance and carcass characteristic. Iraqi Journal of Agricultural Science, 46(1),21-26. https://www.iasj.net/iasj/download/66dda7746d7ed6aa

4. Al-Hassani, D. H., K. M. Abdul-Latif, and B. G. Al-Khatib. 2002. Effect of adding different levels of vitamin E with drinking water on some hematological characteristics of broilers. Iraqi Journal of Agricultural Science, 33(3),197-202. file:///C:/Documents%20and%20Settings/VET/Desktop/3332002.pdf

5. Ali, N. K. and D. H. Al-Hassani. 2020. Temperature and some blood traits response to organic and inorganic selenium added to the broiler diet reared at high temperatures. Iraqi Journal of Agricultural Science, 51(3),734-743.

6. Al-Shamery, N. J., and M. B. S. Al-Shuhaib. 2015. Effect of *in-ovo* injection of various nutrients on the hatchability, mortality ratio and weight of the broiler chickens. IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS), 8(2), 30-33. doi: 10.9790/2380-08123033

7. Anonymous. 2008. Statistical Analysis Systems. Software, User's Guide: Statistics, V. 9.2, 2nd ed., SAS Institute, Cary, North Carolina, USA

8. Araújo, I. C., M. B. Café, R. A. Noleto, J. M. Martins, C. J. Ulhoa, G. C. Guareshi, M. M. Reis and N. S. Leandro. 2019. Effect of vitamin E in ovo feeding to broiler embryos on hatchability, chick quality, oxidative state, and

performance. Poultry Science, 98(9), 3652-3661. https://doi.org/10.3382/ps/pey439
9. Babacanoğlu, E., M. R. Karageçili, and F. Karadaş. 2018. Effects of egg weight and in ovo injection of α-tocopherol on chick development, hatching performance, and lipid-soluble antioxidant concentrations in quail chick tissues. Archiv Fuer Tierzucht, 61(2),179. https://doi.org/10.5194/aab-61-179-2018
10. Bednarczyk M. 2016. Some aspects of poultry biotechnology: A review. The 4th International Scientific Conference "Animal Biotechnology". Slovak Journal of Animal Science,49(4),157-159
11. Boostani, A., A. A. Sadeghi, S. N. Mousavi, M. Chamani, and N. Kashan. 2015. Effects of organic, inorganic, and nano-Se on growth performance, antioxidant capacity, cellular and humoral immune responses in broiler chickens exposed to oxidative stress. Livestock Science, 178, 330-336
12. Boyd, R. 2011. Selenium stories. Nature chemistry, 3(7),570-570. https://doi.org/10.1038/nchem.1076
13. Chan, K. M., E. A. Decker, and C. Feustman. 1994. Endogenous skeletal muscle antioxidants. Critical Reviews in Food Science and Nutrition,34(4),403-426. https://doi.org/10.1080/10408399409527669
14. Deniz, G., S. S. Gezen, and I. I. Turkmen. 2005. Effects of two supplemental dietary selenium sources (mineral and organic) on broiler performance and drip-loss. Revue de médecine vétérinaire, 156(8/9), 423. https://www.revmedvet.com/2005/RMV156_423_426.pdf
15. Downs, K. M., J. B. Hess, and S. F. Bilgili. 2000. Selenium source effect on broiler carcass characteristics, meat quality and drip loss. Journal of Applied Animal Research, 18(1), 61-71
16. Duncan, D. B. 1955. Multiple range and multiple F-test. Biometric. 11: 1-42. https://doi.org/10.2307/3001478
17. El-Said, E. A. and T. H. Tag-El-Din. 2017. Effect of *in-ovo* injection with different levels of selenium nano-particles (SeNPs) on physiological, histological and immune responses and intestinal bacterial population in broiler chickens. International Egyptian Czech Conference for Nanotechnology Applications in Agriculture Sector (IECCNA). (Pp 1-16).
18. Etaki, A.O., M. A. AL Jader, and R. H. Absi. 2019. Comparison of the effect of organic and inorganic selenium on the quality of local quail eggs. Syrian Journal of Agricultural Research-SJAR 6(1),36-50.
19. Etaki, A.O., M. A. AL Jader, R. H. Absi and A. A. AL Marrawi. 2019. The effect of selenium on the digestion factors and their relationship to egg production chains of local quail (*Coturnix coturnix*). Syrian Journal of Agricultural Research – SJAR 6(2), 70-80. http://agri-research-journal.net/?p=2849
20. Gao, J., H. Lin, X. J. Wang, Z. G. Song, and H. C. Jiao. 2010. Vitamin E supplementation alleviates the oxidative stress induced by dexamethasone treatment and improves meat quality in broiler chickens. Poultry Science, 89(2), 318-327. https://doi.org/10.3382/ps.2009-00216
21. Goel, A., S. K. Bhanja, V. Pande, M. Mehra, and A. Mandal. 2013. Effects of in ovo administration of vitamins on post hatch-growth, immunocompetence and blood biochemical profiles of broiler chickens. Indian J Anim Sci, 83(9), 916-921.https://www.researchgate.net/publication/258255366
22. Gore, A. B., and M. A. Qureshi. 1997. Enhancement of humoral and cellular immunity by vitamin E after embryonic exposure. Poultry Science, 76(7), 984-991. https://doi.org/10.1093/ps/76.7.984
23. Habibian, M., S. Ghazi, M. M. Moeini, and A. Abdolmohammadi. 2014. Effects of dietary selenium and vitamin E on immune response and biological blood parameters of broilers reared under thermoneutral or heat stress conditions. International journal of biometeorology, 58(5), 741-752.
24. Ibrahim, N. S., E. M.Sabic, M. M. Wakwak, I. E. El-Wardany, Y. M. El-Homosany, and N. E. D. Mohammad. 2020. *In-ovo* and dietary supplementation of selenium nano-particles influence physiological responses, immunological status and performance of broiler chicks. Journal of Animal and Feed Sciences, 29(1), 46-58. https://doi.org/10.22358/jafs/118209/2020
25. Kadam, M. M., M. R. Barekatain, , S. K. Bhanja, , and P. A. Iji. 2013. Prospects of in

ovo feeding and nutrient supplementation for poultry: The science and commercial applications—A review. Journal of the Science of Food and Agriculture, 93(15), 3654-3661. https://doi.org/10.1002/jsfa.6301

26. Kaiser, M. G., S. S. Block, C. Ciraci, W. Fang, M. Sifri, and S. J. Lamont. 2012. Effects of dietary vitamin E type and level on lipopolysaccharide-induced cytokine mRNA expression in broiler chicks. Poultry Science, 91(8), 1893-1898. https://doi.org/10.3382/ps.2011-02116

27. Menati, J. K. and A. S. Ahmed. 2014. The effect of in ovo-probiotics injection on some productive performance of broilers. Iraqi Journal of Agricultural Science, 45(6),584-592.

28. Min, Y. N., Z. Y. Niu, T. T. Sun, Z. P. Wang, P. X. Jiao, B. B. Zi, D. L. Chen, F. Z. Tian, and F. Z. Liu. 2018. Vitamin E and vitamin C supplementation improves antioxidant status and immune function in oxidative-stressed breeder roosters by up-regulating expression of GSH-Px gene. Poultry Science, 97(4), 1238-1244. https://doi.org/10.3382/ps/pex417

29. Mohamed, H. S., Y. S. Rizk, A. E. Elslamony, A. A. Soliman, and A. F. Ebrahim. 2016. Study the relationship between selenium and heat shock proteins under heat stress for local sinai chickens strain. Egyptian Poultry Science Journal, 36(1), 337-354.

30. Ohta, Y., M. T. Kidd, and T. Ishibashi. 2001. Embryo growth and amino acid concentration profiles of broiler breeder eggs, embryos, and chicks after in ovo administration of amino acids. Poultry Science, 80(10), 1430-1436. https://doi.org/10.1093/ps/80.10.1430

31. Peebles, E. D. 2018. In ovo applications in poultry: a review. Poultry Science, 97(7), 2322-2338.

32. Perez, T. I., M. J. Zuidhof, R. A. Renema, J. M. Curtis, Y. Ren, and M. Betti. 2010. Effects of vitamin E and organic selenium on oxidative stability of ω-3 enriched dark chicken meat during cooking. Journal of Food Science, 75(2), T25-T34.

33. Puthpongsiriporn, U., S. E. Scheideler, J. L. Sell, and M. M. Beck. 2001. Effects of vitamin E and C supplementation on performance, in vitro lymphocyte proliferation, and antioxidant status of laying hens during heat stress. Poultry Science,80(8), 1190-1200. https://doi.org/10.1093/ps/80.8.1190

34. Raza, M., C. Deo, A. B. Mandal, P. Tyagi, and N. A. Mir. 2018. Effect of dietary supplementation of selenium and vitamin E on growth performance and hematology of broiler chicken under heat stress. Indian Journal of Poultry Science, 53 (2), 171-176. https://doi.org/10. 1007/s12011-015-0404-6

35. Sahin, N., K. Sahin, and O. Kucuk. 2001. Effects of vitamin E and vitamin A supplementation on performance, thyroid status, and serum concentrations of some metabolites and minerals in broilers reared under heat stress (32^{o} C). Veterinarni Medicina-Praha-, 46(11/12), 286-292. https://doi.org/10.17221/7894-VETMED

36. Salary, J., F. Sahebi-Ala, M. Kalantar, and H. R. H. Matin. 2014. In ovo injection of vitamin E on post-hatch immunological parameters and broiler chicken performance. Asian Pacific Journal of Tropical Biomedicine, 4, S616-S619.

37. Schaal, T. P. 2008. The Effects of in ovo Feeding of Fatty Acids and Antioxidants on Broiler Chicken Hatchability and Chick Tissue Lipids. [dissertation]. Corvallis: Oregon State University, University Honors College. http://hdl.handle.net/1957/8770

38. Selim, N. A., N. L. Radwan, S. F. Youssef, T. S. Eldin, and S. A. Elwafa. 2015. Effect of inclusion inorganic, organic or nano selenium forms in broiler diets on: 2-Physiological, immunological and toxicity statuses of broiler chicks. International Journal of Poultry Science, 14(3), 144.

39. Selim, N. A., S. F. Youssef, A. F. Abdel-Salam, and Sh. A. Nada. 2013. Evaluations of some natural antioxidant sources in broiler diets: 1-effect on growth, physiological and immunological performance of broiler chicks.International Journal of Poultry Science, 12, 561-571.

40. Selim, S. A., K. M. Gaafar, and S. S. El-ballal. 2012. Influence of *in-ovo* administration with vitamin E and ascorbic acid on the performance of Muscovy ducks. Emirates Journal of Food and Agriculture. 24(3):264–271

41. Singh, C. D., A. K. Gohain, R. Bhuyan, C. P. Dixit, and S. Haloi. 2020. Effect of supplementation of certain antioxidants (Vitamin E, vitamin C and selenium) on the growth performance of broiler chicken during heat stress. Journal of Entomology and Zoology Studies; 8(6):2066-2071. https://www.entomoljournal.com/archives/2020/vol8issue6/PartAB/8-6-39-911.pdf

42 Surai P. F. 2020. Antioxidants in Poultry Nutrition and Reproduction: An Update. Antioxidants (Basel, Switzerland), 9(2),105.https://dx.doi.org/10.3390%2Fantiox9020105

43. Surai, P. F. 2000. Effect of selenium and vitamin E content of the maternal diet on the antioxidant system of the yolk and the developing chick. British poultry Science, 41(2), 235-243. https://doi.org/ 10.1080/ 713654909

44. Surai, P. F. 2002. Natural Antioxidants in Avian Nutrition and Reproduction. Nottingham University Press, Nottingham, UK. (Pp. 5-9

45. Surai, P. F. 2006. Selenium in Nutrition and Health. Nottingham: Nottingham University Press, Nottingham, UK. https://doi.org/10.1093/ajcn/86.1.270

46. Surai, P. F. 2007. Natural antioxidants in poultry nutrition: new developments. In Proceedings of the 16th European symposium on poultry nutrition (Pp. 26-30). World Poultry Science Association.

47. Surai, P. F. 2017. Antioxidant defences: Food for thoughts. EC Nutrition, 10, 65-66.

48. Surai, P. F., and I. I. Kochish. 2019. Nutritional modulation of the antioxidant capacities in poultry: The case of selenium. Poultry Science, 98(10), 4231-4239. https://doi.org/10.3382/ps/pey406

49. Surai, P. F., V. I. Fisinin, and F. Karadas. 2016. Antioxidant systems in chick embryo development. Part 1. Vitamin E, carotenoids and selenium. Animal Nutrition, 2(1), 1-11

50. Tayeb, I. and G. Qader. 2012. Effect of feed supplementation of selenium and vitamin E on production performance and some hematological parameters of broiler. Tarim ve Doga Dergisi, 15(3), 46. https://dergipark.org.tr/en/download/article-file/212021

51. Tufarelli, V., E. Ceci, and V. Laudadio, 2016. 2-Hydroxy-4-methylselenobutanoic acid as new organic selenium dietary supplement to produce selenium-enriched eggs. Biological trace element research, 171(2), 453-458. https://doi.org/10.1007/s12011-015-0548-4

52. Uni, Z., and R. P. Ferket. 2004. Methods for early nutrition and their potential. World's Poultry Science Journal, 60(1), 101-111. https://doi.org/10.1079/WPS20038

53. Yang, Y. R., F. C. Meng, P. Wang, Y. B. Jiang, Q. Q. Yin, J. Chang, R. Y. Zuo, Q. H. Zheng, and J. X. Liu. 2012. Effect of organic and inorganic selenium supplementation on growth performance, meat quality and antioxidant property of broilers. African Journal of Biotechnology, 11(12), 3031-3036. https://www.ajol.info/index.php/ajb/article/view/100694