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The Effects of Dietary Vitamin E and Organic Selenium on the Levels of Some Bioelements in Tissues of Laying Hens*

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Absract: This experiment was conducted to evaluate the effects of dietary vitamin E (α-tocopherol acetate), organic selenium (selenomethionine) and their combination on some bioelements in some tissues of laying hens. Ninety-six white Lohman LSL laying hens aged of 24 weeks were randomly divided into 4 equal groups. The groups were fed with the diets consisting of basal diet (Control), basal diet + 250 mg/kg Vit-E (Trial-I), basal diet + 0.9mg/kg Se (Trial-II) and basal diet + 250 mg/kg Vit-E + 0,9 mg/kg Se (Trial-III), respectively. At the end of the experiment, 12 laying hens from each group were sacrificed, and aforementioned tissues were collected and stored at -20 ºC until analyzes. In tissue samples bioelements levels were determined by ICP-MS. It was found out that when the treatment and control groups were compared in terms of bioelements in some important tissues while Se, Zn and Fe levels increased ($P<0.05$) and the levels of Cu decreased in the tissues of treatment groups ($P<0.05$). In addition, the levels of Ca and Mg were not affected by treatment in present study. Organic selenium and vitamin E, consideration of antagonist and synergistic effects of bioelement levels changes in tissues.

Keywords: Bioelements, Laying hen, Organic selenium, Tissue samples, Vitamin E.

Diyetsel Vitamin E ve Organik Selenyumun Yumurta Tavuğu dokularındaki Bazı Biyoelement Düzeyleri Üzerine Etkileri

Özet: Bu çalışma, diyetsel vitamini E (a-tokoferol asetat), organik selenyum (selenomethionine) ve bunların kombinasyonlarının yumurta tavuklarının bazı dokularında bazı biyoelementlerin düzeyleri üzerine etkilerini değerlendirmek amacıyla yapıldı. Çalışmada, 96 adet beyaz Lohman yumurta tavuğu, eşit sayıda 4 gruba ayrıldı. Gruplar sırasıyla bazal yem (Kontrol), bazal yem + 250 mg/kg Vit-E (D-I), bazal yem + 0.9 mg/kg Se (DII) ve bazal yem + 250 mg/kg Vit-E+ 0.9 mg/ kg Se (D-III) içeren rasyonlarla beslendi. Deneme sonunda, her gruptan 12 hayvan kesildi, belirtilen dokular alındı, analiz edilinceye kadar -20 ºC'de saklandı ve biyoelement düzeyleri ICP-MS ile analiz edildi. Deneme gruplarının dokularındaki Se, Zn ve Fe düzeyleri kontrol grubuna göre önemli derecede artarken ($P<0.05$), Cu düzeylerinin azaldığı ($P<0.05$), Ca ve Mg düzeylerinin ise muameleden etkilenmediği bulundu. Organik selenyumun ve vitamin E'nin, dokularda biyoelement düzeylerindeki değişmelerin oluşturacağı antagonist ve sinerjik etkilerinin dikkate alınması önerilmektedir.

Anahtar Kelimeler: Biyoelementler, Doku örnekleri, E vitamini, Organik selenyum, Yumurta tavuğu.

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INTRODUCTION

Bioelements play a role in many important physiological functions such as vitamin synthesis in organism, hormone production, and enzyme activity, regulation of cell osmotic pressure, tissue synthesis, energy production and growth (1,2). The levels of minerals in the body are affected by stress levels, species, race, age, gender, age of somatic development, pregnancy, lactation, disease, chemical form, interaction with other nutrients, rational quantities and other minerals (3,4). Bioelements increase resistance to diseases in living beings and deficiencies and excesses cause serious health problems (5,6). Selenium is the cofactor of many enzymes that play a role, especially glutathione peroxidase, functions of nucleic acids, synthesis of prostaglandins and metabolism of essential fatty acids and an antioxidant that nerves the immune system of hens and protects the membranes (7-12). The amount of Se in vegetable and animal products shows a wide variation and the amount of Se in plants is influenced by the amount of selenium in the feed sources used while the amount of Se in animal foods is influenced by the amount of selenium in the animal sources (13). Functions of vitamin E in the body; preventing free radicals from entering the cell membrane and acquiring DNA management therefrom, providing lipid peroxidation (7,14), by decreasing the oxidation of vitamin A and carotene, to protect the biological systems in the body and to reduce the loss of Ca in the bone caused by oxidizing agents (15). Vitamin E has an increasingly important role in increasing biological antioxidant and reproductive power in the poultry diet, increasing resistance to infectious mechanisms, resistance to infectious and metabolic diseases, increased meat quality, meat colour effect and shelf life (16). Urso et al. (17) reported that hatchability of the eggs of breeders fed with diet inluding 120 mg vitamin E/kg feed was higher than those fed with diet containing 30 mg vitamin E/kg of feed. Most of the earlier studies report that dietary vitamin E supplementation of a balanced poultry ration significantly supports reproductive functions, including semen volume, sperm concentration, sperm viability, sperm motility, and sperm capacity, in avian species (14,18). The vitamin E is getting substantial interest in poultry nutrition due to its key-role as a dietary antioxidant to prevent oxidative stress (19). Vitamin E is a well-documented fat-soluble antioxidant and has been shown to inhibit free radical-induced damage to sensitive cell membranes (14,20). Vitamin E is supplemented to the diet to maintain and enhance performance in layers, broilers, broiler breeders, and turkey (21,22). The results obtained from the earlier studies varied depending upon the level and duration of feeding diets supplemented with vitamin E, genetic stocks, age, assessment criteria and stress conditions (20,21). The aim of this study was to investigate the effects of vitamin E, organic selenium and their combination added into diets of laying hens on the bioelement levels of different tissues.

MATERIALS and METHODS

The Research Animal Ethic Committee of Atatürk University approved all procedures under this experimental protocol (dated 31.07.2017 and numbered 36643897-128). A total of 96 White Lohman hens aged 24 weeks were randomly assigned to one of the four experimental diets for a periods of 12 weeks (n=24). Within a given group, 6 subgroups of 4 hens each were constituted and hens were housed in 50x46x46 cm^3cages. Hens of the group-I served as control and received the basal diet (2770 kcal/kg ME and 17% crude protein). Whereas other groups (T-II, T-III, T-IV) were fed with 3 different rations consisting of basal diet + 250 mg/kg vitamin E (Vit-E, α-tocopherol acetate) (trial-II), basal diet + 0.9 mg/kg selenium (selenomethionine) (Trial-III) and basal diet + 250 mg/kg Vit-E + 0.9 mg/kg Se (Trial-IV), respectively. Experiment lasted for 12 weeks. Diet and water were provided as ad libitum (Table I). At

the end of the study, 12 hens from each group were sacrificed and aforementioned tissues were collected and stored at -20 ºC until analyzes. In tissue samples, bioelement levels were analyzed by ICP-MS (Perkin-Elmer, Optima 2100 DV, ICP / OES, Shelton, CT 06484-4794, USA). Data obtained from the experiment were analyzed using the SPSS Statistics 17.0 program. Statistical significance and significance levels were determined by the "One-way analysis of variance (ANOVA)" test, and $P<0.05$ was considered significant. Duncan test was applied for multiple comparisons.

Table 1. Ingredients and nutrient levels of the rations used in the experiment.
Tablo 1. Araştırmada kullanılan rasyonların besin madde kompozisyonları ve bileşimleri.

Raw Materials	Vitamin E and Se (mg / kg)			
	Control	Trial-I	Trial-II	Trial-III
Vitamin E (α-tocopherol acetate) (mg/kg)	------	250	-------	250
Selenium (mg/kg) (selenomethionine)	-------	-------	0,9	0,9
Wheat Bran	8.00	8.00	8.00	8.00
Corn	51.81	51.81	51.81	51.81
Soybean Meal	17.13	17.13	17.13	17.13
Full Fat Soy	1.65	1.65	1.65	1.65
Sunflower Meal	7.50	7.50	7.50	7.50
Corn Gluten	2.04	2.04	2.04	2.04
Soybean Oil	1.60	1.60	1.60	1.60
Ground Limestone	6.82	6.82	6.82	6.82
Salt	0.30	0.30	0.30	0.30
DCP (Dicalcium Phosphate)	2.65	2.65	2.65	2.65
Methionine	0.15	0.15	0.15	0.15
Lisin	0.10	0.10	0.10	0.10
Vitamin-Mineral Mixture[a]	0.25	0.25	0.25	0.25
Calculated Values (on dry matter basis)				
Metabolic Energy(Kcal/kg feed)	2770	2770	2770	2770
Crude Protein (%)	17.00	17.00	17.00	17.00

[a]: Each kg mineral–vitamin premix contained: vitamin A, 5,500 IU; vitamin D3, 1,100 IU; vitamin E, 10 IU; riboflavin, 4.4 mg; vitamin B_{12}, 12 mg; nikotinik acit, 44 mg; menadione, 1.1 mg; biotin, 0.11 mg; tiyamin, 2.2 mg; ve ethoxyquin, 125 mg, Mn, 120 mg; Zn, 100 mg; Fe, 60 mg; Cu, 10 mg; Se, 0.17 mg; I, 0.46 mg; ve Ca, 150-180 mg.

RESULTS

The results related to mineral concentrations of liver, kidney, heart, breast, tibia and feather tissues of laying hens fed with diets including vitamin E, Se or their combination were presented in Table II, Table III and Table IV. The mineral concentrations except for Ca and Mg in the tissues of the experimental groups compared to the control group were statistically different ($P<0.05$). Whereas the Ca and Mg concentrations in all groups were not affected by the treatment. Both selenium and vitamin E supplementation significantly increased Se concentrations in aforesaid tissues. Also, dietary selenium and vitamin E supplementation significantly increased Zn and Fe concentrations in all of the tissues. Both dietary organic selenium and vitamin E supplementation significantly decreased Cu concentrations in all studied tissues of experimental groups when compared to control group.

Table 2. Effect of vitamin E and selenium supplement on Se, Cu, Zn, Fe, Ca and Mg levels of liver and kidney tissues of laying hens.
Tablo 2. Vitamin E ve selenyum ilavesinin yumurtacı tavukların karaciğer ve böbrek dokularında Se, Cu, Zn, Fe, Ca ve Mg düzeylerine etkisi.

	Liver Tissue (n=12)				Kidney Tissue (n=12)					
Groups	CO	T-I	T-II	T-III	CO	T-I	T-II	T-III	SEM	P
Se (ppm)	26.7^{d}	36.2^{c}	42.8^{a}	40.8^{b}	14.3^{d}	16.8^{c}	52.4^{a}	40.7^{b}	2.98	*
Cu (ppm)	16.5^{b}	14.5^{a}	13.8^{a}	13.7^{a}	2.43^{a}	1.46^{b}	1.50^{b}	1.58^{b}	3.02	*
Zn (ppm)	245.8^{b}	255.7^{a}	285.0^{a}	275.0^{a}	31.3^{c}	35.8^{b}	44.5^{a}	43.2^{a}	38.0	*
Fe (ppm)	105.7^{c}	124.3^{b}	126.8^{a,b}	127.2^{a}	76.8^{b}	86.8^{a}	86.2^{a}	87.9^{a}	18.5	*
Ca (mg/g)	11.7	11.6	11.8	11.9	15.9	14.9	15.3	15.5	0.25	NS
Mg (mg/g)	32.6	31.7	32.3	32.4	3.39	3.17	3.55	3.36	8.5	NS

CO (control): Basal diet, T-I (Trial-I): Basal diet + 250 mg/kg Vit-E, T-II (Trial-II): Basal diet + 0.9 mg/kg Se, T-III (Trial-III): Basal diet + 250 mg/kg Vit-E + 0.9 mg/kg Se, $^{a, b, c}$: Different superscripts in each row shows the significant difference between the groups *P<0.05, NS: Not significant. Se (selenyum), Cu (bakır), Zn (çinko), Fe (Demir), Ca (kalsiyum), Mg (magnezyum).

TABLE 3. Effect of vitamin E and selenium supplement on Se, Cu, Zn, Fe, Ca and Mg levels of heart and breast tissues of laying hens.
Tablo 3. Vitamin E ve selenyum ilavesinin yumurtacı tavukların kalp ve göğüs dokularında Se, Cu, Zn, Fe, Ca ve Mg düzeylerine etkisi.

	Heart Tissue (n=12)				Breast Tissue (n=12)					
Groups	CO	T-I	T-II	T-III	CO	T-I	T-II	T-III	SEM	P
Se (ppm)	14.6^{c}	21.9^{b}	44.4^{a}	42.6^{a}	3.50^{b}	4.02^{a}	5.65^{a}	4.70^{a}	3.21	*
Cu (ppm)	2.23^{a}	1.66^{b}	1.60^{b}	1.68^{b}	22.7^{a}	17.1^{b}	15.0^{c}	16.2^{b}	0.15	*
Zn (ppm)	21.3^{c}	22.6^{b}	24.5^{a}	23.2^{b}	123^{d}	133^{c}	150^{a}	143^{b}	0.46	*
Fe (ppm)	76.8^{b}	86.8^{a}	86.2^{a}	86.9^{a}	116^{c}	125^{b}	140^{a}	127^{b}	1.54	*
Ca (mg/g)	15.9	14.9	15.3	15.6	11.9	11.7	11.8	11.7	0.93	NS
Mg (mg/g)	3.31	3.17	3.55	3.36	23.5	22.9	23.1	22.6	0.49	NS

CO (control): Basal diet, T-I (Trial-I): Basal diet + 250 mg/kg Vit-E, T-II (Trial-II): Basal diet + 0.9 mg/kg Se, T-III (Trial-III): Basal diet + 250 mg/kg Vit-E + 0.9 mg/kg Se, $^{a, b, c}$: Different superscripts in each row shows the significant difference between the groups *P<0.05, NS: Not significant. Se (selenyum), Cu (bakır), Zn (çinko), Fe (Demir), Ca (kalsiyum), Mg (magnezyum).

Table 4. Effect of vitamin E and organic selenium supplement on Se, Cu, Zn, Fe, Ca and Mg levels of tibia and feather tissues of laying hens.
Tablo 4. Vitamin E ve selenyum ilavesinin yumurtacı tavukların tibia ve tüy dokularında Se, Cu, Zn, Fe, Ca ve Mg düzeylerine etkisi.

	Tibia Tissue (n=12)				Feather Tissue (n=12)					
Groups	CO	T-I	T-II	T-III	CO	T-I	T-II	T-III	SEM	P
Se (ppm)	0.71^{d}	1.14^{c}	5.36^{a}	5.36^{a}	0.50^{d}	1.02^{c}	5.46^{a}	2.20^{b}	0.13	*
Cu (ppm)	38.0^{a}	30.3^{b}	25.8^{c}	25.8^{c}	3.67^{a}	2.19^{b}	1.04^{c}	2.25^{b}	0.28	*
Zn (ppm)	225^{c}	235^{b}	247^{a}	247^{a}	22.8^{c}	27.8^{b}	31.9^{a}	30.9^{a}	0.08	*
Fe (ppm)	260^{c}	275^{b}	285^{a}	285^{a}	56.6^{c}	67.2^{b}	73.9^{a}	68.7^{b}	0.90	*
Ca (mg/g)	11.7	11.6	11.6	11.6	4.9	4.77	4.75	4.67	0.28	NS
Mg (mg/g)	7.25	7.33	7.40	7.37	7.54	7.33	7.10	7.42	2.35	NS

CO (control): Basal diet, T-I (Trial-I): Basal diet + 250 mg/kg Vit-E, T-II (Trial-II): Basal diet + 0.9 mg/kg Se, T-III (Trial-III): Basal diet + 250 mg/kg Vit-E + 0.9 mg/kg Se, $^{a, b, c}$: Different superscripts in each row shows the significant difference between the groups *P<0.05, NS: Not significant. Se (selenyum), Cu (bakır), Zn (çinko), Fe (Demir), Ca (kalsiyum), Mg (magnezyum).

DISCUSSION and CONCLUSION

The selenium's antioxidant properties, high biocompatibility, accumulation rates in tissues and low toxicity have made it more advantageous to use as organic selenium (selenomethionine) (22-24). The findings about mineral concentrations of liver, kidney, heart, breast, tibia and feather tissues of laying hens fed with diets including vitamin E, Se or their combination were given in Table 2, Table 3 and Table 4. In this study, Ca and Mg concentrations of

liver, kidney, heart, breast, tibia and feather were not affected by dietary treatment and remained relatively similar in all groups. Because the metabolisms of macro-minerals such as Ca and P are regulated by hormonal metabolism like PTH, calcitonin and vit-D_3 (25). Whereas both selenium and vitamin E supplementation increased Se concentration in experimental groups when compared with control group. Specially vitamin E supplementation into the diet markedly increased Se concentration in tissues of treatment groups. Some authors reported that vitamin E supplementation into diets of laying hens and broiler promoted Se concentration in some tissues. Vitamin E has the effect of saving Se and this effect may be related to vitamin E achieving the effect of elevating Se concentrations by providing less use of glutathione peroxidase by primarily inhibiting lipid peroxidation in cells membrane from free radicals and oxidative damage. Also it is well known that vitamin E has vanguard antioxidant effect that protects cells membrane from free radicals by primarily inhibiting lipid peroxidation (26,27). These results were in agreement with previous studies (28-32) conducted on laying hens, broilers and rats. Bou et al. (28) studied the effects of different levels of Se and a Zn supplementation into diets of hens and determined a significant increase of Se concentration in tissues with the addition of both minerals. Dagdas et al. (29) reported that different levels of seleno-methionine (0, 0.25, 0.50 and 1.0 mg/kg) and Vit-E (0, 200, 400 IU/kg) supplementation into broiler diets significantly increased the serum and pancreatic Se concentration. Surai (30) reported that Se concentrations of liver and egg yolk were elevated in hens fed on diets supplemented with organic Se and vitamin E. Also Pan et al. (31) and Petrovic et al. (32) noted that the additions of organic Se (0, 0.2, 0.50 and 1.0 mg/kg) to laying hen diets significantly increased Se concentrations in liver, kidney, spleen, heart and breast muscles. At the same time diets including both Se and vitamin E significantly increased Zn and Fe concentrations and decreased Cu concentration in all tissues. In present study, Se and vitamin E significantly increased Zn and Fe concentrations. This increase may cause to a decrease in Cu concentration. Some researchers (33-39) reported that there was a negative relationship not only between Zn and Cu both also Fe and Cu. Sahin et al. (33,34,35), noted that there was an inverse relationship between copper and vitamin E in liver. Also, Ozcelik et al. (36), Skrivanov et al. (37,38) and Spears (39) observed that copper supplementation in poultry diets showed a negative relationship not only between Cu and Zn but also Cu and Fe in tissues. It has been reported that the negative relationship among minerals in the tissues is due to the fact that the minerals form insoluble complexes in the digestive system among themselves (38). These results were in agreement with findings of Harsini (40) who expressed that vitamin E and Se addition to broiler diets significantly increased serum Se and Fe concentrations but decreased Cu concentrations. Kotyzoxa et al. (41) noted an increase in hepatic Cu concentration in the absence of long-term Se in rats. Pappas et al. (42) found that organic Se added into diets had a positive effect on Se in blood, chest and liver specimens, and positively correlated with Zn, Cu and Fe levels. A positive correlation between Se-Zn in present study is consistent with finding of some researchers (28,42). This positive Se-Zn correlation may be attributed to metallothionein-induced synthesis by Zn and other cations (43). In general, Results from present study indicate that the addition of organic selenium and vitamin E to the diets of laying hens significantly increased the concentration of selenium in poultry products, and the products of these animals being important in terms of Se source for human showed that vitamin E had Se saving properties. Supplementing dietary vitamin E and Se have such beneficial effects when trace elements were carefully used in poultry diets. Because deficiency or supplementation of one of them may alter their balance and trigger antagonistic or synergistic effects, alteration of trace element balance may

effect the antioxidant defense system. In addition, several trace elements (Se, Fe, Cu and Zn) are integral part of various antioxidant enzymes.

In Conclusion, organic selenium and vitamin E, consideration of antagonist and synergistic effects of bioelement levels changes in tissues and increasing the quality of animal products and extending the shelf life, it is suggested to add laying hens rations alone and in combination.

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