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Effect of Dietary Vitamin E, Selenium and Their Combination on Concentration of Selenium, MDA, and Antioxidant Enzyme Activities in Some Tissues of Laying Hens

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ABSTRACT

This experiment was conducted to investigate the effects of dietary vitamin-E (α-tocopherol acetate), selenium (selenomethionine) and their combination on Se concentration in liver, heart, kidney and breast muscle. In addition, malondialdehyde (MDA) concentration and enzyme activities of glutathioneperoxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) in liver, heart, breast and thigh tissues of laying hens were determined in present study. A total of 96 White Lohman laying hens, aged 24 weeks, were randomly divided into 4 groups (n=24), each of which was composed of 6 subgroups. The control group received the basal diet (T-1), treatment groups were fed on the the basal diet plus the three experimental diets included one of the followings: 125 mg/kg vitamin E + basal diet (T-2); 0.5 mg/kg Selenium + basal diet (T-3) and 125 mg/kg vitamin E plus 0.5 mg/kg selenium + basal diet (T-4), respectively. Experiment lasted for 12 weeks. In this study, supplementation of diets with Vit-E, selenium (Se) and their combination significantly increased Se concentration in all examined tissues of treatment groups when compared to the control group. Supplementation of Vit-E, Se and their combination significantly ($P<0.05$) decreased the concentration of MDA, but, significantly ($P<0.05$) incrased GSH-Px, SOD and CAT activities in treatment groups. In conclusion, results indicated that Vitamin E and selenium supplementation of laying hen diets could protect these animals from detrimental effect of free radicals by increasing activity of antioxidant enzymes.

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INTRODUCTION

Trace elements are minerals that are requires in minute quantities for the proper growth, development and physiology of the organism (Pappas *et al*., 2008). The essential trace mineral, selenium (Se), is of fundamental importance to human and animals health (Rayman, 2004). Selenium is known to have important roles in reproductive functions, development immunocompetence and ageing (Ševčíková *et al*., 2006).

In poultry nutrition selenium was described in reviews by Surai (2002a, b). Selenium is recognized as an essential element that plays an important role in antioxidant system as a component of Se dependent glutathione peroxidase. It is together with SOD and CAT protects cells against damage caused by free radicals and lipoperoxides (Yoon *et al*., 2007; Harsini *et al*., 2012; Sayiner and Karagul 2017).

Selenium also plays an important roles in the regulation of various metabolic processes in the body, being an integral part of at least 25 selenoproteins via their actions, protect the organism from harmful actions of free radicals (Pappas *et al*., 2008). Moreover, Selenium enhances the actions of vitamin E in reducing peroxy radicals in chickens. Absorption of vitamin E is impaired by severe Se deficiency and Se alleviates such deficiency by promoting higher levels of vitamin E to be absorbed (Harsini *et al*., 2012).

The antioxidant effect of vitamin E has been reported in many studies related to poultry nutrition (Bolukbasi *et al*., 2006; Harsini *et al*., 2012; Kaya and Turgut, 2012; Kaya *et al*., 2013). Vitamin E is the most active natural antioxidant agent used present in the cell mebrane and plays an important role as a chain-breaking lipid antioxidant and free radicals scarvenger in the membranes of cell and subcellular organs (Young *et al*., 2003). Vitamin E exhibits an antioxidant agent activity at low and high concentrations of preoxidant activities concentration (Bollengier-Lee *et al*., 1998; Chen *et al*., 2006).

Surai (2008) indicated that an increased vitamin E supplementation of the meternal diet can substantially increase vitamin E concentration in the developing tissue of chick and significantly decreases their susceptibility to lipid peroxiation. Harsini *et al*. (2012) determined that the combined supplementary level of vitamin E and Selenium

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had increased activity of SOD and decreased MDA levels in skeletal muscles of chick. Grau *et al*. (2001) and Ryu *et al*. (2006) reported that feeding poultry with higher level of dietary vitamin E increased lipid oxidatif stability of poultry meat. Nomerous experiment have been performed on laying hens fed with diets supplemented with different levels of vitamin E, from 20-60 mg/kg (Kirunda *et al*., 2001) to 100-200 mg/kg (Cortinas *et al*., 2004; Zduńczyk *et al*., 2011) and higher than those values (Bou *et al*., 2006).

The aim of this study was to investigate the effects of dietary vitamin E and organic Selenium individually and in their combination into laying hens diet on the Se, MDA concentrations, and the activities of antioxidant enzymes in some tissues of hens.

MATERIALS AND METHODS

The Research Animal Ethic Committee of Atatürk University approved of procedures under this experimental protocol (dated 04.03.2015 and numbered 366438897-59). A total of 96 white lohman laying hens, aged 24 weeks, were randomly assigned to one of the four groups as one control and three treatments. Experiment lasted for 12 weeks. Within a given group, 6 subgroups of 4 hens each were constituted and hens were housed in 50 ×46× 46 cm^3 cages. Control group (T-1) received the basal diet (2770 kcal/kg ME and 17% crude protein). Whereas other 3 groups (T-2, T-3, T-4) were fed with basal diet + 125mg/kg vitamin E (α-tocopherol acetate) (T-2), basal diet + 0.5 mg/kg Selenium (selenomethionine) (T-3) and basal diet + 125 mg/kg Vit E + 0.5 mg/kg Se (T-4), respectively (Table I). Diet and water were provided as ad libitum. At the end of experiment 6 hens were sacrificed from each groups, and liver, heart, kidney and breast muscle samples collected from each subgroups were used for measurment of Se concentrations. Also, thigh muscle in addition to liver, heart, breast samples were used to determine MDA (Malondialdehyde) concentrations and enzyme activities of GSH-PX (Glutathione peroxidase), SOD (superoxide dismutase) and CAT (catalase).

Concentrations of Se in tissue samples were analyzed by IPC-MS (Perkin–Elmer, Optima 2100 DX, ICP/OES, Selton, CT 06484-4 794, USA).

The activities of GSH-PX, SOD, CAT enzymes and the concentration of MDA were measured in liver, heart, breast and thigh muscle tissues, and analysis were performed with spectrophometer, bioassay sysytems using commercial kits according to methods described by Sun *et al*. (1988), Goth (1991) and Ohkawa *et al*. (1979).

Statistical analysis

The 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, at $p<0.05$. Duncan (1955) test was applied for multiple comparisons.

Table I.- Ingredients and calculated analysis of experimental diets .

Feed raw materials (%)	Vitamin E and Se ratio in diets (mg/kg)			
	T-1	T-2	T-3	T-4
Vitamin E (α-tocopherol acetate) (mg/kg)	-	125	-	125
Selenium (mg/kg) (selenomethionie)	-	-	0.5	0.5
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 soybean	1.65	1.65	1.65	1.65
Sunflower seed 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
Marble powder	6.82	6.82	6.82	6.82
Salt	0.30	0.30	0.30	0.30
DCP	2.65	2.65	2.65	2.65
DL-methionine	0.15	0.15	0.15	0.15
L-lysine	0.10	0.10	0.10	0.10
Vitamin-mineral mixture	0.25	0.25	0.25	0.25
Calculated protein and ME				
Metabolic energy (Kcal/kg feed)	2770	2770	2770	2770
Crude Protein (%)	17.00	17.00	17.00	17.00

a-Mineral-vitamin premix provided the following per kilogram of diet: vitamin A, 5.500 IU; vitamin D3, 1,100 IU; vitamin E, 10 IU; riboplavin, 4.4 mg; vitamin B_{12}, 12 mg; nikotinik acit, 44 mg; menadione, 1.1 mg; biotin, 0.11 mg; tiyamin, 2.2 mg; 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

Selenium concentration of tissues

Dietary treatments significantly increased Se concentration in T-2, T-3 and T-4 groups compared to the control group (T-1) ($P< 0.05$). Both selenium and vitamin E supplemented diets versus basal diet had higher Se concentrations in organs and tissues in T-3 and T-4 than T-1 and T-2, but T-4 showed the highest concentration in liver and kidney tissues among the dietary treatments. The highest Se concentration was observed in kidney of T-4.

Antioxiant statues in tissues of laying hens

MDA concentartions in all tissues were varied

according to Vit-E and Se supplementation and type of tissue. Dietary treatments in contrast with basal diet induced a significant effect (P<0.05) on tissue enzymes activity, with substantial reduction MDA concentration.

Vit-E supplementation significantly (P<0.05) decrased MDA concentration in all examined tissues compared to T-1. Also Se supplementation significantly decreased MDA concentration in all tissues compared to the control group (P<0.05). T-4 group fed with diet including the combination of Vit E and Se showed the lowest concentration of MDA in all of the experimental tissues, as well as significiantly lower than T-1 group.

In this study, the activities of antioxiant enzymes (GSH-Px, SOD, CAT) showed significant differences among treatment groups (P<0.05), compaired to control group. The GSH-Px activity was higher in all of Se supplemented groups than those T-1 and T-2 groups. The highest activity was observed in T-4 group. Also, T-4 showed the highest activity in heart tissue.

CAT activity was significantly influenced by dietary treatments (P<0.05), Vit-E and Se supplementation significantly incrase this enzyme activity in experimental tissues compaired to control group (P<0.05). The highest activity was observed in liver of T-4 group.

As to SOD activity, also the SOD activity was significantly influenced by dietary treatments compaired to control group (P<0.05). Supplementation of Vit-E, Se and their combination significantly increased SOD activity of T-3 and T-4 and showed higher SOD activity than T-1 and T-2 (P<0.05). The hens fed on combinated supplementary diet had the highest activity of SOD in all of examined tissues.

Table II.- Selenium concentration (ppm/g) in tissues of laying hens fed Vit-E and Selenium (n=6).

Tissues	T-1	T-2	T-3	T-4	P
Liver	26.7±4.5d	36.2±3.9c	42.8±4.8a	40.8±4.7b	*
Kidney	14.3±1.5d	18.8±1.7c	52.4±4.5a	40.7±5.5b	*
Heart	14.6±1.6c	21.9±2.5b	44.4±5.5a	42.6±4.5a	*
Breast	3.50±0.5b	4.02±0.7a	5.65±1.0a	4.7±0.9a	*

CO(control), Basal diet; T-1 (Trial-1), Basal diet + 250 mg/ kg Vit-E; T-2 (Trial-2), Basal diet + 0.9 mg/kg Se; T-3 (Trial-3), 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.

Table III.- Effect of dietary supplemention of Vitamin E and selenium on antioxidant statues in liver, heart, breast and thigh muscle of laying hens (n=6).

Tissues	Diets	MDA (nmol/mg)	GSH-Px (nmol/min/mg)	SOD (nmol/min/mg)	CAT (nmol/min/mg)
Liver	T-1	37.28 ± 3.5a	15.28 ± 2.5c	6.29 ± 5.5c	372.5 ± 25c
	T-2	35.79 ± 3.5b	17.76 ± 1.7b	6.99 ± 1.5b	374.25 ± 27b
	T-3	36.16 ± 3.7b	19.91 ± 1.9a	7.16 ± 1.9b	375.00 ± 25b
	T-4	30.48 ± 3.8c	20.75 ± 2.5a	7.78 ± 1.5a	426.50 ± 23a
	P	*	*	*	*
Heart	T-1	13.48 ± 1.3a	39.01 ± 2.7b	1.5 ± 0.2 c	38.25 ± 3.3d
	T-2	11.28 ± 1.3b	41.03 ± 4.5a	2.15 ± 0.3 b	61.00 ± 6.8c
	T-3	11.79 ± 1.9c	42.84 ± 3.9a	2.92 ± 0.5 a	87.25± 8.5b
	T-4	11.16 ± 1.1b	43.25 ± 4.5a	3.06 ± 0.8 a	94.00 ± 8.7a
	P	*	*	*	*
Breast	T-1	19.48 ± 1.5a	10.95 ± 1.5c	7.08 ± 2.5c	234.75 ± 31c
	T-2	11.28 ± 1.3c	12.14 ±1.2b	9.03 ± 1.8b	250.00 ± 29b
	T-3	12.79 ± 1.5b	13.30 ± 1.4b	9.06 ± 1.7b	274.25 ± 33ab
	T-4	11.15 ± 1.2c	13.56 ± 1.5a	9.25 ± 2.3a	284.00 ± 32a
	P	*	*	*	*
Thigh	T-1	20.48 ± 2.5 a	9.14 ± 1.1 c	12.02 ± 2.1 c	204.25 ±22 d
	T-2	15.79 ± 1.5 c	9.84 ± 1.2 b	12.38 ± 2.5 b	231.75 ± 21 c
	T-3	16.28 ± 1.8 b	10.18 ± 1.2 a	12.83 ± 2.8 b	240.25 ± 25 b
	T-4	15.16± 1.7 c	10.78 ± 1.0 a	13.36 ± 1.9 a	256.00 ± 28 a
	P	*	*	*	*

T-1 (control), basal diet; T-2, basal diet+250 mg/kg vit-E; T-3, basal diet +0.9 mg/kg Se; T-4, basal diet+250 mg/kg Vit-E+0.9 mg/kg+Se. a, b, c, d, different superscipts in each row shows the significant difference between the groups.

DISCUSSION

Both vitamin E and selenium supplementation into diets increased Se concentration in treatment groups when compared with control group (P<0.05). Vitamin E supplementation into the diet markedly incrased Se concentration in tissues of treatment groups (P<0.05). Se concentration in T-2, T-3 and T-4 groups were higher than control group. Hens fed with diet including combined supplementary Vit-E and Se had the highest Se concentration in all the experimental groups. Hartmann and Wilhelmson (2001) reported that Vit-E supplementation to laying hens and broilers diets promoted Se concentration in some tissues. Vit-E has the effect of saving Se and this effect may be related to Vit-E achieving the effect of elevating Se concentration by providing less use of GSH-Px by primarily inhibiting lipid peroxidation in cell membrane from free radicals and oxidative damage. Also, it is well known that Vit-E has vanguard antioxidant effect that protects cells membrane from free radicals by primarly inhibiting lipid peroxidation.

These results were in agreement with previous studies conducted by some researchers (Wakebe, 1998; Surai, 2002b; Bou *et al*., 2006) on laying hens and broilers. Ševčíková *et al*. (2006) informed that the supplemental organic Se enriched yeast and alga chlorella in diets of broiler significantly increased Se concentration in liver and body muscles. Choct *et al*. (2004) observed that an increasing Se supplementation rate from 0,1 mg/kg to 0,25 mg/kg into brolier diets significantly increased Se concentration in breast muscle.

Also, Pan *et al*. (2007) noted that the addition of organic Se (0.2, 0.5 and 1 mg/kg) to laying hens diets sinificantly increased Se concentrations in liver, kidney, spleen heart and breast muscles (P<0.05).

MDA, GSH-Px, SOD and CAT values were significantly influenced by dietary treatments (P<0.05). Supplementation of vitamin E, selenium and their combination decreased MDA concentration and increased the activities of GSH-Px, SOD and CAT in all tissues of T-2, T-3 and T-4 groups compared to the control group (P<0.05). Hens that received supplemental Vit-E and Se had the lowest MDA concentration in aforesaid tissues. Se together vit-E supplementation had synergistic effect on MDA concentration. MDA considered a marker of oxidative stress is one of the final products of cell poly unsaturated fatty acid peroxidation, increase in MDA indicated peroksidation enhancement caused by tissue damage and reduced antioxidant mechanisms, and also could indirectly reflect the degree of cell damage (Cuzzocrea and Reiter, 2001).

The activities of GSH-Px, SOD and CAT were significantly (P<0.05) influenced by dietary vitamin E and selenium. The activities of these enzymes were higher in T-2, T-3 and T-4 groups than control group. These enzymes are the main enzymatic antioxidants aganist toxic oxygen reduction metabolities. The highest activities of GSH-Px, SOD and CAT were observed in hens fed with Se and Vit E combinated diet. Dietary Vit-E showed a significant pozitive effect on these enzymes and increased their activities in both supplemented groups (T-2, T-4) in all examined tissues.

The highest activities of GSH-Px, SOD and CAT were obtained from all studied tissues of hens fed with diet including Vit-E plus Se, because Vit-E has the effect of saving Se, this effect may be related to Vit-E achieving effect of elevating Se concentration by providing less use of GSH-Px by primarily inhibiting lipid peroxidation in cells membrance from free radicals and oxidative damage (Leeson and Caston, 2003). Also, previous studies reported that addition of Vit-E and Se into diets of laying hens, broilers and rats significantly increase concentration of Se, Zn and Fe in different tissues (Bou *et al*., 2006; Noberg, 2009; Kotyzová *et al*., 2010; Harsini *et al*., 2012). These minerals are necessary for activities of GSH-Px, SOD and CAT, respectively. For example, Se is essential part of a family enzymes called GSH-Px and GR, Zn is integral part of SOD and Fe is essential part of the CAT can be synthesised in sufficient amounts of animal body and the antioxiant enzymes (Surai, 2008).

There are numerous studies were conducted to investigate the effects of dietary Vit-E and Se on antioxidant statues in laying hens, broilers and pigeons. The findings related to MDA concentration and antioxidant enzyme activities in tissues from present study were consistent with earlier results determined by Zduńczyk *et al*. (2013), Zuberbuehler *et al*. (2006), Şahin *et al*. (2002) and Öztürk *et al*. (2001). Also, they stated that in hens given Se and Vit-E, erythrocyte GSH-Px, SOD and CAT increased dramatically than in the control group. In additionally, the results from present study, were smilar to the findings of some rearchers (Chen *et al*., 2006; Jiang *et al*., 2009; Gao *et al*., 2010; Harsini *et al*., 2012; Boostani *et al*., 2015; Dalia *et al*., 2017).

CONCLUSION

These results from present study also showed that when dietary vitamin E and Se combination had synergistic effect on the activities of all the three enzymes, Se concentration and MDA values. In conclusion, results indicated that Vitamin E and selenium supplementation of laying hen diets could protect these animals from detrimental effect of free radicals by increasing activity of

antioxidant enzymes.

Statement of conflict of interest

The authors declare no conflict of interest.

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