

Influence of Vitamin E and Organic Selenium Supplementation on Antioxidant Enzymes Activities in Blood and Egg Samples of Laying Hens

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ABSTRACT: This experiment was conducted to evaluate the effects of dietary vitamin E (α -tocopherol acetate) and selenium (selenomethionine) and a combination of the two, on the activities of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) and malondialdehyde (MDA) levels in serum, egg yolk and egg white of laying hens. Ninety-six white Lohman laying hens aged of 24 weeks were randomly divided into 4 groups. The groups were fed with the diets that consisted of basal diet (2770 kcal/kg metabolic energy and 17 % crude protein) (Control), basal diet + 250 mg / kg Vit-E (Trial-1), basal diet + 0.9 mg/ kg Se (Trial-2) and basal diet + 250 mg / kg Vit-E + 0.9 mg/ kg Se (Trial-3) respectively for 12 weeks. It was found out that when the treatment and control groups were compared in terms of enzymes in serum and egg samples while SOD, CAT and GSH-Px activities increased ($p<0.05$) and the levels of MDA decreased in the samples of treatment groups ($p<0.05$). The results of the present study, is recommended that organic selenium and vitamin E supplemental of laying hen diets, alone or together, increased activities of antioxidant enzymes and decreased MDA concentrations in serum and egg.

Key words: Selenium, laying hens, antioxidant enzymes activities, serum, egg

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INTRODUCTION

Bioelements are required in little quantities for growth, development and physiology of the organism (Pappas et al., 2008). Selenium (Se), is of fundamental importance to animals and human health (Rayman 2004). Selenium is a component of several important selenoproteins and enzymes required for functions including antioxidant defence, reduction of inflammation, thyroid hormone production, DNA synthesis, fertility and reproduction (Soydan and Utlu 2018; Surai et al., 2016, Zduńczyk et al., 2013). It is found in nature in two forms, inorganic (selenite, selenate and selenide) and organic (selenomethionine, selenomethionine). Selenium is recognized as an essential element that plays an important role in antioxidant system as a component of glutathione peroxidase. Additionally, it is together with SOD and CAT protects cells against damage caused by free radicals and lipoperoxides (Harsini et al., 2012, Sayiner and Karagul, 2017). Moreover, Selenium plays an important role in the regulation of various metabolic processes in the body, being an integral part of at least 25 selenoproteins, enhances the actions of vitamin E in reducing peroxy radicals in chickens (Pappas et al., 2008). Absorption of vitamin E is impaired by 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 (Harsini et al., 2012; Kaya et al., 2013). Vitamin E is one of the biological antioxidants widely used in poultry diets and has been proposed as a major antioxidant in plasma membranes of all cells, functioning as a chain-breaker and free radical scavenger (Rengaraj and Hong, 2015), reproductive power, increasing resistance to infectious mechanisms, resistance to infectious and metabolic diseases, increased meat quality, meat colour effect and shelf life (Leeson et al., 2008). 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 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 all procedures under this experimental protocol (dated 26.01.2015 and numbered 36643897-23). A total of 96 White Lohman hens aged 24 weeks were randomly assigned to one of the four experimental diets for a period of 12 weeks. Within a given group, 6 subgroups of 4 hens each were constituted and hens were housed in 50x46x46 cm³ cage. The chickens were fed for 12 weeks with the rations given on the Table 1. Diet and water were provided as ad libitum. Blood samples were collected from the vena jugularis of 12 animals from each group, were centrifuged, serum samples were separated and stored at -30°C until analyses. Egg samples were collected 12 pieces from each group. The white and yolk parts of the eggs were separated and stored at -30°C until analyses. Malondialdehyde levels (Ohkawa et al., 1979) and catalase (Goth, 1991), superoxide dismutase (Sun et al., 1988) and glutathione peroxidase (Beutler, 1975) activities in samples were analyzed by spectrophotometrically. Statistical analysis of the data obtained in the experiment was made 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.

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Table 1. Ingredients and nutrient levels of the rations used in the experiment

Feedstuffs	Composition of diets (mg/kg)			
	Control	Trial-1	Trial-2	Trial-3
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 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
Limestone	6.82	6.82	6.82	6.82
Salt	0.30	0.30	0.30	0.30
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	0.25	0.25	0.25	0.25
Calculated nutrient composition				
Metabolic energy(Kcal/kg)	2770	2770	770	2770
Crude protein (%)	17.00	17.00	17.00	17.00

Mineral–vitamin premix provided the following per kilogram of diet: vitamin A, 5,500 IU; vitamin D3, 1,100 IU; vitamin E, 10 IU; riboflavin, 4.4 mg; vitamin B₁₂, 12 mg; nicotinic acid, 44 mg; menadione, 1.1 mg; biotin, 0.11 mg; thiamine, 2.2 mg; and ethoxyquin, 125 mg, Mn, 120 mg; Zn, 100 mg; Fe, 60 mg; Cu, 10 mg; Se, 0.17 mg; I, 0.46 mg; and Ca, 150-180 mg

RESULTS AND DISCUSSION

In this study, organic Se and Vit E supplemented groups significantly increased the activities of antioxidant enzymes (GSH-Px, SOD, CAT) in serum, egg white and egg yolk of the laying hens ($p < 0.05$) compared with the control group. Additionally, organic Se and Vit E supplemented groups significantly decreased the level of MDA in serum, egg white and egg yolk of the laying hens ($p < 0.05$) compared with the control group. When antioxidant enzyme activities and MDA levels were compared in all samples, it was determined that although there were different results between the experimental groups, it was not significant (Table 2, Table 3 and Table 4).

Table 2. Effect of vitamin E and organic selenium supplementation on CAT, SOD and GSH-PX activities and MDA levels of serum of laying hens.

Groups	CO	T-1	T-2	T-3	SEM	P
SOD (mmol/dakika/ml)	1,35 ^b	1,71 ^a	1,70 ^a	1,72 ^a	0.24	*
CAT(mmol/dakika/ml)	0,11 ^b	0,15 ^a	0,14 ^a	0,15 ^a	0.01	*
MDA (nmol/ml)	11,20 ^a	9,18 ^b	9,49 ^b	9,67 ^b	0.46	*
GSH-Px(mmol/dakika/ml)	0,33 ^b	0,37 ^a	0,37 ^a	0,38 ^a	0.03	*

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

* $P < 0.05$, SEM: Standart error of the mean.

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Table 3. Effect of vitamin E and organic selenium supplementation on CAT, SOD and GSH-Px activities and MDA levels of egg white of laying hens.

Groups	CO	T-1	T-2	T-3	SEM	P
SOD (mmol/dakika/mg)	1,47 ^b	1,63 ^a	1,64 ^a	1,65 ^a	0.07	*
CAT(mmol/dakika/mg)	0,074 ^b	0,078 ^a	0,077± ^a	0,079 ^a	2x10 ⁻⁴	*
MDA (nmol/mg)	10,29 ^a	9,01 ^b	9,02 ^b	8,64 ^b	0.52	*
GSH-Px (mmol/dakika/mg)	0,42 ^b	0,48 ^a	0,49 ^a	0,47 ^a	0.02	*

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, SEM: Standart error of the mean.

Table 4. Effect of vitamin E and organic selenium supplementation on CAT, SOD and GSH-Px activities and MDA levels of egg yolk of laying hens.

Groups	CO	T-1	T-2	T-3	SEM	P
SOD (mmol/dakika/mg)	0,48 ^b	0,75 ^a	0,76 ^a	0,78 ^a	0.11	*
CAT (mmol/dakika/mg)	0,40 ^b	0,56 ^a	0,58 ^a	0,59 ^a	0.06	*
MDA (nmol/mg)	22,48 ^a	16,16 ^{bc}	17,28 ^b	15,79 ^c	0.9	*
GSH-Px (mmol/dakika/mg)	0,71 ^b	0,82 ^a	0,79 ^a	0,82 ^a	0.04	*

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/kgSe

^{a, b, c}: Different superscripts in each row shows the significant difference between the groups

*P<0.05, SEM: Standart error of the mean.

GSH-Px, a selenoprotein, play a critical role in antioxidative defense in poultry. They are widely distributed in the body to protect the structure and function of cell membranes by catalyzing toxic peroxides into non-toxic hydroxyl compounds (Leeson et al., 2008; Czech et al., 2012; Ahmad et al., 2012). The activity center of GSH-Px is selenocysteine, it is generally believed that GSH-Px activity can reflect the selenium levels in the body. The most important metabolic function of Se is shown through activity of GSH-Px enzymes and other reductases in cell protection from peroxidation by means of which free radicals manifest their damaging activity. Se level in blood is an important regulator of GSH-Px activity. Significantly higher GSH-Px activity was observed in blood of hens that consumed more organic Se and as GSH-Px activity is taken as an indicator of Se absorption efficiency, obtained results were as expected. (Ahmad et al., 2012; Dalia et al., 2017). SOD and CAT are the first oxidative barrier against free radicals in the body and are of vital importance for removal of free radicals and maintaining cell energy metabolism. Changes in antioxidative enzyme activity are related to tissue type, animal age, stress and animal status referring to the amount of Se in its organism (Surai 2000a). Animal antioxidant system is greatly influenced by animal nutrition, and dietary Se supplementation is necessary to up-regulate its Se-containing antioxidant enzymes (Jiang et al., 2009). Another important factor affecting blood antioxidant status is the activity of antioxidant enzymes (Ting et al., 2011). Accordingly, dietary organic Se can improve antioxidant system and increase GSH-Px activity in all tissues of broiler chicken (Zhang et al., 2014; Yasin et al., 2012; Khan 2011).

Petrovič et al. (2006) and Placha et al (2014) reported that dietary Se supplementation could significantly increase GSH-Px activity in serum of poultry. Pan et al (2008) reported that supplemented Se for laying hens can significantly improve the SOD activity in plasma. Li et al. (2018) reported that

while the addition of organic Se significantly increased GSH-Px activities in the sera of chickens, there was no change in SOD and CAT activities and MDA levels. Leng et al. (2003) pointed out a positive correlation between the amount of Se in a diet and GSH-Px activity in broiler blood. Besides blood, GSH-Px is also an important indicator of Se absorption in eggs. Wakebe (1999) and Surai (2000b) stated that hens which consumed diets with organic Se had increased GSH-Px activity in eggs, which positively affected the preservation of egg freshness. In the previous studies reported GSH-Px activity significantly higher in blood of hens fed on diet supplemented organic Se (Zhang et al., 2008; Mohapatra et al., 2014; Nadia et al., 2015). Wang et al. (2010) reported that adding 0.3 ppm Se from Se yeast to the hens diet significantly increased GSH-Px activity and reduced the MDA level in yolk compared with the control group. Increasing supplement of Se up to 0.4 ppm from Se yeast to laying hens diets resulted in production of eggs enriched with Se and a prolonged period of egg freshness (Gajcevic et al., 2009; Wang et al., 2010). Dalia et al. (2017) reported Se supplementation in contrast to control diet induced a notable elevation in serum GSH-Px and CAT activity, while substantial decreased in MDA concentration. Additionally, Se-enriched yeast as organic source enhanced antioxidative status of broilers by increasing antioxidant enzyme levels compared to control group (Jiang et al., 2009). Besides that, a study by Chen et al. (2014) showed that organic Se supplementation in broiler chicken increased the activity of serum GSH-Px and SOD. According to Boostani et al. (2015) Se supplementation raised GSH-Px activity and lowered MDA in comparison with the control group. On the contrary, Payne and Southern (2005) reported that GSH-Px activity was unaffected by organic of Se. Zduńczyk et al. (2013) found increased dietary vitamin E levels from 30 to 60 mg/kg contributed to a significant increase in serum SOD activity of blood plasma. Additionally, increased dietary Se levels from 0.15 to 0.30 mg/kg led to an increase in the serum activity of catalase and SOD of hens. In some researches were reported that GSH-Px activity significantly higher, in addition to free radical inhibition and lower MDA content in blood of hens receiving 0.3 ppm Se of organic Se compared with the control group. (Zhou and Wang 2011, Cai et al., 2012; Dalia et al., 2017). Lipid peroxidation is the most important oxidative stress caused by oxygen free radicals. MDA, an important indicator of lipid peroxidation, is one of the final products of cell polyunsaturated fatty acid peroxidation (Gaweł et al., 2004). Nadia et al. (2015) reported that Se supplementation at 0.25 ppm in the diet significantly decreased the MDA level in yolk of the fresh and storage eggs compared with the control group. Thus less the rate of lipid oxidation and improved oxidative stability in the eggs. A decrease of the MDA level in yolk is related to increase Se content in egg, may be due to the enhancement of GSH-Px activity resulting from increasing supplemental dietary Se. A reduction of MDA level in yolk can be explained by Huang et al. (2003) who indicated that small size Nano-Se with the large surface area had greater ability to transfer electrons to radicals, with a high efficacy for scavenging various free radical. Dalia et al. (2017) reported that supplementation of organic Se also caused a significant decrease in serum MDA level compared to dietary control group. MDA, considered a marker of oxidative stress, is one of the final products of cell polyunsaturated fatty acid peroxidation and considered a marker of oxidative stress, is one of the final products of cell polyunsaturated fatty acid peroxidation (Gaweł et al., 2004). Therefore, the decreasing of MDA by organic Se is due to the presence of Se-Met and Se-Cys which are more bioavailable and can raise the levels of antioxidants and decrease the production of lipid peroxidation products.

CONCLUSION

The results of the present study, is recommended that organic selenium and vitamin E supplemental of laying hen diets, alone or together, increased activities of antioxidant enzymes and decreased MDA concentrations in serum and egg.

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