IJAS Iranian Journal of Applied Animal Science

Comparison of Different Selenium Sources and Vitamin E in Laying Hen Diet and Their Influences on Egg Selenium and Cholesterol Content, Quality and Oxidative Stability

Research Article

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Received on: 15 Aug 2016
Revised on: 23 Oct 2016
Accepted on: 31 Oct 2016
Online Published on: Mar 2017

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Online version is available on: www.ijas.ir

ABSTRACT

An experiment was carried out to compare the effects of laying hen's diet supplemented with inorganic and different organic sources of selenium (Se) on quality and oxidative stability of eggs during storage. A total of 81, (35-week old) laying hens of Lohmann LSL-White were assigned to cages in a completely randomized design with 9 groups of treatment and 3 replicates of 3 birds. Hens in each group were fed their corresponded diet included the basal diet supplemented with sodium selenite, Se-enriched yeast, Cytoplex-selenium and Selenomax at two different levels of 0.3 and 0.6 mg/kg, or 200 mg/kg vitamin E. To prevent brand judgment challenge, A, B and C letters were applied for different organic source of Se. After 56 days of feeding experimental diets, eggs were collected from the hens to analysis. Egg weight loss during storage at 4 ˚C was lower ($P<0.05$) in the group fed 0.3 mg of B source Se/kg of feed. Vitamin E and Se supplemented groups had lower malondialdehyde values than those from the non-supplemented ($P<0.01$). The C Source of organic Se resulted in lower malondialdehyde compared with the other sources of Se or control. The supplementation of Se in diet increased ($P<0.01$) yolk Se concentration, with the effect being more significant by C source of Se. Selenium and vitamin E supplementation decreased serum and yolk cholesterol content ($P<0.01$). The results demonstrate the better efficacy of the C source of organic Se to increase Se deposition in egg and improved egg quality compared with the other sources of Se.

KEY WORDS egg quality, laying hen, oxidative stability, selenium, vitamin E.

INTRODUCTION

Selenium (Se) is an important natural antioxidant which is essential in many metabolic processes in living organisms. It is found in nature in two forms of inorganic and organic. Inorganic Se refers to different minerals such as selenite, selenate and selenide, and organic Se is related to seleno-amino acids such as selenomethionine (SeMet) and seleno-cysteine (SeCys). Selenium deficiency in birds, especially if combined with the lack of vitamin E, causes the occurrence of exudative diathesis and encephalomalacia (Leeson S. and Summers, 2001). In human, Se intake is often lower than the recommended daily allowance (Tanguy *et al*. 2012); therefore, there is a need to increase Se consumption in the common human foods. Se-enriched eggs can be produced by adding inorganic or organic Se compounds to the hen diet. In the recent years, different sources of organic Se such as Se-enriched yeast, Se-proteinate and Se-amino acids, have been introduced to the animal feed industries. A Se-enriched yeast product has been produced by cultivation

of *Saccharomyces cerevisiae* in Se-enriched media, thus yeast can accumulate large amounts of Se and incorporate them into organic Se-containing compounds, mainly SeMet (Demirci, 1999; Schrauzer, 2001). Another organic Se source, Se-proteinate, is one of these organic Se sources which is produced with enzymatically hydrolyzed soy protein. Organic Se supplements have been reported to increase egg Se content more than inorganic Se (Payne *et al.* 2005; Mohiti-Asli *et al.* 2008). In addition, it has been shown that addition of organic Se to laying hens diet can improve the quality of stored eggs (Mohiti-Asli *et al.* 2008; Jlali *et al.* 2013).

Mohiti-Asli *et al.* (2008) reported that the diets supplemented with sodium selenite, seleno-yeast or vitamin E, improved egg quality, oxidative stability and fatty acid composition during the storage without any negative effect on hen performance. However, the efficacies of numerous organic Se products have yet to be evaluated (Jang *et al.* 2010; Tufarelli *et al.* 2015). Hence, having a lot of varieties of Se in market and getting confused about choosing the products, the objective of this experiment was to evaluate the effect of three organic Se products compared with inorganic sodium selenite and vitamin E in the diet of laying hens on serum and yolk cholesterol content, egg quality, Se content and oxidative stability.

MATERIALS AND METHODS

Egg quality measurements and laboratory analysis

Egg quality characteristics including shell thickness, shell resistance, shell weight, albumen quality (Haugh unit score; HU) and yolk weight, were measured in two eggs collected from each replicate at the end of 8-wk experimental period. Eggshell strength was tested on the Egg Shell Force Gauge (Robot mation Co. Ltd., Tokyo, Japan; Mohiti-Asli *et al.* (2008)), and egg weight, albumen height, and HU, were measured with the Egg Multi Tester EMT-5200 (Robot mation Co. Ltd.; Mohiti-Asli *et al.* (2008)). The yolk and albumen were separated and weighed to determine the yolk weight and albumen weight. Eggshell was weighed with eggshell membranes giving eggshell weight. Eggshell thickness was measured at the blunt, equatorial and sharp regions to get the average value using an Ultrasonic Thickness Gauge (Echometer 1062, Robot mation Co. Ltd., Japan) as indicated by Mohiti-Asli *et al.* (2008). From each replicate, two eggs were collected at the last week of the experiment in order to measure the yolk cholesterol content by method of Pasin *et al.* (1998). Eggs collected at the last week of experiment were stored in two different temperatures (4 ˚C and 25 ˚C) for 14 days to determine egg quality and oxidative stability. To study egg oxidative stability, malondialdehyde (MDA), was measured as a secondary oxidation product according to the thiobarbituric acid (TBA) method described by Botsoglou *et al.* (1994) using third derivative spectrophotometry (UV-visible S2100, Scinco, Korea). Tetraethoxy propane (1, 1, 3, 3- Tetraethoxy propane, T9889, 97%, Sigma, St. Louis, MO 63103) was used as MDA precursor in the standard curve. At the end of the experiment, 3.0 mL of blood was drawn from the brachial vein of two hens in each cage. The sera were separated and serum cholesterol was determined using a commercial diagnostic kit enzyme method (Pars Azmoon Co., Iran).

Table 1 Composition and calculated analysis of the basal diet

Ingredients	% in diet
Yellow corn grain	62.20
Soybean meal (44%)	23.87
Soybean oil	2.20
Oyster shell	9.20
Dicalcium phosphate	1.54
Common salt	0.34
DL-Methionine	0.15
Vitamin premix[1]	0.25
Mineral premix[2]	0.25
Calculated analysis	
Metabolizable energy (kcal/kg)	2800
Crude protein (%)	16.02
Lysine (%)	0.79
Methionine (%)	0.42
TSAA (%)	0.67
Calcium (%)	3.73
Available phosphorous (%)	0.40
Linoleic acid (%)	2.58
Se (mg)	0.21

[1] Vitamin premix provided per kilogram of diet: vitamin A: 10000 IU; vitamin D_3: 2500 IU; vitamin E: 20 mg; vitamin K_3: 3 mg; vitamin B_1: 1 mg; vitamin B_2: 4 mg; Pantothenic acid: 10 mg; vitamin B_6: 3 mg; Niacin: 30 mg; vitamin B_{12}: 0.025 mg; Folic acid: 0.5 mg; Biotin: 0.05 mg and Cholin chloride: 400 mg.
[2] Mineral premix provided per kilogram of diet: Manganese: 100 mg; Iron: 25 mg; Copper: 5 mg; Iodine: 0.5 mg; Selenium: 0.16 mg and Zinc: 60 mg.

Statistical analysis

All data were analyzed as a completely randomized design with eight treatments using the General Linear Model procedure of SAS (SAS, 2002). The following model was fitted:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:
Y_{ij}: trait of interest for hens.
μ: overall mean.
T_i: treatment effect.
e_{ij}: residual error.

Normal distribution of residuals and variance homogeneity of the data were tested by UNIVARIATE procedure and the Levene's test, respectively. The experimental unit was the collected eggs for egg quality measurements, constituting of two observations in each replicate.

For all the remaining studied traits including egg production, egg weight and feed conversion ratio (FCR), the cage was used as the experimental unit. Differences were considered significant at ($P<0.05$). Significant differences between means were separated by Duncan's multiple range test.

RESULTS AND DISCUSSION

Performance

To prevent brand judgment challenge, A, B and C letters were applied for different organic source of Se (see the Tables). Hen performance parameters, including egg production, egg weight, egg mass and FCR values, were not significantly affected by the vitamin E or any of the supplement sources of in the diets. However, feed consumption was higher in Se-supplemented groups than that of the control group (Table 2).

Egg quality

Neither vitamin E nor organic and inorganic sources of Se affected any internal or external quality characteristics of eggs stored at 4 ˚C (Table 3; $P>0.05$). However, egg and shell weights were significantly higher in hens fed B source of Se (0.3 mg/kg of Se) than the control. Quality traits of eggs stored for 14 days at 25 ˚C (including yolk weight, shell thickness, shell resistance, shell and egg weights) were not influenced by any treatments (Table 4; $P>0.05$). On contrary, the Haugh unit (HU) value was significantly higher in hens fed vitamin E supplemented diet. The other experimental treatments also showed higher HU values than the control group ($P<0.05$).

Dietary supplementation of vitamin E, organic sources and inorganic Se decreased MDA content of the egg yolks in the fresh and stored eggs, which were more pronounced with vitamin E and the C source of organic Se (Table 5). Eggs from hens fed C source of Se in diet had lower MDA in yolk in both levels of 0.3 and 0.6 mg/kg compared with the other sources of Se and the control. All sources of selenium supplements increased yolk Se content (Table 6; $P<0.01$). Organic Se from B and C sources (in the level of 0.6 mg/kg of the diet) increased yolk Se content more than inorganic Se and source A of Se ($P<0.01$). Yolk Se was higher in eggs from hens fed higher dietary Se level, however 0.6 mg/kg Se from C source did not increase yolk Se content than 0.3 mg/kg. Selenium and vitamin E decreased yolk cholesterol content with the effect being more significant by vitamin E (Table 5; $P<0.01$). Also, serum cholesterol was lower in hens fed vitamin E in diet than those fed the control diet ($P<0.01$).

Many previous reports suggested that the dietary Se supplementation has no effect on feed intake, egg production and egg weight (Patton *et al*. 2000; Puthpongsiriporn *et al*. 2001; Dvorska *et al*. 2003; Jiakui and Xialong, 2004; Payne *et al*. 2005; Mohiti-Asli *et al*. 2008; Jlali *et al*. 2013). It could be supposed that Se dependent enzymes with a key role in the antioxidant systems maybe not involved in egg production (Jiakui and Xiaolong, 2004). However, some reports indicated that the effect of Se on egg production was only significant when Se content in the diet was below the requirement (Cantor and Scott, 1994). In the current study, adding organic source of Se to diet influenced weight of the stored eggs at 4 ˚C with the effect being more significant for B source of organic Se. Several factors including shell porosity, albumen quality and egg size, could affect loss of egg weight during storage. Therefore, a probable reason for minor weight loss in stored eggs from hens fed organic source of Se might be related to their better shell quality parameters such as higher shell thickness, shell resistance and shell weight. In the current study no significant difference was detected in shell thickness and resistance, although B source of organic Se had the highest shell weight among the treatments.

Reis *et al*. (2009) reported that egg weight was not different in broiler breeder hens fed different levels of sodium selenite or Zn-SeMet. Payne *et al*. (2005) found a linear increase in egg weight by supplementation of Se yeast from 0 to 3 mg/kg. They proposed that higher Se concentrations in diet could affect protein synthesis and consequently egg weight.

Organic Se incorporation into the egg has been suggested to be in a large fraction as SeMet (Surai, 2002). It has been shown that methionine supplementation per se affected egg weight positively (Calderon and Jensen, 1990). A similar response for SeMet, which leads to increased egg weight, could be expected if SeMe methionine were fully incorporated into egg proteins.

Eggs from hens fed vitamin E or 0.6 mg/kg B source of organic Se supplemented-diets had higher HU after 14 days of storage at 25 ˚C compared to the other groups. It has been reported that vitamin E (Mohiti-Asli *et al*. 2008) and organic Se (Pappas *et al*. 2005) reduced deterioration of egg albumen. However, the effect of Se on albumen quality was not consistent in the previous studies. Arnold *et al*. (1973) reported that HU of eggs were improved by sodium selenite. Payne *et al*. (2005) reported that HU values of eggs stored at 23-27 ˚C was similar in either dietary levels of sodium selenite or seleno-yeast. The concentration of MDA was increased in eggs stored for 14 days at 4 ˚C or 25 ˚C. The least concentration of MDA was found in hens fed diet supplemented with vitamin E. Several reports indicated that dietary administration of supplemental levels of α-tocopherol improved the stability of egg yolk lipids (Galobart *et al*. 2001; Mohiti-Asli *et al*. 2008).

Table 2 Effect of Se sources and vitamin E on laying hen performance[1]

Treatment	Egg production (% hen day)	Egg weight (g)	Egg mass (g/day)	Feed intake (g/hen/day)	FCR
Control	95.6±0.15	59.5±0.97	56.8±1.56	106.2±1.33^{c}	1.87±0.05
Selenite, 0.3 mg/kg	93.6±0.61	57.8±0.32	53.9±1.01	114.7±1.75^{a}	2.13±0.09
A, 0.3 mg/kg	95.5±0.95	59.0±0.89	56.4±0.91	115.1±1.03^{a}	2.05±0.06
B, 0.3 mg/kg	95.0±0.98	60.1±0.59	57.2±1.91	111.8±1.01^{ab}	1.96±0.05
C, 0.3 mg/kg	93.4±0.43	58.5±0.83	54.6±1.30	112.5±1.85^{ab}	2.07±0.14
A, 0.6 mg/kg	92.7±0.63	58.6±0.28	54.3±1.80	112.4±1.14^{ab}	2.08±0.10
B, 0.6 mg/kg	94.0±0.77	59.3±0.92	55.7±1.11	111.4±1.54^{ab}	2.01±0.03
C, 0.6 mg/kg	94.7±0.17	58.8±0.28	55.8±0.86	115.2±1.81^{a}	2.07±0.05
Vitamin E	91.9±0.19	57.6±0.88	52.4±1.41	108.4±1.74^{bc}	2.08±0.07
Effect	NS	NS	NS	*	NS

[1] All data reported in Table are Means ± SE.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05).
NS: non significant.
FCR: feed conversion ratio.

Table 3 Effect of Se source and vitamin E on egg quality characteristics (Means±SE) stored at 4 ˚C for 14 days

Treatment	Egg weight (g)	Shell weight (g)	Albumen weight (g)	Yolk weight (g)	Haugh unit	Shell thickness (mm×10^{2})	Shell resistance (kg/cm^{2})
Control	56.4±1.63^{bc}	5.28±0.14^{cd}	34.06±1.62	17.11±0.68	66.3±1.97	31.39±0.29	3.21±0.40
Selenite, 0.3 mg/kg	56.2±1.02^{bc}	5.31±0.11^{cd}	32.73±1.72	18.19±0.87	71.4±1.89	31.29±0.61	3.40±0.18
A, 0.3 mg/kg	57.8±1.21^{bc}	5.63±0.14^{abc}	34.28±1.27	17.92±0.47	69.1±1.72	31.53±0.91	3.32±0.08
B, 0.3 mg/kg	61.6±1.78^{a}	5.86±0.25^{a}	36.27±1.56	19.52±0.28	72.8±1.01	31.63±0.95	3.57±0.10
C, 0.3 mg/kg	54.6±1.80^{c}	4.90±0.43^{e}	32.23±1.11	17.45±0.26	67.8±1.86	29.28±0.62	3.59±0.20
A, 0.6 mg/kg	59.1±1.48^{ab}	5.65±0.13^{abc}	35.11±.76	18.36±0.48	71.8±1.59	31.68±0.29	3.35±0.26
B, 0.6 mg/kg	58.2±1.21^{abc}	5.46±0.06^{bcd}	33.93±1.80	18.81±0.24	71.8±1.89	31.81±0.45	3.78±0.25
C, 0.6 mg/kg	56.7±1.41^{bc}	5.23±0.15^{de}	34.65±1.58	16.86±0.60	68.7±1.50	29.83±0.54	3.53±0.58
Vitamin E	57.8±1.46^{bc}	5.71±0.12^{ab}	33.75±1.49	18.37±0.68	66.9±1.80	31.39±0.48	2.96±0.28
Effect	*	**	NS	NS	NS	NS	NS

The means within the same column with at least one common letter, do not have significant difference (P>0.01 and P>0.05).
* (P<0.05) and ** (P<0.01).
NS: non significant.

Table 4 Effect of Se source and vitamin E on egg quality characteristics (Means±SE) stored at 25 ˚C for 14 days

Treatment	Egg weight (g)	Shell weight (g)	Albumen weight (g)	Yolk weight (g)	Haugh unit	Shell resistance (kg/cm^{2})	Shell thickness (mm×10^{2})
Control	53.73±1.94	5.40±0.21	29.00±0.91	19.94±0.33	40.63±0.96^{d}	3.21±0.40	29.33±0.51
Selenite, 0.3 mg/kg	54.68±1.84	5.51±0.05	28.66±1.42	20.50±0.12	43.20±1.86^{bcd}	3.40±0.18	31.60±0.47
A, 0.3 mg/kg	55.25±1.26	5.72±0.17	30.19±1.62	19.31±0.61	42.72±1.21^{bcd}	3.32±0.08	32.65±0.50
B, 0.3 mg/kg	56.63±1.62	5.73±0.12	31.28±1.96	19.61±0.38	41.49±1.42^{cd}	3.57±0.10	31.02±0.10
C, 0.3 mg/kg	52.51±1.72	5.45±0.35	28.14±1.68	18.92±0.52	44.19±1.48^{bcd}	3.59±0.20	30.76±0.49
A, 0.6 mg/kg	53.00±1.78	5.33±0.52	28.98±.1.65	18.66±0.94	47.08±1.02^{abc}	3.35±0.26	30.81±0.21
B, 0.6 mg/kg	53.79±1.42	5.57±0.08	29.30±1.29	18.92±0.25	45.15±1.89^{abcd}	3.78±0.25	31.56±0.11
C, 0.6 mg/kg	55.71±1.62	5.61±0.24	31.00±0.92	19.01±0.48	47.91±1.52^{ab}	3.53±0.58	30.55±0.77
Vitamin E	55.16±1.00	5.57±0.12	29.41±1.28	20.17±0.95	50.21±1.91^{a}	2.96±0.28	31.32±0.28
Effect	NS	NS	NS	NS	*	NS	NS

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05).
NS: non significant.

This result is due to the antioxidant property of vitamin E and the role of Se in glutathione peroxidase activity. Galobart *et al*. (2001) reported that adding 100 or 200 mg α-tocopherol per kg diet of laying hens decreased TBA in eggs stored for 6 months and concluded that dietary vitamin E in this level has antioxidant role. Eggs from hens fed C source of Se had lower concentration of MDA in yolk compared with the other sources of Se and the control.

Therefore, the C source of organic Se in the current study had more potential to increase oxidative stability of eggs. Dvorska *et al*. (2003) reported increased glutathione peroxidase activity in eggs after laying hens were fed diets containing Se and vitamin E. This increase in glutathione peroxidase activity would protect the egg from damage by free radicals, resulting in decreased potential of cellular damage to the shell or fluid egg.

Table 5 Effect of Se sources and vitamin E on MDA of fresh eggs and eggs stored at 4 ˚C and 25 ˚C for 14 days[1]

Treatment	Fresh egg	Stored at 4 ˚C	Stored at 25 ˚C
Control	1.31±0.05^{a}	1.74±0.05^{a}	3.35±0.05^{a}
Selenite, 0.3 mg/kg	1.15±0.03^{b}	0.93±0.02^{f}	2.18±0.03^{bc}
A, 0.3 mg/kg	1.08±0.03^{bc}	1.53±0.05^{c}	2.06±0.07^{c}
B, 0.3 mg/kg	1.13±0.05^{b}	1.05±0.05^{e}	2.07±0.05^{c}
C, 0.3 mg/kg	0.97±0.05^{cd}	1.49±0.03^{c}	1.90±0.03^{d}
A, 0.6 mg/kg	1.16±0.05^{b}	1.65±0.05^{b}	2.21±0.05^{bc}
B, 0.6 mg/kg	1.03±0.05^{c}	1.16±0.03^{d}	2.28±0.05^{b}
C, 0.6 mg/kg	0.71±0.05^{d}	0.88±0.03^{fg}	1.78±0.03^{d}
Vitamin E	0.71±0.05^{d}	0.81±0.03^{g}	1.51±0.02^{e}
Effect	**	**	**

The means within the same column with at least one common letter, do not have significant difference (P>0.01).
** (P<0.01).
NS: non significant.

Table 6 Effect of Se sources and vitamin E on eggs Se and cholesterol content (Means±SE)

Treatment	Yolk Se content (ng/g)	Serum cholesterol (mg/dL)	Yolk cholesterol (mg/g)
Control	1.12±0.02^{c}	172.1±1.17^{a}	12.64±0.56^{a}
Selenite, 0.3 mg/kg	1.37±0.03^{b}	142.0±1.22^{b}	10.70±0.47^{dc}
A, 0.3 mg/kg	1.21±0.03^{b}	148.9±1.23^{b}	10.59±0.60^{dc}
B, 0.3 mg/kg	1.43±0.01^{b}	175.5±1.04^{a}	11.18±0.44^{bc}
C, 0.3 mg/kg	1.80±0.01^{a}	140.2±1.07^{b}	10.61±0.59^{dc}
A, 0.6 mg/kg	1.41±0.09^{b}	178.2±1.12^{a}	11.16±0.29^{bc}
B, 0.6 mg/kg	1.88±0.06^{a}	141.9±1.34^{b}	10.54±0.35^{dc}
C, 0.6 mg/kg	1.90±0.03^{a}	143.6±1.15^{b}	10.67±0.56^{dc}
Vitamin E	-	143.6±1.18^{b}	9.24±0.55^{e}
Effect	**	**	**

The means within the same column with at least one common letter, do not have significant difference (P>0.01).
** (P<0.01).
NS: non significant.

They reported that storing the eggs for 14 days at 20 ˚C caused yolk lipid peroxidation and increased MDA content in the yolk. However, lipid peroxidation significantly decreased in eggs by Se supplementation in diet.

Dietary supplementation of Se increased yolk Se content, with the effect being more for the C source of organic Se. However, no difference was generated in yolk Se content either by 0.3 or 0.6 mg of C source or by 0.6 mg of B source of organic Se per kg of diet. Many of researchers have reported that increasing Se content in diet increased Se content of eggs (Ort and Latshaw, 1978; Mohiti-Asli *et al.* 2008; Cobanova *et al.* 2011). Payne *et al.* (2005) reported that a Se-enriched yeast diet was more effective than a sodium selenite diet for increasing the Se content of eggs. Utterback *et al.* (2005) reported that adding seleno-yeast to the laying-hen diet yielded a 4.8-fold increase in eggs Se content compared with a 2.8-fold increase for the sodium selenite diet over the un-supplemented diet. Se content in serum and yolk were higher in some organic Se sources than the others, probably due to various bioavailability of different sources of Se.

Dietary supplementation of selenium and vitamin E decreased serum and yolk cholesterol content, with the effect being more significant for vitamin E.

Sahin *et al.* (2002) reported that higher vitamin E in diet resulted in a decrease in serum cholesterol concentrations of Japanese quails. El-Demerdash (2004) reported that vitamin E or Se alone significantly decreased the levels of cholesterol in rats. Mohiti-Asli and Zaghari (2010) reported that dietary supplementation of vitamin E reduced egg yolk cholesterol content. Hens could eliminate considerable amounts of cholesterol in the egg (Andrews *et al.* 1968). Moreover, Andrews *et al.* (1968) showed that egg cholesterol originates from serum cholesterol. But, other reporters (Washburn and Nix, 1974; Shivaprasad and Jaap, 1979) indicated that the plasma cholesterol concentration is not closely associated with the concentration of yolk cholesterol. Laying hen VLDLy resists the lipolytic activity of LPL (Bacon *et al.* 1978) and provides triacylglycerol and cholesterol for egg yolk. Therefore, yolk cholesterol is not related to serum low density lipoprotein (LDL) and high density lipoprotein (HDL).

Vitamin E affected hepatic synthesis and catabolism of cholesterol in the chicken (Sklan, 1983). The activity of hepatic cholesterol 7α-hydroxylase was reduced in vitamin E-deficient rabbits (Chupukcharoen *et al.* 1985) and paradoxically, the activity of this enzyme was also reduced in rats fed high levels of vitamin E (Kritchevsky *et al.* 1980).

CONCLUSION

It can be concluded that Se supplementation, especially in organic forms, to laying hens diet can increase Se contents of eggs. The source of organic Se supplemented to the diets is also important for egg enrichment and its physiological and antioxidant properties.

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