Selenium and α-tocopherol content in eggs produced by hens that were fed diets supplemented with selenomethionine, sodium selenite and vitamin E

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ABSTRACT: The effect of supplementing dietary selenium (Se) and vitamin E was investigated in 330 24-week-old laying hens. The hens were fed a basal diet containing Se and α-tocopherol at 0.11 and 26 mg/kg, respectively, or a diet supplemented with Se at 0.3 mg/kg and vitamin E between 0 and 625 mg/kg. Se was supplied as Se-methionine or sodium selenite. The eggs were collected for analysis during the third, seventh and eleventh weeks of the experiment. Supplementation of either form of Se significantly increased the Se concentration in egg yolks and whites, with a more pronounced effect caused by Se-methionine. The egg yolk α-tocopherol concentration paralleled the dietary α-tocopherol concentration. At a high dietary α-tocopherol concentration (632 mg/kg), the retinol content in egg yolks from hens fed Se-methionine increased significantly. Supplementation of Se-methionine significantly increased the α-tocopherol content in the eggs in the third and seventh weeks of the experiment. A moderate decrease in yolk cholesterol was observed in hens fed Se-methionine and α-tocopherol at 119 mg/kg. The concentration of products from lipid peroxidation (thiobarbituric acid-reactive substances, TBARS) in egg yolks increased marginally during the refrigerated storage of the eggs for 2 weeks. The effect of dietary vitamin E on TBARS formation was generally small, although a more significant effect was observed at the highest dose tested.

Keywords: eggs; selenium; selenomethionine; selenite; vitamin E; retinol; cholesterol; oxidative stability

Selenium (Se), an essential trace element, is a component of numerous selenoproteins required for many vital functions in the animal body. To date, several dozen selenoproteins have been identified, serving purposes ranging from antioxidant defence, thyroid hormone deiodination, and reduction of disulphides, to DNA synthesis and other functions (Surai, 2006). The effect of selenium and vitamin E on white muscle disease was described by Muth et al. (1958). The nutritional requirement for Se in poultry is quite low (0.15 mg/kg diet; NRC, 1994). However, Se has often been added to poultry diets in order to increase the Se content in meat and eggs. In most cases, Se has been added to diets as sodium selenite (Na_2SeO_3), which is a less efficient but cheaper source of Se than Se-yeast (e.g., Payne and Southern, 2005; Payne et al., 2005; Utterback et al., 2005).

Dietary supplementation of Se influences quality traits of poultry products (Skřivan et al., 2006; Arpášová et al., 2009). Se is a structural component of glutathione peroxidase (GSH-Px, E.C.1.11.1.9), which catalyzes the reduction of hydrogen peroxide and organic hydroperoxides, thus protecting the cells from oxidative damage. Additionally, through its role in the biosynthesis of GSH-Px, Se interacts

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with vitamin E, which is the main protector against *in vivo* lipid peroxidation (Tappel, 1980). Authors of several papers have concluded that dietary Se has a sparing effect on vitamin E in poultry (Thompson and Scott, 1970; Dean and Combs, 1981; Surai, 2000; Skřivan et al., 2008a,b). Surai et al. (2000) have demonstrated that Se supplemented as Se-yeast (at 0.2 and 0.4 mg/kg of poultry diet) significantly increased the vitamin E concentration in the yolk. However, supplementation did not influence yolk concentrations of vitamin A and carotenoids. Organic sources of Se (Se-enriched yeast and the alga, *Chlorella*) were found to be more effective in increasing Se and vitamin E contents in eggs than selenite (Skřivan et al., 2008a). The main form of Se in yeast is Se-methionine, which is either non-specifically incorporated into animal proteins in place of methionine or converted to selenocysteine and specifically incorporated into Se-enzymes. In yeast, Se-methionine is accompanied by several other Se-amino acids (Rayman, 2004).

This study sought to evaluate the effect of dietary Se and vitamin E supplementation on Se content in eggs, α-tocopherol, retinol and cholesterol concentrations in egg yolks, and on the oxidative stability of yolks. Se-methionine was used instead of Se-yeast to eliminate any possible influence of other Se-amino acids. Given that sodium selenite is a common component of many animal feeds, the effects of Na_2SeO_3 and Se-methionine were compared.

MATERIAL AND METHODS

Diets and husbandry

Three hundred and thirty 19-week-old ISA Brown laying hens were obtained from a commercial farm. The hens were all housed in the same air-conditioned facility. The room temperature was kept at 20–22°C and the light cycle consisted of 15 h of light and 9 h of darkness (incandescent lighting, 10 lx). The hens were housed at 10 hens per cage, and the cages were randomly allocated to one of 11 diets. In accordance with EC directive No. 1999/74,

Table 1. Ingredients and chemical composition of the control diet[a] (g/kg)

Ingredients		Analysed nutrient composition	
Wheat	258	dry matter	885
Maize	350	crude protein	176
Soybean meal	200	crude fat	52
Rapeseed oil	30	crude fibre	34
Fish meal	15	calcium	38
Lucerne meal	20	phosphorus	5.8
Wheat bran	25	selenium (mg/kg)	1.1
Limestone	83	vitamin E (mg/kg)	26
Dicalcium phosphate	10	AME_N, MJ/kg (calculated)	11.46
Sodium chloride	2	methionine (calculated)	0.36
Vitamin-mineral premix[b]	5		
Wheat meal[a]	1.2		
DL-Methionine	0.8		

[a]diet "C" in Tables 2–6; experimental diets were supplemented with Se at 0.3 mg/kg and vitamin E at 0, 40, 100, 250 and 625 mg/kg

[b]premix provided per kg of diet: 8 000 IU vitamin A, 2 250 IU vitamin D3, 1.5 mg vitamin K, 1.5 mg thiamine, 4 mg riboflavin, 2 mg pyridoxine, 0.01 mg vitamin B12, 20 mg niacin, 6 mg calcium pantothenate, 0.06 mg biotin, 0.4 mg folic acid, 250 mg choline chloride, 50 mg betaine, 50 mg butylated hydroxytoluene, 0.3 mg Co, 6 mg Cu, 30 mg Fe, 0.7 mg I, 60 mg Mn, 50 mg Zn

the cages were equipped with a nest box, perch, dust bath and equipment for sharpening of claws. Each treatment was replicated 3 times. Table 1 presents the ingredients and chemical composition of the basal (control) wheat-maize-soybean meal diet. Experimental diets were supplemented with Se at 0.3 mg/kg, and with vitamin E at either 0, 40, 100, 250 or 625 mg/kg. Se-methionine and sodium selenite were provided by Sigma (Prague, Czech Republic) and DL-α-tocopherol acetate (Rovimix E-50) by Hoffman La Roche, Ltd. (Basel, Switzerland). The basal diet was fed for 5 weeks, followed by experimental diets for 12 weeks. Feed and fresh water were provided *ad libitum*. The protocol was approved by the Ethical Committee of the Institute of Animal Science.

Data collection and sampling

The feed intake (per cage) and laying performance were recorded weekly. For the analyses, the eggs were collected daily in the third, seventh and eleventh week of the experiment. For the Se determination in the egg yolk and white, as well as for other analyses, 8 eggs were collected from each cage in each period of the experiment (3 960 eggs in all). The pooled samples prepared from 3 yolks and whites were stored at –70°C until further analyses.

Analysis

The dry matter content of the feed was determined by oven drying at 105°C, ash by burning at 550°C, and fat by extraction with petroleum ether in a Soxtec 1043 apparatus (FOSS Tecator AB, Höganäs, Sweden). The protein content in the feed was determined using a Kjeltec Auto 1030 Analyzer from the same company. Feed calcium (Ca) and phosphorus (P) were determined in ashed samples: Ca by atomic absorption spectrometry (Solaar M6 instrument, TJA Solutions, Cambridge, UK), and P colorimetrically by a molybdate reagent (Huxtable and Bressler, 1973). To determine Se, the samples of feed, egg yolks and egg whites were digested in a mixture of HNO_3 and H_2O_2 (trace analysis grade, Analytika Ltd., Prague, Czech Republic) in teflon high-pressure vessels in an MDS-2000 microwave oven (LabX, Midland, ON, Canada). After mineralisation, Se was quantified by electrothermic atomisation in a graphite cuvette, employing the Solaar M6 atomic absorption spectrometer. The analytical procedure was validated by analysis of the certified reference material NIST Whole Egg Powder 8415. The α-tocopherol and retinol contents of the egg yolks were determined in accordance with the EN 12822 (2000) by HPLC (Shimadzu, VP series) equipped with a diode-array detector. In order to determine cholesterol in

Table 2. Concentrations of Se and α-tocopherol in the diets of hens

Diet	Se supplement (0.3 mg Se/kg)	Vitamin E supplement (mg/kg)	Analysed concentration (mg/kg)	
			Se	α-tocopherol
C	–	0	0.11	26
1	Se-methionine	0	0.37	29
2		40	0.35	60
3		100	0.40	119
4		250	0.38	262
5		625	0.36	632
6	Na_2SeO_3	0	0.38	28
7		40	0.39	64
8		100	0.40	120
9		250	0.39	259
10		625	0.40	630

average values ± SEM

Table 3. Concentration of Se in egg yolk and egg white (mg/kg DM) in diet C

Se supplement (0.3 mg Se/kg)	α-tocopherol in feed (mg/kg)	Yolk			White		
		phase of experiment			phase of experiment		
		3rd week	7th week	11th week	3rd week	7th week	11th week
–	26	0.51 ± 0.03[a]	0.43 ± 0.05[a]	0.48 ± 0.05[a]	0.36 ± 0.03[a]	0.40 ± 0.03[a]	0.40 ± 0.04[a]
Se-methionine	29	1.46 ± 0.07[b]	1.42 ± 0.09[b]	1.39 ± 0.10[b]	1.16 ± 0.06[b]	1.02 ± 0.07[b]	1.25 ± 0.08[b]
	60	1.50 ± 0.09[bc]	1.43 ± 0.09[b]	1.40 ± 0.10[b]	1.27 ± 0.08[b]	1.16 ± 0.07[bc]	1.22 ± 0.08[b]
	119	1.39 ± 0.07[b]	1.40 ± 0.09[b]	1.46 ± 0.10[b]	1.43 ± 0.09[b]	1.33 ± 0.07[c]	1.47 ± 0.08[bc]
	262	1.60 ± 0.08[bc]	1.58 ± 0.10[b]	1.57 ± 0.13[b]	1.31 ± 0.01[b]	1.14 ± 0.06[bc]	1.47 ± 0.08[bc]
	632	1.72 ± 0.10[c]	1.60 ± 0.10[b]	1.54 ± 0.11[b]	1.41 ± 0.10[b]	1.25 ± 0.08[bc]	1.58 ± 0.09[c]
Na_2SeO_3	28	1.11 ± 0.06[d]	0.96 ± 0.07[c]	0.91 ± 0.09[c]	0.50 ± 0.06[c]	0.55 ± 0.02[d]	0.58 ± 0.04[d]
	64	1.05 ± 0.07[d]	0.92 ± 0.06[c]	0.98 ± 0.09[c]	0.56 ± 0.05[c]	0.58 ± 0.05[d]	0.62 ± 0.04[d]
	120	1.14 ± 0.07[d]	1.03 ± 0.09[c]	1.02 ± 0.09[c]	0.57 ± 0.04[c]	0.56 ± 0.06[d]	0.62 ± 0.03[d]
	259	1.18 ± 0.10[d]	1.04 ± 0.09[c]	0.96 ± 0.08[c]	0.60 ± 0.05[c]	0.64 ± 0.03[d]	0.66 ± 0.05[d]
	630	1.16 ± 0.07[d]	1.01 ± 0.11[c]	0.94 ± 0.09[c]	0.59 ± 0.05[c]	0.61 ± 0.06[d]	0.66 ± 0.04[d]
		differences in least square means between Se sources					
	Se-methionine	1.53	1.49	1.47	1.32	1.18	1.40
	Na_2SeO_3	1.13	0.99	0.96	0.56	0.59	0.63
	estimate	0.40	0.50	0.51	0.76	0.59	0.77
	SEM	0.07	0.09	0.09	0.06	0.05	0.07
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

average values ± SEM; means were calculated for 8 average samples per egg collection within a treatment
one average sample consisted of 3 yolks or whites
[a–d]values in the same column with different letters are significantly different ($P < 0.05$)

Table 4. Concentrations of α-tocopherol and retinol in egg yolk (mg/kgDM) in diet C

Sesupplement (0.3mgSe/kg)	α-tocopherol in feed (mg/kg)	α-tocopherol			Retinol		
		phase of experiment			phase of experiment		
		3rd week	7th week	11th week	3rd week	7th week	11th week
–	26	137 ± 8[a]	122 ± 7[a]	128 ± 9[a]	14.5 ± 0.5[a]	13.3 ± 0.6[a]	13.8 ± 0.7[a]
	29	156 ± 9[b]	148 ± 7[b]	157 ± 10[b]	17.5 ± 0.9[b]	15.1 ± 0.9[ab]	14.7 ± 0.7[ab]
	60	319 ± 11[c]	303 ± 14[c]	321 ± 17[c]	14.5 ± 0.7[ab]	14.3 ± 0.9[ab]	14.0 ± 0.7[a]
Se-methionine	119	512 ± 36[d]	523 ± 39[d]	518 ± 39[d]	15.6 ± 0.8[ab]	14.8 ± 0.9[ab]	15.2 ± 0.6[ab]
	262	1 088 ± 77[e]	1 068 ± 78[e]	1 091 ± 82[e]	16.1 ± 1[ab]	16.0 ± 0.7[b]	15.7 ± 0.6[ab]
	632	2 315 ± 117[f]	2 368 ± 119[f]	2 304 ± 127[f]	17.6 ± 0.8[b]	16.6 ± 0.9[b]	16.3 ± 0.8[b]
	28	154 ± 10[ab]	138 ± 10[ab]	142 ± 8[ab]	15.9 ± 1.1[ab]	14.7 ± 0.8[ab]	15.7 ± 0.8[ab]
	64	306 ± 18[c]	296 ± 17[c]	300 ± 17[c]	15.1 ± 0.6[a]	14.9 ± 0.9[ab]	14.3 ± 0.7[ab]
Na_2SeO_3	120	501 ± 32[d]	472 ± 30[d]	529 ± 33[d]	14.1 ± 0.9[a]	14.1 ± 0.8[a]	15.3 ± 0.7[ab]
	259	998 ± 61[e]	983 ± 60[e]	957 ± 64[e]	15.7 ± 0.9[ab]	15.0 ± 0.6[ab]	15.6 ± 0.6[ab]
	630	2 186 ± 109[f]	2 142 ± 102[f]	2 326 ± 130[f]	15.0 ± 0.9[a]	15.3 ± 1[ab]	15.4 ± 0.8[ab]
	differences in leasts quare means between Se sources						
	Se-methionine	878	882	878	16.3	15.4	15.2
	Na_2SeO_3	829	806	851	15.2	14.8	15.3
	estimate	49	76	27	1.1	0.6	–0.1
	SEM	38	36	49	0.8	0.7	0.7
	P-value	0.014	0.010	0.120	0.010	0.042	0.81

average values ± SEM; means were calculated for 8 average samples per egg collection within a treatment
one average sample consisted of 3 yolks
[a–f]values in the same column with different letters are significantly different ($P < 0.05$)

yolks, lipids were saponified and the unsaponified matter extracted with diethyl ether in accordance with ISO 3596 (1988). Silyl derivatives were prepared using TMCS and HMDS silylation reagents (Sigma), and quantified on a gas chromatograph equipped with a SAC-5 capillary column (Supelco, Bellefonte, USA), operated isothermally at 285°C. Lipid peroxidation in the yolks of fresh eggs and eggs stored for 14 days at 4°C was measured using the method previously described by Piette and Raymond (1999), and was expressed as thiobarbituric acid-reactive substances (TBARS) in mg of malondialdehyde/kg.

The data were statistically evaluated by the analysis of variance. The assumption of homogeneity of variances was confirmed by Levene's homogeneity test. Significant treatment effects were determined by Scheffe's test. The value $P < 0.05$ was chosen as the limit for statistical significance. Statistics were performed using SAS (2002–2003).

RESULTS

No effect of treatment on egg production was observed. The average laying intensities in individual groups during the third, seventh, and eleventh weeks of the experiment were 96–98%, 96–97% and 95–97%, respectively. Both forms of dietary Se supplementation significantly increased Se concentration in egg yolks and egg whites, with a more pronounced effect seen with Se-methionine compared to sodium selenite (Table 3). In hens fed Se-methionine, the average Se concentrations in egg yolks and egg whites were 1.50 and 1.30 mg/kg DM, respectively. Corresponding Se concentrations in hens fed sodium selenite were 1.03 and 0.59 mg per kg DM. Vitamin E supplementation caused a small, mostly insignificant increase in Se concentration in eggs from hens fed diets with Se-methionine. Egg yolk α-tocopherol concentrations paralleled the concentrations of dietary α-tocopherol (Table 4). Dietary supplementation with Se increased the α-tocopherol content in egg yolk. However, only the effect of Se-methionine was statistically significant. Vitamin E supplementation tended to increase the retinol concentration in egg yolks. In hens fed diet No. 5 (with the combined supplementation of Se-methionine and vitamin E at 625 mg/kg), the increased retinol concentration was statistically significant. The egg yolks from hens fed the diet supplemented with Se-methionine and vitamin E at 100 mg/kg (α-tocopherol content of 119 mg/kg) contained significantly less cholesterol than those from control hens (Table 5). The egg yolks from hens fed sodium selenite and vitamin E at the same level contained significantly less cholesterol only in the third and the eleventh weeks of the experiment.

The effect of Se supplementation on the concentration of products of lipid peroxidation in the yolks of fresh eggs and eggs stored for 14 days was small and statistically insignificant (Table 6). A significant reduction in yolk TBARS was observed at the highest concentration of dietary vitamin E.

DISCUSSION

Selenium and α-tocopherol concentration in eggs

Selenium increased the α-tocopherol content in egg yolks, as shown in previous studies by Surai (2000) and Skřivan et al. (2008b). The effect of Se-methionine on α-tocopherol concentration was more pronounced than that of sodium selenite, and was statistically significant. The sparing effect of both antioxidants was to some extent additive, as the dietary supplementation of vitamin E at 625 mg/kg increased deposition of Se in egg yolks and whites. The concentration of α-tocopherol in egg yolks increased linearly with dietary vitamin E concentration, an effect which has been observed by several other authors (Jiang et al., 1994; Sünder and Flachowsky, 2001; Franchini et al., 2002).

Retinol and cholesterol concentration in eggs

Surai (2000) reported that vitamin E at 200 mg per kg diet and organic Se at 0.4 mg/kg diet had no effect on vitamin A concentration in the egg yolk. At very high dietary concentrations of vitamin E (up to 20 g/kg), the vitamin A content in eggs yolks was reduced (Sünder and Flachowsky, 2001). In the present study no effect of α-tocopherol on the retinol concentration in egg yolks was observed at 119 mg/kg diet, but a moderate increase of the yolk retinol content was found in hens fed 632 mg of α-tocopherol/kg diet. This effect was observed only in hens fed diets supplemented with Se-methionine. The feeding diets containing 119 mg α-tocopherol/kg resulted in significantly lower cholesterol

Table 5. Concentration of cholesterol in egg yolk in diet C

Se supplement (0.3 mg Se/kg)	α-tocopherol in feed (mg/kg)	Cholesterol (g/kg DM)		
		phase of experiment		
		3rd week	7th week	11th week
–	26	12.2 ± 0.8[a]	12.5 ± 0.6[a]	11.6 ± 0.6[a]
Se-methionine	29	10.8 ± 1.1[ab]	12.2 ± 1[ab]	10.1 ± 0.7[ab]
	60	11.8 ± 0.9[ab]	11.6 ± 0.8[ab]	12.3 ± 0.7[a]
	119	9.5 ± 0.8[b]	10.5 ± 0.5[b]	9.9 ± 0.3[b]
	262	10.5 ± 1.0[ab]	12.5 ± 0.1[ad]	10.6 ± 0.5[ab]
	632	10.7 ± 0.7[ab]	12.2 ± 1.1[ab]	10.9 ± 0.6[ab]
Na_2SeO_3	28	11.6 ± 0.9[ab]	12.2 ± 1[ab]	9.8 ± 0.7[ab]
	64	10.0 ± 0.7[ab]	11.0 ± 0.5[ab]	9.4 ± 0.7[b]
	120	9.6 ± 0.9[b]	11.5 ± 0.8[ab]	9.9 ± 0.5[b]
	259	10.4 ± 0.6[ab]	10.9 ± 0.5[ab]	10.3 ± 0.4[ab]
	630	11.2 ± 0.9[ab]	10.8 ± 0.9[ab]	10.1 ± 0.5[ab]
	differences in least square means between Se sources			
	Se-methionine	10.7	11.8	10.8
	Na_2SeO_3	10.6	11.3	9.9
	estimate	0.1	0.5	0.9
	SEM	0.7	0.8	0.5
	P-value	0.710	0.063	0.050

average values ± SEM; means were calculated for 8 average samples per egg collection within a treatment
one average sample consisted of 3 yolks
[a,b]values in the same column with different letters are significantly different ($P < 0.05$)

concentration in the yolk. The exceptions to this were eggs laid by hens fed inorganic Se in the seventh week of the experiment. Possible mechanisms that may explain the association of dietary Se and α-tocopherol with yolk cholesterol concentration is not clear and relevant information in the literature is limited. Sahin et al. (2006) reported that in Japanese quails fed supplemental lycopene and vitamin E, separately or in combination, decreased yolk cholesterol concentration and increased serum and egg yolk vitamin E and A.

Oxidative stability of eggs and concluding remarks

Franchini et al. (2002) reported that egg yolk was resistant to oxidative deterioration during extended refrigerated storage. Indeed, during storage at 4°C for 2 weeks, concentrations of TBARS in egg yolks were essentially stable in all groups. No improvement in oxidative stability was observed with increased dietary vitamin E. This contrasts with the significant effect of dietary vitamin E on the oxidative stability of beef (O'Grady et al., 2001) and veal (Skřivanová et al., 2007).

It can be concluded that Se-methionine is a more readily available Se source in laying hens than sodium selenite. However, Se is a controversial trace element due to a narrow gap between its essentiality and its toxicity (Surai, 2006). The alimentary intake of Se increased in developed countries as a result of Se supplementation to both human foodstuffs and food-animal dietary formulations. Recent findings from observational studies and clinical trials have suggested an association between moderate

Table 6. Concentration of malondialdehyde in yolks of eggs stored at 4°C (mg/kg) – control

Se supplement (0.3 mg Se/kg)	α-tocopherol in feed (mg/kg)	Fresh eggs			Eggs stored for 14 days		
		phase of experiment			phase of experiment		
		3rd week	7th week	11th week	3rd week	7th week	11th week
–	26	1.36 ± 0.05^{a}	1.26 ± 0.03^{a}	1.32 ± 0.05^{a}	1.48 ± 0.04^{a}	1.41 ± 0.03^{a}	1.37 ± 0.02^{a}
	29	1.24 ± 0.06^{ab}	1.14 ± 0.06^{ab}	1.16 ± 0.06^{ab}	1.32 ± 0.02^{ab}	1.23 ± 0.06^{ab}	1.22 ± 0.04^{bc}
	60	1.23 ± 0.05^{ab}	1.16 ± 0.06^{ab}	1.02 ± 0.04^{b}	1.30 ± 0.03^{ab}	1.25 ± 0.03^{ab}	1.17 ± 0.03^{c}
Se-methionine	119	1.21 ± 0.06^{ab}	1.13 ± 0.04^{ab}	0.98 ± 0.04^{b}	1.28 ± 0.04^{bc}	1.19 ± 0.04^{ab}	1.11 ± 0.04^{b}
	262	1.23 ± 0.05^{ab}	1.19 ± 0.04^{ab}	1.03 ± 0.05^{b}	1.28 ± 0.03^{bc}	1.26 ± 0.06^{a}	1.15 ± 0.03^{c}
	632	1.10 ± 0.04^{b}	1.01 ± 0.03^{b}	0.96 ± 0.05^{b}	1.15 ± 0.03^{c}	1.12 ± 0.05^{b}	1.06 ± 0.03^{c}
	28	1.32 ± 0.06^{a}	1.22 ± 0.05^{a}	1.17 ± 0.04^{ab}	1.34 ± 0.02^{ab}	1.32 ± 0.03^{a}	1.21 ± 0.03^{bc}
	64	1.31 ± 0.06^{a}	1.21 ± 0.03^{a}	1.20 ± 0.03^{ab}	1.35 ± 0.05^{ab}	1.27 ± 0.07^{ab}	1.22 ± 0.03^{b}
Na_2SeO_3	120	1.21 ± 0.05^{ab}	1.12 ± 0.05^{ab}	1.14 ± 0.08^{ab}	1.28 ± 0.06^{bc}	1.17 ± 0.04^{ab}	1.19 ± 0.03^{bc}
	259	1.19 ± 0.06^{ab}	1.13 ± 0.06^{ab}	1.09 ± 0.05^{b}	1.29 ± 0.03^{bc}	1.21 ± 0.05^{ab}	1.14 ± 0.03^{bc}
	630	1.11 ± 0.07^{b}	1.00 ± 0.04^{b}	0.98 ± 0.03^{b}	1.16 ± 0.05^{c}	1.12 ± 0.05^{b}	1.07 ± 0.03^{bc}
	differences in least square means between Se sources						
	Se-methionine	1.20	1.13	1.03	1.27	1.21	1.14
	Na_2SeO_3	1.23	1.14	1.12	1.28	1.22	1.17
	estimate	–0.03	–0.01	–0.09	–0.01	–0.01	–0.03
	SEM	0.06	0.04	0.05	0.03	0.04	0.02
	P-value	0.062	0.30	0.033	0.30	0.60	0.041

average values ± SEM; means were calculated for 8 average samples per egg collection within a treatment
one average sample consisted of 3 yolks
$^{a–c}$values in the same column with different letters are significantly different ($P < 0.05$)

to high Se exposure and adverse cardio-metabolic effects (reviewed by Stranges et al., 2010), as well as an increased risk of diabetes (Laclaustra et al., 2009). As selenocysteine, Se is incorporated into Se-enzymes. At a range above that of Se intake at which the activities of Se-enzymes are optimized, Se is non-specifically incorporated as Se-methionine into proteins, with no further increase in the activity of Se-enzymes (Duffield et al., 1999). From this aspect, the benefit of more expensive organic Se-sources over selenite is limited.

REFERENCES

Arpášová H., Petrovič V., Mellen M., Kačániová M., Čobanová K., Leng L. (2009): The effects of supplementing sodium selenite and selenized yeast to the diet for laying hens on the quality and mineral content of eggs. Journal of Animal and Feed Sciences, 18, 90–100.

Council Directive 1999/74/EC. (1999): Laying down minimum standards for the protection of laying hens. Official Journal L 203, 03/08/1999, 53–57.

Dean W.F., Combs G.F. Jr. (1981): Influence of dietary selenium on performance, tissue selenium content, and plasma concentrations of selenium-dependent glutathione peroxidase, vitamin E, and ascorbic acid in ducklings. Poultry Science, 60, 2655–2663.

Duffield A.J., Thomson C.D., Hill K.E., Williams S. (1999): An estimation of selenium requirements for New Zealanders. American Journal of Clinical Nutrition, 70, 896–903.

EN 12822 (2000): Foodstuffs – Determination of vitamin E by high performance liquid chromatography – measurement of α-, β-, γ-, and δ-tocopherols. European Standard. European Committee for Standardization, Brussels, Belgium.

Franchini A., Sirri F., Tallarico N., Minelli G., Iaffaldano N., Meluzzi A. (2002): Oxidative stability and sensory and functional properties of eggs from laying hens fed supranutritional doses of vitamins E and C. Poultry Science, 81, 1744–1750.

Huxtable R., Bressler R. (1973): Determination of orthophosphate. Analytical Biochemistry, 54, 604–608.

ISO 3596 (1988): Animal and Vegetable Fats and Oils. Determination of Unsaponifiable Matter, Part 1: Method using diethyl ether extraction. Czech Standards Institute, Prague, Czech Republic.

Jiang Y.H., McGeachin R.B., Bailey C.A. (1994): Alpha-tocopherol, beta-carotene, and retinol enrichment of chicken eggs. Poultry Science, 73, 1137–1143.

Laclaustra M., Navas-Acien A., Stranges S., Ordovas J.M., Guallar E. (2009): Serum selenium concentrations and diabetes in US adults: National Health and Nutrition Examination Survey (NHANES) 2003–2004. Environmental Health Perspectives, 117, 1409–1413.

Muth O.H., Oldfield J.E., Remmert L.F., Schubert J.R. (1958): Effects of selenium and vitamin-E on white muscle disease. Science, 128, 1090–1090.

NRC (1994): Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, USA.

O'Grady M.N., Monahan F.J., Fallon R.J., Allen P. (2001): Effect of dietary supplementation with vitamin E and organic selenium on the oxidative stability of beef. Journal of Animal Science, 79, 2827–2834.

Payne R.L., Southern L.L. (2005): Comparison of inorganic and organic selenium sources for broilers. Poultry Science, 84, 898–902.

Payne R.L., Lavergne T.K., Southern L.L. (2005): Effect of inorganic versus organic selenium on hen production and egg selenium concentration. Poultry Science, 84, 232–237.

Piette G., Raymond Y. (1999): A comparative evaluation of methods to determine rancidity in processed meat. Fleischwirtschaft, 7, 69–73. (in German)

Rayman M.P. (2004): The use of high-selenium yeast to raise selenium status: how does it measure up? British Journal of Nutrition, 92, 557–573.

Sahin N., Sahin K., Onderci M., Karatepe M., Smith M. O., Kocuk O. (2006): Effects of dietary lycopene and vitamin E on egg production, antioxidant status and cholesterol levels in Japanese quail. Asian-Australian Journal of Animal Sciences, 19, 224–230.

SAS (2002–2003): SAS System for Windows, Release 9.1 (TS1M3), SAS Institute, Inc., Cary, NC, USA.

Skřivan M., Šimáně J., Dlouhá G., Doucha J. (2006): Effect of dietary sodium selenite, Se-enriched yeast and Se-enriched Chlorella on egg Se concentration, physical parameters of eggs and laying hen production. Czech Journal of Animal Science, 51, 163–167.

Skřivan M., Dlouhá G., Masata O., Ševčíková S. (2008a): Effect of dietary selenium on lipid oxidation, selenium and vitamin E content in the meat of broiler chickens. Czech Journal of Animal Science, 53, 306–311.

Skřivan M., Marounek M., Dlouhá G., Ševčíková S. (2008b): Dietary selenium increases vitamin E contents of egg yolk. British Poultry Science, 49, 482–486.

Skřivanová E., Marounek M., De Smet S., Raes K. (2007): Influence of dietary selenium and vitamin E on quality of veal. Meat Science, 76, 495–500.

Stranges S., Laclaustra M., Ji C., Cappucio F.P., Navas-Acien A., Ordovas J.M., Rayman M., Guallar E. (2010): Higher selenium status is associated with adverse blood

lipid profile in British adults. Journal of Nutrition, 140, 81–87.

Sünder A., Flachowsky G. (2001): Influence of high vitamin E dosages on retinol and carotinoid concentration in body tissues and eggs of laying hens. Archives of Animal Nutrition, 55, 43–52.

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, 235–243.

Surai P.F. (2006): Selenium in Nutrition and Health. Nottingham, UK, Nottingham University Press.

Tappel A.L. (1980): Vitamin E and selenium protection from *in vivo* lipid peroxidation. Annals of the New York Academy of Sciences, 355, 18–31.

Thompson J.N., Scott M.L. (1970). Impaired lipid and vitamin E absorption related to atrophy of the pancreas in selenium-deficient chicks. Journal of Nutrition, 100, 797–809.

Utterback P.L., Parsons C.M., Yoon I., Butler J. (2005): Effect of supplementing selenium yeast in diets of laying hens on egg selenium content. Poultry Science, 84, 1900–1901.

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