

*Original study*

## The effect of dietary vitamin E and selenium supplements on the fatty acid profile and quality traits of eggs

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### Abstract

The present study investigated the effect of supplementing a diet containing 5 % soybean oil, which provided 30 g/kg of polyunsaturated fatty acids (PUFAs), with two levels of vitamin E (30 or 60 mg/kg, as-is basis) in combination with two levels of selenium (0.15 or 0.30 mg/kg, as-is basis) on hen performance, the fatty acid profile and antioxidant status of the yolk. A 12-week study was performed on 32 Lohmann Brown hens with 8 birds in each group.

Dietary treatments had no influence on average egg weight and the yolk/albumen ratio, whereas an increased selenium content of diets reduced the intensity of yolk colour ( $P=0.019$ ). Increased dietary levels of vitamin E had no effect on the fatty acid profile of yolk lipids. In the yolk with a higher selenium content, the concentration of n-3 PUFAs was significantly higher (C18:3n-3,  $P=0.001$ ; C22:5n-3,  $P=0.004$ ), whereas the amount of n-6 PUFAs was significantly lower (C18:2n-6,  $P=0.015$ ; C20:4n-6,  $P=0.025$ ). A higher vitamin E content of diets significantly ( $P=0.028$ ) increased lipid-soluble antioxidative capacity (ACL), whereas increased dietary selenium levels had no effect on ACL. The noted differences in the chemical composition and physicochemical properties of eggs had no influence on their sensory attributes. In conclusion, increasing the inclusion levels of vitamin E and selenium in a diet with a high PUFA content has no effect on egg production and quality, but it increases the concentrations of both antioxidants and retinol in the yolk as well as ACL values and improves the n-6/n-3 PUFA ratio in the yolk.

**Keywords:** hens, vitamin E, selenium, egg quality, antioxidant capacity

**Abbreviations:** ACL: antioxidative capacity, FAME: fatty acid methyl ester, PUFA: polyunsaturated fatty acid, QDA: quantitative descriptive analysis, TBARS: thiobarbituric acid reactive substances

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## Introduction

The group of dietary factors known to affect the chemical composition and nutritional value of eggs (Naber 1979) includes lipids, fat-soluble vitamins and minerals, which can be easily modified through diet modification. Poultry diets are often supplemented with vegetable oils to increase their energy density and the concentrations of polyunsaturated fatty acids (PUFAs), in particular n-3 PUFAs, in the final product (Grashorn 2005, Bou *et al.* 2009). Lipids are the major constituents of the egg yolk (Ahn *et al.* 1997) and the composition and stability of the lipid fraction affects the quality and sensory properties of eggs (Franchini *et al.* 2002).

Poultry diets with an increased vegetable oil content should be supplemented with antioxidants which provide protection to both birds and poultry products including meat and eggs (Barroeta 2007). Numerous experiments have shown that increased PUFA concentrations in eggs enhance lipid peroxidation (Grashorn 2005, Mohiti-Asli *et al.* 2008), thus deteriorating the sensory properties and overall quality of eggs (Hayat *et al.* 2010). Therefore, PUFA-rich poultry diets should contain increased levels of antioxidants, in particular vitamin E. In many experiments, the effects of different dietary vitamin E levels from 100 to 200 mg/kg and more (Cherian & Sim 1997, Kirunda *et al.* 2001, Franchini *et al.* 2002, Grobas *et al.* 2002, Sahin *et al.* 2006, Hayat *et al.* 2010), were evaluated in laying hens in view of various dietary factors especially PUFA concentrations in the ration. According to Barroeta (2007), when the polyunsaturation level is low (15 g of PUFA/kg), 60 mg of dietary  $\alpha$ -tocopherol per kg of feed is necessary to assure lipid stability, whereas at a high level of dietary PUFAs (30 g/kg) 200 mg of  $\alpha$ -tocopherol per kg of feed are necessary. Such a recommendation is controversial due to the high cost of supplemental vitamin E.

The application of highly bio-available sources of dietary selenium can reduce vitamin E levels in the diet. In a number of experiments where selenium doses ranged from 0.1 to 0.3 mg/kg (Paton *et al.* 2000, Grobas *et al.* 2002, Pál *et al.* 2002, Pappas *et al.* 2005) and more (Payne *et al.* 2005, Haug *et al.* 2007, Chantiratikul *et al.* 2008, Mohiti-Asli *et al.* 2010), the vitamin E content of the diet was 10–60 mg/kg. This indicates that the optimum ratio of these antioxidants in the diet of laying hens remains unknown.

The aim of this study was to determine the effect of supplementing a diet containing 5% soybean oil, which provided 30 g/kg of PUFAs, with two levels of vitamin E (30 or 60 mg/kg) in combination with two levels of selenium (0.15 or 0.30 mg/kg) on egg production, the physicochemical properties, antioxidant capacity and sensory properties of eggs.

## Material and methods

### *Birds and diets*

The experimental materials comprised 32 Lohmann Brown laying hens aged 18 to 30 weeks. Before starting the experiment, all birds were weighed individually and divided into four experimental groups (eight replications per group). The hens were kept in battery cages with two birds per cage (30 cm  $\times$  60 cm) under controlled environmental conditions and with 14 h lightness and 10 h darkness per day. All birds had free access to feed and water. The experimental procedure was approved by the Local Animal Experimentation Ethics Committee in Olsztyn.

One basal iso-protein and iso-energetic diet (Table 1), whose nutritional value was consistent with NRC (1994), was used. The basal diet without vitamin E and selenium was supplemented with two different levels of vitamin E and two different levels of selenium to obtain four experimental diets:  $S_{0.15}E_{30}$  (supplemented with 0.15 mg selenium and 30 mg vitamin E per kg, as-is basis),  $S_{0.15}E_{60}$  (supplemented with 0.15 mg selenium and 60 mg vitamin E per kg, as-is basis),  $S_{0.30}E_{30}$  (supplemented with 0.30 mg selenium and 30 mg vitamin E per kg, as-is basis) and  $S_{0.30}E_{60}$  (0.30 mg selenium and 60 mg vitamin E per kg, as-is basis). Alkosel and tocopheryl acetate were used as sources of selenium and vitamin E, respectively. The fatty acid composition of the basal diet and soybean oil is shown in Table 2.

Table 1  
Composition and nutritional value of the basal diet

Composition	%	Nutritional value	%
Wheat	50.40	ME, MJ/kg	11.94
Maize	10.00	Total protein	16.51
Soybean meal	15.50	Crude fat	6.74
Sunflower meal	5.00	Crude fiber	4.01
Alfalfa meal	4.00	Crude ash	2.81
Soybean oil	5.00	Lysine	0.71
Salt	0.36	Methionine	0.36
Limestone	8.71	Methionine + cysteine	0.66
Monocalcium phosphate	0.43	Ca	3.60
DL-methionine (99%)	0.10	P	0.41
Mineral-vitamin premix <sup>1</sup>	0.50	Na	0.16

<sup>1</sup>Composition of the mineral-vitamin premix: vitamin A – 2 400 000 IU, vitamin D3 – 600 000 IU, vitamin E – 0.0 mg, vitamin K – 1 000 mg, vitamin B1 – 600 mg, vitamin B2 – 2 400 mg, vitamin B6 – 1 000 mg, vitamin B12 – 6 mg, folic acid – 400 mg, biotin – 60 mg, nicotinic acid – 10 000 mg, calcium pantothenate – 2 600, Mn – 24 g, Zn – 20 g, Fe – 10 g, Cu – 2 g, J – 400 mg, Se – 0 mg, Co – 30 mg, phytase – 750 FTU

Egg production was monitored for ten weeks after a two-week adjustment period. The following indices of productive performance were determined: feed intake, number of eggs laid, initial and final body weights of hens, average egg weight and feed conversion ratio (calculated as kg feed intake per kg eggs laid). Towards the end of the experiment, 12 eggs from each group were picked to determine their chemical composition and physicochemical properties and 12 eggs were collected for a sensory evaluation. The eggs were weighed and broken to determine the ratio of yolk to albumen and yolk colour on the Roche scale (yolk colour fan). Egg protein quality was estimated based on albumen height measured with an egg analyser (Orka Food Technology, Ramat Asharon, Israel). The Haugh unit (HU) was calculated from the following formula:

$$HU = \log_{10} (H - 1.7 W^{0.37} + 7.56) \quad (1)$$

where  $H$  is albumen height (mm) and  $W$  is egg weight (g).

Table 2

Fatty acid composition (% of total fatty acids) of the basal diet and soybean oil

Composition	Basal diet	Soybean oil
C12:0	0.10	–
C14:0	0.19	0.10
C14:1 (n-5)	0.02	–
C15:0	0.11	0.04
C16:0	17.88	12.64
C16:1 (n-7)	0.18	0.14
C17:0	0.67	0.43
C17:1 (n-7)	0.09	0.06
C18:0	4.62	4.16
C18:1 (n-9)	29.32	23.25
C18:2 (n-6)	42.23	52.03
C18:3 (n-3)	2.98	5.94
C20:0	0.52	0.43
C20:1 (n-9)	0.32	0.22
C20:2 (n-6)	0.03	0.04
C20:4 (n-6)	0.04	–
C22:0	0.70	0.52
Total SFA <sup>1</sup>	24.79	18.32
Total MUFA <sup>2</sup>	29.93	23.67
Total PUFA <sup>3</sup>	45.28	58.01

<sup>1</sup>SFA: C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0, <sup>2</sup>MUFA: C14:1 + C16:1 + C17:1 + C18:1 + C20:1,<sup>3</sup>PUFA: C18:2 + C18:3 + C20:2 + C20:4

### Chemical analysis

The content of dry matter and fat in the egg yolk was determined using official methods of analysis 934.01 and 930.09, respectively, of the Association of Official Analytical Chemists (AOAC 2005). Lipid-soluble antioxidative capacity (ACL) in yolk samples was determined using the Analytik Jena Photochem system (Analytik Jena AG, Jena, Germany). The ACL of the sample was quantified by comparison with a standard (construction of a calibration curve using Trolox) and was expressed in corresponding equivalent concentration units. The selenium content of the lyophilized yolk was determined by electrothermal atomic absorption spectrometry at the Institute of Agricultural and Food Biotechnology in Warsaw, using a Varian SpectrAA 880Z spectrophotometer (Varian, Palo Alto, CA, USA) after sample mineralization with HNO<sub>3</sub> in the Microwave Accelerated Reaction System CEM Mars 5 (CEM GmbH, Kamp-Lintfort, Germany) (Jędrzejczak *et al.* 1999). Retinol and tocopherols were measured in the egg yolk according to the method proposed by Rettenmaier & Schüep (1992).

The concentrations of fatty acids in the yolk were determined in fresh eggs. Fat was extracted from samples by the Soxhlet method. Four or five drops (50-60 mg) of extracted fat were transferred to 2 cm<sup>3</sup> ampoules, 1.5 cm<sup>3</sup> of a methanol-chloroform-concentrated

sulphuric acid (100 : 100 : 1 v/v) mixture were added and the ampoules were sealed by melting their tops with an open flame. Sealed vials were placed in a dryer at 80-90 °C and were heated for 2-3 h. During heating, the ampoules were shaken every 15 min. After solvent evaporation and ampoule cooling, 0.5-1 ml of hexane was added to each sample and thoroughly mixed. The resulting fatty acid methyl esters (FAMEs) were analysed using a Varian CP-3800 gas chromatograph (Varian, USA) with flame-ionization detection. Fatty acid methyl ester were injected at 225 °C, separated on a capillary column (50 m, 0.25 mm, 0.25 µm; Varian, USA) using a temperature gradient programme (3 min at 50 °C, from 50 ° to 200 °C in 40 min) and detected via flame-ionization at 250 °C. The peaks of fatty acids were identified by comparing their relative retention times with those of individual FAME reference standards (Supelco, Sigma Aldrich group) diluted in hexane (1 : 1, 1 : 2, 1 : 3, 1 : 4 v/v).

The percentaged content of fatty acids and the total pool of PUFAs, including n-3 PUFAs (C18:3n-3, C20:5n-3 C22:5n-3 and C22:6n-3) and n-6 PUFAs (C18:2n-6, C20:2n-6, C20:4 n-6), were determined by relative fatty acid quantification and % of the total fatty acid peak area was calculated.

#### *Sensory evaluation*

A six-member trained panel (ISO 8586-2:1994) experienced in a descriptive analysis of different food products and familiarized with the sensory quality of eggs performed the assessments. The assessments were carried out at a sensory laboratory room in individual booths equipped with a computerized system for data collection and processing (FIZZ, Biosystemes, Counternon, France) as described elsewhere (Horszwald *et al.* 2009). The laboratory room fulfilled the requirements of the ISO standard (ISO 8589:1998).

For a sensory evaluation, eggs were cooked in boiling water for 12 min, cooled to an external temperature of about 20 °C, shelled and cut in half. The samples were presented in random order to the assessors from three-digital coded transparent plastic containers covered by lids. Together with the samples, the panellists received a cup of spring water at room temperature for cleaning their palates.

A quantitative descriptive analysis (QDA) was used to determine the sensory characteristics of the samples. The panellists, together with the panel leader, established the descriptions of the main sensory attributes of eggs using a standard procedure (ISO/DIS 13299:1998). Nine attributes related to the colour, aroma, taste and mouth feel (adhesiveness) of eggs were selected and thoroughly defined for profiling. The panellists evaluated the intensity perceived for each of the attributes on a continuous unstructured graphical scale. The scale was 10 cm long and verbally anchored at each end. The left side of the scale corresponded to the lowest intensity and the right side to the highest intensity of the attribute. The results were automatically converted to numerical values (from 0 to 10 units) by a computer. All samples were evaluated in three replications (three sessions) preceded by an introductory session.

#### *Statistical analyses*

The STATISTICA software package v. 8.0 (StatSoft Corp., Cracow, Poland) was used to determine whether variables differed among treatment groups. A two-way ANOVA was performed

to assess the effects of the supplementation level of vitamin E (30 and 60 mg/kg of diet), the supplementation level of selenium (0.15 and 0.30 mg/kg of diet) and the interaction between vitamin E and selenium dosages (Vitamin E×Se). When the ANOVA indicated significant treatment effects, means were separated using Duncan's multiple range test. In the tables, results are presented as mean values with pooled standard errors. Data were checked for normal distribution before the statistical analysis was performed. Differences were considered to be significant at  $P \leq 0.05$ . In the case of the sensory analysis, the data were analysed by the one-way ANOVA statistic module.

## Results

In the basal diet given to all experimental groups, PUFAs accounted for 45.3 % of total fatty acids (Table 2) and their levels were lower than PUFA concentrations in soybean oil (58.0 %) which was added to the diet.

Diet supplementation with selenium and vitamin E had no influence on the body weights of hens, the number of eggs laid, average egg weight and feed conversion ratio (Table 3). Different dietary levels of selenium and vitamin E had no significant effect on the yolk to albumen ratio and egg protein quality (HU). The two-way ANOVA showed that an increased selenium content of diets reduced the intensity of yolk colour ( $P=0.019$ ), while supplemental vitamin E had no impact on this trait (Table 4).

Dietary treatments affected the fatty acid profile of yolk lipids (Table 5) and the differentiating factor was selenium content. A higher selenium content of diets significantly increased the concentrations of C18:3n-3 ( $P=0.001$ ) and C22:5n-3 ( $P=0.004$ ) and decreased the percentage amounts of C18:2n-6 ( $P=0.015$ ) and C20:4n-6 ( $P=0.025$ ) in the total fatty acid pool. As a result, the yolks of eggs laid by hens fed diets with increased selenium levels had a higher n-3 PUFA content ( $P=0.001$ ), a lower n-6 PUFA content ( $P=0.009$ ) and a lower n-6/n-3 fatty acid ratio ( $P=0.001$ ). A significant interaction between the experimental factors was noted for C22:5n-3 ( $P=0.031$ ) and total unsaturated fatty acids ( $P=0.033$ ). Higher levels of both antioxidants significantly increased the concentrations of C22:5n-3. A higher content of selenium or vitamin E in diets increased the total unsaturated fatty acid pool which was not affected by the inclusion of both antioxidants in diets.

An increase in the selenium content of diets led to a significant ( $P=0.008$ ) increase in selenium concentration in the yolk (Table 6). The levels of retinol and  $\alpha$ -tocopherol in the yolk increased in response to feeding supplemental selenium ( $P=0.010$  and 0.001, respectively) and vitamin E ( $P=0.004$  and  $P=0.001$ , respectively). Different dietary selenium levels had no effect on ACL, whereas a higher vitamin E content of diets significantly increased ACL values ( $P=0.028$ ).

A QDA employed in a sensory evaluation elicited nine attributes as follows: one for yolk colour, three for aroma, four for taste and one for mouth feel. The mean sensory ratings for the samples and the analysis of variance are presented in Table 7. The ANOVA showed that there were no significant differences in the intensity of the analysed attributes. The mean values of samples show that the sensory profiles of eggs were highly similar with respect to the intensity of all analysed attributes. In all samples, the dominant sensory notes were positive such as egg-like aroma and egg-like taste.

Table 3

Effect of dietary selenium and vitamin E level on the performance of laying hens

Group	Body weight, kg		Diet intake, g/day	Egg number	Average egg weight, g	FCR, kg/kg eggs
	Initial	Final				
Se <sub>0.15</sub> E <sub>30</sub>	1.53	1.72	132.0	53.9	54.2	2.54
Se <sub>0.15</sub> E <sub>60</sub>	1.53	1.72	129.3	53.6	56.7	2.39
Se <sub>0.30</sub> E <sub>30</sub>	1.57	1.79	132.9	54.7	57.5	2.37
Se <sub>0.30</sub> E <sub>60</sub>	1.57	1.77	131.4	55.0	56.7	2.36
SEM	0.022	0.022	1.192	0.427	0.749	0.034
Selenium (Se)						
0.15	1.53	1.72	130.6	53.7	55.5	2.46
0.30	1.57	1.78	132.1	54.9	57.1	2.37
P	0.401	0.196	0.567	0.224	0.307	0.169
Vitamin E						
30	1.55	1.76	132.4	54.3	55.8	2.45
60	1.57	1.75	130.3	54.3	56.7	2.38
P	0.978	0.836	0.422	0.978	0.576	0.242
Se × E	0.935	0.923	0.827	0.754	0.304	0.296

Se: selenium, E: vitamin E, Se × E2: interaction Se × vitamin E level, FCR: feed conversion ratio

Table 4

Effect of dietary selenium and vitamin E level on egg white and egg yolk characteristics

Group	Albumen			Yolk			
	Weight, %	Height, mm	Haugh units	Weight, %	Dry matter, %	Ether extract, %	Colour
Se <sub>0.15</sub> E <sub>30</sub>	66.4	5.0	69.9	23.6	26.65	28.00	8.7
Se <sub>0.15</sub> E <sub>60</sub>	66.2	5.2	69.2	24.3	26.87	27.16	7.9
Se <sub>0.30</sub> E <sub>30</sub>	66.5	4.9	66.3	23.9	26.17	27.23	6.8
Se <sub>0.30</sub> E <sub>60</sub>	66.6	5.6	74.3	24.6	25.87	27.04	7.6
SEM	0.270	0.135	1.384	0.250	0.229	0.168	0.245
Selenium (Se)							
0.15	66.3	5.1	69.5	24.0	26.77	27.61	8.3 <sup>a</sup>
0.30	66.6	5.3	70.3	24.2	26.02	27.10	7.2 <sup>b</sup>
P	0.638	0.524	0.787	0.634	0.114	0.177	0.019
Vitamin E (E)							
30	66.5	5.0	68.1	23.7	26.41	27.58	7.7
60	66.4	5.4	71.7	24.5	26.38	27.13	7.8
P	0.864	0.131	0.188	0.158	0.946	0.122	0.929
Se × E	0.782	0.387	0.119	0.979	0.558	0.320	0.094

Se: selenium, E: vitamin E, Se × E2: interaction Se × vitamin E level, <sup>ab</sup>Means in the same column without common superscripts differ significantly at P≤0.05

Table 5

Effect of dietary selenium and vitamin E level on fatty acids (% of the total fatty acid peak area) of yolk lipids

Group, factor	C14:0 n-5	C14:1 n-5	C15:0	C16:0	C16:1 n-7	C17:0 n-7	C17:1 n-7	C18:0 n-9	C18:1 n-6	C18:2 n-3	C20:0 n-9	C20:1 n-6	C20:2 n-6	C20:4 n-3	C20:5 n-3	C22:0 n-3	C22:5 n-3	C22:6 n-3	Total UFA n-6	Total n-6	Total n-3	n-6/ n-3	
<b>Group</b>																							
Se <sub>0.15</sub> E <sub>30</sub>	0.33 <sup>ab</sup>	0.05 <sup>ab</sup>	0.07	24.71 <sup>a</sup>	2.27 <sup>b</sup>	0.26	0.19	7.36	35.58	24.69	1.48	0.02	0.19	0.20	1.67	0.05	0.04	0.13 <sup>bc</sup>	0.71	67.22 <sup>b</sup>	26.56	2.37	11.19
Se <sub>0.15</sub> E <sub>60</sub>	0.30 <sup>bc</sup>	0.04 <sup>b</sup>	0.77	23.47 <sup>b</sup>	2.13 <sup>b</sup>	0.25	0.21	7.20	36.03	25.74	1.58	0.02	0.20	0.21	1.68	0.04	0.04	0.10 <sup>b</sup>	0.70	68.65 <sup>a</sup>	27.62	2.43	11.41
Se <sub>0.30</sub> E <sub>30</sub>	0.30 <sup>c</sup>	0.05 <sup>b</sup>	0.07	22.90 <sup>b</sup>	2.40 <sup>ab</sup>	0.22	0.23	6.86	37.40	23.79	2.86	0.02	0.20	0.20	1.54	0.03	0.04	0.14 <sup>c</sup>	0.75	69.58 <sup>a</sup>	25.53	3.78	7.26
Se <sub>0.30</sub> E <sub>60</sub>	0.34 <sup>a</sup>	0.06 <sup>a</sup>	0.07	23.22 <sup>b</sup>	2.64 <sup>a</sup>	0.22	0.21	6.89	36.31	23.16	3.93	0.02	0.21	0.20	1.51	0.02	0.04	0.17 <sup>ac</sup>	0.80	69.21 <sup>a</sup>	24.86	4.92	6.20
SEM	0.005	0.002	0.002	0.203	0.056	0.007	0.006	0.109	0.217	0.364	0.288	0.001	0.006	0.005	0.032	0.003	0.002	0.007	0.022	0.251	0.366	0.306	0.511
<b>Selenium (Se)</b>																							
0.15	0.31	0.05 <sup>b</sup>	0.08 <sup>a</sup>	24.09	2.20 <sup>b</sup>	0.25 <sup>a</sup>	0.20	7.28	35.80 <sup>b</sup>	25.21 <sup>a</sup>	1.53 <sup>b</sup>	0.02	0.20	0.20	1.67 <sup>a</sup>	0.05 <sup>a</sup>	0.04	0.12 <sup>b</sup>	0.70	67.93 <sup>b</sup>	27.09 <sup>a</sup>	2.40 <sup>b</sup>	11.30 <sup>a</sup>
0.30	0.32	0.05 <sup>a</sup>	0.07 <sup>b</sup>	23.06	2.52 <sup>a</sup>	0.22 <sup>b</sup>	0.22	6.87	36.85 <sup>a</sup>	23.47 <sup>b</sup>	3.39 <sup>a</sup>	0.02	0.20	0.20	1.52 <sup>b</sup>	0.03 <sup>b</sup>	0.04	0.16 <sup>a</sup>	0.78	69.4 <sup>a</sup>	25.19 <sup>b</sup>	4.35 <sup>a</sup>	6.73 <sup>b</sup>
P	0.552	0.021	0.071	0.005	0.002	0.017	0.104	0.073	0.01	0.015	0.001	0.768	0.548	0.487	0.025	0.003	0.871	0.004	0.103	0.001	0.009	0.001	0.001
<b>Vitamin E (E)</b>																							
30	0.31	0.05	0.07	23.80	2.33	0.24	0.21	7.11	36.49	24.24	2.17	0.02	0.20	0.20	1.61	0.04	0.041	0.14	0.73	68.4	26.04	3.08	9.22
60	0.32	0.05	0.07	23.35	2.38	0.23	0.21	7.04	36.17	24.45	2.75	0.02	0.20	0.20	1.59	0.03	0.039	0.14	0.75	68.93	26.24	3.67	8.81
P	0.415	0.718	0.861	0.187	0.595	0.558	0.784	0.769	0.34	0.758	0.227	0.820	0.589	0.496	0.757	0.212	0.677	0.967	0.685	0.205	0.769	0.248	0.508
Se × E	0.001	0.011	0.421	0.029	0.051	0.983	0.113	0.669	0.053	0.223	0.312	0.479	0.965	0.291	0.807	0.903	0.691	0.031	0.496	0.033	0.203	0.290	0.313

Se: selenium, E: vitamin E, Se × E: interaction Se × vitamin E level, UFA: unsaturated fatty acid (MUFA+PUFA), n-3 PUFA: C18:3+C20:5+C22:5+C22:6, n-6 PUFA: C18:2+C20:2+C22:4,

<sup>ab</sup>Means in the same column without common superscripts differ significantly at P≤0.05

Table 6

Effect of dietary selenium and vitamin E on selected antioxidants and antioxidant capacity of egg yolk

	Selenium, µg/g	Retinol, µg/g	α-tocopherol, µg/g	ACL, µg/g
<b>Group</b>				
Se <sub>0.15</sub> E <sub>30</sub>	1.082	3.381	134.2	0.155
Se <sub>0.15</sub> E <sub>60</sub>	1.052	3.986	202.9	0.192
Se <sub>0.30</sub> E <sub>30</sub>	1.352	3.910	158.6	0.167
Se <sub>0.30</sub> E <sub>60</sub>	1.422	4.576	257.8	0.186
SEM	0.062	0.123	9.623	0.006
<b>Selenium (Se)</b>				
0.15	1.067 <sup>b</sup>	3.683 <sup>b</sup>	168.5 <sup>b</sup>	0.174
0.30	1.387 <sup>a</sup>	4.243 <sup>a</sup>	208.2 <sup>a</sup>	0.177
<i>P</i>	0.008	0.010	0.001	0.807
<b>Vitamin E (E)</b>				
30	1.217	3.645 <sup>b</sup>	146.4 <sup>b</sup>	0.161 <sup>b</sup>
60	1.237	4.281 <sup>a</sup>	230.3 <sup>a</sup>	0.189 <sup>a</sup>
<i>P</i>	0.847	0.004	0.001	0.028
Se × E	0.629	0.881	0.125	0.464

Se: selenium, E: vitamin E, Se × E: interaction Se × vitamin E level, <sup>ab</sup>Means in the same column without common superscripts differ significantly at *P*≤0.05

Table 7

Average intensity values of egg sensory attributes

Attribute	Group			
	Se <sub>0.15</sub> E <sub>30</sub>	Se <sub>0.15</sub> E <sub>60</sub>	Se <sub>0.30</sub> E <sub>30</sub>	Se <sub>0.30</sub> E <sub>60</sub>
<b>Appearance</b>				
Yolk color	6.4	6.4	6.4	6.4
<b>Odour</b>				
Egg-like	7.3	6.8	6.9	6.9
Buttery	2.5	2.5	2.5	2.6
Sulfur-like	1.0	1.0	1.0	1.0
<b>Taste</b>				
Egg-like	7.0 <sup>a</sup>	6.9	6.8	6.9
Buttery	2.4	2.4	2.2	2.5
Sulfur-like	0.3	0.3	0.3	0.3
Aftertaste	6.7	6.5	6.6	6.6
<b>Texture</b>				
Adhesiveness	5.8	5.7	5.7	6.0

Se: selenium, E: vitamin E, <sup>ab</sup>Means in the same column without common superscripts differ significantly at *P*≤0.05

## Discussion

A natural consequence of vegetable oil supplementation of poultry diets is an increase in the percentage amounts of PUFAs in the total fatty acid pool (Grashorn 2005, Barroeta 2007, Bou *et al.* 2009). In our experiment, the concentration of PUFAs in the basal diet was 45.3%, because the added soybean oil was diluted with lipids from grain and soybean. The dose of PUFAs added to the diet with soybean oil was about 30 g/kg and such an amount of PUFAs requires high antioxidant protection (Barroeta 2007).

In the current experiment, an increase in the content of vitamin E and selenium in hen diets from 30 to 60 mg/kg or/and from 0.15 to 0.30 mg/kg, respectively, had no effect on the number of eggs laid, average egg weight, the yolk to albumen ratio and the fat content of yolk. Our results corroborate the findings of other authors who demonstrated that egg production and egg weight are insignificantly affected by the dietary levels of vitamin E (Melluzi *et al.* 2000, Grobas *et al.* 2002, Mohiti-Asli *et al.* 2010) and selenium (Jiakui & Xiaolong 2004, Chantiratikul *et al.* 2008, Mohiti-Asli *et al.* 2010). In the experiments cited above, supplemental antioxidants had no effect on yolk weight relative to the total egg weight. In another experiment (Skřivan *et al.* 2006), egg yolk weight was lower in groups of laying hens fed diets supplemented with organic selenium as compared with mineral selenium.

In the present study, an increase in the vitamin E content of hen diets from 30 to 60 mg/kg increased the concentrations of  $\alpha$ -tocopherol and retinol in the yolk and significantly increased ACL values but it did not affect the fatty acid profile of yolk lipids.

The observed increase in vitamin E concentrations in the yolk was consistent with the results of other experiments (Grobas *et al.* 2002, Scheideler *et al.* 2010), while the increase in retinol concentrations was more pronounced than that reported by other authors. Pál *et al.* (2002) demonstrated that supplemental vitamin E increased vitamin A concentrations in the egg yolk when added to hen diets at 30 mg/kg, but diet supplementation with 60 mg vitamin E significantly decreased yolk retinol concentrations. The authors of previous studies (Abawi *et al.* 1985, Bárdos *et al.* 1996) also noted that low and moderate doses of vitamin E more effectively increased the retinol content of the yolk than high doses.

In our study, higher dietary levels of vitamin E increased ACL values, which is consistent with the findings of other authors (Puthpong Siriporn *et al.* 2001) who expressed the antioxidant effect of increased vitamin E doses in terms of a reduction in thiobarbituric acid (TBA) values, as an indicator of lipid peroxidation. In an experiment where laying hens were fed diets supplemented with pumpkin seed oil, thiobarbituric acid reactive substances (TBARS) values increased in eggs relative to the control group, if the oil was fed together with 30 mg/kg vitamin E and decreased when vitamin E dose was increased to 60 mg/kg (Pál *et al.* 2002). A substantial increase in vitamin E concentrations in the diet from 100 to 200  $\mu$ g/g did not affect TBARS values in fresh and refrigerated eggs (Franchini *et al.* 2002).

In the present study, the fatty acid profile of yolk lipids was not influenced by increased dietary levels of vitamin E. Meluzzi *et al.* (2000) found that different doses of dietary vitamin E (0, 50, 100 and 200 mg/kg) slightly affected the fatty acid composition of the yolk. In an experiment performed by Cherian *et al.* (1996), dietary tocopherols resulted in a significant increase ( $P<0.05$ ) in the content of C20:5n-3 and C22:6n-3 in the yolk, most probably due to the beneficial effect of tocopherols on n-3 fatty acid synthesis via desaturation of n-6 PUFAs.

In other studies (Cortinas *et al.* 2004, Zduńczyk *et al.* 2011), the inclusion of supplemental vitamin E in broiler chicken diets had no influence on the fatty acid profile of meat.

In the present study, an increase in the selenium content of hen diets from 0.15 to 0.30 mg/kg significantly increased the concentrations of selenium,  $\alpha$ -tocopherol and retinol concentrations in eggs and increased the percentage amount of n-3 PUFAs, thus increasing the concentrations of C18:3n-3 and C22:5n-3 and decreasing the concentrations of C18:2n-6 and C20:4n-6 in the total fatty acid pool.

Our results corroborate the findings of other authors who demonstrated that diet supplementation with selenium increased the content of this element in the yolk (Pappas *et al.* 2005, Chantiratikul *et al.* 2008, Scheideler *et al.* 2010) and modified the concentrations of selected fatty acids in poultry products including an increase in C22:6n-3, a decrease in C20:4n-6 in the yolk (Pappas *et al.* 2005) and an increase in the concentrations of long-chain n-3 fatty acids, EPA (20:5), DPA (22:5) and DHA (22:6) in broiler chicken meat Haug *et al.* (2007). The main factor decreasing C20:4n-6 levels could be a selenium-dependent increase in the activity of glutathione peroxidase and enhanced biosynthesis of prostaglandins with the involvement of precursors including arachidonic acid (Hong *et al.* 1989).

In our experiment, the intensity of yolk colour decreased in response to selenium addition, which is difficult to explain because antioxidants delay or prevent the oxidation of carotenoid pigments contained in feed (Mohiti-Asli *et al.* 2010). The results of studies investigating the effects of feeding supplemental antioxidants to laying hens on yolk colour are, however, inconclusive. In one experiment, selenium and vitamin E levels did not affect egg yolk colour (Mohiti-Asli *et al.* 2008), while in another study the addition of vitamin E (20 and 40 mg/kg) to hen diets reduced yolk colour intensity in comparison with the control group (Ell-Mallah *et al.* 2011).

In the present study, a higher selenium content of diets had no effect on ACL values. Feeding organic selenium to turkeys decreased the levels of TBARS in the yolk (Jankowski *et al.* 2011). In other experiments, increased dietary selenium levels affected neither the total antioxidant status of leg muscles in broiler chickens (Haug *et al.* 2007) nor the antioxidant properties of turkey seminal plasma (Słowińska *et al.* 2011).

In the present experiment, a significant interaction between vitamin E and selenium levels was noted for C22:5n-3 and total unsaturated fatty acids. Similar results were obtained in previous experiments: dietary tocopherols resulted in a significant increase in the content of C20:5n-3 and C22:6n-3 in the yolk (Cherian *et al.* 1996, Cherian & Sim 1997) and high intake of organic selenium resulted in increased concentrations of C22:6n-3 in the egg yolk (Pappas *et al.* 2005) and increased concentrations of long-chain n-3 fatty acids in broiler chicken meat (Haug *et al.* 2007). The above could be due to a beneficial influence of dietary tocopherols on n-3 fatty acid synthesis via desaturation of n-6 PUFAs (Cherian *et al.* 1996) and a direct effect of selenium on the activity of glutathione peroxidase and biosynthesis of prostaglandins with the involvement of precursors including n-3 fatty acids (Hong *et al.* 1989).

The QDA used in the present experiment for a sensory evaluation of eggs is a sophisticated tool employed to study a variety of food products (Stone & Sidel 1993, Lawless & Heymann 1999). No significant differences in the sensory properties of eggs including appearance, aroma, taste and texture were observed between groups. The sensory attributes of eggs were not affected by dietary antioxidants in other experiments where hen diets were supplemented

with vitamin E (Parpinello *et al.* 2006), vitamin E and butylated hydroxytoluene (Hayat *et al.* 2010) or vitamins E and C (Franchini *et al.* 2002). In a study by Haug *et al.* (2007), supplemental selenium fed to broiler chickens had no effect on the sensory quality parameters of muscles, which could suggest that the sensory properties of poultry products are not affected by dietary antioxidants.

It can be concluded that laying hens' diets with a high PUFA content (about 30 g/kg) and different levels of vitamin E (30 or 60 mg/kg, as-is basis) and selenium (0.15 or 0.30 mg/kg, as-is basis) have no effect on egg production and the physicochemical and sensory properties of eggs, but higher levels of the antioxidants increase the concentrations of vitamin E, selenium and retinol in the yolk. Higher concentrations of vitamin E increase ACL values, higher concentrations of selenium improve the n-6/n-3 PUFA ratio and both antioxidants increase the concentration of C22:5n-3 in the yolk.

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