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Original article

The effect of different dietary levels of vitamin E and selenium on antioxidant status and immunological markers in serum of laying hens

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Abstract

The effect of different dietary levels of selenium (Se) and vitamin E on egg production, the antioxidant status and the immune system response of hens was investigated in the current study. A total of 32 Lohman Brown hens were divided into four groups and were fed diets with 5% of soybean oil and two levels of Se (0.15 and 0.30 mg/kg) and vitamin E (30 and 60 mg/kg). During 10 weeks of experimental feeding, the body weights of hens and egg production were similar in all dietary treatments, but a higher Se content of diets contributed to a significant increase in egg weight. A higher vitamin E level significantly increased α-tocopherol concentrations (2.71 vs. 2.05 μg/mL, $p = 0.001$), superoxide dismutase (SOD) activity (43.3 vs. 39.9 U/mL, $p = 0.049$) and the ferric reducing ability of serum (FRAP) (123.0 vs. 105.7 μmol/L, $p = 0.029$). A higher Se content increased the concentrations of ascorbic acid (0.309 vs. 0.073 μg/L, $p = 0.001$), retinol (1.48 vs. 1.15 μg/mL, $p = 0.001$) and α-tocopherol (2.86 vs. 1.90 μg/mL, $p = 0.001$), the activity levels of catalase (3.40 vs. 2.98 U/L, $p = 0.010$) and SOD (43.4 vs. 30.8 U/mL, $p = 0.040$) as well as the total antioxidant status (TAS) of serum (0.38 vs. 0.28 mmol/L, $p = 0.026$). There were no significant differences in the concentrations of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) between treatments, while a higher vitamin E content of diets increased serum immunoglobulin A (IgA) concentrations (370.6 vs. 321.3 μg/mL, $p = 0.026$). Higher dietary levels of vitamin E and Se increased the serum concentrations of retinol (1.66 vs. 2.20 μg/mL, $p = 0.013$) and α-tocopherol (3.33 vs. 1.71, $p = 0.014$), but they had no effect on the other physiological parameters. It is recommended that the levels of both supplements, Se and vitamin E, be increased in laying hen diets as they have a beneficial effect on the serum concentrations of retinol and α -tocopherol.

Key words: laying hens, selenium, vitamin E, antioxidant status, IgA, IL-6, TNF-α.

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Introduction

There has been a steady increase in the vegetable oil content of poultry diets over the past ten years. Such diets have a higher energy density (Leeson et al. 1996), in particular if the additional energy source is fat rich in unsaturated fatty acids (Crespo and Esteve-Garcia 2001). Other important consideration includes a ban on the feeding of farmed animals of a given species with processed animal protein derived from the bodies or parts of bodies of animals of the same species. With that problem is also connected a rising consumer demand for poultry meat products with increased concentrations of polyunsaturated fatty acids (PUFAs) (Bou et al. 2009, Jia et al. 2010). PUFAs are highly prone to oxidation, therefore PUFA-supplemented diets should contain antioxidants such as vitamin E (Barroeta 2007, Czech et al. 2012). Diet supplementation with antioxidants also stimulates avian immunity (Muir et al. 2002, Sahin et al. 2010).

Numerous experiments have been performed on laying hens fed diets supplemented with different doses of vitamin E, from 20-60 mg/kg (Kirunda et al. 2001) to 100-200 mg/kg (Cortinas et al. 2004, Zduńczyk et al. 2011), and higher (Chung et al. 2005). Some authors (Cortinas et al. 2004, Bou et al. 2009) question the effectiveness of this dietary strategy due to high feed costs and inconclusive or even contradictory results of studies investigating the effects of high vitamin E levels in hen diets. The recommended vitamin E content of hen diets is 5 mg/kg (NRC, 1994). In Poland, diets for laying hens contain higher amounts of vitamin E, from 20 to 60 mg/kg (Smulikowska and Rutkowski 2005). According to Barroeta (2007), when the polyunsaturation level is low (15 g PUFA/kg) 60 mg of dietary tocopherol per kg of feed is necessary to assure lipid stability.

The vitamin E content of rations for laying hens should be reduced if the diet contains also other antioxidants such as Se (Barroeta 2007). Commercial rations for egg laying hens contain 0.15 mg Se per kg feed and this value is three-fold higher than the recommended Se intake (NRC 1994). Research findings show that dietary Se, in particular organic Se, improves the health status and productivity of laying hens as well as the quality of stored eggs (Payne et al. 2005, Leeson et al. 2008). In recent years, highly bioavailable organic selenium sources have been widely used in poultry nutrition. Thus, it seems important to establish the optimal ratio of Se to vitamin E in layer diets.

Vitamin E and selenium have complementary but independent roles as antioxidants in the protection of cells against the damaging effects of lipid peroxides (Brigelius-Flohe and Traber 1999). In view of the above, the objective of this study was to determine the effect of increased dietary levels of vitamin E (30 and 60 mg/kg) and/or Se (0.15 and 0.30 mg/kg) on egg production, the antioxidant status and the immune system response of laying hens.

Materials and Methods

Hens and diets

The experimental material comprised 32 Lohman Brown laying hens aged 18 weeks. Before the experiment, all birds were weighed individually, and then they were divided into four experimental groups (eight hens per group). The hens were kept in battery cages, two birds per cage (30 cm x 60 cm), under controlled environment conditions, with 14 hours light and 10 hours dark. All birds had free access to feed and water. The experimental procedure was approved by the Local Animal Experimentation Ethics Committee in Olsztyn.

Experimental iso-protein and iso-energetic diets contained different levels of vitamin E and Se in organic form. The basal diet, whose composition is given in Table 1, was supplemented with 50% tocopheryl acetate and Alkosel® R397 (Lallemand Animal Nutrition), as a source of Se, predominantly in the form of selenomethionine.

In the control diet ($Se_{0.15}E_{30}$), the content of Se (0.15 mg/kg) and vitamin E (30 mg/kg) corresponded to the lower levels of the above antioxidants recommended in Poland (Smulikowka and Rutkowski 2005). In experimental diets, the levels of both antioxidants varied, as follows: Se content was increased to 0.30 mg/kg (group $Se_{0.30}E_{30}$) or vitamin E content was increased to 60 mg/kg (group $Se_{0.15}E_{60}$) or Se and vitamin content was increased to 0.30 mg/kg and 60 mg/kg, respectively (group $Se_{0.30}E_{60}$).

Egg production was monitored for 10 weeks, after a two-week adjustment period. Feed conversion ratio (FCR) was calculated as kg feed intake per kg eggs laid. The hens were weighed again after 12 weeks of the experiment and blood was sampled to determine biochemical parameters, including the antioxidant status.

Biochemical analysis

Serum uric acid levels were determined with a Helios Alpha spectrophotometer (Unicam) and Cormay mono tests. Birds blood plasma was also examined to measure spectrophotometrically the activity of superoxide dismutase (SOD) using the adrenaline assay method according to MISRA (Heikkila 1985). The SOD activity is determined measuring the rate of auto-oxidation of adrenaline at 30°C on the basis of the increase of absorbance at 320 nm (that corresponds to monitoring of the increase of concentration of various products of adrenaline oxidation). Besides, the activity of catalase (CAT) was estab-

Table 1. Composition and nutritional value of the basal diet.

Composition	%	Calculated 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[1]	0.50	Na	0.16

[1] Compositin of the mineral-vitamin premix: vitamin A – 2 400 000 IU, vitamin D3 – 600 000 IU, vitamin E – 0.0 mg, vitamin K – 1000 mg, vitamin B1 – 600 mg, vitamin B2 – 2400 mg, vitamin B6 – 1000 mg, vitamin B12 – 6 mg, folic acid – 400 mg, biotin – 60 mg, nicotinic acid – 10 000 mg, calcium pantothenate – 2600, Mn – 24 g, Zn – 20 g, Fe – 10 g, Cu – 2 g, J – 400 mg, Se – 0 mg, Co – 30 mg, phytase – 750 FTU

Table 2. Performance of laying hens fed diets with different levels of Se and vitamin E.

	Final body weight, kg	Daily egg production, %	Total egg weight, g/hen
Treatment			
$S_{0.15}E_{30}$	1.72	96.2	2 917
$S_{0.15}E_{60}$	1.72	95.7	3 033
$S_{0.30}E_{30}$	1.79	97.7	3 141
$S_{0.30}E_{60}$	1.77	98.2	3 119
SEM	0.022	0.762	34.49
Se, mg/kg			
0.15	1.72	95.9	2 975[b]
0.30	1.78	98.0	3 130[a]
p	0.196	0.224	0.021
Vitamin E, mg/kg			
30	1.76	96.9	3 029
60	1.75	96.9	3 076
p	0.836	1.00	0.438
Se x vit.E interaction	0.923	0.754	0.259

a,b Means in the same column without common superscripts differ significantly at $p \leq 0.05$.

lished, following the Clairborne method (1985). The analysis was based on measurement of a substrate decomposition rate (hydrogen peroxide) catalyzed by this enzyme.

Plasma total antioxidant status (TAS) was measured using Randox kit, according to the procedure of Miller at al. (1993). The total antioxidant potential of the plasma (FRAP) was recorded following Benzie and Strain (1996). The method utilized the antioxidant power to cause Fe^{+3} to Fe^{+2} reduction which forms a colored complex with 2,4,6 tripyridyltriazine (TPTZ) present in the solution. The absorbance increase of the TPTZ- Fe^{+2} complex is proportional to antioxidant amount in the test tube.

Vitamin C content in the blood serum of was estimated by the method of Omaye et al. (1979), concentrations of tocopherols and retinol were determined by the method described by Rettenmaier and Schüep (1992) and hydrogen peroxide concentrations (H_2O_2) – as described by Gay and Gębicki (2002).

Immunological parameters

The immune system response of laying hens was determined based on the serum levels of IgA and selected cytokins: IL-6 and TNF-α, using the Bigenet UMV340 blood cell reader, USCN Life Science Inc. kits (IL-6 and IgA) and Cusabio Biotech Co. Ltd. kits (TNFα).

Table 3. Concentrations of selected vitamins in the blood serum of laying hens.

	Retinol μg/mL	α-tocopherol μg/mL	Vitamin C μg/L
Treatment			
$S_{0.15}E_{30}$	1.20[b]	1.71[c]	0.068
$S_{0.15}E_{60}$	1.11[b]	2.10[b]	0.079
$S_{0.30}E_{30}$	1.31[b]	2.39[b]	0.315
$S_{0.30}E_{60}$	1.66[a]	3.33[a]	0.299
SEM	0.054	0.118	0.022
Se, mg/kg			
0.15	1.15[b]	1.90[b]	0.073[b]
0.30	1.48[a]	2.86[a]	0.309[a]
p	0.001	0.001	0.001
Vitamin E, mg/kg			
30	1.25	2.05[b]	0.192
60	1.36	2.71[a]	0.189
p	0.109	0.001	0.802
Se × vit. E interaction	0.013	0.014	0.242

a,b Means in the same column without common superscripts differ significantly at $p \leq 0.05$.

Statistical analysis

The STATISTICA software package version 8.0 (StatSoft Corp., Cracow, Poland) was used to determine whether variables differed between treatment groups. Two-way ANOVA was performed to assess the effects of the supplementation levels of vitamin E (30 and 60 mg/kg of diet), the supplementation levels of Se (0.15 and 0.30 mg/kg of diet) and the interaction between vitamin E and Se dosages (Se × vit E) (Snedecor and Cochran 1989). 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 $p \leq 0.05$.

Results

As shown by two-way ANOVA, the experimental factors, i.e. diet supplementation with Se and vitamin E, had no significant effect on the body weights of hens and egg production, which were similar in all dietary treatments (Table 2), but a higher Se content of diets contributed to a significant increase in egg weight ($p = 0.021$).

Blood vitamin levels in laying hens were affected by dietary treatments (Table 3). Two-way ANOVA revealed that serum retinol concentrations were influenced by both experimental factors – the effect of dietary Se levels was significant ($p = 0.001$), while the effect of dietary vitamin E levels was close to the limit of statistical significance ($p = 0.109$). The Se x vitamin E interaction was significant ($p = 0.013$) – a higher increase in serum retinol concentrations was noted in hens fed diets with an increased content of both vitamin E and Se. Both experimental factors contributed to a significant ($p = 0.001$) increase in serum α-tocopherol levels, which was highest when both antioxidants were administered at higher doses. A significant ($p = 0.001$) increase in serum vitamin C levels was noted in layers fed diets with an increased Se content.

Diet supplementation with Se and vitamin E did not lead to differences in the serum concentrations of uric acid in laying hens (Table 4). Serum catalase activity was significantly higher in hens fed diets with an increased Se content ($p = 0.010$). The Se x vitamin E interaction was significant ($p = 0.001$), indicating that a higher Se content was effective only at a lower vitamin E dose. SOD activity was significantly enhanced in hens receiving diets with increased levels of Se ($p = 0.040$) and vitamin E ($p = 0.049$), and the interaction between the experimental factors was non-significant. The experimental factors had no significant effect on the serum concentrations of lipid peroxides. TAS values were significantly higher in hens fed diets with an increased Se content, and FRAP values were higher in hens fed diets with an increased vitamin E content.

There were no significant differences in the concentrations of TNF-α and the proinflammatory cytokine IL-6 between treatments, while a higher vitamin E content of diets (Table 5) increased serum IgA concentrations in laying hens ($p = 0.026$).

Table 4. Serum total antioxidant status in laying hens.

	Uric acid µmol/l	Catalase U/L	SOD U/mL	H_2O_2 µmol/L	TAS mmol/L	FRAP µmol/L
Treatment						
$S_{0.15}E_{30}$	523.9	2.80[b]	38.1	1.28	0.28	104.0
$S_{0.15}E_{60}$	550.5	3.17[b]	41.6	1.29	0.28	116.6
$S_{0.30}E_{30}$	605.0	3.83[a]	41.8	1.37	0.35	107.5
$S_{0.30}E_{60}$	541.0	2.97[b]	45.0	1.47	0.41	129.4
SEM	22.95	0.101	0.899	0.056	0.022	3.978
Se, mg/kg						
0.15	537.2	2.98[b]	39.8[b]	1.29	0.28[b]	110.3
0.30	573.0	3.40[a]	43.4[a]	1.42	0.38[a]	118.4
p	0.452	0.010	0.040	0.249	0.026	0.284
Vitamin E, mg/kg						
30	564.4	3.31	39.9[b]	1.32	0.31	105.7[b]
60	545.7	3.07	43.3[a]	1.38	0.35	123.0[a]
p	0.694	0.128	0.049	0.627	0.451	0.029
Se × vit. E interaction	0.342	0.001	0.912	0.637	0.402	0.536

a,b Means in the same column without common superscripts differ significantly at $p \leq 0.05$.

Table 5. The immune system response of laying hens.

	TNF-α pg/mL	IL-6 pg/mL	IgA µg/mL
Treatment			
$S_{0.15}E_{30}$	12.6	32.2	313.9
$S_{0.15}E_{60}$	13.8	45.2	366.5
$S_{0.30}E_{30}$	13.0	35.7	328.8
$S_{0.30}E_{60}$	14.0	31.8	374.8
SEM	0.541	3.43	10.98
Se, mg/kg			
0.15	13.2	38.7	340.2
0.30	13.5	33.7	351.8
p	0.781	0.481	0.586
Vitamin E, mg/kg			
30	12.8	33.9	321.3[b]
60	13.9	38.5	370.6[a]
p	0.355	0.517	0.026
Se × vit.E interaction	0.932	0.231	0.877

a,b Means in the same column without common superscripts differ significantly at $p \leq 0.05$.

Discussion

In the present experiment, the applied dietary treatments had no effect on the body weights of laying hens and egg production, while some indicators of blood antioxidant status and immune system responses were depend on the level of vitamin E and selenium in the diet.

The results of earlier studies conducted by other authors are inconclusive with regard to the correlation between the vitamin E content of hen diets and laying performance. In some experiments, an increase in the vitamin E content of hen diets from 20-25 mg/kg to 60-65 mg/kg significantly improved daily egg production (Kirunda et al. 2001), while in other studies even high dietary levels of vitamin E (100 and 200 mg/kg) did not increase egg production, as compared with non-supplemented diets and diets supplemented with a low (20 mg/kg) amount of vitamin E (Mohiti-Asli et al. 2010). Studies investigating the effects of different Se levels in hen diets have also produced ambiguous results. Many authors demonstrated that dietary Se supplementation did not affect daily egg production and egg weight (Jiakui and Xialong 2004, Chantiratikul et al. 2008, Mohiti-Asli et al. 2010). Payne et al. (2005), whose results are consistent with our findings, observed a linear increase in egg weight as dietary Se levels increased. In a study by Stępińska et al. (2012), the average egg weight was higher in turkey hens fed a diet with organic selenium than in layers receiving inorganic selenium.

In the present experiment, increased dietary

vitamin E levels from 30 to 60 mg/kg contributed to a significant increase in serum α-tocopherol concentrations, SOD activity and the antioxidant capacity of plasma measured by the FRAP assay but did not increase total antioxidant status (TAS) of blood plasma.

Sahin et al. (2006) reported that an increase in the dl-α-tocopheryl acetate content of Japanese quail diets, from 1.25 to 250 mg/kg, significantly increased the serum concentrations of vitamins A, C and E, and decreased serum malondialdehyde concentrations. In a study by Ni et al. (2012), diets fed to broiler breeder hens were supplemented with the antioxidant daidzein which led to a significant increase in glutathione-peroxidase activity, and an insignificant increase in SOD activity and the total antioxidant status of serum. A significant increase in serum α-tocopherol concentrations denoted in our study was consistent with the results of experiments cited.

In the present experiment a higher vitamin E content of diets did not increase TAS, but it increased FRAP values. FRAP values are known to be considerably affected by the levels of uric acid, ascorbic acid and bilirubin, and – to a lower degree – by α-tocopherol content (Benzie and Strain 1996). Another important factor affecting blood antioxidant status is the activity of antioxidant enzymes (Ting et al. 2011). In our study, hens fed diets with an increased vitamin E content were characterized by higher serum SOD activity.

In the present experiment, different dietary levels of E had no effect on the concentrations of TNF-α and the proinflammatory cytokine IL-6, while a higher vitamin E content of hen diets increased serum IgA concentrations. The increase in serum IgA concentrations, observed in our study, corroborates the findings of Muir et al. (2002) who demonstrated that dietary supplementation with vitamin E stimulated IgA production in the intestines, thus boosting avian intestinal immunity.

Increased dietary levels from 0.15 to 0.30 mg/kg led to an increase in the serum concentrations of vitamin E and A, the activity levels of catalase and SOD as well as the serum TAS of hens.

In another experiment (Zuberbuehler et al. 2006), an increase in the Se content of hen diets had no effect on vitamin E concentrations and the activity levels of aspartate aminotransferase and creatine kinase in serum. Perhaps it was due to the large increase in the concentration of Se, 0.15 to 1.45 mg/kg, resulting in increased activity of selenium-dependent enzymes.

The inclusion of supplemental Se in organic bound in turkey diets enhanced SOD activity, but the antioxidant capacity of blood remained unchanged (Mikulski et al. 2009, Jankowski et al. 2011). In another experiment (Słowińska et al. 2011), dietary Se supplementation did not affect the antioxidant properties of turkey seminal plasma, however it increased the volume and concentration of sperm.

Our results, similarly as the findings of other authors (Sahin et al. 2006, Ni et al. 2012), indicate that higher dietary Se levels increase TAS values in laying hens. It was probably due to increase in the concentration of vitamin E and A in the blood of hens fed a diet with a higher content of Se.

In the present experiment, different dietary levels of Se had no effect on the concentrations of serum IgA, TNF-α and the proinflammatory cytokine IL-6. The available literature found no information on the impact of selenium on the analyzed parameters of the immune system response. In a study by Sahin et al. (2010), supplementation with other antioxidant element (chromium) added to quail diets did not affect TNF-α and IL-6 concentrations.

Conclusions

It can be concluded that in addition to the beneficial influence of increased dietary level of Se or vitamin E on the serum antioxidant status of laying hens, both supplements applied at higher doses exerted additional positive physiological effect as demonstrated by increased serum retinol and α-tocopherol concentrations and enhanced SOD activity, in comparison with the remaining dietary treatments.

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