

Effects of Dietary Selenium Source and Storage on Internal Quality of EggsGordana Kralik¹, Zlata Gajčević¹, Pavel Suchý², Eva Straková², Danica Hanžek¹¹ Josip Juraj Strossmayer University of Osijek, Faculty of Agriculture, Osijek, Croatia² University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

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Abstract

A 4-week experiment was carried out on 360 laying hens of the Hy Line Brown hybrid. Laying hens were divided into three groups (C, E₁ and E₂) with 120 hens in each group and kept in 24 cages. Hens were fed layer diets containing 18% of crude protein and 11.60 MJ ME. Hens in the control group C were fed diets that contained 0.2 mg/kg of inorganic selenium (sodium selenite). Experimental groups E₁ and E₂ were given diets with increased concentrations of selenium as follows: E₁ = 0.4 mg/kg of selenium (sodium selenite), E₂ = 0.4 mg/kg of organic selenium (Sel-Plex). Selenium concentration in diets affected significantly the content of selenium in albumen ($P < 0.001$) and yolk ($P < 0.05$). The highest concentration of selenium was determined in albumen and yolk of eggs produced in group E₂ (345 ng/g and 783 ng/g, respectively), then in eggs of group E₁ (230 ng/g and 757 ng/g, respectively), and group C had the lowest concentration of selenium in albumen and yolk (181 ng/g and 573 ng/g, respectively). After 28 days of storage at 4 °C, the eggs containing organic selenium had more freshness (VN: C = 32.9, E₁ = 2.60, E₂ = 2.11). It was concluded that higher concentration of organic selenium in eggs was a limiting factor in metabolic processes, which positively affected the indicators of egg freshness.

Organic selenium, inorganic selenium, albumen, yolk, egg freshness

Selenium is found in two forms in nature: inorganic and organic. Inorganic selenium refers to different minerals such as selenite, selenate and selenide, and organic selenium is related to amino acids, methionine and cysteine. Outdoor living animals that eat plants take selenium in the form of selenomethionine (SeMet) in concentrations that depend on selenium concentration in soil, which can vary considerably according to area (Reilly 1996). Klapeč et al. (2004) reported low concentrations of selenium in soil on the territory of Croatia because of which the selenium content in plant and animal foodstuffs was relatively low (egg = 52.5 ng/g; chicken meat = 115.3 ng/g; onion = 12.4 ng/g; potato = 7.2 ng/g). As a microelement, selenium has manifold importance in animal feed. Its antioxidant properties help protect animals from free radicals caused by oxygen metabolism. Moreover, selenium strengthens the immune system and boosts growth and feathering. As a dietary supplement, selenium reduces negative effects of stress in chickens kept in intensive production conditions, reduces mortality rate of one-day chickens and enhances quality of poultry products. One of the possibilities of enriching feed with selenium is to supplement it with plants fertilized with selenium (MacLeod et al. 1998). Another possibility is to supplement selenium preparations to commercial mixture fed to animals in order to design animal products that differ in their nutritive values from other conventional products available on the market.

The aim of our study was to determine the effects of supplementation of organic and inorganic selenium in increased concentrations to the hens' diet and to assess deposition of selenium in egg yolk and albumen as well as the effects of selenium on preservation of egg freshness.

Materials and Methods

The experiment was carried out on 360 Hy Line Brown laying hens of the age of 26–30 weeks. Hens were divided into three experimental groups; each group consisted of 24 repetitions. Hens were kept in cages by groups of five and fed commercial mixture containing 18% of crude protein and 11.60 MJ ME. The experiment was set up in three different feeding treatments. The control group had diets supplemented with 0.2 mg/kg of selenium

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from an inorganic source (sodium selenite). Groups E₁ and E₂ were given 0.4 mg/kg of selenium (E₁: inorganic source of selenium; E₂: organic source of selenium, selenomethionine - Sel-Plex[®], Alltech, Inc.). On the 30th day of experiment five eggs were selected randomly from each group. The eggs were broken, and yolks and albumens were prepared for selenium analysis. The analysis was carried out as follows: The egg was broken and albumen and yolk were separated carefully from each other. Separated egg parts were homogenized. Around 0.5 g of homogenized sample was put in a Teflon flask for destruction. The sample was mixed with 5 ml of concentrated HNO₃ and 1 ml of H₂O₂. After 15 min, the tubes were closed and heated in a microwave oven (CEM, Mars 5 model). After digestion, the tube content was transferred to a 25-ml flask and filled up with distilled water up to the line. Prior to sample preparation, all laboratory utensils were kept in a 10%-solution of HNO₃ for 24 h. Selenium content in egg albumen and yolk was determined by the method of electrothermic atomic absorption spectrometry (Perkin Elmer, AAnalyst 600). Egg freshness was assessed over three different time periods (fresh eggs, eggs stored at 4 °C for 14 and 28 days) by determining values of the ageing rate (AR) and value number (VN) using the method of Kralik et al. (1990).

The AR was obtained by the following mathematical expression:

$$AR = (1.4184 - \eta_y) \cdot 1000;$$

where: η_y = refractive index of examined yolk, 1.4184 = refractive index of standard yolk obtained from a sample of 20 eggs at a temperature of 25 °C.

The VN was calculated by the following expression:

$$VN = 1000 \cdot (\eta_y - \eta_a);$$

where: η_y = refractive index of examined yolk, η_a = refractive index of examined albumen.

Yolk and albumen refraction was determined using Refracto 30PX device. In order to calculate AR and VN, 75 eggs from each group were analyzed over three time periods. The influence of the main factors (concentration and source of selenium) on the assessed traits was determined using MANOVA (Main effects ANOVA). When the selenium source or concentration had a significant ($P < 0.05$) effect on the assessed traits, differences between the groups were tested by the Fisher's LSD-test. Analyses were performed using Statistica v.7.1 software (StatSoft, Inc., 2005).

Results and Discussion

Table 1. Content of selenium in egg albumen and yolk (n = 5)

Content of selenium	Groups ($\bar{X} \pm SD$)			P value	
	C	E ₁	E ₂	Source	Concentration
Albumen (ng of selenium/g)	181 ± 11.8 ^C	230 ± 17.4 ^B	345 ± 11.3 ^A	< 0.001	< 0.001
Yolk (ng of selenium/g)	573 ± 36.2 ^b	757 ± 89.1 ^a	783 ± 90.9 ^a	< 0.05	< 0.001

^{a, b} $P < 0.05$; ^{A, B, C} $P < 0.001$ Values within the row differ significantly

C = 0.2 mg/kg of inorganic selenium, E₁ = 0.4 mg/kg of inorganic selenium, E₂ = 0.4 mg/kg of organic selenium

Data presented in Table 1 show that the source and concentration of selenium supplemented to the hens' diet affected selenium deposition in albumen ($P < 0.001$). The content of selenium in albumen of eggs of group E₂ was higher than in groups E₁ and C (345 ng of selenium/g; 230 ng of selenium/g; 181 ng of selenium/g, respectively). The concentration of selenium in the hens' diet has greater influence ($P < 0.001$) on the selenium content in egg yolk. The source of selenium had slightly less but still significant influence ($P < 0.05$) on the selenium content in egg yolk. Sample analysis showed that the eggs of the control group contained less selenium in yolks than groups E₁ and E₂ (573 ng of selenium/g : 757 ng of selenium/g : 783 ng of selenium/g, respectively). Based on the results stated above it was concluded that plants should be enriched with selenium in order to provide animal diets with higher concentrations of organic selenium as it is absorbed through membranes of the intestinal tract and actively accumulated in the organism cells. Humans and animals cannot synthesize selenomethionine in their organisms, so it is necessary to ensure selenium intake through diets (Schrauzer 2000). Producers usually supplement sodium-selenite (inorganic source of selenium) to animal feed; however, inorganic selenium cannot be fully used by an organism and is mostly excreted. Therefore, it is important to emphasize advantages of organic selenium in comparison to inorganic selenium.

Cantor et al. (1996) studied the effects of selenium (inorganic and organic; 0.3 mg/kg) supplemented to the hens' diet on the selenium content in egg and reported a significant effect of selenium concentration in diet on its content in egg, which corresponds with our results. Moreover, Payne et al. (2005) pointed out that different sources of selenium (inorganic or organic) and concentrations of selenium in the feed (0; 0.15; 0.3; 0.6 and 3 mg/kg) significantly affected ($P < 0.01$) content of selenium in egg. The authors stated that the concentration of selenium in eggs was increased in proportion to the concentration of selenium in the diet ($P < 0.01$). Their results are also in accordance with ours. Surai (2000) stated that supplementation of organic selenium to the hens' diet could significantly increase the selenium content in egg yolk and albumen ($P < 0.01$). He also reported a high correlation coefficient between selenium concentrations in the hens' diet and in eggs. Cantor (1997) and Paton et al. (2000) also confirmed relations between source and concentration of selenium in diets and selenium concentration in eggs.

Table 2. Ageing rate and value number for eggs stored for 28 days at 4 °C (n = 75)

Groups	Time period of egg storage at 4 °C					
	1 day		14 days		28 days	
	AR	VN	AR	VN	AR	VN
C	1.07 ± 1.27	60.20 ± 2.17	1.82 ± 1.23	59.44 ± 1.63	3.29 ± 1.35 ^a	57.51 ± 1.35
E ₁	1.24 ± 1.42	60.50 ± 1.53	2.30 ± 2.10	59.37 ± 2.06	2.60 ± 1.41 ^{ab}	58.22 ± 2.07
E ₂	0.94 ± 1.06	60.60 ± 1.66	1.55 ± 1.48	60.30 ± 2.62	2.11 ± 1.29 ^b	58.44 ± 2.04
<i>P</i> value	Source		Concentration			
	> 0.05	> 0.05	> 0.05	> 0.05	< 0.05	> 0.05
	> 0.05	> 0.05	> 0.05	> 0.05	< 0.01	> 0.05

^{a, b} $P < 0.01$ Values within the row differ significantly

C = 0.2 mg/kg of inorganic selenium, E₁ = 0.4 mg/kg of inorganic selenium, E₂ = 0.4 mg/kg of organic selenium

Egg freshness is related to its quality. It depends on the egg storage period (counted in days) and on conditions under which the eggs are stored (temperature and relative air humidity). There are many indicators that show changes occurring as a consequence of egg storage. Longer period of storage increases AR and decreases VN. Table 2 shows changes in AR and VN of eggs of all three groups over the period of 28 days. In our experiment, the AR values were increased over the 28-day period; however, the differences between groups in were not significant ($P > 0.05$) for the first two periods (1st and 14th day). After storing eggs at 4 °C for 28 days, the highest AR was determined for group C (3.29), followed by group E₁ (2.60), and the least AR was exhibited by group E₂ (2.11). Determined differences regarding AR of investigated eggs after 28-day storage were significant. The source and concentration of selenium in hens' diets influenced the determined differences in AR ($P < 0.05$ and $P < 0.01$, respectively). In the course of egg ageing, the concentration of egg mass is reduced. Water contained in albumen permeates the yolk, and some nutrients contained in yolk can permeate albumen. These osmotic tracks and changes in albumen and yolk concentrations can be measured by the refractometric method. Metabolic processes in eggs of group E₂ were less intensive, so AR in that group was the lowest over the whole storage period, and the VN was the highest. Kralik et al. (1990) measured the mean of AR of fresh eggs 0.92, which corresponds to AR of group E₂. Rotruck et al. (1973) proved selenium to be a part of glutathione peroxidase enzymes (GSH-Px), which confirmed its role in cellular antioxidative metabolism. Wakebe (1999) emphasized positive effects of dietary supplementation with organic selenium at the concentration of 0.3 mg/kg of feed on the activity of GSH-Px enzymes. Its presence reduces occurrence of free radicals that intensify metabolic processes in cells (oxidation of lipids and proteins) and reduce activity of enzymes of polymerase, nuclease, ligase, etc. Changes that occur through intensified metabolic processes in the egg have negative effects on preservation of egg freshness. In

our study, the highest VN was measured in fresh eggs of group E₂, then of group E₁, and C group had the least VN (60.60 : 60.50 : 60.20, respectively), which is in accordance with the results of Kralik et al. (1990). On the 14th and 28th day of egg storage, VN was still the highest in group E₂. The influence of source and concentrations of selenium were not significant ($P > 0.05$) regarding VN of the examined groups. Sources and different concentrations of selenium in hens' diets resulted in the increase of the selenium content of egg albumen ($P < 0.001$) and yolk ($P < 0.05$ and $P < 0.001$, respectively). Analysis of AR and VN of eggs exhibited more favourable values for eggs produced by hens that were fed diets containing organic selenium compared to eggs from hens fed diets with inorganic selenium. The source and concentration of selenium in hens' diets did not influence the differences in VN between groups, however, both factors had a significant effect on AR of eggs after they were stored for 28 days at 4 °C ($P < 0.05$ and $P < 0.01$, respectively).

Vliv dietních zdrojů selenu na skladování a kvalitu vajec

Čtyřtýdenní pokus se uskutečnil na 360 nosnicích hybridu Hy Line Brown. Nosnice byly rozděleny po 120 kusech do tří skupin (C, E₁ a E₂) a drženy v 24 klecích. Byly krmeny dietami, které obsahovaly 18 % dusíkatých látek a 11,6 MJ ME. Nosnice kontrolní skupiny byly krmeny dietou, která obsahovala 0,2 mg/kg anorganického selenu (selenit sodný). Pokusné skupiny E₁ a E₂ měly v dietě vyšší koncentraci selenu; skupina E₁ 0,4 mg/kg selenitu sodného, skupina E₂ 0,4 mg/kg organického selenu (přípravek Sel-Plex). Selen v dietách ovlivnil významně hladiny selenu ve vaječném bílku ($P < 0,001$) a vaječném žloutku ($P < 0,05$). Nejvyšší koncentrace selenu byly nalezeny ve vaječném bílku a žloutku vajec skupiny E₂ (345 a 783 ng/g), následovaly vejce skupiny E₁ (230 a 757 ng/g), nejnižší hodnoty byly zjištěny u kontrolní skupiny (181 a 573 ng/g). Po 28 dnech skladování při teplotě 4 °C byla čerstvost nejpříznivější u vajec obsahující organicky vázaný selen (VN: C = 32,9, E₁ = 2,60, E₂ = 2,11). Vyšší koncentrace organického selenu byla limitujícím faktorem metabolických procesů, které pozitivně ovlivnily indikátory čerstvosti vajec.

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