Effects of organic selenium supplemented to layer diet on table egg freshness and selenium content

Zlata Gajčević¹, Gordana Kralik¹, Elizabeta Has-Schön², Valentina Pavić²

¹Faculty of Agriculture. University J.J. Strossmayer, Osijek, Croatia
²Department of Biology. University J.J. Strossmayer, Osijek, Croatia

Corresponding author: Prof. Gordana Kralik. Faculty of Agriculture. Josip Juraj Strossmayer University of Osijek. Trg sv. Trojstva 3, 31000 Osijek, Croatia - Tel. +385 31 224102 - Fax: +385 31 207015
Email: gkralik@pfos.hr

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ABSTRACT

The aim of the research was to determine the effects that organic selenium supplemented in layer diets has on its content in the edible part of egg, on egg freshness and activity of glutathione peroxidase (GSH-Px) in hens’ blood.

The experiment lasted for 30 days and was completed on 240 laying hens of the Hy Line Brown hybrid. Hens were divided into 2 experimental groups (E₁ and E₂), each consisting of 120 hens housed in 24 cages. Hens were fed a commercial diet containing 18% of crude protein and 11.60MJ ME/kg. The experiment was set up in two different feeding treatments. The E₁ group had diets supplemented with 0.2 ppm of selenium, and E₂ had diets with 0.4 ppm of selenium (organic selenium Sel-Plex®, Alltech, inc.).

The GSH-Px activity was higher in blood of hens in the E₂ group than in the E₁ (P<0.05). Furthermore, statistically the E₂ group had a significantly higher portion of selenium in egg yolks and albumen than the E₁ group (P<0.05). Analysis of effects that feeding treatments have on egg freshness over three examined periods (fresh eggs, eggs stored for 14 and for 28 days at 4ºC) showed better results for HU (Haugh units) and TBARS (thiobarbituric acid reactive substances) in the E₂ eggs than in the E₁ eggs.

Key words: Laying hens, Organic selenium, Freshness of eggs, Glutathione peroxidase.

RIASSUNTO

EFFETTI DI SELENIO ORGANICO ADDIZIONATO ALLA DIETA DI GALLINE OVAIOLE SU FRESCHEZZA E CONTENUTO DI SELENIO DELLE UOVA DA CONSUMO

Lo scopo di questo lavoro è stato quello di determinare gli effetti di selenio organico addizionato a die di galline ovaiole ibride Hy Line Brown. Le galline sono state suddivise in due gruppi sperimentali (E₁ e E₂) di 120 animali ciascuno e stabulate in 24 gabbie. Gli animali sono stati alimentati con una dieta commerciale contenente il 18% di proteina...
grezza e 11,60MJ EM/kg. L’esperimento è stato suddiviso in due diversi trattamenti alimentari. Al gruppo E₁ sono state somministrate diete contenenti una concentrazione di selenio pari a 0, ppm, mentre il gruppo E₂ è stato alimentato con diete contenenti un supplemento di selenio di 0,4ppm (selenio organico Sel-Plex®, Alltech, inc.).

L’attività glutatone-perossidasica nel sangue è risultata maggiore nel gruppo E₂ rispetto al gruppo E₁ (P<0,05), inoltre è stata rilevata una quantità di selenio più alta sia nel tuorlo che nell’albumine nel gruppo E₁ (P<0,05). Analisi relative alla freschezza del prodotto nei tre momenti esaminati (uova fresche, uova conservate per 14 e 28 giorni a 4°C) hanno evidenziato, attraverso TBARS e Haugh units, risultati migliori nel gruppo E₂ rispetto al gruppo E₁.

Parole chiave: Galline ovaiole, Selenio organico, Freschezza delle uova, Glutatione perossidasi.

Introduction

Selenium (Se) is an important natural antioxidant that is essential in many metabolic processes in humans and animals. It is found in nature in two forms, inorganic and organic. Inorganic selenium refers to different minerals such as selenite, selenate and selenide, and organic selenium is related to amino acids such as methionine and cysteine. Outdoor living animals that eat plants take Se in the form of selenomethionine (SeMet) (Combs and Combs, 1986) in concentrations that depend on Se concentration in soil, which can vary considerably according to area (Reilly, 1996). Due to the mentioned metabolic processes, there is a constant need for supplementation of mostly inorganic Se to the animal diet. The allowed concentration of inorganic Se was determined through various studies, as its dietary supplementation depends on its toxicity, interaction with other minerals and vitamins, and on relatively low absorption of Se taken in by animals. Taking into consideration Se toxicity, a limit of inorganic Se supplementation was determined through various scientific studies. Todorović et al. (1999) determined a reduced daily gain of chickens fed diets supplemented with inorganic Se in the amount of 5mg/kg of feed. Sodium selenite exhibits strong cytotoxicity because in reaction with glutathione it enables synthesis of super-hydrogen radicals (Seko and Imura, 1997). Selenomethionine is not toxic for cells; on the contrary, it has proven to be a strong antioxidant (Vinson et al., 1998). Moreover, absorption of inorganic Se from food is relatively low if compared to organic Se, thus resulting in production of animal foodstuffs containing low concentration of selenium (Payne et al., 2005; Yoon et al., 2007).

Moreover, reaction with reduced glutathione confirmed the prooxidative action of sodium selenite (Yan and Spalholtz, 1993). This compelled scientists to produce Se in organic form, which is metabolized as methionine (Wolfram, 1999) and better used by an organism. Se deficiency in birds, especially if combined with the lack of vitamin E, causes the occurrence of exudative diathesis (Bartholomew et al., 1998) and encephalomalacia (Combs and Hardy, 1991). Insufficient immunity, lowering of production ability, lower fertility and laying capacity, decreased feathering of chickens and increased embryo mortality were also reported (Combs and Combs, 1984). Surai (2000a) pointed out that supplementation of Se and vitamin E to the hens’ diet increased their concentrations in eggs, as well as in the liver of day-old chickens. The same author claimed that it was possible to track absorption of Se from food to tissue by measuring the GSH-Px activity. In their research on production traits, physical parameters of eggs and Se content in the edible part
of eggs, Skrivan et al. (2006) reported that organic Se in the diet of the experimental group significantly increased Se content in the edible part of the egg, and had a better effect on egg freshness parameters (HU and yolk and albumen height), when compared to the control group.

Taking into consideration all stated facts, the aim of present study was to examine the effects that organic Se supplemented to hens’ diet in various concentrations (0.2ppm and 0.4ppm) has on egg freshness and Se content in the edible part of the egg. Furthermore, our research focused on the determination of GSH-Px activity in the hens’ blood, as this is taken as an indicator of Se absorption from food.

Material and methods

The experiment was carried out on 240 Hy Line Brown laying hens in the age range of 26 - 30 weeks. Hens were divided into two groups, housed five per cage and fed a commercial diet containing 18% of crude protein and 11.60MJ ME/kg. Both groups had 0.08ppm Se in their basic diets, which was shown to be a satisfactory level for laying hens (NRC, 1994). In accordance with the aim of the present study to increase the Se content in eggs, the experiment was set up in two different feeding treatments. Group E1 was given diets with 0.2ppm, and group E2 was given diets with 0.4ppm of organic Se (Sel-Plex®, Alltech, inc.). Diet composition is outlined in Table 1. At the end of the experiment (on the 30th day), 5 birds were randomly selected from each group and blood samples were taken from their wing vein by means of sterile puncture for the purpose of determining enzyme activity of glutathione peroxidase (GSH-Px) in blood. Enzyme activity was measured at 340nm, in units that present oxidation of 1μmol NADPH per minute in 1ml of blood (U/l). The GSH-Px activity was determined in the blood sample using commercial “RANSEL” kit (RANDOX Laboratories Ltd, London, UK). The method of GSH-Px determination is based on catalysis of glutathione oxidation (GSH) by means of cumene hydroperoxide. If glutathione reductase (GR) and NADPH are present, oxidized glutathione (GSSG) is instantly reduced, which is followed by oxidation of NADPH into NADP+

Test tubes were without enzymatic extracts (Paglia and Valentine, 1967). The GSH-Px activity was measured by the UV/Visible Jenway model 6305 (Bibby Scientific T/As Jenway, Ltd, England) spectrophotometer. Samples taken for determination of Se content in albumen and yolk were destroyed by concentrated nitric acid in a microwave oven (CEM, Mars 5 model), applying the method of microwave digestion (Matek and Blanuša, 1998). Content of Se in the edible part of the egg was determined by a method of electrothermic atomic absorption spectrometry ET AAS. The device used was Perkin Elmer, AAnalyst 600 Zeeman, equipped with transversally heated graphite test tubes and Zeeman system for correction of non-specific signal and system for automatic supply of a sample AS-800. A non-electrode discharge lamp and ‘end cup’ platform graphite test tubes were also used in the experiment. A solution of 0.15% Pd/0.1% Mg(NO3)2 was used as a matrix modifier. This solution was prepared by diluting 10g/l of solution of Pd(NO3)2 and Mg (NO3)2x6H2O (Merck, Darmstandt, Germany) in deionized water. A standard selenium solution, 1000mg/l (Merck, Darmstandt, Germany) was used as a basic standard. Calibration of the device was completed by a method of adding the standard on one of both albumen and yolk samples. The calibration line was in the area 5-35μg/l. Instrumental conditions for determination of Se in prepared samples, and temperature program are presented in Tables 2 and 3. Measurement
precision (RSD) was ≤4% within one day, and ≤6% between days. Detection limit was 0.6μg/l (obtained as 3 standard deviations (3σ) from 10 measurements of blind test). Determination limit was 1.8μg/l (obtained as 10 standard deviations from 10 measurements of blind test). At the end of experiment (on the 30th day) 93 eggs were randomly selected from each group. Haugh units (HU) were analyzed on 75 eggs per group, and 18 eggs per group were used for the determination of thiobarbituric acid reactive substanc-
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Egg freshness was analyzed for three different periods (fresh egg, 14th and 28th day of storing eggs at 4°C). Values for HU were measured by the egg multi tester EMT-5200 (Robotmation, Co. Ltd, Japan) device, while TBARS were analyzed in yolk samples by a method of Heath and Packer (1968). Oxidation number was determined in yolks of fresh eggs, as well as in eggs that were kept in a refrigerator at 4°C for 14 and 28 days. A sample for analysis was prepared as follows: 0.5g of yolk was weighed, 4.5ml of 3.86% HClO₄ was added to the yolk. The sample was homogenized and centrifuged for 10 minutes at 9000g. After centrifugation, 2ml of supernatant was mixed with 2ml of 20mM thiobarbituric acid, slightly shaken and then boiled for 30min in a water bath at 95°C. The sample was then cooled down on ice for 10min. Extract extinction was measured in the UV/Visible Jenway model 6305 (Bibby Scientific T/As Jenway, Ltd, England) spectrophotometer at 531nm opposite to the blind experiment that contained the fraction.

Values for TBARS were expressed in mMxg⁻¹ of yolk.

Obtained results were processed in a computer software Statistica 7.1 (StatSoft, 2005), presented in tables, and conclusions on observed occurrences were discussed. Research results were processed in ANOVA by application of the GL model.

Table 2. Instrumental conditions for selenium determination in solutions of albumen and yolk samples (Perkin-Elmer A Analyst 600 Zeeman).

| Source of radiation       | mA  | Non-electrode discharge lamp (EDL) |
|---------------------------|-----|-----------------------------------|
| Electric force            |     | 300                               |
| Wave length               | nm  | 196.0                             |
| Gap width                 |     | 2.0                               |
| Time for adjustment of base line | s  | 3                                 |
| Initial signal integration time |   | 0                                 |
| Signal integration time   |     | 5                                 |
| Volume of solution injection | µl | 10                                |
| Volume of matrix modifier injection |    | 5                                 |

Table 3. Temperature program for selenium determination in solutions of albumen and yolk samples (Perkin-Elmer A Analyst 600 Zeeman).

| Indicator  | Temperature (°C) | Time of temperature increase (s) | Duration of steps (s) | Flow of argon (ml/min) |
|------------|------------------|----------------------------------|-----------------------|------------------------|
| Drying     | 110              | 1                                | 20                    | 250                    |
| Drying     | 130              | 15                               | 30                    | 250                    |
| Pyrolysis  | 1250             | 10                               | 20                    | 250                    |
| Atomization| 1950             | 0                                | 5                     | 0                      |
| Cleaning   | 2450             | 1                                | 4                     | 250                    |
Results and discussion

Resorbed selenomethionine is deposited in tissue proteins building up Se reserves necessary for antioxidative protection and other functions in an organism. The most important metabolic function of Se is shown through activity of GSH-Px enzymes and other reductases in cell protection from peroxidation by means of which free radicals manifest their damaging activity. Se content in blood and tissue is an important regulator of GSH-Px activity. Indicators of GSH-Px enzyme activity in hens’ blood are presented in Table 4. According to mean values, significantly higher (P<0.05) GSH-Px activity was observed in blood of hens that consumed more organic Se. As GSH-Px activity is taken as an indicator of Se absorption efficiency, obtained results were as expected. GSH-Px enzyme activity depended on the concentration of Se in an organism as aminoacid selenocystein maintains active the centre of an enzyme. Changes in antioxidative enzyme activity are related to animal age, tissue type, exposure to stress and animal status referring to the amount of Se in its organism (Surai, 2000a). As laying hens in our experiment were of the same age and kept in the same conditions, it is assumed that the concentration of Se supplemented to layer diets was the only reason for obtaining different values of GSH-Px in blood. Leng et al. (2003) pointed out a positive correlation between the amount of Se in a diet and GSH-Px activity in broiler blood. Increased GSH-Px activity in blood as a result of organic Se dietary supplementation can have positive effects on strengthening the hens’ immunity (Edens, 2002).

Besides blood, GSH-Px is also an important indicator of Se absorption in eggs. Wakebe (1999) and Surai (2000b) stated that hens which consumed diets with organic Se had increased GSH-Px activity in eggs, which positively affected the preservation of egg freshness. Table 5 presents values of Se content in albumen and yolk produced by hens in different feeding treatments. Analysis of edible parts of eggs proved that the albumen of the E2 group had significantly more Se than the albumen of the E1 group (345ng/g and 231.5ng/g, respectively, P<0.05). Moreover, similar changes in Se content were observed also in yolks. Yolks of the E1 group that was treated with lower amounts of Se contained less Se than yolks of the E2 group treated with higher amounts of Se in the layer diet (584.8ng/g and 779.5ng/g, respectively, P<0.05). In their study on designed eggs, Surai and Sparks (2001) stated that supplementation of 0.2ppm and 0.4ppm of organic Se to the layer diet resulted in four and eight times more accumulated Se in albumen if compared to eggs produced by hens fed commercial diets (albumen 50.7ng/g: 193.7ng/g: 403.7ng/g). Moreover, the authors proved Se

| Table 4. Indicators of GSH-Px enzyme activity in hens’ blood. |
|-----------------|-----------------|-----------------|-----------------|
| Concentration   | Experimental groups | SEM            |
|                 | E1               | E2               |                 |
| n               | 5                | 5                |                 |
| GSH-Px U/l      | 9622.48b         | 13381.80a        | 654.92          |

GSH-Px: glutathione peroxidase; E1: 0.2ppm of Se; E2: 0.4ppm of Se.

a, b: estimated mean values between groups differ statistically significantly (P<0.05).
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content in yolks of designed eggs to be two-fold and threefold higher than in yolks of eggs produced by hens which were given a commercial diet (yolks 298.3ng/g; 605.3ng/g; 854.0ng/g). Their results are in accordance with ours.

Kenyon et al. (2003) also stated that supplementation of 0.2ppm of organic Se to layer diets affected the increase of Se in albumen and yolk, and Yaroshenko et al. (2003) pointed out that supplementation of 0.4ppm Se to layer diets resulted in production of eggs enriched with Se and a prolonged period of egg freshness.

Freshness of eggs is a quality parameter influenced by storage time (expressed in days) and conditions (temperature and relative humidity). Egg age refers to the period from egg laying to its consumption. The most common indicators of egg freshness are air bubble height, pH of albumen, ageing rate, oxidation intensity of yolk lipids and Haugh units (HU). In the present study, the following two parameters of egg freshness were evaluated: oxidation intensity of yolk lipids and HU values. Determination of TBARS concentration indicates the level of fatty acid peroxidation. If TBARS concentration in blood or in other tissue is high, the level of lipid peroxidation will be also high. Figure 1 presents the intensity of yolk lipid peroxidation measured over three periods. Fresh eggs and eggs kept for 14 days at 4°C did not exhibit statistically significant differences related to the intensity of yolk lipid peroxidation. In both measurements yolks of the E2 group exhibited lower TBARS values than E1 (E2=3.1mMxg⁻¹, E1=3.4mMxg⁻¹; E2=3.3mMxg⁻¹, 3.6mMxg⁻¹). Analysis of TBARS showed statistically significant difference (P<0.05) between examined groups after 28 days of storing eggs at 4°C. It is to assume that higher dietary content of Se absorbed by yolk influenced less intensive lipid peroxidation in yolks of the E2 group.

Figure 2 presents HU for E1 and E2 eggs measured over three periods. Fresh eggs of the E2 group had higher HU values than those of the E1 (90.23HU and 88.66HU, respectively). In comparison to E1, declination trend of the E2 eggs was less intensive over the storage period. Such trend of HU values can be explained by reduced metabolic processes in eggs of the E2 group, the cause of which can be the higher content of Se in eggs of that group and its antioxidative effect. Many factors affect the quality of the edible part of the egg: period and temperature of storage, age of laying hens, layer diets, health condition of a flock, dietary supplements, etc. (Roberts, 2004). Over the storage period, the concentration of egg mass is reduced and dry matter percentage is increased due to evaporation of water through egg pores. Water from albumen penetrates into yolk and vice versa, and some nutrients contained in yolk penetrate into albumen. Because of CO₂ loss, pH of albumen in-

| Table 5. Content of selenium in egg yolk and albumen. |
|------------------------------------------------------|
| Content                                      | Experimental groups |
|                                            | E1  | E2  | SEM |
| n                                               | 6   | 6   |     |
| Albumen Se ng/g                                | 231.5<sup>a</sup> | 345.0<sup>a</sup> | 17.7 |
| Yolk                                           | 584.8<sup>b</sup> | 779.5<sup>a</sup> | 34.2 |

E1: 0.2 ppm of Se; E2: 0.4 ppm of Se.
<sup>a, b</sup>: estimated mean values between groups differ statistically significantly (P<0.05).
Figure 1. Effect of feeding treatments E1 and E2 on intensity of lipid peroxidation in yolks (TBARS, mMxg-1).

Figure 2. Effect of feeding treatments E1 and E2 on changes of HU for eggs over a storage period at 4°C.

\( a, b \ p < 0.05 \).
creases and viscosity decreases (Silversides and Scott, 2001). Average values of HU in both experimental groups (E1 88.66HU, E2 90.23HU) were higher than the minimum required (70) for extra quality fresh eggs, as defined in the Regulation on quality of eggs in the Republic of Croatia (2006). Results similar to ours were reported by Rutz et al. (2003). They pointed out a weaker declination trend of HU over a 14-day long period of storing eggs laid by hens fed diets with a higher content of Se in comparison with those produced by hens fed diets with a lower content of Se. In their research, Payne et al. (2005) stated that eggs produced by hens fed a diet with organic Se (0.15; 0.30; 0.60 and 3.00ppm) had higher HU values than eggs of hens fed a commercial diet.

The importance of our research is also found in the fact that modified eggs are useful in human nutrition. Due to the modern way of living and irregular nutrition, the human body is constantly exposed to stress. Taking into consideration the significant role of Se in metabolic processes, the American Health Organization calculated daily allowances of Se as 70μg for adult men and 55μg for adult women (Surai and Sparks, 2001). It should be stressed that the daily consumption of only one egg from the E2 treatment satisfies around 50% of the daily required amount of Se.

**Conclusions**

Based on the analyzed results, it can be concluded that dietary supplementation with a higher amount of Se resulted in increased GSH-Px activity in the hens’ blood (P<0.05). Furthermore, a higher amount of Se in layer diets affected a statistically significant (P<0.05) increase in Se content in the albumen and yolk, thus resulting in the production of eggs enriched with Se desirable in human nutrition. It should be also pointed out that eggs with a higher amount of organic Se remain fresh for a longer period of storage.

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