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The effects of supplementing sodium selenite and selenized yeast to the diet for laying hens on the quality and mineral content of eggs*

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ABSTRACT

In this experiment the effects of supplementing the diet for laying hens with sodium selenite (SS) or selenized yeast (SY) on the quality and content of some minerals of eggs were studied. Hy-Line Brown chickens were randomly divided on the day of hatching into 4 groups (12 birds per group). The birds were fed from day 1 of life to 9 months of age with diets differing in amounts and/or forms of selenium. The control group received a basal diet (BD) containing selenium naturally occurring in feeds (0.1 mg Se/kg of dry matter (DM)). The first and second experimental groups were fed the same BD enriched with Se at a dose of 0.4 mg/kg DM from sodium selenite or selenized yeast, respectively. The feed for the third experimental group consisted of BD supplemented with selenized yeast to a final selenium content of 1.0 mg/kg DM. The results showed that supplementation of SY to the diet significantly affected egg weight, egg yolk weight, egg albumen weight and Haugh units. Significantly lower egg shell weights and egg shell ratios were found in the experimental group with sodium selenite. Also, the concentration of Se in blood as well as that of some trace- and macroelements in eggs of laying hens appeared to be significantly influenced by selenium supplementation.

KEY WORDS: laying hens, sodium selenite, selenized yeast, egg quality, microelements, macroelements

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INTRODUCTION

Among many minerals, selenium is the most controversial trace element. Indeed, the narrow gap between essentiality and toxicity and environmental issues on the one hand, and global selenium deficiency on the other, fuels research in this field (Surai, 2006). Feedstuffs are routinely supplemented with various selenium sources to the final approved dose of 0.5 mg Se/kg DM. Although sodium selenite is absorbed from the gastrointestinal tract by a simple diffusion process, little is retained in the tissues of birds. Excessive intake of sodium selenite can cause toxicity and objectionable interactions with other minerals. Both sources of Se are reduced, however, to selenide, which is subsequently used in *de novo* synthesis of selenocysteine. As a structural component of the active centre of specific selenoenzymes (including glutathione peroxidases, iodothyronine deiodinases, thioredoxin reductases, selenophosphate synthetase and many others) selenocysteine is responsible for their catalytical activity. The best understood selenoenzyme is cytosolic glutathione peroxidase, which works as an antioxidant by removing hydrogen peroxides and organic peroxides (Kyriakopoulos and Behne, 2002). There are two major sources of Se for poultry - organic selenium, mainly in the form of selenomethionine (SeMet), which can be found in any feed ingredient in varying concentrations, and inorganic selenium, mainly selenite or selenate, which are widely used for dietary supplementation. The digestive system of animals, including birds, has adapted to metabolize organic Se from plant-based feedstuffs during evolution. Therefore, inclusion of selenite or selenate in the diet is not a "natural situation" and differences in assimilation, distribution and accumulation of Se in tissues depend on the source of Se. Se deficiency or surplus in modern poultry production is very rare. In general, adequate Se supplementation is considered to be a crucial factor in maintaining the high productive and reproductive characteristics of commercial poultry (Surai, 2002). Selenomethionine (the so-called 21st amino-acid) increases egg production, improves fertility, antioxidant status, accelerating growth after feather loss in young laying hens and has a positive influence on the storage ability of eggs (Edens, 2002). Supplementation of feed with selenized yeast has been shown to be more effective in decreasing lipid peroxidation in liver tissue as well as in formation of Se muscle deposits in laying hens than with sodium selenite (Petrovič et al., 2006).

The nutritional interrelations between selenium and other elements are not entirely understood. Increased contents of selenium in plants decrease the amounts of nitrogen, phosphorus and sulphur and act synergistically on the intake of manganese, tin, copper, iron and cadmium (Richter and Hlusek, 1994).

The aims of this study were to determine the effects of supplementing diets with sodium selenite and selenized yeast on the physical quality parameters of laying hen eggs, as well as to assess the concentration of some macro- and trace-elements in egg yolk and albumen.

MATERIAL AND METHODS

Animals, diets and treatments

Hens (n=48) of laying breed Hy-Line Brown were randomly divided on the day of hatching into 4 groups (n=12) and fed for 9 months with diets containing different amounts and forms of selenium. The composition of the basal diet (BD) fed to the laying hens from week 23 up to the age of 9 months is shown in Table 1.

Table 1. The composition of the basal diet fed to the laying hens from 23rd week to 9 months of age

Component	g/kg
Wheat, 10.5% CP	366
Barley, 12% CP	200
Maize, 8.3% CP	50
Soyabean meal, 45% CP, 1.5% fat	90
Limestone	82
Vitamin-mineral premix	35
Soyabean oil	7
Soyax, 35% CP, 20% fat	170

1 kg of basal diet contained, IU: vit. A 13469; vit. D_3 3106; mg: vit. E, 19; vit. K 2.49; tiamine, 5.6; riboflavin 6.6; pyridoxine 6.1; niacin, 59; pantothenic acid 13.86; biotin 0.09; folic acid 0.86; Se 0.1; Zn 64.2; I 0.77; Co 0.06; Mn 100.13; Cu 13.96; Fe 192.55; g: lysine 8.7; metionine 4.267; µg: cyanocobalamin 0.35

Chickens in the control group were fed the BD with a native content 0.1 mg Se/kg of dry matter (DM). The first and second experimental group received the same BD supplemented with an equivalent dose of Se 0.4 mg/kg DM of either sodium selenite (SS) or selenized yeast (SY) (Sel-Plex, Alltech Inc., USA), respectively. The BD for the third experimental group was supplemented with SY at a dose 0.9 mg Se/kg DM. The diets for all experimental groups were fortified with corresponding amounts of the yeast extract without Se (NUPRO, Alltech, USA), to obtain the same final levels of yeast extract as in the diet for the third experimental group (81.9 g per 100 kg of feed).

At the beginning of the experiment, the chickens were placed in a single-level cage battery in groups. After rearing to the age of 4 months, the birds were kept in a three-stage cage battery for laying hens. Rearing of the chickens started with a lighting regime 23L:1D which was adjusted to 16L:8D after three weeks of life. A light regime of 16L:8D was maintained during egg production. The initial room temperature 32-33°C was reduced every week by 3°C to a final temperature of 23°C. All birds had free access to water and feed. The experiment was carried out in accordance with established standards for laying hens.

Eggs were collected regularly twice a month (n=30 per group) and were assessed immediately after collection.

The protocol was approved by the local ethics and scientific authorities.

Sample analysis

At the age of 9 months, the hens were anaesthetized with an intraperitoneal injection of xylazine (Rometar 2% SPOFA, Czech Republic) and ketamine (Narkamon 5% SPOFA, Czech Republic) at doses of 0.6 and 0.7 ml/kg body weight, respectively. After laparotomy, blood was collected into heparinized tubes by intracardial punction. Samples of blood were stored at -65°C until analysed.

The following parameters of egg quality were measured: egg weight (g), egg albumen weight, egg albumen content, egg albumen index and Haugh units (HU), egg yolk weight, egg yolk content, egg yolk index, egg yolk colour (°HLR), egg shell weight, egg shell content, egg shell specific weight, egg shell strength (N/cm^2) and average egg shell thickness, using standard methods (Arpasova et al., 2007).

The concentration of selenium in blood was measured fluorimetrically (Rodriquez et al., 1994). The concentrations of copper, zinc, iron, calcium and manganese in egg albumen and yolk were determined by flame AAS (Perkin Elmer, A Analyst 100) after preceding wet mineralization in an MLS 1200 microwave oven. A kit (Randox, UK) was used for spectrophotometric assessment of phosphorus in egg yolk.

Statistical analysis

Statistical analysis was done using one-way analysis of variance (ANOVA) and the post hoc Tukey's multiple comparison test.

RESULTS

The concentration of Se in the blood of hens fed with selenium-enriched diets was significantly higher in comparison with the control group (Table 2). The highest concentration was in the group receiving Se-yeast supplementation at 0.9 mg/kg. Table 3 presents the changes in egg weight, egg albumen, egg yolk and egg shell quality caused by Se supplementation.

Table 2. The effects of sodium selenite and Se-yeast supplementation into basal diet (BD) for laying hens on concentrations of selenium in blood, µmol/l

Hen breed	Dietary treatment, mg/kg DM				SEM
	BD Se 0.1	BD + Se 0.4 selenite	BD + Se 0.4 Se-yeast	BD + Se 0.9 Se-yeast	
Hy-Line Brown	1.7^{a}	4.3^{b}	4.2^{b}	5.2^{b}	0.31

^{a,b} means with different letters within a row were significantly different (P<0.001); n=6

Table 3. The changes in egg weight, egg yolk, egg albumen and egg shell quality caused by supplementation of sodium selenite and Se-yeast into basal diet (BD) for laying hens

Parameter	Dietary treatment, mg/kg DM				SEM
	BD Se 0.1	BD + Se 0.4 selenite	BD + Se 0.4 Se-yeast	BD + Se 0.9 Se-yeast	
Egg weight, g	60.6^{a}	60.9^{a}	62.3^{b}	62.1^{b}	0.33
Egg yolk, g	14.2^{a}	14.5^{a}	14.9^{b}	14.8^{b}	0.11
Egg yolk, %	23.6	23.8	23.9	23.7	0.19
Egg yolk index	52.5	52.5	52.7	53.3	0.32
Egg yolk colour, °HLR	7.1	7.2	7.2	7.2	0.07
Egg albumen, g	40.5^{a}	40.8^{a}	41.6^{b}	41.5^{b}	0.29
Egg albumen, %	66.9	66.7	66.7	66.8	0.20
Egg albumen index	96.3	97.4	97.2	97.2	0.76
Haugh units, HU	85.5^{a}	86.7^{a}	87.0^{b}	87.1^{b}	0.32
Egg shell, g	5.8^{a}	5.7^{b}	5.9^{a}	5.9^{a}	0.03
Egg shell, %	9.6^{a}	9.3^{b}	9.4^{ab}	9.4^{ab}	0.05
Egg shell specific weight, g/cm^3	2.0	2.0	2.0	2.0	0.009
Egg shell strength, N/cm^2	29.9^{a}	26.7^{b}	27.1^{b}	28.0^{b}	0.49
Average egg shell thickness, µm	380	371	375	380	2.89

^{a,b} means with different letters within a row were significantly different (P<0.05); n=150; °HLR - coloured Hoffman La Roche scale

The average weight of the analysed eggs in both groups receiving selenized yeast-enriched diets was higher (P<0.05) than of eggs from control and sodium selenite-supplemented groups. Egg yolk weight was also significantly higher in the experimental groups with Se-yeast. On the other hand, no statistically significant differences among the groups were found in the yolk percentage ratio, egg yolk index, and average values of yolk colour assessed in °HLR. A significant difference in egg albumen weight was found between the experimental groups in which birds were fed selenized yeast-supplemented diets and the control group (P<0.05).

The supplementation of Se into the diet for hens did not change the egg albumen ratio or the egg albumen index. The Haugh units (HU) score revealed a higher quality of egg albumen in the groups of birds fed the selenized yeast-supplemented diet (P<0.05).

Supplementation of SS into the diet significantly decreased egg shell weight and egg shell weight ratio. The egg shell specific weight was not significantly influenced by selenium supplementation.

Egg shell strength was significantly lower in all groups supplemented with selenium, but Se supplementation did not affect the average egg shell thickness.

The influences of dietary SS or SY supplementation on the concentration of copper, iron and zinc in egg albumen are presented in Table 4. The concentration of copper in egg albumen was significantly lower in the third experimental group that received Se-yeast at an amount of 0.9 mg/kg compared with the sodium selenite experimental group. An effect of both forms of supplemented Se on the concentration of iron in egg albumen was found.

Table 4. The effect of sodium selenite and Se–yeast supplementation into basal diet (BD) for laying hens on the concentration of copper, iron and zinc (mg/kg DM) in egg albumen

Element	Dietary treatment, mg/kg DM				SEM
	BD Se 0.1	BD + Se 0.4 selenite	BD + Se 0.4 Se-yeast	BD + Se 0.9 Se-yeast	
Copper	0.83^{ab}	0.95^{a}	0.87^{ab}	0.58^{b}	0.09
Zinc	1.40	1.34	1.01	1.06	0.24
Iron	15.09^{a}	65.44^{c}	46.49^{b}	31.51^{ab}	4.18

^{a,b} means with different letters within a row were significantly different ($P<0.05$); n=10

The effects of supplementing SS or SY into the diet for laying hens on the concentration of some trace- and macroelements in egg yolk are shown in Table 5. A similar pattern was revealed in the concentrations of copper and iron in egg yolk compared with the same parameters in egg albumen. The concentration of zinc was statistically significantly lower, and concentration of iron was significantly higher, in the group supplemented with sodium selenite. The concentration

Table 5. The effects of sodium selenite and Se-yeast supplementation (mg/kg DM) into basal diet (BD) for laying hens on the concentration of copper, iron, zinc, manganese, calcium and phosphorus in egg yolk

Element	Dietary treatment, mg/kg DM				SEM
	BD Se 0.1	BD + Se 0.4 selenite	BD + Se 0.4 Se-yeast	BD + Se 0.9 Se-yeast	
Copper, mg	1.10^{ab}	1.46^{a}	0.95^{ab}	0.86^{b}	0.13
Zinc, mg	73.4^{a}	57.5^{b}	61.2^{ab}	63.4^{ab}	3.70
Iron, mg	111.9^{a}	198.5^{b}	131.7^{a}	119.2^{a}	16.63
Manganese, mg	1.96^{a}	1.50^{ab}	1.42^{b}	1.81^{ab}	0.12
Calcium, g	1.8^{ab}	1.61^{a}	1.79^{b}	1.87^{b}	0.42
Phosphorus, g	10.4	10.25	9.88	10.04	2.10

^{a,b} means with different letters within a row were significantly different ($P<0.05$); n=10 of manganese was significantly decreased in the experimental group with Se-yeast at 0.4 mg/kg compared with the control group. A significantly higher concentration of calcium in egg yolk was detected in groups receiving Se-yeast compared with those given only sodium selenite. Supplementation of Se did not significantly affect the concentration of phosphorus in egg yolk.

DISCUSSION

In our experiment, the highest concentration of selenium was found in the blood of hens fed the basal diet enriched with selenized yeast at a dose 0.9 mg Se/kg DM. However, the concentration of both forms of Se in the blood of hens fed selenium-enriched diets was significantly higher in comparison with the control group, which is in accordance with Petrovič et al. (2006).

The higher average egg weight in the groups with SY in our experiment corresponds with the results of several authors. Payne et al. (2005) found a positive influence of Se-yeast supplementation on egg weight, however, they simultaneously reported a higher occurrence of egg shell defects. They did not note a higher laying rate. A similar increase of egg weight after administration of SY was found by Skrivan et al. (2006), however, egg shell strength was not influenced by higher egg weight. Partial or full replacement of sodium selenite by organic selenium was shown to increase egg components and weight of egg parts, including yolk, albumen and shell in an experiment by Xavier et al. (2004). Sahin et al. (2003) observed a beneficial effect on egg weight of supplementing inorganic selenium with vitamin E into the diet for laying hens.

In our experiment, egg yolk weight was significantly higher in the experimental groups with Se-yeast. Similarly, Xavier et al. (2004) found increased egg yolk weight in response to organic selenium supplementation. Skrivan et al. (2006) detected a significantly lower difference in the group supplemented with selenized yeast compared with the control group. Fernandes et al. (2008) reported a significantly higher ratio of fresh and dried yolk yields with dietary inclusion of an organic mineral blend at 0.250 and 0.500 kg/ton.

Yolk colour was not significantly influenced in our experiment by the form or amount of added selenium. In contrast, a significant improvement in yolk colour was observed as a result of organic selenium supplementation in the experiment of Pan and Rutz (2003).

The egg weight increase in the groups receiving organic selenium at both doses is related to the higher weights of egg albumen, yolk and shell in these groups. Increased albumen weight was also observed in the experiments of Pan and Rutz (2003), Xavier et al. (2004) and Skrivan et al. (2006).

It is well established that specific selenoenzymes/selenoproteins are able to reduce the harmful effects of free radicals. Free radicals are initiators of uncontrolled oxidation, which affects primarily lipids, causing peroxidation of unsaturated fatty acids. The damage to lipids can induce further damage of proteins and DNA (Kelly et al., 1998).

Egg freshness is one of the most important parameters determining consumer perception and demand. Egg freshness decreases during storage. Aging results in the egg albumen becoming thinner and the egg yolk not keeping a globular shape. This process is associated with biochemical changes in the composition and structure of egg membranes. Inclusion of organic selenium in a commercial diet significantly increased the Se level in the perivitelline membrane (Surai, 2002). Therefore, this could be an additional mechanism of the positive effect of organic selenium on egg freshness during storage. This effect probably depends on the age of hens, diet composition, and egg storage conditions (Surai, 2006). According to these parameters, eggs laid by hens fed organic selenium remained fresh longer and their evaluation by Haugh units was more favourable.

The Haugh unit scores in our experiment were higher in all groups supplemented with selenium, however, only in those groups given Se-yeast were the differences statistically significant in comparison with the control group. Our results disagree, however, with those of Payne et al. (2005) who reported that the albumen quality of eggs (determined by Haugh unit score) stored at 22.2°C was improved in hens fed with SS compared with those fed with SY, but albumen quality was not affected by diet in eggs stored at 7.2°C. The supplementation of inorganic selenium with vitamin E into the diet for quails beneficially affected HU of eggs (Sahin et al., 2003). Paton et al. (2000) reported that SS or SY supplementation at 0.30 ppm had no effect on Haugh unit values in eggs on d 0, 21, or 42 compared with eggs from hens fed the basal diet. However, in a second experiment by Paton et al. (2000), SS supplementation at 0.10, 0.20, or 0.30 ppm improved Haugh unit scores compared with eggs from hens fed by SY at the same dietary levels.

Egg shell structure and, consequently, the rate of diffusion through shell pores, is affected by age and laying stage. Trace minerals may affect egg shell quality due to their catalytic properties as constituents of key enzymes involved in the processes of membrane and egg shell synthesis or by direct interaction with calcium crystals during egg shell formation (Fernandes et al., 2008).

Egg shell weight and egg shell weight ratio were significantly lower in our experiment in the groups with supplemented with only sodium selenite. Improvement of egg shell weight by organic selenium was reported by Xavier et al. (2004). Similarly, replacement of 50% selenite in a laying hen diet by organic selenium was associated with a significant increase in egg shell weight in the experiment of Klecker et al. (2001).

Xavier et al. (2004) noted improvement of egg shell specific weight. These results are not in agreement with our experiment. We found insignificant differences among the groups in this respect.

The highest egg shell strength in our experiment was in the control group. Skrivan et al. (2006) found only a tendency towards improving egg shell strength when Se from an organic source was supplemented into the diet for laying hens.

In the experiment of Payne et al. (2005) the hen-day production percentage and percentage of shell-less eggs were not affected by the source or level of Se. However, the percentages of dirty and cracked eggs were higher from hens fed SY compared with those fed SS. The occurrence of cracked eggs was more than 2.5 times greater from hens supplemented with SY compared with those supplemented with SS. Paton et al. (2000) reported no difference in egg breaking strength from hens fed supplemental SS or SY in 2 experiments, but observed an increase in egg breaking strength from hens supplemented with SY relative to SS in a third experiment. In contrast, Fernandes et al. (2008) observed positive effects of organic trace mineral supplementation on the percentage of cracked and thin shell eggs. Similarly, Siske et al. (2000) reported increased egg shell strength when organic forms of Mn, Se, and Zn were substituted into the diet for one-half of the inorganic forms of these minerals.

The average egg shell thickness in our experiment was not significantly influenced by selenium supplementation.

Heavier eggs often have thinner and thus less strong egg shells. The egg shell of heavier eggs was less resistant to pressure at non-destructive strength measurements, but the differences were not significant (Skrivan et al., 2006). In contrast, egg shell thickness was positively influenced by organic selenium (Klecker et al., 2001) and also by inorganic selenium and vitamin E supplementation (Sahin et al., 2003).

A significantly lower concentration of copper in egg albumen in our experiment was found in the third experimental group with Se-yeast compared with the experimental group given sodium selenite. The concentration of iron in egg albumen was influenced by Se supplementation in both forms. A similar tendency in the egg-yolk concentrations of copper and iron was found as in the same parameters in egg albumen. In the group supplemented with sodium selenite, significantly lower concentrations of zinc were found. The concentration of iron was significantly higher, however, in this experimental group. The higher concentrations of copper, zinc and iron in egg yolk in comparison with egg albumen indicate their importance in the development of bird embryos. All of these elements are considered to be antioxidants, Fe (as a part of catalase), Cu and Zn (superoxide dismutase) and have interdependent roles with Se (glutathion peroxidase) in the antioxidant protection of the embryo. The available information about the nutritional interrelationships between selenium, cooper, zinc and iron in birds are very limited. Synergic interactions between dietary Cu and Se have been found (Hartmann and van Ryssen, 1997). The concentrations of zinc, iron and manganese in egg albumen were significantly higher in quails fed an SS- and vitamin E-supplemented diet (Sahin et al., 2003). The higher amount of copper in the diet is able to reduce the incorporation of selenomethionine into the protein fraction of egg albumen (Davis et al., 1996).

In conclusion, the results of this experiment show that the majority of parameters of egg quality may be positively influenced by supplementation of laying hens with selenium, especially in the form of Se-yeast. Additional selenium also seems to influence the content of some trace- and macro-elements in eggs.

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