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EFFECTS OF DIETARY SUPPLEMENTATION OF SODIUM SELENITE AND SELENIZED YEAST ON SELECTED QUALITATIVE PARAMETERS OF LAYING HENS EGGS

H. ARPÁŠOVÁ[1]*, M. MELLEN[1], M. KAČÁNIOVÁ[1], P. HAŠČÍK[1], V. PETROVIČ[3], K. ČOBANOVÁ[2], L. LENG[2]

[1] Slovak University of Agriculture, Nitra, Slovak Republic; [2]Slovak Academy of Sciences, Košice, Slovak Republic; [3]University of Veterinary Medicine, Košice, Slovak Republic

ABSTRACT

In this experiment the effects of supplementation of the diet of laying hens with sodium selenite (SS) or selenized yeast (SY) on quality of eggs were studied. The chickens of Shaver Starcross hybrid were randomly divided at the day of hatching into 4 groups (n=12 in each group). The birds were fed from Day 1 of life till 9 months of age with diets differing in amounts and/or forms of selenium. Control group received basal diet (BD) containing selenium naturally occurring in feeds (0.1 mg Se/kg of dry matter (DM)). First and second experimental group of chickens were fed with a same BD enriched with equivalent dose of Se 0.4 mg/kg DM in the form of SS or SY, respectively. The BD for the third experimental group was supplemented with SY at a dose 0.9 mg Se/kg DM. The supplementation of both forms of selenium into diet had a significant effect on the concentration of Se in blood of laying hens. Egg weight and egg albumen weight were significantly higher in the experimental groups with SY supplementation only. The supplementation of both forms of selenium into the diet of laying hens significantly affected the width of egg, egg albumen high, Haugh units (HU), egg yolk weight as well as egg yolk ratio. These results indicate that selenium supplementation into laying hens' basal diet significantly influences the concentration of Se in blood of laying hens and most of the physical qualitative parameters of eggs.

Key words*:* laying hens, sodium selenite, selenized yeast, blood, egg weight, egg albumen, egg yolk

INTRODUCTION

Selenium (Se) is considered to be one of the most controversial trace elements. On the one hand, it is toxic at high doses and there is a great body of information related to environmental issues of Se contamination. On the other hand, Se deficiency is a global problem related to an increased susceptibility to various diseases of animals and humans and decreased productive and reproductive performance of farm animals (Lyons et al., 2007). Adequate intake of selenium is needed for immunocompetence (Rayman, 2000). Inadequate Se supply in combination with low vitamin E status causes deficiency symptoms in many species (Zuberbuehler et al., 2002). In nature, it can be found in the elemental form as well as incorporated into both inorganic and organic compounds (Schrauzer, 2000).

Se metabolism depends on the chemical form of Se in the diet, and some forms are better for some actions (e.g., cancer reduction) than other forms. Food may contain different amounts and chemical forms of Se (Finley, 2007). There are two major sources of Se for poultry: organic Se, mainly in the form of selenomethionine (SeMet), which can be found in any

Correspondence: E-mail: Henrieta.Arpasova@uniag.sk
Slovak University of Agriculture, Tr. A. Hlinku 2,
949 76 Nitra, Slovak Republic; tel. 00421 37 6414314

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feed ingredient in varying concentrations and inorganic selenium, mainly selenite or selenate, which are widely used for dietary supplementation (Surai, 2002). Se in the form of another amino acid, selenocysteine, is the central structural component of specific selenoenzymes including glutathione peroxidases, iodothyronine deiodinases, thioredoxin reductases, selenophosphate synthetase and many others. The best understood selenoenzyme is cytosolic glutathione peroxidase (cGPx), which acts as an antioxidant by removing reactive oxygen species (Kyriakopoulos and Behne, 2002).

In most EU countries the natural Se content of grains and forages, which consists mainly of the selenoamino acids selenomethionine and selenocysteine in plant proteins, is only 0.03–0.12 mg/kg with values more commonly at the lower end of this range. Intake of such feed may result in serious Se deficiency and health problems, especially in highly productive animals. For this reason, feedstuffs are routinely supplemented with various Se sources at 0.2-0.3 mg Se/kg of dry matter.

Eggs are a good source of nutrients and play an important role as a functional food in human nutrition (Seuss-Baum, 2005; Sparks, 2006). The increase of Se content in the eggs of commercial laying hens express in stronger antioxidant protection of yolk, prolonged stability and nutrition value of eggs (Hess et al. 2003). Selenomethionine (so–called 21st amino acid) increases egg production, antioxidant status, accelerate feathers growth after feather loss of layers at saddle and back sections (Edens, 2002), and has positive influence on storage ability of eggs. Se concentration in eggs was markedly higher in birds fed with with organic selenium (Vukasinovic et al., 2006; Surai et al., 2006; Pan et al., 2007). Replacement of sodium selenite by organic Se in the laying hen diet was shown to: improve feed conversion ratio, shell quality, egg freshness during storage, and decrease lipid peroxidation during storage.

Krška et al. (2000) found positive effect of organic Se additive on increase of capacity of the antioxidant defensive system in the experimental group of pigs fed with Se and vitamin E additives together. Foltys et al. (2001) reported decrease of somatic cells counts and increase of Se content in cow´s milk. In their experiment Marounek et at. (2006) supplemented the diet with Se-enriched yeast to increase Se concentration to 0.50 mg/kg. The authors found higher selenium concentration of Se-enriched yeast supplementation in meat, faeces and hair of calves. Glutathione peroxidase activity was significantly increased in the liver tissue of Se-supplemented calves, but not in the muscle.

The aim of this study was to compare the effects of feed supplementation with SS and SY on concentration of Se in blood of laying hens, egg weight and physical qualitative parameters of egg yolk and egg albumen.

MATERIAL AND METHODS

Animals, Diets and Treatments

Hens (n=48) of the laying hybrid Shaver Starcross 288 were randomly divided at the day of hatching into 4 groups (n=12) and fed for 9 months with diet containing different amounts and forms of Se. The appropriate diets were fed *ad libitum* during the rearing and breeding period for healthy development of laying hens. BD was fortified with Premix HYD–04 for a period 0–6 weeks, Premix HYD–05 for the period 7–16 weeks, Premix HYD–06 for the period 17–22 weeks and Premix HYD–10 for the period 23–36 weeks, respectively. The composition of the final BD with Premix HYD-10 fed to the laying hens from the 23rd week up to the age of 9 months is presented in Table 1.

Table 1: Composition of the basal diet fed to the laying hens for the last 4 months

Component	g / kg
Ground wheat, 10.5% crude protein (CP)	366
Ground barley (12% CP)	200
Ground corn (8.3% CP)	50
Soybean extracted ground meal (45% CP, 1.5% fat)	90
Limestone	82
Premix HYD-10	35
Soybean oil	7
Pulverized soya fat, Soyax-FORTA (35% CP, 20% fat)	170

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

Chickens in control group were fed with BD with native content of 0.1 mg Se/kg of dry matter (DM). First and second experimental groups received the same BD supplemented with equivalent dose of Se 0.4 mg/kg DM of either SS or SY (Sel–Plex, Alltech Inc., USA), respectively. Content of Se (0.4 mg/kg DM) with Se occurring naturally in feed (0.1 mg Se/kg of DM) represents 0.5 mg Se/kg of dry matter - the final approved dose for selenium content in animal feed according to EU. The BD for the third experimental group was supplemented with SY at a dose of 0.9mg 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 (81.9g per 100kg of feed).

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

Sample Analysis

The concentration of Se in blood was measured using the fluorimetric method (Rodriquez et al., 1994)

Eggs of laying hens of SS 288 hybrid were collected twice every month (n= 30 per group) and were assessed immediately after collection. Five analyses were carried out altogether. Egg weight (g), length of egg (mm), width of egg (mm), egg albumen weight (g), egg albumen content (%), egg albumen high (mm), Haugh units (HU), egg yolk weight (g), egg yolk content (%), egg yolk high (mm) and egg yolk colour (°HLR) were determined. All these parameters were detected using routine methods (Arpášová et al., 2007).

Weight parameters were detected using analytical weighing machine and the growth intensity and percentage contents were calculated from weight data. Haught units (HU) detected egg quality in relation to albumen weight and egg weight [100 log.(dense albumen height – 1.7x egg weight$^{0.37}$ + 7.6)]. Yolk color was evaluated using Hoffman-La Roche colour scale (Hoffman–La Roche, Switzerland).

At the age of 9 months, the hens were anaesthetized with intraperitoneal injections of xylazine (Rometar 2% SPOFA, Czech Republic) and ketamine (Narkamon 5% SPOFA, Czech Republic) at doses 0.6 and 0.7 ml/kg of body weight, respectively. After laparotomy, blood was collected into heparinized tubes by intracardial punction. All blood samples were stored at –65°C to await analysis.

Statistical Analysis

Statistical analysis was done using one–way analysis of variance (ANOVA) with the post hoc

Duncan's multiple comparison test with the help of statistical programme Statgraphics Centurio XV.

RESULTS AND DISCUSSION

The effect of sodium selenite (SS) or selenized yeast (SY) supplementation into the diets for laying hens on the concentration of selenium in blood is presented in Table 2. The concentration of Se in hen blood fed with Se enriched diets was significantly higher in comparison with control group of birds, which is in accordance with Jiakui and Xiaolong (2004) and Pan et al. (2007). The highest concentration of Se was found in the group of hens fed with BD enriched with SY at a dose of 0.9 mg Se/kg DM.

The changes in whole egg quality caused by SS and SY supplementation are presented in Table 3. The average weight of analyzed eggs in all groups receiving Se enriched diet was 59.57±4.16^{a}, 59.27±4.43^{a}, 60.63±4.67^{b}, 60.99±4.16^{b}, respectively. A similar increase of egg weight after administration of Se was reported by Payne et al. (2005) and Utterback et al. (2005). The length of egg was significantly lower in the group with SS. The width of egg was statistically ($P<0.05$) higher in experimental groups with Se supplementation in both forms. Sahin et al. (2003) observed the beneficial effect of supplementation of inorganic Se with vitamin E into the diet for laying hens on the egg weight.

The present explanation was based on the antioxidant features of Se, which is through seleno–enzymes able to reduce the production of harmful free radicals. Free radicals are initiators of uncontrolled oxidation processes which primarily affect lipids, causing lipid peroxidation of unsaturated fatty acids. The damage of lipids can induce further damage of proteins and DNA (Kelly et al., 1998).

Table 2: Effects of sodium selenite and Se–yeast supplementation into basal diet (BD) of laying hens on concentrations of selenium in blood (µmol/l)

Hen strain	BD (Se 0.1 mg/kg DM)	BD + Se 0.4 mg/kg DM (selenite)	BD + Se 0.4 mg/kg DM (Se-yeast)	BD + Se 0.9 mg/kg DM (Se-yeast)
Shaver Starcross 288	1.71±0.16a	4.59±0.16b	5.28±0.28bc	5.77±0.26c

Distinct letters in superscripts within a row mean significant differences ($p<0.001$). Values are means±S.D; n=7 hens in each group

Table 3: Changes in whole egg quality caused by supplementation of sodium selenite and Se–yeast into basal diet (BD) for SS 288 laying hens

Group	BD Se 0.1 mg/kg of dry matter (DM)	BD + Se 0.4 mg/kg DM (selenite)	BD + Se 0.4 mg/kg DM (Se-yeast)	BD + Se 0.9 mg/kg DM (Se-yeast)	P value
Egg weight (g)					
n	150	150	150	150	
$\bar{x}$	59.57a	59.26a	60.63b	60.98b	0.0012
SD	4.16	4.43	4.69	4.16	
CV (%)	6.98	7.48	7.70	6.82	
minimum	50.9	50.7	51.0	50.5	
maximum	82.1	78.3	82.1	69.6	
Length of egg (mm)					
n	150	150	150	150	
$\bar{x}$	56.12bc	55.43a	55.86b	56.35c	0.0001
SD	2.06	1.40	1.53	1.68	
CV (%)	3.68	2.53	2.74	2.99	
minimum	51.0	52.0	52.0	52.0	
maximum	67.0	62.0	65.0	62.0	
Width of egg (mm)					
n	150	150	150	150	
$\bar{x}$	42.61a	43.0b	43.21b	42.98b	0.0002
SD	1.06	1.33	1.23	1.21	
CV (%)	2.49	3.08	2.84	2.82	
minimum	39.0	41.0	40.0	39.0	
maximum	48.0	47.0	47.0	47.0	

Distinct letters in superscripts within a row mean significant differences (p<0.05). Values are means±SD; n=150 pieces of analyzed eggs in each group

Changes in egg albumen quality are presented in Table 4. Albumen weight was significantly higher in the group of birds fed with diets enriched with SY in amount 0.9 mg/kg of DM compared to the control group or the first experimental group (38.65±3.31^{b}, 37.81±3.45^{a}, 38.95±3.41^{bc}, 39.57±3.34^{c}; P<0.05), respectively.

The supplementation of Se into the diet for hens did not change the percentage of egg albumen ratio. Egg albumen high was statistically higher (P<0.05) in the experimental groups with SS and SY supplementation, too. Also, the egg stability analyzed by Haugh units (HU) revealed higher quality of egg albumen in the groups of birds fed with Se in both forms supplemented diet (85.63±3.91^{a}, 86.69±3.92^{b}, 87.05±4.13^{b}, 87.12±3.62^{b}; P<0.05), respectively. The supplementation of inorganic Se with vitamin E into the diet of quails beneficially affected HU of eggs (Sahin et al. 2003). Pappas et al. (2005) also found a positive correlation between Se intake and Haugh units.

The changes in dynamics of yolk quality caused by Se supplementation are presented in Table 5. The weight of egg yolk unveiled a similar pattern, but Skrivan et al. (2006) did not detect any significant differences in this parameter when hens were fed with Se enriched diet. The supplementation of Se into the diet for hens did not affect the egg yolk high as well as yolk colour counted in °HLR. Results of our experiment are consistent with those of Pribilincova and Maretta (1996).

The results showed that the supplementation of SY into the diet significantly affected the egg weight. The concentration of Se in blood as well as most of the physical qualitative parameters of eggs appeared to be significantly influenced by Se supplementation in both forms into laying hens' basal diet.

Higher level of Se (0.9 mg/kg DM) beyond EU limit was used in the experiment for the purpose of increasing and comparing the contents of organic form of Se by means of SY in yolk, albumin and in the whole egg in the group fed with a dose of 0.5 mg/kg DM and a over limit dose of 0.9 mg/kg DM, in consideration with well known low daily intake of Se by the experimental population and markedly higher recommended daily needs.

Advantages of SY application are their better bio-efficiency and ability of selenomethionine from this source to create body deposit in hens' tissues and thus

Table 4: Changes in egg albumen quality caused by supplementation of sodium selenite and Se–yeast into basal diet (BD) for SS 288 laying hens

Group	BD Se 0.1 mg/kg of dry matter (DM)	BD + Se 0.4 mg/kg DM (selenite)	BD + Se 0.4 mg/kg DM (Se-yeast)	BD + Se 0.9 mg/kg DM (Se-yeast)	P value
Egg albumen weight (g)					
n	150	150	150	150	
$\bar{x}$	38.65b	37.81a	38.95bc	39.57c	0.0001
SD	3.31	3.45	3.41	3.34	
CV (%)	8.57	9.12	8.76	8.44	
minimum	32.2	31.6	30.9	31.1	
maximum	50.4	50.1	46.7	46.3	
Egg albumen %					
n	150	150	150	150	
$\bar{x}$	64.93	64.07	64.30	64.88	0.1535
SD	4.65	3.84	3.52	3.74	
CV (%)	7.17	5.99	5.47	5.76	
minimum	51.79	51.85	51.45	48.64	
maximum	95.27	82.55	81.55	78.74	
Egg albumen high (mm)					
n	150	150	150	150	
$\bar{x}$	7.30a	7.48b	7.61b	7.64b	0.0001
SD	0.64	0.67	0.69	0.60	
CV (%)	8.79	8.96	9.01	7.86	
minimum	6.0	6.0	6.0	7.0	
maximum	9.5	9.0	10.0	9.0	
Haugh units (HU)					
N	150	150	150	150	
$\bar{x}$	85.53a	86.69b	87.01b	87.11b	0.0012
SD	3.91	3.92	4.12	3.62	
CV (%)	4.57	4.53	4.74	4.16	
minimum	73.52	77.14	76.24	81.17	
maximum	96.63	96.18	99.76	96.32	

Distinct letters in superscripts within a row mean significant differences ($p<0.05$). Values are means±SD; n=150 pieces of analyzed eggs in each group. HU – Haugh units

incorporate to their products – eggs, meat etc. The use of over limit dose of Se in the form of SY yeast to the feed did not have a negative effect on the quality parameters of inner content of market eggs. In consideration of above mentioned advantages of organic form of Se and also from the results of this study, we can recommend larger use of this form of Se in practice.

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Table 5: Changes in egg yolk quality caused by supplementation of sodium selenite and Se–yeast into basal diet (BD) for SS 288 laying hens

Group	BD Se 0.1 mg/kg of dry matter (DM)	BD + Se 0.4 mg/kg DM (selenite)	BD + Se 0.4 mg/kg DM (Se-yeast)	BD + Se 0.9 mg/kg DM (Se-yeast)	P value
Egg yolk weight (g)					
n	150	150	150	150	
$\bar{x}$	14.81a	15.42b	15.61b	15.28b	0.0002
SD	1.79	1.35	1.74	1.63	
CV (%)	12.12	8.80	11.12	10.69	
minimum	10.8	12.4	11.5	10.8	
maximum	25.1	21.8	27.8	27.8	
Egg yolk %					
n	150	150	150	150	
$\bar{x}$	24.86a	26.05b	25.75b	25.06a	0.0001
SD	2.35	1.79	1.93	2.13	
CV (%)	9.47	6.88	7.51	8.50	
minimum	17.62	21.59	21.37	18.50	
maximum	31.03	31.73	33.86	29.38	
Egg yolk high (mm)					
n	150	150	150	150	
$\bar{x}$	20.23	20.11	20.14	20.27	0.7411
SD	1.33	1.13	1.22	1.87	
CV (%)	6.56	5.60	6.03	9.21	
minimum	17.0	17.0	17.0	17.0	
maximum	25.0	23.0	22.0	23.0	
Egg yolk colour (°HLR)					
n	150	150	150	150	0.0621
$\bar{x}$	7.12	7.20	7.19	7.19	
SD	0.70	0.74	0.85	0.91	
CV (%)	9.83	10.27	11.82	12.66	
minimum	6	6	6	6	
maximum	9	9	9	9	

Distinct letters in superscripts within a row mean significant differences ($p<0.05$). Values are means±SD; n=150 pieces of analyzed eggs in each group. °HLR – colored Hoffman La Roche scale

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