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# Antioxidant and selenium status of laying hens fed with diets supplemented with selenite or Se-yeast*

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ABSTRACT

The experiment was designed to investigate the effects of feed supplementation with selenite or selenized yeast on parameters of antioxidant and selenium status of laying hens.

Hens of laying breed Shaver Starcross 288 were randomly divided at the day of hatching into 4 groups and fed for 9 months on diets which differed only in amounts or forms of selenium supplemented. Group 1 was fed the basal diet (BD) with native Se content 0.1 mg·kg$^{-1}$ DM. Groups 2 and 3 were fed the BD diets supplemented with equivalent Se dose 0.4 mg·kg$^{-1}$ DM of either sodium selenite or Se-yeast, respectively. The diet for group 4 was supplemented with Se-yeast at Se dose 0.9 mg·kg$^{-1}$ DM. The activities of glutathione peroxidase (GPx) in blood and tissues of liver, kidney and duodenal mucosa were significantly increased by Se supplementation, but no differences due to form or dose of Se were observed. Both Se sources resulted in significant reduction of superoxide dismutase (SOD) activity in erythrocytes. Malondialdehyde (MDA) content in kidney tissue was reduced by both Se sources, but its production in liver tissue was inhibited by Se-yeast only. Selenium supplementation did not influence the levels of MDA and -SH groups in plasma. Altrough both Se significantly raised Se concentrations in blood and tissues of liver, kidney, spleen, hearth and duodenal mucosa, significant Se deposition into muscles appeared in hens given Se-yeast only. The presented results suggest that Se-yeast is more effective in maintenance of antioxidant and selenium status of laying hens than selenite.

KEY WORDS: poultry, selenium, lipid peroxidation, antioxidant, enzyme

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## INTRODUCTION

Selenium is an essential trace element with very similar chemical and physical properties to sulphur. Both inorganic and organic compounds occur in nature. In most EU countries, the natural selenium content of grain and forages used in animal feedstuffs is only 0.03-0.12 of dry matter, with values more commonly at the lower end of this range. Intake of such feeds may result in serious selenium deficiency with subsequent impaired animal efficiency and/or health problems. Another situation may be induced by a marginal shortage of Se, which usually does not exert apparent clinical signs of Se deficiency, but may cause delayed development of immunocompetence and hence raise susceptibility of animals to infectious diseases. For this reason, feedstuffs in the EU are routinely supplemented with inorganic selenium sources like selenite and selenate up to maximum total approved Se contents 0.5 $mg \cdot kg^{-1}$.

Selenium in the form of amino acid selenocysteine is the central structural component of specific selenoenzymes such as glutathione peroxidases, iodothyronine deiodinases, thioredoxin reductases or selenophosphate synthetase. To date about 30 selenoproteins have been identified but only half of them are functionally described. The best understood selenoenzyme appears to be cytosolic glutathione peroxidase (cGPx) which works as an antioxidant by removing hydrogen peroxides and organic hydroperoxides (Behne and Kyriakopoulos, 2001). Adequate intake of bioavailable forms of selenium is therefore critical for maintaining the appropriate Se and antioxidant status of animals (Pavlata et al., 2001; Zuberbuehler et al., 2005).

The most striking finding in the use of sodium selenite as a feed supplement concerns its pro-oxidative properties. Its ability to form superoxide anion ($O_2^{*-}$) has been demonstrated under *in vitro* conditions and this process is presumed to run *in vivo* too (Kobayashi et al., 2001). Free radicals are involved in uncontrolled chain reactions which primarily affect phospholipids, causing lipid peroxidation (LPO). The $O_2^{*-}$ anion has been designated as the initiator of toxic chain radical reactions. Lipid hydroperoxides, peroxyl radicals and hydroperoxides generated within the initiation and propagation of LPO can induce further damage to proteins and DNA (Tirosh and Reznick, 2000).

In practical terms, Se in the form of selenomethionine (SeMet) incorporated into the proteins of *Saccharomyces cerevisiae* represents some 60-80% of the total selenium content of selenized yeast (Rayman, 2004). Moreover, *in vitro* experiments with free SeMet give indications of its antioxidant properties due to its ability to inhibit peroxyl radical formation and thus protect against the damaging effects of peroxynitrite (Sies et al., 1998).

To estimate the level of oxidative stress, the activities of antioxidant enzymes such as superoxide dismutases (SOD), gluthatione peroxidases (GPx) as well as

lipid peroxidation based on measurement of thiobarbituric acid reactive substances like malondialdehyde in various tissues are generally employed.

The aim of this study was to compare the effects of feed supplemented with sodium selenite and Se-yeast on SOD activity in red blood cells, cytosolic GPx activities and lipid peroxide formation in liver, kidney and duodenal mucosa tissues, as well as the parameters of Se metabolism in laying hens.

## MATERIAL AND METHODS

### *Animal, diets and treatments*

Sixty females of the laying strain Shaver Starcross 288 were randomly divided at the day of hatching into 4 groups (n=15) and fed for 9 months on diets which differed only in amounts or forms of selenium supplemented. Appropriate diets for growth and development of laying breed hens were used from day 0 until the birds were aged 21 weeks (HYD-04 for period 0 to 6 weeks, HYD-05 for period 7-16 weeks and HYD-06 from 17 to 22 weeks). The composition of the last basal diet HYD-10 fed to the hens from the 23rd week up to the age of 9 months is presented in Table 1.

Table 1. The composition of the basal diet fed to hens for the last 4 months before tissue sampling

| Component | g/kg |
|---|---|
| Wheat ground, 10.5% of crude protein (CP) | 366 |
| Soyabean oil | 7 |
| Maize ground (8.3% CP) | 50 |
| Soyabean extracted ground meal (45% CP, 1.5% fat) | 90 |
| Limestone | 82 |
| Premix HYD-10 | 35 |
| Barley ground (12% CP) | 200 |
| Pulverized soya fat, Soyax-FORTA (35% CP, 20% fat) | 170 |

1 kg of basal diet contained: IU: vit. A, 13469; vit. D3, 3106; mg: vit. K, 2.49; thiamine, 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; µg: cyanocobalamin, 0.35; g: lysine, 8.7; methionine, 4.267

All basal diets (BDs) fed to group 1 had selenium contents 0.1 $mg \cdot kg^{-1}$ of dry matter (DM) arising from native dietary components only. Groups 2 and 3 were fed BDs supplemented with Se dose 0.4 $mg \cdot kg^{-1}$ DM of either sodium selenite or selenized yeast (Sel-Plex, Alltech Inc., USA), respectively. The BDs for group 4 was supplemented with Se-yeast selenium dose 0.9 $mg \cdot kg^{-1}$ DM, resulting in final Se content 1.0 $mg \cdot kg^{-1}$ DM. The diets for groups 1, 2 and 3 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 group 4 (81.9 g per 100 kg of feed). The contents of vitamin E in BDs HYD-04, HYD-05, HYD-06 and HYD-10 were 41.9, 43.4, 30.5 and 19.0 $mg \cdot kg^{-1}$ of diet, respectively. The lower value content of vitamin E in BD HYD-10 fed to the hens for the last 4 months was preferred due to better unmasking of Se source and/or dose effects on the parameters of oxidative stress. The differences in the final contents of Cu and Zn in the experimental diets caused by the additions of various amounts of Se-yeast or NUPRO were less than 0.01% due to their minimal proportions into diets.

At the beginning of the experiment, the hens were placed in large pens with wood shavings. After reaching the age of 4 months, the birds were then kept in battery cages for laying hens. Rearing of the chickens started with a lighting regimen of 23L:1D which was adjusted to 16L:8D after three weeks of life. The constant lighting regimen 16L:8D was kept during egg production too. 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 use of animals. The protocol was approved by the local ethical and scientific authorities.

At age 9 months, the hens were anaesthetized with intraperitoneal injection of xylazine (Rometar 2%, SPOFA, Czech Republic) and ketamine (Narkamon 5%, SPOFA, Czech Republic) at doses 0.6 and 0.7 $ml \cdot kg^{-1}$ of body weight, respectively. After laparotomy, blood was collected into heparinized tubes by intracardial punction and centrifuged for plasma specimens at 1180 *g* for 15 min. Samples of blood and plasma for analysis were frozen and stored at -65ºC. Following euthanasia, samples of liver, kidney, spleen, duodenal mucosa, heart and breast muscle tissues were collected and stored also at -65ºC until analysed.

*Sample analysis*

The concentrations of selenium in diets, blood and tissues were measured using the fluorimetric method of Rodriguez et al. (1994). The standard procedure of tissue drying at 105°C was used to determine the dry matter content of diets and tissues.

Activity of blood glutathione peroxidase (GPx, EC 1.11.1.9) was determined using the method of Paglia and Valentine (1967) with a Ransel kit (Randox, UK). To analyse the activities of GPx in liver, kidney and duodenal mucosa, pieces of tissues were homogenized in phosphate buffer saline pH 7.4 containing 0.372 g of $Na_2EDTA \cdot 2H_2O$. Homogenates were centrifuged at 13680 g at 4ºC, for 20 min. Enzyme activity in the supernatant was measured by monitoring oxidation of $NADPH+H^+$ at 340 nm as described by Paglia and Valentine (1967) and modified by Zagrodzki et al. (1998).

Tissues samples of liver, kidney and duodenal mucosa for malondialdehyde (MDA) determination were homogenized with deionized distilled water and 50 µl of butylated hydroxytoluene. The MDA concentrations in plasma and homogenates were measured with the modified fluorimetric method according to Jo and Ahn (1998).

Haemoglobin (Hb) content of blood and superoxide dismutase (SOD, EC 1.15.1.1) activities (Arthur and Boyne, 1985) in erythrocytes were analysed using kits from Randox (UK). The protein concentrations in tissues examined were measured by spectrophotometric method of Bradford (1976). Ellman's method (1958) was used to determine -SH groups in plasma.

*Statistical analysis*

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

## RESULTS

The concentrations of Se in blood, liver, kidney, spleen, duodenal mucosa and heart tissue were significantly increased in all groups of birds fed on diets supplemented with both forms of selenium. No response in Se content was found in the breast muscle of the hens given selenite, whereas Se-yeast resulted in significant and dose dependent increases in this parameter. The equivalent dose of Se-yeast induced a significantly higher level of Se in heart tissue than selenite did. Changes of Se levels in liver, spleen and heart tissue also showed a dose dependent pattern in hens fed diets supplemented with selenized yeast (Table 2).

Table 2. The effects of supplementation of basal diet (BD) with Se from selenite or Se-yeast on Se concentrations in blood (µmol·l$^{-1}$) and tissues (µmol·kg$^{-1}$ DM) of laying hens aged 9 months (n=7 in each group)

| Item | BD 0.1 mg Se per kg DM | BD + Se 0.4 mg·kg$^{-1}$ DM selenite | BD + Se 0.4 mg·kg$^{-1}$ DM Se-yeast | BD + Se 0.9 mg·kg$^{-1}$ DM Se-yeast | SEM |
|---|---|---|---|---|---|
| Blood | 1.7$^{a}$ | 4.6$^{b}$ | 5.3$^{bc}$ | 5.8$^{c}$ | 0.27 |
| Liver | 19.7$^{a}$ | 32.4$^{b}$ | 35.9$^{b}$ | 42.0$^{c}$ | 1.41 |
| Kidney | 28.3$^{a}$ | 49.7$^{b}$ | 54.7$^{bc}$ | 58.0$^{c}$ | 2.09 |
| Spleen | 27.3$^{a}$ | 40.0$^{b}$ | 39.0$^{b}$ | 49.1$^{c}$ | 1.42 |
| Duodenal mucosa | 20.0$^{a}$ | 34.0$^{b}$ | 37.7$^{b}$ | 37.8$^{b}$ | 1.42 |
| Breast muscle | 5.0$^{a}$ | 6.7$^{a}$ | 17.0$^{b}$ | 26.1$^{c}$ | 1.40 |
| Heart | 13.9$^{a}$ | 19.9$^{b}$ | 28.8$^{c}$ | 34.5$^{d}$ | 1.50 |

specific superscripts within a row = significant differences ($P<0.05$)

The activities of GPx in blood and in each tissue examined were significantly increased by feed supplementation with selenium. No differences due to form or dose of Se added to diets were observed (Table 3).

Table 3. The effects of selenite or Se-yeast selenium supplementation of basal diet (BD) on activities of glutathione peroxidase in blood (in U·g$^{-1}$ hemoglobin) and tissues (in U·g$^{-1}$ of protein) of liver, kidney and duodenal mucosa in laying hens aged 9 months (n=7 in each group)

| Item | BD 0.1 mg Se per kg DM | BD + Se 0.4 mg·kg$^{-1}$ DM selenite | BD + Se 0.4 mg·kg$^{-1}$ DM Se-yeast | BD + Se 0.9 mg·kg$^{-1}$ DM Se-yeast | SEM |
|---|---|---|---|---|---|
| Blood | 177.1$^{a}$ | 430.4$^{b}$ | 414.2$^{b}$ | 411.5$^{b}$ | 25.2 |
| Liver | 10.9$^{a}$ | 23.8$^{b}$ | 19.7$^{b}$ | 22.2$^{b}$ | 1.08 |
| Kidney | 12.4$^{a}$ | 24.4$^{b}$ | 22.8$^{b}$ | 24.0$^{b}$ | 1.12 |
| Duodenal mucosa | 5.6$^{a}$ | 12.5$^{b}$ | 12.8$^{b}$ | 12.2$^{b}$ | 0.66 |

specific superscripts within a row = significant differences (P<0.05)

Se supplementation of diets resulted in significant reduction of SOD activity in erythrocytes. Birds with the highest dietary contents of selenized yeast tended to have the lowest values of SOD activity, but the difference was not significant. No effects of selenium supplementation on concentrations of MDA and -SH groups in the hens' plasma were determined (Table 4).

Table 4. The effects of basal diet (BD) supplementation with selenium in the form of selenite or Se-yeast on activity of superoxide dismutase (SOD) in red blood cells and levels of malondialdehyde (MDA) and -SH groups in plasma of laying hens aged 9 months (n=7 in each group)

| Parameter | BD 0.1 mg Se per kg DM | BD + Se 0.4 mg·kg$^{-1}$ DM selenite | BD + Se 0.4 mg·kg$^{-1}$ DM Se-yeast | BD + Se 0.9 mg·kg$^{-1}$ DM Se-yeast | SEM |
|---|---|---|---|---|---|
| SOD, U·g$^{-1}$ Hb | 2150$^{a}$ | 1204$^{b}$ | 1317$^{b}$ | 929$^{b}$ | 122 |
| MDA, µmol·l$^{-1}$ | 0.73 | 0.67 | 0.85 | 0.66 | 0.05 |
| -SH groups, mmol·l$^{-1}$ | 0.28 | 0.38 | 0.48 | 0.27 | 0.04 |

specific superscripts within a row = significant differences (P<0.05)

Comparing to control hens fed BD without any Se supplements, the liver MDA concentration did not change when selenite was added to feed. On the other hand, diet with equivalent dose of Se-yeast significantly reduced MDA content in liver tissue (P<0.014). Nevertheless, the difference in liver MDA levels between groups 2 and 3 was slightly under the statistical significance level (P<0.074). Kidney tissue showed significant decrease in MDA concentrations in both selenite and Se-yeast supplemented groups with no differences due to treatment. No effects of dietary Se on MDA levels were noted in duodenal mucosa (Figure 1).

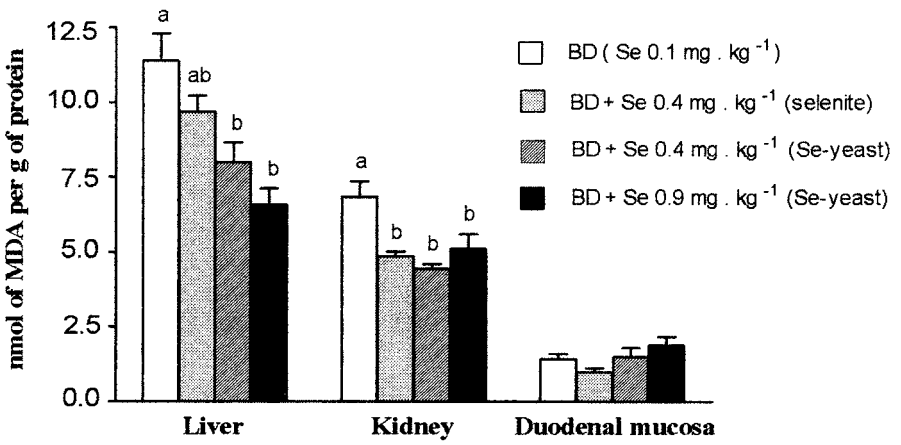


Figure 1. The effects of selenite or Se-yeast selenium supplementation of basal diet (BD) on malondialdehyde (MDA) concentrations in tissues of liver, kidney and duodenal mucosa of laying hens aged 9 months. Values are means ± SEM, n=7 in each group. Distinct letters above columns mean significant differences between treatments ($P<0.05$)

## DISCUSSION

The results of this experiment reveal that the concentrations of selenium in the hens' blood and all tissues except breast muscle were significantly increased by both selenite and selenized yeast supplemented diets. Regarding breast muscle, only diets supplemented with Se-yeast resulted in significant increase of its selenium content. Moreover, this Se accumulation in the hens' muscles seemed to be dose dependent.

Tetrameric GPx and selenoprotein W have been shown to be the main forms of Se in the muscle tissue of birds fed on diets containing Se of native origin only (Daun et al., 2004). The activity of GPx as well as Se content in muscle tissue are very low compared to those found in liver or kidney (Behne and Wolters, 1983). For this reason it is generally supposed that the proportion of selenomethionine (SeMet) escaping metabolism to $H_2Se$ is incorporated non-specifically into the structural proteins of muscles. SeMet replaces the common amino acid methionine there, which contains sulphur in its molecule instead of selenium. The reason is that $tRNA^{Met}$ is not able to distinguish between these two amino acids (Schrauzer, 2000).

The striated muscle mass represents about 52-56% of body weight in poultry. That is why muscle tissue of birds fed on selenized yeast becomes the most significant body deposit of selenium. It has been suggested that SeMet deposited in the muscle tissue of animals fed with Se-yeast may account for more than

50% of total selenium in the body (Daniels, 1996). Taken altogether it means the substantially larger formation of Se body deposits from selenized yeast than from selenite. The possible benefits of SeMet deposited in body tissues is that it may serve as quantitatively important storage capable of releasing Se during episodes of insufficient dietary selenium supply (Zuberbuehler et al., 2005).

The activity of GPx in the hens' blood, liver, kidney and duodenal mucosa tissue was found to be significantly increased by Se dietary supplementation, but no differences due to the form or amount of selenium delivered by feed were recorded. It is known that GPx mRNA is regulated by the absorbed Se at a post-transcriptional step (Toyoda et al., 1990). The linear correlation between Se concentration and GPx activities of blood and/or various tissues has been well demonstrated (Pavlata et al., 2001). It seems that GPx activity in the blood and examined tissues of nine-month-old hens is already saturated at a total Se dose 0.5 $mg \cdot kg^{-1}$ of feed. As in this experiment, our previous work (Kuricová et al., 2003) did not show any relation between the form of Se added to feed and GPx activity. The rational explanation for the lack of different effects of Se source on GPx activities is based on the well-known fact that all Se compounds must be split into $H_2Se$ before selenocysteine is synthetized *de novo* for its incorporation into an active centre of selenoenzymes (Schrauzer, 2000).

Superoxide anions are a specific substrate for dismutation catalyzed by SOD yielding hydrogen peroxide. The significantly decreased SOD activity in erythrocytes of all hens fed on diets enriched with selenium seems to be associated with reduced formation of superoxide anions due to increased Se delivery. Our explanation can be based on the sparing effects of Se on α-tocopherol. Selenoenzyme thioredoxin reductase activity was found to be significantly increased in broilers given feed supplemented with $Na_2SeO_3$ or Se-yeast. This enzyme is responsible for recycling of ascorbic acid, which in turn is able to reduce (i.e. to recycle) tocopheroxyl radicals. Larger recycling of α-tocopherol may thus provide more efficient scavenging of superoxide anions and other reactive oxygen radicals (Edens and Gowdy, 2004).

It has been reported that lower GPx activity is generally accompanied with increase of MDA concentration (Balogh et al., 2004). Feeding of Se-yeast significantly reduced MDA production in liver tissue, which was not the case in hens given diets supplemented with equivalent selenite dose. As already stated in results, the difference in liver MDA levels between selenite group of hens and group given equivalent dose of Se-yeast was closely under the statistical significance level ($P<0.07$). The digestive tract itself is considered be a major site of free-radical production in animals (Surai, 2002) and some of them might be delivered *via* portal blood system into liver. Moreover, the reduction of absorbed selenite into $H_2Se$ by glutathione in cells is considered to be a source of superoxide production (Kobayashi et al., 2001) which in

turn may induce even larger formation of MDA in liver. The explanation of different responses in liver MDA level to equivalent doses of selenite and Se-yeast on the base of antioxidant properties of SeMet (Sies et al., 1998) absorbed and delivered by portal blood into liver would be a speculation only.

On the other hand, kidney tissue which is supplied with blood already filtered by the liver did not show any differences in MDA levels related to the source of supplemented Se. Anyway, the MDA production was reduced due to both sources of Se supplemented. As already mentioned, GPx activity in kidney tissue was significantly increased by Se supplementation with no differences due to the source of feed selenium. No effects of Se supplementation on MDA levels in plasma were observed in our hens. A similar lack of plasma MDA response to Se-yeast feeding was found by Arai et al. (1994).

In conclusion, supplementation of feed with selenized yeast showed to be more effective in decreasing of lipid peroxidation in liver tissue as well as in formation of Se muscle deposits in laying hens than with sodium selenite.

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