

Antioxidant enzyme activities in liver tissue of chickens fed diets supplemented with various forms and amounts of selenium*

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ABSTRACT

The effects of feed supplementation with anorganic and organic forms of Se on the activities of liver enzymes, glutathione peroxidase (cGPx) and superoxide dismutase (SOD), was examined in young growing chickens. The birds were divided to 4 groups on the day of hatching. The first group received the basic diet (BD) with a Se content 0.12 mg·kg⁻¹ of dry matter (DM) only. Diets for groups 2, 3 and 4 consisted of BD supplemented with sodium selenite, 0.2 mg·kg⁻¹ of DM; Se-enriched yeast, 0.2 and 0.7 mg·kg⁻¹ of DM, respectively. Analysis of the liver tissue from 2- and 4-week-old chickens showed no effects of the various Se intake or effects of the form supplemented to the diet on cGPx activity. The 2-week-old birds showed no significant effects of diets on SOD activity in the liver tissue. On the other hand, liver SOD activity appeared to be dose-dependent in 4-week-old chickens, but no effects of the form of Se applied were found. Lipid peroxide formation measured as the appearance of thiobarbituric acid-reactive substances (TBARS) showed no dependence on the amount or form of Se supplemented to the ration, but its content in the liver of 4-week-old chickens was higher than in the 2-week-old ones. The selenium level in the liver tissue was clearly related to its contents in the feeds, but no effects of the form of selenium supplemented on this parameter were noticed after 2 or 4 weeks of dietary intake. The presented results suggest that dietary supplementation with Se-yeast to a level reaching even 0.7 mg·kg⁻¹ of DM does not induce oxidative stress in young growing chickens. The synthesis of liver cGPx of 2- to 4- week-old chickens of a laying strain already seemed to be saturated at a dietary intake of Se at a level of 0.1 mg·kg⁻¹ of DM.

KEY WORDS: chicken, liver, selenium, glutathione peroxidase, superoxide dismutase

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INTRODUCTION

Selenium (Se) is a nutritionally essential (Schwarz and Foltz, 1957) trace element. In animals several disorders related to selenium deficiency have been characterized, including muscular dystrophy (white muscle disease), liver necrosis, cardiac myopathy and exudative diathesis. All these syndromes appear to be essentially related to insufficient protection against oxidative stress (Wolffram, 1999).

Body Se is incorporated mainly into different kinds of selenoproteins having specific functions (Zachara 1993; Wolffram, 1999; Zagrodzki et al., 2001). Selenium exerts its best known biological role *via* the glutathione peroxidases (GPxs). The activity of this enzyme in the blood pGPx is frequently used as an indicator of Se status (Hafeman et al., 1974; Murray et al., 1987). The biochemical basis for Se deficiency in animals was explained by a discovery that the amino acid selenocysteine formed a structural component of the active center of cGPx (Rotruck et al., 1973). This enzyme provides a major defense mechanism against oxidative damage and accumulation of free radical products in tissues through the reduction of hydrogen peroxide, lipid hydroperoxides and other organic hydroperoxides (Tappel et al., 1982). Accordingly, inadequate levels of Se can lead to a reduction in cGPx activity and, consequently, to a decreased ability to degrade H₂O₂ (Kelly et al., 1998). Lipid peroxidation elevated by 32% was recently found in erythrocytes of Se-depleted chickens (Bozkaya et al., 2001). Deficient cGPx and increased H₂O₂ levels have both been linked to auto-oxidation of cell membranes, microtubular and DNA damage, impairment of immune cell function and bactericidal activity (Spielberg et al., 1979; McCallister et al., 1980). Thus, homeostatic regulation of optimal levels of Se appears critical for protecting tissues from H₂O₂-induced oxidative damage and for maintaining overall health.

From a nutritional point of view, the inorganic compounds selenate and selenite and the seleno-amino acids selenomethionine and selenocysteine are the most important chemical forms of selenium (Wolffram, 1999). The Se content of the plant components grown in Central Europe and used to complete the feeds for livestock does not exceed 0.1 mg·kg⁻¹ of dry matter (DM). Therefore selenium is widely added to feedstuffs in the form of sodium selenite to reach a final Se level from 0.3 to 0.5 mg·kg⁻¹ of DM. Recently a trend to use organic forms of selenium supplements such as high Se-yeast (Falkowska et al., 2000) or seleninotriglycerides (Zagrodzki et al., 2000) in the feeds of farm animals instead of Na₂SeO₃ has appeared.

It is well established that selenide is a key product of the metabolism of both selenite and selenomethionine absorbed from the digestive tract (Schrauzer, 2000). Recently published results of Kobayashi et al. (2001) demonstrated that Se bound in the form of selenide to albumin is susceptible to oxidation, yielding selenite *in vitro*. These researchers suggested that this process might also occur *in vivo*. Selenite *per se* was shown to have considerable pro-oxidative features (Spallholz,

1997). In other words, this means that any excessive intake of both anorganic and organic Se-compounds might result in increased production of reactive oxygen species with the relevant consequences.

This study investigated the effects of various dietary Se levels and its supplementation in the forms of sodium selenite or Se-yeast on the activities of the antioxidant enzyme cGPx and superoxide dismutase in the liver tissue. Concomitantly lipid peroxide formation and Se contents in the chicken liver were determined. The liver tissue was selected because it is known to have a considerable potential for a high rate of free radical generation due to its metabolic activities.

MATERIAL AND METHODS

Animals, diets and treatment

Forty eight female chickens of the laying strain Isa Brown were obtained on the day of hatching from LP-Párovské Háje a.s. (Slovak Republic). They were divided to 4 groups of 12 birds each and kept in large pens with wood shavings. From the first day of life until the age of four weeks the chickens were fed diets that differed either in total selenium content or only in the form of supplemented Se. The first group was given the basic diet (BD) with a Se content of $0.12 \text{ mg}\cdot\text{kg}^{-1}$ of dry matter (DM) coming only from the natural occurrence of Se in the dietary components. The diet for the second group of chickens was the same BD plus a Se supplement of $0.2 \text{ mg}\cdot\text{kg}^{-1}$ of DM in the form of Na_2SeO_3 resulting in a final selenium level of $0.32 \text{ mg}\cdot\text{kg}^{-1}$ of DM. The third group received a BD supplemented with 0.2 mg of Se in the form of selenium yeast extract (Sel-Plex, Alltech, USA) yielding the same final Se level of $0.32 \text{ mg}\cdot\text{kg}^{-1}$ as in the diet for the 2nd group of birds. The diet for the 4th group of chickens consisted of BD that was enriched with selenium ($0.7 \text{ mg}\cdot\text{kg}^{-1}$ of DM) in the form of a Se-yeast extract (Sel-Plex, Alltech, USA) providing its total concentration of $0.82 \text{ mg}\cdot\text{kg}^{-1}$. The diets for the 1st, 2nd and 3rd groups were also fortified with adequate amounts of the yeast extract without Se (NUPRO, Alltech, USA) to obtain the same final levels of the yeast extract as in the 4th diet (63.7 g in 100 kg of feed). The mineral and vitamin premix for mixing into diets was supplied by Biotika a.s. (Slovak Republic). The final content of vitamin E in diets was $159 \text{ mg}\cdot\text{kg}^{-1}$ of DM. The differences in the final contents of Cu and Zn in experimental diets caused by the additions of various amounts of Sel-Plex or NUPRO were less than 0.01% due to their minimal portion in diets. The final contents of Cu and Zn were 13.58 and $113.07 \text{ mg}\cdot\text{kg}^{-1}$ of DM in feed for each group, respectively. The composition of BD is presented in Table 1.

TABLE 1

The composition of the basic diet for chickens	
Component	%
Maize ground (9% CP)	53.0
Solvent extracted soyabean meal (3.5% crude fibre, 48% CP)	23.0
Fish meal (65% CP)	4.0
Wheat, ground (11% CP)	11.45
Premix BR 1 Forte ¹	1.0
Dicalcium phosphate (20% P)	0.3
Salt	0.25
Meat-and-bone meal with a middle contents of fat (48% CP)	5.0
Pulverised fat, BERGAFAT HPL 106	2.0

¹ supplied per kg of diet: vit. A, 12500 IU; vit. D₃, 3500 ICU; vit. E, 150 mg; vit. K₃, 4 mg; thiamine, 3 mg; riboflavin, 9 mg; pyridoxine, 6 mg; cyanocobalamin, 40 µg; niacin, 60 mg; pantothenic acid, 15 mg; betaine, 277 mg; biotin, 0.2 mg; folic acid, 2 mg; L-lysine, 1.2 g; DL-methionine, 1 g; I, 1 mg; Co, 0.4 mg; Mn, 100 mg; Cu, 8 mg; Zn, 80 mg; Fe, 80 mg

Chickens began to be reared with a lighting regimen of 23L:1D that was adjusted to 15L:9D after reaching three weeks of age. 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.

Six chickens from every group were slaughtered at the end of week 2 and 4 and their livers were collected and immediately frozen at -24°C.

Sample analysis

The tissue extractions of liver were washed two times with cooled physiological solution, cut into pieces, and homogenized in Ultra-Turrax T-25 homogenizer to make a 25% (w/v) homogenate in 5 mM Tris-HCl buffer, pH 7.8 containing 0.15 M KCl, 1 mM EDTA, and 2 mM GSH. Homogenates were centrifuged 60 min at 105,000 x g using a Beckman L8-60 ultracentrifuge, and were stored at -50°C until use for later assays. All procedures were performed at 4°C.

Superoxide dismutase (E.C. 1.15.1.1; SOD) was measured according to Flohé and Ötting (1984), at 550 nm (25°C), through the inhibition of cytochrome c reduction using a xantine-xantine oxidase O₂ generating system. One unit of SOD was defined as the amount of enzyme that inhibits the rate of cytochrome c reduction by 50%, under the specified conditions.

Glutathione peroxidase (E.C. 1.11.1.9; cGPx) was measured according to Flohé and Günzler (1984) in a coupled assay with glutathione reductase using cumene hydroperoxide (37°C, 340 nm). Enzyme activities are expressed in U/mg of protein.

Lipid peroxide formation was measured as the appearance of malondialdehyde and other aldehydes by reaction with thiobarbituric acid yielding coloured products called

thiobarbituric acid reactive substances (TBARS) that absorb at 535 nm (Gutteridge, 1984). The content of TBARS was expressed as absorbance/mg of protein.

The selenium concentration in the liver tissue was analysed in triplicate by the fluorimetric method of Rodriguez et al. (1994) and expressed in μmol per kg of dry matter. The standard procedure of tissue drying in an oven at 105°C was used to determine the dry matter content of the liver.

The protein concentration was determined by the method of Bradford (1976), using bovine serum albumin as a standard.

All reagents, of the highest purity, were from Sigma, Merck and Boehringer.

Statistical procedure

Statistical analysis was done by one-way analysis of variance (ANOVA) with the *post hoc* Tukey multiple comparisons test. The results are quoted as means \pm SEM.

RESULTS

The measured selenium content in the liver tissue is depicted on Figure 1. The feeding of the diet with the basic level of Se (group 1) resulted in the significantly lowest liver Se concentration, while the diet supplemented with Se in yeast extract, $0.7\text{ mg}\cdot\text{kg}^{-1}$ (group 4) yielded the highest values in both 2- and 4-week-old chickens.

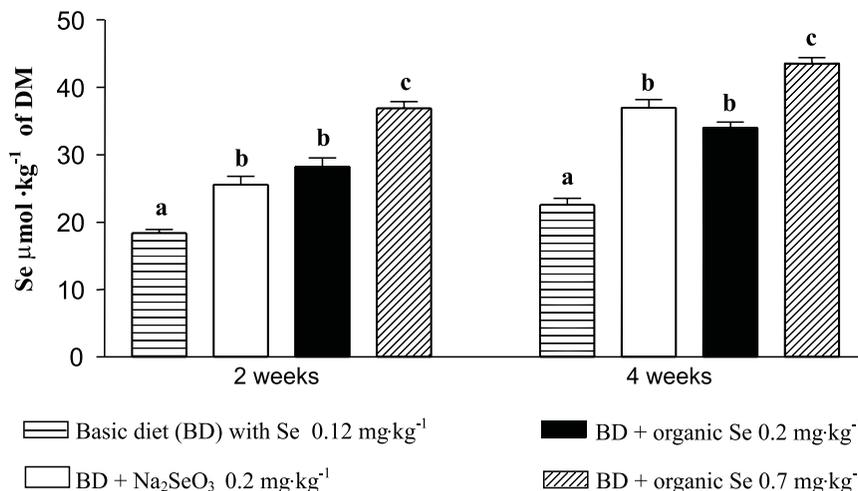


Figure 1. The selenium concentration in the liver tissue (in $\mu\text{mol}\cdot\text{kg}^{-1}$ of dry matter) of chickens aged 2 and 4 weeks which were fed diets supplemented with anorganic (Na_2SeO_3) or organic forms (Se-yeast) of Se. Values are means \pm SEM. Different letters above the column = significant differences ($P < 0.05$)

The dose response was evident during the whole experiment. No differences in Se tissue levels due to the addition of the same amounts of anorganic (group 2) or organic forms of Se (3rd group) could be observed in this short experiment. The liver selenium contents tended to increase with the age of the birds, but it was significantly elevated in group 2 only.

Though selenocysteine is a structural component of the active centre of glutathione peroxidase (cGPx), the activity of this enzyme was not found to be directly related to the tissue level of selenium (Figure 2). No effects of the Se forms used to supplement the feeds could be detected in the activities of this selenoenzyme. With the exception of group 2 fed a diet with an anorganic selenium supplement, a time response resulting in a significantly higher specific cGPx activity in 4-week-old chickens than in the 2-week-old ones was observed.

The activities of liver superoxide dismutase showed no differences between diets in 2-week-old chickens. On the other hand, the enzyme activity appeared to be dose dependent in 4-week-old chickens, but no effects of the form of Se applied could be found.

The appearance of thiobarbituric acid reactive substances (TBARS) in the liver significantly increased with age but no response to the amount or form of supplemented Se was found.

DISCUSSION

Studies on a number of species demonstrated that cGPx activity was directly related to blood and tissue Se concentration and to its dietary intake (Oh et al., 1976; Yoshida et al., 1999). Similarly, Behne et al. (1988) found that selenium content and glutathione peroxidase activity in the blood decrease rapidly during selenium depletion. Plasma and erythrocyte values are generally considered indicators of an inadequate selenium intake but not of the actual degree of deficiency or the levels of the element in most tissues. According to Wolfram (1999), a good correlation between tissue accumulation of selenium, e.g. in muscle or liver and the activity of cGPx exists in a situation of deficient or marginal selenium supply, whereas the correlation clearly weakens with nutritionally adequate selenium intake.

One of the possible explanations for a poor response of liver tissue cGPx to Se intake in our experiment appears that only a small proportion of Se received from feed was incorporated into cGPx. The other possibility was that animals already received a sufficient amount of Se from the basic diet (1st group) to satisfy the Se requirement for synthesis of cGPx. Zachara et al. (1993) observed that the enzyme activity in the plasma of lambs fed a diet with 0.25 ppm Se was almost at the same level as in higher Se-supplemented groups. This indicated that the Se requirement of lambs for different organs and tissues can be met by a daily dose of approximately

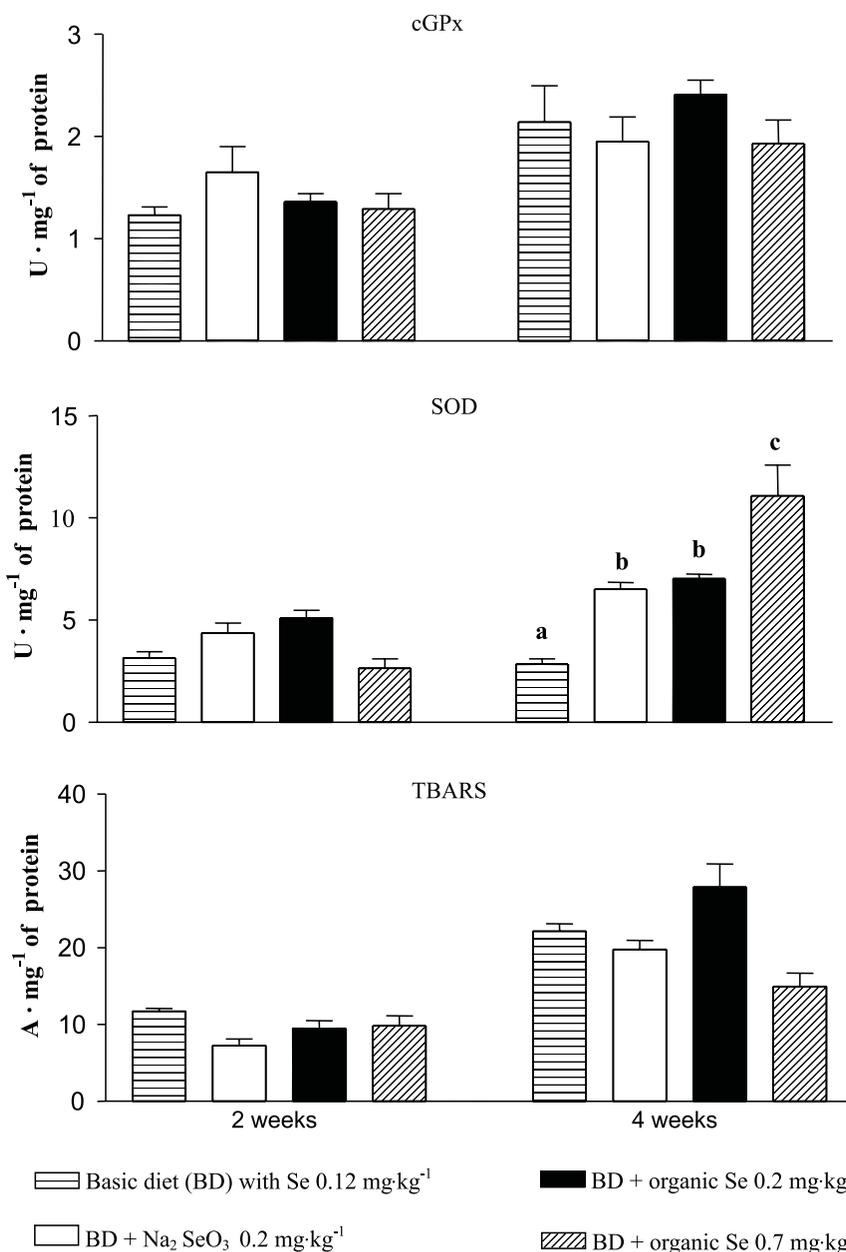


Figure 2. The activities of glutathione peroxidase (cGPx), superoxide dismutase (SOD) and the content of thiobarbituric acid reactive substances (TBARS) in the liver tissue of chickens aged 2 and 4 weeks. Birds were fed from the day of hatching the diets supplemented with anorganic (Na₂SeO₃) or organic forms (Se-yeast) of selenium. Values are means ± SEM. Different letters above the column = significant differences (P < 0.05). A = absorbance, U = units

0.25 ppm. The idea that selenium transferred by eggs from mothers (Paton et al., 2002) influenced the measured values of cGPx too could be excluded because the differences in liver Se contents due to its various intake were significant even in two-week-old chickens. Likewise Hafeman et al. (1974) investigated the dietary selenium effect on liver and erythrocyte cGPx in rats. They suggested that a level of 0.1 ppm Se appeared to have met this requirement since increasing dietary Se from 0.1 to 0.5 or 1 ppm induced only very small increases in cGPx.

The relatively narrow range between the beneficial effects of selenium and selenium toxicity is well known. Concentrations of 0.1 to 0.5 ppm on a dry matter basis are generally accepted as safe and adequate for livestock and poultry (Oldfield, 1992; Edmondson et al., 1993). It is known that toxicity of Se is based on the production of the superoxide anion and other ROS which result in increased levels of lipid peroxidation and DNA strand breaks (Kitahara et al., 1993; Yan and Spallholz, 1993). This might be an explanation for our findings that the diet with the highest Se supplement (group 4) also induced the largest SOD activity in liver tissue. As can be seen from Figure 2, SOD activity was significantly increased in all three groups of chickens fed Se-supplemented diets for 4 weeks (groups 2, 3 and 4) compared with the birds kept on the diet with the basic Se content only (group 1).

The changes in these enzyme activities led us to study whether the larger dietary intake of Se could evoke oxidative stress. We analyzed malondialdehyde and other lipid peroxidation products by determining the levels of thiobarbituric acid reactive substances in liver tissue. There was no significant response to the amount of Se supplemented to diet.

The main reason for using organic sources of selenium in chicken nutrition is based on the higher bioavailability of Se-methionine, the main Se-compound of Se-yeast (Schrauzer, 2000). We have recently shown that selenium from Se-yeast, which was not used in the synthesis of specific selenoproteins, could escape reduction processes by its incorporation directly into the muscle protein of chicken (Čekonová et al., 2001). The creation of Se-methionine deposits in muscle tissue would not yield as much selenide in the circulation and, in turn, this would also mean lower production of pro-oxidative selenite. Finally, the evident good health of chickens in every group supported the idea that oxidative stress was not evoked in our experiment.

In conclusion, data from this experiment show that dietary supplementation with Se-yeast to a level reaching even $0.7 \text{ mg}\cdot\text{kg}^{-1}$ does not induce oxidative stress in young growing chickens. The dietary intake of Se already at a level of $0.1 \text{ mg}\cdot\text{kg}^{-1}$ of DM seems to saturate the requirements of 2- to 4-week-old chickens of a laying strain for the synthesis of liver cGPx.

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STRESZCZENIE

Aktywność enzymów antyoksydacyjnych w tkance wątrobowej kurcząt żywionych dietami uzupełnionymi różnymi związkami i różną ilością selenu

Badano wpływ dodatku nieorganicznych i organicznych związków Se na aktywność enzymów wątroby: peroksydazy glutationowej (cGpx) oraz tlenu dysmutazy (SOD) u rosnących kurcząt brojlerów. Ptaki podzielono na 4 grupy w dniu wylęgu. Pierwsza grupa otrzymywała dietę kontrolną (BD) zawierającą 0,12 mg·kg⁻¹ Se w s.m. Diety 2, 3 i 4 składały się z diety kontrolnej uzupełnionej (w s.m.): seleninem sodu, 0,2 mg·kg⁻¹, drożdżami wzbogaconymi w Se: 0,2 i 0,7 mg·kg, odpowiednio.

Nie stwierdzono wpływu ani rodzaju ani ilości Se na aktywność cGPx w tkance wątrobowej kurcząt 2- i 4-ro tygodniowych, a także na aktywność SOD u kurcząt 2-tygodniowych. Aktywność SOD u kurcząt 4-ro tygodniowych wydaje się jednak być zależna od ilości Se, a nie od jego formy. Powstawanie peroksydazy lipidowej mierzone pojawieniem się reaktywnych związków kwasu triobarbiturowego (TBARS) nie zależało ani od ilości ani od formy dodatku Se, lecz jej zawartość w wątrobie 4-ro tygodniowych kurcząt była większa niż u 2-tygodniowych. Poziom Se w tkance wątrobowej wyraźnie zależał od jego zawartości w paszy, natomiast rodzaj związków nie miał na to wpływu u 2- i 4-ro tygodniowych kurcząt.

Otrzymane wyniki wskazują, że uzupełnienie dawki drożdżami wzbogaconymi w Se w ilość 0,7 mg·kg⁻¹s.m. nie wywołuje stresu tlenowego u młodych rosnących kurcząt. Synteza wątrobowej cGPx u kurcząt 2- i 4-ro tygodniowych linii kur nieśnych wydaje się osiągać nasycenie już przy dziennym pobraniu Se w ilości 0,1 mg·kg⁻¹s.m.