

The effect of dietary selenium sources and levels on performance, selenium content in muscle and glutathione peroxidase activity in broiler chickens

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ABSTRACT: The study examined the effect of dietary supplements of sodium selenite (SS), selenium-enriched yeast (Sel-Plex[®], SP) and selenium-enriched alga *Chlorella* (SCH) on growth traits, carcass analysis, selenium content in breast meat, glutathione peroxidase (GSH-Px) activity in breast and thigh meat and liver of chickens. The experiment was realized with seven hundred thirty-five cockerels Ross 308 randomly divided into 7 dietary treatments with 3 replications in each treatment. Chickens were fed a diet supplemented with 0 (control), 0.15 or 0.30 mg of selenium/kg in the form of sodium selenite (SS), Sel-Plex[®] (SP) and selenium-enriched alga *Chlorella* (SCH). Selenium addition influenced body weight at 21 ($P \leq 0.001$) and 35 ($P \leq 0.05$) days of age. Significantly higher body weight at 35 days of age was determined in chickens receiving 0.15 mg of selenium from SP (2 122 g) and 0.3 mg of selenium from SCH (2 116 g) contrary to dietary treatment with a lower level of selenium from SCH (2 010 g) per kg of feed. The selenium content in breast muscle was increased ($P \leq 0.001$) by both the lower and higher selenium concentration in the form of SP (0.6 and 0.85 mg/kg dry matter) and SCH (0.6 and 0.82 mg/kg dry matter) in comparison with the control (0.31 mg/kg dry matter). A significant increase ($P \leq 0.001$) was ascertained even in SS treatments, but no significant differences were found between both levels. The selenium source and level, including SS, significantly ($P \leq 0.001$) influenced the GSH-Px activity in breast and thigh meat.

Keywords: selenium source; performance; GSH-Px activity; broiler cockerels

Selenium (together with vitamin E) is one of the basic essential nutritional elements whose function consists in the protection of cells and tissues from oxidation damage (Schwarz and Foltz, 1957). Selenium has a specific anticarcinogenic effect (Schrauzer, 2003) and influences the parameters of immunity as a component of numerous selenoproteins and enzymes. It is important for the brain and thyroid

function. Its main physiological role is mediated by the glutathione peroxidase group (GSH-Px), which has selenium as an integral part (Mills, 1957; Flohe et al., 1973; Rotruck et al., 1973). The basic function of GSH-Px is the removal of hydrogen peroxide (H₂O₂) excess from the cell cytoplasm (Burk, 1997).

Schwarz and Foltz (1957) reported that selenium contained in yeast could protect geese against he-

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patic necrosis and broiler chickens against exudative diathesis.

The acceptable amount of selenium in diet for poultry in the European Union, including the Czech Republic, is 0.5 mg/kg (EU Directive, 2004).

The addition of selenium from selenomethionine to feed mixture increased body weight in chickens (Skřivan et al., 2008a). Dlouhá et al. (2008) also recorded higher body weight after selenium supplementation in organic form. Conversely, Niu et al. (2009) found an insignificant effect of selenium on body weight, while feed conversion was improved at a concentration of selenium supplement 0.2 mg/kg. Peric et al. (2007) or Yoon et al. (2007) obtained similar results of an insignificant increase in growth.

The study of dietary sodium selenite or selenium-enriched yeast supplement showed that the organic form of selenium was more efficiently stored in chicken breast meat compared to the inorganic form (Kuricova et al., 2003; Choct et al., 2004; Payne and Southern, 2005). Accordingly, Dlouhá et al. (2008) or Skřivan et al. (2008a) confirmed that organic sources of selenium increased its content in breast meat. The organic form of selenium is deposited to a greater extent than the inorganic form. Previous studies (Skřivan et al., 2006; Ševčíková et al., 2006; Dlouhá et al., 2008) showed the higher utilization of selenium-enriched alga *Chlorella* in laying hens or broiler chickens compared with sodium selenite.

It is clear from the literature that each source of selenium is utilizable in a different way. The question is whether different levels of different resources will function similarly.

The aim of the experiment was to compare the effect of selenium-enriched alga *Chlorella* and sodium selenite supplement at various concentrations on growth, carcass analysis, selenium content in breast meat and GSH-Px activity in breast meat, thigh meat and liver of chickens.

MATERIAL AND METHODS

Seven hundred thirty-five 0-day-old cockerels Ross 308 were randomly assigned to 7 dietary treatments containing 105 chicks. Each dietary treatment was replicated three times (35 chickens per pen). The basal diet composition fed as control treatment is shown in Table 1. Other dietary

treatments were supplemented with 0.15 mg and 0.30 mg of selenium, respectively, in the form of sodium selenite (SS; Na₂SeO₃), Sel-Plex® (SP) and selenium-enriched alga *Chlorella* (SCH) per kg of feed. Maize-wheat-soybean granulated basal diet contained 22.67% of crude protein, 12.87 MJ/kg of ME_N and 50 mg/kg of vitamin E. Feed and water were provided *ad libitum*. Broiler chickens were housed in pens (1.98 m × 0.9 m) on wooden shavings with 24-h lighting schedule. Body weight at 0, 21st and 35th day by individual weighing, feed con-

Table 1. Composition of basal diet^a

Ingredient	(g/kg)
Maize	309
Wheat	284.5
Soybean meal	300
Fish meal	28.5
Rapeseed oil	41.2
Limestone	12
Dicalcium phosphate	12
Sodium chloride	2.8
Vitamin/mineral premix ^b	10
Analysed chemical composition	
Dry matter	890.1
Crude protein	226.7
Fat	59.9
Crude fibre	24.1
Calcium	10.2
Total phosphorus	6.9
Selenium	79.10 ⁻⁶
ME _N by calculation (MJ/kg)	12.87

^aexperimental diets were supplemented with 0.15 or 0.3 mg of selenium per kg

^bthe vitamin/mineral premix provided per kg of diet: retinyl acetate 4.5 mg; cholecalciferol 0.15 mg; α-tocopheryl acetate 50 mg; menadione 4 mg; thiamine 6 mg; riboflavin 8 mg; pyridoxine 5 mg; cyanocobalamin 0.02 mg; niacinamide 60 mg; calcium pantothenate 18 mg; biotin 0.2 mg; folic acid 2 mg; choline chloride 300 mg; betaine 100 mg; L-lysine 1.6 g; DL-methionine 1.8 g; cobalt 0.4 mg; copper 20 mg; iron 60 mg; iodine 1 mg; manganese 120 mg; zinc 100 mg; molybdenum 1 mg

Characterization of Sel-Plex® 2000

Product description	Selenium content
Selenium-enriched yeast species <i>Saccharomyces cerevisiae</i> CNCM I-3060 (No. 3b8.10)	At least 2 000 mg Se/kg

sumption and mortality were monitored during the experiment. Cockerels were slaughtered at 35 days of age. Eight chickens from each dietary treatment representing the average live weight were selected for carcass analysis.

Analyses

The breast fillets ($n = 8$ per treatment) were stored in plastic bags at -20°C for dry matter and selenium content analysis. Feed or meat dry matter was assayed by oven drying at 105°C , ash by ashing at 550°C (AOAC, 1997), crude protein using a Kjeltec Auto 1030 (Tecator Comp., Sweden) and fat by extraction with petroleum ether in a Soxtec 1045 apparatus (Tecator Comp., Sweden). After ashing of feed, calcium content was determined with a Solaar M-6 atomic absorption spectrometer (JTA Solutions, UK) and phosphorus photometrically with a Specol 11 spectrophotometer (Carl Zeis, Jena). Selenium in feed and meat was measured with a Millenium Excalibur atomic fluorescence spectrometer (PS Analytical, UK) after mineralization in a closed system by a microwave digestion technique in the presence of HNO_3 and H_2O_2 in Milestone Ethos TC (Milestone S.r.l., Italy) equipment with temperature and pressure sensor. The analytical procedure was verified by the analysis of certified reference material RM 8414 Bovine Muscle (NIST).

The determination of GSH-Px activity was carried out in breast and thigh meat and liver ($n = 8$ per treatment) frozen at -80°C after thawing and mincing. The activity of GSH-Px was measured with tert-butyl hydroperoxide as substrate by a coupled assay, recording the oxidation of NADPH by the decrease in absorbance at 340 nm. The activity was expressed as $\mu\text{mol NADHP oxidized min/g meat tissue}$ (DeVore and Greene, 1982).

The resultant values were evaluated by the analysis of variance (ANOVA) using the General Linear Models (GLM) procedure of the SAS 9.2 software (SAS, 2008). Significant treatment effects were detected by Scheffé's test. All differences were considered insignificant at $P > 0.05$.

RESULTS

The selenium supplement significantly influenced body weight at 21 ($P \leq 0.001$) and 35 ($P \leq 0.05$) days of age (Table 2). The highest values of body weight were reached at 21 days of age in chickens fed 0.3 mg/kg of SS (1 050 g) and at 35 days in dietary treatments with 0.15 mg/kg of SP (2 122 g) and 0.3 mg/kg of SCH (2 116 g). The lowest body weight at 21 days of age was determined in chicks receiving 0.15 mg/kg of SP (943 g) and at 35 days in broiler chickens with 0.15 mg/kg of SCH in feed mixture. No significant differences among dietary treatments were determined in feed conversion and mortality. Mortality was under 5% in all dietary treatments.

As shown in Table 3, the dietary selenium supplement did not influence the characteristics of carcass analysis such as carcass weight, breast meat, thigh meat, heart, gizzard and abdominal fat share and dressing percentage. However, significantly ($P \leq 0.05$) higher liver weight was determined in chickens from dietary treatment with 0.3 mg/kg of SS (3.8%) in comparison with SCH at both selenium levels (3.1 and 3.0%).

Selenium supplements in the form of Sel-Plex®, selenium-enriched alga *Chlorella* and sodium selenite significantly ($P \leq 0.001$) influenced selenium content in the breast meat of broiler chickens (Table 4). These forms of selenium achieve higher values at a concentration of 0.3 mg/kg in mixture (0.85, 0.82 and 0.42 mg Se/kg dry matter, respectively), but lower values were recorded in the control treatment (0.31 mg Se/kg dry matter). No differences in selenium content in breast meat were found when using both levels of SS. Breast and thigh meat GSH-Px activity in experimental dietary treatments was significantly ($P \leq 0.001$) different from the control. Higher GSH-Px activity was registered in the case of 0.3 mg/kg of SCH (0.31 U/g for breast and 0.42 U/g for thigh meat). The GSH-Px activity in liver was not significantly influenced either by selenium source or by selenium level. However, a higher value was determined in broilers receiving 0.15 mg of SP (2.39 U/g).

Table 2. Effect of selenium source on the growth performance in broiler chickens

Characteristic	Dietary selenium supplementation and the source						Probability	
	0.0 mg control	0.15 mg SP	0.3 mg SP	0.15 mg SS	0.3 mg SS	0.15 mg SCH		0.3 mg SCH
BW (0 day; g)	38	38	37	39	39	38	39	NS
BW (21 st day; g)	1 013 ^{ab}	946 ^b	975 ^{ab}	970 ^{ab}	1 050 ^a	968 ^{ab}	972 ^{ab}	**
BW (35 th day; g)	2 111 ^{ab}	2 122 ^a	2 066 ^{ab}	2 098 ^{ab}	2 069 ^{ab}	2 010 ^b	2 116 ^a	*
F:G (35 th day; g:g)	1.85	1.88	1.92	1.87	1.91	1.93	1.86	NS
Mortality (%)	4.76	2.86	3.81	2.86	4.76	1.9	4.76	NS

^{a,b} means with different superscripts differ significantly, determined by Scheffe's test** $P \leq 0.001$; * $P \leq 0.05$; NS = not significantSP = Sel-Plex; SCH = selenium-enriched *Chlorella*; SS = sodium selenite; BW = body weight; F:G = feed:gain

Table 3. Effect of dietary selenium source on some indicators of carcass composition and dressing percentage

Characteristic	Dietary selenium supplementation and the source ¹							Probability
	0.0 mg control	0.15 mg SP	0.3 mg SP	0.15 mg SS	0.3 mg SS	0.15 mg SCH	0.3 mg SCH	
Carcass weight (g)	1 420	1 442	1 341	1 473	1 396	1 331	1 412	NS
Breast (%)	24.8	25.7	25.8	26.2	25.7	25.06	24.8	NS
Thigh (%)	23.6	22.7	23.5	23.2	24.1	23.3	23.6	NS
Liver (%)	3.3 ^{ab}	3.2 ^{ab}	3.6 ^{ab}	3.3 ^{ab}	3.8 ^a	3.1 ^b	3.0 ^b	*
Heart (%)	0.7	0.7	0.8	0.8	0.7	0.8	0.6	NS
Gizzard (%)	1.7	1.8	1.7	1.7	1.8	1.8	1.8	NS
Abdominal fat (%)	1.7	1.9	1.7	1.9	2.1	1.8	2.0	NS
Dressing percentage (%)	73.7	72.8	71.4	73.4	72.6	73.9	74.1	NS

^{a,b} means with different superscripts differ significantly, determined by Scheffe's test* $P \leq 0.05$; NS = not significantSP = Sel-Plex; SCH = selenium-enriched *Chlorella*; SS = sodium selenite¹ $n = 8$ per treatment

Table 4. Effect of dietary selenium source on selenium content (mg/kg DM) in breast meat and activity of glutathione peroxidase (U/g)

Characteristic	Dietary selenium supplementation and the source ¹						Probability	
	0.0 mg control	0.15 mg SP	0.3 mg SP	0.15 mg SS	0.3 mg SS	0.15 mg SCH		0.3 mg SCH
Se in breast meat	0.31 ^d	0.6 ^b	0.85 ^a	0.42 ^c	0.42 ^c	0.6 ^b	0.82 ^a	**
GSH-Px in breast meat	0.16 ^b	0.27 ^a	0.29 ^a	0.28 ^a	0.29 ^a	0.28 ^a	0.31 ^a	**
GSH-Px in thigh meat	0.18 ^b	0.32 ^a	0.39 ^a	0.39 ^a	0.4 ^a	0.35 ^a	0.42 ^a	**
GSH-Px in liver	1.62	2.39	2.13	2.25	1.99	2.29	1.84	NS

^{a,b,c,d} means with different superscripts differ significantly, determined by Scheffe's test

** $P \leq 0.001$; NS = not significant

SP = Sel-Plex; SCH = selenium-enriched *Chlorella*; SS = sodium selenite

¹ $n = 8$ per treatment

DISCUSSION

Similarly to this experiment, many authors described the influence of selenium supplement on body weight. Skřivan et al. (2008a) found out the positive effect of organic and inorganic selenium form on an increase in body weight and Ševčíková et al. (2006) or Dlouhá et al. (2008) also reported the positive effect of organic selenium source. But other authors (Choct et al., 2004; Yoon et al., 2007) did not observe any significant differences in the final body weight of chickens after addition of selenium in organic form. The same results were obtained in chickens receiving an inorganic selenium source. Miller et al. (1972) did not reveal any differences in the body weight of chickens fed various concentrations (0–0.5 mg/kg) of selenium from SS or selenomethionine. Similar findings were reported by Yoon et al. (2007) using different levels (0–0.3 mg/kg). The insignificant effect of selenium supplementation on carcass weight in this experiment is not in accordance with some studies (Choct et al., 2004; Payne and Southern, 2005) which found a higher carcass share in chickens receiving the organic form of selenium. The present study showed the insignificant effect of organic and inorganic selenium on an increase in the breast meat share. On the other hand, Choct et al. (2004), Payne and Southern (2005) or Ševčíková et al. (2006) determined higher breast meat weight in broiler chickens fed the organic form of selenium. Similarly, Ševčíková et al. (2006) did not ascertain any significant effect of organic source of selenium on an increase in thigh meat weight.

The increase in selenium concentration in breast meat due to dietary selenium supplementation is in agreement with findings presented by Ševčíková et al. (2006), Dlouhá et al. (2008) and Skřivan et al. (2008a). Little information about the effect of SCH and SP on selenium content in breast meat and GSH-Px activity was published. Inconsistent results were also obtained for selenium deposition in poultry meat after SS addition. Our results correspond with the findings of some authors (Cantor et al., 1982; Payne and Southern, 2005; Ševčíková et al., 2006; Dlouhá et al., 2008; Skřivan et al., 2008a,b), who showed an increase in selenium in the breast meat of poultry fed selenomethionine, SCH and selenium-enriched yeast. The published results are ambiguous in the case of SS. Shan and Davis (1994) reported an increase in selenium concentration in the breast meat of chickens that

received SS. Whereas, Cantor et al. (1982), Payne and Southern (2005) or Dlouhá et al. (2008) ascertained no significant differences in breast muscle selenium content after SS addition. In our study, selenium content in breast meat increased after SS addition, but no significant differences were found between the levels.

Recent research has shown that less selenium is maintained in chicken tissue when the inorganic form of selenium is used compared to the organic selenium source. Mahan and Parrett (1996) and Dlouhá et al. (2008) found that SS was retained at a lower concentration in muscle tissue, was absorbed less efficiently and was excreted at a higher rate than the organic selenium source.

The GSH-Px activity was balanced in all experimental dietary treatments without depending on the concentration and form of selenium, but an insignificantly higher enzyme activity in breast and thigh muscle was after addition of 0.3 mg of selenium from SCH. It was probably caused by differences in metabolic pathways (Forstrom et al., 1978). The observed differences in GSH-Px activity in our study are in agreement with results published by other authors (Cantor et al., 1982; Hassan et al., 1988; Spears et al., 2003; Dlouhá et al., 2008), who found that selenium supplementation increased GSH-Px activity. In addition, GSH-Px activity was significantly higher in the case of using SS in comparison with SCH. In this study, the effect of dietary selenium supplement was not recorded in GSH-Px activity in liver. Whereas, Pappas et al. (2005) indicated that selenium addition contributed to an increase of GSH-Px activity in liver, blood and breast muscle. Selenium, regardless of its form, must be converted to selenocysteine before its incorporation into the enzyme pGPX3. Sunde and Hoekstra (1980) found that inorganic SS was efficiently metabolizable into selenocysteine, while Henry and Ammerman (1995) determined that selenomethionine has slower transfer efficiency into selenocysteine.

This study adds published data on selenium enrichment of animal products. The results confirmed the identical effect of SP and SCH in broiler chickens. The new benefit is the finding that addition of sodium selenite at both levels of 0.15 and 0.30 mg Se/kg in diets may have the same effect. Furthermore, the organic selenium supplement (SP, SCH) was effectively absorbed into muscles of chickens contrary to SS. Selenium-enriched alga *Chlorella* could be applied in commercially pro-

duced premixes as a potential source of organic selenium form.

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