Effect of dietary selenium sources on growth performance, breast muscle selenium, glutathione peroxidase activity and oxidative stability in broilers

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ABSTRACT: This study examined the effects of supplementation of dietary sodium selenite and sodium enriched alga *Chlorella* on growth performance, selenium concentration in breast meat and excreta, activity of glutathione peroxidase in meat, and oxidative stability of meat in broilers. Sexed broiler cockerels Ross 308 were allotted to 3 dietary treatments, each comprising 100 chickens. The basal diet was supplemented with 0 (control) or 0.3 mg/kg Se from sodium selenite (SS) or Se-*Chlorella* (SCH). Dietary supplementation with SCH increased ($P < 0.05$) body weight. The breast muscle Se concentration was increased ($P < 0.05$) by SCH (0.70 mg/kg DM; 0.36 mg/kg DM in control) supplementation, but not ($P > 0.05$) by SS (0.49 mg/kg DM) supplementation. The concentration of Se in excreta was highest in the SS group. The activity of GSH-Px in breast meat was significant $P < 0.05$) in all treatments (0.16 U/g in control, 0.30 U/g in SS and 0.23 U/g in SCH group). The inclusion of SCH in the diet enhanced the oxidative stability of meat expressed as reduced malondialdehyde (MDA) values in breast meat after 0; 3 and 5 days storage in refrigerator at 3 to 5°C.

Keywords: broilers; selenium; alga *Chlorella*; glutathione peroxidase; lipid oxidation

Selenium is known to have important roles in reproductive functions and development, immunocompetence and ageing. As a constituent of selenoproteins, selenium has structural and enzymic functions, in the latter context being best-known as an antioxidant and catalyst for the production of an active thyroid hormone. Selenium occurs both in the organic and inorganic form, with the organic form predominantly found in grains, fish, meat, poultry, and dairy products (Klein, 2004). Selenium has been shown to have an analogous function as an essentials component of Se-dependent glutathione peroxidase (Combs, 1981). The primary function of GSH-Px enzymes is to detoxify hydrogen peroxide and to convert lipid hydroperoxides to nontoxic alcohols (Jenkinson et al., 1982). Chan and Decker (1994) suggested that an increase in tissue Se content might not always be accompanied by a corresponding increase in GSH-Px activity. This implies an influence of dietary Se levels on the oxidative stability of skeletal muscle. The effects of dietary vitamin E and Se deficiencies on promoting the lipid peroxidation of biological membranes, either independently or combined, are well described (Stolz et al., 1994). Unfortunately there is little information available on the effects of Se supplementation on lipid oxidation in broiler chickens (Ryu et al., 2005). Studying diets that have been supplemented with either sodium selenite and Se enriched yeast (Kuricova et al., 2003; Choct et al., 2004; Payne and Southern, 2005), it was found that organic selenium was deposited more effectively in broiler

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breast muscle than inorganic selenium. Previous research (Skřivan et al., 2006) proved higher utilization of the Se-enriched alga *Chlorella* in laying hens compared to sodium selenite. *Chlorella kessleri* is a freshwater alga containing 60% proteins and 10% fat. The concentration of inorganic Se is 2% of total Se content. Ševčíková et al. (2006) reported positive effects of Se-*Chlorella* on Se deposition in chicken meat.

The objectives of this experiment were to compare the influence of dietary sodium selenite (SS) and Se-enriched *Chlorella* (SCH) on growth performance, selenium concentration and activity of glutathione peroxidase in breast meat, selenium in excrements, and oxidative stability of breast meat lipids in broilers.

MATERIAL AND METHODS

Three hundred male broiler chicks (Ross 308, 0 day old) were randomly assigned to 3 pens each containing 100 chicks. The 3 treatments were a maize-wheat-soybean meal basal diet with no supplemental Se (Table 1), the basal diet supplemented with 0.3 mg/kg from SS (Na_2Se0_3), and the basal diet supplemented with 0.3 mg/kg from SCH supplied by the Institute of Microbiology, Academy of Sciences of the Czech Republic. Feed and water were provided *ad libitum*. Broilers were kept in pens of 2 m × 3.3 m on wood shavings, with gas heating, ventilation with a temperature controlled fan, and 24 h lighting program, at the Institute of Animal Science. Each pen was equipped with 7 nipple drinkers, 3 pan feeders and a feed hopper. At the age of five weeks samples of excreta were collected for the analysis of selenium content. With termination of the experiment at 42 days of age, 20 broiler chickens representing the average live weight in each group were selected from each pen and sacrificed at a slaughtering plant. Ten breast fillets (*M. pectoralis maior* and *minor)* from each group were stored in plastic bags at –20°C before analysis for dry matter, crude protein, fat, ash and selenium. The remaining 10 breast fillets were minced and frozen at –70°C before thiobarbituric acid-reactive substances (TBARS) analysis. The maximum freezer storage time was 45 days. After thawing, parts of muscles were stored at 3 to 5°C for 0, 3 or 5 days before the determination of malondialdehyde. GSH-Px activity was assayed from the meat immediately after mincing.

Analyses

Feed, meat and excreta dry matter was determined by oven drying at 105°C, ashing at 550°C (AOAC, 1997), and fat by extraction with petroleum ether in a Soxtec 1045 apparatus (Tecator Comp., Sweden). Crude protein in feed and meat was determined using a Kjeltec Auto 1030 (Tecator Comp., Sweden), Ca after ashing of samples by atomic absorption spectrometry (Solaar M-6, JTA Solutions, U.K.) and P colorimetrically (Huxtable and Bressler, 1973). Se in meat and excreta was measured by atomic absorption spectrometry (Solaar M-6 instrument) after feed and excreta samples were mineralized using a microwave di-

Table 1. Composition of basal diet[a]

Ingredient	g/kg
Maize	299
Wheat	290
Soybean meal	320
Fish meal	20
Rapeseed oil	40
DL-methionine	2
Limestone	12
Dicalcium phosphate	10
Sodium chloride	2
Vitamin/mineral supplement[b]	5
Analysed chemical composition	
Dry matter	903
Crude protein	225
Crude fat	67
Crude fibre	32
Ash	55
Calcium	8.8
Phosphorus	6.2
Selenium	15.10^{-5}
ME_N by calculation (MJ/kg)	12.51

[a]experimental diets were supplemented with Se at 0.3 mg/kg

[b]the vitamin/mineral supplement provided per kg of diet: vitamin A 12 000 IU; cholecalciferol 500 IU; vitamin E 50 mg; menadione 3 mg; thiamine 3 mg; riboflavin 5 mg; pyridoxine 4 mg; vitamin B_{12} 0.04 mg; niacin 40 mg; calcium pantothenate 12 mg; biotin 0.15 mg; folic acid 1.5 mg; choline chloride 250 mg; ethoxyquin 100 mg; copper 12 mg; iron 50 mg; iodine 1 mg; manganese 80 mg; zinc 60 mg

Table 2. Effect of selenium source on growth performance[1]

Parameter	Control	SS	SCH	PSEM
Day – 0 BW (g)	42.0	41.1	42.0	0.19
Day – 21 BW (g)	634[a]	582[b]	719[c]	7
Day – 42 BW (g)	2 671[a]	2 591[a]	2 870[b]	19
F:G, 0 – 42, (g:g)	1.65	1.58	1.61	
Mortality (%)	1	1	4	

[a,b,c]treatment means with different superscripts differ ($P < 0.05$)
[1]SS = sodium selenite; SCH = selenium enriched *Chlorella*; BW = body weight; F:G = feed:gain

gestion technique in a closed system (Milestone Ethos TC, Italy) in the presence of HNO_3 and H_2O_2. The analytical procedure was validated by the analysis of a certified reference material RM 8414 Bovine Muscle (NIST).

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 340nm. The activity was expressed as μmol NADHP oxidized min/g meat tissue (DeVore and Greene, 1982). Lipid oxidation in minced meat samples was measured by the thiobarbituric acid method of Piette and Raymond (1999), and results were expressed as TBARS in mg of malondialdehyde/kg muscle.

By using the GLM procedure of SAS software (SAS, 2003), the data were subjected to the ANOVA analysis for a randomized complete block design. The results are presented as the least-squares means (LSM) with pooled standard errors.

RESULTS

The supplementation of the diet with SCH increased live weight at 21 and 42 days ($P < 0.05$) compared to the control diet (Table 2). Se supplementation did not affect ($P > 0.05$) the protein and fat contents in dry matter of meat (Table 3). Whereas the breast meat Se concentration was increased ($P < 0.05$) by SCH, no difference ($P > 0.05$) was found between the control and supplemental SS group (Table 3). Both dietary Se sources increased ($P < 0.05$) the concentration of Se in excreta, with a more pronounced effect caused by sodium selenite compared to Se-*Chlorella*. GSH-Px activity differed ($P < 0.05$) in all treatments, with the highest activity of GSH-Px caused by dietary SS. The influence of dietary supplementation with Se on lipid oxidation during meat storage is presented in Table 4. Lipid oxidation, as measured by MDA formation, was decreased ($P < 0.05$) in

Table 3. Effect of Se source on crude protein and intramuscular fat contents of breast meat (g/kg dry matter), diet, breast meat and excreta selenium concentration (mg/kg DM), and the activity of glutathione peroxidase in meat (U/g)[1]

Parameter	Control	SS	SCH	PSEM
Crude protein	918.00	910.00	912.00	1.90
Fat	22.80	22.60	27.60	1.60
Se in diet	0.15	0.40	0.42	
Se in breast meat[2]	0.36[a]	0.49[a]	0.70[b]	0.03
Se in excreta[3]	0.49[a]	1.58[b]	0.76[c]	0.04
GSH-Px in breast meat[2]	0.16[a]	0.30[b]	0.23[c]	0.01

[a,b,c]treatment means with different superscripts differ ($P < 0.05$)
[1]SS = sodium selenite; SCH = sodium enriched *Chlorella*
[2]n = 10 per treatment
[3]n = 4 per treatment

Table 4. Effect of storage in refrigerator (3 to 5°C) on the concentration of malondialdehyde (mg/kg) in breast muscle[1,2]

Time of storage	Control	SS	SCH	PSEM
Day – 0	0.82[a]	0.72[a,b]	0.63[b]	0.02
Day – 3	1.00[a]	0.87[a,b]	0.72[b]	0.03
Day – 5	1.21[a]	1.09[a,b]	0.81[b]	0.04

[a,b]treatment means with different superscripts differ ($P < 0.05$)

[1]n = 10 per treatment

[2]SS = sodium selenite; SCH = sodium enriched *Chlorella*

meat samples after 0, 3, and 5 days cooler storage by SCH supplementation.

DISCUSSION

An increase in live weight due to dietary SCH supplementation found in the present study is in agreement with the results of Ševčíková et al. (2006). In contrast Choct et al. (2004) observed no differences in final live weight of chickens with supplement Se-yeast. The same result was found with dietary supplementation of inorganic Se. Miller et al. (1972) reported no differences in live weight gain of broilers fed various concentrations (0 to 0.5 ppm) of Se from sodium selenite or selenomethionine.

In agreement with previous results (Ševčíková et al., 2006) SCH supplementation increased the breast Se concentration. No literature could be found on the effects of SCH on Se deposition in broiler tissue or excreta or GSH-Px activity and lipid oxidation. Contradictory results on the influence of dietary SS on Se concentration in poultry meat have been reported. Present results are in agreement with Cantor et al. (1982) and Payne and Southern (2005), who reported an increase in breast Se concentrations in poults or chickens fed selenomethionine or Se-yeast, and no difference found when including SS in the diet. However, Shan and Davis (1994) presented an increase in the breast Se concentration due to the feeding of SS. Differences in analytical techniques or composition of diets could contribute to differences between the studies.

The present study proves than chickens receiving inorganic Se retained less Se in tissues than those receiving SCH. Mahan and Parrett (1996) reported that SS was retained at a lower concentration in muscle tissue, was less efficiently absorbed and was excreted at a higher rate than organic Se. This could probably be related to differences in metabolic pathways between organic and inorganic forms of selenium. Inorganic selenium is passively absorbed from the intestine by a simple diffusion process, whereas organic selenium is actively absorbed through an amino acid transport mechanism (Wolfram et al., 1989).

The differences in GSH-Px activity due to Se source are in agreement with the results presented by Cantor et al. (1982), Hassan et al. (1988) and Spears et al. (2003), who reported that Se supplementation increased plasma GPX3 activity. Less pronounced increases in GSH-Px activity by the supplementation of SCH compared to SS are probably due to the above-mentioned differences in metabolic pathways (Forstrom et al., 1978). Se, regardless of its form, has to be converted to selenocysteine before incorporation into the pGPX3 enzyme. Sunde and Hoekstra (1980) reported that inorganic SS was effectively metabolized into selenocysteine, whereas Henry and Ammerman (1995) indicated that selenomethionine was converted into selenocysteine at a lower rate of efficiency. Using MDA as a measurement of lipid oxidation in breast meat, it was found that the addition of SCH to diets decreased the oxidation of lipids in all storage periods.

It can be concluded from this study that SCH supplementation of diets for broilers is effective to increase the selenium contents of broiler meat. Furthermore, dietary supplementation of SCH is effective to enhance the oxidative stability of chicken meat during storage in refrigerator.

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