The use of selenium-enriched alga *Scenedesmus quadricauda* in a chicken diet

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ABSTRACT: The selenium-enriched *Scenedesmus* biomass of patented selenium-resistant strain SeIV was tested in a broiler chicken diet in comparison with sodium selenite supplementation. Feed conversion, mortality and live weight were not significantly influenced by the source of selenium. Supplementation of either form of selenium significantly increased the Se concentration in breast muscle with a more pronounced effect ($P < 0.001$) caused by selenium enriched *Scenedesmus* (635 μg/kg DM, 229 μg/kg DM in control). Breast meat of chickens fed a diet with sodium selenite or Se-*Scenedesmus* had a significantly ($P < 0.001$) higher value of glutathione peroxidase activity (0.329 μmol/g, 0.361 μmol/g) compared to the basal diet (0.190 μmol/g). The inclusion of Se-*Scenedesmus* biomass in the diet enhanced ($P = 0.021$) oxidative stability of meat expressed as reduced malondialdehyde in breast meat after 10-day cooler storage. Se-enriched *Scenedesmus* can be a good source of organic selenium for the production of Se-enriched chicken meat for further use in human diets. Furthermore, the Se-resistant strain SeIV was found advantageous for its fast and easy production of Se-enriched biomass.

Keywords: broiler chicken; green alga; *Scenedesmus*; meat quality; selenium

The role of selenium in human health and disease has been discussed recently (Rayman, 2009) and selenium deficiency is recognized as a global problem. Selenium enriched chicken, eggs, pork or beef can be produced using organic selenium as a component of the diet and serves as an important delivery system for this trace element to humans. As an organic source of selenium in poultry and farm animals, Se-enriched yeast, alga *Chlorella*, garlic, bean, sprout and cabbage were tested (Seo et al., 2008; Trávníček et al., 2008; Wang and Xu, 2008; Chinrasri et al., 2009; Mikulski et al., 2009; Svoboda et al., 2009b; Upton et al., 2009). Selenium in poultry nutrition was described and reviewed by Surai (Surai, 2002a,b). The selenium concentration in poultry tissues and in edible egg components is generally a reflection of nutrient levels, and feeds supplemented with Se-enriched yeast were more effective for Se utilization and formation of mobile body deposits containing this microelement than by supplementation with sodium selenite (Kuricova et al., 2003; Bobček et al., 2004). The selenium con-

Supported by Czech Scientic Foundation (Grant No. 525/09/0102), Ministry of Education, Youth and Sports of the Czech Republic (Project EUREKA No. OE0906), Ministry of Agriculture of the Czech Republic (No. Project MZE 0002701404) and by Institutional Research Concept (No. AV0Z50200510).

tent of eggs can be easily manipulated by organic selenium supplementation of a hen's diet at levels from 0.3 to 0.5 mg Se/kg of feed (Fisinin et al., 2009).

Efficient broiler production is facilitated by the feeding of well-balanced diets to highly productive lines of birds. Natural antioxidants play an important role in maintaining bird health, productivity, and reproductive characteristics. The supplementation of selenium by Se-yeast and *Chlorella* in the diet of broiler chickens increased the microelement concentration in muscles (Ševčíková et al., 2006) and vitamin E and Se content in egg yolk and chicken meat (Skřivan et al., 2008b). The use of Se-*Chlorella* in animal nutrition is reviewed by Doucha et al. (2009).

The selenium-enriched *Scenedesmus* biomass of selenium-resistant strain SeIV was tested in a broiler chicken diet. Feed conversion, mortality and live weight were examined. Lipid oxidation, GPX (glutathione peroxidase) activity and nutritive characteristics were determined.

MATERIAL AND METHODS

Experimental organism, culture growth conditions

The selenium-resistant strain SeIV (resistant to selenite) selected in our laboratory is deposited as a patented strain in Culture Collection of Autotrophic Microorganisms (CCALA) (Doušková et al., 2009). CCALA is a member of World Federation for Culture Collections, reg. number CCALA WDCM905 (http://wdcm.nig.ac.jp/CCINFO/CCINFO.xml?905). Cultures of *S. quadricauda* were cultivated in a liquid mineral medium: (KNO_3 2.021 g/l; K_2HPO_4 0.140 g/l; KH_2PO_4 0.340 g/l; $MgSO_4 \times 7H_2O$ 0.988 g/l; $CaCl_2 \times 2H_2O$ 0.011 g/l; Fe/NaEDTA 0.018 g/l; H_3BO_3 3.10 mg/l; $ZnSO_4 \times 7H_2O$ 1.43 mg/l; $MnSO_4 \times 4H_2O$ 1.20 mg/l; $CuSO_4 \times 5H_2O$ 1.24 mg/l; $CoSO_4 \times 7H_2O$ 1.40 mg/l; $(NH_4)_6Mo_7O_{24} \times 4H_2O$ 1.84 mg/l) in an outdoor photobioreactor for 7 days. The cultures were aerated with air containing 2% carbon dioxide (v/v).

Pilot scale outdoor photobioreactor

The cells were grown in an outdoor open thin-layer solar photobioreactor with 24 m^2 of culture area (length 24 m, slope 1.7%), and a 6–7 mm-thick layer of algal culture. The suspension volume in the bioreactor was 250 l. The cultivation was performed in batch regime. The algal suspension was illuminated approximately 11 h a day; during nights the suspension was kept in the retention tank and aerated. The experimental culture unit was located at 49°N, Czech Republic. The principles and details of the cultivation system are described in Doucha and Lívanský (2006).

Selenium treatment

Selenium was added as sodium selenite in the concentration of 10 mg Se/l to nutrient medium at the beginning of cultivation. Three replicate samples were used for all analyses and measurements.

Diet and husbandry

Eight hundred and ten broiler cockerels (Ross 308, 0 day old) were randomly assigned to 9 pens containing 90 chickens. The 3 dietary treatments with 3 replicates were as follows: basal diet with no supplemental Se (Table 1), basal diet supplemented with 0.3 mg/kg of Se from sodium selenite (Na_2SeO_3), and basal diet supplemented with 0.3 mg/kg of Se from selenium enriched alga *Scenedesmus*. Each pen was equipped with nipple drinkers and pan feeders. Feed and water were provided *ad libitum*. Broiler chicks were kept under a 24-h constant lighting schedule. Feed conversion, mortality and live weight were examined. The chicks were weighed at 0, 14 and 35 days of age. Termination of the experiment was at 35 days of age. Nine breast fillets from each group were chosen for chemical analysis.

Analyses

Lipid peroxidation in breast meat samples was measured by the method of Piette and Raymond (1999), and results were expressed as thiobarbituric acid-reactive substances (TBA) in milligrams of malondialdehyde (MDA) per kilogram of muscle. The breast meat was minced and frozen at –70°C. Before analysis, the samples were thawed and stored in a refrigerator at temperatures ranging from 2.5 to 4°C for 0, 5 or 10 days. The activity of GPX was measured immediately after mincing

Table 1. Ingredients and chemical composition of the basal diet (g/kg)[a]

Ingredients	(g/kg)
Wheat	304.2
Maize	300
Soybean meal	310
Fish meal	15
Rapeseed oil	40
Limestone	12
Dicalcium phosphate	10
Sodium chloride	2
Vitamin-mineral premix[b]	5
DL-methionine	1.8
Analyzed nutrient composition	
Dry matter	892
Crude protein	212
Crude fat	67
Crude fibre	39
Calcium	9.2
Phosphorus	6.1
Selenium (mg/kg)	0.11
AME_N MJ/kg (calculated)	12.45

[a]experimental diets were supplemented with Se of 0.3 mg/kg, analyzed content of selenium in sodium selenite group was 0.404 mg/kg and in Se-*Scenedesmus* group 0.398 mg/kg

[b]premix provided per kg of diet: retinyl acetate 3.6 mg; cholecalciferol 13 μg; α-tocopheryl acetate 50 mg; menadione 3 mg; thiamine 3 mg; riboflavin 5 mg; pyridoxine 4 mg; cyanocobalamin 40 μg; 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

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 μmol NADPH oxidized per min/g meat tissue (DeVore and Greene, 1982). For determination of nutritive characteristics, breast meat was stored in plastic bags at –20°C. Fat content in the basal diet and breast meat was determined by extraction with petroleum ether in a Soxtec 1045 apparatus (Tecator Comp., Sweden) and crude protein was analyzed using a Kjeltec Auto 1030 (Tecator Comp., Sweden). Dry matter was determined by oven drying at 105°C, and ash by burning at 550°C. Ca content in feed was measured after ashing of samples by absorption spectrometry (Solaar M-6, JTA Solutions, UK) and P was determined colorimetrically (Huxtable and Bressler, 1973). Feed and meat samples for Se determination were mineralized using a microwave digestion technique in a closed system (Milestone Ethos TC, Italy) in the presence of HNO_3 and H_2O_2. Se content was measured by atomic absorption spectrometry (Solaar M-6 with GF 90 Zeeman graphite cuvette). Hydroxyproline content was determined spectrophotometrically using the procedure of Bergman and Loxley (1963).

Results were statistically analyzed by analysis of variance (ANOVA) using the GLM procedure of SAS (SAS Institute Inc., 2003). The results are presented as the least squares means with standard errors (SEM).

RESULTS

Se-enriched *Scenedesmus quadricauda* cultivation

After 7 days of cultivation of selenium-resistant strain SeIV with the addition of 10 mg Se/l in the outdoor photobioreactor we obtained 950 g of selenium enriched biomass. The dry weight was 8.37 g/l. The total selenium content was 641 mg/kg. The biomass contained 98 mg/kg of selenomethione and 50 mg/kg of selenocysteine, which made 23% of the total selenium content. The biomass was used in a feed experiment.

Use of Se-enriched *Scenedesmus* biomass in a chicken diet

Se-enriched biomass as a source of organically bound selenium was used in a feeding experiment. Table 2 shows the effect of dietary selenium supplementation on performance traits and mortality. No significant differences in body weight and feed conversion were determined between the groups. Chicken mortality was similar in all groups. The highest value ($P = 0.024$) for dry matter (260.1 g/kg BM) was obtained in the Se-*Scenedesmus* group (Table 3). In addition, a higher ($P = 0.026$) crude

Table 2. Effect of sodium selenite and selenium enriched alga *Scenedesmus* on broiler performance

Item	Basal	SS	SA	SEM	*P*
BW (0 day; g)	43.40	43.10	43.20	0.120	NS
BW (14th day; g)	391.80	398.20	392.80	2.100	NS
BW (35th day; g)	2 174.60	2 177.60	2 177.50	9.250	NS
F:G (35th day; g/g)	1.85	1.88	1.87	0.014	NS
Mortality (%)	3.70	4.43	2.87	0.588	NS

SS = basal diet supplemented with sodium selenite (0.3 mg Se/kg)
SA = basal diet supplemented with selenium enriched alga *Scenedesmus* (0.3 mg Se/kg)
BW = body weight; F:G = feed:gain; NS = not significant; *P* = probability

protein content was present in the breast muscle of chicks fed either mixture with elevated levels of selenium (236.1 g/kg BM, 234.2 g/kg BM) compared with the basal diet (228.4 g/kg BM). The selenium concentration both in raw breast meat and in dry matter was markedly ($P < 0.001$) increased in the Se-*Scenedesmus* group (165.1 µg per kg BM, 634.9 µg/kg DM) even when compared with the sodium selenite group (107.9 µg/kg BM, 418.8 µg/kg DM). Fat and hydroxyproline content in breast muscle or in dry matter was not significantly affected. Glutathione peroxidase activity and lipid oxidation in breast muscle are compared in Table 4. The addition of selenium, using either Se-*Scenedesmus* or sodium selenite, promotes significantly ($P < 0.001$) increased GPX activity (0.361 µmol/g and 0.329 µmol/g) in comparison with the control (0.190 µmol/g). Lipid oxidation, expressed as thiobarbituric acid-reactive substances of malondialdehyde, was the highest ($P = 0.021$) in breast meat from the group without selenium supplementation (1.025 mg/kg), stored at a temperature of 2.5–4°C for 10 days. Selenium supplementation, using either selenium enriched alga or sodium selenite, decreased lipid oxidation (0.721 mg/kg and 0.763 mg/kg).

Table 3. Selected nutritive characteristics in raw breast muscle (BM) and dry matter (DM)

Item	Basal	SS	SA	SEM	*P*
Dry matter (g/kg BM)	252.2^{b}	257.7^{ab}	260.1^{a}	1.25	0.024
Fat (g/kg BM)	6.2	6.9	5.5	0.29	NS
Crude protein (g/kg BM)	228.4^{b}	234.2^{a}	236.1^{a}	1.25	0.026
Ash (g/kg BM)	11.7	11.6	11.6	0.05	NS
Hydroxyproline (g/kg BM)	0.778	0.785	0.798	0.0140	NS
Selenium (µg/kg BM)	57.6^{c}	107.9^{b}	165.1^{a}	8.66	< 0.001
Fat (g/kg DM)	24.7	26.8	21.3	1.13	NS
Crude protein (g/kg DM)	905.7	908.9	907.5	1.20	NS
Ash (g/kg DM)	46.6^{a}	44.9^{b}	44.6^{b}	0.22	< 0.001
Hydroxyproline (g/kg DM)	3.09	3.05	3.07	0.059	NS
Selenium (µg /kg DM)	228.6^{c}	418.8^{b}	634.9^{a}	32.78	< 0.001

SS = basal diet supplemented with sodium selenite (0.3 mg Se/kg)
SA = basal diet supplemented with selenium enriched alga *Scenedesmus* (0.3 mg Se/kg)
^{a,b,c}means with different superscripts differ significantly; NS = not significant; *P* = probability

Table 4. Activity of glutathione peroxidase (GPX) and malondialdehyde (MDA) content in raw breast meat (TBA 0) after 5 and 10 days (TBA 5; TBA 10) of storage

Item	Basal	SS	SA	SEM	*P*
GPX (μmol /g)	0.190[b]	0.329[a]	0.361[a]	0.0164	< 0.001
TBA 0 (mg/kg)	0.438	0.349	0.296	0.0266	NS
TBA 5 (mg/kg)	0.782	0.594	0.609	0.0627	NS
TBA 10 (mg/kg)	1.025[a]	0.763[b]	0.721[b]	0.0497	0.021

SS = basal diet supplemented with sodium selenite (0.3 mg Se/kg)

SA = basal diet supplemented with selenium enriched alga *Scenedesmus* (0.3 mg Se/kg)

[a,b]means with different superscripts differ significantly; NS = not significant; *P* = probability

DISCUSSION

Se-enriched *Scenedesmus quadricauda* cultivation

The cultivation of selenium-resistant strain SeIV in an outdoor photobioreactor confirmed the advantage of the strain if compared with the wild type. When the wild type is cultivated to obtain Se-enriched biomass, selenium has to be added in several low doses during the cultivation to avoid the toxic effect of selenium (Doucha et al., 2009). In the case of SeIV strain the selenium was added in a sufficiently high dose at the very beginning of the cultivation without any toxic effect on algal cells. After 7 days of cultivation we obtained 950 g of selenium enriched biomass with good total selenium content (641 mg/kg). The amount of organically bound selenium in the biomass was 23%, which is comparable with 24% and 39% of organic selenium in *Chlorella* reported by Neumann et al. (2003) and much higher when compared with SeMet content (in the range of ng/g) found by Larsen et al. (2001).

Use of Se-enriched *Scenedesmus* biomass in a chicken diet

Our results showed that Se-enriched biomass, as a source of organically bound selenium, caused no significant differences in body weight, feed conversion and survival rate between the experimental groups. The results are in agreement with the findings of other authors, who reported no differences in gain, feed efficiency or mortality of broiler chickens fed diets containing 0.2 or 0.3 ppm Se from sodium selenite or an organic source of selenium (Ševčíková et al., 2006; Yoon et al., 2007; Wang and Xu, 2008). Niu et al. (2009) reported improved feed conversion with 0.2 ppm Se-supplementation for broiler chickens. Our data confirmed that there was no toxic effect of 0.3 ppm Se-*Scenedesmus* in a chicken diet, making it an equally good source of organically bound selenium for the production of selenium enriched chicken meat.

The high selenium content in breast muscle of the Se-*Scenedesmus* group (165.1 μg/kg BM, 634.9 μg per kg DM) even when compared with the selenite group (107.9 μg/kg BM, 418.8 μg/kg DM) proved the good bioavailability of organically bound selenium. Similar good bioavailability of selenium was shown for Se-enriched garlic and cabbage (Seo et al., 2008), Se-enriched alga *Chlorella* (Ševčíková et al., 2006; Dlouhá et al., 2008) and Se-enriched yeast Sel-Plex (Perić et al., 2009). The increased levels of selenium in muscle tissues by organic selenium supplementation were also observed in other organisms, e.g. lambs (Juniper et al., 2009), turkeys (Mikulski et al., 2009) and pigs (Svoboda et al., 2009a). Mahan and Parret (1996) reported that inorganic Se (sodium selenite) was retained at a much lower concentration in muscle tissue, was less efficiently absorbed and was excreted at a higher rate than organic Se due to their different metabolic pathways. In laying hens, egg weight was significantly higher in the Se-enriched alga *Chlorella* and Se-yeast groups compared to the basal diet and the diet with sodium selenite (Skřivan et al., 2006). Supplementation of Se-enriched yeast to a hen's diet resulted in higher egg production and significantly higher whole-egg Se concentration (Pavlović et al., 2009).

An effective way to estimate the bioavailability of selenium is by determination of GPX activity (Favier, 1993). In the present study, GPX activity was highest in the Se-*Scenedesmus* group (0.361 μmol/g), implying good bioavailability of organic selenium. In broiler chickens fed the Se-yeast diet (0.2–0.3 ppm), plasma and liver GPX activity was increased significantly (Yoon et al., 2007; Wang and Xu, 2008). The organic form improved the selenium and redox status in broilers, leading to greater resistance to oxidative stress than when the inorganic form of selenium was fed (Upton et al., 2009). Blood plasma GPX activity was significantly reduced in Se-deficient hens (Hassan, 1990). The lipid oxidation in the raw breast meat was lowest (0.296 mg/kg) in the Se-*Scenedesmus* group. After 10 days of storage, the difference in lipid oxidation between basal and Se-*Scenedesmus* group was even more significant (1.025 mg/kg, 0.721 mg per kg respectively). This confirms the positive effect of selenium on oxidative stability of meat, which was previously observed in broilers (Dlouhá et al., 2008; Skřivan et al., 2008a; Perić et al., 2009) and turkeys (Mikulski et al., 2009).

CONCLUSION

To conclude, we have shown that the SeIV strain of *Scenedesmus quadricauda* is suitable for large-scale cultivation of Se-enriched biomass. The use of Se-enriched *Scenedesmus* in a chicken diet led to an increase of selenium content in breast muscle, the enhancement of GPX activity and the reduction of lipid oxidation in meat without any toxic effect on the experimental animals. This suggests that Se-*Scenedesmus* may be a promising alternative source of organic selenium.

Acknowledgement

We thank Dr. Jiří Machát, Masaryk University, Brno, Czech Republic, for ICP-MS analyses of selenium content in the biomass.

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Received: 2010–07–26
Accepted after corrections: 2010–11–29

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