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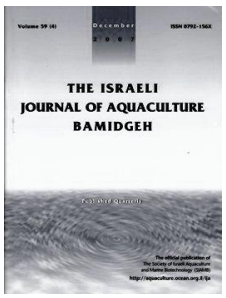


# Improving the Nutritional Value of Nile Tilapia Fillet by Dietary Selenium Supplementation

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Key words: selenium, *Oreochromis niloticus*, antioxidant capacity

**Abstract**

Selenium (Se) supplementation in animal feeds for producing feasible foods was successfully experimented with in different meat-producing animals including fish. In the present study, Nile tilapia were fed Se-fortified diets (0.5, 2.0, or 4.0 mg/kg) *ad lib* for six weeks. In addition to traditional production traits, the antioxidant parameters glutathione (GSH), glutathione peroxidase (GPx), and malondialdehyde (MDA) in the blood, liver, and muscle of the tilapia, Se accumulation in the fillet, and body composition were determined. The highest Se value in the fillet was obtained with the diet containing 2 mg/kg supplementation, where the actual Se content of 2.47 mg/kg Se induced 128 µg/kg Se in the fillet. Selenium incorporation had a cubic relationship with the dietary Se content.

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## Introduction

Selenium functions within mammalian systems primarily in the form of selenoproteins that perform a variety of physiological roles (Holben and Smith, 1999). Because of the potential of selenoproteins to protect against oxidative stress, selenium is being examined for the prevention of chronic diseases in humans, including cancer, cardiovascular disease, and type 2 diabetes (Stranges et al., 2010). In some areas, e.g., the northeastern part of China, Finland, and New Zealand, Se soil levels are very low (<0.05 ppm). Strategies to improve human selenium intake include selenium supplements, increasing the selenium content of soils, and production of selenium-rich foods (Navarro-Alarcon and Cabrera-Vique, 2008; Zhang et al., 2010). Dietary selenium supplementation increases the selenium concentration of the meat of beef, pigs, calves, broiler chickens, rabbits, and even eggs (Dalle Zotte and Szendrő, 2011) because the Se content of animal products reflects the Se level in their consumed diet (Navarro-Alarcon and Cabrera-Vique, 2008).

Dietary selenium deficiency can cause tissue damage as a result of the impaired activity of selenoenzymes such as glutathione peroxidase, while a high Se concentration can be toxic (Mézes and Balogh, 2009). Thus, the line between Se deficiency and Se toxicity is very fine (Elia et al., 2011). Albeit under special conditions (e.g., to reduce heavy metal toxicity), high concentrations (225-300 mg/kg diet) of selenium can be used (James, 2011), the European Commission limits the Se concentration in feed to a maximum of 0.5 mg Se/kg (70/524/EEC). Selenium concentrations in experiments concerning production of functional foods in aquaculture range 1.22-4.42 mg/kg in hybrid striped bass (*Morone chrysops* x *M. saxatilis*; Cotter et al., 2008) and 1.9-8.5 mg/kg in African catfish *Clarias gariepinus* Burchell (Mierke-Klemeyer et al., 2008; Schram et al., 2008).

High Se concentrations can cause toxic effects involving pro-oxidative reactions. Selenium can interact with cellular sulfydryls leading to a depletion of glutathione (GSH) and an increase of lipid peroxidation (Elia et al., 2011). Alkanals such as malondialdehyde (MDA) are meta-stable end-products of *in vivo* lipid peroxidation (Tappel and Dillard, 1981). During this process, fatty acids break down and small molecular weight products such as MDA emerge, making MDA a marker of *in vivo* lipid peroxidation (Janero, 1990). Antioxidant defense systems in aquatic organisms comprise specific antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione reductase, and water-soluble (vitamin C, reduced GSH, carotenes) and fat-soluble (vitamins A and E) low-molecular weight free radical scavengers (Atencio et al., 2008).

The aim of the present study was to determine the effect of different levels of organic selenium (seleno yeast) supplementation on the production traits, fillet selenium content, and *in vivo* antioxidant capacity of tilapia. For the latter purpose, all specimens were evaluated for MDA, GSH, and GPx activity in the liver, muscle, and blood.

## Materials and Methods

*Experimental fish, culture facilities, nutrition.* Nile tilapia (*Oreochromis niloticus*) from Tuka Fish Farm, Hungary (335.5±29 g), were introduced to twelve 500-l tanks (three tanks per treatment, 30 fishes in each) in a recirculation system with a total volume of 20 $m^3$. The stocking density was 20 $kg/m^3$ and the water temperature was 23.5±1.0°C. Three experimental diets with 0.5, 2.0, or 4.0 mg/kg Se supplementation (resulting in Se contents of 1.15, 2.47, and 4.66 mg/kg, respectively) plus one control diet containing 0.81 mg/kg Se were fed to the tilapia *ad libitum* for 42 days. Experimental diets were formulated using a commercial tilapia feed (control) containing 88.0% dry matter, 45.0% crude protein, 6.4% crude fat, and 1.8% crude fiber, plus organic selenium (SelenoYeast®, Alltech, Lexington, KA).

*Sampling and chemical analyses.* On the first day of the experiment, five randomly chosen fish were sacrificed to determine initial values. On day 43 of the experiment, five fish from each treatment group were selected, over-anesthetized with clove oil (0.025 ml/l for 2 min), and processed to obtain samples. Blood (2 ml/fish), liver, and muscle

samples were taken from each fish to measure antioxidant parameters. Blood samples were stored at 4°C, then the plasma was separated from the blood cells by centrifugation. The red blood cells were lysed with a nine-fold volume of deionized water and by freezing and thawing. Small amounts of liver samples (0.5 g) were homogenized in a nine-fold volume of 0.65% NaCl solution. Samples were stored at -20°C until further analysis. Fillets were stored frozen (-70°C) until analysis.

MDA concentrations in blood samples were determined by the method of Placer et al. (1966) and in liver samples by the method of Mihara et al. (1980). Both methods are based on the reaction of the lipid peroxidation end-product MDA (that serves as a marker for lipid peroxidation) with 2-thiobarbituric acid. Tetramethoxypropane was used as the standard to generate MDA for setting up a calibration curve. The reduced GSH concentration in the blood plasma, red blood cell (RBC) hemolysate, and 10000 × *g* supernatant fraction of liver homogenate was measured by the method of Sedlak and Lindsay (1968). GPx activity was measured by the method of Lawrence and Burk (1976). Enzyme activity is given as units/g protein (one unit = 1 nanomol of reduced glutathione oxidation per min at 25°C). Protein contents in the plasma and RBC hemolysate were determined by the method of Weichselbaum (1948). Total protein concentrations in the 10,000 × *g* supernatant fractions of liver homogenates were determined by the method of Lowry et al. (1951), using bovine serum albumin as the standard.

Selenium concentrations in the samples were determined fluorometrically by 2,3-diaminonaphthalene with a spectro-fluorometer (Shimadzu RF-1501) after digestion in nitric acid and perchloric acid as described by Watkinson (1966). Dry matter in muscle samples was determined after drying in a vacuum oven at 103°C until a constant weight was reached. Nitrogen contents were determined in fresh samples by Kjeldahl analysis. Crude fat contents were determined by extraction of freeze-dried samples with petroleum-ether and drying the extract at 103°C to a constant weight.

*Measurements.* Total body, fillet, liver, and total viscera weights were measured individually. Growth was quantified by specific growth weight, calculated as SGR = $100(\ln_{\text{final wt}} - \ln_{\text{initial wt}})/\text{days}$. Feed consumption was measured. Feed conversion ratio was calculated as FCR = total feed consumption x fish biomass increment, hepatosomatic index as HSI = 100(liver wt)(body wt), viscero-somatic index as VSI = 100(carcass wt)(body wt), and fillet yield as 100(fillet wt)(body wt).

## Results

No mortalities were recorded throughout the study period. The specific growth rate ranged 0.54-0.70%/day but values did not significantly differ (Table 1). The feed conversion ratios in the 0.5 and 4.0 diets were significantly better than in the control. Fillet yields in fish fed the 0.5 and 4.0 diets were significantly better than initially.

The selenium content of the fillet showed a moderate positive cubic relationship with

Table 1. Production and body composition of tilapia fed selenium-supplemented diets for 42 days (means±SD).

| | *Diet (mg Se/kg diet)* | | | | | *p* = |
|---|---|---|---|---|---|---|
| | *Initial* | *Control* | *0.5* | *2.0* | *4.0* | |
| Specific growth rate (%/day) | - | 0.56±0.28 | 0.65±0.15 | 0.54±0.07 | 0.70±0.20 | NS |
| Feed consumption (kg) | - | 7.46±1.1 | 7.71±0.9 | 7.49±0.4 | 7.34±0.8 | NS |
| Feed conversion ratio | - | 3.88±0.57[b] | 2.58±0.30[a] | 3.01±0.33[ab] | 2.30±.06[a] | 0.039 |
| Hepatosomatic index (%) | 1.86±0.46 | 2.12±0.45 | 2.20±0.46 | 2.72±0.45 | 2.59±0.18 | NS |
| Viscero-somatic index (%) | 8.84±0.93 | 9.97±2.95 | 8.46±1.39 | 10.82±2.19 | 11.22±1.76 | NS |
| Fillet yield (%) | 14.80±0.67[a] | 16.50±1.19[ab] | 17.58±1.29[b] | 16.63±1.32[ab] | 17.51±0.41[b] | 0.001 |
| Dry matter (%) | 23.25±0.57 | 23.78±1.47 | 23.48±2.32 | 25.64±0.81 | 25.54±0.73 | NS |
| Protein (%) | 18.53±0.51 | 17.86±0.50 | 17.79±0.98 | 18.57±0.47 | 18.40±0.33 | NS |
| Fat (%) | 0.71±1.42[a] | 3.20±0.86[b] | 3.03±1.32[b] | 4.42±0.66[b] | 4.40±0.66[b] | 0.001 |

NS = not significant, $p>0.05$.

the selenium content of the feed, described as $Se_{Fillet}$ = 75.6 + (46.8 × $Se_{Feed}$) - (12.3 × $Se_{Feed}^2$) + (0.72 × $Se_{Feed}^3$), where $r^2$ = 0.65 and $p$ = 0.001 (Fig. 1). The Se level in fish fed the 2.0 diet was 1.6 times higher than the initial value.

Selenium supplementation had a significant effect on MDA content in the muscle and liver but did not significantly affect GSH content because of high individual variance (Table 2). GPx activity in the blood plasma was significantly higher in fish fed the 0.5 diet.

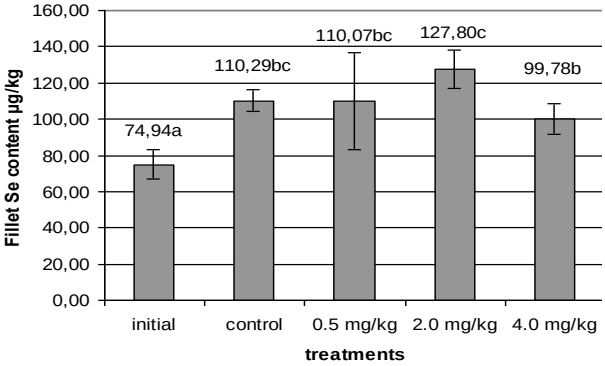


Fig. 1. Se content in fillets of Nile tilapia fed diets supplemented with different levels of selenium.

Table 2. Glutathione (GSH), malondialdehyde (MDA), and glutathione peroxidase (GPx) activity in the liver, muscle, and blood of Nile tilapia fed diets containing different levels of selenium (means±SD, n = 5).

| *Parameter*[1] | *Diet (mg Se/kg diet)* | | | | *p =* |
|---|---|---|---|---|---|
| | *Control* | *0.5* | *2.0* | *4.0* | |
| *Glutathione (µmol/g protein)* | | | | | |
| Liver | 0.95±0.09 | 0.96±0.04 | 0.94±0.05 | 0.89±0.09 | NS |
| Muscle | 1.14±0.06 | 1.41±0.78 | 1.07±0.09 | 1.19±0.21 | NS |
| Plasma | 10.38±2.52 | 10.95±3.16 | 10.08±2.31 | 9.29±1.83 | NS |
| Red blood cells | 19.19±6.02 | 17.75±1.78 | 20.54±4.71 | 18.20±2.66 | NS |
| *Malondialdehyde (nmol/ml; µmol/g)* | | | | | |
| Liver | 11.25±0.90$^{b}$ | 8.97±0.52$^{a}$ | 10.10±1.72$^{ab}$ | 9.87±0.57$^{ab}$ | 0.045 |
| Muscle | 3.36±1.35$^{ab}$ | 1.95±0.37$^{a}$ | 2.88±0.90$^{ab}$ | 3.84±1.11$^{b}$ | 0.05 |
| Plasma | 18.37±2.95 | 19.05±2.52 | 19.56±1.43 | 19.56±4.21 | NS |
| Red blood cells | 17.16±1.51 | 18.37±0.47 | 17.27±3.63 | 18.53±3.32 | NS |
| *Glutathione peroxidase (U/g protein)* | | | | | |
| Liver | 1.00±0.25 | 0.92±0.10 | 0.90±0.04 | 0.90±0.13 | NS |
| Muscle | 0.95±0.49 | 0.92±0.18 | 0.87±0.15 | 0.97±0.44 | NS |
| Plasma | 6.19±1.12$^{a}$ | 9.22±0.72$^{b}$ | 6.00±1.57$^{a}$ | 6.83±0.49$^{a}$ | 0.004 |
| Red blood cells | 25.36±7.63 | 24.56±3.39 | 24.78±8.74 | 22.19±2.67 | NS |

NS = not significant, $p$<0.05

## Discussion

Although tilapia growth was not affected by the level of dietary selenium in the present study, the FCR and fillet yields were better in the 0.5 and 4.0 mg/kg groups. In contrast, growth was improved by an increase in dietary Se in channel catfish (*Ictalurus punctatus*; Gatlin and Wilson, 1984), crucian carp (*Carassius auratus gibelio*; Zhou et al., 2009), and rainbow trout (*Oncorhynchus mykiss*; Hunt et al., 2011) but even the highest dietary Se concentration (4.42 mg/kg) did not have an impact on the growth of hybrid striped bass (Cotter et al., 2008). While a high Se concentration in diets did not impact growth in carp, it caused significant Se accumulation in the kidney, liver, and muscles (Elia et al., 2011).

The most important action of selenium is its antioxidant effect. Selenium is part of the active center of selenoenzymes such as GPx. Although the GSH content did not significantly differ in any of the sampled tissues in the present study, blood plasma GPx activity was higher and muscle and liver MDA lower in the 0.5 mg/kg group, suggesting that this level of organic Se supplementation might increase the endogenous antioxidant capacity of tilapia. Dietary sodium selenite supplementation of tilapia (*O. niloticus*) seems to confer some protection against oxidative stress induced by microcystin-producing cyanobacterial cells, since the highest dose (6 µg Se/g dry diet) induced lipid peroxidation and protein oxidation in non-intoxicated fish (Atencio et al., 2008).

Selenium supplementation enhances the endogenous antioxidant capacity of carp (*Cyprinus carpio*) by increasing the glutathione content in tissues, but supplementation levels above 1.0 mg/kg might worsen the antioxidant condition (Elia et al., 2011). GPx activity was significantly higher and liver MDA significantly lower in rainbow trout (*O. mykiss)* fed Se-supplemented diets but, despite the protective role of GPx against hydroperoxide-induced lipid peroxidation, hepatic MDA was not reduced in stressed rainbow trout (Hunt et al., 2011). That hepatic MDA levels did not drop can be explained by the mobilization of stored hepatic selenite and its pro-oxidant properties (Rider et al., 2009).

Fortified meat products have great potential for delivering important nutrients to humans. Muscle selenium concentrations in beef, pork, and chicken can be increased by dietary selenium supplementation (Decker and Park, 2010). In the present study the highest Se value in fillet was achieved with the 2 mg/kg supplementation, where the actual 2.47 mg/kg dietary Se content resulted in 128 µg/kg Se in the fillet. However, the accumulation of selenium in the fillet had a cubic relationship with the dietary Se content, resulting in a lower accumulation of Se in the fillet of fish fed the diet with the highest level of supplementation. The increase of muscle Se had a linear relationship to dose in hybrid striped bass where the highest tissue concentration at six weeks (1.09 mg/kg) was obtained with a diet supplemented by 3.2 mg/kg organic selenium (Cotter et al., 2008). Se build-up after six weeks was similar in African catfish where the highest level of total selenium in the fillet was 0.87 mg/kg, resulting from a total dietary selenium level of 8.5 mg/kg (Schram et al., 2008). Production of Se-fortified fillets requires feeding an Se-supplemented finishing diet for 6-8 weeks or less (Cotter et al., 2008). The present study confirms this finding.

The recommended dietary allowance (RDA) of selenium for humans, determined according to the maximum activity of GPx, is 55 µg selenium per day (Pedrero and Madrid, 2009). According to our results, a 100-g portion of tilapia fillet, fortified with 2.0 mg/kg Se, covers approximately 25% of the RDA of Se.

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