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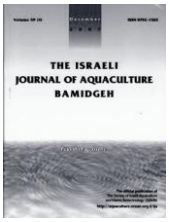

Effects of Organic Selenium on Growth, Muscle Composition, and Antioxidant System in Rainbow Trout

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Abstract

This experiment was conducted to understand the effects of organic selenium on rainbow trout, *Oncorhynchus mykiss*. Fish (33.47±0.15 g; n = 216) were randomly assigned to four treatment groups consisting of three replicates of 18 fish each in 100 x 100 x 125 cm cages. The fish were fed a basal diet supplemented with 2, 3, or 4 mg/kg organic Se (Sel-Plex®) supplementation for 8 weeks. Muscle of fish fed the unsupplemented control diet had a lower (p<0.05) selenium content (4.78±0.12 µg/g) than muscle of fish fed the supplemented feeds (6.45±0.18, 7.51±0.17, and 8.23±0.11 µg/g for the 2, 3 and 4 mg/kg diets, respectively). The highest (p<0.05) weight gain and specific growth rate were obtained in fish fed the 3 mg/kg diet. There were no significant differences (p>0.05) in proximate composition except for protein content in fish fed the 3 mg/kg diet. Glutathione peroxidase (GSH-Px), catalase (CAT), and superoxide dismutase (SOD) activity was significantly higher (p<0.05) and liver malondialdehyde (MDA) concentration significantly lower in fish fed the supplemented diets than in the control. Results show that 3 mg/kg Se supplementation is most effective for growth and hepatic antioxidant enzyme activity in rainbow trout.

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Introduction

Trace elements are essential for maintaining health, growth, and crucial biochemical-physiological functions. Selenium (Se) is an essential trace element for human and animal nutrition. As a constituent of selenoproteins, Se has structural and enzymatic functions, particularly as an antioxidant and catalyst for the production of active thyroid hormone (Pappas et al., 2008). Fish, which are a good dietary source of the element for humans, accumulate significant amounts of selenium (WHO, 1987).

Selenium aids the antioxidant defense system and is an essential part of glutathione peroxidase (GSH-Px), an antioxidant that protects cellular membranes and organelles from oxidative and peroxidative damage that may be caused by superoxide radicals. There are two main sources of dietary Se: organic (selenomethionine, selenocystein) and inorganic (selenite or selenate), both of which may be added to commercial feed (Jaramillo et al., 2009). Selenoprotein plays an essential role in physiological functions including reproduction, immune system, growth, and development (Watanabe et al., 1980; Pappas et al., 2008). Organic selenium has an advantage in reducing oxidative stress in comparison to inorganic forms and incorporates into skeletal muscles, kidney, liver, and gastrointestinal mucosa proteins as selenomethionine and selenocysteine and is an essential micronutrient for fish (Martinez-Alvarez et al., 2005). Selenomethionine is the major selenocompound in Se-enriched yeast (Sel-Plex®, Alltech, USA) which is used as a natural form of Se for dietary supplementation. Se yeast is increasingly used in animal nutrition; its use is FDA-approved for several animal species (Schrauzer, 2006).

The requirements for Se in farmed fish might be elevated due to the low availability of Se from diets containing fishmeal and the effects of physical and environmental stressors. Dietary Se in commercial trout diets may not meet the requirements of intensively raised fish. Tissue Se levels of cultivated salmonids are markedly lower than in their wild counterparts, the cause of which is yet unknown (Rider et al., 2009). The discrepancy may be due to a combination of factors including differences in growth rates and feeding regimes and Se losses resulting from stressors associated with intensive fish farming. Thus, Se supplementation might be necessary for aquaculture species fed fishmeal-based diets which do not contain adequate amounts of Se (Gaber, 2008; Rider et al., 2009).

In general, organic minerals have higher rates of absorption and retention than inorganic forms. Organic Se has higher bioavailability than inorganic Se for Atlantic salmon (Bell and Cowey, 1989), channel catfish (Wang and Lowel, 1997), and striped bass (Cotter et al., 2008). Deteriorative oxidative reactions in meat lead to losses of nutritional value and food quality. To increase the oxidative stability of meat, antioxidants such as Se have been added to the feed of farm animals, leading to improved meat quality (Schrauzer, 2006).

Oxygen in its molecular state, O_2, is essential for many metabolic processes that are vital to aerobic life. This dependence on oxygen forces aerobic life to withstand considerable toxicity (Martinez-Alverez et al., 2005). Whereas oxygen concentration in the atmosphere is relatively constant, it

often fluctuates in the aquatic medium due to temperature variations and the energy burn-up of fish. Consequently, fish are frequently exposed to oxygen stress produced by an abrupt increase in oxygen concentration. Further, fish are often subjected to prooxidant effects of pollutants present in the aquatic environment. To a certain extent, environmental conditions have led to the development of defense mechanisms that protect fish against reactive oxygen species (Jovanovic et al., 1997).

Dietary modifications are among the most preferable and practical methods of improving the effects of environmental stressors and farming methods on the growth of fish. The current study aimed to evaluate the effects of different ratios of organic Se supplementation on growth performance, muscle composition, and oxidative stress in farmed rainbow trout, *Oncorhynchus mykiss*.

Materials and Methods

Fish. The experiment was carried out at the outdoor installation of the Fresh Water Fish System Culture Unit of the Faculty of Fisheries at Cukurova University in Adana, Turkey, from November to February. Five hundred rainbow trout (30 g) were obtained from a trout farm in Kozan, Adana, Turkey. The fish were stocked into three 1000-l concrete ponds and allowed to acclimate to the experimental systems two weeks before the start of the experiment. During acclimation, fish were fed a commercial diet (48% crude protein, 22% crude lipid; Çamli Yem, İzmir, Turkey) at a level of 3% body weight. After acclimatization, 216 fish were individually weighed, randomly distributed into twelve cages at 18 fish/cage, and allowed to acclimatize for two days without feeding.

Experimental system. The experimental system consisted of 12 experimental cages located inside big concrete ponds and connected to each other on one side of the cage. Wooden walkways connected the cages to the pond bank. Water depth in the pond was maintained at 100 cm throughout the experiment by continuously adding water. Fresh water was supplied from the Seyhan Dam drainage system by pipes. Cages were cleaned every two weeks after fish sampling. Dissolved oxygen was measured every other day using YSI model 58 oxygen meters (Yellow Springs Instrument Company, Yellow Springs, OH), pH twice a week using an electronic pH meter (pH pen, Fisher Scientific, Cincinnati, OH), total NO_3, NO_2, and NH_4 once a week using a Merck-Spectroquant® Nova 60 A, and water temperature daily at 13:00 using a mercury thermometer suspended at a depth of 30 cm. Average water quality parameters were temperature 14.5±0.8°C, dissolved oxygen 8.3±0.07 mg/l, pH 8.3±0.15, NO_3 0.4±0.1 mg/l, NO_2 0.02±05 mg/l, and NH_4 0.06±0.5 mg/l.

During the experiment, fish were fed an unsupplemented basal diet (control) or the basal diet supplemented with 2 mg/kg, 3 mg/kg, or 4 mg/kg of organic Se (Sel-Plex®, Alltech Inc., Nicholasville, KY, USA). The measured Se concentrations were 0.55, 2.70, 3.50, and 4.3 mg/kg in the basal, 2 mg/kg, 3 mg/kg, and 4 mg/kg diets, respectively (Table 1). All known

nutritional requirements of rainbow trout were met by the experimental feeds (NRC, 1993). Fish were fed by dividing the daily feed portion (3% of the body weight) into two equal portions (08:00, 16:00). The fish from each cage were weighed biweekly and the daily food rations adjusted after weighing. On the days of weighing no feed was offered.

Proximate analysis. At the end of the study, 24 fish (six fish per treatment) were randomly chosen from each treatment. Fish fillets were analyzed for moisture, protein, lipid, and ash (AOAC, 1996): moisture by heating at 60°C to a constant weight, protein by estimating the Kjeldahl nitrogen (N × 6.25) in an automated distillation unit, lipid by chloroform/ methanol extraction, and ash by incinerating in a muffle furnace at 550°C for 18 h. All analyses were done in triplicate. Se concentration was determined by hydride generation atomic absorption spectrophotometer (AA6501, Shimadzu Ltd, Japan) according to the method described by Tinggi (1999).

Table 1. Ingredients and proximate composition of the basal rainbow trout diet.

Ingredient	*g/kg*
Fishmeal	460
Wheat flour	201
Yellow corn meal	130
Soybean meal	100
Fish oil	64
Mineral mixture (Se free)[1]	30
Vitamin mixture[2]	10
Binder (lignobond)	5
Proximate composition	*(% as fed)*
Moisture	8.6
Crude protein	48.4
Crude lipid	22.2
Crude fiber	5.23
Ash	8.30
NFE[3]	7.27

[1] mg/kg diet): Na (as NaCl) 197; Mg (as $MgSO_4/7H_2O$) 735; Fe (as $FeC_6H_5O_7/5H_2O$) 258; Zn (as $ZnSO_4/7H_2O$) 40; Mn (as $MnSO_4/5H_2O$) 18; Cu (as $CuSO_4/5H_2O$) 3.9; Al (as $AlCl_3/6H_2O$) 0.56; Co (as $CoCl_2/6H_2O$) 0.15; I (as KIO_3) 0.89; α-cellulose carrier

[2] per kg diet: thiamin hydrochloride 60 mg; riboflavin 100 mg; pyridoxine hydrochloride 40 mg; cyanocobalamin 0.1 mg; ascorbic acid 5000 mg; niacin 400 mg; calcium pantothenate 100 mg; inositol 2000 mg; biotin 6 mg; folic acid 15 mg; p-aminobenzoic acid 50 mg; vitamin K_3 50 mg; vitamin A acetate 9,000 IU; vitamin D_3 9,000 IU

[c] nitrogen free extract = 100 - (%moisture + %protein + %lipid + %fiber + %ash)

Determination of antioxidant enzymes and lipid peroxidation. Twenty-four fish (six fish per treatment) were sacrificed for analysis of antioxidant enzymes and lipid peroxidation. Total GSH-Px (EC 1.11.1.9) activity was assayed by the method of Jocely (1970), using H_2O_2 and NADPH as substrates. The conversion of nicotinamide adenine dinucleotide phosphate (NADPH) to nicotinamide adenine dinucleotide phosphate (NADP) was followed by recording the changes in absorption intensity at 340 nm, and one unit was expressed as one mole of NADPH consumed per min, using a molar extinction coefficient of $6.22 \times 10^{6.}$

Catalase (CAT; EC 1.11.1.6) activity of tissues was determined according to the method of Aebi (1974). The enzymatic decomposition of H_2O_2 was followed directly by the decrease in absorbance at 240 nm. The difference in absorbance per unit time was used as a measure of CAT activity. The enzyme activity is given in U/mg protein.

Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured by the inhibition of nitroblue tetrazolium (NBT) reduction due to O_2 generated by the xanthine/xanthine oxidase system (Sun et al., 1988). One unit of SOD activity was

defined as the amount of protein causing 50% inhibition of the NBT reduction rate.

The level of malondialdehyde (MDA) on homogenized tissue, an index of lipid peroxidation, was determined by thiobarbituric acid reaction using the method of Yagi (1998). Tissue protein content was determined according to the method developed by Lowry et al. (1951) using the bovine serum albumin as standard.

Statistical analysis. All results were analyzed by one-way analysis of variance (ANOVA). When ANOVA identified differences among groups, multiple comparisons among means were made with Duncan's new multiple range tests. Data differences were considered significant at $p<0.05$. Statistical tests were performed using SPSS 10.0 for Windows (SPSS Inc., Chicago, Illinois).

Results

No mortalities were recorded during the 60-day feeding trial. There were no obvious effects of Se on water quality. Fish fed the 3 mg Se diet had the significantly highest daily growth rate, specific growth rate, and feed conversion ratio (Table 2).

Table 2. Growth, feed utilization, and proximate composition (mean±SEM) of rainbow trout fed diets containing different amounts of selenium yeast (n = 3).

		Selenium supplemented diets		
	Control	*2 mg/kg*	*3 mg/kg*	*4 mg/kg*
Initial wt (g)	33.49±0.16	33.61±0.09	33.48±0.19	33.33±0.16
Final wt (g)	100.54±1.19^a	97.62±1.68^a	105.71±0.91^b	89.71±1.86^c
Live wt gain (%)	200.19±1.91^a	190.44±4.26^a	215.78±4.51^b	169.19±2.64^c
Daily wt gain (g)	1.12±0.07^a	1.07±0.02^a	1.20±0.05^b	0.94±0.04^c
SGR[1]	1.84±0.07^a	1.78±0.02^a	1.95±0.05^b	1.64±0.05^c
FCR[2]	1.59±0.05^a	1.72±0.01^b	1.53±0.03^a	1.79±0.03^b
Proximate composition (% wet weight basis)				
Moisture (%)	70.89±0.06^a	71.83±0.7^a	71.33±0.3^a	70.76±0.0^a
Crude protein (%)	16.69±0.25^a	16.97±0.1^a	19.22±0.7^b	16.49±0.0^a
Crude lipid (%)	6.02±0.17^a	6.75±0.66^a	6.50±0.41^a	5.83±0.10^a
Crude ash (%)	1.73±0.10^a	1.88±0.09^a	1.72±0.11^a	1.87±0.07^a
Se (µg/g)	4.78±0.12^a	6.45±0.18^b	7.51±0.17^c	8.23±0.11d

Values in a row with different superscripts significantly differ ($p<0.05$).
[1] Specific growth rate = ln final body wt - ln initial body wt/time × 100
[2] Feed conversion ratio = dry feed fed/wt gain

GSH-Px activity in rainbow trout fed the 3 mg/kg Se diet (40.74±1.33 U/mg) was significantly higher than in the control group (18.17±1.76 U/mg), CAT activity was significantly higher in fish fed the 3 mg/kg Se diet (62.37±2.80 U/mg) than in the control (42.30±2.04 U/mg), 2 mg/kg (52.57±2.05 U/mg), and 4 mg/kg (50.58±2.45 U/mg) groups, SOD activity was significantly highest in fish fed the 4 mg diet, and the MDA level

significantly lower in fish fed the supplemented diets, especially the 3 mg/kg Se diet (Fig. 1).

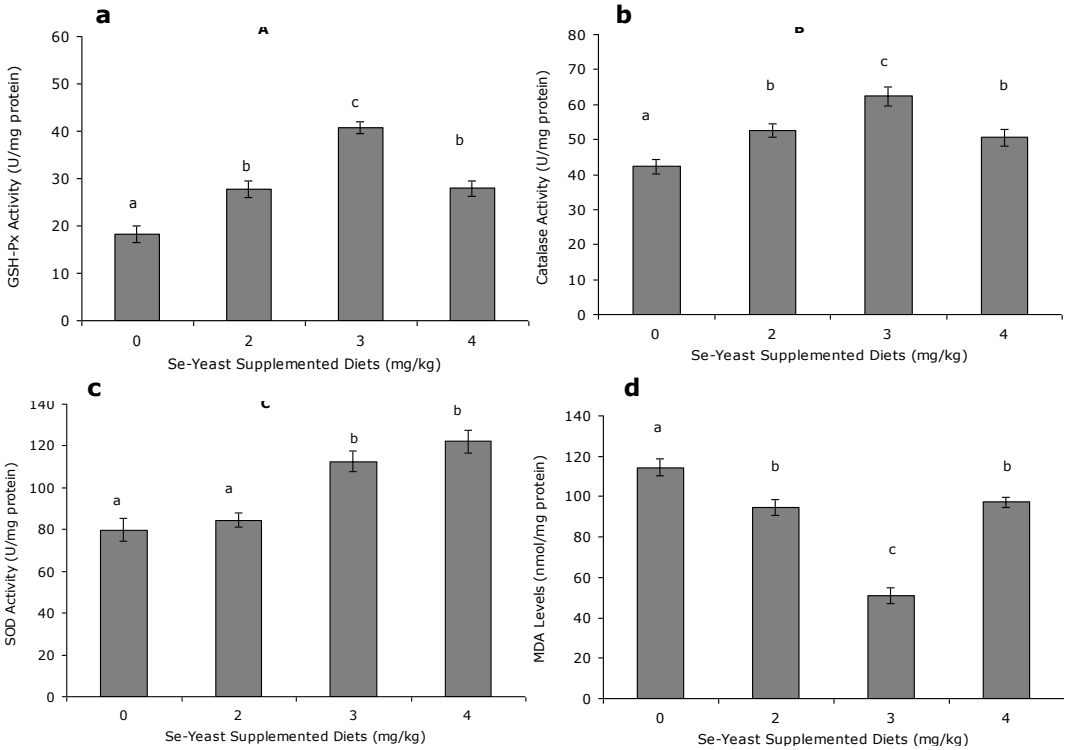

Fig. 1. Activity of the antioxidant enzymes (a) glutathione peroxidase (GSH-Px), (b) catalase (CAT), and (c) superoxide dismutase (SOD),; and (d) malondialdehyde (MDA) content in liver tissues of rainbow trout fed diets containing 0, 2, 3, or 4 mg organic Se per kg diet. Values represent means±SEM (n = 6). Different letters indicate significant difference between diet groups (p<0.05).

Discussion

The present study was carried out to investigate the effects of different levels of dietary Se supplementation on growth, muscle Se, oxidative stress markers (GSH-Px, CAT, SOD activity), and determination of lipid peroxidation level (MDA) in rainbow trout. Results indicate that supplementation of 3 mg Se per kg basal diet significantly affects fish growth as observed in channel catfish, *Ichtalurus punctatus* (Gatlin and Wilson, 1984), *Cyprinus carpio* (Gaber, 2008), and *Carassius auratus gibelio* (Zhou et al., 2009). Se deficiency can result in growth depression and increased mortality, whereas high levels of Se can have toxic effects resulting in reduced growth, SGR, feed efficiency, and FCR (Bell et al., 1987; Kim et al., 2003). There is a fine partition between deficiency and toxicity. High cellular concentration of Se can be utilized instead of sulfur, causing errors in protein synthesis and compromising the functionality of proteins. High selenium concentration may cause

bioaccumulation in the trophic chain, representing a risk for the organism (Dörr et al., 2008). On the other hand, long-term feeding of deficient trace elements, especially Se, can lead to cataracts and short body dwarfism in juvenile fish (Watanabe et al., 1980). Some minerals, like Se, play a crucial role in preventing oxidative stress and are essential micronutrients in fish (Martinez-Alvarez et al., 2005). Se regulates the metabolism of thyroid hormones, which are important for normal growth and development. Selenium supplementation in fish diets increased thyroid hormone activation which contributed to better growth, feed efficiency, and an improvement of meat quality (Cotter et al., 2008).

There was a linear relationship between Se concentration in the fish muscle and level of Se supplementation, as in Atlantic salmon (Lorentzen et al., 1994), *Penaeus vannamei* (Wang et al., 2006), rainbow trout (Küçükbay et al., 2009), common carp (Gaber, 2008), hybrid striped bass (Jaramillo et al., 2009).

The main antioxidative enzymes for the detoxification of reactive oxygen species (ROS) in all organisms are GSH-Px, CAT, and SOD. GSH-Px is the most important peroxidase for the detoxification of hydroperoxides (Dörr et al., 2008). CAT primarily occurs in peroxisomes and detoxifies H_2O_2 to O_2 and water. SOD plays an important role in the body's antioxidant system, intervening in the first transformation by dismuting the superoxide free radical, O_2^- into the most reactive form of oxygen, H_2O_2 (Dörr et al., 2008). MDA is the main oxidation product of peroxidized polyunsaturated fatty acids; a raised MDA level is an important indicator of lipid peroxidation (Elia et al., 2002). Antioxidants can protect organisms from free radicals and ROS, and slow down the progress of many chronic diseases as well as lipid peroxidation (Kalaiselvi and Panneerselvam, 1998). The antioxidant defense system is a multicomponent mechanism with enzymatic and non-enzymatic elements. Antioxidant defenses in fish are dependent on factors such as feeding behavior and nutrition (Martinez-Alvarez et al., 2005). While dietary micronutrients contribute to the antioxidant defense system and are being used more widely in animal feeds, safety and negative consumer perception restrict their application. Antioxidants such as Se-yeast may be added to the feed of farm animals to increase the oxidative stability and quality of meat (Schrauzer, 2006).

GSH-Px, CAT, and SOD activity increased in fish fed the supplemented diets, while MDA concentration decreased. The protective role of GSH-Px against hydroperoxide induced lipid peroxidation and hepatic MDA, a product of lipid peroxidation, was reduced by the Se supplementation. Serum and muscle concentrations of MDA are significantly lower in rainbow trout receiving Se (Küçükbay et al., 2009). GSH-Px activity in liver tissue of allogynogenetic crucian carp fed Se was remarkably higher than in the control (Wang et al., 2007). SOD and CAT activity was significantly increased in erythrocytes and liver tissues of carp fed supplemented Se (Jovanovic et al., 1997). The activity of the enzymes (GSH-Px, SOD, CAT) was dose dependant, all were significantly higher in fish fed the supplemented diets than in those

fed the unsupplemented control, similar to results in the shrimp, *Neocaridina heteropoda* (Wang et al., 2009).

Se has a large number of biological functions in animals including fish. The most important and known action is its antioxidant effect because it forms selenoprotein, part of the active center of GSH-Px (Wang et al., 2007; Zhou et al., 2009). Organic Se-yeast is a potential source of microelements such as copper, zinc, and iron which are essential for the synthesis of CAT and SOD (Jovanovic et al., 1997; Varga and Maraz, 2002). As a result, the increased enzyme activity in fish fed the Se-supplemented diets might be explained by the microelements contained in Se-yeast. The increased activity of GSH-Px, CAT, and SOD, together with the decreased MDA content in the liver, indicate that dietary Se yeast depresses lipid peroxidation and resistance to oxidative stress in rainbow trout.

In conclusion, this study shows that 3 mg/kg Se supplementation improves growth performance and FCR for rainbow trout and that supplementation in excess of this amount decreases the growth rate. Organic Se yeast had a positive effect on the accumulation of Se in the muscle and was effective in preventing oxidative stress, improving enzymatic antioxidant capacity, and preventing lipid peroxidation. This study shows that Se yeast antioxidant has ideal characteristics and may be a dietary source of a natural antioxidant agent in fish feed. Further studies are needed to examine the mechanism of Se function as an antioxidant in other cultured fish species.

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