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Effects of Accumulated Selenium on Some Physiological Parameters and Oxidative Stress Indicators in Tilapia Fish (*Oreochromis* spp.)

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Abstract: Samples of Tilapia fish (*Oreochromis* spp.) are used to evaluate bioaccumulation and the toxicity of Selenium (Se) through oxidative stress. Fish were exposed to sodium selenite (Na_2SeO_3) and physiological stress response and stress-related parameters (plasma cortisol, glucose, T3, T4, gill Na^+/K^+-ATPase, condition factor and HSI) and hepatic oxidative stress indicators (GSH, GPx and LPO) were measured after 96 hrs (acute exposure to 4.32 mg/L Se) and 8 weeks (sub-chronic exposure to 0.432 mg/L Se). Acute exposure to sodium selenite significantly increased plasma cortisol levels and plasma glucose levels, but gill Na^+/K^+-ATPase activities, plasma T3 and T4 levels, condition factor and HSI were unchanged. The 96 hrs acute selenium exposure decreased hepatic GSH. LPO and GPx activities significantly varied with treatment. The 8 weeks sub-chronic exposure increased plasma cortisol, T3 and T4, but there was no effect on plasma glucose levels, gill Na^+/K^+-ATPase activity, condition factor and HSI. The sub-chronic exposure to selenium did not alter antioxidant activities or LPO levels. It was found that Se accumulated in fish muscle in two-three folds higher than the control fish.

Key words: Selenium · *Oreochromis* spp. · Physiology · Oxidative stress · Accumulation

INTRODUCTION

Selenium (Se) is an essential trace element required in the ration for normal growth and physiological function of animal, including fish [1-6]. This element is required for normal development, growth and maintenance of homeostatic functions at trace concentrations [6]. It is a part of the antioxidant defense system and is involved in thyroid hormone metabolism, in spermatogenesis and probably in other processes unidentified to date [6, 7]. Se is involved in many functions such as moderation of the immune system and prevention of cancer, acting directly as a support for the organismal health [6, 8]. It is widely distributed throughout the environment and is found in most ground and surface waters at concentrations between 0.1 and 0.4 μg/L [9, 10]. Agricultural drain water, sewage sludge, fly ash from coal-fired power plants, oil refineries and mining of phosphates and metal ores are all sources of selenium contamination of the aquatic environment [1, 11, 12]. Se is a suspected carcinogen and teratogen [4, 13] and becomes very toxic to fish when it is elevated above a threshold concentration [1, 4]. The difference between nutritional requirement and toxic levels is very narrow for Se. For most fish, the requirement range is 0.25-0.70 μg Se/g diet [14, 15] and the toxic levels with prolonged exposure can be as low as 3 μg Se/g diet [2]. The U.S. Environmental Protection Agency (USEPA) proposed a chronic criterion for selenium at a whole body fish concentration of 7.91 μg/g dry weight [9, 10]. However, there is still controversy regarding the proposed selenium threshold for the protection of fish populations [16]. The most significant effect of excess Se in fish is growth inhibition, tissue damage, damage on most biomolecules (namely lipids, proteins and DNA), reproductive impairment, larval deformities and mortality [17, 18]. Other documented effects in fish include skin lesions, cataracts, swollen gill filament lamellae, myocarditis and liver and kidney necrosis [12].

Most of previous studies on Se toxicity have focused on the reproductive effects [11, 17, 19], but at present, very little is known about the toxicological profiles of Se on other systems, such as the physiological stress response (PSR) in fish. The PSR enables fish to maintain the internal homeostasis that is required for survival, growth and reproduction in a changing environment.

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The objectives of this study were to determine the bioaccumulation of Se in Tilapia (*Oreochromis* spp.) fingerlings fish muscle and the effect of selenium on the PSR and stress-related responses.

MATERIAL AND METHODS

Fish: Tilapia (*Oreochromis* spp.) fingerlings (average weight = 35.1±4.3 g) were obtained from the Arabian Fisheries Company Hatchery, Abbassa, Abou-Hammad, Sharkia Governorate, Egypt and kept in holding fiberglass tanks (1000 L tank) for one week before stocking in glass aquaria (40X35X80 cm) for recovery from any transportation stress or mortalities. Fish were fed extruded floating steelhead food (5% with 27% protein).

Experiments

Determination of Acute Lethal Concentration Dose (LC_{50}): To determine lethal concentration dose, 5 groups (10 fish per group) were used. Group I served as a control. Other 4 groups exposed to a graded series of sodium selenite (Na_2SeO_3) for consecutive 96 hrs. Cumulative mortality within the 96 hrs after exposure was used for the calculation of median lethal concentration (LC_{50}).

Acute Exposure: Fish were exposed to 100% of LC_{50} sodium selenite. 12 fish were randomly assigned to five aquaria (112 Liter). Using the acute concentration (96 hrs Lc_{50}) in fishes is commonly used to assess the effect of high concentration of the pollutants on fishes in addition to measure the physiological and biochemical changes which will happen in a short term exposure.

Subchronic Exposure: Fish were exposed to sodium selenite for 8 weeks at 1/10 of the 96 hrs LC_{50}. 60 fish were randomly assigned to each treatment; each treatment consisted of five aquaria with 12 fish per aquaria. More fish were used in the subchronic exposure than the acute exposure, as recommended for longer term studies [20].

Sampling: Fish were sampled every 24 hrs during the four days of acute exposure and every 2 weeks during subchronic exposure. Fish samples were lightly anesthetized with lidocaine (80 mg/L). Blood samples were drawn from the caudal vein. Whole blood was centrifuged at 12000 rpm for 15 min to obtain plasma. Fish gills, liver and parts of muscles were obtained for subsequent analyses.

Water Analysis: Water samples of the treatments were analyzed daily in case of acute exposure and weekly in case of subchronic exposure. The oxygen content was measured by using oxygen meter (YSI model 58). The pH was measured using pH meter (digital mini-pH meter model 55). Total hardness and bicarbonate alkalinity were determined by standard methods [21]. Conductivity was measured using a conductivity bridge. Temperature and salinity were measured by salinity meter (YSI model 57).

Growth Indices and Physiological Analyses: Length, weight and liver weight were recorded to calculate the condition factor [K=(weight (g) /length^3 (cm))x 100] and liver somatic index [HSI = (liver weight (g)/body weight (g)) ×100)]. Cortisol was measured in plasma by a simple radioligand assay as described by Levesque *et al.* [22]. The concentration of plasma glucose was measured according to Trinder [23]. T3 and T4 were measured with radioimmunoassay kits as described in Levesque *et al.* [22]. Gill Na^+/K^+-ATPase activity, expressed as µmol PO_4 liberated per mg of protein in a gill homogenate, was measured as described by Levesque *et al.* [22].

Oxidative Stress Parameters: A portion of the liver was homogenized in 10 volumes of 50 mM potassium phosphate buffer with 1 mM EDTA, pH 7.4 for GSH and LPO determination [12]. A second portion of liver was homogenized in 10 volumes of 50 mM Tris-HCl buffer with 5 mM EDTA and 1 mM 2-mercaptoethanol for GPx determination as described by Miller *et al.* [12]. Lipid peroxidation (LPO) was determined using the Bioxytech LPO-596 kit [12].

Se Bioaccumulation: Fish muscle samples were processed, extracted and analyzed as described by Cottenie *et al.* [24] in Evaluation and Treatment of Soil and Plant Pollution Unit, National Research Center, Dokki, Egypt. Se was determined using aa, ae atomic absorption spectrophotometer (Model IL 57).

Statistical Analysis: Data were analyzed statistically by analysis of variance using SPSS (version 14.0) statistical software package (SPSS, Inc., Chicago, Illinois, USA). Differences were considered significant at an alpha level of 0.05.

RESULTS

Water Characteristics: The observed water characteristics revealed that, water quality parameters in

the subchronic selenium exposures were similar to those measured in the acute exposures (Table 1).

Determination of Acute Lethal Concentration Dose (LC_{50}): Toxicity experiments revealed that, the acute lethal concentration dose was 4.32 mg/L during the 96 hrs post-exposure.

Physiological Stress Response: Plasma glucose levels (Table 2) were greater in all times of sampling ($P<0.05$) than the control group in the 96 hrs exposures. Plasma cortisol levels were significantly higher ($P<0.05$) in fish exposed to 4.32 mg/L selenium for 96 hrs than in the control during the entire experimental period (Table 2).

Plasma T3 and T4 (Table 3) and Gill Na^+/K^+-ATPase (Table 4) were not significantly influenced by a 96 hrs exposure to Se. Condition factor and HSI (Table 5) were also not significantly affected by the acute selenium exposure.

After the 8 weeks (subchronic) selenium exposure, plasma cortisol levels were higher ($P<0.05$) in fish exposed to 0.432 mg/L Se (Table 2). The obtained results showed no significant differences between treatment groups in plasma glucose levels after 8 weeks. Selenium did not significantly change gill Na^+/K^+-ATPase activity after 8 weeks (Table 4). Fish exposed to 0.432 mg/L of selenium had significantly higher plasma T3 and T4 levels ($P<0.05$) than the control on the end of subchronic exposure (Table 3). Condition factor and HSI were not different among treatment groups (Table 5).

Oxidative Stress Bioindicators: Hepatic oxidative stress biomarkers were modified by acute (96 hrs) selenium exposure. Lipid peroxidation levels were significantly ($P<0.05$) lower in fish exposed to 4.32 mg/L selenium than in the control group at the end of exposure time (Table 4). Tilapia had significantly less hepatic GSH reserves ($P<0.05$) in the 4.32 mg/L exposures compared to the control treatment (Table 6). Hepatic GPx activity was significantly lower ($P<0.05$) in the group exposed to 4.32 mg/L Se than in the control (Table 6). Hepatic oxidative stress parameters did not significantly change (Tables 4 and 6) with 8 weeks of subchronic Se exposure.

Bioaccumulation of Selenium (Se): After 96 hrs of exposure, it was observed that Se concentrations in fish muscles increased dramatically (Table 7), which were significantly ($P<0.05$) and two-three folds higher than the control. After 8 weeks of the experimental period, Se

Table 1: Water characteristics during all experiments

Parameter	Value	Parameter	Value
Temperature (°C)	27.7±0.11	Electric conductivity (mmohs/cm)	0.41±0.09
Dissolved oxygen (DO) (mg/L)	7.2±0.36	Total dissolved solids (mg/L)	240.00±8.7
pH	7.4±0.06	Ammonium (NH_4^+) (mg/L)	0.71±0.05
Total alkalinity (mg/L as $CaCO_3$)	182.0±7.8	Ammonia (NH_3) (mg/L)	0.03±0.001
Salinity (mg/L)	0.1±0.01	Nitrite (NO_2^-) (mg/L)	0.01±0.001
Total hardness (mg/L as $CaCO_3$)	106.0±13.4	Nitrate (NO_3^-) (mg/L)	1.10±0.6

Table 2: Effect of selenium on glucose (mg/dl plasma) and cortisol (ng/ml plasma) concentrations of Tilapia spp. during the period of experiments (Mean±S.E.)

Glucose (mg/dl plasma)				Cortisol (ng/ml plasma)			
Acute		Chronic		Acute		Chronic	
Control	88.6±3.16^a	Control	88.6±3.16^a	Control	4.9±0.8^a	Control	4.9±0.8^a
1 day	129.3±5.8^b	2 weeks	94.1±4.9^{ab}	1 day	6.4±1.6^b	2 weeks	5.1±0.9^a
2 days	156.5±8.18^c	4 weeks	103.8±7.8^b	2 days	6.6±1.4^b	4 weeks	5.2±0.6^a
3 days	186.6±11.2^d	6 weeks	97.2±9.14^{ab}	3 days	7.3±0.9^c	6 weeks	4.8±1.2^a
4 days	210.4±9.3^e	8 weeks	93.5±12.3^a	4 days	8.1±0.7^d	8 weeks	5.8±1.7^a

Means with the same letter at the same column are not significantly different ($P>0.05$)

Table 3: Effect of selenium on T3 (ng/ml plasma) and T4 (ng/ml plasma) concentrations of Tilapia spp. during the period of experiments (Mean±S.E.)

T3 (ng/ml plasma)				T4 (ng/ml plasma)			
Acute		Chronic		Acute		Chronic	
Control	0.90±0.07^a	Control	0.90±0.07^a	Control	6.2±1.3^a	Control	6.2±1.3^a
1 day	0.94±0.16^a	2 weeks	1.20±0.07^{bc}	1 day	5.9±1.6^a	2 weeks	7.3±0.93^{ab}
2 days	0.88±0.12^a	4 weeks	1.40±0.08^c	2 days	6.3±1.8^a	4 weeks	7.9±1.2^b
3 days	0.93±0.50^a	6 weeks	1.90±0.11^d	3 days	7.1±2.6^{ab}	6 weeks	9.8±1.7^c
4 days	1.01±0.14^{ab}	8 weeks	1.13±0.09^b	4 days	6.7±1.8^{ab}	8 weeks	10.7±1.7^c

Means with the same letter at the same column are not significantly different ($P>0.05$)

Table 4: Effect of selenium on Lipid Peroxidation (LPO) (U/mg protein) concentrations in Liver and Gill Na^+/K^+-ATPase concentrations of Tilapia spp. during the period of experiments (Mean±S.E.)

Gill Na+/K+ -ATPase				LPO (U/mg protein)			
Acute		Chronic		Acute		Chronic	
Control	1.01±0.03[a]	Control	1.01±0.03[a]	Control	0.06±0.001[a]	Control	0.06±0.001[a]
1 day	1.14±0.02[ab]	2 weeks	1.11±0.06[ab]	1 day	0.05±0.003[a]	2 weeks	0.07±0.007[ab]
2 days	1.20±0.14[b]	4 weeks	1.17±0.03[b]	2 days	0.06±0.006[a]	4 weeks	0.05±0.004[b]
3 days	1.09±0.04[ab]	6 weeks	1.08±0.06[ab]	3 days	0.04±0.003[b]	6 weeks	0.06±0.001[ab]
4 days	0.97±0.03[a]	8 weeks	1.05±0.08[a]	4 days	0.03±0.003[b]	8 weeks	0.04±0.004[c]

Means with the same letter at the same column are not significantly different ($P>0.05$)

Table 5: Effect of selenium on liver somatic index (HSI) and condition factor (K) values of Tilapia spp. during the period of experiments (Mean±S.E.)

Acute			Chronic		
Time	HSI	K	Time	HSI	K
Control	2.12±0.15[a]	1.48±0.04[a]	Control	2.12±0.15[a]	1.48±0.04[a]
1 day	2.14±0.12[a]	1.39±0.03[a]	2 weeks	2.17±0.12[a]	1.53±0.08[a]
2 days	2.21±0.18[a]	1.52±0.05[a]	4 weeks	2.11±0.18[a]	1.47±0.06[a]
3 days	2.16±0.11[a]	1.48±0.04[a]	6 weeks	2.12±0.11[a]	1.42±0.07[a]
4 days	2.13±0.13[a]	1.46±0.02[a]	8 weeks	2.14±0.13[a]	1.50±0.05[a]

Means with the same letter at the same column are not significantly different ($P>0.05$)

Table 6: Effect of selenium on GSH Reductase (µmol/ml protein) and Glutathione Peroxidase (GPx) (mU/mg protein) concentrations in Liver of Tilapia spp. during the period of experiments (Mean±S.E.)

GSH Reductase (µmol/ml protein)				GPx (mU/mg protein)			
Acute		Chronic		Acute		Chronic	
Control	24.3±2.5[a]	Control	24.3±2.5[a]	Control	4.2±0.06[a]	Control	4.2±0.06[a]
1 day	21.9±1.9[ab]	2 weeks	22.8±1.8[ab]	1 day	4.1±0.29[a]	2 weeks	3.7±0.18[a]
2 days	20.3±2.7[b]	4 weeks	21.9±3.1[b]	2 days	3.7±0.28[b]	4 weeks	4.3±0.27[a]
3 days	23.8±3.6[a]	6 weeks	25.4±3.3[ab]	3 days	4.7±0.41[c]	6 weeks	4.2±0.25[a]
4 days	19.7±2.9[c]	8 weeks	21.6±4.3[b]	4 days	3.2±0.16[d]	8 weeks	4.2±0.40[a]

Means with the same letter at the same column are not significantly different ($P>0.05$)

Table 7: Selenium concentrations (mg/l) in the muscles of Tilapia spp. during the period of experiments (Mean±S.E.)

Se concentrations (mg/l)			
Acute		Chronic	
Control	5.50±0.17[a]	Control	5.50±0.17[a]
1 day	10.40±0.01[b]	2 weeks	7.70±0.50[b]
2 days	10.25±0.45[b]	4 weeks	8.85±0.55[c]
3 days	12.10±0.20[c]	6 weeks	8.80±0.10[c]
4 days	10.90±0.40[b]	8 weeks	8.00±0.01[bc]

Means with the same letter at the same column are not significantly different ($P>0.05$)

concentrations in muscles reduced to some extent, where it was observed that there were statistically significant ($P<0.05$) convergences to control group values.

DISCUSSION

One of the most important functions of Se is related to its antioxidant role and participation in the antioxidant defense system [6, 25]. But, since the margin between salutary and toxic effects of selenium is very narrow [7], so, potential toxic effects are important questions to be addressed when the role of selenium is considered.

The lowest Se exposure used in this study, during the subchronic (8 weeks) exposures, was 0.432 mg/L. This concentration approaches the Se concentrations measured in water of streams in many parts of the world where developmental deformities have been observed in the resident fish population [19]; therefore, the Se concentrations used in present subchronic laboratory study are environmentally relevant.

In the present study, acute (96 hrs) and subchronic (8 weeks) exposures to selenium activated the PSR in Tilapia fingerlings. Plasma cortisol levels increased significantly in the 0.432 mg/L exposure group, although

the acute exposure raised cortisol higher. This indicates exposures to Se for 96 hrs are more stressful to the fish than 0.432 mg Se/L for 8 weeks. Many previous studies have reported that acute exposures to contaminants such as heavy metals [26, 27] and herbicides [28], increase plasma cortisol in fish. Similar findings were reported by Miller *et al.* [12] who reported that elevated Se activates the PSR in fish and increases plasma cortisol. Hontela [20] and Barton [29] mentioned that, in chronic exposures to contaminants, an increase in plasma cortisol is generally followed by a decrease as the fish acclimates, or, as has been reported for cadmium, the cortisol secretory response becomes impaired. In the present study, plasma cortisol increased in the subchronic exposure (0.432 mg/L). This pattern suggests Se is still activating the stress response after 8 weeks and the fish did not acclimate to Se before the end of the exposure. The obtained results also indicate that the interrenal cells were not impaired by the 8 weeks exposure, since the ability of the head kidney to secrete cortisol was not significantly altered by Se exposure [12].

Plasma glucose levels increased during the acute (96 hrs) but not affected during the subchronic (8 weeks) exposure to Se. Rise of glucose level indicated the presence of stressful stimuli eliciting rapid secretion of both glucocorticoids and catecholamines from the adrenal tissue and accompanied by cortisol elevation [27, 30]. On the contrary, plasma T3 and T4 levels were not affected during the acute (96 hrs) but increased during the subchronic (8 weeks) exposure to Se. Exposure to Se increased plasma cortisol and it has been documented that cortisol influences thyroid hormone metabolism [31]. Other stress-related responses (gill Na^+/K^+-ATPase activity, condition factor and HSI) were not affected along the period of all experiments. The maintenance of liver weight and HSI in fish exposed to acute and chronic Se concentrations may be due to the contribution of Se to hepatocyte proliferation, leading to hepatic regeneration, which is a critical step to prevent liver injury [6]. Also, the maintenance of condition factor may be due to the suggestion reported by Zhu *et al.* [32] who mentioned that, the induction of oxidative stress may have an inhibitory effect on fish growth.

Selenium decreased liver LPO in the acute and chronic exposures, although only the acute exposure was significantly lower than the control group. The present results disagree with findings of Miller *et al.* [12], Di Giulio *et al.*, [33], Oakes *et al.* [34] and Dorval *et al.* [35] who reported that most contaminants including hydrocarbons, pulp and paper effluents, agricultural runoff and metals increase LPO in fish tissues. GPx activity was significantly changed in fish exposed to acute selenium exposure. Similar results was obtained by Orun *et al.* [36] who found that hepatic GPx increased in rainbow trout at exposures over 4 mg/L of sodium selenite. Other studies suggested that the response of GPx is toxicant-dependent, with some toxicants decreasing it [35, 37] and others increasing its activity [12, 38, 39].

GSH is a powerful antioxidant and anti-toxicant as it binds to many different toxicants and inactivating them [12]. Therefore, decreases in the GSH content make the fish cells more susceptible to attack by toxic electrophilic compounds [6]. In the present study, Se decreased liver GSH concentrations in the acute exposure (96 hrs) groups. Holm [40] observed depletion in GSH levels in rainbow trout fed Se-methionine during chronic laboratory experiment. The GSH concentrations also decreased with exposure to heavy metals [38, 41, 42], endosulfan [43] and agricultural runoff [35].

The most important implication of elevated environmental selenium is its propensity to accumulate in the aquatic food chain, potentially causing adverse effects on fish populations [1, 16]. The major finding of the present study was the accumulation and biomagnification of selenium in muscle of Tilapia fingerlings and this may be the reason of high toxicity of Se. Biomagnifications of selenium has been reported by some investigators [16, 44].

It could be concluded that, Selenium (Se) appears to play a dichotomous role in fish because it is both a nutrient and a toxicant where at higher levels (similar to those present in the 96 hrs acute exposure) Se may begin to alter antioxidant status, but at lower levels it may protect the liver from damage. So, the particular pattern of the effects of Se reported in the present study requires further investigation to identify the threshold at which Se ceases to be an essential element and becomes a toxicant.

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