# RESPONSE OF NILE TILAPIA, *OREOCHROMIS NILOTICUS* (L.) TO ENVIRONMENTAL CADMIUM TOXICITY DURING ORGANIC SELENIUM SUPPLEMENTATION

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***Abstract***

This study was carried out to evaluate the resistance of Nile tilapia, *Oreochromis niloticus* (L.), fed on organic selenium (OS)-supplemented diet to water-born cadmium (Cd) toxicity. Fish (14.8±1.3 g) were randomly distributed at a density of 10 fish in 100-L aquarium. Fish in three treatments were fed a diet (30% crude protein) containing 0.5 g OS/kg diet (T1 - T3), while three other treatments (T4 - T6) were fed a basic diet without OS. During the feeding trial, fish in all groups were then exposed to 0.0, 1.116, or 2.232 mg $Cd^{2+}$/L for 6 weeks. The fish performance and all the biochemical aspects were affected significantly by both Cd concentration, Se supplementation, and their interaction. OS supplementation enhanced fish growth, survival, and feed utilization, meanwhile the exposure to Cd reduced them (T1 vs T4, T2 vs T5, and T3 vs T6). All fish body constituents except moisture content were significantly affected by OS supplementation, Cd level, and their interaction. Crude protein and total lipids in fish body decreased, while ash content and Cd residue increased significantly with increasing Cd levels. OS reduced significantly Cd residues in fish body. Moreover, OS supplementation reduced creatinine, uric acid, AST, and ALT to below those of Cd-exposed treatments. Serum lipids increased, while protein decreased only in Cd-exposed groups (T5 and T6). Glutathione peroxidase (GPX) activity increased significantly with increasing Cd level and it was higher in T1 vs T4, T2 vs T5, and T3 vs T6. It could be concluded that OS supplementation may reduce the harmful effect of water-born Cd in fish, which in turn improves the growth, survival, and feed efficiency.

**Keywords:** Nile tilapia, organic selenium, growth, feed utilization, cadmium toxicity, physiological aspects.

## INTRODUCTION

Heavy metals are among the most dangerous substances in the aquatic environment because they persist and are harmful to aquatic organisms. Cadmium (Cd) is one of these heavy metals that have become widely distributed in the aquatic environment as an industrial waste produced from electroplanting and plastic industry. Although WHO (1989) reported that the permissible level of Cd in water and fish muscles are 0.01 ppm and 1.0 mg/g, respectively, fish can accumulate Cd to levels ten

to one-thousand times higher than its level in ambient water (Fleischer *et al.*, 1974). The freshwater quality criteria values of Cd for the protection of aquatic organisms in North America are less than 1 μg/L at low hardness (USEPA, 2001; CCME, 2002). Freshwater fish exposed to waterborne Cd at total concentrations well below 100 μg/L exhibit substantial pathophysiology (Wood, 2001). The effect of Cd on aquatic organisms may be affected by many environmental factors (Wicklund and Runn, 1988; Dutta and Kaviraj, 1996; Kaviraj and Dutta, 2000) and the presence of dissolved organic matter (Meinelt *et al.*, 2001, 2007; Burnison *et al.,* 2006). The actual Cd level may exceed these levels to the limits which can induce respiratory or osmoregulatory dysfunctions (Thurberg *et al.*, 1973; Bjerregaard and Vislie, 1985; Zyadah and Abdel-Baky, 2000), lipid peroxidation, DNA damage, and glutathionylation of proteins (Stohs and Bagchi, 1995; Risso-de Faverney *et al.*, 2001; Silvestre *et al.*, 2006).

Selenium (Se) is an essential trace element required in the diet for normal growth and physiological function of fish (Hilton *et al.*, 1980; Bell *et al.,* 1985; Wang and Lovell, 1997). Se is a component of the enzyme glutathione peroxidase, which catalyzes reactions that may protect cell membrane against oxidative damage (Rotruck *et al.,* 1973). The use of organic selenium (OS) such as selenomethionine and selenoyeast to improve the Se availability has been examined because of their potentially higher bioavailability than inorganic forms (Bell and Cowey, 1989; Lorentzen *et al.,* 1994; Wang and Lovell, 1997; Mahan, 1999; Schram *et al.,* 2008). Specifically, OS has been shown to have a protective effect for fish exposed to toxic levels of heavy metals (Lin and Shiau, 2007; Abdel-Tawwab *et al.*, 2007a). Therefore, the purpose of this study was to investigate the influence of OS supplementation on the reduction of water-born Cd toxicity by evaluating the growth performance, feed efficiency, and the biochemical aspects of Nile tilapia, *O. niloticus* (L.).

## MATERIALS AND METHODS

### Experimental Procedures

Healthy Nile tilapias, *O. niloticus* (L.), were collected from the nursery ponds at the Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharqia, Egypt. Fish (14.8 ± 1.3 g) were acclimated to laboratory conditions in indoor tanks for 2 weeks. Prior to the feeding trial, the lethal concentration of Cd after 96 hour (96-h $LC_{50}$) of exposure was calculated according to Behreus and Karber (1953). Briefly, forty healthy fish were randomly distributed in eight aquaria, 100 L each. Cadmium sulfate ($3CdSO_4.8H_2O$, M wt = 769.51; produced by BDH Chemicals Ltd., Poole, England) was dissolved in distilled water and add to each aquarium's water to produce

1, 3, 5, or 10 mg $Cd^{2+}$/L. Fish were exposed to the above concentrations for 96 hour and each Cd concentration was represented by two replicates. The dead fish were recorded daily and removed from the aquaria. The 96-h $LC_{50}$ was 11.16 mg Cd/L and the implicated doses were 0.0, 0.1, or 0.2 $LC_{50}$ i.e. 0.0, 1.116, or 2.232 mg Cd/L, respectively.

The acclimated fish were randomly divided into two groups; the first group was fed a diet containing 30% crude protein and 4.51 kcal/g. A 0.5% of Sel-Plex® (produced by All-Tech Feed, Lexington, Kentucky, USA) was added to the first group representing 5.54 mg Se/kg (+ OS) and the second group was fed a basic diet without OS (- OS) supplement. Eighteen 100-L aquaria were randomly allocated with three-replicate aquaria per treatment and stocked with 10 fish per aquarium. The design of the experiment was as follows:

| Treatment | OS supplemented | Water-borne Cd concentration |
|---|---|---|
| T1 | Yes | 0 mg Cd/L |
| T2 | Yes | 1.116 mg Cd/L |
| T3 | Yes | 2.232 mg Cd/L |
| T4 | No | 0 mg Cd/L |
| T5 | No | 1.116 mg Cd/L |
| T6 | No | 2.232 mg Cd/L |

Each aquarium was supplied with compressed air via air-stones from air pumps. The ambient temperature throughout this study ranged from 26 to 28 $^{o}$C. Dead fish were removed and recorded daily. Fish were offered a 30% crude protein diet at a rate of 3% of live body weight daily divided into 2 equal meals; 5 days a week for 6 weeks. Three quarters of aquarium's water was siphoned daily along with fish excrement and replaced with an equal volume of water maintaining the same Cd concentration per each aquarium. Fish were anaesthetized using tricane methane sulfonate (20 mg/L) and weighed at the beginning of the experiment and at weekly intervals. Group weights were determined to the nearest 0.1 g.

**Water Chemistry Analysis**

Water samples for chemical analyses were monitored biweekly. Dissolved oxygen and temperature were measured on site using a YSI model 58 oxygen meter (Yellow Spring Instrument Co., Yellow Springs, Ohio, USA). The pH was measured

using a pH-meter (Fisher Scientific, Denver, Colorado, USA). Unionized ammonia, total alkalinity, and total hardness were measured by titration as described by Boyd (1984).

In all treatments, dissolved oxygen concentrations ranged from 6.3 to 7.1 mg/L. The ambient water temperature was approximately stable for the experimental duration and ranged from 26 to 28 °C, pH ranged from 7.6 to 7.9, and unionized ammonia range was 0.18 – 0.28 mg/L. Total alkalinity and total hardness ranges were 135 - 180 mg/L as $CaCO_3$, and 125 - 175 mg/L as $CaCO_3$, respectively. Cadmium and selenium were measured in aquaria water but were below detection limits. All the water parameters were within the acceptable range for fish growth (Boyd, 1984).

**Growth Parameters**

Growth performance was determined and feed utilization was calculated as follows:

Weight gain = $W_2 - W_1$;

Specific growth rate (SGR) = 100 ($\ln W_2 - \ln W_1$) / T; where $W_1$ and $W_2$ are the initial and final fish weight, respectively, and T is the experimental period in days;

Feed conversion ratio (FCR) = feed intake calculated on dry matter basis / weight gain.

**Biochemical Measurements**

Fish blood samples were collected with a hypodermic syringe from the caudal vessels. The extracted blood was left to clot at 4 C and centrifuged at 5000 rpm for 5 min at room temperature. The collected serum was stored at – 20 C for further assays. Glucose was determined colorimetrically according to Trinder (1969). Total protein was determined colorimetrically according to Henry (1964). Total lipids were determined colorimetrically according to Joseph *et al.* (1972). Uric acid was measured according to Barham and Trinder (1972) and creatinine was measured colorimetrically as described by Henry (1974). Activities of aspartate amninotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically according to Reitman and Frankel (1957). Glutathione peroxidase (GPX) activity was assayed by the method of Paglia and Valentine (1967) with modifications according to Lawrence and Burke (1978).

**Proximate Chemical Analyses**

After growth trial, the proximate chemical analyses of whole-fish body collected from each treatment were done according to the standard methods of AOAC (1990) for moisture, protein, fat, and ash. Moisture content was estimated by drying the

samples to constant weight at 85 C. (GCA, model 18EM, Precision Scientific group, Chicago, Illinois, USA). Nitrogen content was measured using a microkjeldahl apparatus (Labconco, Labconco Corporation, Kansas, Missouri, USA) and crude protein was estimated by multiplying nitrogen content by 6.25. Lipid content was determined by ether extraction in multi-unit extraction Soxhlet apparatus (Lab-Line Instruments, Inc., Melrose Park, Illinois, USA) for 16 hours and ash was determined by combusting dry samples in a muffle furnace (Thermolyne Corporation, Dubuque, Iowa, USA) at 550 C for 6 hours.

**Metal Residue**

For measuring Cd and Se residues in diet and whole-fish body, samples were oven-dried at 85 C until constant weight. Afterwards, one gram of dry sample was ashed in muffle furnace at 550 C four 6 hours and was digested with concentrated $HNO_3$, and diluted with 2N HCl to a constant volume. A 100 ml of aquaria's water was filtered via Millipore filter paper (0.45 μm) by using Millipore apparatus and the filtrate was used for measuring the heavy metals. Cd and Se concentrations were measured using an atomic absorption spectrophotometer (Thermo 6600, Thermo Electron Corporation, Cambridge, UK).

**Statistical Analysis**

The obtained data were subjected to two-way ANOVA to test the effect of water-born cadmium and OS supplementation as the two factors simultaneously tested. The differences between means were done by using Duncan's Multiple Range test that was used as a post-hoc test to compare between means at $P \leq 0.05$. The software SPSS, version 10 (SPSS, Richmond, Virginia, USA) was used as described by Dytham (1999).

## RESULTS

Fish performance, survival, and feed utilization were significantly affected by both OS supplementation, and Cd concentration ($P < 0.05$; Table 1). OS supplementation enhanced fish growth, survival, and feed utilization, meanwhile the exposure to Cd reduced them. However, fish groups fed OS exhibited better growth at low and high Cd concentration than those were not fed OS. Fish survival decreased significantly with increasing the Cd concentration ($P < 0.05$) and it was 80% vs 66.7% in T2 vs T5 and 73.3% vs 53.3% in T3 vs T6, respectively (Table 1).

Feed intake and FCR were significantly affected by OS supplementation as well as Cd concentration ($P < 0.05$). Feed intake decreased, while FCR increased significantly with increasing Cd concentration ($P < 0.05$; Table 1). Feed intake and FCR in T1, T2, and T3 were better than those of T4, T5, and T6.

TABLE 1. Composition and proximate chemical analyses (%; on DM bases) of the experimental diets containing different levels of organic selenium (OS).

| Ingredients | OS levels (g $kg^{-1}$ diet) | |
|---|---|---|
| | 0.0 | 0.5 |
| Herring fish meal | 9.0 | 9.0 |
| Soybean flour | 52.5 | 52.5 |
| Corn flour | 19.5 | 19.5 |
| Starch | 7.0 | 6.5 |
| Corn oil | 2.0 | 2.0 |
| Cod liver oil | 2.0 | 2.0 |
| Vitamin premix (1) | 2.0 | 2.0 |
| Mineral premix (2) | 2.0 | 2.0 |
| α-cellulose | 3.0 | 3.0 |
| Carboxy-methyl-cellulose | 1.0 | 1.0 |
| Sel-Plex® | 0.0 | 0.5 |
| Total | 100 | 100 |
| Chemical analysis (%) | | |
| Dry matter | 91.7± 0.45 | 91.70± 0.57 |
| Crude protein | 30.6± 0.33 | 30.5± 0.54 |
| Ether extract | 9.1± 0.25 | 9.2± 0.27 |
| Ash | 8.1± 0.15 | 8.2± 0.12 |
| Se level (mg $kg^{-1}$) | 1.04± 0.045 | 5.54± 0.15 |
| Cd level (mg $kg^{-1}$) | 0.21±0.013 | 0.22±0.018 |
| Crude fibers | 5.5± 0.15 | 5.4± 0.13 |
| Nitrogen free extract (3) | 46.7 | 46.8 |
| Gross energy (kcal/g) (4) | 4.51 | 4.52 |

(1) Vitamin premix (per kg of premix): thiamine, 2.5g; riboflavin, 2.5g; pyridoxine, 2.0g; inositol, 100.0g; biotin, 0.3g; pantothenic acid, 100.0g; folic acid, 0.75g; para-aminobenzoic acid, 2.5g; choline, 200.0g; nicotinic acid, 10.0g; cyanocobalamine, 0.005g; α-tocopherol acetate, 20.1g; menadione, 2.0g; retinol palmitate, 100,000 IU; cholecalciferol, 500,000 IU.

(2) Mineral premix (g per kg of premix): $CaHPO_4.2H_2O$, 727.2; $MgCO_3.7H_2O$, 127.5; KCl 50.0; NaCl, 60.0; $FeC_6H_5O_7.3H_2O$, 25.0; $ZnCO_3$, 5.5; $MnCl_2.4H_2O$, 2.5; $CuCl_2$, 0.785; $CoCl_3.6H_2O$, 0.477; $CaIO_3.6H_2O$, 0.295; $CrCl_3.6H_2O$, 0.128; $AlCl_3.6H_2O$, 0.54.

(3) Nitrogen free extract (NFE) = 100 – (protein% + lipid% + ash% + crude fiber%)

(4) Gross energy (GE) was calculated as 5.65, 9.45 and 4.11 kcal/g for protein, lipid and NFE, respectively (NRC 1993).

Table 2. Growth performance (mean ± SE) of Nile tilapia exposed to 0, 1.116, or 2.232 mg Cd/L and fed a diet with (+ OS) and without (- OS) organic selenium for 6 weeks.

| Treatments | | Initial weight (g) | Final weight (g) | Weight gain (g) | SGR (%/day) | Survival (%) | Feed intake (g feed/fish) | FCR |
|---|---|---|---|---|---|---|---|---|
| + OS – Cd | T1 | 15.2±0.88 a | 35.3±0.58 a | 20.1±0.51 a | 2.01±0.027 a | 93.3±3.33 a | 20.7±0.55 a | 1.03±0.029 c |
| + OS + 1.116 mg Cd/L | T2 | 15.1±0.58 a | 28.4±0.73 b | 13.3±0.67 b | 1.50±0.049 b | 80.0±3.33 b | 19.3±1.36 ab | 1.45±0.159 bc |
| + OS + 2.232 mg Cd/L | T3 | 15.2±0.88 a | 26.6±0.61 c | 11.4±0.52 c | 1.33±0.041 c | 73.3±5.77 bc | 18.4±1.07 ab | 1.61±0.087 b |
| - OS – Cd | T4 | 15.2±0.88 a | 30.4±0.63 b | 15.2±0.67 b | 1.66±0.057 b | 93.3±5.77 a | 19.6±1.18 ab | 1.29±0.137 bc |
| - OS + 1.116 mg Cd/L | T5 | 15.1±0.67 a | 26.2±0.32 c | 11.1±0.32 c | 1.31±0.029 c | 66.7±3.33 c | 18.0±1.04 ab | 1.62±0.142 b |
| - OS + 2.232 mg Cd/L | T6 | 15.3±0.67 a | 22.8±0.29 d | 7.5±0.35 d | 0.95±0.038 d | 53.3±3.33 d | 17.1±0.93 b | 2.28±0.028 a |

Means and SE were calculated from n = 3 aquaria.

Means having the same letter in the same column are not significantly different at $P < 0.05$.

Table 3. Proximate chemical analysis on wet weight basis (mean ± SE) of whole-body of Nile tilapia exposed to 0, 1.116, or 2.232 mg Cd/L and fed a diet with (+ OS) and without (- OS) organic selenium for 6 weeks.

| Treatments | | Crude protein (g/100 g) | Total lipids (g/100 g) | Ash (g/100 g) | Cd residue (µg/g DW) | Se residue (µg/g DW) |
|---|---|---|---|---|---|---|
| + OS – Cd | T1 | 14.3±0.37 a | 2.7±0.073 a | 5.1±0.27 d | 2.9±0.021 e | 32.5±1.31 a |
| + OS + 1.116 mg Cd/L | T2 | 13.9±0.55 a | 2.2±0.014 b | 7.3±0.29 b | 29.7±0.718 d | 30.08±1.52 b |
| + OS + 2.232 mg Cd/L | T3 | 11.8±0.27 b | 2.0±0.012 c | 8.6±0.47 a | 57.8±1.164 b | 30.2±1.37 b |
| - OS – Cd | T4 | 14.1±0.42 a | 2.7±0.036 a | 4.8±0.25 d | 3.1±0.035 e | 10.19±0.41 c |
| - OS + 1.116 mg Cd/L | T5 | 12.7±0.34 b | 1.8±0.017 c | 6.3±0.29 c | 41.2±0.936 c | 8.51±0.98 d |
| - OS + 2.232 mg Cd/L | T6 | 10.6±0.28 c | 1.1±0.047 d | 7.0±0.33 b | 69.9±2.97 a | 8.18±0.89 d |

Means having the same letter in the same column are not significantly different at $P < 0.05$.

Table 4. Changes in serum glucose, total protein, total lipids, albumin, and globulin (mean ± SE) of Nile tilapia exposed to 0, 1.116, or 2.232 mg Cd/L and fed a diet with (+ OS) and without (- OS) organic selenium for 6 weeks.

| Treatments | | Glucose ($g\ L^{-1}$) | Total protein ($g\ L^{-1}$) | Albumin ($g\ L^{-1}$) | Globulin ($g\ L^{-1}$) | Total lipids ($g\ L^{-1}$) |
|---|---|---|---|---|---|---|
| + OS – Cd | T1 | 0.987±0.015 c | 23.00±0.20 a | 14.97±0.88 a | 8.83±0.12 a | 16.52±1.83 e |
| + OS + 1.116 mg Cd/L | T2 | 1.022±0.036 c | 19.48±0.23 b | 12.17±0.67 b | 7.31±0.23 b | 17.63±2.48 d |
| + OS + 2.232 mg Cd/L | T3 | 1.162±0.026 b | 16.10±0.10 c | 10.23±0.32 c | 5.87±0.24 b | 19.76±2.74 c |
| - OS – Cd | T4 | 0.992±0.023 c | 19.87±0.39 b | 12.47±0.19 b | 7.40±0.23 b | 15.93±1.79 e |
| - OS + 1.116 mg Cd/L | T5 | 1.197±0.017 b | 15.63±0.27 c | 10.90±0.10 c | 4.73±0.32 c | 22.31±2.38 b |
| - OS + 2.232 mg Cd/L | T6 | 1.389±0.016 a | 11.33±0.33 e | 8.23±0.18 d | 3.10±0.32 d | 26.47±1.85 a |

Means having the same letter in the same column are not significantly different at $P < 0.05$.

Table 5. Changes in serum creatinine, uric acid, AST, ALT, and GPX (mean ± SE) of Nile tilapia exposed to 0, 1.116, or 2.232 mg Cd/L and fed a diet with (+ OS) and without (- OS) organic selenium for 6 weeks.

| Treatments | | Creatinine (mg/L) | Uric acid (mg/L) | AST (IU/L) | ALT (IU/L) | GXP (μmol/min/mg protein) |
|---|---|---|---|---|---|---|
| + OS – Cd | T1 | 5.87±0.20 e | 15.53±0.20 d | 17.8±1.98 d | 15.1±0.230 e | 4.56±0.13 b |
| + OS + 1.116 mg Cd/L | T2 | 7.23±0.22 d | 17.17±0.33 c | 28.7±1.21 c | 18.7±0.454 d | 5.13±0.16 ab |
| + OS + 2.232 mg Cd/L | T3 | 8.50±0.15 c | 17.67±0.12 bc | 32.2±1.92 b | 21.4±0.225 c | 5.47±0.21 a |
| - OS – Cd | T4 | 6.20±0.12 e | 16.23±0.42 d | 17.4±1.22 d | 15.3±0.676 e | 3.31±0.13 d |
| - OS + 1.116 mg Cd/L | T5 | 9.43±0.29 b | 18.13±0.13 b | 32.4±2.02 b | 28.7±0.243 b | 4.12±0.18 c |
| - OS + 2.232 mg Cd/L | T6 | 14.27±0.23 a | 22.73±0.28 a | 39.7±2.08 a | 37.6±0.276 a | 4.73±0.21 b |

Means having the same letter in the same column are not significantly different at $P < 0.05$.

All fish body constituents except moisture content were significantly affected by OS supplementation, Cd level, and their interaction ($P < 0.05$; Table 2). Crude protein and total lipids decreased significantly with the increase of Cd concentration (T2 vs T5 and T3 vs T6). Ash content and Cd residues in T1 - T3 were significantly ($P < 0.05$) lower than those of T4 - T6, whereas Se residue in fish body was significantly higher in T1 – T3 those of T4 - T6 ($P < 0.05$). No significant changes in Se residue were observed following Cd exposure ($P > 0.05$).

All biochemical parameters were significantly affected by OS supplementation, Cd level, and their interaction ($P < 0.05$; Tables 3 and 4). There was a significant ($P < 0.05$) increase in glucose levels in T5 vs T2 and T6 vs T3, whereas total protein, albumin, and globulin decreased significantly with the increase of Cd toxicity. Fish fed OS exhibited high protein, albumin, and globulin, compared to fish that were not fed OS ($P < 0.05$). On the other hand, serum lipids increased significantly with the increased Cd concentration ($P < 0.05$), and were significantly ($P < 0.05$) lower in fish fed OS.

Creatinine, uric acid, AST, and ALT were significantly affected by both OS supplementation and Cd concentration ($P < 0.05$; Table 4). While these parameters increased significantly with the increase of Cd concentration ($P < 0.05$), fish fed OS (T2 and T3) exhibited lower values of creatinine, uric acid, AST, and ALT compared to those without OS supplements (T5 and T6). The GPX activity was significantly affected by OS supplementation as well as Cd concentration ($P < 0.05$; Table 4). However, GPX activity increased significantly ($P < 0.05$) with the increase of Cd concentration. OS supplementation enhanced GPX activity T1 vs T4, T2 vs T5, and T3 vs T5.

## DISCUSSION

Se is an essential micronutrient for aquatic animals (Hilton *et al.*, 1980; Bell *et al.*, 1985). The inorganic form of Se as sodium selenite or sodium selenate is commonly used as a Se supplement in livestock diets. The organic form of Se as selenoyeast or selenomethonine may have benefits that are superior to those provided by the inorganic forms (Wang and Lovell, 1997; Mahan, 1999). The present study clearly indicates that the supplementation of 0.5 g OS/kg diet (5.54 mg Se/kg diet) enhanced tilapia growth and feed efficiency (T1 vs T4). The Se level herein (5.54 mg Se/kg) is comparable to that reported for Nile tilapia (4.6 mg Se/kg; Ahmad *et al.*, 2006) and African catfish (3.67 mg Se/kg; Abdel-Tawwab *et al.*, 2007a), and higher than that reported for rainbow trout (0.38 mg Se/kg, Hilton *et al.*, 1980), channel catfish (0.25 mg Se/kg, Gatlin and Wilson, 1984), and grouper ( 0.77 mg Se/kg; Lin

and Shiau, 2005). The differences among the above studies may be related to the difference in Se sources and Se concentration in the rearing water.

The fish growth was improved in T2 vs T5 and T3 vs T6 suggesting that dietary Se could play a role in reducing Cd toxicity. Se is known to act against metal toxicity by forming Se–metal protein and selenide–metal complexes (Levander, 1986; Rana and Boora, 1992; Rana and Verma, 1997). Previously researchers have suggested that an insoluble Cd–Se compound could be formed in the liver after absorption for subsequent excretion through the bile which in turn reduces the bioavailability of dietary Se (Lorentzen *et al.*, 1998). Lin and Shiau (2007) investigated the effects of diets supplemented with selenium at rate of 0, 0.8, or 1.6 mg Se/kg on the oxidative stress of grouper, *Epinephelus malabaricus* fed 20 mg Cu/kg for 8 weeks. They found that the supplementation of 1.6 mg Se/kg reduced the Cu stress and improved the immune response of the fish. Also, Abdel-Tawwab *et al.* (2007a) reported that African catfish, *Clarias gariepinus* fed 0.3 g OS/kg diet for 12 weeks could resist water-born Cu toxicity.

In the present study, OS supplementation played a role in enhancing feed intake with a subsequent enhancement of the fish body composition. The low feed utilization in Cd-challenged groups (T5 and T6) may have been due to the fact that the Cd levels may led to reduction in fish appetite or complete fish fasting resulting in reduced growth. Abdel-Tawwab *et al.* (2007b) reported that Cu toxicity lead to a reduced fish growth and feed utilization. An alternative hypothesis is that due to the reduced intake, the energy requirements were met via the decomposition of the storage-deposited nutrients (Abdel-Tawwab *et al.*, 2006), which is supported in the current study by the significant decrease in the body contents of crude protein and total lipids.

Performing blood chemistry analyses often provide vital information aiding the diagnosis for health assessment and management of cultured fish (Pincus, 1996; Cnaani *et al.*, 2004; Řehulka *et al.*, 2004; Abdel-Tawwab *et al.*, 2007a, b). In the present study, glucose and serum lipids were significantly increased during Cd exposure in the absence of supplemental OS. These results are in agreement with those of Saeed (1989), Arias (1990), and Diab *et al.* (1996) who reported that the intensity of hyperlipemedia may reflect the degree of stress imposed on the fish under the influence of toxic agents and environmental pollutants.

Measurement of albumin, globulin, and total protein in serum or plasma is of considerable diagnostic value in fish, as it relates to general nutritional status (Schaperclaus *et al.*, 1992). Serum protein, albumin, and globulin were significantly

lower in T5 vs T2 and T6 vs T3. These results may be due to the disturbances in the liver protein metabolism due to Cd toxicity, as was found to be the case with other contaminants (Dange and Masurekar 1984; Abdel-Tawwab *et al.* 2007a, b). On the other hand, Nguyen (1999) reported that a low albumin may result from impaired synthesis, loss through urine or feces, or increased catabolism.

Creatinine and uric acid levels are indicators of kidney function. In the present study, creatinine and uric acid showed a significant increase in fish exposed to Cd. These results may be due to the action of heavy metal on glomeruli filtration rate (El-Bagori, 2001; Abbass *et al.,* 2002) and/or Cd may cause pathological changes to the kidney resulting in dysfunction. AST and ALT were significantly high in T5 vs T2 and T6 vs T2 suggesting that such liver damage might have occurred in T5 and T6 and hence leading to the leakage of these enzymes into the blood (Casillas *et al.*, 1983; Chen *et al.,* 2004; Abdel-Tawwab *et al.*, 2007b).

GPX activity increased with the increase of Cd level. This result may be due to the increase in defense mechanism against Cd toxicity. In this regard, Shaik *et al.* (1999) and Siraj Basha and Usha Rani (2003) reported that the chronic Cd administration in *Oreochromis mossambicus* resulted in a gradual rise in hepatic antioxidant defense. They also reported that the increase in hepatic GPX shows a possible shift toward a detoxification mechanism under long-term exposure to Cd. On the other hand, GPX activities in OS-fed groups were higher in T1 vs T4, T2 vs T5, and T3 vs T6 because Se is incorporated in the structure of GPX enzyme as selenocysteine (Rotruck *et al.,* 1973).

It could be concluded that OS supplementation played a role in reducing the harmful effect of water-born Cd on fish, which in turn improved the growth, survival, and feed utilization. Also, OS supplementation decreased Cd residue found in fish body, thereby mitigating potential hazards to human health.

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