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The selenium accumulation and its effect on growth, and haematological parameters in red sea bream, *Pagrus major*, exposed to waterborne selenium

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ABSTRACT

Juvenile *Pagrus major* (mean length 15.8 ± 1.6 cm, and mean weight 90.4 ± 4.7 g) were exposed for 4 weeks with waterborne selenium concentration (0, 50, 100, 200, and 400 μg/L). The profile of Se accumulation among tissue in *P. major* is dependent on the exposure periods and Se concentration. After 4 weeks, the highest accumulation of Se was observed in the kidney, and the order of Se accumulation in tissues was kidney ≈ liver > spleen > intestine > gill > muscle. Se decreased the growth rate, and there was an inverse proportion between growth and Se concentration. The major hematological findings were significant decrease in the RBC count, Ht value, and Hb concentration exposed to ≥ 100 μg/L Se concentrations. Se exposure (≥ 100 μg/L) led to significant increase in the glucose, GOT, and GPT levels, whereas the levels of calcium, magnesium, cholesterol, and total protein did not increase. The results suggest that waterborne Se exposure can induce significant Se accumulation in tissues, inhibition of growth, and hematological alterations.

1. Introduction

Selenium is an essential micronutrient in animals for normal growth and development, such as the elements zinc or copper. The metabolic functions of the selenoenzyme are vital for cells, because it is a part of the mechanism responsible for the metabolism and detoxification of oxygen (Nève, 1991). Selenium deficiency is related with elevated lipid peroxidation which alters the integrity of cell membranes, and influences cell functions (Stadtman, 1990). However, the excessive amounts of selenium can be very toxic to organisms like fish and wildlife (Lemly, 2002a), because it has a narrow range between baseline nutritional requirements and toxic dietary levels, which is distinguished from other toxic elements (Kobayashi, et al., 2002).

Selenium is a naturally occurring trace element that can be concentrated and released in the waste materials from certain mining, agricultural, petrochemical, and industrial manufacturing operations. Lemly (1997a) reported elevated selenium residues in the Belews Lake and associated biological impacts on fish were still present a decade later, whereas the power plant stopped disposing of selenium-laden water into the lake.

In the perspective of biochemical basis of selenium, the selenium toxicity has the major symptoms that lead to important deficiency in the process of protein synthesis. Surfer is a basic component of proteins, and it is essential to protein molecules. Cells do not differentiate between surfer and selenium, because the basic chemical and physical features are similar. Therefore, the high concentration of selenium erroneously replaces the position of surfer with selenium such as the formation of triselenium linkage (Se–Se–Se) and selenotrisulfide linkage (S–Se–S), and it prevents the formation of the essential disulfide bonds (S–S) (Reddy and Massaro, 1983; Sunde, 1984).

Bioaccumulation is affected by the interaction between environmental factors like hardness, temperature, salinity, and pH, as well as species-specific factors such as feeding, metabolism, growth dilution, digestion, and excretion efficiency, in addition to the bioavailability of the chemical in the water (McCarty and Mackay, 1993). Excess Se exposure in fish can cause selenium accumulation which has adverse effects on the reproduction such as teratogenic deformities and reproductive disorders (Kennedy et al., 2000; Lemly, 1997b; Lemly, 2002a), as well as the inhibition of growth, tissue damage, and mortality (Lemly, 1993a; May et al., 2008). It is also known that large amounts of toxicants from their living environment can be accumulated in fish tissues (Suhaimi et al., 2005). The toxicants uptake is positively linked to the metabolic rate in marine fish, and it can be supposed that toxicants accumulation would be highest in young fish,

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which is attributed to the higher metabolic activity in young individuals (Canli and Furness, 1993). In a low concentration of selenium in aquatic environments, aquatic consumers can be accumulated in their tissues at the high level of selenium by dietary intake (Hamilton, 2004; Stewart et al., 2004). Therefore, fish tissues can be valuable indicators for evaluating aquatic pollution.

Fish blood parameters are also considered possible indicators of physiological and pathological alterations in toxicological and environmental research to assess the effects on toxicants (Adhikari et al., 2004). The hematological values such as ion concentrations, hemoglobin, and hematocrit can be an indicator to demonstrate a physiological response to a contaminated environment (Dethloff et al., 2001). The elevated selenium exposure to fish caused hematological changes and gill damage that reduced respiratory capacity, on the contrary to increasing respiratory demand and oxygen consumption (Lemly, 1993b).

Pagrus major, the red sea bream, is an economically important fish that is commonly cultured in Korea in marine net cages, but little information is available on the effect of Se. Therefore, through this present study for assessing the accumulation of selenium (Se) in red sea bream and its toxic effects on growth and hematological parameters, we can suggest an indicator for selenium toxicity and effects on the fish.

2. Materials and methods

2.1. Experimental fish and conditions

Red sea breams were obtained from a local fish farm in Tongyeong, Korea. The fish were acclimatized for 2 weeks under laboratory conditions was evaluated prior to selenium exposure. During the acclimation period, the fish were fed a commercial diet twice daily (Woosungfeed, Daejeon City, Korea) and maintained on a 12-h:12-h light/dark cycle and constant condition at all times (Table 1). After acclimatization, 120 fishes (body length, 15.8 ± 1.6 cm; body weight, 90.4 ± 4.7 g) were selected for the study. Selenium exposure took place in 20 L glass tanks containing 6 fish per treatment group in triplicates. Sodium selenite (Sigma, St. Louis, MO, USA) solution was dissolved in the respective glass tanks. The selenium concentrations in the glass tanks were 0, 50, 100, 200, and 400 μg/L. An extremely high dose of 400 μg/L is a highly improbable occurrence in a real environment, but it provided an opportunity to evaluate selenium toxicity in the experimental fish. The glass tank water was thoroughly exchanged once per two days, and made the same concentration in the respective glass tank. At the end of each period (at 2 and 4 weeks), fish were anesthetized in buffered 3-aminobenzoic acid ethyl ester methanesulfonate (Sigma Chemical, St. Louis, MO), and length and weight were measured.

2.2. Selenium analysis

The tissue samples of liver, kidney, spleen, intestine, gill, and muscle were performed with freeze-dried to measure dry weight of the samples. The freeze-drying samples were digested by wet digestion method. The dried samples were digested in 65 percent (v/v) HNO3, and re-dried at 120 °C. The procedure was repeated until total digestion. The entirely digested samples were diluted in 2 percent (v/v) HNO3. The samples were filtered through a 0.2 μm membrane filter (Advantec mfs, Ins.) under pressure for analysis. For determination of total selenium concentrations, the digested and extracted solutions were analyzed by ICP-MS. The ICP-MS measurements were performed using an ELAN 6600DRC ICP-MS instrument with argon gas (Perkin-Elmer). Total selenium concentrations were determined by external calibration. ICP multi-element standard solution VI (Merck) was used for standard curve. The selenium bioaccumulation in tissue samples was expressed μg/g dry wt.

2.3. Growth

The weight and length of *P. major* was measured just before exposure, at 2 weeks, and at 4 weeks. Daily length gain, daily weight gain, and condition factor were calculated by the following method.

Daily growth gain = $W_f - W_i$/day.
W_f = Final length or weight.
W_i = Initial length or weight.
Condition factor (percent) = $(W/L^3) \times 100$.
W = weight (g), L = length (cm).

Table 1
The chemical components of seawater and experimental condition used in the experiments.

Item	Value
Temperature (°C)	21.0 ± 1.0
pH	8.1 ± 0.5
Salinity (‰)	33.5 ± 0.6
Dissolved oxygen (mg/L)	7.1 ± 0.3
Chemical oxygen demand (mg/L)	1.13 ± 0.1
Ammonia (μg/L)	12.5 ± 0.7
Nitrite (μg/L)	1.3 ± 0.3
Nitrate (μg/L)	11.48 ± 1.0

2.4. Blood samples and hematological assay

Blood samples were collected within 35–40 s through the caudal vein of the fish in 1-mL disposable heparinized syringes. The blood samples were kept at 4 °C until the blood parameters were completely studied. The total red blood cell (RBC) count, hemoglobin (Hb), concentration, and hematocrit (Ht) value were determined immediately. Total RBC counts were counted using optical microscope with hemocytometer (Improved Neubauer, Germany) after diluted by Hendrick's diluting solution. The Hb concentration was determined using Cyan-methemoglobin technique (Asan Pharm. co., Ltd.). The Ht value was determined by the micro-hematocrit centrifugation technique. The blood samples were centrifuged to separate serum from blood samples at 3000 g for 5 min at 4 °C. The serum samples were analyzed for inorganic substances, organic substances, and enzyme activity using clinical kit (Asan Pharm. Co., Ltd.). In inorganic substances assay, calcium and magnesium were analyzed by the *o*-cresolphthalein-complexon technique and xylidyl blue technique. In organic substances assay, glucose and total protein were analyzed by GOD/POD technique and biuret technique. In enzyme activity assay, glutamic oxalate transaminase (GOT) and glutamic pyruvate transaminase (GPT) were analyzed by Kind-king technique.

2.5. Statistical analysis

The experiment was conducted in two exposure periods (2 weeks and 4 weeks) and performed triplicate. Statistical analyses were performed using the SPSS/PC+statistical package (SPSS Inc, Chicago, IL, USA). Significant differences between groups were identified using one-way ANOVA and Duncan's test for multiple comparisons or Student's *t*-test for two groups (Duncan, 1955). The significance level was set at $P < 0.05$.

3. Results

3.1. Selenium accumulation

Selenium accumulation in liver, kidney, spleen, intestine, gill, and muscle tissues of *P. major* is shown in Fig. 1. Se accumulation profile in liver depended upon the waterborne Se concentration. After 4 weeks of exposure, Se accumulation values were 18.86 ± 1.62 and 22.56 ± 2.12 μg/g for exposure to 200 and 400 μg/L, respectively. For kidney, Se accumulation increased considerably with time and concentration during the 4-week experiment. Se accumulation at 4 weeks increased sharply at 50, 100, 200, 400 μg/L exposures. Finally, after 4 weeks the highest Se accumulation value in the kidney was 23.81 ± 2.54 μg/g for exposure to 400 μg/L. Se accumulation in spleen increased significantly, but Se accumulation level was observed lower than the liver and kidney. Se accumulation in the spleen increased sharply, reaching a value 19.34 ± 1.32 μg/g at 400 μg/L dose. For intestine and gills, a similar increase trend with the above tissues was observed. The highest Se accumulation values were 13.93 ± 0.83 and 4.69 ± 0.26 μg/g for exposure to 400 μg/L, respectively. No notable Se accumulation was observed in the muscle, excluding a slight increase at 400 μg/L. After 4 weeks of exposure, the order of Se accumulation in tissues was kidney ≈ liver > spleen > intestine > gill > muscle.