Thesis for the Degree of Master of Science

Effects of Selenium on the Responses of Antioxidant Enzyme, Acetylcholinesterase Activity, Non-specific Immune, and Bioaccumulation in Red seabream, *Pagrus major*.

by
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셀레늄 노출에 따른 참돔 *Pagrus major*의 항산화효소, AChE, 비특이적 면역의 변화 및 생체축적

Advisor: Prof. Ju-Chan Kang

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CONTENTS

셀레늄 노출에 따른 참돔 Pagrus major의 항산화효소, AChE, 비특이적 면역의 변화 및 생체축적

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요 약

셀레늄은 동물의 성장과 발달에 필수적인 미량영양소이다. 하지만, 높은 수준의 셀레늄 노출은 어류와 야생동물에게 심각한 독성으로 작용할 수 있다. 셀레늄은 생체막에 쉽게 침투할 수 있으며, 생체축적 뿐만 아니라, 항산화·면역반응의 변화 및 아세틸콜린에스테라아제 활성의 억제를 초해할 수 있다. 이번 연구의 목적은 셀레늄 노출에 따른 참돔 *Pagrus major*의 생체축적, 생화학적 생물지표, 비특이적 면역의 반응을 평가하는데 있다. 이러한 요인들의 농도 별 노출 반응을 평가하기 위해, 실험에 사용된 참돔은 대조구와 함께, 4개의 농도 구간 (50, 100, 200, and 400㎍/L)에 4주간 노출되었다.

본 연구에서, 참돔의 간, 신장, 비장, 장, 그리고 아가미 조직에서 상당한 셀레늄의 생체축적이 200㎍/L과 400㎍/L의 셀레늄 노출농도에서 관찰된 반면, 뇌와 근육의 생체축적은 다른 장기에 비해 현저하지 않았다. 생물지표에서, 비만도 지수와 간 중량은 높은 농도에서 약간의 감소를 제외하고 유의적 변화를 나타내지 않은 반면, 일일전장성장량과 일일체중성장량은 셀레늄 노출에 따라 크게 감소하였다. 혈액분석에서 셀레늄 노출농도가 증가함에 따라 적혈구수, hematocrit 값, 헤모글로빈 수치에서 상당한 감소를 나타냈다. 혈청분석에서 글루코즈, GOT, GPT는 상당한 증가를 나타낸 반면, 알부민과 alkaline phosphatase는 큰 감소를 나타냈다. 하지만, 혈청 칼슘, 마그네슘, 콜레스테롤, 총인에서는 큰 유의적 변화는 없었다. 간과 아가미 조직에서의 항산화효소 활성에서 superoxide dismutase는 2주 4주 모두 400㎍/L 농도에서 상당히 높은 유의적 증가를 나타냈다. glutathione S-transferase 활성은 간조직에서 400㎍/L 셀레늄 노출농도에서 2주 4주 모두 높은 증가를 나타냈고, 아가미조직에서는 2주에서 유의적 변화는 없었지만, 4주의 100㎍/L 이상의 셀레늄 노출농도에서 유의적 증가를 나타내었다. 간과 아가미 조직에서의 glutathione 함량은 2·4주 모두 400㎍/L의 농도에서 유의적 증가를 나타냈다. 아세틸콜린

에스테라아제 활성은 뇌조직의 200㎍/L 이상의 농도에서 2·4주 모두 상당한 억제와 근육에서 400㎍/L 농도에서 2·4주 모두 상당한 억제와 간에서는 4주 후의 400㎍/L 농도에서 상당한 감소를 나타내었다. 하지만, 장에서는 유의적 변화는 나타나지 않았다. 혈청과 신장에서 라이소자임 활성은 50㎍/L 이상의 농도에서 상당히 증가했지만, 혈청 peroxidase 활성과 anti-protease 활성은 높은 농도에서 낮은 유의적 감소를 나타냈다.

Ⅰ. Introduction

The number of synthesized chemicals has been sharply increased and exposed to the environment. The number of chemical that people use is over a hundred thousand, and new chemicals have been continuously produced over a thousand a year. Although these potential hazards of synthetic and natural chemicals in the environment have been constantly raised, the variety of study about these contaminations has been insufficient.

Among chemical substances, selenium is a chemical nonmetal element discovered in 1817 by Jöns Jakob Berzelius with symbol Se and atomic number 34. It 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. Selenium is widely used today in photo cells, glassmaking, pigments, and electronics. Selenium is an essential micronutrient in animals for normal growth and development such as the elements of zinc or copper. Dietary selenium requirements to fish for growth and development were reported in many articles. Furthermore, many researchers also observed that selenium influences both the innate "nonadaptive and the acquired, "adaptive" immune systems. These immune systems can be improved by selenium supplementation (Arthur, 2003), and WHO also recommended intake of selenium to 6-21 ㎍/day for infants and children, 26-30 ㎍/day for adolescents, and 26-35 ㎍/day for adults.

The selenium deficiency to fish cause increased pyruvate kinase which may be due to damaged cells (Bell, 1986). However, selenium can be highly toxic to fish and wildlife at the higher concentration than a permissible amount, because it can rapidly bioaccumulate and reach toxic

level in the aquatic environment (Lemly, 2002). For those reasons, South Korea has also controled selenium as one of toxic agents and strictly regulated selenium level below the concentration of 10 ㎍/L with the same as WHO regulation standard for drinking water (Korea ministry of environment, 2002).

It is one of selenium toxicity mechanisms that forming CH_3Se^- from selenium enters a redox cycle, and generates superoxide and oxidative stress (Spallholz J.E., Hoffman D.J., 2002), though moderate selenium concentrations for organisms help improve the immune system with the action to protect neutrophils from oxygen-derived radicals (Arthur J.R., McKenzie R.C., Beckett G.J., 2003).

The accumulation of selenium in fish may mostly occur through their gill, and be eliminate by gills and bile (Kleinow, 1986). The major influence of selenium on fish is teratogenesis of developing embryos. It was also observed that elevated concentrations of selenium caused deformity to larva and fry of fish in both the laboratory and field through various studies. Meanwhile, 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, A.D., 1993). In addition, toxic symptoms such as swollen gill lamellae, increased lymphocytes, decrease of hematocrit and hemoglobin (anemia), corneal cataracts, protruding eyeballs, pathological alterations in the tissues of liver, kidney, heart, and ovary, reproductive failure, and teratogenic deformities of the spine, head, mouth, and fins were observed in the studies over two decades with the resident fish community (20 species) at Belews Lake, North Carolina which was contaminated by selenium in wastewater from a coal-fired power plant during the mid-1970s (Lemly, A.D., 2001).

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) (Ganther, 1974; Stadtman, 1974; Reddy and Massaro, 1983; Sunde, 1984).

Aerobic organisms require mechanisms that prevent or limit cellular damage which is caused by reactive oxygen species (ROS), and cells have evolved an interdependent antioxidant defence system (Cavaletto, 2002). Oxidative stress is highly associated with toxic substances, and antioxidant activities occur against oxidative stress within intracellular space. Selenium species generate reactive oxygen species (ROS) reacting with GSH, and it can cause oxidative damage when not eliminated by antioxidants (Miller, 2006). Superoxide dismutase (SOD) decomposes superoxide anion to hydrogen peroxide (H_2O_2), and catalase (CAT) decomposes H_2O_2 to molecular oxygen and water, and glutathione peroxidases (GPx) reduce both H_2O_2 and lipid hydroperoxide, as three of the principal antioxidant enzymes (Almeida, 2007). Glutathione (GSH) is the most plentiful intracellular thiol-based antioxidant, and function as a sulfhydryl buffer. It also has the function of detoxifying compounds via conjugation reactions catalyzed by glutathione S-transferases (Nordberg, 2001). Glutathione S-transferase (GST) functions the detoxification enzyme existing all aerobic organisms, and catalyze the nucleophilic attack of the sulfur atom of the tripeptide glutathione. Through this process, the substrate is conjugated to reduced glutathione as well as reduced and isomerized with the concomitant production of oxidized glutathione (Kim, 2001).

Acetylcholine play a major role in both central and peripheral nervous system as one of the most important neurotransmitter. Acetylcholine functions as activator of muscles in the peripheral nervous system and

has a major role in the enhancement of sensory perceptions in the central nervous system (Wikipedia). Acetylcholinesterase (AChE) is a principal component of cholinergic system in fish and control the nervous impulse transmission in cholinergic synapses. Considerably high enzyme activity of AChE occur in central nervous system of fish especially in brain (Chuiko, 1997). A significant reduction in acetylcholinesterase activity is commonly observed in fish exposed to various toxic substances such as organophosphorous compounds, heavy metals, and chemicals (Modesto, 2010). Therefore, the inhibition of acetylcholinesterase can be a biomarker for the neurotoxicity (Manzo, 1995).

The fish immune parameters can be affected by aquatic pollutants such as pesticides, hydrocarbons, metals, and other chemicals. Many proofs of disrupted immune function in fish exposed to contaminated coastal waters have been reported by several field studies (Arkoosh, 1991; Hutchinson, 2003; Pulsford, 1995). Lysozyme is one of the principal element in non-specific humoral defenses in fish. Lysozyme also has functions of a direct antibacterial effect by splitting peptidoglycan layers in the cell wall and a promotor of phagocytosis by other immune cells as an opsonin (Yano, 1996). Lysozyme have been positively correlated with disease resistance in different fish species and in some cases. The peroxidase activity can be considered an immunological parameter as an indicator of leucocyte activation (Tapia-Paniagua, 2011). Anti-proteases are one of the elements of non-specific immunity of the vertebrates that may play a role in restricting the ability of bacteria to invade and grow in vivo (Ellis, 2001).

To infer ecologically useful and desirable results from aquatic toxicity experiment, appropriate organisms should be used as well as proper experiments. If possible, it is desirable to use endemic organisms which be studied well, as a represented organism (Rand et al., 1995). Red seabream is widely distributed over subtropics sea area such as Southeast Asia, Taiwan, the South China Sea, and Japan including littoral sea of

South Korea. Particularly in South Korea, red seabream comes into the spotlight as one of the most highest price raw material of sliced raw fish and Sushi, as well as it is currently third largest farming fishing species followed by flatfish and rockfish.

There has been little research about red seabream exposed to selenium toxicity. In consideration of the harmful toxic effects exposed to selenium and the commercial value of red seabream, much more various studies of red seabream exposed to selenium should be conducted. The aim of this study is to assess the effects of the selenium toxicity on red seabream, *Pagrus major*, with the selenium concentrations at 0, 50, 100, 200, and 400 ㎍/L.

II. Materials and Methods

1. Experimental fish and conditions

Red seabreams, *Pagrus major*, were obtained from a local fish farm in Tongyeong, Korea. The fish were acclimatized for 2 weeks under laboratory conditions and their health status was evaluated prior to status was evaluated prior to selenium exposure (Table 1). During the acclimation period, the fish were fed a commercial diet twice daily and maintained on a 12-h:12-h light/dark cycle at all times. After acclimatization, several fish (body length, 15.8±1.6cm; body weight, 90.4±4.7g) were selected for further study.

Selenium exposure took place in 20L glass tanks containing 6 fish per treatment group. 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 ㎍/L. Blood and tissue samples were taken to examine several hematological assay, bioaccumulation, acetylcholinesterase, and non specific immune response at 2 and 4 weeks after exposure.

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

Item	Value
Temperature (℃)	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 (㎍/L)	1.13±0.1
Ammonia (mg/L)	12.5±0.7
Nitrite (mg/L)	1.3±0.3
Nitrate (mg/L)	11.48±1.0

2. bioaccumulation

The tissue samples were performed with freeze-drying to measure dry weight of the samples. The freeze-drying samples were digested by wet digestion method. The dried samples were digested in 65%(*v*/*v*) HNO_3, and redried at 120℃. The procedure was repeated until entire digestion. The entirely digested samples were diluted in 2%(*v*/*v*) HNO_3. The samples were filtered through a 0.2㎛ 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 (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 ㎍/g dry wt.

3. Bioindicator

The weight and length of red seabream was measured just before exposure, 2weeks, and 4weeks, and the liver wet weight of red seabream was measured immediately after dissection at 2 weeks and 4 weeks. Daily length gain, daily weight gain, condition factor, and hepatosomatic index were calculated by the followed method.

Daily growth gain = $W_f - W_i$/day
W_f = Final length or weight
W_i = Initial length or weight

Condition factor (%) = $(W/L^3) \times 100$
W = weight (g), L = length (cm)

HSI (%) = liver wet weight / weight *100

4. Haematological and plasma 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℃ 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 hemo-cytometer (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 microhematocrit centrifugation technique.

The blood sample were centrifuged to separate serum from blood samples at 3000 g for 5 min at 4℃. 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 o-cresolphthalein-complexon technique and xylidyl blue technique.

In organic substances assay, albumin, glucose, and total protein were analyzed by bromcresol green (BCG) technique, GOD/POD technique, and biuret technique.

In enzyme activity assay, glutamic oxalate transminase (GOT), glutamic pyruvate transminase (GPT), and alkaline phosphatase were analyzed by Reitman-Frankel technique and Kind-king technique.

5. Antioxidant enzyme analysis

Liver and gill tissues were excised and homogenized with 10 volumes of ice-cold homogenization buffer using teflon-glass homogenizer (099CK4424, Glass-Col, Germany). The homogenate was centrifuged at 10,000 g for 30 min under refrigeration and the obtained supernatants were stored at -80℃ for analysis.

5-1. Superoxide dismutase (SOD)

Superoxide dismutase (SOD) activity was measured with 50% inhibitor rate about the reduction reaction of WST-1 using SOD Assay kit (Dojindo Molecular Technologies, Inc.). One unit of SOD is defined as the amount of the enzyme in 20 ㎕ of sample solution that inhibits the reduction reaction of WST-1 with superoxide anion by 50%. SOD activity was expressed as unit mg protein^{-1}.

* WST-1 = 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)- 2*H*-tetrazolium, monosodium salt

5-2. Glutathione s-transferase (GST)

Glutathione-S-transferase(GST) activity was measured according to the method of modified Habig (1974). The reaction mixture consisted of 0.2 M phosphate buffer (pH 6.5), 10 mM GSH (Sigma) and 10 mM 1-chloro-2,-dinitrobenzene, CDNB (Sigma). The change in absorbance at 25 ℃ was recorded at 340 nm and the enzyme activity was calculated as 340 nm and the enzyme activity was calculated as nmol min^{-1} mg protein^{-1}.

5-3. Glutathione (GSH)

Reduced glutathione was measured following the method of Beutler et al.(1963). Briefly, 0.2ml fresh supernatant was added to 1.8 ml distilled water. Three ml of the precipitating solution (1.67 g metaphosphoric acid, 0.2 g EDTA and 30 g NaCl in 100 ml distilled water) was mixed with supernatants. The mixture was centrifuged at 4500 g for 10 min. 1.0mL of supernatant was added to 4.0ml of 0.3M $NaHPO_4$ solution and 0.5mL DTNB (5,5`-dithiobis-2-nitrobenzoic acid) was then added to this solution. Reduced glutathione was measured as the difference in the absorbance values of samples in the presence and the absence of DTNB at 412 nm. GSH value was calculated as μmol mg protein^{-1} in the tissues.

6. Acetylcholinesterase activity

Acetylcholinesterase activity was determined muscle(1:10), brain(1:25), liver(1:10), and intestine(1:10) homogenate in 0.1M phosphate buffer, pH 8.0. The homogenate were centrifuged 10,000 g for 20 min at 4℃. The supernatant was removed and used to test acetylcholinesterase activity. Acetylcholinesterase activity was normalized to protein content and expressed as nmol min^{-1} mg $protein^{-1}$. Protein concentration was determined using Bradford's method (1976), with a bovine serum albumin (Sigma, USA) as standard.

7. Non-specific immune responses

The serum for analysis were separated from the blood sample. Kidney tissues were excised and homogenized with 10 volumes of ice-cold homogenization buffer (0.004M phosphate buffer, pH6.6) using teflon-glass homogenizer (099CK4424, Glass-Col, Germany). The homogenate was centrifuged at 10,000 g for 10 min under refrigeration and the obtained supernatant was stored at -70℃ (MDF-U53V, SANYO Electric Co. Ltd., Japan) for analysis.

Protein content was determined by the Bio-Rad Protein Assay Kit (Bio-Rad Laboratories GmbH, Munich, Germany) based on the Bradford dye-binding procedure, using bovine serum albumin as standard.

7-1. Lysozyme activity

Lysozyme concentration was calculated through the measure of its enzyme activity. Lysozyme activity was determined by a turbidimetric method (Ellis, 1990) using Micrococcus lysodeikticus (Sigma) as substrate (0.2 mg/ml 0.05M phosphate buffer, pH 6.6 for kidney sample and pH 7.4 for plasma). A standard curve was made with lyophilized hen egg white lysozyme (sigma) and the rate of change in turbidity was measured at 0.5-min and 4.5-min intervals at 530 nm. The results were expressed as ㎍/ml and ㎍/g equivalent of hen egg white lysozyme activity.

7-2. Peroxidase activity

The peroxidase activity was measured as an indicator of leukocyte activation by a colorimetric method (Quade MJ, Roth JA, 1997). Briefly, 5 μl of serum were diluted with 50 μl of HBSS in flat-bottomed 96-well plates. Serum samples were mixed with the peroxidase substrate (80 μM

3,3',5,5'-tetramethylbenzidine hydrochloride (TMB; Sigma) and 2.5mM H_2O_2). The colour-change reaction was stopped after 2 min by adding 50 µl of 2M sulphuric acid and the optical density was read at 450 nm in a plate reader (BMG, Fluoro Star Galaxy). Standard sample without serum was used as blank, The peroxidase activity was determined defining as one unit the peroxidase that produces an absorbance change of 1 OD.

7-3. Anti-protease activity

A modification of the method described by Ellis (1990) was used (Magnadóttir *et al.*, 1999). Briefly, 20 µl of serum were incubated with the same volume of standard trypsin solution (Sigma T-7409, 1000-2000 BAEE) for 10 min at 22℃. To this, 200 µl of 0.1M phosphate buffer, pH7.0 and 250 µl 2% azocasein (Sigma A-2765) were added and incubated for 1h at 22℃. Then, 500 µl of 10% trichloro acetic acid (TCA) was added and incubated for 30 min at 22℃. The mixture was centrifuged at 6000g for 5 min. One hundred microlitres of the supernatant was transferred to a 96 well non-absorbent microtray (Nunc) containing 100 µl $well^{-1}$ of 1 N NaOH. The OD was lead at 430 nm. The blank was phosphate buffer in place of serum and trypsin and the reference sample was phosphate buffer in place of serum. The percentage inhibition of trypsin activity compared to the reference sample was then calculated for each serum sample. All the samples collected were analysed in duplicate.

8. Statistical analysis

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 $P < 0.05$.

Ⅲ. Results

1. Bioaccumulation

The bioaccumulation of brain, liver, kidney, spleen, intestine, gill, and muscle tissues exposed to selenium are shown at Fig. 1 - Fig. 7.

No significant differences in brain tissues were observed with the exception of slight increases at concentrations of 400 ㎍/L after 2 weeks and 4 weeks. In the tissues of liver, the considerable increases were presented at 400 ㎍/L after 2 weeks and at the higher concentrations of 200 ㎍/L after 4 weeks. In the tissues of kidney, the bioaccumulation is constantly increased with the higher exposure concentration levels. Among the increases, a marked increase was observed at 400 ㎍/L after 4 weeks. The tissues of spleen showed the similar increase trend with kidney tissues. In the tissues of intestine, the clear increases were observed at all concentrations at 2 weeks and 4 weeks. The substantial increases in the gill tissues were presented at the higher concentrations of 200 ㎍/L after 2 weeks and at 50 and 400 ㎍/L after 4 weeks. However, no significant bioaccumulation in muscle tissues were observed, despite of slight increases at 400 ㎍/L after 2 weeks and at the excess concentrations of 100 ㎍/L after 4 weeks.

Overall, the notable bioaccumulation was observed in the tissues of liver, kidney, spleen, intestine, and gill. However, no considerable increases were shown in brain and muscle tissues, compared to other tissues.

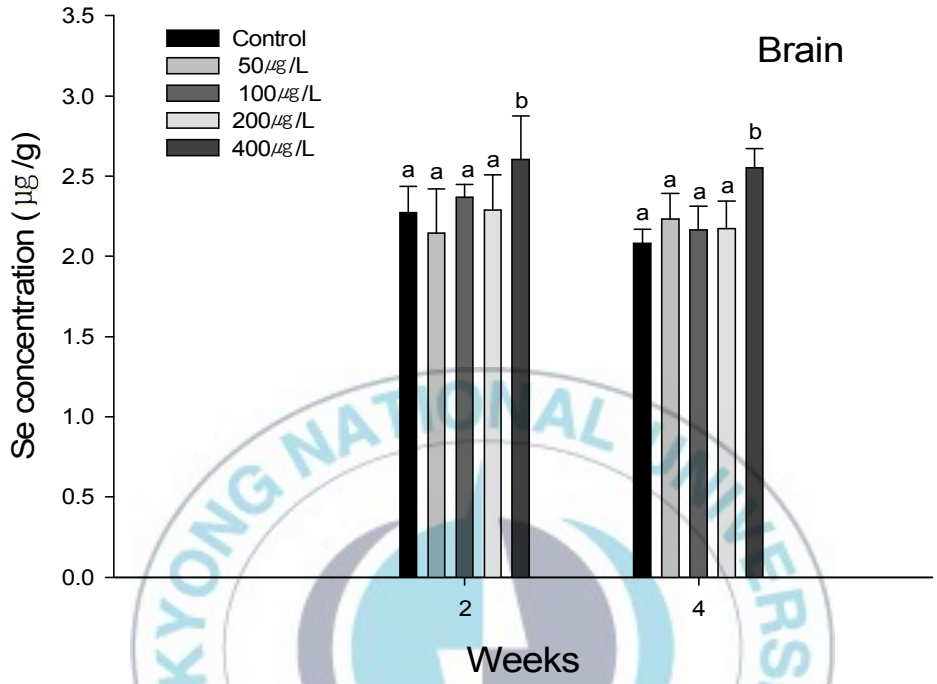

Figure. 1 Se concentration in brain of red seabream, *Pagrus major* exposed to the different concentration of selenium. Vertical bar denotes a standard error. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

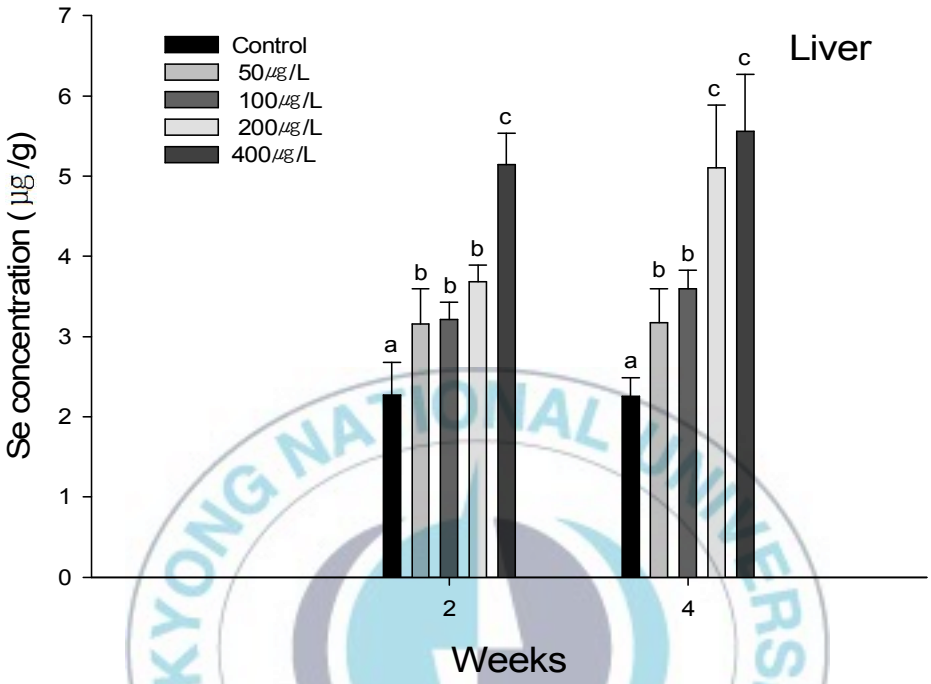

Figure. 2 Se concentration in liver of red seabream, *Pagrus major* exposed to the different concentration of selenium. Vertical bar denotes a standard error. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

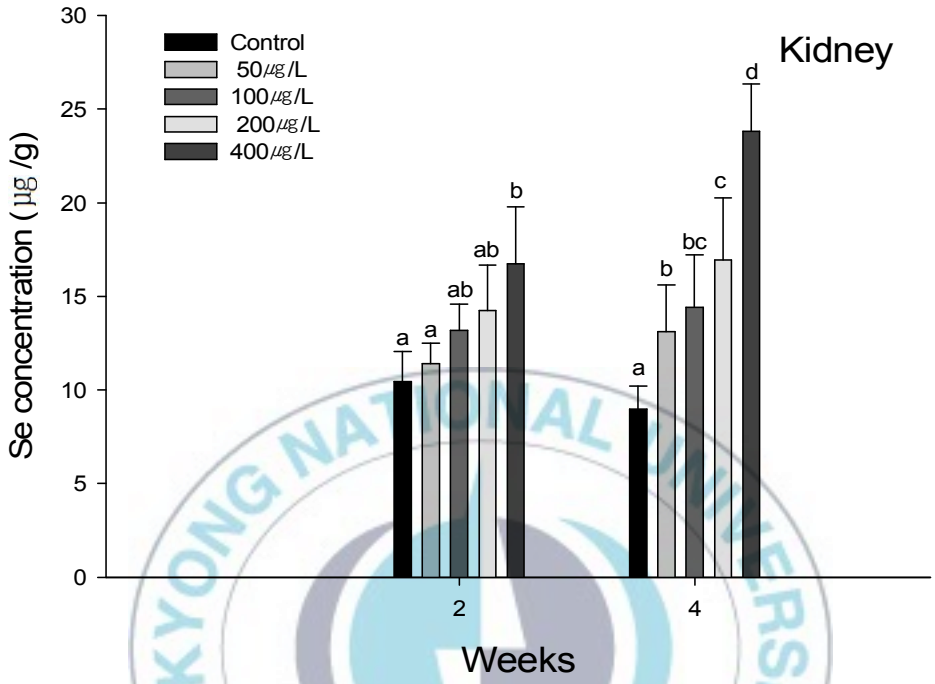

Figure. 3 Se concentration in kidney of red seabream, *Pagrus major* exposed to the different concentration of selenium. Vertical bar denotes a standard error. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

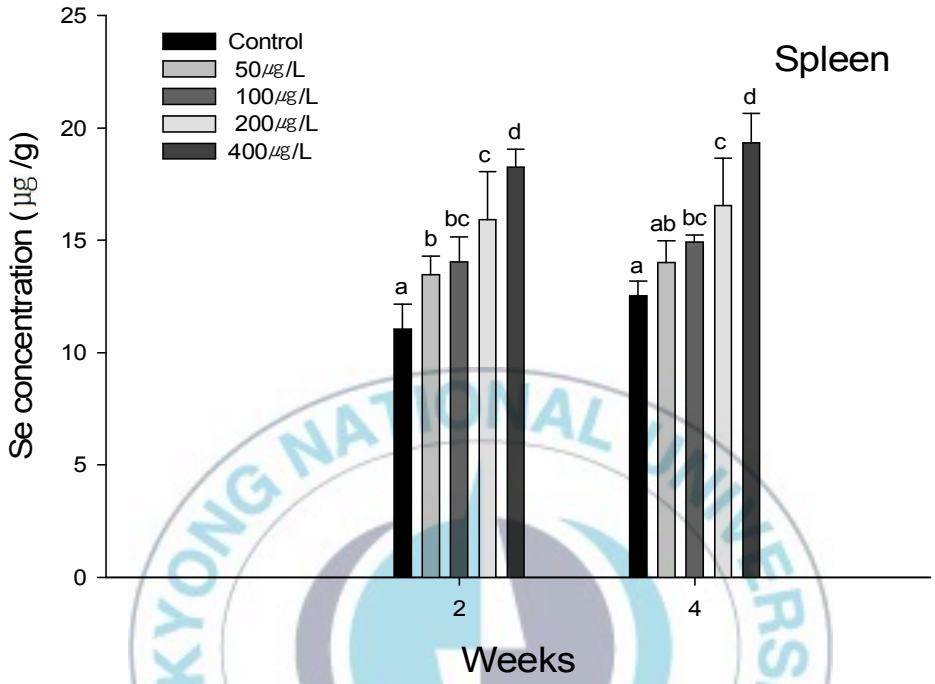

Figure. 4 Se concentration in spleen of red seabream, *Pagrus major* exposed to the different concentration of selenium. Vertical bar denotes a standard error. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

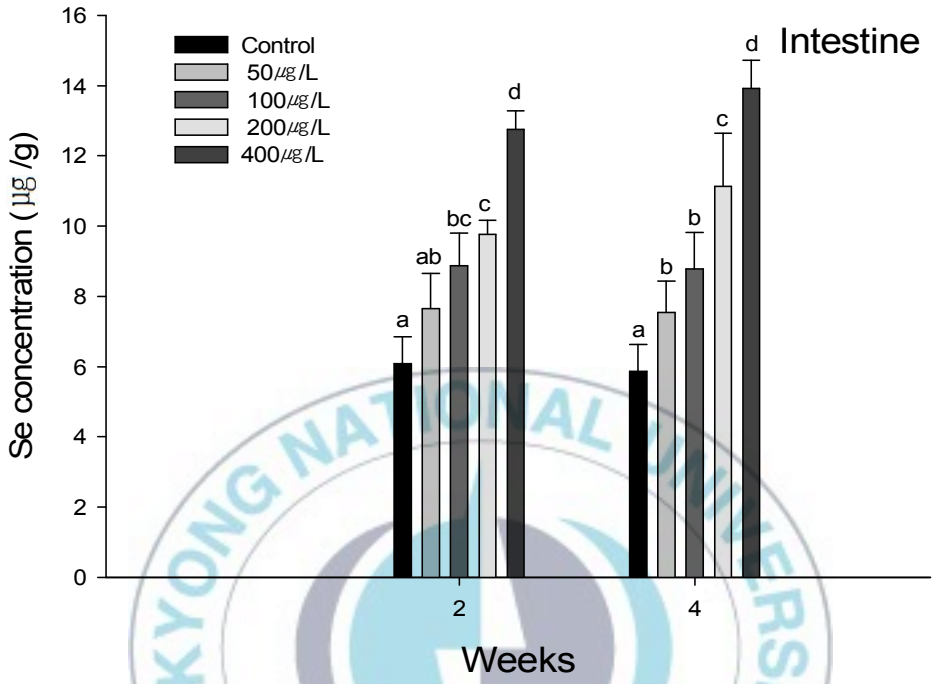

Figure. 5 Se concentration in intestine of red seabream, *Pagrus major* exposed to the different concentration of selenium. Vertical bar denotes a standard error. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

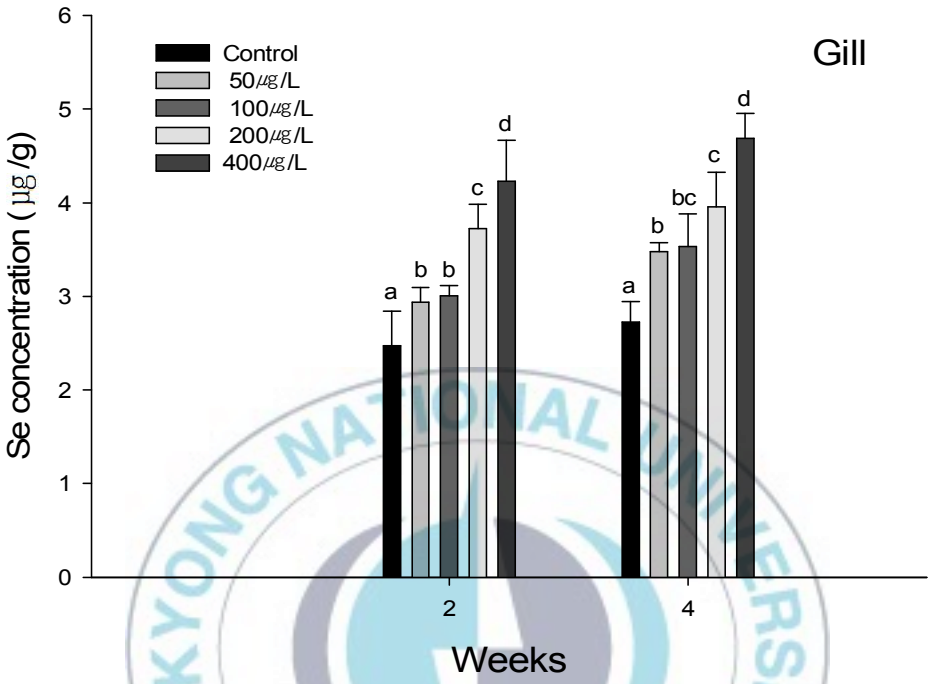

Figure. 6 Se concentration in gill of red seabream, *Pagrus major* exposed to the different concentration of selenium. Vertical bar denotes a standard error. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

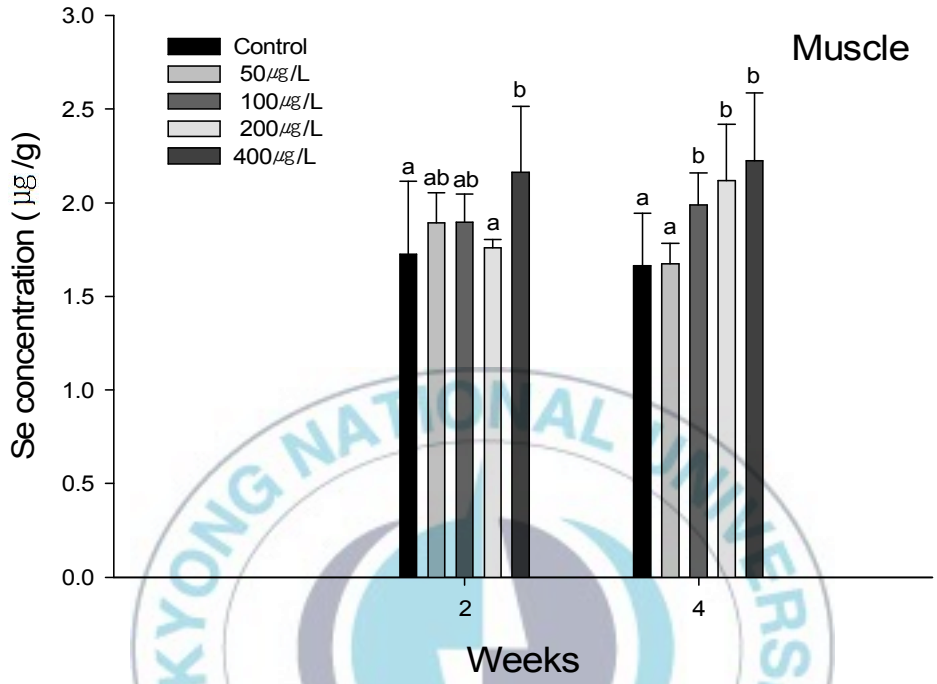

Figure. 7 Se concentration in muscle of red seabream, *Pagrus major* exposed to the different concentration of selenium. Vertical bar denotes a standard error. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

2. Bioindicator

Daily length gain and daily weight gain are presented in **Fig. 8-9**. In daily length gain, a clear decreasing trend noted in the concentration of 400 ㎍/L at 2 weeks, and same was observed higher than 50 ㎍/L of concentration after 4 weeks, compared to controls ($P < 0.05$). Daily weight gain in fish exposed to the concentrations higher than 200 ㎍/L at 2 weeks and 4 weeks had significantly decreased compared to the controls ($P < 0.05$).

Condition factor and hepatosomatic index are shown in Fig. 10-11. The significant changes in condition factor were not generally observed. However, slight decreases were observed at 200 ㎍/L and 400 ㎍/L after 4 weeks. Hepatosomatic index was considerably decreased at 400 ㎍/L after 2 weeks and 4 weeks.

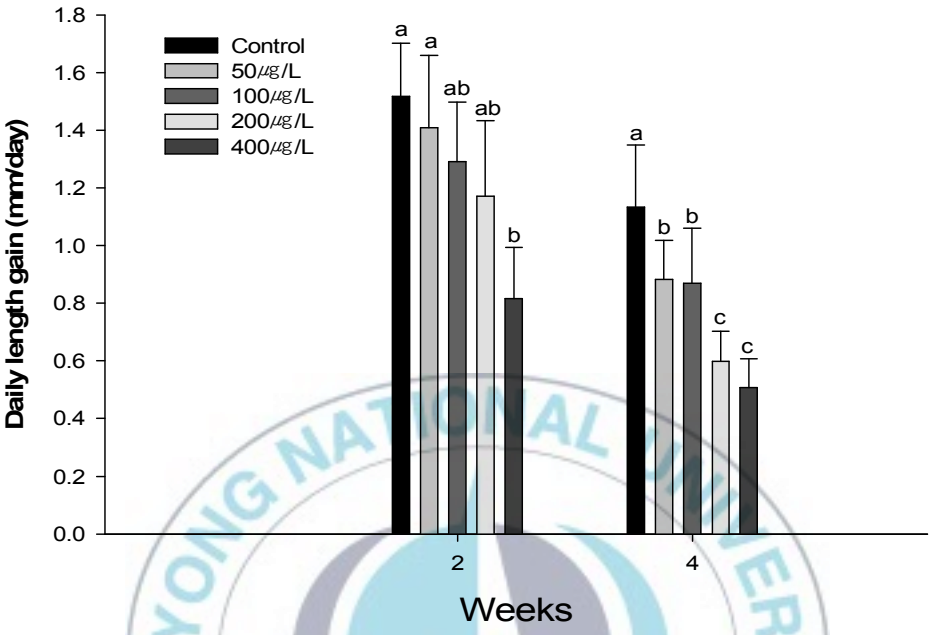

Figure. 8 Daily length gain of red seabream, *Pagrus major* exposed to the different concentration of selenium. Vertical bar denotes a standard error. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

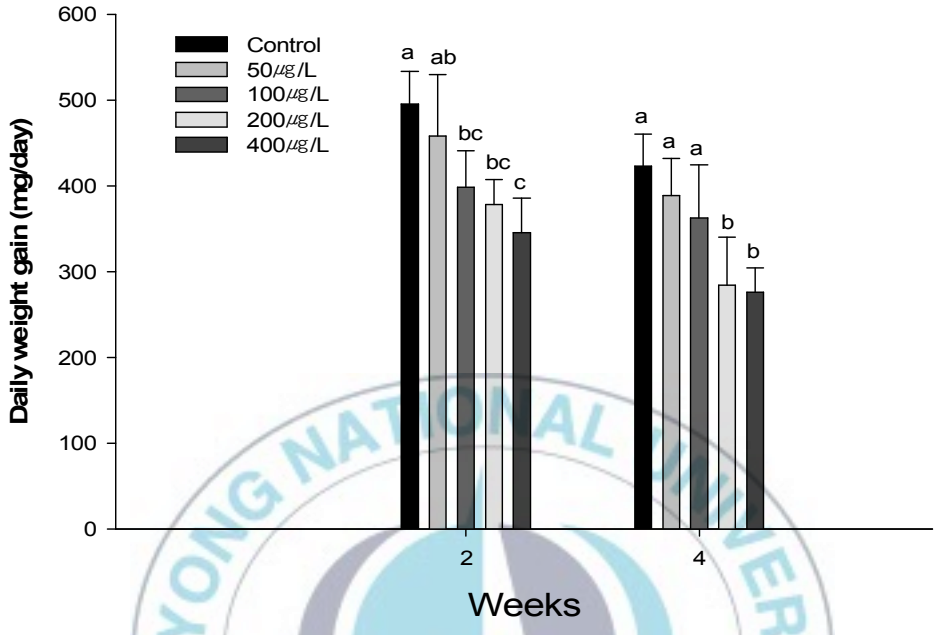

Figure. 9 Daily weight gain of red seabream, *Pagrus major* exposed to the different concentration of selenium. Vertical bar denotes a standard error. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

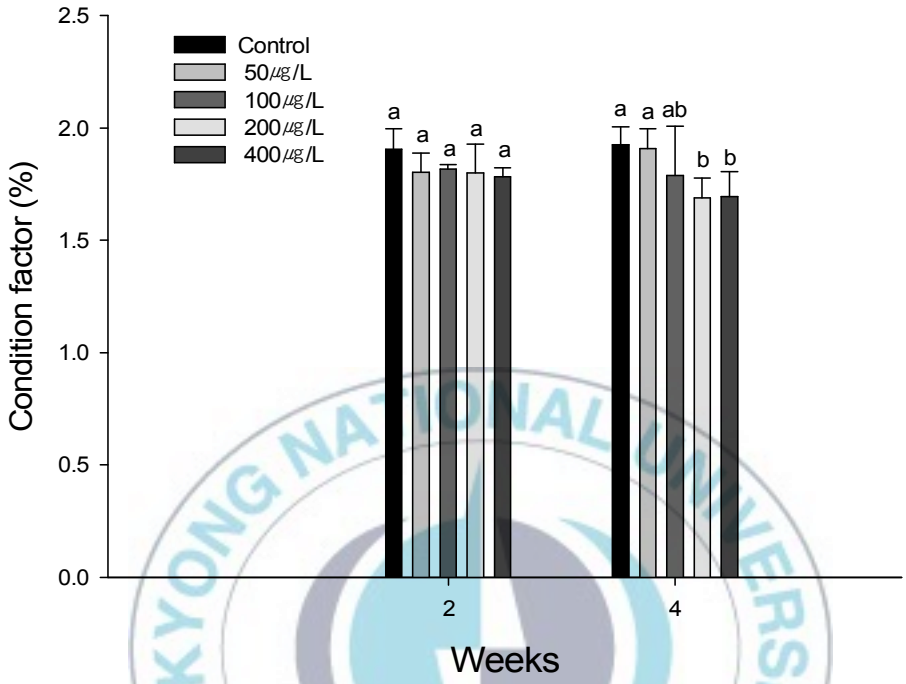

Figure. 10 Condition factor of red seabream, *Pagrus major* exposed to the different concentration of selenium. Vertical bar denotes a standard error. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

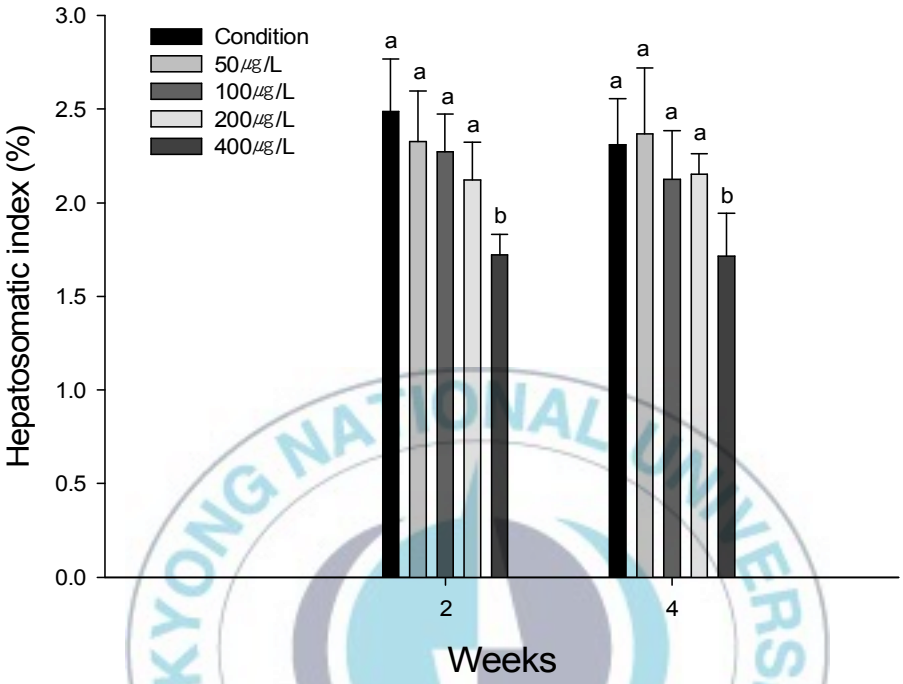

Figure. 11 Hepatosomatic index of red seabream, *Pagrus major* exposed to the different concentration of selenium. Vertical bar denotes a standard error. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

3. Hematological and plasma assay

The RBC count, Hb concentration, and Ht value of red seabream exposed to different levels of selenium are summarized in Table 2. The major hematological findings were significant decreases in RBC count and Ht value in red seabream exposed to 50 ㎍/L compared with the control group at 2 weeks and 4 weeks ($P < 0.05$). The Hb concentration following selenium exposure for 4 weeks was considerably decreased at 400 ㎍/L compared with the control group ($P < 0.05$).

The blood serum components of red seabream treated with selenium are shown in Table 3-5.

In inorganic components, it has no considerable change in calcium at 2 weeks and 4 weeks. Magnesium has no considerable change at 2 weeks, but slight decreases were observed at 200 ㎍/L and 400 ㎍/L after 4 weeks.

In organic components, serum glucose was significantly increased at 400 ㎍/L after 2 weeks of exposure to selenium and at the higher concentrations of 50 ㎍/L at 4 weeks, whereas a striking decrease in the excess of 50 ㎍/L in 2 weeks and 4 weeks was observed in albumin. It has no noticeable change in cholesterol and total protein.

In the change of enzyme components, GOT and GPT were gradually increased by growing the concentration of selenium. A striking increase at 2 weeks and 4 weeks was observed at 400 ㎍/L of GOT and at 50 ㎍/L of GPT. However, it has a constant decrease in alkaline phosphatase at all concentration section.

Table. 2 Changes of RBC count, Hematocrit and Hemoglobin in red seabream, *Pagrus major* exposed to selenium for 4 weeks.

Parameters	Period (week)	Selenium concentration (μg/L)				
		0	50	100	200	400
RBC count ($\times 10^4 mm^3$)	2	268.6±9.3^{a}	246.3±12.2^{b}	232.7±11.8^{c}	223.3±6.7^{c}	226.4±6.8^{c}
	4	308.4±21.7^{a}	268.7±12.9^{b}	246.9±8.6^{c}	225.3±15.2^{c}	236.7±9.7^{c}
Hematocrit (%)	2	42.33±1.64^{a}	37.35±4.61^{b}	36.50±2.38^{b}	36.18±2.47^{b}	36.28±2.17^{b}
	4	43.75±1.18^{a}	36.20±1.62^{bc}	37.32±4.61^{b}	32.33±3.27^{c}	33.41±3.28^{bc}
Hemoglobin (g/dL)	2	10.62±0.26^{a}	10.05±0.77^{a}	10.33±0.90^{a}	10.14±1.16^{a}	7.63±0.13^{b}
	4	10.59±0.26^{a}	9.74±1.37^{ab}	9.58±0.12^{ab}	9.25±1.15^{b}	7.46±0.48^{c}

Values are mean±S.E. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

Table. 3 Changes of serum calcium and magnesium in red seabream, *Pagrus major* exposed to selenium for 4 weeks.

Parameters	Period (week)	Selenium concentration (㎍/L)				
		0	50	100	200	400
Calcium (mg/dL)	2	11.28±1.67[a]	10.60±0.64[a]	11.09±0.12[a]	11.69±1.05[a]	10.84±1.17[a]
	4	14.08±0.37[a]	13.71±3.49[a]	13.74±1.47[a]	12.38±1.09[a]	12.41±0.30[a]
Magnesium (mg/dL)	2	9.41±1.47[a]	9.32±0.41[a]	9.41±0.40[a]	9.17±0.77[a]	9.08±0.67[a]
	4	9.96±0.36[a]	9.13±0.54[ab]	9.11±0.78[ab]	7.86±0.39[b]	8.03±1.09[b]

Values are mean±S.E. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

Table. 4 Changes of serum glucose, albumin, cholesterol, and total protein in red seabream, *Pagrus major* exposed to selenium for 4 weeks.

Parameters	Period (week)	Selenium concentration (μg/L)				
		0	50	100	200	400
Glucose (mg/dL)	2	126.7±9.4^{a}	138.8±12.0^{ab}	143.4±5.5^{ab}	153.5±8.6^{b}	207.7±14.2^{c}
	4	141.8±4.8^{a}	176.0±26.9^{b}	181.8±14.0^{b}	201.2±13.8^{bc}	223.3±11.2^{c}
Albumin (g/dL)	2	0.80±0.12^{a}	0.59±0.09^{b}	0.38±0.06^{c}	0.34±0.05^{c}	0.38±0.03^{c}
	4	0.87±0.21^{a}	0.60±0.12^{b}	0.63±0.05^{b}	0.45±0.06^{bc}	0.39±0.09^{c}
Cholesterol (mg/dL)	2	163.7±38.0^{a}	197.9±12.4^{a}	198.1±45.5^{a}	179.1±11.0^{a}	175.0±5.7^{a}
	4	157.2±10.5^{a}	146.6±12.8^{a}	160.9±11.3^{a}	157.1±21.2^{a}	155.5±22.1^{a}
Total protein (g/dL)	2	4.89±0.58^{a}	4.70±0.15^{a}	4.58±0.27^{a}	4.44±0.64^{a}	4.55±0.51^{a}
	4	5.16±0.78^{a}	4.68±0.50^{a}	4.80±0.76^{a}	4.90±0.12^{a}	4.77±0.19^{a}

Values are mean±S.E. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

Table. 5 Changes of serum GOT, GPT and ALP in red seabream, *Pagrus major* exposed to selenium for 4 weeks.

Parameters	Period (week)	Selenium concentration (μg/L)				
		0	50	100	200	400
GOT (karmen unit)	2	90.6±6.0^{a}	101.7±18.3^{ab}	113.9±17.8^{ab}	115.6±14.1^{b}	139.2±16.5^{c}
	4	111.7±13.3^{a}	122.8±10.1^{ab}	132.8±19.4^{bc}	146.1±25.5^{bc}	169.4±18.9^{c}
GPT (karmen unit)	2	42.6±10.0^{a}	53.3±5.4^{ab}	61.1±10.5^{bc}	67.6±7.0^{c}	73.6±10.1^{c}
	4	56.6±4.0^{a}	71.6±8.7^{b}	73.3±3.3^{b}	83.6±8.4^{bc}	88.2±13.4^{c}
ALP (K-A)	2	5.38±0.36^{a}	5.07±0.18^{ab}	4.65±0.53^{bc}	4.17±0.14^{cd}	3.94±0.09^{d}
	4	5.88±0.42^{a}	5.68±0.21^{a}	5.34±0.11^{ab}	4.86±0.44^{bc}	4.51±0.24^{c}

Values are mean±S.E. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

4. Antioxidant enzyme analysis

4-1. Superoxide dismutase (SOD) activity

The SOD activity of liver and gill tissues exposed to selenium is presented at Fig. 12-13.

In SOD activity of liver tissues, the noticeable increases were observed at the concentration in excess of 200 ㎍/L after 2 weeks and 100 ㎍/L after 4 weeks. The SOD activity in accordance with increasing selenium concentrations at 4 weeks was generally higher than the activity at 2 weeks.

The SOD activity of gill tissues considerably increased the concentrations in excess of 50 ㎍/L at 2 weeks. The noticeable increases were observed at all concentrations in accordance with increasing concentrations at 4 weeks.

The relationship between SOD activity of liver and gill tissues and the selenium bioaccumulation of liver and gill tissues is shown at Fig. 14-15. A significant relationship was found for all correlations.

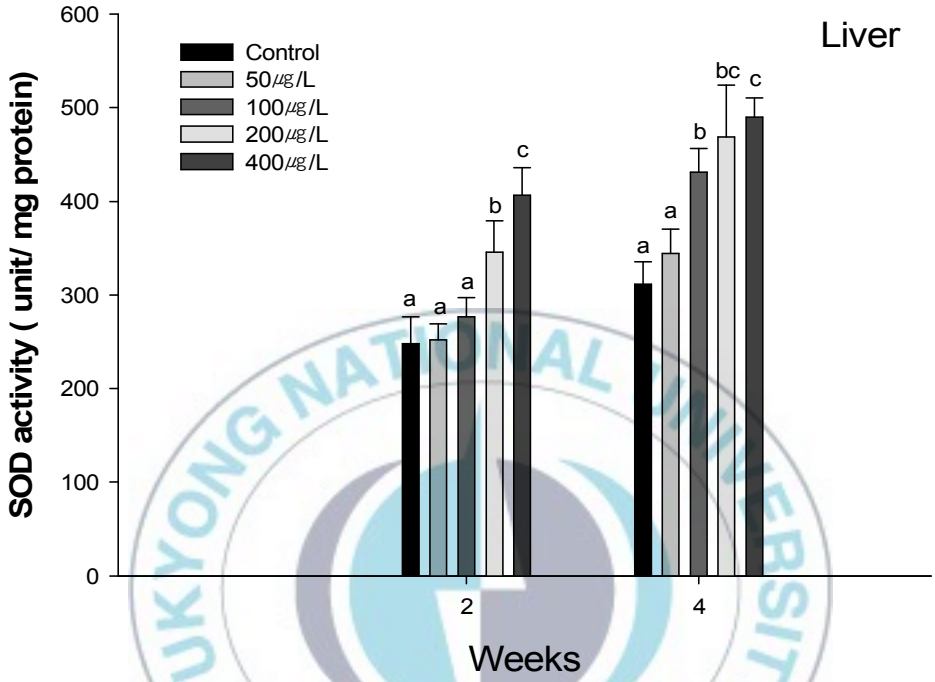

Figure. 12 Changes of SOD activity in liver of red seabream, *Pagrus major* exposed to the different concentration of selenium. Vertical bar denotes a standard error. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

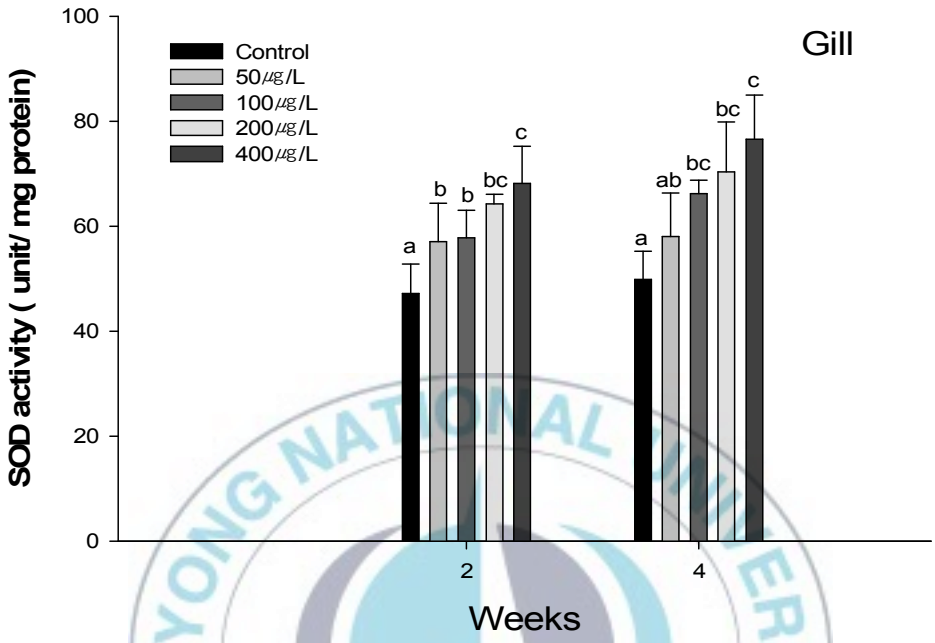

Figure. 13 Changes of SOD activity in gill of red seabream, *Pagrus major* exposed to the different concentration of selenium. Vertical bar denotes a standard error. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

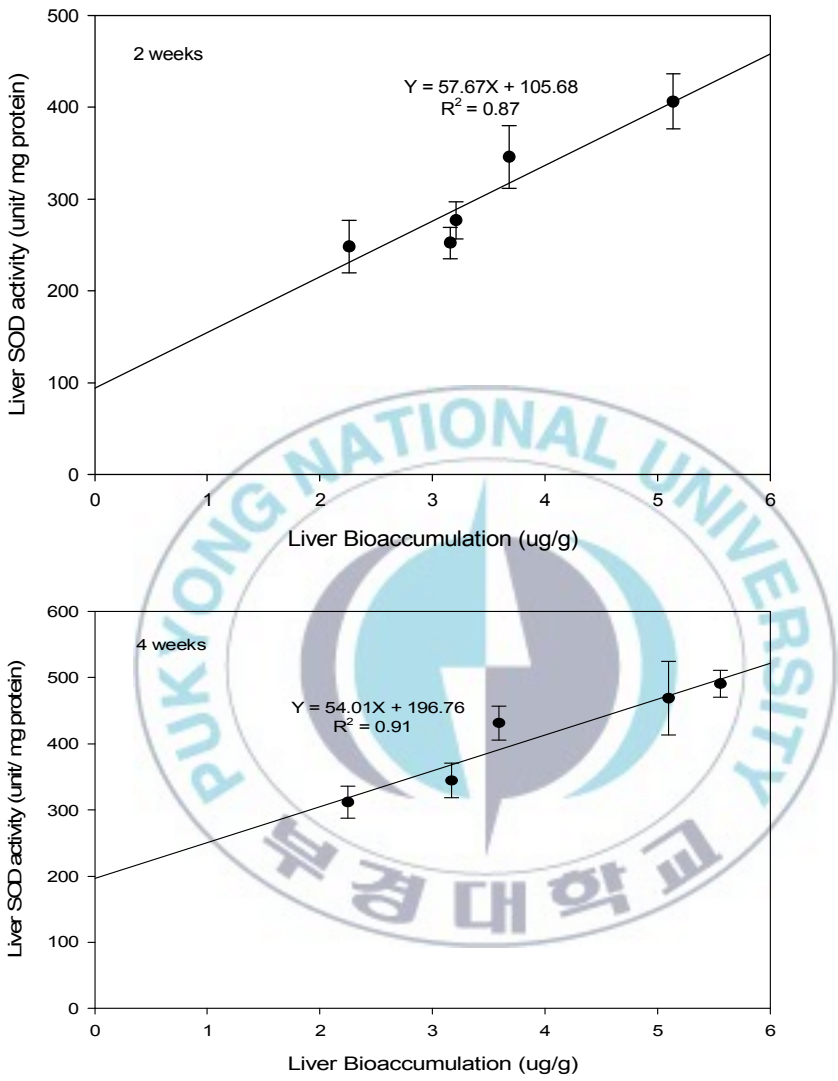

Figure. 14 The relation between liver SOD activity and liver bioaccumulation of Se at 2 weeks and 4 weeks.

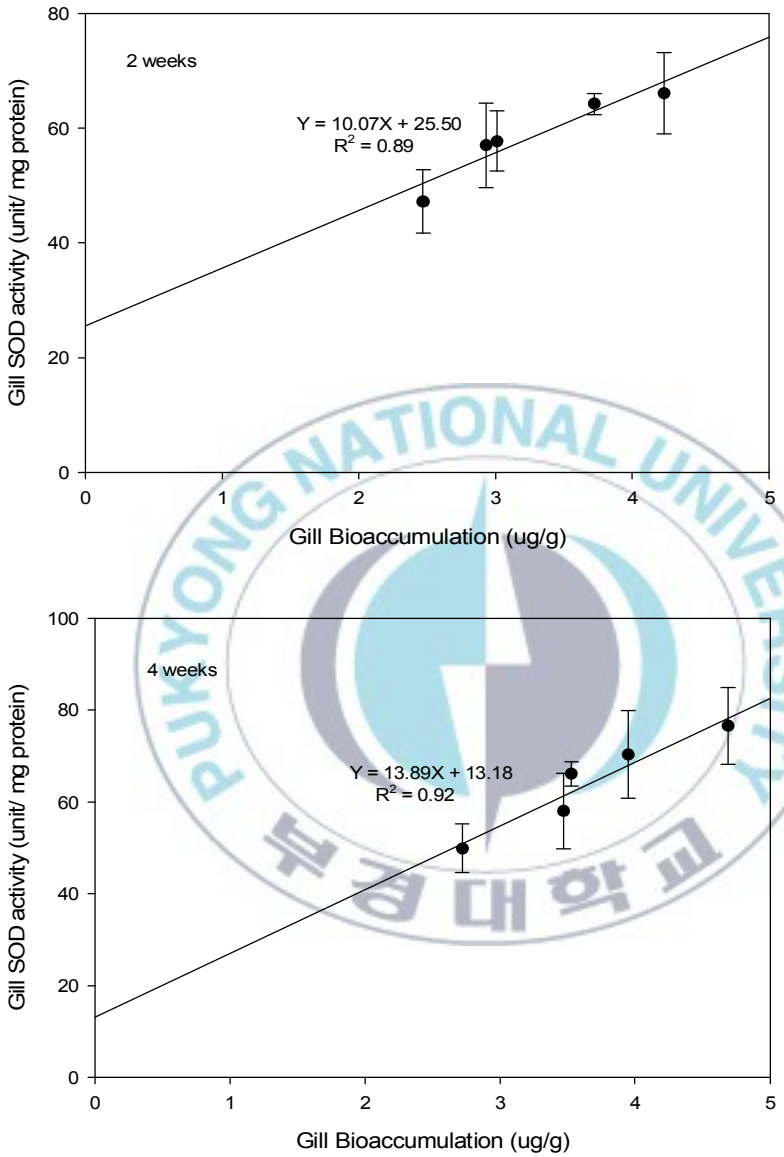

Figure. 15 The relation between gill SOD activity and gill bioaccumulation of Se at 2 weeks and 4 weeks.

4-2. Glutathione s-transferase (GST) activity

The GST activity of liver and gill tissues exposed to selenium are presented at **Fig. 16-17**.

In GST activity of liver tissues, no considerable change was observed at the concentrations from control to 200 ㎍/L after 2 weeks. However, the remarkable increase appeared at the concentration of 400 ㎍/L after 2 weeks. At 4 weeks, GST activity was slightly increased at the concentration from control to 200 ㎍/L. The most noticeable increase was observed at 400 ㎍/L after 4 weeks.

The GST activity of gill tissues had no change at 2 weeks, but the activity was sharply increased at the concentration over 100 ㎍/L at 4 weeks.

The relationship between GST activity of liver and gill tissues and the selenium bioaccumulation of liver and gill tissues is demonstrated at **Fig. 18-19**. Significant relations of liver and gill tissues at 2 weeks were found, but considerable relations of liver and gill tissues at 4 weeks were not observed.

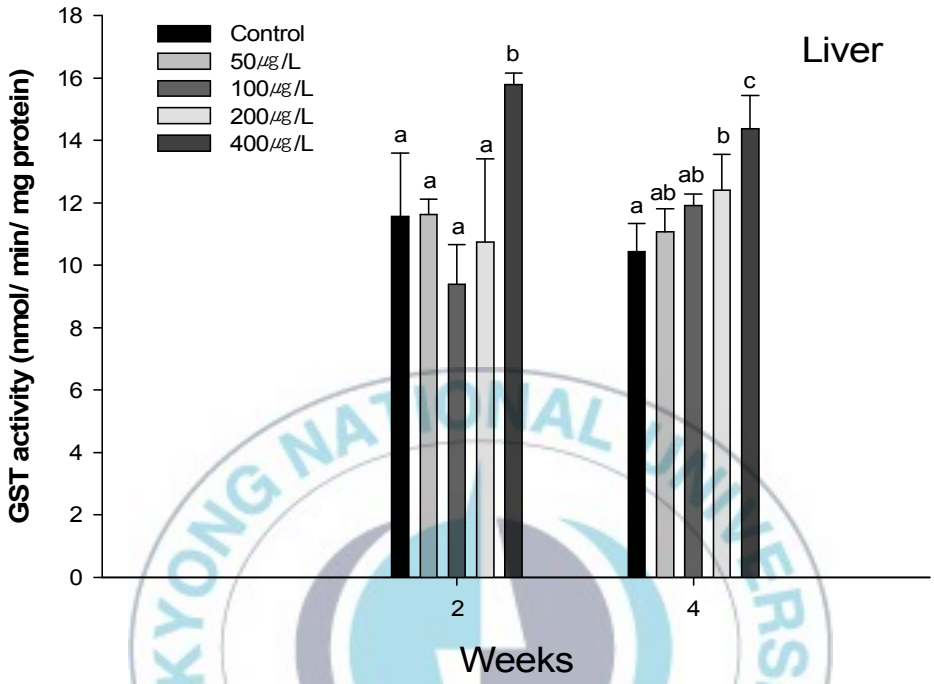

Figure. 16 Changes of GST activity in liver of red seabream, *Pagrus major* exposed to the different concentration of selenium. Vertical bar denotes a standard error. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

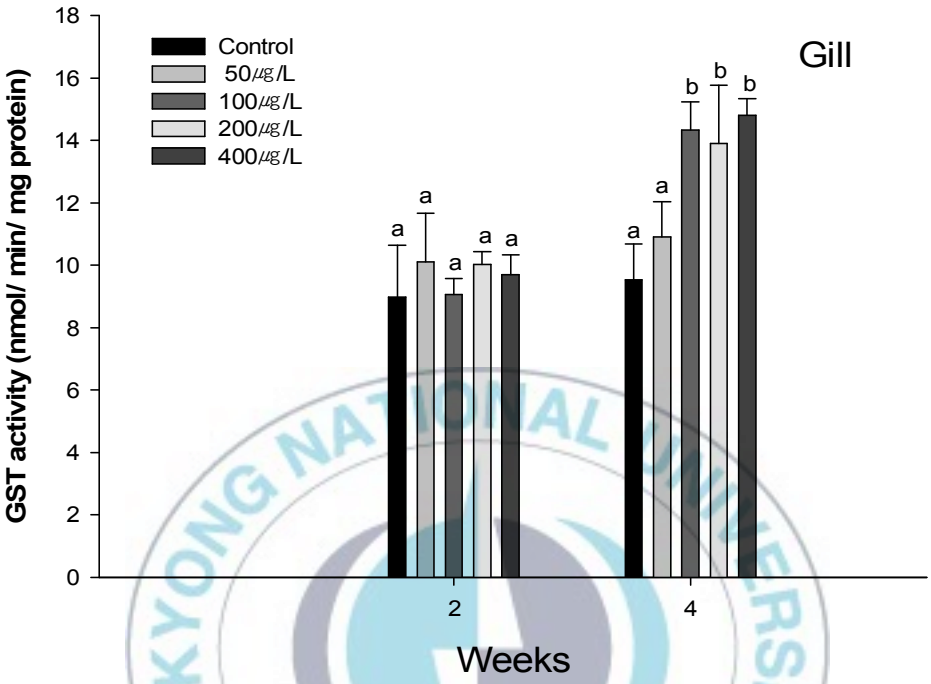

Figure. 17 Changes of GST activity in gill of red seabream, *Pagrus major* exposed to the different concentration of selenium. Vertical bar denotes a standard error. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

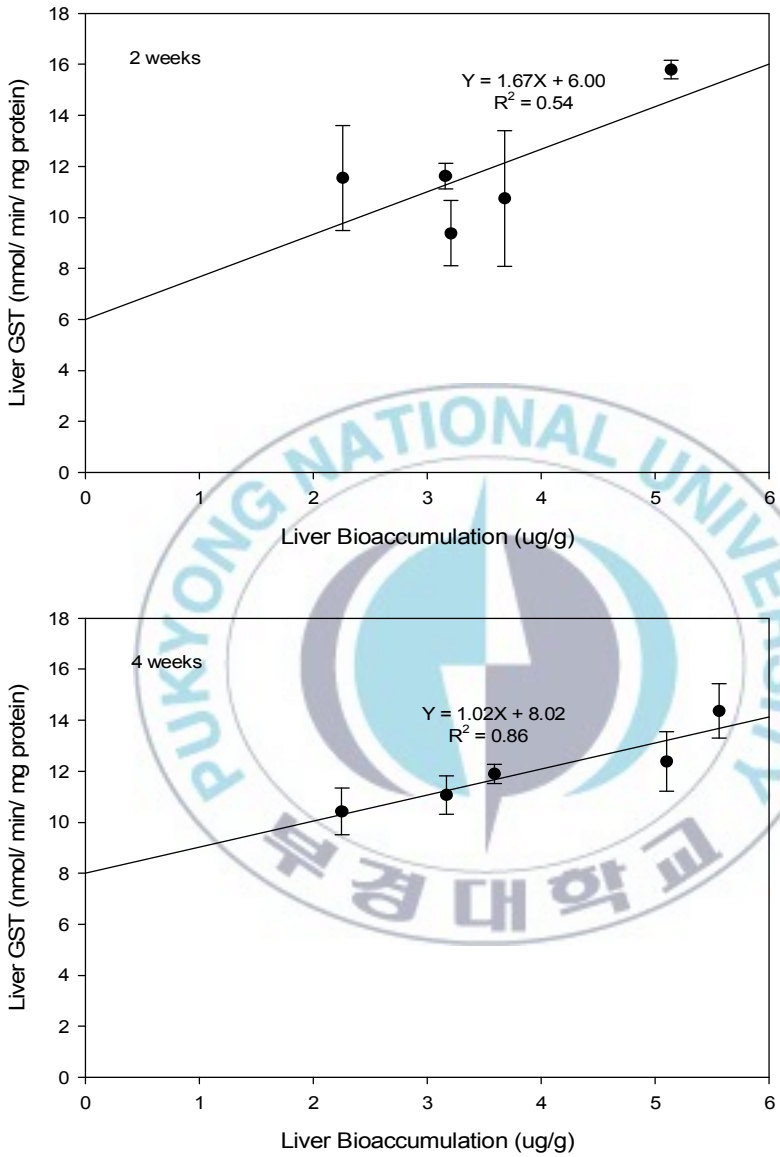

Figure. 18 The relation between liver GST activity and liver bioaccumulation of Se at 2 weeks and 4 weeks.

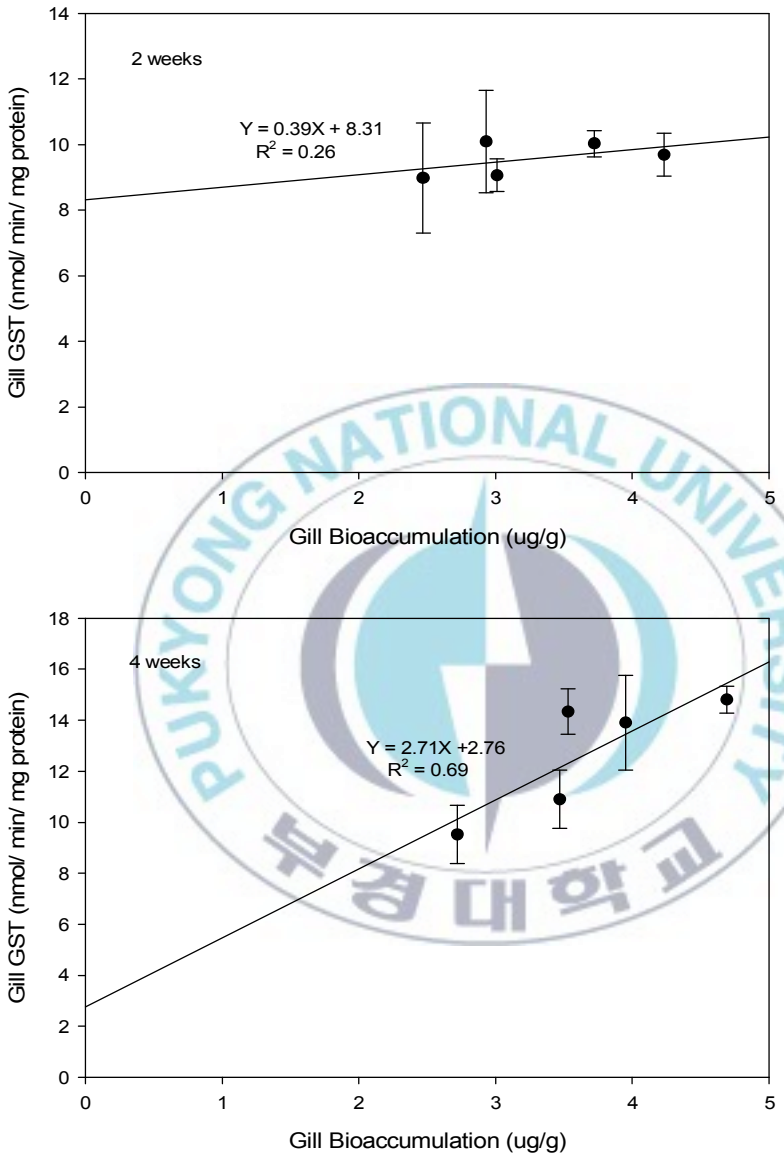

Figure. 19 The relation between gill GST activity and gill bioaccumulation of Se at 2 weeks and 4 weeks.

4-3. Glutathione (GSH) level

The GSH level of liver and gill tissues exposed to selenium are shown at **Fig. 20-21**.

The GSH level of liver tissues at 2 weeks had no considerable change except 400 ㎍/L. The noticeable increase was observed at the concentration at 400 ㎍/L after 2 weeks. At 4 weeks, the concentrations at 50 and 100 ㎍/L indicated slight increases, and a slight decline was observed at 200 ㎍/L. The most considerable increase was observed at 400 ㎍/L after 4 weeks.

In GSH level of gill tissues, slight increases were shown at all concentrations. The most remarkable difference was at 400 ㎍/L after 2 weeks. At 4 weeks, no difference was observed except for the concentration at 400 ㎍/L which was the most considerable increase at 4 weeks.

The relationship between GSH level of liver and gill tissues and the selenium bioaccumulation of liver and gill tissues is presented at **Fig. 22-23**. A significant relationship was found for all correlations except for liver tissues at 4 weeks.

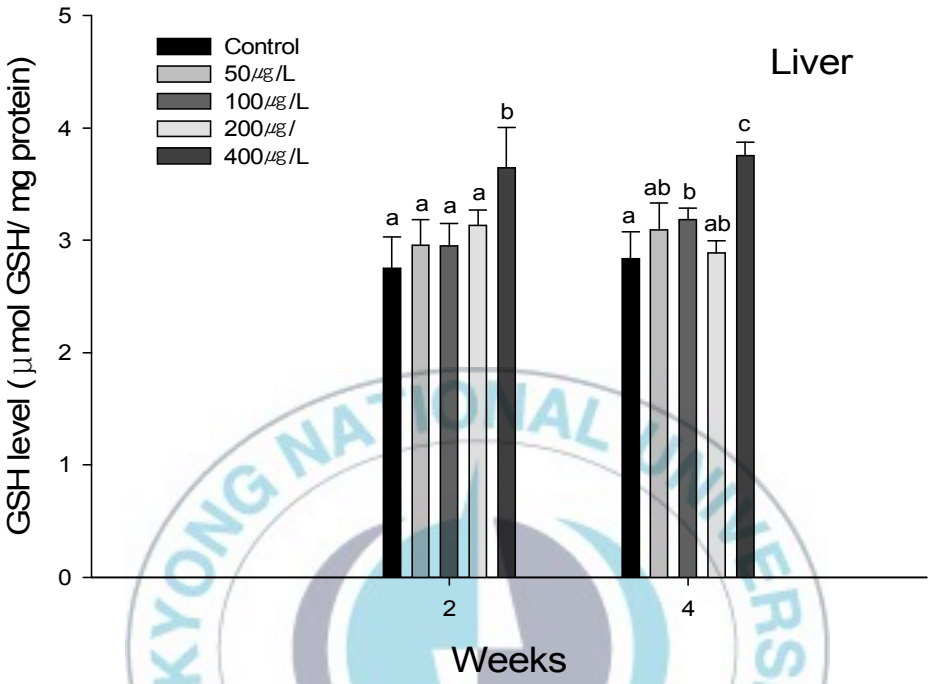

Figure. 20 Changes of GSH level in liver of red seabream, *Pagrus major* exposed to the different concentration of selenium. Vertical bar denotes a standard error. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

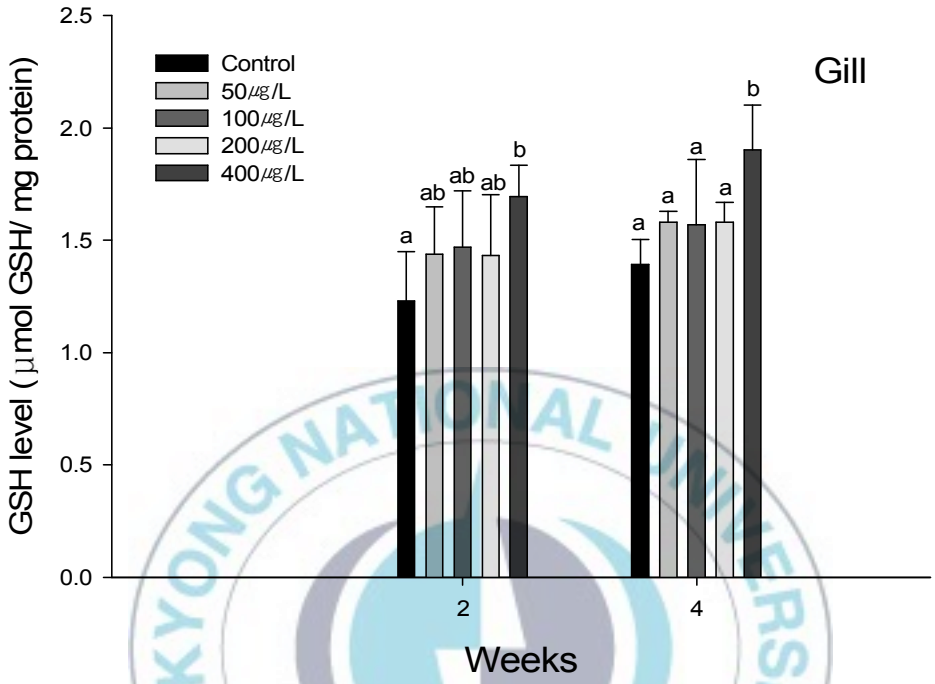

Figure. 21 Changes of GSH level in gill of red seabream, *Pagrus major* exposed to the different concentration of selenium. Vertical bar denotes a standard error. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

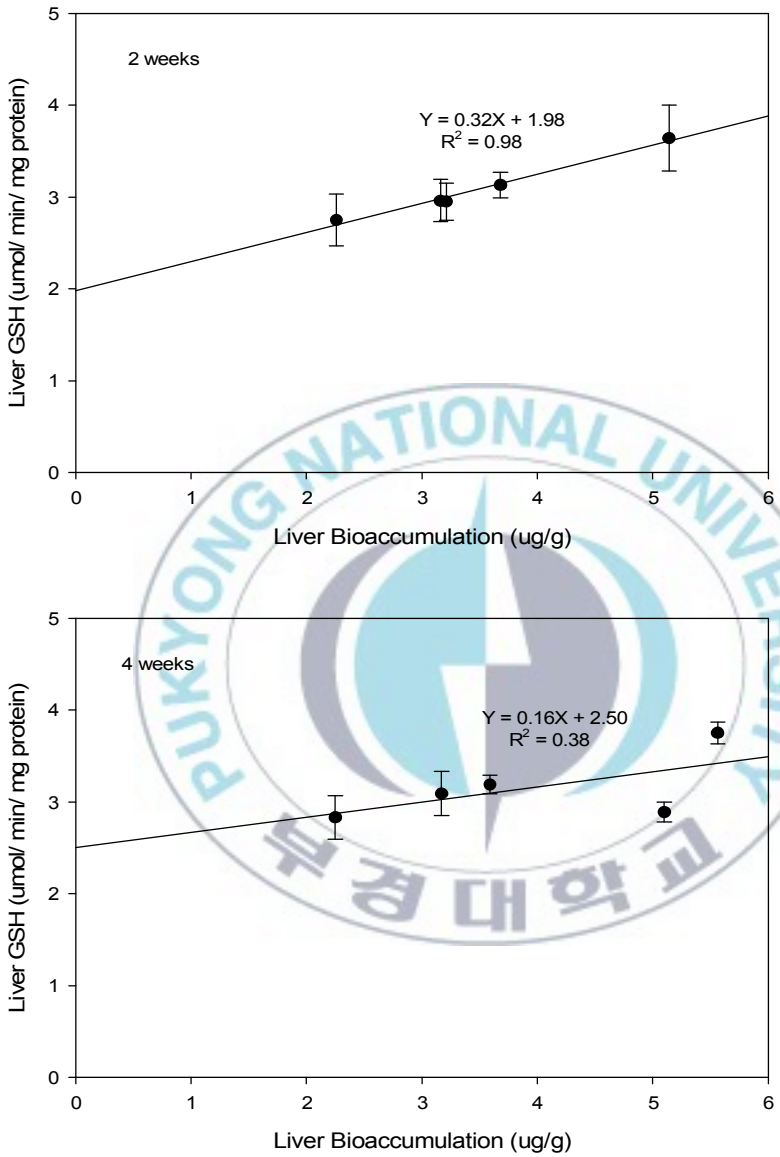

Figure. 22 The relation between liver GSH level and liver bioaccumulation of Se at 2 weeks and 4 weeks.

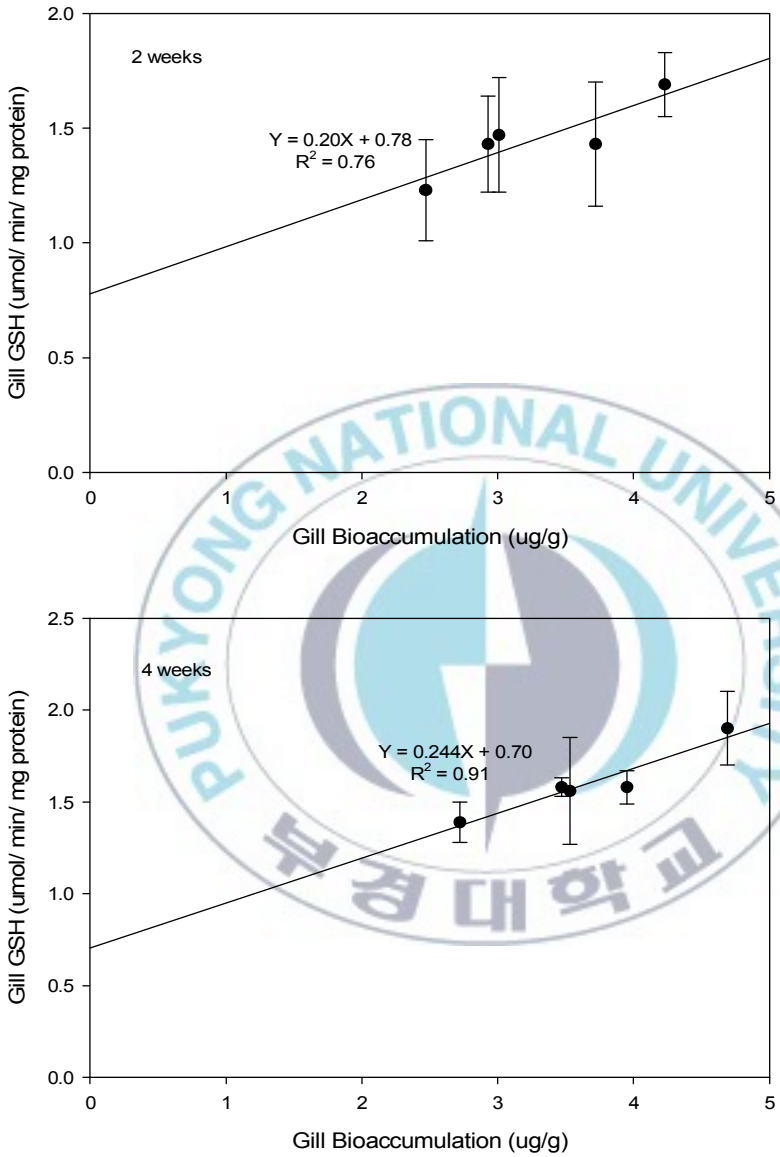

Figure. 23 The relation between gill GSH level and gill bioaccumulation of Se at 2 weeks and 4 weeks.

6. Acetylcholinesterase activity

The acetylcholinesterase activities of muscle, brain, liver, and intestine tissues of red seabream exposed to selenium are shown in Fig 24-27. The major finding was a significant decrease of the acetylcholinesterase activity in brain tissues. Acetylcholinesterase activity was noticeably inhibited in brain tissue, compared to other tissues. The amount of acetylcholinesterase activity in brain and muscle was relatively higher than liver and intestine tissues.

In the acetylcholinesterase activity of muscle tissues, it had a considerable decrease in the concentration of 400 ㎍/L at 2 weeks and 4 weeks. The acetylcholinesterase activity of brain tissues was the most notable among the experimented tissues, especially in the concentration of 200 ㎍/L and 400 ㎍/L at 2 weeks and 4 weeks. The most noticeable decrease in the brain tissues was presented at 400 ㎍/L after 4 weeks.

In the acetylcholinesterase activity of liver tissues, it had no large change, but it has a considerable decrease in the concentration of 400 ㎍ /L at 4 weeks. There was no activity change in the intestine tissues.

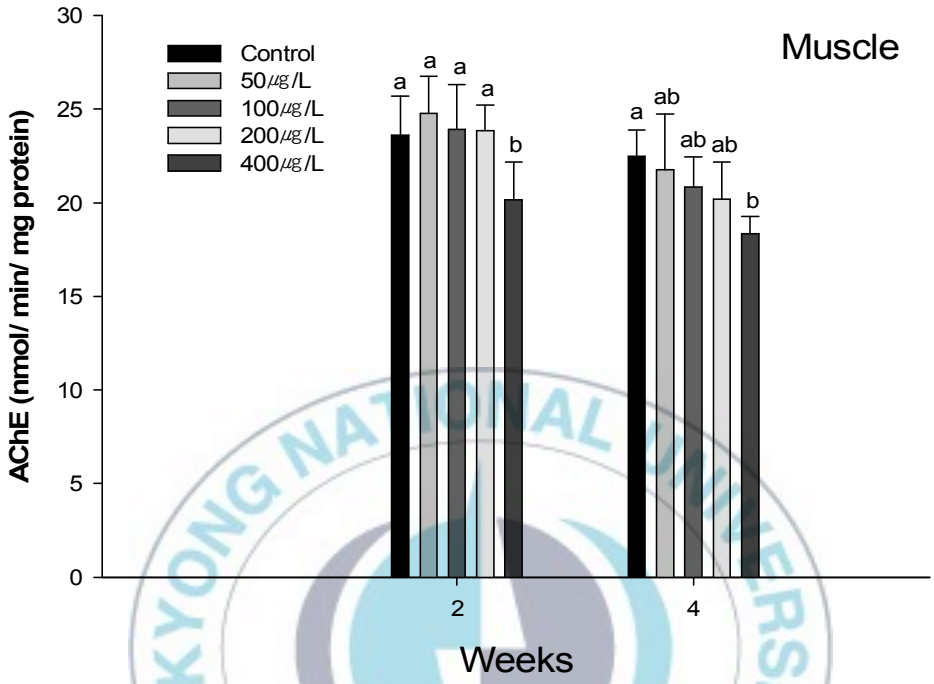

Figure. 24 Changes of acetylcholinesterase activity in muscle of red seabream, *Pagrus major* exposed to the different concentration of selenium. Vertical bar denotes a standard error. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

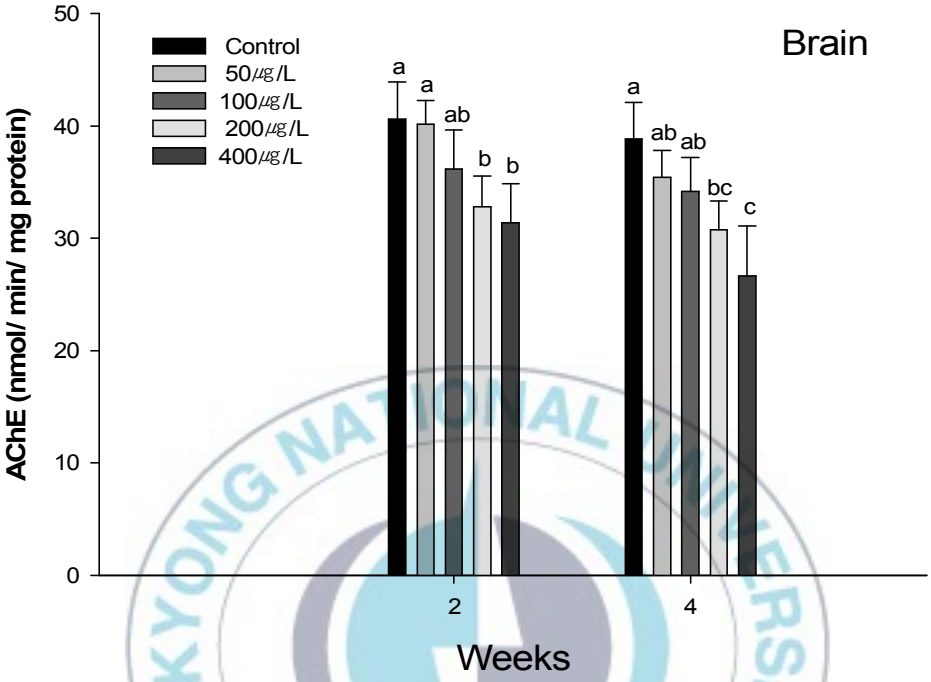

Figure. 25 Changes of acetylcholinesterase activity in brain of red seabream, *Pagrus major* exposed to the different concentration of selenium. Vertical bar denotes a standard error. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

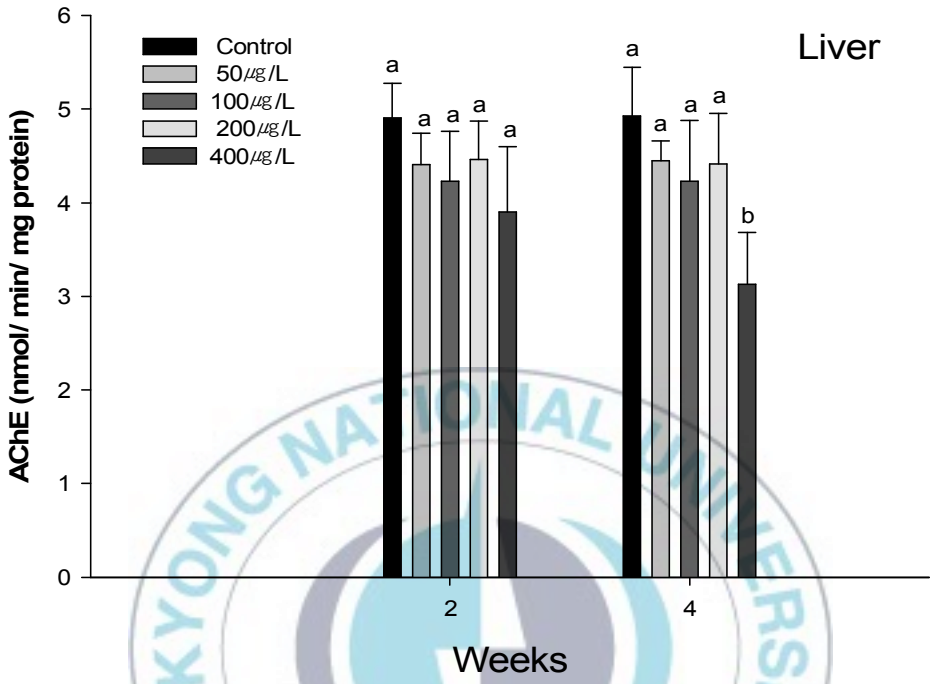

Figure. 26 Changes of acetylcholinesterase activity in liver of red seabream, *Pagrus major* exposed to the different concentration of selenium. Vertical bar denotes a standard error. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

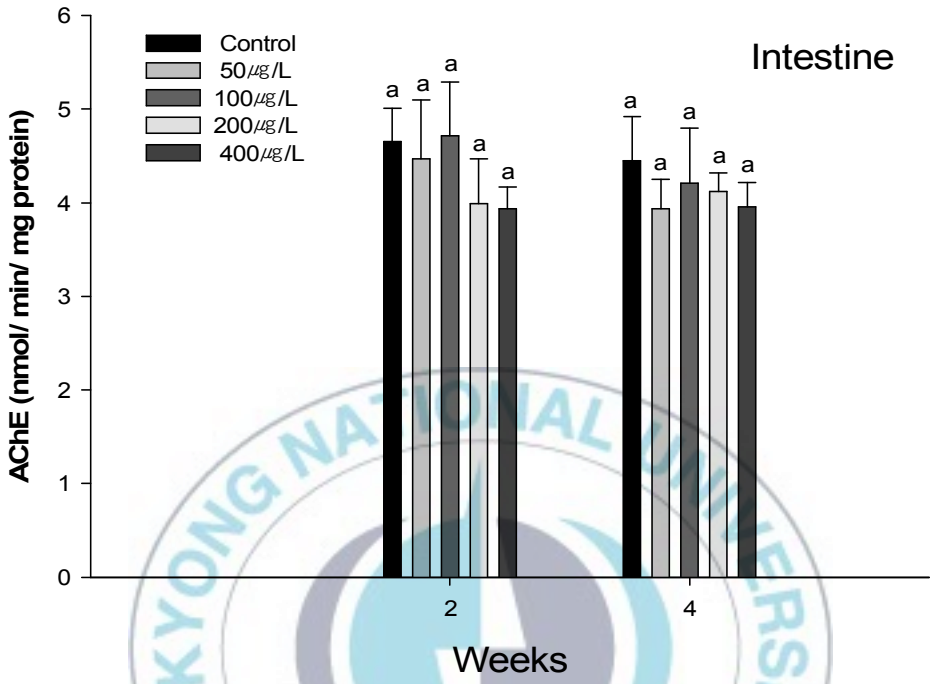

Figure. 27 Changes of acetylcholinesterase activity in intestine of red seabream, *Pagrus major* exposed to the different concentration of selenium. Vertical bar denotes a standard error. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

5. Non-specific immune response

5-1. Lysozyme activity

The lysozyme activities of plasma and kidney tissues of red seabream exposed to selenium are shown in Fig. 28 and Fig 29. In the lysozyme activity of plasma, it had a increasing trend, significantly at 400 ㎍/L after 2 weeks and 4 weeks. The lysozyme activity of kidney was significantly increased at 50 ㎍/L after 2 weeks and 4 weeks. The greatest activities were observed at 400 ㎍/L in the highest concentration.

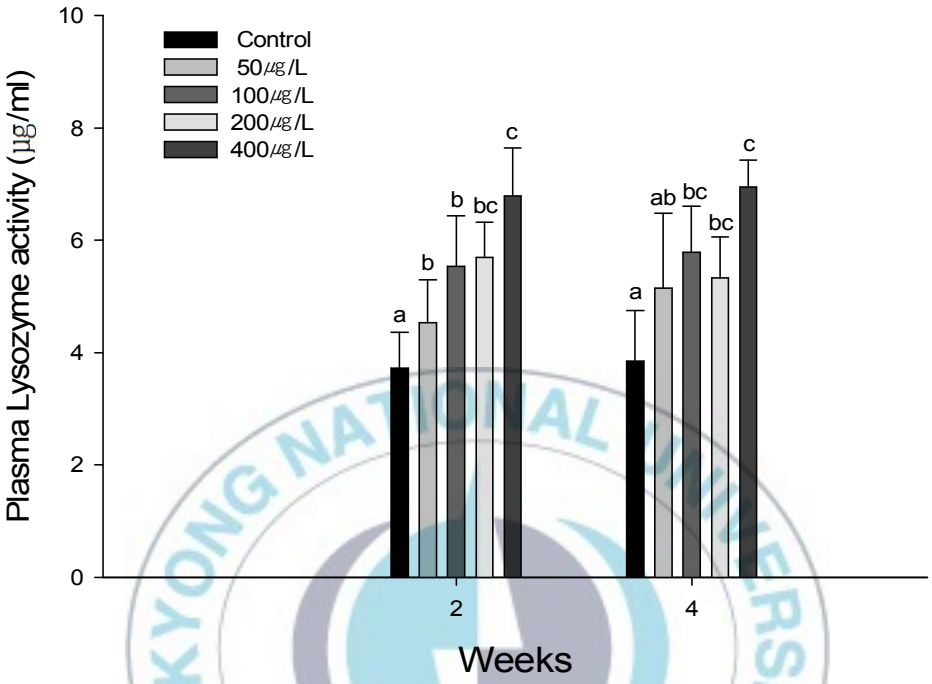

Figure. 28 The lysozyme activity in plasma of red seabream, *Pagrus major* exposed to the different concentration of selenium. Vertical bar denotes a standard error. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

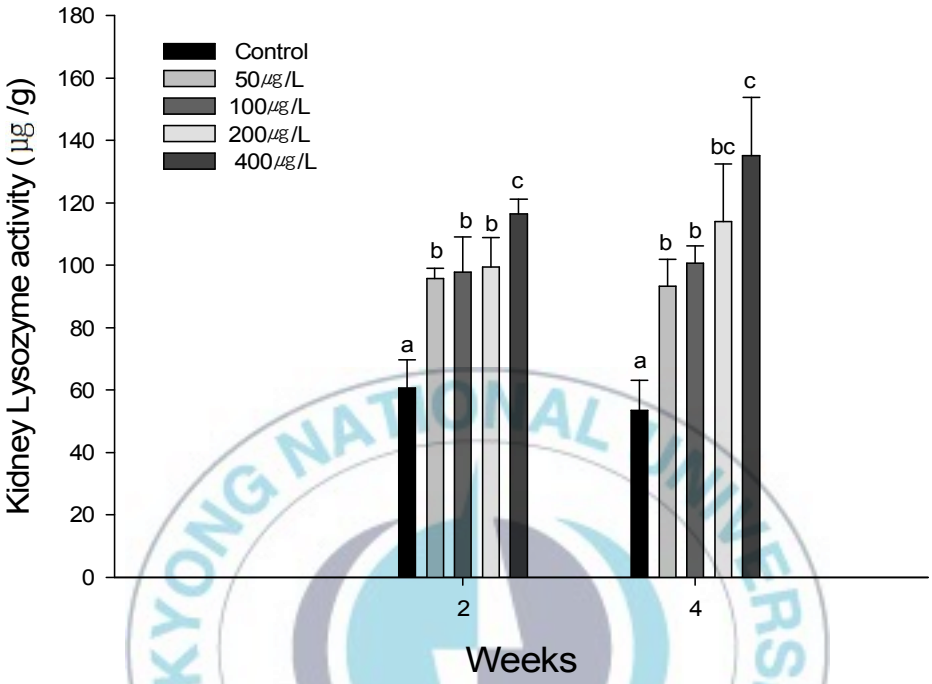

Figure. 29 The lysozyme activity in kidney of red seabream, *Pagrus major* exposed to the different concentration of selenium. Vertical bar denotes a standard error. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

5-2. Peroxidase activity

The peroxidase activity of red seabream exposed to selenium was shown in Fig 30. It had no considerable change in the concentrations of 50 ㎍/L to 200 ㎍/L at 2 weeks and the concentrations of control to 200 ㎍/L at 4 weeks. However, the noticeable decreases were observed at 400 ㎍/L of 2 weeks and 4 weeks.

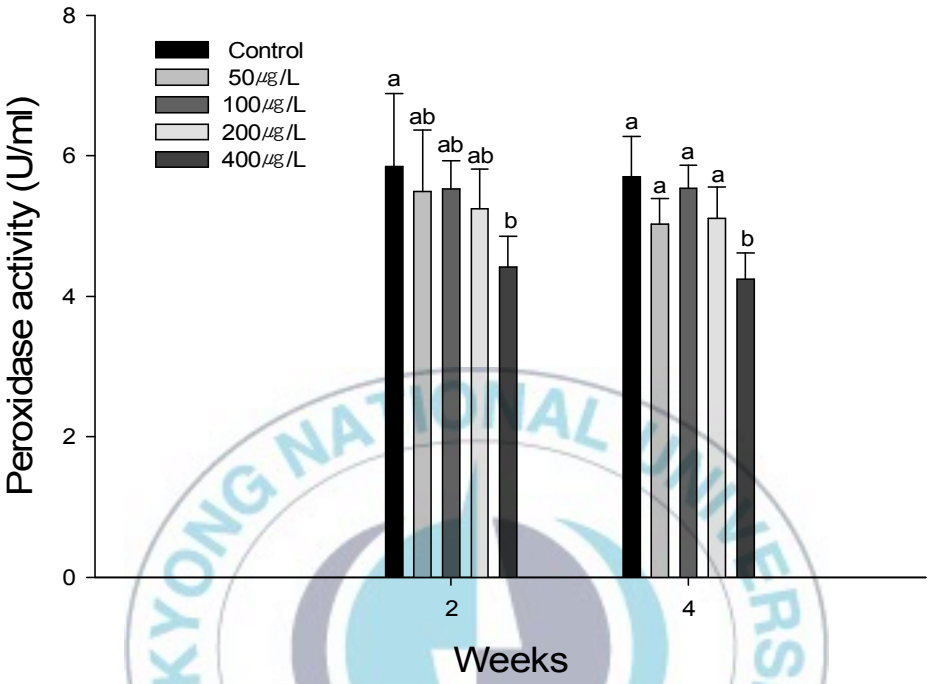

Figure. 30 Peroxidase activity in serum of red seabream, *Pagrus major* exposed to the different concentration of selenium. Vertical bar denotes a standard error. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

5-3. Anti-protease activity

The anti-protease activity of red seabream exposed to selenium was presented in Fig 31. It had no change in the concentrations at 2 weeks. At 4 weeks, it had no noticeable change in the concentrations of control to 200 ㎍/L. However, slight decreases were observed at 200 ㎍/L and 400 ㎍/L after 4 weeks.

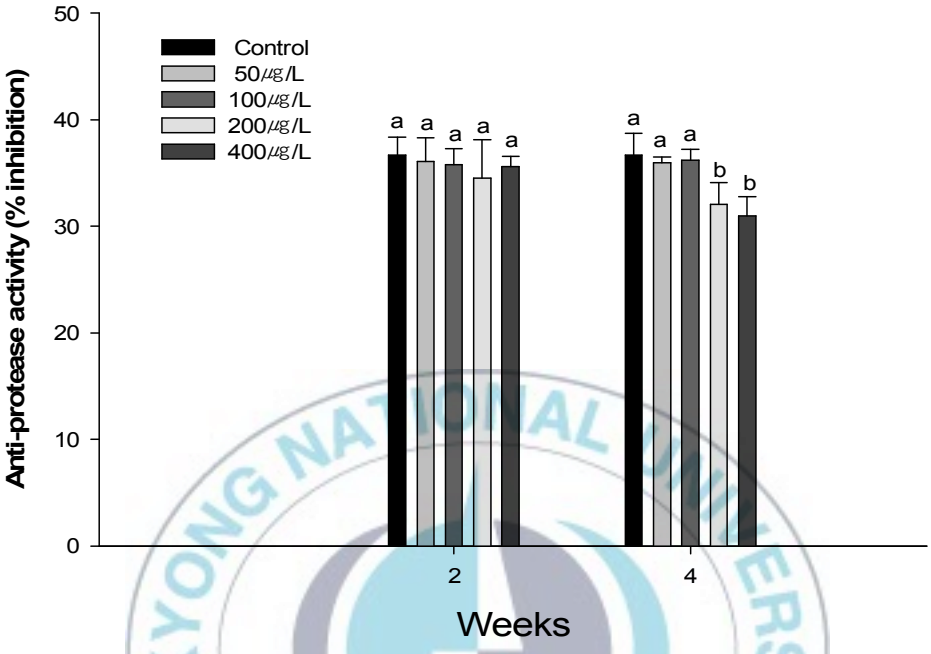

Figure. 31 Anti-protease activity in serum of red seabream, *Pagrus major* exposed to the different concentration of selenium. Vertical bar denotes a standard error. Values with different superscript are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

Ⅳ. Discussion

The essential trace elements for animals such as selenium, zinc, and cupper are necessary for growth, development, and the homeostatic maintenance of physical response. However, it can be toxic at concentrations higher than those required for homeostasis with the structural disorder of intracellular space and the disability of homeostatic function, which bring about a result in negative influence in physiological activity (Kawai., 1959; Miller, L.L., 2009). Selenium has a narrow range of safety between baseline nutritional requirements and toxic dietary levels, which is distinguished from other toxic elements (Stadman, 1974).

Bioaccumulation is affected by the interaction between the environment factors like hardness, temperature, salinity, and pH and the 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, 1978; Hakanson, 1980; Bendell-Young, 1989). It is known that large amounts of toxic contaminations from their living environment can be accumulated in fish tissues (Suhaimi, 2005). The contaminant uptake is positively linked to the metabolic rate in marine organisms, and it can be supposed that toxic substance accumulation would be highest in young fish, which is attributed to the higher metabolic activity in young individuals.

Generally, the high selenium bioconcentration is commonly shown in the tissue of spleen, liver, and kidney on the contrary to muscle tissue which appears to accumulate the least (Adams, 1976; Hodson, 1980; Sato, 1980; Lemly, 1982; Goldstein, 1999; Kojadinovic, 2007; Waska, 2008). Elia et al. (2011) reported the juvenile carp exposed to selenium accumulated the following order: kidney > liver > muscle. In this study, the relative bioaccumulation of *Pagrus major* exposed to selenium in specific tissues

was: kidney > spleen > intestine > liver > gill > muscle > brain, which is similar pattern with previous studies. The most amount of selenium bioaccumulation was the tissue of kidney. Except for the brain and muscle tissues, the selenium bioaccumulation was significantly increased according to the increased selenium exposure of the concentrations in rearing aquarium. The high selenium bioconcentration level in the liver and kidney tissues is caused by the main site of detoxification and excretion organ, as well as main bioaccumulation sites. In the case of marine fishes, they drink seawater for osmoregulation, and water uptake occur in their intestine, which is considered the high bioaccumulation of intestine tissues. The bioaccumulation of gill tissue seems to occur by the direct contact with breeding water and the ion exchange. Even though slight increases in muscle and brain were observed at the high selenium exposure concentrations, no notable increase was shown in the muscle and brain tissues. Through this study, it is observed that the vital organs such as kidney, spleen, intestine, liver, and gill tissues has considerable selenium bioaccumulation, while the brain and muscle tissues has no notably large bioaccumulation.

The fish growth can be affected by various factors such as temperature, sex, nutrient, and pollutants. Furthermore, the fish growth are typically inhibited by the contaminants exposure to sublethal dose. In this study, the growth of red seabream exposed to selenium was significantly decreased by increasing the selenium exposure concentration. In daily length gain, the figure started to fall in the higher concentration of 100 ㎍/L at 2 weeks and 50 ㎍/L at 4 weeks. In daily weight gain, significant decreases were observed in the higher concentration of 100 ㎍/L at 2 weeks and 200 ㎍/L at 4 weeks. Condition factor slightly decreased at 200 ㎍/L and 400㎍/L after 4 weeks. In hepatosomatic index, considerable decreases were observed at 400 ㎍/L after 2 weeks and 4 weeks.

The hematological parameters are widely used to measure toxicity stress caused by environmental pollutants. Many studies observed anemia and

substantial reductions in hematocrits, hemoglobin levels, and erythrocyte counts in vertebrates exposed to selenium toxicity (Lillie, 1940; Moxon, 1943). In this study, significant decreases in RBC count, hematocrit, and hemoglobin were also observed at the high selenium concentration, compared to control. The affinity of selenium for hemoglobin causes cell death resulting in the reduction of respiratory capacity in blood.

The inorganic components, organic components, and enzyme components in plasma were observed in this study. The inorganic components in plasm, calcium and magnesium, increase or decrease according to the change of osmotic pressure in serum (Waring, 1996; Chang, 2001; Hur, 2001). In this study, there is no considerable change in calcium and magnesium except for slight decreases at the higher concentration of 200 ㎍/L after 4 weeks. Considering the result of inorganic components, slight decreases at the high concentrations were deemed to affect the osmoregulation of fish in some degree. The organic components in plasma such as glucose, albumin, cholesterol, and total protein were measured. In case of the blood glucose, it generally increases because carbohydrate metabolism increase under stress (Hontela, 1996; Kennedy, 1995). In this study, the blood glucose values indicated continually considerable increases by increasing the selenium exposure concentration. At the concentration of 400 ㎍/L after 2 weeks and 4 weeks, the blood glucose was the highest, and the glucose values at 4 weeks were much higher than the values at 2 weeks. In contrast with glucose, albumin significantly decreased. In cholesterol and total protein, no notable change was observed. The glutamic oxalate transminase (GOT), glutamic pyruvate transminase (GPT), alkaline phosphatase (ALP) were measured to observe the enzyme components in plasma. The components of GOT and GPT are frequently used to gauge the sublethal toxic influence of fish exposed to the environmental contaminants, and it can be increased by the histological damages of liver and pancreas tissues (Blasco, 1999). In this study, the considerable increases were

observed by increasing the selenium concentration. The increases at high exposure concentration were more significant than low concentration, and the increases at 4 weeks were more considerable than the increases at 2 weeks. Alkaline phosphatase (ALP) is a membrane-bound enzyme which is relevant to the transport of various metabolites (Lin, 1976), and it also proposed as a biomarker in ecotoxicology (Boge, 1992). In this study, ALP was notably decreased by increasing the selenium concentration. Considering the results of the enzyme components, the selenium exposure should significantly affect the experimented fish.

Oxidative stress becomes accepted as an important aspect of toxicology, and has received increasing attention in recent years. When reactive oxygen species (ROS) overwhelm the cellular defences, oxidative stress occurs, and damage proteins, membranes, and DNA (Kelly, 1998). As a defense mechanism, antioxidant enzymes are increased by oxidative substances in cells. It is one of the main mechanisms for selenium toxicity that the high accumulation of selenium in liver is a major cause of reactive oxygen species (ROS) generation (Spallholz, 2004). Superoxide anions are generally dismutated by superoxide dismutase (SOD) to H_2O_2, which is the first defense mechanism against oxygen toxicity. In this study, SOD activity both in liver and gill was significantly increased by increasing selenium exposure concentration, and the highest activity was at the concentration of 400 ㎍/L after 2 weeks and 4weeks. In general, many studies reported that SOD activity is increased by the exposure to toxicant substances or heavy metals. Misra et al. (2009) also reported the SOD activity increase of rainbow trout (*Oncorhynchus mykiss*) exposed to selenite. Glutathion S-transferases (GST) function a main role in detoxification of deleterious electrophilic xenobiotics such as environmental pollutants. In this study, a significant increase of GST activity in liver was observed at 400 ㎍/L after 2 weeks and 4 weeks, and a considerable increase in gill tissues was observed at the higher concentration of 100 ㎍/L after 4 weeks, whereas there was no notable change at 2 weeks. Silva

et al. reported a dose-dependent increase in GST activity exposed to different diesel oil concentrations. Glutathione (GSH) is the most abundant intracellular thiol-based antioxidant. In general, glutathione depletion is considered a biomarker of environmental stress in fish exposed to chemical and natural pollutants (Vaglio, 1999; Peña-Llopis, 2001). However, GSH can be increased by the synthesis of hepatic GSH, and it is induced by the pollutant interaction with GCL (glutamate:cysteine ligase) (Peña, 2001). In this study, GSH level had no considerable change in liver and gill tissues, but notable increases was shown at the concentration of 400 ㎍/L at 2 weeks and 4 weeks. These increases are caused by the GSH synthesis growth against the oxidative stress. Lange et al. (2002) reported the GSH increase in the rainbow trout exposed cadmium and zinc. In addition, many studies reported the increases of GSH level to protect the fish exposed to toxic substances from cytotoxic (Tort, 1996; Paris-Palacios, 2000). In the antioxidant enzymes of this study such as SOD, GST, and GSH, the significant increases were overall observed, and the increases may result from increased activity against oxidative stress by selenium exposure. These increases may be according to the activation of fish defense mechanisms.

Acetylcholine is one of the most important neurotransmitter in either central or peripheral nervous system, and the inhibition of acetylcholinesterase has been proposed as a biomarker of the neurotoxicity (Manzo, 1995). A significant reduction in acetylcholinesterase activity is commonly observed in fish exposed to toxic substances (Modesto, 2010). Miron et al. (2005) reported the AChE inhibition of the silver catfish exposed to different herbicide concentrations. In addition, Jebali (2006) et al. also reported significant AChE decrease of *Seriola dumerilli* exposed to cadmium. In this study, the high AChE activity in muscle and brain was observed, as other species also has the high activity of AChE in muscle and brain tissues (Bocquenet, 1990). AChE activity was considerably inhibited in brain tissues treated with higher

concentrations than 200 ㎍/L at 2 weeks and 4 weeks. In muscle tissues, considerable inhibition was observed at 400 ㎍/L both 2 weeks and 4 weeks. In the liver, the inhibition of AChE was observed only at 400 ㎍/L after 4 weeks. However, there was no notable AChE change in the intestine tissues. Considering significant AChE inhibition, selenium exposure may substantially affect the experimented fish as neurotoxicity. The accumulation of acetylcholine according to the inhibition of AChE activity may influence the fleeing and reproductive behavior of fish, which can be interfering immediately in the survival of the fish species (Bretaud, 2000).

The immune system can provide potential biomarkers that may be sensitive enough to assess environmental contamination (Bainy, 2003). Lysozyme is an important parameter in the immune defence. Lysozyme have been positively correlated with disease resistance in different fish species and in some cases (Fevolden, 1994). Secombes et al. (1997) observed elevated lysozyme levels in plaice exposed to sewage sludge. Exposure to toxic substances such as heavy metals modulates lysozyme levels causing alterations in immuno-regulatory functions (Bols, 2001). Sub-lethal mercury exposure reduced plasma lysozyme activity in plaice (Fletcher, 1986), whereas mercury exposure to blue gourami increased kidney lysozyme activity (Low, 1998). Apart from these studies, there are various increase or decrease results of lysozyme activity. In this study, significant increases of the lysozyme activities in plasma and kidney started to be shown at 50 ㎍/L after 2 weeks and 4 weeks. These cosiderable increases of lysozyme activity may represent an immunological stimulation. The peroxidase activity is an indicator of leucocyte activation (Tapia-Paniagua, 2011). The pseroxidase is an major enzyme which use oxidative radicals to produce hypochlorous acid to kill pathogens. In this study, the considerable change of the peroxidase activity in this study was observed at 400 ㎍/L after 2 weeks and 4 weeks. Anti-proteases are one of the non-specific immunity components in the vertebrates. Fish plasma

contains a number of protease inhibitors which may play an important role in inhibiting the ability of bacteria to invade and grow (Ellis, 2001). In this study, anti-protease activity was no considerable change except for slight decreases at higher concentration of 200 ㎍/L after 4 weeks. An decrease in antiprotease activity might be expected to decrease disease resistance in the high concentrations exposed to selenium. Magnadóttir et al. (2006) mentioned the activity generally seemed to be unaffected by immunization or infection. Thilagam (2009) reported the decreased level of protease inhibitors in japanese sea bass exposed to 17β-estradiol. Through the results of these non-specific parameters such as lysozyme, peroxidase activity, and anti-protease activity, selenium exposure may substantially affect the experimented fish.

To conclude, the toxic substances exposure such as selenium exposure can affect the human health by taking fish exposed to selenium toxicity, as well as the harmful effects to sea farming. Through this study, the waterborne selenium can highly affect organisms at the high concentration exposure through this study. However, there have been little studied about selenium toxicity. Therefore, many studies related to selenium toxicity should be conducted in various aspects.

Ⅴ. Summary

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. Even though the selenium deficiency to fish cause damaged cells, selenium can be highly toxic to fish and wildlife at the higher concentration than a permissible amount, because it can rapidly bioaccumulate and reach toxic level in the aquatic environment. Red seabream is a prevalent fish species in South Korea, and it is also commercially important regarding its popularity.

Therefore, the aim of this study is to measure the heamatological change, bioaccumulation, antioxidant enzyme, acetylcholinesterase, non-specific immune responses of red seabream exposed to selenium at the concentrations of 0, 50, 100, 200, and 400 ㎍/L.

The bioaccumulation of red seabream exposed to selenium present a similar increasing pattern except for the brain and muscle tissues. The bioaccumulation order was kidney, spleen, intestine, liver, gill, muscle, brain. Daily length gain and daily weight gain was significantly decreased at high selenium exposure concentrations. Condition factor was slightly decrease at 200 and 400 ㎍/L after 4 weeks. A considerable decrease in hepatosomatic index was observed at 400 ㎍/L after 2 weeks and 4 weeks. In hematological components, all components of RBC count, hematocrit, and hemoglobin observed a significant decrease. In inorganic components, there was no change in calcium, but a slight decrease was observed at 200 and 400 ㎍/L after 4 weeks. In organic components, glucose was increased, whereas albumin was considerably decreased. There was no change in cholesterol and total protein. In enzyme components, GOT and GPT were increased, whereas ALP was decreased. In antioxidant enzyme analysis, SOD activity in liver and gill was significantly increased. GST activity in liver was considerably increased at

400 ㎍/L after 2 weeks and 4 weeks, and the activity in gill was significantly increased at the higher concentration of 200 ㎍/L after 4 weeks. GSH level in liver and gill was slightly increased at 400 ㎍/L after 2 weeks and 4 weeks. Acetylcholinesterase activity was significantly inhibited in brain and muscle, whereas there was no change in intestine tissues. In liver tissues, a significant decrease was observed at 400 ㎍/L after 4 weeks. In non-specific immune response, lysozyme activity in plasma and kidney was considerably increased at the higher concentration of 50 ㎍/L after 2 weeks and 4 weeks. Peroxidase activity was slightly decreased at 400 ㎍/L after 2 weeks and 4 weeks. Anti-protease activity was slightly decrease at 200 ㎍/L and 400 ㎍/L after 4 weeks.

Ⅵ. References

Adams, W.J. (1976) The toxicity and residue dynamics of selenium in fish and aquatic invertebrates. Ph.D. dissertation. Michigan State Univ., East Lansing.

Akerboom, T.P.M., Sies. H. (1981) Assay of glutathione disulfide and glutathione mixed disulfide and glutathione mixed disulfide in biological samples. Methods Enzymol. 71, 373-382.

Almeida, E.A., Bainy, A.C.D., Loureiro, A.P.M., Martinez, G.R., Miyamoto, S., Onuki, J., Barbosa, L.F., Garcia, C.C.M., Prado, F.M., Ronsein, G.E., Sigolo, C.A., Brochini, C.B., Martins, A.M.G., Medeiros, M.H.G., Mascio, P.D. (2007) Oxidative stress in Perna perna and other bivalves as indicators of environmental stress in the Brazilian marine environment: Antioxidants, lipid peroxidation and DNA damage. Comparative Biochemistry and Physiology 146, 588-600.

Arkoosh, M.R., Casillas, E., Clemons, E., McCain, B., Varanasi, U. (1991) Suppression of immunological memory in juvenile chinnok salmon (Oncorhynchus tshawytscha) from an urban estuary. Fish and Shellfish Immunology 1, 261-278.

Arthur, J.R., Mckenzie R.C. & Beckett, G.J. (2003) Selenium in the Immune System. The journal of nutrition 133: 1457S-1459S.

Atencio, L., Moreno, I., Jos, Á., Prieto, A.I., Moyano, R., Blanco, A., Cameán, A.M. (2009) Effects of dietary selenium on the oxidative stress and pathological changes in tilapia (Oreochromis niloticus) exposed to a microcystin-producing cyanobacterial water bloom.

Toxicon 53, 269-282.

Atli, G., Canli, M. (2007) Enzymatic resposes to metal exposures in a freshwater fish *Oreochromis niloticus*.

Bainy, A.C.D., Marques, M.R.F. (2003) Global analysis of biomarker responses in Aquatic Organisms Exposed to Contaminants. Comments on Toxicology 9, 271-278.

Bayne, C.J., Gerwick, L. (2001) The acute phase response and innate immunity of fish. Developmental and Comparative Immunology 25, 725-743.

Bell, J., Pire, B., Adron, J., Cowey, C. (1986) Some effects of selenium deficiency on glutathione peroxidase activity and tissue pathology in rainbow trout (*Salmo gairdneri*). British Journal of Nutrition 55, 305-311.

Bendell-Young, L. and Harvey, H.H. (1989) Concentrations and distribution of Fe, Zn and Cu in Tissues of the White Sucker in relation to elevated levels of metals and low pH. Hydrobiologia. 176/177, 349-354.

Benifey T.J. and Biron M. (2000) Acute stress response in triploid rainbow trout (*Onchrhynchus mykiss*) and brook trout (*Salvelinus fontinalis*). Aquaculture. 184. 167-176.

Besser, J.M., Giesy, J.P., Brown, R.W., Buell, J.M., Dawson, G.A. (1996) Selenium bioaccumulation and hazards in a fish community affected by coal fly ash effluent. Ecotoxicology and Environmental Safety 35, 7-15.

Bhaskaram, P. (2002) Micronutrient malnutrition, infection, and immunity:

an overview. Nutr. Rev. 60: S40-S45.

Blasco, J., Puppo, J (1999) Effect of heavy metals (Cu, Cd and Pb) on aspartate and alanine aminotransferase in Ruditapes philippinarum (Mollusca: Bivalvia). Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology 122(2), 253-263.

Bocquene, G., Galgani, F., Truquet, P. (1990) Characterization and assay conditions for use of AChE acticvity from several marine species in pollution monitoring. Marine Environmental Research 30, 75-89.

Boge, G., Leydet, M., Houvet, D. (1992) The effects of hexavalent chromium on the activity of alkaline phosphatase in the intestine of rainbow trout (*Oncorhynchus mykiss*). Aquatic Toxicology 23, 247-260.

Bols, N.C., Brubacher, J.L., Ganassin, R.C., Lee, L.E.J. (2001) Ecotoxicology and innate immunity in fish. Developmental and Comparative Immunology 25, 853-873.

Bretaud, S., Toutant, J.P., Saglio, P. (2000) Effects of carbofuran, diuron and nicosulfuron on acetylcholinesterase activity in goldfish (Carassius auratus). Ecotoxicology and Environmental Safety 47, 117-124.

Brix, K. V., Deforest, D.K., Fairbrother, A., Adams, W.J. (2000) Critical review of tissue-based selenium toxicity thresholds for fish and birds.

Canton, S.P., Van Derveer, W.D. (1997) Selenium toxicity to aquatic life: An argument for sediment-based water quality criteria. Environmental toxicology and Chemistry, Vol. 16, No. 6, pp. 1255-1259.

Cardwell, R.D., Foreman, D.G., Payne, T.R., Wilbur, D.J. (1976) Acute

toxicity of selenium dioxide to freshwater fishes. Archives of Environmental Contamination and Toxicology Vol. 4, 129-144.

Cavaletto, M., Ghezzi, A., Burlando, B., Evangelisti, V., Ceratto, N., Viarengo, A. (2002) Effect of hydrogen peroxide on antioxidant enzymes and metallothionein level in the digestive gland of *Mytilus galloprovincialis*. Comparative Biochemistry and Physiology Part C 131, 447-455.

Chang, Y.J., Hur, J.W., Lim, H.K., Lee, J.K. (2001) Stress in olive flounder (Oaralichthys olivaceus) and fat cod (Hexagrammos otakii) by the sudden drop and rise of water temperature. J. Korean. Fish. Soc. 34, 91-97.

Chuiko, G.M., Zhelnin, Y., Pod'gornaya, V.A. (1997) Seasonal fluctuations in brain acetylcholinesterase activity and soluble protein content in Roach (*Rutilus rutilus* L.): A Freshwater fish from Northwest Russia. Comp. Biochem. Physiol. 117(3) 251-257.

Crane, M., Flower, T., Holmes, D., Watson, S. (1992) The toxicity of selenium in experimental freshwater ponds. Archives of Environmental Contamination and Toxicology. 23, 440-452.

Cuesta, A., Rodríguez, A., Esteban, M.Á., Meseguer, J. (2005) In vivo effects of propolis, a honeybee product, on gilthead seabream innate immune responses. Fish & Shellfish Immunology 18, 71-80.

Dalmo, R.A., Ingebrigtsen, K., Bøgwald, J. (1997) Non-specific defence mechanisms in fish, with particular reference to the reticuloendothelial system (RES). Journal of Fish Diseases 20, 241-273.

Davies, K.J.A. (2000) Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems. IUBMB Life 50, 279-289.

Dörr, A.J.M., Pacini, N., Abete, M.C., Prearo, M. (2008) Effects of a selenium-enriched diet on antioxidant response in adult crayfish (*Procambarus clarkii*). Chemosphere 73, 1090-1095.

Elia, A.C., Prearo, M., Pacini, N., Dörr, A.J.M., Abete, M.C. (2011) Effects of selenium diets on growth, accumulation and antioxidant response in juvenile carp. Ecotoxicology and Environmental Safety 74, 166-173.

Ellis, A.E. Lysozyme assay. In: Stolen JS, Fletcher TC, Anderson DP, Roberson BS, van Muiswinkel WB, editors. Techniques in fish immunology. NJ: SOS Publications, 1990:197.

Ellis, A.E. (1999) Immunity to bacteria in fish. Fish & Shellfish Immunology 9, 291-308.

Ellis, A.E. (2001) Innate host defense mechanisms of fish against viruses and bacteria. Development and Comparative Immunology 25, 827-839.

Ellis, A.E. (2001) Innate host defense mechanisms of fish against virus and bacteria. Developmental & Comparative Immunology 25(8-9), 827-839.

Elia, A.C., Dörr, A.J.M., Mastrangelo, C., Prearo, M., Abete, M.C. (2006) Glutathione and antioxidant enzymes in the hepatopancreas of crayfish *Procambarus clarkii* (Girard, 1852) of Lake Trasimeno (Italy). B. Fr. Peche. Piscic. 380-381, 1351-1361.

Ellaman, G. L., Courtney, K. D., Andres, V., Featherstone, R. M. (1961) A

new and rapid colormetric determination of acetylcholinesterase activity. Biochemical Pharmacology. 7, 88-95.

Flammarion, P., Migeon, B., Garric, J. (1996) Joint effects of copper sulphate and Methidathion on Rainbow Trout (*Oncorhynchus mykiss*) EROD and AChE activity. Bull. Environ. Contam. Toxicol. 56, 440-445.

Fletcher, T.C. Modulation of nonspecific host defenses in fish. Vet Immunol Immunopathol 12, 59-67.

Frankenberger, W. T., Jr, Benson SL (eds) (1994) Selenium in the Environment. Marcel Dekker Inc, New York.

Ganther, H.E. (1974) Biochemistry of selenium. In: Zingaro, R.A., Cooper, W.C. (Eds.), Selenium. Van Nostrand Rein-hold, New York, NY, pp. 546-614.

Goldstein, R.M., DeWeese, L.R. (1999) Comparison of trace element concentrations in tissue of common carp and implications for monitoring. Journal of the American Water Resources Association.

Habig, W.H., Pabst, M.J., Jakoby, W.H. (1974) Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130-7139.

Hakanson, L. (1980) The quantitative impact of pH, bioproduction and Hg-contamination on the Hg-contant of fish (pike). Environ. Poll. 18, 285-304.

Halter, M.T., Adams, W.J., Johnson, H.E. (1980) Selenium toxicity to Daphnia magna, Hyallela azteca, and the Fathead Minnow in Hard

Water. Environmental contamination and Toxicology. 24, 102-107.

Hamilton, S.J., Lemly, A.D. (1999) Commentary Water-Sediment Controversy in setting environmental standards for selenium. Ecotoxicology and Environmental Safety 44, 227-235.

Hamilton, S.J., Holley, M., Buhl, K.J., Bullard, F.A., Weston, L.K., McDonald, S.F. (2002) Toxicity of selenium and other elements in food organisms to razoback sucker larvae. Aquat. Toxicol. 59, 253-281.

Hamilton, S.J. (2003) Review of selenium toxicity in the aquatic food chain. Science of the Total Environment 326 (2004) 1-31.

Hamilton, S.J. (2004) Review of selenium toxicity in the aquatic food chain. Science of the Total Environment 326:1-31.

Hoang, T.C., Klaine, S.J. (2008) Characterizing the toxicity of pulsed selenium exposure to *Daphnia magna*. Chemosphere 71, 429-438.

Hodson, P.V., Spray, D.J., Blunt, B.R. (1980) Effects on rainbow trout (Salmo gairdneri) of a chronic exposure to waterborne selenium. Canadian Journal of Fisheries and Aquatic Sciences 37(2), 233-240.

Hodson, P.V., Hilton, J.W. (1983) The nutritional requirements and toxicity to fish of dietary and waterborne selenium. Environmental Biogeochemistry 35, 335-340.

Hodson, P.V., Hilton, J.W., Slinger, S.J. (1986) Accumulation of waterborne selenium by rainbow trout (*Salmo gairdneri*), eggs, fry and juveniles. Fish Physiology and Biochemistry vol.1 no. 4 pp 187-196.

Holland, H.T., Coppage, D.L., Butler, P.A. (1967) Use of fish brain acetylcholinesterase to monitor pollution by Organophosphorus Pesticides. Bulletin of Environmental Contamination & Toxicology 2(3).

Hontela, A., Daniel, C., Richard, A.C. (1996) Effects of acute and subacute exposure to cadmium on the interrenal and thyroid function in rainbow trout (*Oncorhynchus mykiss*). Aquatic Toxicology 35, 171-182.

Hur, J.W., Chang, Y.J., Lim, H.K., Lee, B.K. (2001) Stress responses of cultured fishes elicited by water level reduction in rearing tank and fish transference during selection process. J. Korean. Fish. Soc. 34, 465-472.

Hutchinson, T.H., Manning, M.J. (1996) Seasonal trends in serum lysozyme activity and total protein concentration in dab (*Limanda limanda* L.) sampled from Lyme Bay, U.K. Fish & Shellfish Immunology 6, 473-482.

Hutchinson, T.H., Field, M.D.R., Manning, M.J. (2003) Evaluation of non-specific immune functions in dab, *Limanda limanda* L., following short-term exposure to sediments contaminated with polyaromatic hydrocarbons and/or polychlorinated biphenyls. Marine Environmental Research 55, 193-202.

Jebali, J., Banni, M., Guerbej, H., Almeida, E.A., Bannaoui, A., Boussetta, H. (2006) Effects of malathion and cadmium on acetylcholinesterase activity and metallothionein levels in the fish *Seriola dumerilli*. Fish Physiol Biochem 32, 93-98.

Jovanovic, A., Grubor-Lajsic, G., Djukic, N., Gardinovacki, G., Matic, A., Spasic, M. (1997) The effect of selenium on antioxidant system in

erythrocytes and liver of the carp (*Cyprinus carpio*L.). Crit. Rev. Food. Sci. 37, 443-448.

Karrow, N.A., Boermans, H.J., Dixon, D.G., Hontella, A., Solomon, K.R., Whyte, J.J.,Bols, N.C. Characterizing the immunotoxicity of creosote to rainbow trout (Oncorhynchus mykiss): a microcosm study. Aquatic Toxicology 45, 223-239.

Kawai, K. (1959) The cytochrome system in marine lamellibranch tissues. Biol Bull 117:125.32.

Kelly, S.A., Havrilla, C.M., Brady, T.C., Abramo, K.H., Levin, E.D. (1998) Oxidative stress in toxicology: established mammalian and emerging piscine model systems. Environ. Health Persp. 106, 375-384.

Kennedy, C.J., Sweeting, R.M., Farrell, A.P., Mckeown, B.A. (1995) Acute effects of chlorinated resin exposure on juvenile rainbow trout, Oncorhynchus mykiss. Environmental Toxicology Chemistry 14, 977-982.

Kennedy, C.J., McDonald, L.E., Loveridge, R., Strosher, M. (2000) The effect of bioaccumulated selenium on mortalities and deformities in the eggs, larvae, and fry of a wild population of Cutthroat Trout (*Oncorhynchus clarki lewisi*). Archives of Environmental Contamination and Toxicology. 39, 46-52.

Kim, H.G., Park, K.N., Cho, Y.W., Park, E.H., Fuchs, J.A., Lim, C.J. (2001) Characterization and regulation of glutathione S-transferase gene from *Schizosaccharomyces pombe*. Biochemica et Biophysica Acta 1520, 179-185.

Kojadinovic, J., Potier, M., Corre, M.L., Cosson, R.P., Bustamante, P. (2007) Bioaccumulation of trace elements in pelagic fish from the Western Indian Ocean. Environmental Pollution 146, 548-566.

Lan, W.G., Wong, M.K., Chen, N., Sin, Y.M. (1995) Effect of combined copper, zinc, chromium and selenium by orthogonal array design on alkaline phosphatase activity in liver of the red sea bream, *Chrysophrys major*. Aquaculture 131, 219-230.

Lange, A., Ausseil, O., Segner, H. (2002) Alterations of tissue glutathione levels and metallothionein mRNA in rainbow trout during single and conbined exposure to cadmium and zinc. Comparative Biochemistry and Physiology Part C 131(3), 231-243.

Lange, S., Gudmundsdottir, B.K., Magnadottir, B. (2001) Humoral immune parameters of cultered Atlantic halibut (*Hippoglossus hippoglossus* L.). Fish & Shellfish Immunology 11, 523-535.

Lawrence, G.S., Chapman, P.M. (2007) Human health risks of selenium-contaminated Fish: A case study for risk assessment of essential elements. Human and Ecological Risk Assessment, 13:1192-1213.

Lawrence, R.A., Bu가, R.F. (1976) Glutathione peroxidase activity in selenium deficient rat liver. Biochem. Biophys. Res. Commun. 71, 592-598.

Lee, S., Lee, J.H., Bai, S.C. (2008) A preliminary study on effects of different dietary selenium (Se) levels on growth performance and toxicity in Juvenile Black Seabream, *Acathopagrus schlegeli* (Bleeker). Asian-Aust. J.Anim. Sci. 21(12), 1794-1799.

Lee, S., Lee, J., Bai, S.C. (2010) Evaluation of the Dietary Toxic Level of Selenium (Se) in Juvenile Olive Flounder, *Paralichthys olivaceus*. Journal of the world aquaculture society 41, 245-254.

Lemly, A.D. (1982) Response of juvenile centrarchids to sublethal concentrations of waterborne selenium. Aquatic Toxicology 2, 235-252.

Lemly, A.D. (1992) Guidelines for evaluating selenium data from aquatic monitoring and assessment studies.

Lemly, A.D. (1993) Metabolic stress during winter increases the toxicity of selenium to fish. Aquatic Toxicology 27, 133-158.

Lemly, A.D. (1999) Selenium Impacts on fish : An insidious time bomb. Human and Ecological Risk Assessment 5(6), 1139-1151.

Lemly, A.D. (1995) A Protocol for Aquatic Hazard Assessment of Selenium. Ecotoxicology and Environment Safety 32, 280-288.

Lemly, A.D. (2001). Symptoms and implications of selenium toxicity in fish: the Belews Lake case example. Aquatic Toxicology 57(2002) 39-49.

Lemly, A.D. (2002) Selenium assessment in aquatic ecosystems - A quide for hazard evaluation and water quality criteria. New York: Springer-Verlag.

Lemly, A.D. (2004) Aquatic selenium pollution is a global environmental safety issue. Ecotoxicology and Environmental Safety 59, 44-56.

Li, H., Zhang, J., Wang, T., Luo, W., Zhou, Q., Jiang, G. (2008) Elemental selenium particles at nano-size (Nano-Se) are more toxic to Medaka (Oryzias latipes) as a consequence of hyper-accumulation of selenium: A comparison with sodium selenite. Aquatic Toxicology 89, 251-256.

Lillie, R.D., Smith, M.I. (1940) Histogenesis of hepatic cirrhosis in chronic food selenosis. Am.J.Path. 16, 223.

Lin, C.W., Sasaki, M., Orcutt, M.L., Migayama, H., Singer, K.M. (1976) Plasma phosphatases in the Hela cells. J. Histochem. Cytochem. 23, 659-664.

Lopes, P.A., Pinheiro, T., Santos, M.C., Mathias, M.D.L., Collares-Pereira, M.J., Viegas-Crespo, A.M. (2001) Response of antioxidant enzymes in freshwater fish populations (*Leuciscus alburnoides* complex) to inorganic pollutants exposure. Science of The Total Environment 280, 153-163.

Low, K.W., Sin, Y.M. (1998) Effects of mercuric chloride and sodium selenite on some immune responses of blue gourami, *Trichogaster trichopterus* (Pallus) The Science of the Total Environment 214, 153-164.

Magnadóttir, B., Jónsdóttir, H., Helgason, S., Björnsson, B., Jørgensen, T.& Pilström, L. (1990). Humoral immune parameters in Atlantic cod (Gadus morhua L.) I. The effects of environmental temperature. Comparative Biochemistry and Physiology 112B, 173-180.

Magnadóttir, B., Jónsdóttir, H., Helgason, S., Björnsson, B., Jørgensen, T.Ø., Pilström, L. (1999) Humoral immune parameters in Atlantic cod

(Gadus morhua L.) Ⅰ. The effects of environmental temperature. Comparative Biochemistry and Physiology Part B 122, 173-180.

Magnadóttir, B., Jónsdóttir, H., Helgason, S., Björnsson, B., Solem, S.T., Pilström, L. (2001) Immune parameters of immunised cod (*Gadus morhua* L). Fish & Shellfish Immunology 11, 75-89.

Magnadóttir, B. (2006) Innate immunity of fish (overview). Fish & Shellfish Immunology 20, 137-151.

Maier, K.J., Nelson, C.R., Bailey, F.C., Klaine, S.J., Knight, A.W. (1998) Accumulation of selenium by the aquatic biota of a watershed treated with seleniferous fertilizer. Environmental Contamination and Toxicology, 60:409-416.

Maier, K.J., Knight, A.W. (1993) Comparative acute toxicity and bioconcentration of selenium by the midge *Chironomus decorus* exposed to selenate, selenite, and seleno-DL-methionine. Archives of Environmental Contamintaion and Toxicology 25, 365-370.

Manzo, L., Castoldia, A.F., Coccinia, T., Rossia, A.D., Nicoteraa, P., Costaa, L.G. (1995) Mechanisms of neurotoxicity: applications to human biomonitoring. Toxicology Letters 77(1-3), 63-72.

Maritim, A.C., Sanders, R.A., Watkins Ⅲ, J.B. (2002) Diabetes, Oxidative stress, and Antioxidants: A Review. J Biochem Molecular Toxicology 17, 24-38.

Martínez-Álvarez, R.M., Morales, A.E., Sanz, A. (2005) Antioxidant defenses in fish: Biotic and abiotic factors. Reviews in Fish Biology and Fisheries 15, 75-88.

Martinez-Tabche, L., Mora, B.R., Faz, C.G., Lopez, E.L., Martinez, M.G. (2001) Hemoglobin concentration and acetylcholinesterase activity of Oligochaetes in ralation to lead concentration in spiked sediments from Ignacio Ramirez Reservoir. Ecotoxicology and Environmental Safety 49, 76-83.

McCarty, L.S., Mackay, D. (1993) Enhancing ecotoxicological modeling and assessment. Body residues and modes of toxic action. Environ. Sci. Techol. 27, 1718-1728.

McKenzie, R.C., Rafferty, T.S., Beckett, G.J. (1998) Selenium: an essential element for immune function. TREMDS Immunology today Vol.19, No.8, pp. 342-345.

Mi-Hee C. (2003) Determination and treatment of selenium in wastewater.

Miller, L.L., Hontela, A. (2011) Species-specific sensitivity to selenium-induced impairment of cortisol secretion in adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*). Toxicology and Applied Pharmacology 253, 137-144.

Miller, L.L., Rasmussen, J.B., Palace, V.P., Hontela, A. (2009) The physiological stress response and oxidative stress biomarkers in rainbow trout and brook trout from selenium-impacted streams in a coal mining region. Journal of Applied Toxicology 29, 681-688.

Miller, L.L., Rasmussen, J.B., Palace, V.P., Hontela, A. (2009) Physiological stress response in white suckers from agricultural drain waters containing pesticides and selenium. Ecotoxicology and

Environmental Safety 72, 1249-1256.

Miller, L.L., Wang, F., Palace, V.P., Hontela, A. (2007) Effects of acute and subchronic exposures to waterborne selenite on the physiological stress response and oxidative stress indicators in juvenile rainbow trout. Aquatic Toxicology 83, 263-271.

Miller, L.L. (2006) The effects of selenium on the physiological stress response in fish.

Miron, D.D.S., Crestani, M., Shettinger, M.R., Morsch, V.M., Baldisserotto, B., Tierno, M.A., Moraes, G., Vieira, V.L.P. (2005) Effects of the herbicides clomazone, quinclorac, and metsulfuron methyl on acetylcholinesterase activity in the silver catfish (*Rhamdia quelen*) (Heptapteridae). Ecotoxicology and Environmental Safety 61, 398-403.

Misra, S., Niyogi, S. (2009) Selenite causes cytotoxicity in rainbow trout (Oncorhynchus mykiss) hepatocytes by inducing oxidative stress. Toxicology in Vitro 23, 1249-1258.

Modesto, K.A., Martinez, C.B.R. (2010) Roundup causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish *Prochilodus lineatus*. Chemosphere 78, 294-299.

Mohamed, S., Kheireddine, O., Wyllia, H.M., Roquia, R., Aicha, D., Mourad, B. (1994) Proportioning of Biomarkers (GSH, GST, Ache, Catalase) Indicator of pollution at *Gambusia affinis* (Teleostei Fish) Exposed to Cadmium.

Moxon, A.L., Rhian, M. (1943) Selenium poisoning. Physiol. Rev. 23, 305.

Nordberg, J., Arnér, E.S.J. (2001) Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radical Biology & Medicine 31(11), 1287-1312.

Ogle, R.S., Maier, K.J., Kiffney, P., Williams, M.J., Brasher, A., Melton, L.A., Knight, A.W. (1988) Bioaccumulation of selenium in aquatic ecosystems. Lake and Reservoir Management 4(2), 165-173.

Ogle, R.S., Knight, A.W. (1996) Selenium bioaccumulation in aquatic ecosystems: 1.Effects of sulfate on the uptake and toxicity of selenate in *Daphnia magna*. Environmental Contamination and Toxicology 30, 274-279.

Olsen, R.E., Sundell, K., Mayhew, T.M., Myklebust, R., Ringø, E. (2005) Acute stress alters intestinal function of rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquaculture 250, 480-495.

Önning, G. (2000) Separation of soluble selenium compounds in different fish species. Food Chemistry 68, 133-139.

Paris-Palacios, S., Biagianti-Risbourg, S., Vernet, G. (2000) Biochemical and (ultra) structural hepatic perturbations of *Brachydanio rerio* (Teleostei, Cyprinidae) exposed to two sublethal concentrations of copper sulphate. Aquatic Toxicology 50, 109-124.

Peña, S., Peña, J.B., Sancho, E., Fernández, C., Ferrando, M.D. (2000) Role of glutathione in thiobencarb resistance in the European eel *Anguilla anguilla*. Ecotoxicology and Environmental Safety 45, 51-56.

Peña-Llopis, S., Peña, J.B., Sancho, E., Fernandez-Vega, C., Ferrando, M.D. (2001) Glutathione-dependent resistance of the European eel

Auguilla anguilla to the herbicide molinate. Chemosphere 45, 671-681.

Peña-Llopis, S., Ferrando, M.D., Peña, J.B. (2003) Fish tolerance to organophosphate-induced oxidative stress is dependent on the glutathione metabolism and enhanced by *N*-acetylcysteine. Aquatic Toxicology 65, 337-360.

Pulsford, A.L., Thomas, M.E., Lemaire-Gony, S., Coles, J., Fossato, V.U., Pipte, R.K. (1995) Studies on the immune systemn of the goby, *Zosterisessor ophiocephalus*, from the Venice Lagoon. Marine Pollution Bulletin 30, 586-591.

Quade MJ, Roth JA (1997) A rapid, direct asssay to measure degranulation of bovine neutrophil primary granules. Vet Immunol Immunopathol 58: 239-248.

Rand, G. M., Wells, P. G. & Mccarthy, L. S. (1995) Introduction to aquatic toxicology. In: G. M. Rand (ed.). Fundamentals of aquatic toxicology: effects, environmental fate and risk assessment. 2nd ed. Taylor & Francis, Washington, DC.

Reddy, C.C., Massaro, E.J. (1983) Biochemistry of selenium: an overview. Fundamental and Applied Toxicology 3, 431-436.

Richetti, S.K., Rosemberg, D.B., Ventura-Lima, J., Monserrat, J.M., Bogo, M.R., Bonan, C.D. (2011) Acetylcholinseterase activity and antioxidant capacity of zebrafish brain is altered by heavy metal exposure. NeuroToxicology 32, 116-122.

Sato, T., Ose, Y., Sakai, T. (1980) Toxicological effect of selenium on fish. Environmental Pollution Series A 21(3), 217-224.

Secombes, C.J., Tahir, A., Stagg, R. (1997) Immunocompetence in flatfish as a measure of the biological effects of exposure to sewage sludge or hydrocarbon contaminated sediments. Ecotoxicology, 281-292.

Silva, A.Z., Zanette, J., Ferreira, J.F., Guzenski, J., Marques, M.R.F., Bainy, A.C.D. (2005) Effects of salinity on biomarker responses in *Crassostrea rhizophorae* (Mollusca, Bivalvia) exposed to diesel oil. Ecotoxicology Environment 62(3), 376-382.

Sorensen, E. M. B., Cumbie, P. M., Bauer, T. L., Bell, J. S. and Harlan C. W. (1984) Histopathological, hematological, condition-factor, and organ weight changes associated with selenium accumulation in fish from Belews Lake, North Carolina. Archieves of Environmental Contamination and Toxicology 13, 153-162.

Spallholz, J.E., Hoffman D.J. (2002) Selenium toxicity: cause and effects in aquatic birds. Aquatic Toxicology 57, 27-37.

Spallholz, J.E., Palace, V.P., Reid, T.W. (2004) Methioninase and selenomethionine but not Se-methylselenocysteine generate methylselenol and superoxide in an in vitro chemiluminescent assay: implications for the nutritional carcinostatic activity of selenoamino acids. Biochemical Pharmacology 67(3), 547-554.

Stadtman, T.C. (1974) Selenium biochemistry. Science 183, 915-922.

Suhaimi F, Wong SP, Lee VLL, Low LK (2005) Heavy metals in fish and shellfish found in local wet markets. Singapore J Primary Ind 32, 1-18.

Sunde, R.A. (1984) The biochemistry of selenoproteins. Journal of the

American Organic Chemistry Society 61, 1891-1900.

Takayanagi, T. (2001) Acute toxicity of waterborne Se(IV), Se(VI), Se(III), and Se(V) on Red Seabream (*Pargus major*). Bull. Environ. Contam. Toxicol. 66, 808-813.

Tallandini, L., Cecchi, R., Boni, S.D., Galassini, S., Ghermandi, G., Gialanella, G., Liu, N., Moro, R., Turchetto, M., Zhang Y. (1996) Toxic levels of selenium in enzymes and selenium uptake in tissues of a marine fish. Biological Trace Element Research 51, 97-106.

Tapia-Paniagua, S.T., Reyes-Becerril, M., Ascencio-Valle, F., Esteban, M.Á., Clavijo, E., Balebona, M.C. (2011) Modulation of the intestinal microbiota and Immune system of farmed Sparus aurata by the administration of the Yeast *Debaryomyces hansenii* L2 in conjunction with inulin. Probiotic & Prebiotic Applications in Aquaculture.

Thilagam, H., Gopalakrishnan, S., Bo, J., Wang, K.J. (2009) Effect of 17β-estradiol on the immunocompetence of japanese sea bass (Lateolabrax japonicus). Environmental Toxicology and Chemistry 28, 1722-1731.

Tinggi. U. (2003) Essentiality and toxicity of selenium and its status in Australia: a review. Toxicology Letters 137, 103-110.

Torre, F.R., Ferrari, L., Salibián, A. (2002) Freshwater pollution biomaker: response of brain acetylcholinesterase activity in two fish species. Comparative Biochemistry and Physiology Part C 131, 271-280.

Tort, L., Kargacin, B., Torres, P., Giralt, M. Hidalgo, J. (1996) The effect of cadmium exposure and stress on plasma cortisol, metallothionein

levels and oxidative status in rainbow trout (Oncorhynchus mykiss) liver. Comparative Biochemistry and Physiology Part C 114, 29-34.

Turner, R. J. & Finch, J. M. (1991) Selenium and the immune response. Proc. Nutr. Soc. 50:275-285.

Vaglio, A., Landriscina, C. (1999) Changes in liver enzyme activity in the teleost *Sparus aurata* in response to cadmium intoxication. Ecotoxicology and Environmental Safety 43, 111-116.

Vinodhini, R., Narayanan, M. (2008) Bioaccumulation of heavy metals in organs of fresh water fish *Cyprinus carpio* (Common carp)

Vuilleumier, S., Pagni, M. (2002) The elusive role of bacterial glutathione S-transferases: new lessons from genomes. Appl. Microbiol. Biotechnol. 58, 138-146.

Vuilleumier, S. (1997) Bacterial glutathione S-transferases: What are they good for. Journal of Bacteriology, 1431-1441.

Wang, W., Wang, A., Zhang, Y. (2006) Effect of dietary higher level of selenium and nitrite concentration on the cellular defense response of *Penaeus vannamei*. Aquaculture 256, 558-563.

Waring, C.P., Stagg, R.M., Poxton, M.G. (1996) Physiological responses to handling in the turbot. J. Fish Biol. 48, 161-173.

Waska, H., Kim, S., Kim, G., Kang, M.R., Kim, G.B. (2008) Distribution patterns of chalcogens (S, Se, Td, and ^{210}Po) in various tissues of a squid, *Todarodes pacificus*. Science of the total Environment 392, 218-224.

Yano, T. (1996) The nonspecific immune system: humoral defense. In: Iwama, G., Nakanishi, T. (Eds.), The fish immune system: Organism, pathogen, and environment. Academic Press, San Diego, 105-157.