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Effects of dietary chromium exposure to rockfish, *Sebastes schlegelii* are ameliorated by ascorbic acid

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ABSTRACT

Juvenile rockfish *Sebastes schlegelii* (mean length 10.8 ± 1.4 cm, and mean weight 31.7 ± 3.6 g) were exposed for 4 weeks with the different levels of dietary chromium (Cr^{6+}) at 0, 120 and 240 mg/L and ascorbic acids (AsA) at 100, 200 and 400 mg/L. Significant accumulation occurred in specific tissues and hematological parameters were altered: red blood cell count, hematocrit, and hemoglobin increased; plasma components were altered including calcium, glucose, cholesterol, total protein, glutamic oxalate transaminase, and glutamic pyruvate transaminase. However, magnesium and alkaline phosphatase concentrations were unchanged. Ascorbic acids reduced both chromium uptake into tissues and altered hematological parameters.

1. Introduction

While chromium (Cr) can be a highly toxic metal, it is also a critical nutrient for aquatic animals. In aquatic environment, Cr exists in two main forms: trivalent chromium [Cr(III)] and hexavalent chromium [Cr(VI)]. Oxidized state, Cr(VI), is highly toxic due to its ability to be absorbed more easily in biological system through anion-exchange carriers than is Cr(III) (Dayan and Paine, 2001; Salnikow and Zhitkovich, 2008).

Hematological factors in aquatic animals have been widely considered as useful indicators of physiological and pathological changes in toxicological and environmental research seeking to evaluate influences of exposure to toxins (Adhikari et al., 2004). Fish blood parameters are good indicators of toxicity of aquatic environment owing to the close relationship between circulatory system and external environment. Moreover, blood parameters can be affected by various factors such as xenobiotic type, target species, and exposure concentration (Chandrasekara and Pathiratne, 2005). Fish physiological status, e.g. hematological factors, is known to be affected by metallic stress. For example, Cr(VI) exposure was shown to significantly affect hematological factors of Indian major carp, *Labeo rohita* (Vutukuru, 2005). Shaheen and Akhtar (2012) also reported blood can be a reliable parameter for measuring environmental toxicity because it is highly susceptible to alterations in the environment, and Cr(VI) has a negative effect on hematological and biochemical factors in aquatic animals. In addition, Mazon et al. (2002) reported that hematological and physiological alterations in fish can suggest the homeostatic status in fish exposed to environmental pollution.

Ascorbic acid (AsA) is a critical nutrient required for the proper metabolic function and immunity of aquatic animals, in addition to their growth and development (Wang et al., 2003). Moreover, AsA supplementation is known to aid in detoxification against contamination by various toxic metals (Kim and Kang, 2015a). AsA is a strong antioxidant, and its supplementation is considerably effective to attenuate Cr(VI) toxicity caused by reactive oxygen species formed during Cr(VI) reduction (Poljsak et al., 2005). AsA supplementation reduced metal retention in tissues caused by metal exposure (Kadrabova et al., 1992). AsA supplementation is also highly effective to attenuate metal-induced alterations in hematological and serum biochemical factors (Yousef, 2004). Ambali et al. (2007) reported that vitamin C has a protective function that can ameliorate damage to hematological factors of mice caused by chlorpyrifos.

Fish biomarkers have been considered useful tools in the following risk assessment procedures: effect, exposure and hazard assessment, risk characterization or classification, and monitoring aquatic ecosystems (Oost et al., 2003). Information on toxic effects of Cr(VI) on bioaccumulation and hematological factors, and detoxification effects of AsA for exposure of aquatic animals to Cr is limited. Given that rockfish, *Sebastes schlegelii*, is a widely consumed species in South Korea, research on toxic effects of Cr on *S. schlegelii* and ameliorative effects of AsA may be useful for identifying potential bioindicators for Cr toxicity in marine environment.

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

Item	Value
Temperature (°C)	19.0 ± 1.0
pH	8.1 ± 0.5
Salinity (‰)	33.2 ± 0.5
Dissolved oxygen (mg/L)	7.3 ± 0.4
Chemical oxygen demand (mg/L)	1.31 ± 0.3
Ammonia (μg/L)	11.0 ± 0.6
Nitrite (μg/L)	1.6 ± 0.3
Nitrate (μg/L)	9.41 ± 1.2

2. Materials and methods

2.1. Experimental fish and conditions

Juvenile *S. schlegelii* were obtained from a local fish farm in Tongyeong, Korea. Fish were acclimatized for 2 weeks under laboratory conditions. During the acclimation period, fish were fed a Cr-free diet twice daily and maintained on a 12-h:12-h light/dark cycle and constant condition at all times (Table 1). After acclimatization, 90 fish (body length, 10.8 ± 1.4 cm; body weight, 31.7 ± 3.6 g) were randomly selected for this study. Dietary chromium exposure took place in 500 L circular tanks with 5 fish per treatment group. Dietary chromium and ascorbic acid concentrations were 0, 120, and 240 mg/kg and 100, 200 and 400 mg/kg (Table 2), and fish were fed each chromium concentration at a rate of 2% body weight daily (as two 1% meals per day). Chromium concentration of 240 mg/kg seems to be unrealistic. However, the chromium concentration in the coast near the Incheon North Harbor, Korea reached 214 ppm (Um et al., 2003). Ascorbic acid requirement in *S. schlegelii* for growth and development is 65 mg/kg based on Standard Manual of Black Rockfish Culture (NFRDI, 2003). However, there is no research about the proper ascorbic acid requirement for detoxification. At the end of each period (at 2 and 4 weeks), fish were anesthetized in buffered 3-aminobenzoic acid ethyl ester methanesulfonate (Sigma Chemical, St. Louis, MO). Anesthetization concentration and time were different to fish species and size. In this study, anesthetization was conducted at 20 ppm concentration for 5 min.

2.2. Feed ingredients and diet formulation

Formulation of diets is shown in Table 2. Potassium dichromate and ascorbic acid were obtained from Sigma Chemical Co., Ltd. All diets contained 33% casein, 23% fish meal, 20% wheat flour, 5% corn starch, 2% vitamin premix (vitamin C-free), and 2% mineral premix. 10% fish oil was added to meet the essential fatty acid (EFA) requirements of *S. schlegelii*. Chromium premix was made up of 2 g chromium with 98 g cellulose. Chromium premix was added at different concentrations in diets for supplementation of different dietary chromium concentrations of 0, 120, and 240 mg/kg diet. Ascorbic acid premix was made up of 2 g ascorbic acids with 98 g cellulose. Three isonitrogenous and isolipidic diets were formulated supplementation of different dietary ascorbic acid concentrations of 100, 200 and 400 mg/kg. All ingredients were blended thoroughly. At last, water was added into the mixture to produce stiff dough. Then the dough was pelleted by experimental feed mill, and dried for 24 h at room temperature. After processing, all diets were packed and kept at −20 °C until use. The actual chromium and AsA concentrations are shown in Table 3. For determination of total chromium concentrations in diets, ICP-MS measurements were performed using an ELAN 6600DRC ICP-MS instrument with argon gas (Perkin-Elmer). Total chromium concentrations were determined by external calibration. ICP multi-element standard solution VI (Merck) was used to develop a standard curve. The chromium bioaccumulation in diet samples was expressed as mg/kg dry wt. To determine of total ascorbic acid concentrations in diets, HPLC measurements were performed using an Agilent 1200 series. The ascorbic acid content in diet samples was expressed mg/kg dry wt.

2.3. Chromium accumulation

Tissue samples of liver, kidney, spleen, intestine, gill, and muscle of *S. schlegelii* were removed using clean techniques and freeze-dried to measure the dry weight. The freeze-drying samples were digested by the wet digestion method (Arain et al., 2008). The dried samples were digested in 65%(*v/v*) HNO3, and re-dried at 120 °C on a hot plate. The procedure was repeated until total digestion. The entirely digested samples were diluted in 2%(*v/v*) HNO3. The samples were filtered through a 0.2 μm membrane filter (Advantec MFS, Ins., CA, USA) under pressure. For determination of total Cr concentrations, the digested and extracted solutions were analyzed by ICP-MS. The ICP-MS measure-

Table 2
Formulation of the experimental diet (% dry matter).

Ingredient (%)	Concentration (mg/kg)								
	M0V1	M0V2	M0V3	M1V1	M1V2	M1V3	M2V1	M2V2	M2V3
Casein[a]	33.0	33.0	33.0	33.0	33.0	33.0	33.0	33.0	33.0
Fish meal[b]	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0
Wheat flour[c]	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Fish oil[d]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Cellulose[a]	4.5	4.0	3.0	3.9	3.4	2.4	3.3	2.8	1.8
Corn starch[c]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin Premix (Vitamin C-free)[e]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Mineral Premix[f]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Chromium Premix[g]	0.0	0.0	0.0	0.6	0.6	0.6	1.2	1.2	1.2
Ascorbic acid Premix[h]	0.5	1.0	2.0	0.5	1.0	2.0	0.5	1.0	2.0

(M0: Pb 0 mg/kg, M1: Pb 120 mg/kg, M2: Pb 240 mg/kg, V1: AsA 100 mg/kg, V2: AsA 200 mg/kg, V3: AsA 400 mg/kg)

[a] United States Biochemical (Cleveland, OH).

[b] Suhyup Feed Co., Ltd., Gyeong Nam Province, Korea.

[c] Young Nam Flour Mills Co., Pusan, Korea.

[d] Sigma Chemical Co., St. Louis, MO.

[e] Vitamin Premix (vitamin C-free) (mg/kg diet): dl-calcium pantothenate, 400; choine chloride 200; inositol, 20; menadione, 2; nicotinamide, 60; pyridoxine·HCl, 44; riboflavin, 36; thiamine mononitrate, 120, dl-a-tocopherol acetate, 60; retinyl acetate, 20000IU; biotin, 0.04; folic acid, 6; vitamin B_{12}, 0.04; cholecalcifero, 4000IU.

[f] Mineral Premix (mg/kg diet): Al, 1.2; Ca, 5000; Cl, 100; Cu, 5.1; Co, 9.9; Na, 1280; Mg, 520; P, 5000; K, 4300; Zn, 27; Fe, 40; I, 4.6; Se, 0.2; Mn, 9.1.

[g] Chromium Premix (mg/kg diet): 20,000 mg Pb/ kg diet.

[h] Ascorbic acid Premix (mg/kg diet): 20,000 mg ascorbic acid/ kg diet.