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Changes in hematological parameters, plasma cortisol, and acetylcholinesterase of juvenile rockfish, *Sebastes schlegelii* supplemented with the dietary ascorbic acid

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ABSTRACT

Juvenile rockfish (mean length 13.6 ± 1.4 cm, and mean weight 53.6 ± 4.2 g) were fed diets containing different levels of ascorbic acid (0, 50, 100, 200, and 400 mg/kg) for 4 weeks. Fish were fed twice daily (08:30 and 18:30). The major hematological findings were significant increases in the red blood cell count, hematocrit value, and hemoglobin level in the rockfish fed diets over 100 mg/kg ascorbic acid. The dietary ascorbic acid supplementation caused significant increases in the glucose and total protein, whereas notable decreases were observed in GOT and GPT. However, alterations in calcium and magnesium were not observed by the ascorbic acid supplementation. Plasma cortisol was substantially decreased over 50 mg/kg ascorbic acid. Acetylcholinesterase activity of the rockfish was significantly increased over 200 mg/kg ascorbic acid. In conclusion, the present study indicates that the dietary ascorbic acid supplementation in the rockfish induces considerable increases in hematological parameters, alterations in plasma components, and increase in AChE activity.

1. Introduction

Ascorbic acid (vitamin C, AsA) is one of the most crucial nutrients for normal, physiological function, and immunity in aquatic animal species (Roberts et al., 1995; Ai et al., 2004; Lin and Shiau, 2005; Ren et al., 2007). A deficient dietary ascorbic acid in fish may cause a lot of negative symptoms such as the malformed spine, internal haemorrhaging, resorbed opercles, hyperplasia in jaw and snout, and late wound repair, as well as the decrease in growth and reduction in food intake and feed utilization (Roberts et al., 1995). Most teleost species need to require the supplementation of exogenous ascorbic acid, because they don't have an enzyme, L-gulonolactone which is required for biosynthesis of ascorbate from glucose (Rosenlund et al., 1990). Correlations between the dietary ascorbic acid supplementation in fish and several defense mechanisms against pathogens including environmental factors and general stress reactions were conducted in studies (Sandnes et al., 1990).

The assessment of hematological parameters in fish can be a good indicator to monitor physiological and pathological changes, because it offers fundamental information about the degrees of stress, metabolic abnormalities, reproductive dysfunctions, and diseases (Clauss et al., 2008; Buscaino et al., 2010). Many authors suggest that the evaluation of hematological values is critical to assess the health status in wild and cultured fish (Knowles et al., 2006; Fanouraki et al., 2007; Tavares-Dias and Moraes, 2007). However, it is difficult to interpret the blood parameters due to variations caused by both internal and external factors such as blood sampling, laboratory techniques, size, sex, population density, and environmental effects (Rey Vazquez and Guerrero, 2007). Cortisol is widely known as an important indicator to assess the stress by the various stressor factors such as toxicants and environment alterations (Barton, 2000). However, insufficient study was conducted on the correlation between ascorbic acid and cortisol.

Studies about ascorbic acid have insufficiently been conducted about the functions on the nervous system, whereas it is well known that dietary ascorbic acid is essential for the immunity in addition to the growth and development of fish. Ascorbic acid has been considered as a neuromodulator both dopamine- and glutamate- mediated neurotransmission (Grunewald, 1993; Rebec and Pierce, 1994). In fish, the ascorbic acid deficiency has influence on neurotransmitters, although clear mechanisms between ascorbic acid and neurotransmitters have not been reported (Harrison and May, 2009). Of various neurotransmitters, acetylcholine is one of the most critical neurotransmitters, and acetylcholinesterase (AChE) is a critical enzyme, which acts as a terminator of neuro-

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

Item	Value
Temperature (°C)	20.0 ± 0.5
pH	8.1 ± 0.5
Salinity (‰)	33.2 ± 0.5
Dissolved Oxygen (mg/L)	7.1 ± 0.3
Chemical Oxygen Demand (mg/L)	1.21 ± 0.1
Ammonia (μg/L)	11.7 ± 0.8
Nitrite (μg/L)	1.6 ± 0.2
Nitrate (μg/L)	10.31 ± 1.0

transmission process so as not to accumulate acetylcholine that may excessively stimulate nicotinic or muscarinic receptors (Taylor and Radic, 1994).

Considering the results of the present study, the dietary ascorbic acid supplementation should affect the hematological indicators and plasma cortisol in addition to the acetylcholinesterase activity. Rockfish, *Sebastes schlegelii* is an economically important fish species cultured in cage-aquaculture of South Korea due to its commercial value. But, the study about rockfish and the dietary ascorbic acid supplementation have not been sufficiently conducted. Therefore, the aim of the present study was to evaluate the effects of the dietary ascorbic acid on the hematological parameters, plasma cortisol, and neurotransmission in juvenile rockfish.

2. Materials and methods

2.1. Experimental fish and conditions

Juvenile rockfish were obtained from a local fish farm in Tongyeong, Korea. The fish were acclimatized for 2 weeks under laboratory conditions (water temperature: 20.0 °C, pH: 8.1, salinity: 33.2‰, dissolved oxygen: 7.1 mg/L, chemical oxygen demand: 1.21 mg/L, ammonia: 11.7 μg/L, nitrite: 1.6 μg/L, and nitrate: 10.31 μg/L). The experiment was conducted in exposure period for 4 weeks. During the experimental period, the fish were fed diets containing different levels of ascorbic acid diet twice daily and maintained on a 12-h:12-h light/dark cycle and constant condition at all times (Table 1). After acclimatization, 60 fish (body length, 13.6 ± 1.4 cm; body weight, 53.6 ± 4.2 g) were randomly selected for the study. The actual dietary ascorbic acid doses were 6.5, 41.8, 84.5, 166.2, and 343.5 mg/kg, and ascorbic acid doses in diets supplemented with L-ascorbic acid were determined by HPLC (Agilent 1200 series). Fish were fed each ascorbic acid feed at a rate of 2% body weight daily (as two 1% meals per day). During the feeding time, the intake state was monitored with the naked eye, and ensured that all fish were fed. Dietary ascorbic acid study took place in 500 L circular tanks containing 3 fish per treatment group in duplicates.

2.2. Feed ingredients and diets formulation

Formulation of the diets is shown in Table 2. AsA was obtained from Sigma Chemical Co., Ltd. All diets contained 33% casein, 23% fish meal, 5% corn starch, 2% vitamin premix (vitamin C free), and 2% mineral premix. 10% fish oil was added to meet the essential fatty acids (EFA) requirements of rock fish. Ascorbic acid premix was made up of 1 g AsA with 99 g cellulose. Five isonitrogenous and isolipidic diets were formulated with supplementation of different dietary AsA levels of 0, 50, 100, 200, and 400 mg AsA/kg diet. 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.

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

Ingredient (%)	Ascorbic acid concentration (mg/kg)				
	0	50	100	200	400
Casein[a]	33.0	33.0	33.0	33.0	33.0
Fish meal[b]	23.0	23.0	23.0	23.0	23.0
Wheat flour[c]	20.0	20.0	20.0	20.0	20.0
Fish oil[d]	10.0	10.0	10.0	10.0	10.0
Cellulose[a]	5.0	4.5	4.0	3.0	1.0
Corn starch[c]	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
Mineral Premix[f]	2.0	2.0	2.0	2.0	2.0
Ascorbic acid Premix[g]	0.0	0.5	1.0	2.0	4.0
Actual vitamin C levels	6.5	41.8	84.5	166.2	343.5

[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] Ascorbic acid Premix (mg/kg diet): 10,000 mg ascorbic acid/kg cellulose.

After processing, all the diets were packed and kept − 20 °C until use.

2.3. Blood samples and hematological assay

Blood samples were collected within 35–40 s through the caudal vein of the fish in 1-ml disposable heparinized syringes. The blood samples were kept at 4 °C until the blood parameters were completely studied. The total red blood cell (RBC) count, hemoglobin (Hb), concentration, and hematocrit (Ht) value were determined immediately. Total RBC counts were counted using optical microscope with 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 samples were centrifuged to separate plasma from blood samples at 3000*g* for 5 min at 4 °C. The plasma samples were analyzed for inorganic substances, organic substances, and enzyme activity using clinical kit (Asan Pharm. Co.,Ltd.). In inorganic substances assay, calcium and magnesium were analyzed by the o-cresolphthalein-complexon technique and xylidyl blue technique. Calcium was analyzed using clinical kit. Briefly, add 20 μL of plasma sample in 1 mL color reagent. Incubate 37 °C for 300 s, and read the optical density at 570 nm. Magnesium was also analyzed using clinical kit. Briefly, add 20 μL of plasma sample in 3 mL color reagent. Incubate at room temperature for 10 min, and read the optical density at 515 or 660 nm. In organic substances assay, glucose and total protein were analyzed by GOD/POD technique and biuret technique. In enzyme activity assay, glutamic oxalate transaminase (GOT) and glutamic pyruvate transaminase (GPT) were analyzed by Kind-king technique.

2.4. Plasma cortisol levels

Plasma cortisol concentration was measured with a monoclonal antibody enzyme-linked immunosorbent assay (ELISA) quantification kit (Enzo Life Sciences, Inc., NY, USA). Briefly, add 100 uL standard (156, 313, 625, 1250, 2500, and 5000 pg/ mL) and 100 μL samples in anti-Mouse Ig G microtiter plate. Add 50 μL assay buffer, 50 μL blue conjugate, and 50 μL yellow antibody, successively. After that, incubate the plate at room temperature on a plate shaker for 2 h at 500 rpm. And then, empty the contents of the wells and

wash by adding 400 μL wash solution 3 times, and dry until no moisture appears. After the final wash, add 5 μL blue conjugate and 200 μL pNpp substrate solution. Incubate at room temperature for 1 h without shaking. After that, add 50 μL stop solution, and read the optical density at 405 nm. The measurements were performed in triplicate.

2.5. Acetylcholinesterase activity

AChE activity was determined brain (1:25) and muscle (1:10) homogenate in 0.1 M phosphate buffer, pH 8.0. The homogenate were centrifuged 10,000*g* for 20 min at 4 °C. The supernatant was removed and used to test AChE activity. AChE 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.

2.6. Statistical analysis

No mortality was observed for the experimental periods. All analysis were performed triplicate (Total number were 60 fish, and the analysis was performed triplicate). Statistical analyses were performed using the SPSS/PC+ statistical package (SPSS Inc, Chicago, IL, USA). Significant differences between groups were identified using one-way ANOVA and Duncan's test for multiple comparisons or Student's *t*-test for two groups (Duncan, 1955). The significance level was set at $P < 0.05$.

3. Results

3.1. Hematological parameters

The RBC count, Ht value, and Hb concentration of the rockfish by the ascorbic acid supplementation are shown in Table 3. The major hematological findings were significant increases in RBC count and Hb concentration in the rockfish over 100 mg/kg AsA after 2 and 4 weeks. The Ht value for 4 weeks was significantly increased at 200 and 400 mg/kg.

The blood plasma components of the rockfish by the ascorbic acid supplementation are shown in Table 4. In inorganic components, it has no notable alteration in calcium and magnesium for 4 weeks. In organic components, glucose was considerably increased at 400 mg/kg after 2 weeks and at the higher concentrations of 200 mg/kg after 4 weeks. A significant increase in the total protein was observed at 400 mg/kg after 2 weeks and over 200 mg/kg after 4 weeks. In enzyme components, the GOT and GPT were significantly decreased by increasing the dietary ascorbic acid supplementation. A considerable decrease in the GOT was observed over 100 mg/kg at 2 and 4 weeks, and the GPT substantially decreased over 100 mg/kg after 2 weeks and 50 mg/kg after 4 weeks.

3.2. Plasma cortisol levels

Plasma cortisol levels in the rockfish by the ascorbic acid supplementation are shown in Fig. 1. Cortisol levels in plasma were decreased over 50 mg/kg after 2 and 4 weeks.

3.3. Acetylcholinesterase activity

AChE activities of the brain and muscle tissues in the rockfish by the ascorbic acid supplementation are demonstrated in Fig. 2. AChE activity in the brain was significantly increased at the higher concentrations of 200 mg/kg after 2 and 4 weeks, compared to control. Brain AChE was increased 19.7% at 400 mg/kg after 2 weeks and 41.9% at 400 mg/kg after 4 weeks, compared to control. Similarly, a significant increase in the muscle AChE activity was observed at 400 mg/kg after 2 weeks and over 200 mg/kg after 4 weeks. Muscle AChE was increased 21.4% at 400 mg/kg after 2 weeks and 34.8% at 400 mg/kg after 4 weeks, respectively.

4. Discussion

The hematological parameters indicate the physiological effects on the dietary ascorbic acid supplementation (Sandnes et al., 1990), and it can be a good indicator to assess the health status in fish (Fazio et al., 2012). In addition, ascorbic acid is a potent antioxidant, which protects most fish tissues against oxidative damage, and it also improves resistance in red blood cell membranes (Sahoo and Mukherjee, 2002; Pearce et al., 2003; Sau et al., 2004). Therefore, red blood cell values such as hemoglobin and RBC count should be a good indicator of oxidative status. Many authors reported a positive correlation between ascorbic acid supplementation and hematological parameters in fish. Andrade et al. (2007) reported a significant increase in the red blood cells count of *Arapaima gigas* by the dietary ascorbic acid supplementation of 800 and 1200 mg/kg, whereas the deficient ascorbic acid supplementation caused the decrease in hematocrit (Chagas and Val, 2003). Henrique et al. (1998) and Montero et al. (2001) reported alterations in the hematological parameters of gilthead seabream, *Sparus aurata* by the vitamin deficient diets. It was also reported that a significant increase in the hematocrit, hemoglobin, and RBC count of *Arapaima gigas* by the dietary ascorbic acid supplementation (Menezes et al., 2006). In the present study, we observed significant increases in the RBC count, Ht value, and Hb concentration of the rockfish. Similar with previous studies, the dietary ascorbic acid supplementation to the rockfish causes considerable increases in the RBC count, Ht value, and Hb concentration.

The inorganic components in plasma, calcium and magnesium can be affected by the alterations of osmotic pressure in plasma (Waring et al., 1996; Hur et al., 2001). But, the change of calcium and magnesium in the plasma of the rockfish was not observed in this study. In the organic components in the plasma of the rockfish, glucose was increased at the high dietary ascorbic acid supplementation, and total protein was increased by the dietary ascorbic acid supplementation. Paolisso et al. (1994) suggested that the dietary ascorbic acid intake increases plasma ascorbic acid levels, and glucose disposal was elevated by vitamin C-mediated improvement of insulin action, which increases non-oxidative glucose metabolism. But, the ascorbic acid supplementation to *S. schlegelii* increased the plasma glucose, which may be a modulation of glucose homeostasis. The dietary ascorbic acid supplementation of the rockfish may affect the modulation of insulin action. The total protein concentration can be an indicator of the non-specific immune response in fish by the dietary ascorbic acid supplementation, because the supplementation increases the protein activity in the complement system (Sahoo and Mukherjee, 2002; Ai et al., 2004; Lin and Shiau, 2005). Therefore, the total plasma level of the rockfish may be influenced by the dietary ascorbic acid supplementation. Menezes et al. (2006) also reported a notable increase in the total plasma of pirarucu, *Arapaima gigas*, whereas no considerable alteration in the total protein levels in fish by the dietary ascorbic acid supplementation was reported (Hardie et al., 1991; Roberts et al., 1995). Plasma GOT and GPT are indicators to assess liver damage. Many authors reported that lower GOT level of Japanese flounder, *Paralichthys olivaceus* by the dietary ascorbic acid supplementation over 500 mg/kg (Gao et al., 2014) and reduced the GOT value of Japanese eel, *Anguilla japonica* by the high dose of dietary ascorbic acid supplementation (Ren et al., 2007). On the other hand, the excess dietary ascorbic acid supplementation can induce the increase in GOT level by a poor condition of liver tissue, because it causes lipid peroxidation (Gao et al., 2014). The dietary ascorbic acid supplementation of the

Table 3
Changes of RBC count, Hematocrit and Hemoglobin in rockfish, *Sebastes schlegelii* fed diets containing different levels of ascorbic acids for 4 weeks.

Parameters	Period (week)	Ascorbic acid concentration (mg/kg)				
		0	50	100	200	400
RBC count ($\times 10^4$ mm^3)	2	197.6 ± 10.5^{a}	214.8 ± 12.5^{a}	246.6 ± 14.8^{b}	259.4 ± 13.1^{b}	261.7 ± 10.4^{b}
	4	183.5 ± 14.1^{a}	205.3 ± 16.4^{ab}	228.7 ± 10.9^{b}	267.5 ± 11.5^{c}	263.8 ± 15.7^{c}
Hematocrit (%)	2	36.4 ± 4.7^{a}	38.4 ± 5.1^{ab}	41.7 ± 4.2^{ab}	45.6 ± 3.5^{b}	46.2 ± 5.2^{b}
	4	34.2 ± 3.9^{a}	35.5 ± 4.2^{a}	42.3 ± 3.5^{ab}	44.3 ± 5.3^{b}	45.7 ± 5.7^{b}
Hemoglobin (g/dL)	2	5.72 ± 0.52^{a}	6.19 ± 0.46^{ab}	6.86 ± 0.33^{bc}	7.35 ± 0.42^{c}	7.48 ± 0.68^{c}
	4	5.81 ± 0.68^{a}	6.32 ± 0.37^{ab}	7.08 ± 0.49^{bc}	7.60 ± 0.38^{c}	7.57 ± 0.41^{c}

Values are mean ± S.E. Values with different superscript are significantly different at 2 weeks and 4 weeks ($P < 0$ 0.05) as determined by Duncan's multiple range test.

Table 4
Changes of plasma parameters in rockfish, *Sebastes schlegelii* fed diets containing different levels of ascorbic acids for 4 weeks.

Parameters	Period (week)	Ascorbic acid concentration (mg/kg).				
		0	50	100	200	400
Calcium (mg/dL)	2	19.4 ± 2.2^{a}	19.8 ± 2.0^{a}	20.2 ± 2.2^{a}	20.4 ± 1.9^{a}	19.8 ± 2.1^{a}
	4	20.3 ± 1.8^{a}	19.9 ± 2.4^{a}	19.6 ± 2.5^{a}	21.3 ± 2.4^{a}	20.1 ± 2.0^{a}
Magnesium (mg/dL)	2	3.71 ± 0.36^{a}	3.70 ± 0.26^{a}	3.94 ± 0.29^{a}	3.85 ± 0.35^{a}	3.87 ± 0.25^{a}
	4	3.76 ± 0.28^{a}	3.72 ± 0.33^{a}	3.84 ± 0.31^{a}	3.82 ± 0.33^{a}	3.83 ± 0.27^{a}
Glucose (mg/dL)	2	76.2 ± 4.8^{a}	73.3 ± 5.4^{a}	76.2 ± 4.6^{a}	79.3 ± 4.3^{ab}	82.8 ± 4.9^{b}
	4	73.9 ± 5.1^{a}	75.7 ± 4.5^{a}	76.6 ± 3.6^{a}	83.2 ± 4.1^{b}	83.6 ± 3.7^{b}
Total protein (g/dL)	2	4.46 ± 0.24^{a}	4.37 ± 0.31^{a}	4.58 ± 0.30^{a}	4.98 ± 0.21^{ab}	5.13 ± 0.21^{b}
	4	4.39 ± 0.18^{a}	4.50 ± 0.27^{a}	4.41 ± 0.24^{a}	5.10 ± 0.18^{b}	5.52 ± 0.29^{b}
GOT (karmen unit)	2	93.6 ± 5.7^{a}	89.4 ± 4.5^{a}	80.6 ± 4.4^{b}	75.6 ± 3.9^{b}	76.4 ± 4.2^{b}
	4	96.4 ± 4.3^{a}	94.9 ± 3.8^{a}	84.1 ± 5.2^{b}	75.1 ± 4.5^{c}	74.7 ± 3.7^{c}
GPT (karmen unit)	2	46.1 ± 2.4^{a}	43.2 ± 2.9^{ab}	40.7 ± 2.0^{bc}	37.5 ± 2.8^{c}	36.2 ± 2.6^{c}
	4	49.3 ± 1.7^{a}	45.7 ± 2.7^{b}	39.3 ± 1.8^{c}	36.4 ± 1.4^{c}	37.8 ± 1.9^{c}

Values are mean ± S.E. Values with different superscript are significantly different at 2 weeks and 4 weeks ($P < 0.05$) as determined by Duncan's multiple range test.

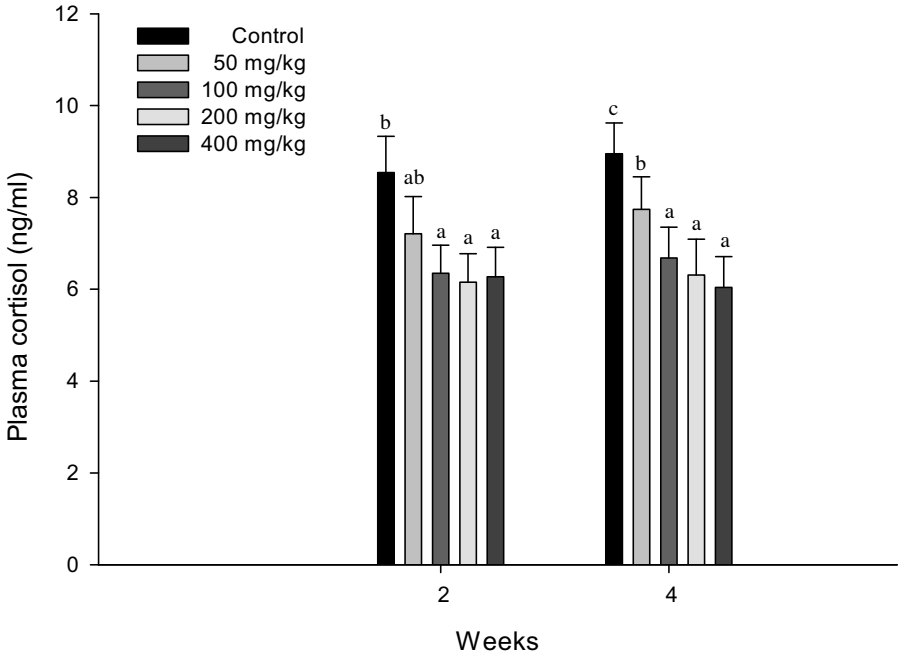

Fig. 1. Plasma cortisol of rockfish, *Sebastes schlegelii* fed diets containing different levels of ascorbic acids for 4 weeks. Vertical bar denotes a standard error. Values with different superscript are significantly different at 2 weeks and 4 weeks ($P < 0.05$) as determined by Duncan's multiple range test.

rockfish caused considerable decreases in the GOT and GPT level. The dietary ascorbic acid supplementation to the rockfish has influenced on the plasma parameters such as glucose, total protein, GOT, and GPT, whereas there was no alteration in calcium and magnesium.

As well as the increase in catecholamine such as adrenaline and noradrenaline, the corticosteroid cortisol increase in plasma is gen-

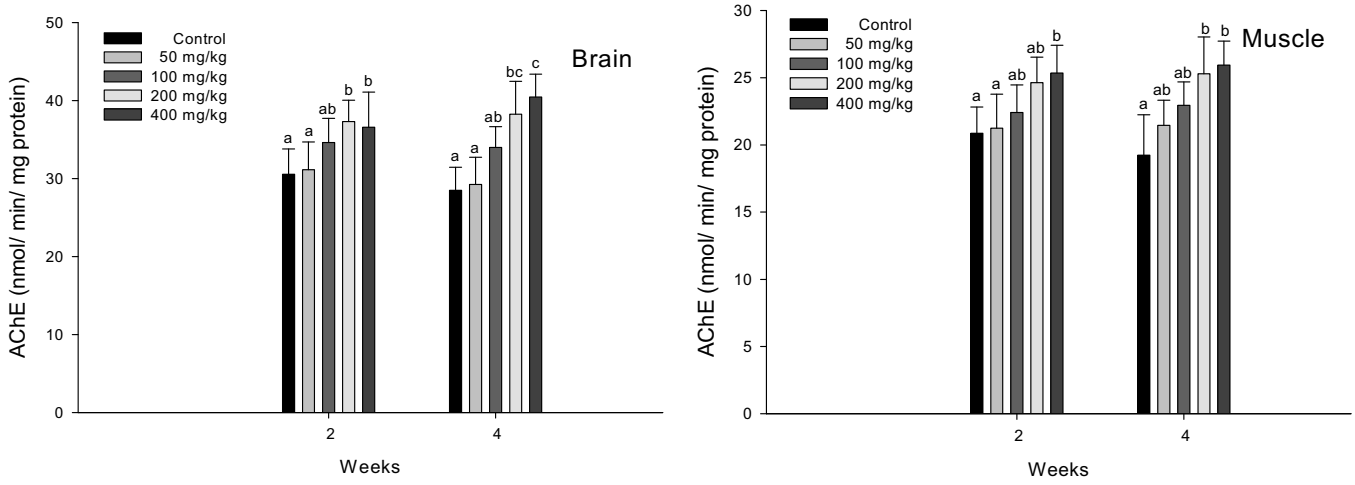

Fig. 2. AChE activity of rockfish, *Sebastes schlegelii* fed diets containing different levels of ascorbic acids for 4 weeks. Vertical bar denotes a standard error. Values with different superscript are significantly different at 2 weeks and 4 weeks ($P < 0.05$) as determined by Duncan's multiple range test.

erally observed by the response to fish stress (Sumpter, 1997). The vitamin deficient diets in fish can induce a significant increase in plasma cortisol. Henrique et al. (1998) reported that a significant cortisol increase by the deficient ascorbic acid supplementation of seabream, *Sparus aurata*. A significant decrease in the plasma cortisol of the rockfish was observed by the dietary ascorbic acid supplementation. The dietary ascorbic acid supplementation may affect the cortisol levels of the experimental fish.

Acetylcholine is a major activator of sensory perceptions in the central nervous system and muscle in the peripheral nervous system, and acetylcholinesterase (AChE) is well known as a modulator to control acetylcholine secretion. Of various ascorbate functions, it is widely known as a role to be concerned in the regulation of acetylcholine release from synaptic vesicles (Kuo et al., 1979; Harrison and May, 2009). Mor and Ozmen (2010) reported that ascorbate also alleviates the AChE inhibition by a toxicant, which is a neuroprotective action of ascorbate. The dietary ascorbic acid supplementation of the rockfish induced the increase in AChE activity, although Dhingra et al. (2006) suggest that ascorbate has been considered in an effective AChE inhibitor.

In this study, the ascorbic acid supplementation over the concentration at 200 mg/kg affected the hematological parameters, plasma cortisol levels, and AChE activity of the rockfish, and this indicates the suitable ascorbic acid supplementation should need to supply commercial culture as well as the experimental rockfish. However, the much higher ascorbic acid supplementation than their requirement can cause unnecessary cost loss, because the ascorbic acid over their needs excrete their urine. Therefore, many studies should be conducted for applications to commercial culture of the rockfish and other fishes.

In conclusion, the dietary ascorbic acid supplementation of the rockfish resulted in considerable alterations in hematological parameters. In addition, the dietary ascorbic acid supplementation of the rockfish significantly decreased the plasma cortisol levels, and the dietary ascorbic acid supplementation induced a notable increase in AChE activity. Considering the results, the dietary ascorbic acid supplementation improves the hematological parameters, and it also reduces the cortisol levels, which may help alleviate the various stress factors. Ascorbic acid supplementation also stimulate the neurotransmitter, AChE. Given that the rockfish are economically important, the more research about ascorbic acid supplementation to the rockfish should be conducted.

Conflict of interest statement

The authors of this paper do not have a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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