

## EFFECTS OF VITAMIN E ON ANTIOXIDANT ENZYME ACTIVITIES AND FATTY ACID COMPOSITIONS IN JUVENILE ABALONE *HALIOTIS DISCUS HANNAI* INO

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**ABSTRACT** A 240-day feeding trial was conducted in a recirculated water system to investigate the effects of dietary vitamin E on the activities of antioxidant enzymes (catalase, CAT; superoxide dismutase, SOD; glutathione peroxidase, GPX) and the composition of fatty acids in abalone, *Haliotis discus hannai* Ino. Triplicate groups of juvenile abalone (initial weight:  $0.71 \pm 0.00$  g; initial shell length:  $15.49 \pm 0.04$  mm) were fed to satiation one of three semipurified diets containing 0, 50, and 5,000-mg/kg vitamin E, respectively. Abalone were sampled on the 120th day and the 240th day, respectively. There were no significant differences in activities of CAT and SOD in soft body of abalone fed with different levels of dietary vitamin E for 120 days ( $P > 0.05$ ), but significantly higher activity of GPX was found with 5,000-mg/kg dietary vitamin E ( $P < 0.05$ ). Activities of CAT and GPX were significantly elevated by dietary vitamin E on the 240th day. The lowest value of 18:1n-9, 18:2n-6 and the highest value of 22:6n-3 in soft body were found with 50 mg/kg dietary vitamin E supplement on the 120th day. On the 240th day, the content of monounsaturated fatty acids (MUFA) in abalone with 50-mg/kg dietary vitamin E supplement was significantly higher than those in the other two treatments ( $P < 0.05$ ). There were no significant effects of dietary vitamin E on the content of polyunsaturated fatty acids (PUFA) in abalone during the two sampling periods ( $P > 0.05$ ). In conclusion, 50-mg/kg dietary vitamin E supplement elevated the activities of antioxidant enzymes and could protect MUFA from peroxidation damage. Excessive dietary vitamin E (5,000 mg/kg) did not serve as an antioxidant any more, but tended to be a pro-oxidant in the soft body of abalone.

**KEY WORDS:** abalone, vitamin E, antioxidant enzymes, fatty acid, *Haliotis discus*

### INTRODUCTION

Highly reactive oxygen species (ROS) are continuously produced during the course of normal aerobic cellular metabolism. Excessive ROS generation leading to oxidative stress and damage of cellular macromolecules (proteins, lipids, and nucleic acids) has been hypothesized to be the major contributor to aging process and many diseases, such as cardiovascular diseases and cancers (Herberg et al. 1998; Drew & Leeuwenburgh 2002). In particular, lipid peroxidation is considered to be a major phenomenon by which ROS can cause tissue damage leading to impaired cellular function and alterations in physicochemical properties of cell membranes, which in turn disrupt vital functions (Rikans & Hornbrook 1997).

Antioxidant enzymes are an important protective mechanism against ROS and, like many other biochemical systems, their effectiveness may vary with the stage of development and other physiological aspects of the organism (Halliwell & Gutteridge 1999, Livingstone et al. 2001). The most important antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) (Halliwell & Gutteridge 1999). A second important factor affecting the potential for oxidative damage is the level of the target molecules, for example polyunsaturated fatty acids (PUFA) are readily oxidized by ROS to lipid peroxides (Di Giulio et al. 1995). Thus, the incidence of lipid peroxidation may depend upon both the level of antioxidant enzymes and the composition of fatty acids in the organisms, the latter of which may also change with aspects of the animal's physiology, including development, age and sex (Parihar & Dubey 1995).

Vitamin E is an essential nutrient for all species of animals (McDowell 1989). As a fat-soluble vitamin, it is the most effective chain-breaking, lipid-soluble antioxidant in biological membranes, where it contributes to membrane stability. It protects critical cellular structures against damage from oxygen free radicals and reactive products of lipid peroxidation (Chan 1998, Devaraj et al. 1996). Vitamin E occurs in several naturally occurring forms, with  $\alpha$ -tocopherol having the highest vitamin E activity (Lee & Shiau 2004).

A number of studies have demonstrated the potential effects of vitamin E on the activities of antioxidant enzymes, fatty acid composition and lipid peroxidation, mainly in vertebrates (Kivanova et al. 1998, O'Neill et al. 1998, Ammouche et al. 2002, Kiron et al. 2004). Up to now, however, there is no relative information available on molluscs. Abalone, *Haliotis discus hannai* are the large algivorous marine molluscs of the genus, *Haliotis* (Gastropoda, Prosobranchia, Archaeogastropoda, Haliotidae), which are the most commercially important gastropods in aquaculture. In our previous studies, it was found that 50–100 mg/kg dietary vitamin E supplement was needed for the optimal growth of abalone *H. discus hannai* (Zhou et al. 2001). The aim of this study is to investigate the effects of vitamin E on the activities of antioxidant enzymes and fatty acid composition in juvenile abalone *H. discus hannai*.

### MATERIAL AND METHODS

#### Experimental Diets

Composition of the casein-gelatin-based diets used in this study is presented in Table 1. Dietary crude protein level was approximately 30.1%, which is considered to be sufficient to

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**TABLE 1.**  
**Ingredient and proximate composition of basal diet**  
**(%, dry-weight basis).**

Ingredient	%
Casein (vitamin-free)*	25.0
Gelatin†	6.0
Dextrin†	34.0
CM-cellulose†	5.0
Sodium alginate†	20.0
Tocopherol-free vitamin mix <sup>1</sup>	2.0
Mineral mix <sup>2</sup>	4.0
SO/MFO <sup>3</sup>	3.5
Choline chloride†	0.5
Proximate analysis	
Crude protein	30.1
Crude lipid	3.3
Ash	10.1

\* Sigma Chemical, St. Louis, MO.

† Shanghai Chemical Co., Shanghai, China.

<sup>1</sup> Tocopherol-free vitamin mix, each 1,000 g of diet contained: Thiamin HCl, 120.0 mg; Riboflavin, 100.0 mg; Folic acid, 30.0 mg; PABA, 400.0 mg; Pyridoxine HCl, 40.0 mg; Niacin, 800.0 mg; Ca pantothenate, 200.0 mg; Inositol, 4,000.0 mg; Biotin, 12.0 mg; Ascorbic acid, 4,000.0 mg; Vitamin B<sub>12</sub>, 180.0 µg; Vitamin A, 500,000 IU; Vitamin D, 10,000 IU.

<sup>2</sup> Mineral mix, each 1000g of diet contained: NaCl, 0.4 g; MgSO<sub>4</sub>·7H<sub>2</sub>O, 6.0 g; NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O, 10.0 g; KH<sub>2</sub>PO<sub>4</sub>, 12.8 g; Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>·H<sub>2</sub>O, 8.0 g; Fe-citrate, 1.0 g; Ca-lactate, 1.4g; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 141.2 mg; MnSO<sub>4</sub>·H<sub>2</sub>O, 64.8 mg; CuSO<sub>4</sub>·5H<sub>2</sub>O, 12.4 mg; CoCl<sub>2</sub>·6H<sub>2</sub>O, 0.4 mg; KIO<sub>3</sub>, 1.2 mg; Na<sub>2</sub>SeO<sub>3</sub>, 0.4 mg.

<sup>3</sup> Soybean oil: Menhaden fish oil = 1:1.

maintain optimum growth for *H. discus hannai* (Mai et al. 1995a). A mixture of soybean oil and menhaden fish oil (1:1) was used as the basal lipid sources. Dietary lipid level was about 3.3%, which was sufficient to support optimum growth and provide enough essential fatty acid (EFA) for abalone (Mai et al. 1995b).

Diets supplemented with three levels of vitamin E (0, 50, and 5,000 mg/kg) were prepared by adding appropriated quantities of DL- $\alpha$ -tocopherol (Sigma Chemicals, St. Louis, MO). The proximate analysis of vitamin E content in experimental diets was 3.5, 52.8, and 4856.8 mg/kg, respectively.

#### Animal Rearing

Abalone juveniles were derived from a spawning at Maidao Fisheries, Qingdao, P. R. China. Prior to initiation of the experiment, animals were placed in glass aquaria (45 × 25 × 35 cm) and conditioned with the basal diet for 2 wk. The growth experiment was conducted in a recirculated water system. Similar size of *H. discus hannai* (initial weight, 0.71 ± 0.00 g; initial shell length: 15.49 ± 0.04 mm) were assigned to the rearing system using a completely random design with 3 triplicated treatments. Abalone were stocked at 55 animals for each rearing unit and were hand-fed the test diets at a rate equaling 5% to 10% of wet body weight once daily at 17:00. Every morning, feces and uneaten feed were removed to maintain the water quality. During the experimental period, water temperature was 17.5–19.0°C, salinity 31–34‰, pH 7.4–7.9. Dissolved oxygen

was not less than 7.0 mg/L. The feeding trial was conducted for 240 days.

#### Sample Collection and Analysis

On the 120th day of the experiment, 20 abalone in each replicate were sampled randomly from the aquaria and were not fed for three days. Then the soft body of abalone were dissected and stored at –70°C for subsequent analysis. At the termination of the feeding trial (on the 240th day), all remaining animals were treated in the same manner as mentioned above.

Analysis of the soft body composition was according to AOAC (1995). The method numbers in AOAC for protein and lipid analysis were 990.03 and 4.5.01, respectively.

Analysis of catalase activity (CAT) in the soft body of abalone was performed by the method of Góth (1991). Superoxide dismutase (SOD) activity was analyzed by the method of Marklund and Marklund (1974). Glutathione peroxidase (GPX) activity analysis was according to the method of Bell et al. (1985).

A HPLC method was used to analyze vitamin E content in diets (Salo-Väänänen et al. 2000). The method involved alkali saponification, extraction of the unsaponifiable matter with n-hexane, washing the extracts with an aqueous 5% NaCl solution, and quantification with HPLC on an ODS Hypersil column (HP; 250 × 4 mm; 5 µm). The mobile phase was methanol at a flow rate of 1 mL/min. UV absorbance detection was operated at wavelength of 280 nm.

The fatty acid compositions in the soft body of abalone were analyzed by the method of Metcalfe et al. (1966) with some modifications. Briefly, an HP 5890 gas chromatograph fitted with a carbowax capillary column (30 mm × Φ 0.25 mm) was used. High purity N<sub>2</sub> was used as the carrier gas at a flow rate of 2 mL/min. Injector and detector temperature was 270°C. The oven was programmed from 150°C to 200°C at 15°C/min, then to 250°C at 2°C/min and held at 250°C until all peaks had appeared. Fatty acid methyl esters were identified by comparing the retention time of experimental samples to standards.

The sum of the saturated fatty acids (SFA) was calculated using the equation:

$$\text{SFA} = 14:0 + 16:0 + 18:0$$

The sum of the monounsaturated fatty acids (MUFA) was calculated using the equation:

$$\text{MUFA} = 16:1 + 18:1n-9 + 18:1n-7$$

The sum of the polyunsaturated fatty acids (PUFA) was calculated using the equation:

$$\text{PUFA} = 18:2n-6 + 18:3n-3 + 20:4n-6 + 20:5n-3 + 22:6n-3$$

#### Statistical Analysis

All percentage data were square-root arcsine transformed before analysis. Data from each treatment were submitted to one-way ANOVA using the SPSS package (version 11.0, SPSS Inc., Chicago). When overall differences were significant at less than the 5% level, Tukey test was used to compare the means (Gill 1978).

## RESULTS

**Soft Body Composition**

Contents of protein and lipid in the soft body of abalone fed with different levels of dietary vitamin E for 120 days and 240 days are presented in Table 2 and Table 3, respectively.

There was no significant effect of dietary vitamin E on the content of protein in the soft body after 120 days feeding ( $P > 0.05$ ). On the 240th day, however, 50 mg/kg dietary vitamin E supplement significantly elevated protein content in the soft body compared with 0 mg/kg and 5,000 mg/kg dietary vitamin E supplements ( $P < 0.05$ ).

Lipid content in the soft body was significantly elevated by dietary vitamin E ( $P < 0.05$ ). The highest values were found as  $7.18 \pm 0.52\%$  and  $7.51 \pm 0.23\%$  in the treatment with 5,000 mg/kg dietary vitamin E supplement after 120 days and 240 days, respectively.

**Activities of Antioxidant Enzymes In Soft Body of Abalone****Catalase (CAT) Activity**

Activities of CAT in soft body of *H. discus hannai* fed with different dietary vitamin E for 120 days and 240 days are presented in Table 2 and Table 3, respectively. The activity of CAT in soft body was not significantly influenced by dietary vitamin E after 120 days feeding ( $P > 0.05$ ), but it was significantly elevated after 240 days feeding ( $P < 0.05$ ). The highest value of CAT activities was found as  $9.11 \pm 0.05$  U/mg pr in soft body of abalone fed with 5,000 mg/kg dietary vitamin E for 240 days.

**Superoxide Dismutase (SOD) Activity**

Activities of SOD in the soft body of *H. discus hannai* fed with different concentrations of dietary vitamin E for 120 days and 240 days are presented in Table 2 and Table 3, respectively. The activity of SOD in the soft body was not significantly influenced by dietary vitamin E after 120 days feeding ( $P > 0.05$ ). After 240 days, however, the SOD activity in the treatment with 50 mg/kg dietary vitamin E supplement was significantly higher than those with 0 mg/kg or 5,000 mg/kg dietary vitamin E supplement ( $P < 0.05$ ). The highest value of SOD activity was found as  $3.89 \pm 0.03$  U/mg pr in the treatment with 50 mg/kg dietary vitamin E supplement, and the lowest

was  $3.19 \pm 0.04$  U/mg pr with 5,000 mg/kg dietary vitamin E supplement.

**Glutathione Peroxidase (GPX) Activity**

Activities of GPX in the soft body of abalone fed with different dietary vitamin E for 120 days and 240 days are presented in Table 2 and Table 3, respectively. The activity of GPX in the soft body of abalone fed with 5,000 mg/kg dietary vitamin E for 120 days was significantly higher than those with 0 and 50 mg/kg dietary vitamin E ( $P < 0.05$ ), and the highest value were found as  $5.97 \pm 0.15$  U/mg pr. After 240 days, the activities of GPX in the treatments with 50 mg/kg and 5,000 mg/kg dietary vitamin E supplements were significantly higher than that with 0 mg/kg dietary vitamin E supplement ( $P < 0.05$ ).

**Fatty Acid Composition in Soft Body of Abalone**

Fatty acid composition in the soft body of abalone fed with dietary vitamin E for 120 days and 240 days are presented in Table 4 and Table 5, respectively. The main fatty acids in the soft body include saturated fatty acids (SFA; 14:0, 16:0, and 18:0), monounsaturated fatty acids (MUFA; 16:1, 18:1n-9, and 18:1n-7) and polyunsaturated fatty acids (PUFA; 18:2n-6, 18:3n-3, 20:4n-6, 20:5n-3, and 22:6n-3).

There were no significant effects of dietary vitamin E on the total SFA, 14:0, 16:0, and 18:0 in the soft body of abalone after either a 120-day or a 240-day feeding trial ( $P > 0.05$ ).

Dietary vitamin E did not significantly affect the total MUFA and 18:1n-7 in the soft body after 120 days ( $P > 0.05$ ). However, 16:1 and 18:1n-9 were significantly affected by dietary vitamin E ( $P < 0.05$ ). Furthermore, the lowest values for these two fatty acids were found in the treatment with 50 mg/kg dietary vitamin E supplement. After 240 days, the content of 16:1 and 18:1n-7 in the soft body with 0 mg/kg dietary vitamin E supplement was significantly lower than those with 50 mg/kg or 5,000 mg/kg dietary vitamin E supplement ( $P < 0.05$ ). And the content of 18:1n-9 in the treatment with 50 mg/kg dietary vitamin E was significantly higher than those with 0 mg/kg or 5,000 mg/kg dietary vitamin E ( $P < 0.05$ ).

The total PUFA in the soft body were not significantly affected by dietary vitamin E for either 120 days or 240 days ( $P > 0.05$ ). During the first 120 days, 18:2n-6, 20:4n-6 and 22:6n-3 in the soft body were significantly affected by dietary vitamin E ( $P < 0.05$ ). The content of 18:2n-6 in the 50 mg/kg dietary vitamin E treatment was significantly lower than those with

TABLE 2.

Survival, proximate compositions and activities of antioxidant enzymes in the soft body of abalone *Haliotis discus hannai* fed diets with different vitamin E levels for 120 days<sup>1</sup>.

Dietary Vitamin E (mg/kg)	Protein (%)	Lipid (%)	CAT <sup>2</sup> (U/mg pr)	SOD <sup>3</sup> (U/mg pr)	GPX <sup>4</sup> (U/mg pr)	Survival (%)
0	71.02 ± 0.62	6.20 ± 0.06 <sup>b</sup>	1.16 ± 0.06	6.61 ± 0.17	4.29 ± 0.14 <sup>b</sup>	90.91 ± 2.58
50	70.38 ± 0.21	6.47 ± 0.17 <sup>ab</sup>	1.29 ± 0.21	6.21 ± 0.13	3.98 ± 0.22 <sup>b</sup>	89.09 ± 2.68
5,000	70.00 ± 0.11	7.18 ± 0.52 <sup>a</sup>	1.13 ± 0.09	6.56 ± 0.09	5.97 ± 0.15 <sup>a</sup>	88.48 ± 2.15

Means in the same column not sharing a common superscript letter were significantly different ( $P < 0.05$ ).

<sup>1</sup> Values are means ± SE,  $n = 3$ .

<sup>2</sup> CAT, catalase.

<sup>3</sup> SOD, superoxide dismutase.

<sup>4</sup> GPX, glutathione peroxidase.

TABLE 3.

Survival, proximate compositions and activities of antioxidant enzymes in the soft body of abalone *Haliotis discus hannai* fed diets with different vitamin E levels for 240 days<sup>1</sup>.

Dietary vitamin E (mg/kg)	Protein (%)	Lipid (%)	CAT <sup>2</sup> (U/mg pr)	SOD <sup>3</sup> (U/mg pr)	GPX <sup>4</sup> (U/mg pr)	Survival (%)
0	70.25 ± 0.06 <sup>b</sup>	5.77 ± 0.14 <sup>b</sup>	6.10 ± 0.04 <sup>c</sup>	3.62 ± 0.03 <sup>b</sup>	1.96 ± 0.03 <sup>b</sup>	81.82 ± 2.58 <sup>a</sup>
50	71.13 ± 0.12 <sup>a</sup>	6.83 ± 0.18 <sup>a</sup>	8.20 ± 0.03 <sup>b</sup>	3.89 ± 0.03 <sup>a</sup>	2.96 ± 0.03 <sup>a</sup>	83.64 ± 2.58 <sup>a</sup>
5000	69.53 ± 0.12 <sup>c</sup>	7.51 ± 0.23 <sup>a</sup>	9.11 ± 0.05 <sup>a</sup>	3.19 ± 0.04 <sup>c</sup>	2.93 ± 0.01 <sup>a</sup>	70.90 ± 2.36 <sup>b</sup>

Means in the same column not sharing a common superscript letter were significantly different ( $P < 0.05$ ).

<sup>1</sup> Values are means ± SE,  $n = 3$ .

<sup>2</sup> CAT, catalase.

<sup>3</sup> SOD, superoxide dismutase.

<sup>4</sup> GPX, glutathione peroxidase.

0 mg/kg and 5,000 mg/kg dietary vitamin E ( $P < 0.05$ ). However, the content of 22:6n-3 had the highest value (3.32 ± 0.02%) in the treatment with 50-mg/kg dietary vitamin E ( $P < 0.05$ ). Dietary vitamin E significantly elevated the content of 20:4n-6 in the soft body ( $P < 0.05$ ), and the highest value was found as 6.11 ± 0.04% in the treatment with 5,000 mg/kg dietary vitamin E. After 240 days, the lowest values of 18:3n-3 (0.70 ± 0.03%) and 20:4n-6 (6.26 ± 0.18%) were found in the 50 mg/kg dietary vitamin E treatment ( $P < 0.05$ ), whereas 22:6n-3 had the highest value (4.07 ± 0.12%) ( $P < 0.05$ ).

#### DISCUSSION

Vitamins directly scavenge ROS and regulate the activities of antioxidant enzymes. Among them, vitamin E has been recognized as one of the most important antioxidants (Topinka et al.

1989). Although a few of studies explicitly show the effects of vitamin E on the activities of antioxidant enzymes, there is no consensus on what might be the responses of antioxidant enzymes to vitamin E, partly because of different feeding behavior and other ecological conditions (Mourente et al. 2002, Giray et al. 2003, Zaidi & Banu 2004). According to a 120-day feeding trial, Wan et al. (2004) pointed out that there were no significant differences in CAT and SOD activities in the serum of adult *H. discus hannai* between the treatments with 0 mg/kg and 50 mg/kg dietary vitamin E. However, GPX activity significantly increased with dietary vitamin E supplements. These results are agreement with the present data during the first 120 days (Table 2). In present study, however, the activities of the antioxidant enzymes (CAT, SOD, and GPX) in abalone fed for 240 days were significantly elevated by dietary vitamin E, excepted that SOD activity decreased in 5,000 mg/kg

TABLE 4.

Fatty acid composition in the soft body of abalone *Haliotis discus hannai* fed diets with different vitamin E levels for 120 days<sup>1</sup>.

Fatty Acid (%)	Dietary Vitamin E (mg/kg)		
	0	50	5000
14:0	2.83 ± 0.04	2.90 ± 0.03	2.94 ± 0.03
16:0	16.30 ± 0.25	16.28 ± 0.03	15.98 ± 0.10
16:1	1.73 ± 0.00 <sup>a</sup>	1.34 ± 0.00 <sup>c</sup>	1.47 ± 0.01 <sup>b</sup>
18:0	6.92 ± 0.14	6.97 ± 0.01	6.94 ± 0.01
18:1n-9	6.36 ± 0.18 <sup>a</sup>	5.50 ± 0.01 <sup>b</sup>	6.35 ± 0.01 <sup>a</sup>
18:1n-7	5.49 ± 0.14	5.64 ± 0.01	5.68 ± 0.01
18:2n-6	4.23 ± 0.14 <sup>a</sup>	3.45 ± 0.03 <sup>b</sup>	4.54 ± 0.01 <sup>a</sup>
18:3n-3	0.84 ± 0.06	0.61 ± 0.06	0.85 ± 0.00
20:4n-6	5.30 ± 0.23 <sup>b</sup>	6.00 ± 0.03 <sup>ab</sup>	6.11 ± 0.04 <sup>a</sup>
20:5n-3	5.55 ± 0.31	5.93 ± 0.01	5.65 ± 0.05
22:6n-3	2.98 ± 0.09 <sup>b</sup>	3.32 ± 0.02 <sup>a</sup>	2.63 ± 0.03 <sup>c</sup>
SFA <sup>2</sup>	26.05 ± 0.43	26.15 ± 0.05	25.86 ± 0.05
MUFA <sup>3</sup>	13.58 ± 0.32	12.47 ± 0.20	13.50 ± 0.02
PUFA <sup>4</sup>	18.89 ± 0.82	19.30 ± 0.03	19.77 ± 0.10

Means in the same line not sharing a common superscript letter were significantly different ( $P < 0.05$ ).

<sup>1</sup> Values are means ± SE,  $n = 3$ .

<sup>2</sup> SFA, saturated fatty acids.

<sup>3</sup> MUFA, monounsaturated fatty acids.

<sup>4</sup> PUFA, polyunsaturated fatty acids.

TABLE 5.

Fatty acid composition in the soft body of abalone *Haliotis discus hannai* fed diets with different vitamin E levels for 240 days<sup>1</sup>.

Fatty Acid (%)	Dietary Vitamin E (mg/kg)		
	0	50	5,000
14:0	2.63 ± 0.04	2.72 ± 0.10	2.84 ± 0.04
16:0	15.67 ± 0.18	15.87 ± 0.30	15.55 ± 0.14
16:1	1.68 ± 0.03 <sup>b</sup>	1.99 ± 0.02 <sup>a</sup>	2.03 ± 0.03 <sup>a</sup>
18:0	7.73 ± 0.01	7.36 ± 0.19	7.72 ± 0.20
18:1n-9	6.76 ± 0.06 <sup>b</sup>	7.07 ± 0.02 <sup>a</sup>	6.13 ± 0.01 <sup>c</sup>
18:1n-7	5.48 ± 0.03 <sup>b</sup>	6.23 ± 0.06 <sup>a</sup>	6.02 ± 0.05 <sup>a</sup>
18:2n-6	5.16 ± 0.12	5.58 ± 0.15	5.34 ± 0.09
18:3n-3	0.72 ± 0.01 <sup>b</sup>	0.70 ± 0.03 <sup>b</sup>	0.88 ± 0.01 <sup>a</sup>
20:4n-6	7.10 ± 0.12 <sup>a</sup>	6.26 ± 0.18 <sup>b</sup>	7.60 ± 0.04 <sup>a</sup>
20:5n-3	5.02 ± 0.07	5.83 ± 0.10	5.64 ± 0.25
22:6n-3	3.80 ± 0.10 <sup>ab</sup>	4.07 ± 0.12 <sup>a</sup>	3.49 ± 0.03 <sup>b</sup>
SFA <sup>2</sup>	26.03 ± 0.23	25.94 ± 0.20	26.11 ± 0.38
MUFA <sup>3</sup>	13.91 ± 0.06 <sup>b</sup>	15.29 ± 0.10 <sup>a</sup>	14.17 ± 0.06 <sup>b</sup>
PUFA <sup>4</sup>	21.79 ± 0.08	22.43 ± 0.48	22.94 ± 0.31

Means in the same line not sharing a common superscript letter were significantly different ( $P < 0.05$ ).

<sup>1</sup> Values are means ± SE,  $n = 3$ .

<sup>2</sup> SFA, saturated fatty acids.

<sup>3</sup> MUFA, monounsaturated fatty acids.

<sup>4</sup> PUFA, polyunsaturated fatty acids.

dietary vitamin E treatment. At this time point, it is suggested that different experimental period might lead to different result about the effect of dietary vitamin E on the activities of antioxidant enzymes.

The fatty acid composition in any tissues reflects protection of fatty acid against damage by free radicals (Fernandes & Venkatraman 1993, Berry 1997). Vitamin E, an antioxidant agent, increased the levels of polyunsaturated n-3 fatty acids and decreased oxidative stress and protected PUFA against damage by free radical (Yilmaz et al. 1997, Chvojková et al. 2001). Clement and Bourre (1993), who observed higher amounts of 18:0 and of total saturated fatty acids and a lower amount of monounsaturated fatty acids and of 18:2n-6 in liver microsomes of vitamin E-deficient rats, suggested that vitamin E deficiency may alter the relation between vitamin E and PUFA. Although the effects of vitamin E on individual fatty acid, such as the EFA (18:2n-6, 18:3n-3 and 20:4n-6) in *H. discus hannai* were not the same during the two sampling periods, the slightly elevated concentration of PUFA in the soft body of abalone caused by dietary vitamin E supplementation, in present study, might be ascribed to a protection of fatty acids against oxidation during absorption and storage.

It is interesting in present study that MUFA in abalone fed with 5,000 mg/kg dietary vitamin E for 240 days was significantly lower than that with 50 mg/kg dietary vitamin E (Table 5). In humans, the high supplementation of vitamin E has been shown to induce a pro-oxidant activity making them react directly with other free radicals or induce lipid oxidation under mild oxidative stress but not under severe situations (Kontush et al. 1996). Kiron et al. (2004) pointed out that the rainbow

trout (*Oncorhynchus mykiss*) were under a mild oxidative stress at the high levels of vitamin E (1,000 mg/kg, tocopheryl acetate), which no longer served as an antioxidant, but tended to be a pro-oxidant. Taking these points into consideration, the possible reason is that excessive dietary vitamin E (5,000 mg/kg), in present study, tended to be a prooxidant, which decreased the MUFA content in the soft body. However, the excessive dietary vitamin E supplement did not result in the significant decrease of PUFA in the soft body. The reason might be that vitamin E functions together with selenium and ascorbic acid in GPX to stop the chain reactions of PUFA peroxidation (Lehninger 1975). GPX activities in the soft body significantly increased with the dietary vitamin E supplements (Table 2 and Table 3), so the content of PUFA in soft body of abalone with excessive dietary vitamin E supplement was not decreased.

### CONCLUSION

As can be seen, 50-mg/kg dietary vitamin E supplement elevated the activities of antioxidant enzymes and could protect MUFA from peroxidation damage. Furthermore, excessive dietary vitamin E (5,000 mg/kg) did not serve as an antioxidant any more but tended to be a pro-oxidant in the soft body of abalone.

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