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Effects of vitamin C deficiency or excess on growth performance, anti-oxidative response and fatty acid composition of juvenile abalone *Haliotis discus hannai* Ino*

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Abstract A 240-day feeding trial was conducted to investigate the effects of dietary vitamin C on growth performance, anti-oxidative response, and fatty acid composition of juvenile abalone *Haliotis discus hannai* Ino (initial body weight: 0.93±0.00 g). Three semi-purified experimental diets were formulated containing 0.00, 94.52, and 9 649.58 mg/kg of vitamin C supplied as L-ascorbyl-2-monophosphate. The results show that there was no significant difference in weight gain ratio, daily increment in shell length, and survival rate among the three treatments. Adding dietary vitamin C (9 649.58 mg/kg) significantly increased the activities of superoxide dismutase (SOD), glutathine peroxidase (GPX), glitathione-S-transferase (GST), glutathione reductase (GR), alkaline phosphatase (AKP), and lysozyme in viscera (P<0.05). In muscle, activities of phenoloxidase, catalase, SOD, GST, GR, and AKP were increased in the treatment with 9 649.58 mg/kg of dietary vitamin C (P<0.05). The highest concentrations of ascorbic acid in viscera and muscle were found in the group with 9 649.58 mg/kg of dietary vitamin C (P<0.05). The contents of crude protein and crude lipid in the soft body were significantly increased in the 9 649.58 mg/kg group (P<0.05). Dietary vitamin C supplementation significantly decreased the contents of saturated fatty acids (14:0, 16:0, and 18:0), and increased the composition of 18:2n-6 and 22:6n-3 in the soft body of abalone (P<0.05). Therefore, although there were no significant effects on the growth performance, dietary vitamin C supplementation improved the anti-oxidation and immune responses, increased specific unsaturated fatty acid (i.e., 16:1, 18:1n-7, 18:1n-9, 18:2n-6 and 22:6n-3), and decreased specific saturated fatty acid (i.e., 14:0, 16:0 and 18:0) contents in the soft body of abalone.

Keyword: abalone; ascorbic acid; anti-oxidative capability; fatty acid

1 INTRODUCTION

Vitamin C, also known as ascorbic acid, is a water-soluble vitamin that involves many physiological processes including growth, collagen formation, iron metabolism and haematology, reproduction, and wound healing (NRC, 2011). It can also scavenge harmful free radicals produced by cells (Bendich et al., 1986; Ames et al., 1993; Shao et al., 2018), and inhibit the destructive effect of reactive oxygen species (ROS) on biological macromolecules (Berger et al., 1997) to protect cells from oxidative damage (Halver, 1995; Chew, 1996). In addition, numerous studies have shown that vitamin C could improve the innate immunity in many ways, such as protecting surrounding phagocyte and promoting serum bactericidal activity (Ren et al., 2008; Chen et al.,

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2015). In aquatic animals, vitamin C can improve the anti-oxidative ability and innate immunity of fish and shrimp, such as Wuchang bream *Megalobrama amblycephala* Yih (Ming et al., 2012), cobia *Rachycentron canadum* (Zhou et al., 2012), loach *Misgurnus anguillicaudatus* Cantor (Zhao et al., 2017) and white shrimp *Litopenaeus vannamei* (López et al., 2003; Wu et al., 2016).

Vitamin C activates the lipid soluble system and protects lipid membranes from free radical peroxidation (Kosutarak et al., 1995; Hwang and Lin, 2002; Wang and Huang, 2015). In aquatic animals, researches have shown that dietary vitamin C increased the lipid contents in yellow drum *Nibea albiflora* (Wang et al., 2017), and effectively prevented the lipid peroxidation in common carp (Hwang and Lin, 2002). Moreover, Gao et al. (2013) observed that incremental dietary vitamin C increased percentages of EPA, DHA, 22∶5n-3, and total n-3 fatty acids in both liver and muscle of red sea bream *Pagrus major*. On the contrary, the percentages of 14∶0, 18∶0, and 18∶1n-9 decreased with increased dietary vitamin C levels.

Abalone of the archaeogastropod genus of mollusca is one of the most important mariculture mollusk species in China, with more than 148 539 tons produced in 2017 (Fishery Bureau, Ministry of Agriculture, People's Republic of China, 2018). Among the abalone farmed in China, *Haliotis discus hannai* Ino is the most important species. A previous study showed that supplementation of vitamin C from 0 to 800 mg/g in diet had no significant effects on the growth of juvenile abalone, but significantly affected the accumulation of ascorbic acid in the soft body (Mai, 1998). The purpose of the present study was to explore the effect of dietary vitamin C deficiency or excess on growth performance, anti-oxidative response and fatty acid composition of juvenile abalone. It provides basic data to better understand the protective effects of vitamin C on abalone.

2 MATERIAL AND METHOD

2.1 Experimental diets

The basal diet formulation was based on Wu et al. (2010, 2011), and the ingredients are shown in Table 1. The basal diet contained 306.8 g/kg of crude protein from casein (vitamin free) and gelatin, and 33.2 g/kg of crude lipid from soybean oil and menhaden fish oil (1∶1). Vitamin C was added to the diets at levels of 0, 100, and 10 000 mg/kg in the form of L-ascorbyl-2-monophosphate, and the corresponding vitamin C contents in diets were 0.00, 94.52, and 9 649.58 mg/kg, respectively.

Table 1 Ingredients and compositions of the basal diet

Ingredient	Content (g/kg)
Casein (vitamin free) *	250.00
Gelatin †	60.00
Dextrin †	340.00
Carboxymethyl cellulose †	50.00
Sodium alginate †	200.00
Vitamin mix ‡	20.00
Mineral mix §	40.00
Choline chloride †	5.00
SO/MFO ¶	35.00
Proximate analysis	
Crude protein	306.80
Crude lipid	33.20
Ash	101.80

* Sigma Chemical, St Louis, MO, USA. † Shanghai Chemical, Shanghai, China. ‡ Vitamin mix (vitamin C free), each 1 000 g of diet contained: thiamin HCl, 120 mg; riboflavin, 100 mg; folic acid, 30 mg; pyridoxine HCl, 40 mg; niacin, 800 mg; Ca pantothenate, 200 mg; inositol, 4 000 mg; biotin, 12 mg; B_{12}, 0.18 mg; vitamin E, 450 mg; menadione, 80 mg; retinol acetate, 100 000 IU; cholecalciferol, 2 000 IU. § Mineral mix, each 1 000 g of diet contained: NaCl, 0.4 g; $MgSO_4 \cdot 7H_2O$, 6.0 g; $NaH_2PO_4 \cdot 2H_2O$, 10.0 g; KH_2PO_4, 20.0 g; $Ca(H_2PO_4)_2 \cdot H_2O$, 8.0 g; Fe-citrate, 1.0 g; $ZnSO_4 \cdot 7H_2O$, 141.2 mg; $MnSO_4 \cdot H_2O$, 64.8 mg; $CuSO_4 \cdot 5H_2O$, 12.4 mg; $CoCl_2 \cdot 6H_2O$, 0.4 mg; KIO_3, 1.2 mg. ¶ Soybean oil and menhaden fish oil (1:1).

2.2 Experimental animals and feeding trial

Abalone juveniles (initial shell length: 1.74±0.03 cm, initial weight: 0.93±0.00 g) were collected from Damai Island in Qingdao. Before the start of the feeding trial, all abalones were acclimated to the experimental conditions for 14 days with normal diet with no vitamin C supplementation. After body weight was measured, abalones were randomly distributed into nine tanks and 55 individuals per tank. Each treatment had three replicates (tanks). The feeding trial was carried out in an indoor recirculating water system. The pre-weighted experimental diets were fed to abalone once a day (17∶00) at a satiation level for 240 days. Feces and excess feeds were removed at 8∶00 every morning to maintain water quality. During the feeding trial, water temperature ranged from 17.5–19.0°C, salinity 31–34, pH 7.4–7.9, and dissolved oxygen was above 7 mg/L.

2.3 Leaching

The leaching test for dietary vitamin C was carried out according to the method used by Zhang et al.

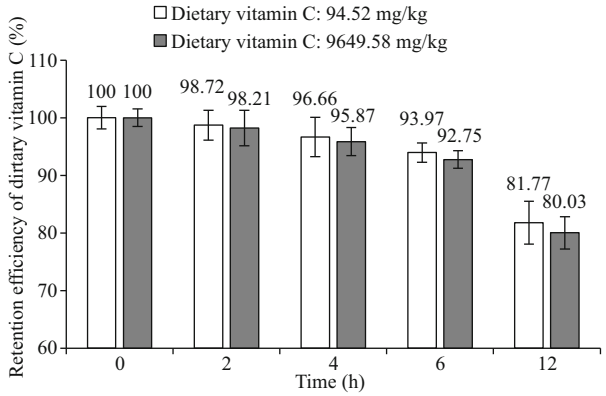

Fig.1 The retention efficiency of vitamin C in diets at different interval immersed in seawater (0, 2, 4, 6, and 12 h)

(2003). Pre-weighed diet was placed onto 100-μm mesh screens and allowed it to the bottom of experimental glass aquaria. Temperature was adjusted to match that of the experiment (18±1°C). At allotted time (0, 2, 4, 6, and 12 h, respectively), the remaining diet was removed from the glass aquaria and lyophilized. Dried diet was submitted to analyses of vitamin C contents. The leaching of dietary vitamin C was expressed as retention efficiency (RE):

$$RE\ (\%)=100V_t/V_0,$$

where V_t and V_0 are final (0, 2, 4, 6, or 12 h) and initial dietary vitamin C content, respectively.

2.4 Sample collection and analysis

All animal care and handling procedures were approved by the Animal Care Committee of Ocean University of China. Before sampling, all the abalones were fasted for 3 days. Abalones in the same tank were counted one by one and weighed together. The viscera and muscle were collected and stored at -80°C for later analysis. The crude protein and crude lipid content of diets and soft body of abalone were quantified by the method of AOAC (1995).

2.4.1 Ascorbic acid analysis

The concentrations of ascorbic acid in abalones and diets were determined by high performance liquid chromatography (HPLC, Agilent 1100). The mobile phase was 0.05 mol/L KH_2PO_4 at pH 2.8 and the flow rate was 0.6 mL/min. Weighed samples were homogenized in 5% cold metaphosphoric acid, homogenates were centrifuged at 2 739×*g* for 6 min, and supernatants were analyzed on HPLC after being filtered through a 0.22-μm pore size syringe filter. The detection wavelength was 254 nm, column temperature was 30°C, and dissolving appropriate amount of ascorbic acid (Sigma Chemicals Co.) in ultrapure water was used as standard sample.

2.4.2 Activities of enzymes

Activities of acid phosphatase (ACP), alkaline phosphatase (AKP), lysozyme(LZ), phenoloxidase (PO), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione S-transferase (GST) and glutathione reductase (GR) were determined by the commercial reagent kits (Nanjing Jiancheng Bioengineering Institute) according to the protocol instructions.

2.4.3 Fatty acid analysis

Fatty acid methyl esters (FAMEs) were prepared by esterification using 2% sulfuric acid methanol as described in Metcalfe et al. (1966). The FAMEs were separated and quantified using HP 5890II gas chromatograph (Agilent) equipped with a fused silica capillary column (007-CW). The temperature program was: initial temperature 150°C, 15°C/min rise to 200°C, then 2°C/min rise to 250°C. Nitrogen was used as carrier gas. The fatty acid quantification was identified by comparison of retention time and peak area with standard FAMEs (Sigma Chemicals Co.).

2.5 Calculations and statistical analysis

The growth performances of abalone were expressed as weight gain ratio (WGR), the daily increment in shell length (DISL) and survival rate (SR). They were calculated as follows:

$$WGR\ (\%)=[(W_t-W_i)/W_i]\times 100,$$

$$DISL\ (\mu m/day)=[(SL_t-SL_i)/t]\times 10\ 000,$$

$$SR\ (\%)=N_t\times 100/N_0,$$

where W_t and W_i are final and initial body weight of abalone respectively; SL_t and SL_i are final and initial shell length (cm) of abalone, respectively; t is feeding trial duration in day; N_t and N_0 are final and initial numbers of abalone in each replicate, respectively.

Statistical analysis was performed using SPSS 25.0 and Microsoft Excel 2010. All the data were presented as means±SE. Data were analyzed by one-way ANOVA. Differences between the means were tested by Tukey's test at level of 0.05.

3 RESULT

3.1 Leaching

The results of the leaching test with experimental diets are illustrated in Fig.1. The retention efficiency

Table 2 Effects of dietary vitamin C on the growth and survival of abalone *Haliotis discus hannai* Ino after a 240-day feeding trial

Item of growth performance	Dietary vitamin C (mg/kg)			ANOVA	
	0	94.52	9 649.58	*F* value	*P* value
Initial body weight (g)	0.93±0.00	0.93±0.00	0.93±0.00	0.462	0.651
Initial shell length (cm)	1.76±0.01	1.74±0.02	1.73±0.02	0.680	0.542
Final body weight (g)	3.11±0.10	2.81±0.06	3.03±0.12	2.417	0.170
Final shell length (cm)	2.88±0.05	2.76±0.05	2.85±0.04	2.023	0.213
WGR (%)	234.38±11.45	201.56±6.21	226.52±13.70	2.471	0.165
DISL (μm/d)	46.90±2.30	42.24±1.33	46.60±1.64	2.091	0.205
Survival rate (%)	72.73±3.79	69.09±2.10	79.39±3.21	2.748	0.142

WGR: weight gain ratio; DISL: daily increment in shell length. All values were presented as the mean±SE (n=3).

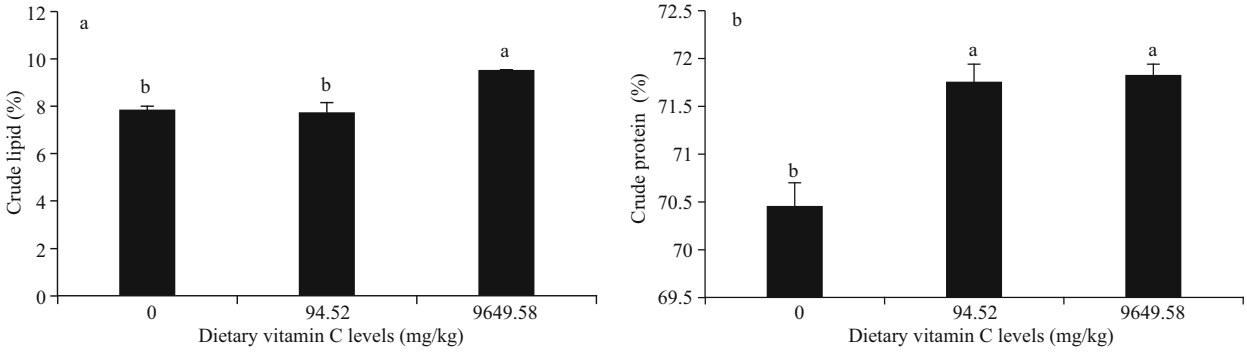

Fig.2 Effects of dietary vitamin C on crude lipid (a) and crude protein (b) contents in the soft body of abalone *Haliotis discus hannai* Ino after a 240-day feeding trial

Data were presented in mean±SE (n=3). Bars bearing different letters are significantly different (P<0.05; Tukey's test).

(RE) of dietary 94.52 mg/kg vitamin C at different interval immersed in seawater for 0, 2, 4, 6, and 12 h were 100%, 98.72%, 96.66%, 93.97%, and 81.77%, respectively. In the diet of 9 649.58 mg/kg vitamin C, the RE at different interval immersed in seawater for 0, 2, 4, 6, and 12 h were 100%, 98.21%, 95.87%, 92.75%, and 80.03%, respectively.

3.2 Growth performance

The results of the growth performance of abalone are shown in Table 2. There were no significant differences in WGR, DISL, and SR among all the groups (P>0.05). The WGR were ranged 201.56%–234.38%, the DISL 42.24–46.90 μm/d, and the SR 69.09%–72.73%.

3.3 Soft body composition

The contents of crude lipid and crude protein in the soft body of abalone are shown in Fig.2. The highest value of crude lipid content was in the group with 9 649.58 mg/kg of dietary vitamin C (P<0.05), but there was no significant difference between the other two groups (P>0.05). The significantly lowest value of crude protein was in the group with 0 mg/kg of dietary vitamin C (P<0.05).

The contents of ascorbic acid in the soft body of abalone are shown in Fig.3. Ascorbic acid contents had the highest value in viscera (449.78 μg/g) and muscle (253.33 μg/g) in the group with 9 649.58 mg/kg of dietary vitamin C (P<0.05). There was no significant difference between the treatments with 0 mg/kg and 94.52 mg/kg of dietary vitamin C (P>0.05).

3.4 Activities of anti-oxidation and immune related enzymes

The activities of AKP, ACP, LZ, and PO in viscera and muscle are shown in Tables 3 & 4, respectively. The highest value of AKP activity in viscera was found to be 23.67 U/g protein in the 9 649.58 mg/kg group (P<0.05). In muscle, the activity of AKP significantly increased with the increasing of dietary vitamin C levels (P<0.05). However there was no significant difference in ACP activity in viscera and muscle among the three treatments (P>0.05).

With the increasing of dietary vitamin C levels, the activity of LZ in viscera significantly increased (P<0.05). In muscle, there was no significant differences in LZ activity among all the groups

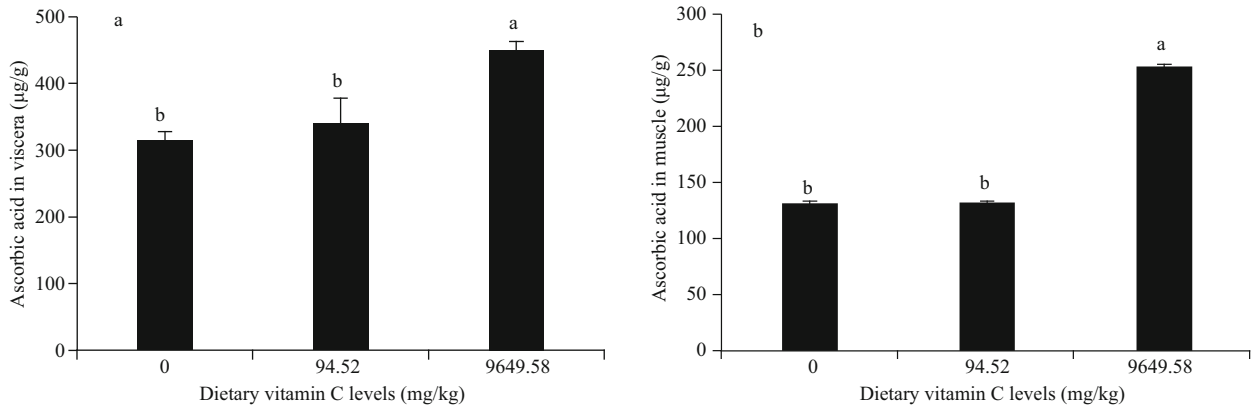

Fig.3 Effects of dietary vitamin C on the contents of ascorbic acid in viscera (a) and muscle (b) of abalone *Haliotis discus hannai* Ino after a 240-day feeding trial

Data were presented in mean±SE (n=3). Bars bearing different letters are significantly different (P<0.05; Tukey's test).

Table 3 Effects of dietary vitamin C on the activity of anti-oxidative enzymes and immune parameters in viscera of abalone *Haliotis discus hannai* Ino after a 240-day feeding trial

Enzyme	Dietary vitamin C (mg/kg)			ANOVA	
	0	94.52	9 649.58	F value	P value
AKP (U/g protein)	16.00±0.12^{b}	14.72±0.07^{c}	23.67±0.15^{a}	720.942	0.000
ACP (U/g protein)	247.86±8.51	222.15±3.17	258.35±13.50	3.929	0.081
LZ (U/mg protein)	4.83±0.10^{c}	7.56±0.28^{b}	12.70±0.46^{a}	160.276	0.000
PO (U/mg protein)	4.96±0.08^{a}	2.89±0.06^{b}	2.63±0.04^{b}	411.274	0.000
CAT (U/mg protein)	1.52±0.08	1.12±0.18	2.40±0.73	2.266	0.185
SOD (U/mg protein)	1.15±0.01^{c}	2.11±0.06^{b}	3.24±0.16^{a}	111.871	0.000
GPX (U/mg protein)	2.66±0.14^{ab}	2.12±0.21^{b}	2.94±0.18^{a}	5.411	0.045
GST (U/mg protein)	44.42±1.40^{b}	36.04±1.09^{c}	58.32±1.22^{a}	82.042	0.000
GR (U/g protein)	6.12±0.07^{b}	5.97±0.05^{b}	8.65±0.05^{a}	701.532	0.000

AKP: alkaline phosphatase; ACP: acid phosphatase; LZ: lysozyme; PO: phenoloxidase; CAT: catalase; SOD: superoxide dismutase; GPX: glutathione peroxidase; GST: glutathione-s-transferase; GR: glutathione reductase. All values were presented as the mean±SE (n=3). Different superscripts a, b, c in the same line indicate significant (P<0.05) difference between different dietary treatments as determined by Tukey's test.

(P>0.05). The addition of dietary vitamin C (94.52 and 9 649.58 mg/kg) significantly increased the activity of PO in muscle (P<0.05). However, in viscera, the highest value of PO activity was 4.96 U/mg protein in the 0 mg/kg group.

The activities of CAT, SOD, GPX, GST, and GR in viscera and muscle are shown in Tables 3 & 4, respectively. There was no significant difference in CAT activity in viscera among the three treatments (P>0.05). Supplementation of dietary vitamin C (94.52 and 9 649.58 mg/kg) significantly increased the activity of CAT in muscle (P<0.05).

With the increasing of dietary vitamin C levels, the activity of SOD significantly increased in viscera (P<0.05). In muscle, the 9 649.58 mg/kg treatment group significantly increased the activity of SOD (P<0.05). There was no significant difference between the groups of 0 and 94.52 mg/kg (P>0.05).

The highest value of GPX activity in viscera was 2.94 U/mg protein in 9 649.58 mg/kg group. There was no significant difference in GPX activity in muscle among all the groups (P>0.05). The activity of GST in viscera and muscle was significantly higher in the 9 649.58 mg/kg group than those of 0 and 94.52 mg/kg groups (P<0.05). The highest value of GR activity in viscera was 8.65 U/mg protein in the 9 649.58 mg/kg group (P<0.05). In muscle, the activity of GR significantly increased in the 94.52 mg/kg group (P<0.05).

3.5 Fatty acid compositions

The fatty acid compositions in soft body of abalone are shown in Table 5. The addition of dietary vitamin C (groups of 94.52 and 9 649.58 mg/kg) significantly decreased the contents of saturated fatty acid (SFA) 14:0, 16:0, and 18:0 in soft body of abalone (P<0.05).

Table 4 Effects of dietary vitamin C on the activity of anti-oxidative enzymes and immune parameters in muscle of abalone *Haliotis discus hannai* Ino after a 240-day feeding trial

Enzyme	Dietary vitamin C (mg/kg)			ANOVA	
	0	94.52	9 649.58	*F* value	*P* value
AKP (U/g protein)	4.95±0.08^{c}	7.65±0.18^{b}	9.46±0.18^{a}	345.624	0.000
ACP (U/g protein)	38.89±1.11	37.22±1.58	42.69±1.10	4.778	0.057
LZ (U/mg protein)	0.38±0.05	0.54±0.05	0.34±0.07	3.447	0.101
PO (U/mg protein)	0.37±0.02^{b}	0.80±0.04^{a}	0.68±0.06^{a}	23.799	0.001
CAT (U/mg protein)	0.69±0.08^{b}	1.38±0.17^{a}	1.37±0.18^{a}	7.447	0.024
SOD (U/mg protein)	3.30±0.03^{b}	3.32±0.06^{b}	4.71±0.05^{a}	54.623	0.000
GPX (U/mg protein)	1.86±0.05	2.00±0.05	1.91±0.04	2.407	0.111
GST (U/mg protein)	32.21±0.87^{b}	32.13±0.33^{b}	53.57±2.61^{a}	59.782	0.000
GR (U/g protein)	8.71±0.05^{c}	14.17±0.04^{a}	12.75±0.02^{b}	780.146	0.000

AKP: alkaline phosphatase; ACP: acid phosphatase; LZ: lysozyme; PO: phenoloxidase; CAT: catalase; SOD: superoxide dismutase; GPX: glutathione peroxidase; GST: glutathione-s-transferase; GR: glutathione reductase. All values were presented as the mean±SE (n=3). Different superscripts a, b, c in the same line indicate significant (P<0.05) difference between different dietary treatments as determined by Tukey's test.

Table 5 Effect of dietary vitamin C on fatty acid composition in the soft body of abalone *Haliotis discus hannai* Ino after a 240-day feeding trial

Fatty acid (%)	Vitamin C (mg/kg)			ANOVA	
	0	94.52	9 649.58	*F* value	*P* value
14:0	3.72±0.05^{a}	2.38±0.02^{b}	2.48±0.11^{b}	123.751	0.000
16:0	17.67±0.32^{a}	15.62±0.22^{b}	16.20±0.48^{b}	22.292	0.020
16:1	1.50±0.04^{b}	2.01±0.04^{a}	1.87±0.01^{a}	71.553	0.003
18:0	7.52±0.12^{a}	7.09±0.03^{b}	6.88±0.04^{b}	20.070	0.018
18:1n-9	5.42±0.05^{b}	5.82±0.07^{a}	5.71±0.05^{ab}	14.249	0.029
18:1n-7	5.53±0.06^{ab}	5.80±0.07^{a}	5.42±0.02^{b}	14.457	0.029
18:2n-6	2.87±0.05^{c}	3.28±0.04^{b}	3.59±0.03^{a}	87.729	0.002
18:3n-3	0.62±0.03	0.74±0.01	0.67±0.07	2.240	0.250
20:4n-6	6.08±0.16	6.43±0.05	6.67±0.99	0.270	0.780
20:5n-3	4.35±0.28	4.46±0.09	5.09±0.25	3.210	0.180
22:6n-3	2.94±0.17^{b}	3.74±0.01^{ab}	3.83±0.20^{a}	11.032	0.041

All values were presented as the mean±SE (n=3). Different superscripts a, b, c in the same line indicate significant (P<0.05) difference between different dietary treatments as determined by Tukey's test.

With the increasing of dietary vitamin C levels, the contents of monounsaturated fatty acid (16:1 and 18:1n-9) and PUFA (18:2n-6 and 22:6n-3) were increased in soft body of abalone (P<0.05). There was no significant difference in 18:3n-3, 20:4n-6, and 20:5n-3 in soft body of abalone among the three treatment groups (P>0.05).

4 DISCUSSION

As shown in the last section, different levels of dietary vitamin C had no significant effects on the growth performance of abalone, which is consistent with a previous study on abalone (Mai, 1998). Dietary vitamin C supplementation significantly increased the content of total lipid in the soft body of abalone (Fig.2), which was consistent with the finding in freshwater prawn *Macrobrachium malcolmsoni* (Asaikkutti et al., 2016). The possible reason is that vitamin C can activate the lipid soluble system, so that triacylglycerol could decrease and the lipid peroxidation could be prevented (Kosutarak et al., 1995; Wang and Huang, 2015). Moreover, dietary vitamin C supplementation significantly increased the content of crude protein in the soft body of abalone, which is consistent with the findings in Japanese eel *Anguilla japonica* (Shahkar et al., 2015). Researches

showed that high levels of vitamin C could stimulate protein production in fish, since diets supplemented with higher vitamin C can increase protein synthesis (Chagas and Val, 2003).

When foreign bodies are phagocytized into cells and fused with lysosome, they are finally digested by various hydrolases to decompose (Roosta et al., 2014). The AKP is a hydrolytic enzyme that can enhance the recognition and phagocytosis ability of the invasive organism (Zhou et al., 2012). In present study, the activity of AKP in viscera and muscle reached the maximum value when excessive dietary vitamin C supplemented. In previous studies, dietary vitamin C supplementation could effectively increase the serum AKP level in Wuchang bream *M. amblycephala* under pH stress (Wan et al., 2014). Zhou et al. (2012) found that dietary vitamin C supplementation enhanced serum AKP activity in cobia *R. canadum*. Our results show that vitamin C could improve the non-specific immunity of abalone, and play vital roles in defending the pathogen.

It is generally believed that LZ has bactericidal effect and is one of the main defense mechanisms of mollusc (Andersen et al., 1998; Ordás et al., 2000). In present study, dietary vitamin C significantly increased the activity of LZ in viscera, which is consistent with the findings in grass carp *Ctenopharyngodon idella* and yellow drum *N. albiflora* (Xu et al., 2016; Wang et al., 2017). The PO is a key enzyme in the immune system of invertebrates (Gollas-Galván et al., 1997; Moreau et al., 2000). It is closely related to the identification of foreign and host defense. In present study, in the 94.52 mg/kg group, the activity of PO in muscle was significantly increased, which is consistent with the findings in grass shrimp *Penaeus monodon* and white shrimp *L. vannamei* (Lee and Shiau, 2002; Qiao et al., 2011).

The anti-oxidative enzymes, such as CAT, SOD, GPX, and GST, can remove excessive damaging ROS, and reduce the damage by lipid peroxidation. The GR could prevent the oxidative decomposition of hemoglobin, and maintain the activity of mercapto group protein to ensure the integrity of cells (Freeman and Crapo, 1982; Yang and He, 2007; Asaikkutti et al., 2016; Liang et al., 2017). The present results showed that compared with vitamin C deficiency, dietary vitamin C supplementation significantly increased the activity of anti-oxidative enzymes in viscera and muscle of abalone. Although the activity of enzymes in different tissues was slightly different, the basic trend was identical. These findings were in agreement with those in yellow catfish *Pelteobagrus fulvidraco* (Liang et al., 2017) and black carp *Mylopharyngodon piceus* (Hu et al., 2013). These data indicate that vitamin C can enhance the anti-oxidative ability of abalone. In addition, it is speculated that the high anti-oxidative response are associated with the accumulation of ascorbic acid in abalone. In this study, with the increasing of dietary vitamin C levels, the content of ascorbic acid in the soft body of abalone was also increased, and the anti-oxidative responses of abalone were improved.

Vitamin C is known affecting the fatty acid composition of *Terapon jarbua* (Chien and Hwang, 2001). Researches showed that the SFA were represented by C14:0, C16:0, and C18:0. The monounsaturated fatty acid (MUFA) were dominated by C18:1n-7, C18:1n-9, and C20:1n-9 in soft body of abalone (Lou et al., 2013). In this study, we found that dietary vitamin C could significantly reduce the content of SFA 14:0 and 16:0 in abalone, while the content of MUFA (16:1 and 18:1n-9) and PUFA (18:2n-6 and 22:6n-3) were increased. A previous study shows that with the increasing of dietary vitamin C levels, the percentages of EPA, DHA, 22:5n-3, and total n-3 fatty acids significantly increased and the percentages of 14:0 and 18:0 decreased in red sea bream *Pagrus major* (Gao et al., 2013), which was consistent with the present study to some extent. However, we found that increasing the levels of dietary vitamin C could decrease the contents of EPA, 22:5n-3, DHA, and n-3 highly unsaturated fatty acid (HUFA) in liver of Japanese flounder *Paralichthys olivaceus* (Gao et al., 2014). A similar result was found by Chien and Hwang (2001) who reported that vitamin C significantly reduced the % of PUFA and increased the % of SFA in the liver lipid of thornfish *T. jarbua*. The mechanism behind the effect vitamin C on fatty acid compositions in abalone needs further research.

5 CONCLUSION

Although there were no significant effects on the growth performance, dietary vitamin C supplementation improved the anti-oxidation and immune responses, significantly increased specific MUFA (i.e., 16:1, 18:1n-7, and 18:1n-9), and PUFA (i.e., 18:2n-6 and 22:6n-3) contents, and decreased the contents of specific SFA (i.e., 14:0, 16:0, and 18:0) in the soft body of abalone. These data suggest that the addition of dietary vitamin C could improve the anti-oxidative response and fatty acid composition of abalone.

6 DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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