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Effects of dietary *n*-3 highly unsaturated fatty acids (HUFAs) on growth, fatty acid profiles, antioxidant capacity and immunity of sea cucumber *Apostichopus japonicus* (Selenka)

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ABSTRACT

The present study was conducted to understand the effects of dietary *n*-3 highly unsaturated fatty acids (HUFAs) on growth, fatty acid profiles, antioxidant capacity and the immunity of sea cucumber *Apostichopus japonicus* (Selenka). Five experimental diets were prepared, containing graded levels of *n*-3 HUFAs (0.46%, 0.85%, 1.25%, 1.61% and 1.95%, respectively), and the 0.46% group was used as control group. The specific growth rates, fatty acid profiles, activities and gene expression of antioxidative enzymes and lysozyme of the sea cucumbers that were fed with the 5 experimental diets were determined. The results showed that the specific growth rate of sea cucumbers in all the treatment groups significantly increased compared to the control group ($P < 0.05$), indicating the positive effects of *n*-3 HUFAs on the growth of sea cucumbers. The contents of eicosapentaenoic acid (EPA; 20:5*n*-3) and docosahexaenoic acid (DHA; 22:6*n*-3) in the body wall of the sea cucumbers gradually increased with the increasing levels of *n*-3 HUFAs in the diets. The suitable supplement of *n*-3 HUFAs in diets improved the activities of superoxide dismutase (SOD) and catalase (CAT) of sea cucumbers by up-regulating the expression of SOD and CAT mRNA in sea cucumbers. However, excess *n*-3 HUFAs in diets caused lipid peroxidation, inhibited the expression of lysozyme (LSZ) mRNA and decreased the activities of LSZ in sea cucumbers. In summary, the suitable supplement levels of *n*-3 HUFAs in diets of sea cucumbers *A. japonicus* were estimated between 0.85% and 1.25% considering the growth performance, cost and the indicators of antioxidant capacity and immunity.

1. Introduction

Sea cucumber *Apostichopus japonicus* has been widely cultured in Asia as an important species for mariculture in recent years owing to its highly nutritional and economic value [1–3]. And studies focused on nutrient requirements of sea cucumbers have been conducted intensively in the past few years [4–6].

N-3 highly unsaturated fatty acids (HUFAs) such as eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3) play significant roles in the growth, development and immune response of aquatic animals [7–9]. Aquatic animals generally have limited ability to synthesize *n*-3 HUFAs [10,11] and accordingly dietary *n*-3 HUFAs are the dominant source of supplements for aquatic animals. The requirements of *n*-3 HUFAs in aquatic animals have been conducted in numerous species [8–11]. The optimum addition level of *n*-3 HUFAs in diets for sea cucumbers has not been quantified in the past years due to the high levels of *n*-3 HUFAs in seaweed *Sargassum thunbergii* which is the traditional and main ingredient of the formulated artificial feed for sea cucumber farming [12,13]. Terrestrial plants such as corn meal and soybean meal, however, have been increasingly used in the formulated feed of sea cucumbers in recent years as a replacement of the traditional feed ingredient *S. thunbergii* of which price has reached 4000 dollars per ton [12–14] due to the rapid expansion of sea cucumber farming. Generally, fatty acid profiles of terrestrial plants were different with those of seaweeds, and, in particular, terrestrial plants were void of *n*-3 HUFAs [12,15]. Hence, the

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addition of terrestrial plants in diets as a replacement of *S. thunbergii*, might lead to the changes in the fatty acid profiles of sea cucumbers. Yu et al. [12] discovered that the sea cucumbers fed diets with increasing inclusion levels of corn meal and soybean meal as the replacement of *S. thunbergii*, exhibited significant decrease in the contents of EPA and DHA due to the absence of EPA and DHA in corn meal and soybean meal. Addition of EPA and DHA in diets might be a possible method to overcome such problem. However, literatures focused on the *n*-3 HUFAs requirements of sea cucumbers or the suitable addition levels of *n*-3 HUFAs in diets of sea cucumbers are limited to the present.

Rapid expansion and high farming density have resulted in serious diseases occurring in this species, and it has negatively affected the development of sea cucumber farming due to huge economical losses [16–20]. Nutritional immunology is a new approach that is used in preventing diseases in aquatic animals after the chemical drugs and vaccines. It does not only avoid the drug resistance and the drug residues, but can also overcome the difficult operation of vaccine prevention [21,22]. Macronutrients in diet such as proteins and fatty acids have been proven to be associated with the immune responses of various aquatic organisms [7]. As such, regulation of immunity and disease resistance of sea cucumbers using dietary *n*-3 HUFAs might be an alternative and more effective method. No previous studies, however, have covered the modulation of antioxidant capacity and the immunity of sea cucumbers induced by dietary *n*-3 HUFAs to the present.

The present study was conducted to determine the suitable supplement levels of *n*-3 highly unsaturated fatty acids (HUFAs) in diets of sea cucumbers, and understand the role of dietary *n*-3 HUFAs as modulators of the antioxidant capacity and immunity of sea cucumber *Apostichopus japonicus* (Selenka). Three enzymes, including superoxide dismutase (SOD), catalase (CAT), lysozyme (LSZ), were chosen as the antioxidant capacity or immunity indices, all of which have been well recognized to be correlated with the antioxidant capacity and immune competency of invertebrates [18,23,24]. And the mRNA expressions of these 3 enzymes were determined by qRT-PCR so as to clarify the role of dietary *n*-3 HUFAs as modulators of the expression of these 3 genes of sea cucumbers.

2. Materials and methods

2.1. Feed ingredients and diet formulation

Ingredients and nutrient composition of the experimental diets are given in Table 1. Five isoproteic (19.4% crude protein) and isolipidic (4.98% crude lipid) diets were formulated containing graded levels of *n*-3 HUFAs (0.46% [the control group], 0.85%, 1.25%, 1.61% and 1.95% dry weight). The fatty acid compositions of the diets were listed in Table 2. Seaweed *Sargassum thunbergii*, fish meal and soybean meal were used as main protein sources. EPA- and DHA-enriched oil was used as the source of *n*-3 HUFAs. All ingredients were ground into fine powder through 200 mm mesh and thoroughly blended. Pellets with size of 2.0 × 2.0 × 3.0 mm were made with pellet-making machine (DZLP-120, Minglun Machinery Company, Shandong province, China) and dried for 12 h in a ventilated oven at 40 °C. The finished feed was packed in double plastic bags and stored at −20 until use.

2.2. Experimental procedures and sample collection

Sea cucumbers were bought from a local sea cucumber rearing farm in Qingdao, China. Prior to the start of the experiment, sea cucumbers were reared in plastic tanks and fed the control diet for two weeks to adapt to experimental conditions and feeds. After acclimation, the sea cucumbers were fasted for 24 h. Then 100 individuals of sea cucumber with similar size (5.00 ± 0.05 g) were selected and randomly distributed into 20 aquariums with size of

Table 1
Proximate composition of trial diets for *A. japonicus* (Dry matter basis).

Items	Dietary *n*-3 HUFA contents (% dry weight)				
	0.46	0.85	1.25	1.61	1.95
Ingredients (%)					
S. thunbergii[a]	30.0	30.0	30.0	30.0	30.0
Soybean meal[a]	10.0	10.0	10.0	10.0	10.0
Corn meal[a]	20.0	20.0	20.0	20.0	20.0
Fish meal[a]	10.0	10.0	10.0	10.0	10.0
Vitamin premix[b]	0.5	0.5	0.5	0.5	0.5
Mineral premix[c]	0.5	0.5	0.5	0.5	0.5
Enriched fish oil[d]	0.0	0.6	1.2	1.8	2.4
Soybean oil	2.4	1.8	1.2	0.6	0.0
Sea mud[a]	26.6	26.6	26.6	26.6	26.6
Proximate composition (%)					
Moisture	10.7	10.7	10.6	10.5	10.5
Crude protein	19.4	19.4	19.4	19.4	19.4
Crude lipid	4.98	4.98	4.98	4.98	4.98
Ash	35.7	35.7	35.7	35.7	35.7
n-3 HUFA (EPA + DHA)	0.46	0.85	1.25	1.61	1.95

[a] Soybean meal (dry matter, %): crude protein 50.30, crude lipid 1.79; corn meal (dry matter, %): protein 10.66, crude lipid 4.54; *Sargassum thunbergii* (dry matter, %): crude protein 19.35, crude lipid 2.00; fish meal (dry matter, %): protein 70.05, crude lipid 8.06; sea mud (dry matter, %): protein 2.74, crude lipid 0.90; These ingredients were obtained from Great seven Bio-Tech (Qingdao, China).

[b] Vitamin premix contained the following amount which were diluted in cellulose (g kg^{-1} premix): L-ascorbic acid, 100; DL-a-tocopheryl acetate, 2; thiamin hydrochloride, 8; riboflavin, 10; pyridoxine hydrochloride, 15; niacin, 45; Ca-D-pantothenate, 18; myo-inositol, 80; D-biotin, 0.3; folic acid, 1.5; menadione, 4; retinyl acetate, 3.2; cholecalciferol, 1; cyanocobalamin, 0.004; ethoxyquin 16.

[c] Mineral premix contained the following ingredients which were diluted in zeolite (g kg^{-1} premix): $MgSO_4$ $7H_2O$, 80.5; Ferric citrate, 16; $ZnSO_4H_2O$, 9; $CuSO_4$ $5H_2O$, 3; $AlCl_3$ $6H_2O$, 6; KIO_3, 0.04; $MnSO_4$ H_2O, 2; $CoCl_2$ $6H_2O$, 0.04.

[d] EPA and DHA enriched fish oil: EPA and DHA contents (determined data) were 323 mg g^{-1} and 325 mg g^{-1}, respectively. Fish oil was obtained from Great seven Bio-Tech (Qingdao, China).

Table 2
Fatty acid compositions of experimental diets (%).

Items	Dietary *n*-3 HUFA contents (%)				
	0.46	0.85	1.25	1.61	1.95
Saturated fatty acid					
14: 0	1.20	1.28	1.36	1.39	1.43
16: 0	19.5	18.7	17.8	16.9	16.2
18: 0	2.04	1.88	1.75	1.62	2.71
20: 0	0.57	0.52	0.48	0.44	0.41
22: 0	0.53	0.45	0.35	0.31	0.23
24: 0	0.18	0.61	0.86	1.11	1.34
Subtotal	24.3	24.1	23.5	23.1	23.6
Monounsaturated fatty acid					
16: 1*n*7	2.02	2.28	2.55	2.81	3.03
18: 1*n*7	0.92	1.59	1.60	1.59	1.61
18: 1*n*9	22.8	19.6	17.3	15.0	12.8
20: 1*n*9	2.48	2.51	2.48	2.52	2.54
22: 1*n*9	2.36	2.28	2.28	2.27	2.31
Subtotal	31.0	28.7	26.7	24.8	22.9
Polyunsaturated fatty acid					
18: 2*n*6c	26.6	21.9	17.5	13.2	8.73
18: 2*n*6t	7.13	5.87	4.60	3.39	2.13
20: 4*n*6	1.71	2.11	2.43	2.80	3.10
20: 5*n*3	5.57	9.49	13.3	16.9	20.1
22: 6*n*3	3.67	7.67	11.7	15.4	19.0
∑*n*-3	9.2	17.3	25.2	32.7	39.6
∑*n*-6	35.5	29.9	24.5	19.4	14.0
n-3 HUFA (EPA + DHA)	9.20	17.2	25.0	32.3	39.1
DHA/EPA	0.7	0.8	0.9	0.9	0.9
Subtotal	44.7	47.2	49.7	52.1	53.5

Notes: ∑*n*-3: Sum of *n*-3 PUFAs; ∑*n*-6: Sum of *n*-6 PUFAs.