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Short communication

Effect of dietary vitamin E on the growth performance and nonspecific immunity in sub-adult turbot (*Scophthalmus maximus*)

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ABSTRACT

This study investigated the growth performance and non-specific immunity in sub-adult turbot fed with graded levels of vitamin E (0, 120, 240, 480 and 960 mg kg^{-1}) for 15 weeks. Results showed that the final weight, specific growth rate, nitro blue tetrazolium positive leucocytes of head kidney, phagocytic index, serum lysozyme activity and superoxide dismutase activity significantly increased with increasing vitamin E levels. The highest values were recorded in the diet with 480 mg kg^{-1} vitamin E. However, no significant differences in the hepatosomatic index, viscerosomatic index and survival rate were found among all dietary treatment. Furthermore, the expression levels of complement component 3 (C3), tumor necrosis factor-α (TNF-α) and interleukine 1β (IL-1β) were significantly upregulated in the fish feed with the vitamin E-supplemented diets. Compared with the basal diet, the diet supplemented with 480 mg kg^{-1} vitamin E significantly augmented the mRNA expression of IL-1β, TNF-α in the spleen and head-kidney, C3 in the liver, respectively. In conclusion, the obtained results indicate the basal diet supplemented with moderate dietary vitamin E (480 mg kg^{-1}) increased the growth, nonspecific immune responses, and expression levels of some immune-related genes in sub-adult turbot. These observations suggest that optimal dietary vitamin E can promote the growth, maintain the health and improve the broodstock management for turbot.

1. Introduction

Aquaculture has been popularized over the last several decades and has provided nearly half of the total fish and shellfish for human consumption, it has been developed as a major source of animal protein and as an important component in food security [1]. The goal of the aquaculture industry is to produce high-quality products. Proper nutrition is essential to avoid deficiency signs, achieve optimal growth rates and maintain the health of cultured fish [2]. Efforts have been exerted over the past two decades to understand the link between nutrition, immune response and diseases resistance in different species, particularly in farmed fish [3,4]. Vitamin E, a lipophilic vitamin, is an essential nutrient for normal physiological functions in fish [5]. Vitamin E can maintain flesh quality, immunity, normal resistance of red blood corpuscles to hemolysis, and permeability of capillaries and heart muscle [6]. Vitamin E functions as a lipid-soluble antioxidant that protects biological membranes, lipoproteins and lipid stores against damage induced by oxygen free radicals and reactive products of lipid peroxidation, thereby stimulating subcellular particle stabilization [5,7,8]. Diet supplementation with vitamin E decreases the levels of lipid peroxidation products and protects the integrity of tissues in farmed fish, including Atlantic halibut [9], black sea bream [10], gilthead sea bream and turbot [11]. Meanwhile, vitamin E is also involved in the regulation of the specific immunity, nonspecific resistance factors, disease resistance capacity [12], and stimulated the survival and growth of fish in captive [13]. Thus, vitamin E can affect several physiological, biochemical and immunological indices in aquaculture.

Vitamin E occurs in eight naturally occurring forms, with α-tocopherol having the highest vitamin E activity [14]. DL-α-Tocopheryl acetate as a stable form of α-tocopherol is the most commonly used vitamin E supplement in animal feeds. A dietary requirement for vitamin E has been demonstrated in different fish species including Atlantic salmon [15], grass carp [16] and guppy [17]. In addition, the requirement of vitamin E increases as the lipid

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level increases. Shiau S and Shiau L (2001) found that the optimum dietary vitamin E requirements of juvenile hybrid tilapia range from 40 mg to 44 mg and from 60 mg to 66 mg in 50 and 120 g lipid per kg diets, respectively [18]. Feeding market-size turbot with at least 550 mg α-tocopheryl acetate kg^{-1} diet at two months prior to slaughter can improve fillet quality [19]. Devesa (1994) already reported that turbot requires less than 300 mg kg^{-1} of vitamin E when dietary lipid ranged 6–15% [20]. Therefore, the dietary requirement of vitamin E is different in fish-specific species and correlated with lipid concentration in diet during farming.

Turbot (*Scophthamus maximus*) with a high economic value is a rapidly growing species widely cultured in Europe and Asia. Numerous studies on the nutritional requirements of this species have been intensively conducted in captive [20–22]. Tocher et al. (2002) found decreased dietary vitamin E led to decreased levels of tissue vitamin E, and generally higher activities of the liver antioxidant enzymes and higher levels of lipid peroxides in juvenile turbot [11]. Meanwhile, the ratio of dietary vitamin E to polyunsaturated fatty acids affects the lipid peroxidation in turbot [23]. The previous study on dietary vitamin E functions mainly focuses on lipid peroxidation and flesh quality in turbot during farming. However, information on the nutritional immunity is sparse. Particularly, in-depth studies on the vitamin E for sub-adult turbot are lacking. Therefore, this study aims to determine the effects of vitamin E on the growth, nonspecific immunological parameters and expression of some immune-related genes [C3, interleukine (IL)-1β and tumor necrosis factor (TNF)-α] in sub-adult turbot.

2. Material and method

2.1. Diet formation and preparation

The basal diet formation and proximate analysis are shown in Table 1. The basal diets contained 3.82% polyunsaturated fatty acids (PUFAs) on a dry matter basis. Of the total lipids, 31.45% were PUFAs and 20.83% were n-3 fatty acid. Dl-all-rac-α-tocopherol acetate (Sigma Chemical) was selected as the source of vitamin E because of its stability and bioavailability to fish. Basal diets were supplemented with Dl-all-rac-α-tocopherol acetate at concentrations of 0, 120, 240, 480 and 960 mg kg^{-1} diet. Basal diet without addition of Dl-all-rac-α-tocopherol acetate served as the control diet.

Table 1
Formulation and proximate composition of basal diets (% dry matter).

Ingredients	%	Proximate compositions	
Fish meal[a]	52.00	Moisture (%)	9.28
Soybean meal[a]	16.00	Crude protein (%)	48.85
Wheat meal[a]	15.20	Crude lipid (%)	14.38
Shrimp meal	3.00	Vitamine E[d] (mg kg^{-1})	28.60
Fish oil	5.00		
Soybean oil	3.00		
Soybean lecithin	2.00		
$Ca(H_2PO_3)_2$	2.00		
Vitamine premix[b]	0.60		
Mineral premix[c]	1.00		
Choline chloride	0.20		
Total	100.00		

[a] Fish meal: crude protein 69.7% dry matter, crude lipid 7.1% dry matter; soybean meal, crude protein 53.3% dry matter, crude lipid 1.9% dry matter; wheat meal, crude protein 14.70 dry matter. These ingredients were supplied by Tech TianBang Bio-Tech (Ningbo, China).

[b] Vitamine premix (mg kg^{-1} diet): thiamin, 25; riboflavin, 45; pyridoxine HCl, 20; vitamin B12, 0.1; vitamin K3, 10; inositol, 800; pantothenic acid, 60; niacin acid, 200; folic acid, 20; biotin, 1.20; retinol acetate, 32; cholecalciferol, 5; choline chloride, 2500; wheat middling, 18.52 g kg^{-1} diet.

[c] Mineral premix (mg kg^{-1} diet): $MgSO_4 \cdot 7H_2O$, 1200; $CuSO_4 \cdot 5H_2O$, 10; $ZnSO_4 \cdot H_2O$, 50; $FeSO_4 \cdot H_2O$, 80; $MnSO_4 \cdot H_2O$, 45; $CoCl_2$ (1%), 50; Na_2SeO_3 (1%), 20; $Ca(IO_3)_2$ (1%), 60.

[d] Total vitamin E content in basal diet.

Ingredients were ground into fine powder through a 246 μm mesh. All ingredients were thoroughly mixed with fish oil and soybean oil, and water was added to produce stiff dough. The dough was then pelleted with an experimental feed mill and dried in a warm air cabinet (40 °C, 48 h). After drying, the diets were sieved into proper pellet size and then kept in a freezer at −20 °C until fed.

2.2. Experiment procedure

Turbot were obtained from Zhuo Yue Aquatic Limited Corporation (Qingdao, Shandong, China). Prior to the experiment, the fish were acclimatized to laboratory conditions for 2 weeks. The basal diets were fed to all fish during the conditioning period. Fish of similar sizes (113.08 ± 2.88 g) were randomly distributed into 15 cylindrical fiberglass tanks (2500 L) with 15 individuals per tank. Each diet was randomly assigned to triplicate tanks. Each tank was provided with a continuous flow of water (6.5 L min^{-1}) and continuous aeration through air stones to maintain dissolved oxygen at or near saturation. The fish were fed two times daily at 07:00 and 18:00. Any uneaten feed was collected 1 h after each meal, dried to constant weight at 70 °C and then reweighed to calculate feed conversion ratio. The feeding trials lasted for 15 weeks. During the experimental period, water temperature ranged from 16.0 to 19.0 °C, salinity from 27‰ to 30‰, pH from 7.5 to 8.0, ammonia nitrogen was lower than 0.1 mg/L, nitrite was lower than 0.1 mg/L, and dissolved oxygen was higher than 6.0 mg/L.

At the end of the experiment, the fish were fasted for 24 h and anesthetized with 100 mg/L tricaine methane sulfonate (MS-222, Sigma, St. Louis, MO) before harvest. The total number and body weight of fish in each tank were measured. After the final weight was noted, blood samples were obtained from the caudal vein of five fish from each cage with 5 mL syringes and allowed to clot at room temperature for 4 h and then at 4 °C for 6 h. Subsequently, the blood was centrifuged at 1000 g for 10 min. The serum was collected and then stored at −80 °C to analysis lysozyme and superoxide dismutase (SOD) activities.

After blood collecting, the fish were sacrificed. The viscera and liver were removed from the fish, and then weighted respectively. In addition, tissues from the spleen, head-kidney and liver were collected from each fish. The specimens were snap-frozen in liquid nitrogen or preserved in RNAstore Reagent (Tiangen Biotech, Beijing, China) and then stored at −80 °C until RNA extraction.

2.3. Immunological parameters and superoxide dismutase (SOD) activity

The lysozyme activity, phagocytic index (PI) and nitro blue tetrazolium (NBT) positive test were measured following a previous method, respectively [24–26]. SOD activity was determined using an SOD detection kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and defined as the amount of required for inhibiting the rate of xanthine reduction by 50% in 1 mL reaction system, specific activity was expressed as units per mL serum [27].

2.4. RNA extraction and real-time quantitative polymerase chain reaction (RT-PCR)

A two-step, real-time RT-PCR was used to measure the expression of C3, IL-1β, and TNF-α. Total RNA was extracted from the spleen, head-kidney and liver of five turbot by Trizol reagent (GIBCO-BRL, Carlsbad, CA, USA). Total RNA (2 μg) was reverse transcribed by a Thermol One step RT-PCR kit according to the manufacture's instructions. The mRNA expression levels of C3, IL-