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Effects of dietary vitamin A on growth, feed utilization, lipid metabolism enzyme activities, and fatty acid synthase and hepatic lipase mRNA expression levels in the liver of juvenile orange spotted grouper, *Epinephelus coioides*

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ABSTRACT

A 10-week feeding trial was conducted to investigate the effects of dietary vitamin A (VA) on growth, feed utilization, lipid metabolism enzyme activities, and fatty acid synthase (FAS), and hepatic lipase (HL) mRNA expression levels in the liver of juvenile *Epinephelus coioides*. Six semi-purified diets were formulated to contain VA as retinyl acetate at concentrations of 0, 1000, 2000, 4000, 8000, and 20,000 IU·kg^{-1}, providing actual dietary values of 387 (control group), 1256, 2242, 4102, 7635, and 18,231 IU VA·kg^{-1} diet, respectively. A total of 540 juvenile *E. coioides* were randomly stocked into 18,500 L fiberglass tanks with 30 fish per tank and 3 tanks per diet. The results showed weight gain rate (WGR), specific growth rate (SGR), and survival rate (SR), significantly increased in fish supplemented with over 1000 IU dietary VA compared with the control group ($P < 0.05$). The feed conversion ratio (FCR) significantly decreased in fish supplemented with dietary VA from 2000 IU·kg^{-1} to 8000 IU·kg^{-1} compared with fish in the other groups ($P < 0.05$). However, supplementing the diet with VA at > 8000 IU·kg^{-1} inhibited the growth of juvenile *E. coioides* ($P < 0.05$). The FAS and HL activities were significantly higher in the livers of the group supplemented with 2000 IU VA·kg^{-1} compared to those of the control group and the group supplemented with 1000 IU VA·kg^{-1} ($P < 0.05$), but dietary VA did not significantly affect whole body lipid deposition in fish ($P > 0.05$). The FAS and HL mRNA expression levels were significantly higher in the groups with dietary VA levels from 4000 IU·kg^{-1} to 8000 IU·kg^{-1} compared to those in the control group ($P < 0.05$). Based on the broken-line regression analysis of WGR and dietary VA levels, the optimal dietary VA requirement is 1775 IU VA·kg^{-1}for juvenile grouper. At the same time, under the condition of this experiment, a dietary VA level of 4000 IU·kg^{-1} promoted lipolytic enzyme activity and the expression of lipid decomposition genes in the liver of groupers.

1. Introduction

Vitamin A (VA), a fat-soluble vitamin, plays an important role in regulating the growth and development, cell proliferation, and differentiation of many animals, including fish (NRC, 2011). VA can affect the visual function in fish through the impact of rhodopsin synthesis on visual capability adjustment (Lamb and Pugh, 2004) and VA levels can affect the immune function of tilapia (*Oreochromis niloticus*) (Guimarães et al., 2014), carp (*Cyprinus carpio* var. Jian) (Yang et al., 2008), and sun bass (*Morone chrysops* × *M. saxatilis*) (Hemre et al., 2004), VA deficiency decreased the immune function in carps (Yang et al., 2008), and octopus (*Octopus vulgaris*) (Villanueva et al., 2009) and also; resulted in growth retardation, liver enlargement, and carcass index reduction in rainbow trout (*Oncorhynchus mykiss*) (Estevez and Kanazawa, 1995; Takeuchi et al., 1995), Atlantic salmon (*Salmo salar* L.) (Qrnsrud et al., 2002) and silver catfish (*Rhamdia quelen*) (Battisti et al., 2017).

In addition to the aforementioned functions of VA, studies in mammals have shown that VA is an important regulator of lipid metabolism (Berry and Noy, 2012), and can regulate the activity of oxidases and mediate lipid peroxidation (Gelain et al., 2008). High VA levels can significantly reduce blood cholesterol levels (Chen et al., 2002) and feeding with 5000 IU VA·kg^{-1} is favorable for lipid synthesis (Ma and Xu, 2002). Studies on the regulation of lipid metabolism in aquatic animals are limited. VA feeding can promote lipase activity and decrease lipid deposition in the livers of Senegalese sole (Darias et al.,

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Table 1
Feed formulation and proximate composition of the basal diet (DM basis).

Ingredients	g·kg^{-1} diet
White fish meal	200.00
Casein(vitamin free)[a]	280.00
Gelatin	70.00
Wheat flour	230.00
Fish oil[b]	30.00
Corn oil[c]	30.00
Soybean lecithin oil	30.00
Vitamin mix[d]	10.00
Mineral mix[e]	10.00
Choline chloride	5.00
l-ascorbat-2-phosphate	0.50
Calcium phosphate primory	10.00
Attractant	1.00
Antioxidant	0.50
Total	100.00
Analysis nutrient composition (g·kg^{-1} in dry matter)	
Crude protein	502.60
Crude lipid	102.80
Crude Ash	48.90
Energy (MJ·kg^{-1})	20.80
Vitamin A (IU·kg^{-1})	387.00

[a] Obtained from Sigma Chemical Co., St. Louis, MO;
[b] Obtained from semi-refined fish oil, Oleaginosa Victoria S.A., Peru;
[c] Obtained from Tai-Tang Industrial, Taiwan;
[d] Vitamin premix supplied the diets with (mg/g dry diet):inositol, 0.5; niacin, 37.5; D-calcium pantothenate, 25; DL-a-tocopheryl acetate, 20; thiamine mononitrate, 2.5; riboflavin, 10; pyridoxine HCl, 2.5; cyanocobalamin, 0.0025. menadione, 2; folic acid, 0.75; biotin, 0.25; cholecalciferol, 0.05;
[e] Mineral premix supplied the diets with(g/kg of premix): $CaCO_3$, 350; $NaH_2PO_4{\cdot}H_2O$, 200; KH_2PO_4,200; NaCl, 12;$MgSO_4.7H_2O$, 10; $FeSO_4.7H_2O$, 2; $MnSO_4.7H_2O$, 2; $AlCl_3.6H_2O$, 1; $CuCl_2.2H_2O$, 1; KF, 1; $Na_2MoO_4.2H_2O$, 0.5; Na_2SeO_3, 0.4; $CoCl_2.6H_2O$, 0.1; KI, 0.1.

2012) and gilthead seabream larvae (Fernández and Gisbert, 2011).

The orange-spotted grouper (*Epinephelus coioides*) is a popular edible fish cultured in the Asia-Pacific region and a potentially important aquaculture species because of its fast growth, efficient feed conversion, good quality, and high economic value (Boonyaratpalin, 1997). However, the nutritional requirements and pathological signs attributed to varying VA levels have not been studied in *E.coioides*. The objective of this study is to determine the pathological signs of VA supplementation, as well as the effects of different VA levels on the growth, feed utilization, lipid metabolism enzyme activities, and FAS and HL mRNA expression levels in the livers of juvenile *E. coioides*.

2. Materials and methods

2.1. Diet formulation and preparation

The basal diet was formulated to provide 50.26% of crude protein (Table 1). White fish meals, casein, and gelatin served as the main protein sources. Fish oil, corn oil, and soybean lecithin oil were the lipid sources and provided a total of 10.28% of crude lipid. Six diets containing 0, 1000, 2000, 4000, 8000, and 20,000 IU VA·kg^{-1} (as retinyl actate, Sigma Chemical Co., St. Louis) were fed to the treatment groups. High-performance liquid chromatography (HPLC) was used to determine the exact VA content of each of the six diets (Driskell et al., 1982). The results are as follows: 387 (a basal diet), 1256, 2242, 4102, 7635, and 18,231 IU·kg-1 diet, respectively.

2.2. Experimental animals and breeding management

In this experiment, juvenile *E. coioides* were obtained from the Guangdong Ocean University Seawater-fish Breeding Laboratory (Zhanjiang, Guangdong province, China). Upon arrival, fish were acclimated to laboratory conditions for two weeks in a cement pool [3 m (w) × 5 m (l) × 2 m (h)] and fed commercial diets (Zhanjiang Yuehai Feed Co. Ltd., Guangdong, China) in the first week, and then fed a non-VA supplemented diet to decrease the VA concentration for the second week until the fish achieved the required specifications. The laboratory conditions during the acclimation period were similar to those at the initiation of the experiment. Before weighing, the fish were starved for 24 h, and then a total of 540 healthy fish with an average initial weight of 7.40 ± 0.01 g were divided into 18 aquariums at a density of 30 tails per repeat in a completely randomized design. Each diet was randomly assigned to triplicate groups. The experiment was conducted in an indoor farming system in the Marine Biological Research Base of Guangdong Ocean University. The experiment lasted for 10 weeks, and 30% to 50% new water was added to the recirculation system every 2 days in the first 4 weeks and then every day in the last 6 weeks. During the experiment, the water temperature was measured daily and varied from 28 °C to 33 °C, while the salinity of seawater ranged from 26 to 28. The aquariums were provided aerated to maintain a level of dissolved oxygen near saturation. The fish were fed twice per day at 09:00 and 17:00 at a rate of 3–5% of their body weight per day. Daily feed allowances were adjusted every two weeks according to sampling weight. From the second week until the end of the experiment, all groups were fed corresponding test diets.

2.3. Sample collection and analysis

At the end of the 10 week period, 24 h after the last feeding, fish in each tank were counted and weighed to determine survival rate (SR), weight gain rate (WGR) and feed conversion ratio (FCR). After the final weighing, blood was collected from eight randomly selected fish from each aquarium with 1 mL sterile syringes, placed in a 1.5 mL Eppendorf tubes, and then stored at 4 °C. The mixture was centrifuged and the supernatant was collected to evaluate the effect of VA on lipid metabolism. The hepatopancreas was removed from three randomly selected fish from each aquarium and stored at − 80 °C for the quantification of the FAS and HL mRNAs by RT-PCR (reverse transcription- polymerase chain reaction).

Three fish were chosen randomly from each dietary replicate and frozen at − 20 °C for subsequent proximate carcass analysis. Analysis of dry matter (dried at 105 °C), crude protein (by Kjeldhal apparatus, nitrogen × 6.25), crude fat (extraction with petroleum ether by Soxhlet apparatus), and ash (incineration at 550 °C) were performed for the carcasses and from the different diet groups (AOAC, 1995).

The hepatopancreas and muscles were removed from the other five fish from each aquarium and pooled to determine VA concentration. The collected hepatopancreas were also used to investigated lipid metabolism. VA concentrations were determined through the methods used in the feed analysis. The lipid metabolism enzyme activities were determined by their corresponding commercial kits (Nanjing Jiancheng Bioengineering institute).

2.4. Real-time quantitative RT-PCR analysis of FAS and HL mRNA expression

Total RNA was extracted from the hepatopancreas of three fish per treatment using the conventional method (TRI Reagent solution, USA). First strand cDNA synthesis in RT was performed using PrimeScript™ RT-PCR Kit (TaKaRa, Japan) according to the manufacturer's instructions. The cDNA was stored at − 20 °C for real-time quantitative RT-PCR (qRT-PCR).

Gene-specific primers were designed with the online Primer 5 software based on the cDNA sequences in GenBank (FAS: FJ196231; HL: EU683733) (Table 2). SYBR@ Premix Ex Taq™ Kit (Takara, Japan) was used for real-time quantitative RT-PCR (qRT-PCR) analysis with an ABI 7500Real-Time PCR System (Lifetech, USA). The $2^{-\Delta\Delta Ct}$ comparative CT method (Livak and Schmittgen, 2001) was used to analyze the expression level of the above genes.