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Effects of dietary phospholipids on survival, growth, digestive enzymes and stress resistance of large yellow croaker, *Larmichthys crocea* larvae

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ABSTRACT

A 30-day feeding experiment was conducted in tanks to determine the effects of dietary phospholipids (PL) on survival, growth, digestive enzyme activity and stress resistance of *Larmichthys crocea* larvae (15 days after hatching, initial average weight: 3.36 ± 0.10 mg, initial average body length: 6.15 ± 0.71 mm). Five practical microdiets were formulated with graded levels of PL (26.0 g kg^{-1}, 38.5 g kg^{-1}, 57.2 g kg^{-1}, 69.5 g kg^{-1} and 85.1 g kg^{-1} dry diet). Live prey (*Artemia sinicia* nauplii and copepods) were used as the control diet. Each diet was randomly assigned to triplicate groups of larvae (2500 larvae per tank). Results showed that the survival rate significantly increased as dietary PL increased from 26.0 to 57.2 g kg^{-1} ($P < 0.05$), and then decreased with further increase of dietary PL. The specific growth rate (SGR) of larvae fed the diet with 69.5 g kg^{-1} PL was significantly higher than that of larvae fed the diets with 26.0 and 38.5 g kg^{-1} PL. Selected digestive enzyme activities (trypsin, alkaline phosphatase and aminopeptidase) increased with increasing dietary PL. Stress resistance of larvae against declining water temperature and salinity alteration increased with dietary PL increasing from 26.0 to 69.5 g kg^{-1} ($P > 0.05$), significantly higher than that in the treatment of live prey ($P < 0.05$). These results showed that relatively higher dietary PL (57.2–85.1 g kg^{-1}) could be beneficial for survival and growth performance of large yellow croaker larvae. Higher dietary PL (69.5–85.1 g kg^{-1}) could generally promote development of digestive tract and stress tolerance of this fish larvae.

1. Introduction

Lipids are the favored source of metabolic energy for fish, especially for marine fish (Sargent et al., 2002). Among lipid components, phospholipids (PL) are important components for maintaining the structure and function of cellular membrane, emulsifying lipids in gut and improving intestinal absorption of long chain fatty acids (Tocher et al., 2008). Furthermore, PL could exert beneficial effects by improving diet qualities and providing essential nutrients, e.g. essential fatty acids, phosphorous, choline and inositol (Halver, 2002; Lall, 2002; Tocher, 1995; Tocher et al., 2008). Since *de novo* synthesis of PL of fish larvae is not sufficient to meet the needs of the comparatively rapid growth of early life stages, it is essential to provide PL in the diets of marine fish larvae (Sargent et al., 2002). PL have been demonstrated to significantly affect survival, growth, stress resistance and malformation in several fish larvae species including ayu (*Plecoglossus altivelis*), European sea bass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*), Japanese flounder (*Paralichthys olivaceus*) and knife jaw (*Oplegnathus fasciatus*) (Cahu et al., 2003; Kanazawa, 1993; Kanazawa et al., 1981; Liu et al., 2002; Tocher et al., 2008). Recommended requirements for PL ranged from 3% to 12% dry diet according to previous studies (Cahu et al., 2003; Kanazawa, 1993; Kanazawa et al., 1981). Since requirements are not inconsistent, but rather quite variable depending on the species of study, it is imperative to determine the PL requirement of large yellow croaker larvae. To our knowledge, no information has been published evaluating the physiological effects and optimal dietary levels of PL in diets for large yellow croaker larvae.

Large yellow croaker is a popular culture species in east ocean of China because of its delicious meat and commercial value. There have been a few studies on the nutrition for larvae of this fish (Ai et al., 2008; Xie et al., 2011; Yu et al., 2012). However, as far as we know, it is still unclear about physiological effects of dietary PL in larvae of this fish species. Thus, the present study was conducted to investigate the effects of dietary PL levels on survival, growth, digestive enzyme activities and body composition of large yellow croaker larvae.

2. Materials and methods

2.1. Experimental diets

White fish meal, krill meal and squid meal, together with hydrolyzed fish meal (Ningbo Chaoxing Halobios Products Co., Ltd, China) were used as the main protein sources. Fish oil and soy lecithin were used as lipid sources. Before formulation, α-starch was precooked in

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order to improve its digestibility and adhesiveness. Lycopene was supplemented in the formulated diets as visual stimulant, and the mixture of betaine, alanine, taurine, glycine, argine and inosine-5′-monophosphoric acid were used as chemical attractants. Five isonitrogenous and isoenergetic microdiets were formulated to contain 26.0 g kg^{-1}, 38.5 g kg^{-1}, 57.2 g kg^{-1}, 69.5 g kg^{-1}, and 85.1 g kg^{-1} PL, respectively, by adding graded levels of fish oil and soybean lecithin (Tables 1 and 2). Diets were prepared by thoroughly mixing dry ingredients with the oil and lecithin. Methodology used to prepare the microbound diets resembled the method of particle-assisted rotational agglomeration (PARA). PARA is a method to produce microbound feed. This method utilizes the marumerizer which was equipped with a charge of inert particles. Wet mash is placed directly into the marumerizer and then spheroid feed particles in a wide size range are produced through the rotation of the marumerizer (Hardy and Barrows, 2002). The particle size of the formulated diets ranged from 150 to 250 μm for fish between 12 and 25 DAH (day after hatch) and 200 to 350 μm for fish thereafter. All formulated diets were packed in separate silver bags and stored at −20 °C until used. Live prey including *Artemia sinicia* nauplii and live copepods were used as the control diet.

Table 1
Formulation and proximate chemical composition of the experimental diets (g kg^{-1} dry matter).

Diet no. (PL g kg^{-1})	Diet 1 (26.0)	Diet 2 (38.5)	Diet 3 (57.2)	Diet 4 (69.5)	Diet 5 (85.1)
LT-white fish meal[a]	450	450	450	450	450
LT-krill meal[b]	140	140	140	140	140
LT-squid meal[c]	120	120	120	120	120
Hydrolyzed fish meal[d]	40	40	40	40	40
LT-yeast	30	30	30	30	30
α-Starch	50	50	50	50	50
Alginate sodium	20	20	20	20	20
Vitamin premix[e]	15	15	15	15	15
Mineral premix[f]	10	10	10	10	10
Ascorbyl polyphosphate	1.5	1.5	1.5	1.5	1.5
Attractant mixture[g]	20	20	20	20	20
Pigment (tomato red)	0.5	0.5	0.5	0.5	0.5
Antioxidant	0.5	0.5	0.5	0.5	0.5
Choline chloride	2	2	2	2	2
Fish oil	100	75	50	25	0
Soybean lecithin[h]	0	25	50	75	100
Proximate composition (g kg^{-1})					
Crude protein	553	549	550	551	550
Crude lipid	170	166	164	162	159
NL/PL[i]	2.73	1.46	1.06	0.88	0.49
Phospholipids	26.0	38.5	57.2	69.5	85.1
n−3HUFA	2.51	2.18	1.79	1.56	1.17

[a] Contained 709 g kg^{-1} crude protein and 76 g kg^{-1} crude lipid (commercially available from American Seafood Co., USA).
[b] Contained 637 g kg^{-1} crude protein and 40 g kg^{-1} crude lipid (commercially available from Haiyun Biotech Co., Ltd., China).
[c] Contained 777 g kg^{-1} crude protein and 73 g kg^{-1} crude lipid (commercially available from Haiyun Biotech Co., Ltd., China).
[d] Contained 875 g kg^{-1} crude protein and 28 g kg^{-1} crude lipid (provided by Ningbo Chaoxing Halobios Products Co., Ltd., China).
[e] Composition of vitamin premix (IU or g kg^{-1}): retinal palmitate, 3000000 IU; cholecalciferol, 1200000 IU; DL-α-tocopherol acetate, 40.0 g; menadione, 8.0 g; thiamin-HCl, 5.0 g; riboflavin, 5.0 g; D-calcium pantothenate, 16.0 g; pyridoxine-HCl, 4.0 g; meso-inositol, 200.0 g; D-biotin, 8.0 g; folic acid, 1.5 g; para-aminobenzoic acid, 5.0 g; niacin, 20.0 g; cyanocobalamin, 0.01 g; ascorbyl polyphosphate (contained 25% ascorbic acid), 100.0 g.
[f] Composition of mineral premix (g kg^{-1} premix): $Ca(H_2PO_4)_2.H_2O$, 675.0; $CoSO_4.4H_2O$, 0.15; $CuSO_4.5H_2O$, 5.0; $FeSO_4.7H_2O$, 50.0; KCl, 50.0; KI, 0.1; $MgSO_4.2H_2O$, 101.7; $MnSO_4.4H_2O$, 18.0; NaCl, 80.0; $Na_2SeO_3.H_2O$, 0.05; $ZnSO_4.7H_2O$, 20.0.
[g] Composition of attractant mixture (g kg^{-1} premix): betaine, 500 g; glycine, 150 g; alanine, 100 g; argine, 100 g; taurine, 100 g; inosine-5′-monophosphoric acid, 50 g.
[h] Contained 620 g kg^{-1} of phospholipids, including 260 g kg^{-1} of phosphatidylcholine, 200 g kg^{-1} of phosphatidylethanolamine and 140 g kg^{-1} of phosphatidylinositol.
[i] NL/PL: Neutral lipid to polar lipid ratio.

2.2. Experimental procedure

Larvae used in this study were obtained and reared at the hatchery of the National Center for Large Yellow Croaker in Xiangshan Bay (Ningbo, China). 45000 larvae of 15 DAH age, with average body weight 3.36 ± 0.10 mg and average body length 6.15 ± 0.71 mm, were used in this study in 18 blue plastic tanks (70 × 50 × 60 cm, 180 l). Each tank was stocked initially with 2500 individuals. All tanks were placed in an indoor concrete pond (800 × 400 ×160 cm). They were supplied with seawater that had been filtered through two-grade sand filter. During the rearing period, water temperature (23 ± 1 °C), pH (8.0 ± 0.2) and salinity (25 ± 2 ‰) were regularly monitored and readjusted when needed. About 50–200% of the water was renewed daily and there was a light permanent agitation by means of air bubbling. Light intensity was 8.5 W m^{-2} maximum during daytime at the water surface. Undissolved surface materials were skimmed with a polyvinylchloride pipe in time and accumulations of feed and feces at the tank bottoms were siphoned twice daily.

Larvae were fed with rotifers *Brachionus plicatilis* (0.5–1.5 × 10^4 individuals l^{-1} seawater) from 3–14 DAH. The rotifers used as feed had been enriched with a mixture of unicellular algae (*Chlorella*) to increase DHA and EPA contents, according to Zheng et al. (1996). Assayed composition of the rotifers was 61.9% crude protein, 13.7% crude lipid and 13.2% ash. The unicellular algae were continuously supplied at a concentration of 20000 to 40000 cell ml^{-1} in the rearing pond before 12 DAH. From 15 to 45 DAH, the larvae were weaned to the 6 dietary treatments (3 tanks per group). The fish were manually fed in large excess for 8 times (06:00, 07:30, 08:30, 09:30, 12:30, 14:00, 15:30, 17:00) per day during the daylight period. Larvae were reared under 14 h light:10 h dark dial cycle photoperiod.

2.3. Sampling

In order to monitor wet body weight (BW), 300 larvae at 15 DAH were randomly sampled from the rearing pond and 200 specimens at 45 DAH were randomly sampled from each tank before morning food distribution. Samples were weighed in a microbalance. To monitor body length (BW), 30 larvae at 15 DAH and 45 DAH were randomly sampled from rearing pond and each blue tank, respectively. Then the length was measured with a Vernier caliper. At the end of the experiment, larval survival rates were determined by counting individuals remaining in each tank. Fifty specimens were collected from each tank and immediately frozen with liquid nitrogen and stored at −80 °C for later analysis of digestive enzyme. The remaining fish from each tank were collected and then freeze-dried for subsequent analysis.

Under a microscope, each specimen was carefully dissected into four parts (head, pancreatic segment, intestinal segment and tail) on a glass maintained at 0 °C. Then the pancreatic segments and intestinal segments were collected for digestive enzyme analysis (Ai et al., 2008; Cahu and Zambonino Infante, 1994; Ma et al., 2005).

2.4. Analytical methods

The chemical composition of diets and fish was determined following the standard procedures (AOAC, 1995). The samples of diets and fish larvae were dried to a constant weight at 105 °C to determine the dry matter content. Protein was determined by measuring nitrogen (N × 6.25) using the Kjeldahl method; Lipids were extracted with a chloroform:methanol (2:1, v/v) according to Folch et al. (1957). Neutral and polar lipids were separated by adsorption chromatography on silica cartridges (Sep-pak, Waters, Milford, MA) as described by Juaneda and Rocquelin (1985). The procedures for analysis of the fatty acid profiles in neutral and polar lipids were performed according to the method described by Metcalfe et al. (1966). Fatty acid methyl esters were prepared