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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.

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## 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, 3 000 000 IU; cholecalciferol, 1 200 000 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). 45 000 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 20 000 to 40 000 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

by transesterification with $BF_3$-methanol and then quantified by HP6890 gas chromatograph (Agilents Technologies Inc., Santa Clara, California, USA) with a fused silica capillary column (007-CW, Hewlett Packard, Palo Alto, CA, USA) and a flame ionization detector. The column temperature was programmed to rise from 150 °C up to 200 °C at a rate of 15 °C $min^{-1}$, from 200 °C to 250 °C at a rate of 2 °C $min^{-1}$. Injector and detector temperature was 250 °C, respectively. The PL content of diets was assayed by the method of colorimetric measurement of phosphorus as molybedenum blue according to Li et al. (2005).

The pancreatic or intestinal segments were put into a clean tube and homogenized with vortex into 5 volumes (v/w) of ice-cold distilled water, and then centrifuged (3300 g, 4 °C, 5 min). The supernatant was used for further assays. Trypsin (EC 3.4.21.4) and amylase (EC 3.2.1.1) activities were assayed according to Holm et al. (1988) and Métais and Bieth (1968), respectively. Purified brush-border membranes (BBM) from the intestinal segment homogenate were collected as described by Crane et al. (1979) and Cahu and Zambonino Infante (1994). Aminopeptidase N (AN, EC 3.4.11.2) and alkaline phosphotase (AP, EC 3.1.3.1) of intestinal segments and BBM were assayed according to Maroux et al. (1973) and Bessey et al. (1946), respectively. Enzyme activities were expressed as specific activity (mU/mg protein). Protein was determined according to Bradford (1976).

### 2.5. Stress test

At the end of the feeding experiment, 30 fish larvae from each replicate were chosen and kept in steel buckets (volume: 8 l). After starved for 24 h, fish larvae were used for temperature and salinity stress test. For temperature stress test, water temperature rose from 22 °C to 34 °C at a rate of 2 °C $h^{-1}$ or declined from 22 °C to 8 °C at a rate of 7 °C $h^{-1}$and then kept at the terminal temperature (34 °C or 7 °C) for 1 h. For salinity stress test, water salinity rose from 26‰ to 44‰ at a rate of 2‰/h or declined from 26‰ to 0.5‰ at a rate of 3‰/h. Survival rate were calculated by recording the number of dead fish during temperature or salinity stress test. For low oxygen stress test, 12 fish larvae from each replicate were kept in an airtight bottle (volume: 600 ml) and then assayed the dissolved oxygen level of water when 50% larvae died.

**Table 2**
Fatty acid composition of the experimental diets (% total fatty acids).

| Diet no. ($PL\ g\ kg^{-1}$) | Diet1 (26.0) | Diet2 (38.5) | Diet3 (57.2) | Diet4 (69.5) | Diet5 (85.1) | LP (49.9) |
|---|---|---|---|---|---|---|
| C14:0 | 4.87 | 4.83 | 3.35 | 2.70 | 2.35 | 1.38 |
| C16:0 | 19.40 | 19.50 | 18.90 | 18.30 | 19.89 | 9.03 |
| C18:0 | 3.96 | 3.72 | 3.87 | 4.03 | 3.98 | 0.11 |
| C20:0 | 1.55 | 1.28 | 0.93 | 0.71 | 0.43 | 0.66 |
| ∑SFA[a] | 29.78 | 29.32 | 27.05 | 25.74 | 26.65 | 11.18 |
| C16:1 | 6.92 | 6.57 | 5.27 | 4.42 | 4.15 | 11.33 |
| C18:1 | 17.91 | 18.27 | 18.68 | 18.28 | 18.66 | 35.33 |
| ∑MUFA[b] | 24.83 | 24.85 | 23.95 | 22.70 | 22.81 | 46.66 |
| C18:2n−6 | 7.14 | 12.50 | 20.23 | 25.78 | 30.86 | 6.86 |
| C20:4n−6 | 4.36 | 3.89 | 3.66 | 3.24 | 2.81 | 0.90 |
| ∑n−6 PUFA[c] | 11.50 | 16.38 | 23.89 | 29.02 | 33.67 | 7.76 |
| C18:3n−3 | 1.66 | 2.09 | 2.81 | 3.35 | 3.51 | 12.69 |
| C20:5n−3 | 7.70 | 6.57 | 5.44 | 4.75 | 3.50 | 7.88 |
| C22:6n−3 | 11.01 | 9.23 | 7.85 | 6.75 | 5.70 | – |
| ∑n−3 PUFA[d] | 20.38 | 17.88 | 16.09 | 14.85 | 11.72 | 20.57 |
| n−3/n−6PUFA | 1.77 | 1.09 | 0.67 | 0.51 | 0.35 | 2.65 |
| n−3HUFA[e] | 18.72 | 15.79 | 13.29 | 11.50 | 9.20 | 7.88 |
| DHA/EPA[f] | 1.43 | 1.41 | 1.44 | 1.42 | 1.34 | – |

[a] SFA: saturated fatty acids.
[b] MUFA: mono-unsaturated fatty acids.
[c] n−6 PUFA: n−6 poly-unsaturated fatty acids.
[d] n−3 PUFA: n−3 poly-unsaturated fatty acids.
[e] n−3 HUFA: n−3 highly-unsaturated fatty acids.
[f] DHA/EPA: 22:6n−3/20:5n−3.

### 2.6. Calculations

The following calculations were performed:

$$\text{Specific growth rate}\left(\text{SGR}, \%\ \text{day}^{-1}\right) = 100 \times (\text{LnW}_f - \text{LnW}_i)/d$$

where $W_f$ was the final wet body weight (g), $W_i$ was the initial wet body weight and d was the experimental duration.

### 2.7. Statistical analyses

The statistical analysis was performed by using SPSS 16.0 for Windows (SPSS Inc., Chicago, Illinois, USA). All percentage data were arcsine transformed prior to statistical analysis. All data were subjected to a one-way analysis of variance (ANOVA) and differences between the means were tested by Tukey's multiple range test. The level of significance was chosen at $P < 0.05$ and the results are presented as mean values with their standard errors.

## 3. Results

### 3.1. Survival and growth

Survival rate increased significantly with dietary PL increasing from 26.0 to 57.2 g $kg^{-1}$, and then decreased with further increase of PL. The survival rate of larvae fed the diet with 26.0 g $kg^{-1}$ PL (13.3%) was significantly lower than that of larvae fed the diet with 57.2 g $kg^{-1}$ PL (25.3%) and that of the live prey group (24.8%) ($P < 0.05$) (Table 3).

Body length and specific growth rate (SGR) increased significantly with dietary PL increasing from 26.0 to 69.5 g $kg^{-1}$, and then decreased. Body length of fish larvae fed the diet with 26.0 g $kg^{-1}$ PL (10.0 mm) were significantly smaller than that of larvae fed the diets with 57.2 g $kg^{-1}$ (11.0 mm) and 69.5 g $kg^{-1}$ PL (11.3 mm). SGR of larvae fed the diets with 57.2 g $kg^{-1}$ (9.2% $day^{-1}$) and 69.5 g $kg^{-1}$PL (9.4% $day^{-1}$) were significantly higher than that of larvae fed the diet with 26.0 g $kg^{-1}$ PL (8.3% $day^{-1}$) ($P < 0.05$) (Table 3).

### 3.2. Activities of enzyme

Activities of trypsin in pancreatic segment (PS) significantly increased as dietary PL increased from 38.5 g $kg^{-1}$ to 85.1 g $kg^{-1}$ ($P < 0.05$) (Table 4). Activity of trypsinin PS of larvae fed live prey (14.99 mU/mg protein) was comparable with that in larvae fed diets with 38.5 g $kg^{-1}$ (15.74 mU/mg protein) and 57.2 g $kg^{-1}$ PL (17.18 mU/mg protein), but significantly lower than that in larvae fed the other artificial microdiets ($P < 0.05$). Activity of trypsin in intestinal segment (IS) significantly increased from 29.49 to 47.43 mU/mg protein when dietary PL levels increased from 26.0 to 38.5 g $kg^{-1}$, and then decreased to 32.98 mU/mg protein with further increase of dietary PL. The lowest activity of trypsin in IS (12.5 mU/mg protein) was found in larvae fed live prey, significantly lower than that in larvae fed microdiets ($P < 0.05$). Activities of amylase in both PS and IS were comparable among the artificial microdiet treatments ($P > 0.05$), but significantly lower than that in the treatment of live prey (Table 4).

Activities of alkaline phosphatase in IS and brush border membrane (BBM) increased significantly with the increase of dietary PL. Activities of alkaline phosphatase in IS of fish larvae fed diets with 26.0 g $kg^{-1}$ (116.4 U/mg protein) and 38.5 g $kg^{-1}$ PL (115.8 U/mg protein) were significantly lower than those in treatments with 57.2 g $kg^{-1}$ (140.6 U/mg protein), 69.5 g $kg^{-1}$ (149.4 U/mg protein) and 85.1 g $kg^{-1}$ PL (175.5 U/mg protein). Fish fed live prey had a relatively higher activities of alkaline phosphatase in IS (163.7 U/mg protein), significantly higher than that fed diets with PL equal to and less than 57.2 g $kg^{-1}$. Activities of alkaline phosphatase in BBM of fish larvae fed diets with 26.0 g $kg^{-1}$ (573.0 U/mg protein) were significantly

**Table 3**
Effects of dietary phospholipids on survival, body length and SGR of *Larmichthys crocea* larvae (45 dph, mean ± S.D., n = 3).[a]

| 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) | LP (49.9) |
|---|---|---|---|---|---|---|
| Survival (%) | 13.3 ± 2.2$^{b}$ | 18.9 ± 2.3$^{ab}$ | 25.3 ± 6.5$^{a}$ | 18.5 ± 4.4$^{ab}$ | 15.7 ± 2.8$^{ab}$ | 24.8 ± 2.9$^{a}$ |
| Body length (mm) | 10.0 ± 0.2$^{b}$ | 10.5 ± 0.1$^{ab}$ | 11.0 ± 0.3$^{a}$ | 11.3 ± 0.6$^{a}$ | 10.9 ± 0.3$^{ab}$ | 10.5 ± 0.3$^{ab}$ |
| Initial weight (mg) | 3.4 ± 0.1 | 3.4 ± 0.1 | 3.4 ± 0.1 | 3.4 ± 0.1 | 3.4 ± 0.1 | 3.4 ± 0.1 |
| Final weight (mg) | 40.9 ± 5.5$^{c}$ | 42.2 ± 3.3$^{bc}$ | 55.4 ± 2.9$^{ab}$ | 56.2 ± 2.1$^{a}$ | 45.6 ± 4.2$^{abc}$ | 47.9 ± 5.7$^{abc}$ |
| SGR (% day$^{-1}$) | 8.3 ± 0.4$^{c}$ | 8.4 ± 0.3$^{bc}$ | 9.2 ± 0.2$^{ab}$ | 9.4 ± 0.1$^{a}$ | 8.7 ± 0.3$^{abc}$ | 8.8 ± 0.4$^{abc}$ |

[a] Data with the same superscript letter in the same row are not significantly different as determined by Tukey's test ($P > 0.05$).

lower than those in treatments with 57.2 g kg$^{-1}$ (828.5 U/mg protein), 69.5 g kg$^{-1}$ (783.6 U/mg protein) and 85.1 g kg$^{-1}$ PL (825.0 U/mg protein). Activity of alkaline phosphatase (682.7 U/mg protein) in BBM of larval fed live prey was lower than that of larvae fed diets with PL equal to or more than 57.2 g kg$^{-1}$, though no significance was detected between live prey and each artificial microdiet ($P > 0.05$) (Table 4).

Activities of leucine aminopeptidase in both IS and BBM showed a similar trend as dietary PL increased. Activities of LAP in IS of fish larval fed diets with 26.0 g kg$^{-1}$ (19.34 mU/mg protein) and 38.5 g kg$^{-1}$ PL (19.25 mU/mg protein) were comparable with those in 69.5 g kg$^{-1}$ (26.5 mU/mg protein) and 85.1 g kg$^{-1}$ PL treatments (28.05 mU/mg protein), but significantly lower than those in 57.2 g kg$^{-1}$ PL treatment (30.05 mU/mg protein) (Table 4).

Activities of LAP in BBM of fish larval fed diets with 26.0 g kg$^{-1}$ (61.15 mU/mg protein) were comparable with those in diets with 38.5 g kg$^{-1}$ PL (127.16 mU/mg protein), but significantly lower than those in 57.2 g kg$^{-1}$ (154.81 mU/mg protein), 69.5 g kg$^{-1}$ (167.70 mU/mg protein) and 85.1 g kg$^{-1}$ PL (183.67 mU/mg protein) treatments. No significant differences were found among activities of LAP in BBM of fish fed live prey and each microdiet treatment (Table 4).

### 3.3. Body composition

The crude protein composition of larvae significantly increased with increasing dietary PL from 26.0 g kg$^{-1}$ to 57.2 g kg$^{-1}$, and then decreased with further increase of dietary PL ($P < 0.05$) (Table 4). The highest crude protein content (612.7 mg g$^{-1}$) was observed in larvae fed diets with 57.2 g kg$^{-1}$ PL ($P < 0.05$), significantly higher than that in larvae fed the control diet (574.0 mg g$^{-1}$) and the diet with the lowest PL supplementation (577.6 mg g$^{-1}$). Crude lipid of larval significantly increased with increasing dietary PL from 26.0 g kg$^{-1}$ to 85.1 g kg$^{-1}$ ($P < 0.05$). Crude lipid of fish larval in 69.5 g kg$^{-1}$ (149.1 mg g$^{-1}$) and 85.1 g kg$^{-1}$ PL treatments (152.2 mg g$^{-1}$) was significantly higher than that in 26.0 g kg$^{-1}$ (101.9 mg g$^{-1}$), 38.5 g kg$^{-1}$ (123.7 mg g$^{-1}$) and 57.2 g kg$^{-1}$ (130.1 mg g$^{-1}$) phospholipid treatment. Ratio of neutral to polar lipid (NL/PL) of whole fish body significantly decreased with the increase of dietary PL. NL/PL of fish fed live prey (0.38) was comparable to that in 57.2 g kg$^{-1}$ (0.51), 69.5 g kg$^{-1}$ (0.42) and 85.1 g kg$^{-1}$ PL treatments (0.37), but significantly lower than that in 26.0 g kg$^{-1}$ (0.92) and 38.5 g kg$^{-1}$ PL treatments (0.75) (Table 5).

C16:0, C18:1n−9 and n−3 HUFA (C20:5n−3 and C22:6n−3) were the most abundant fatty acids of SFA, MUFA and HUFA, respectively, in both polar and neutral lipid fractions of experimental fish. There were no significant differences in SFA, mainly 16:0, 18:0 and 20:0, in both polar and neutral lipid fractions of larvae fed microdiet. Fish larvae fed live prey had a significantly higher SFA composition than larvae fed the microdiets. MUFA in both polar and neutral lipid fractions decreased significantly as dietary PL increased. MUFA of fish larvae in live prey treatment was comparable to that in treatments with 26.0 and 38.5 g kg$^{-1}$ PL, but significantly higher than those in treatments with equal to or higher than 57.2 g kg$^{-1}$ PL. As to PUFA, n − 3 PUFA decreased but n − 6 PUFA increased with the increase of dietary PL in microdiets. The ratio of n − 3/n − 6 PUFA decreased significantly from 4.14 to 0.46 in neutral lipid fraction, 6.06 to 1.04 in polar lipid fraction as dietary PL increased from 26.0 to 85.1 g kg$^{-1}$. The content of n − 3 HUFA in neutral and polar lipid decreased significantly with the increase of dietary PL levels (Tables 6 and 7).

### 3.4. Stress resistance

Fish larvae fed live prey had significantly lower stress resistance compared with larvae fed the microdiets. Survival rate of fish larvae increased significantly from 56.3 to 78.1% in response to decreasing water temperature, from 45.7 to 63.0% in response to increasing salinity and from 50.0 to 69.6% in response to declining salinity as dietary PL increased. No significant differences were observed in stress tolerance of fish larvae in response to increasing water temperature (Table 8).

## 4. Discussion

Addition of PL significantly improved survival and growth performance of ayu (Kanazawa et al., 1981), gilthead sea bream (Liu et al., 2002) and sea bass larvae (Cahu et al., 2003). Improved growth and survival rates were obtained with ayu larvae (10–100 day post

**Table 4**
Effects of dietary phospholipid levels on the main digestive enzymes of *Larmichthys crocea* larvae (45 dph, mean ± S.D., n = 3).[a]

| 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) | LP (49.9) |
|---|---|---|---|---|---|---|---|
| Trypsin[b] | PS | 29.20 ± 2.39$^{ab}$ | 15.74 ± 0.70$^{c}$ | 17.18 ± 1.34$^{bc}$ | 38.27 ± 8.94$^{a}$ | 40.80 ± 10.01$^{a}$ | 14.99 ± 3.14$^{c}$ |
| | IS | 29.49 ± 6.04$^{b}$ | 47.43 ± 10.93$^{a}$ | 41.45 ± 1.74$^{ab}$ | 38.76 ± 1.17$^{ab}$ | 32.98 ± 4.60$^{b}$ | 12.5 ± 0.63$^{c}$ |
| Amylase[c] | PS | 1.11 ± 0.07$^{b}$ | 0.86 ± 0.12$^{b}$ | 1.04 ± 0.12$^{b}$ | 1.14 ± 0.23$^{b}$ | 1.05 ± 0.16$^{b}$ | 1.69 ± 0.20$^{a}$ |
| | IS | 1.29 ± 0.17$^{ab}$ | 1.15 ± 0.18$^{ab}$ | 0.7 ± 0.23$^{b}$ | 0.92 ± 0.18$^{b}$ | 1.04 ± 0.20$^{ab}$ | 1.6 ± 0.49$^{a}$ |
| AKP[c] | IS | 116.4 ± 7.8$^{d}$ | 115.8 ± 5.1$^{d}$ | 140.6 ± 9.3$^{c}$ | 149.4 ± 8.0$^{bc}$ | 175.5 ± 10.1$^{a}$ | 163.7 ± 13.1$^{ab}$ |
| | BBM | 573.0 ± 11.5$^{c}$ | 640.6 ± 62.1$^{bc}$ | 828.5 ± 106.6$^{a}$ | 783.6 ± 62.5$^{ab}$ | 825.0 ± 29.6$^{a}$ | 682.7 ± 73.6$^{abc}$ |
| LAP[b] | IS | 19.34 ± 3.04$^{bc}$ | 19.25 ± 1.37$^{bc}$ | 30.05 ± 4.55$^{a}$ | 26.55 ± 2.24$^{ab}$ | 28.05 ± 2.58$^{ab}$ | 11.45 ± 2.34$^{c}$ |
| | BBM | 61.15 ± 12.00$^{b}$ | 127.16 ± 15.13$^{ab}$ | 154.81 ± 44.88$^{a}$ | 167.7 ± 17.69$^{a}$ | 183.67 ± 20.57$^{a}$ | 136.03 ± 22.69$^{ab}$ |

PS: Pancreatic segments; IS: Intestinal segments; BBM: Brush border membrane of intestine.
[a] Data with the same superscript letter in the same row are not significantly different as determined by Tukey's test ($P > 0.05$).
[b] The unit of enzyme activity is mU/mg protein.
[c] The unit of enzyme activity is U/mg protein.

**Table 5**
Effects of dietary phospholipid levels on the body composition of *Larmichthys crocea* larvae (45 dph, mean ± S.D., n = 3, mg $g^{-1}$ dry weight).[a]

| 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) | LP (49.9) |
|---|---|---|---|---|---|---|
| Crude protein | 577.6 ± 8.4[c] | 592.4 ± 7.9[bc] | 612.7 ± 11.4[a] | 604.5 ± 2.4[ab] | 592.0 ± 3.1[bc] | 574.0 ± 4.3[c] |
| Total lipids | 101.9 ± 0.62[c] | 123.7 ± 0.73[b] | 130.1 ± 0.51[b] | 149.1 ± 0.69[a] | 152.2 ± 0.44[a] | 138.2 ± 3.1[ab] |
| NL/PL | 0.92 ± 0.10[a] | 0.75 ± 0.75[ab] | 0.51 ± 0.08[bc] | 0.42 ± 0.11[c] | 0.37 ± 0.08[c] | 0.38 ± 0.08[c] |

[a] Data with the same superscript letter in the same row are not significantly different as determined by Tukey's test ($P > 0.05$).

hatching) when the diets were supplemented with 3% chicken-egg lecithin, bonito-egg lecithin fraction or soybean lecithin (Kanazawa et al., 1981). In the present study, survival rate and SGR of fish larvae fed diets with 57.2 and 69.5 g $kg^{-1}$ PL were significantly higher than that of larvae fed the diets with 26.0 g $kg^{-1}$ PL. This indicated that biosynthesis could not meet the requirement for PL at least for large yellow croaker larvae (15–45 day post hatchery). The PL requirement for large yellow croaker was a little higher than that for ayu according to results of the present study. This inconsistence could be due to different diet formulation, fish species and developmental stages. Studies have also shown that the requirement for PL decreased as fish grew (Sargent et al., 2002). Indeed, it has been found that fish juveniles do not require additional PL and this could be due to a higher capacity of PL biosynthesis for fish at this stage (Deng et al., 2002).

Due to rapid growth and development, fish larvae require a large amount of PL involved in the formation of new cell components. Thus, PL should be included additionally into diets of fish larvae to meet the need of PL for rapidly dividing cells (Geurden et al., 1995b; Kanazawa, 1993). It has been known that multiple precursors and enzymes are required for the biosynthesis of PL. Scarce of these precursors and rate-limiting enzymes could account for the low biosynthesis of PL (Iritani et al., 1984; Kanazawa et al., 1981). Thus, additional supplementation of PL could be directly or indirectly used for cell membrane formation, maintaining relatively better growth performance and development. PL could promote the absorption of lipids through its emulsifying properties and thus make up for the lack of bile secretion in fish larvae and early juveniles (Salhi et al., 1994). Meanwhile, dietary PL could benefit the maturation of digestive tracts of fish larvae (Zambonino Infante and Cahu, 1999). Dietary PL could increase feed palatability, improve feed properties and water stability, thereby allowing the microdiets more easily captured by fish larvae (Coutteau et al., 1997).

Trypsin activity is mainly affected by dietary protein and amino acid composition (Grendell and Rothman, 1981). Few studies have been reported on the effects of dietary lipid on digestive enzyme activities. In the present experiment, dietary PL significantly affected pancreatic and intestinal trypsin activity of large yellow croaker larvae, with the highest activities observed in the treatment of 85.1 and 38.5 g $kg^{-1}$ PL, respectively. This indicated that activities of digestive enzyme could be affected by dietary PL in addition to protein and amino acid (Ma et al., 2005). With the development of large yellow croaker, pancreatic segmental digestive enzyme activity decreased while intestinal digestive enzyme activity increased according to the findings of Ma et al. (2005). Therefore, different pancreatic and intestinal trypsin activities reflected the development of digestive system of fish larvae fed different levels of PL.

Amylase secretion can be considered as an indicator of the maturation of the exocrine pancreas (Cahu et al., 2003). A decline of amylase is observed during the normal maturation process (Cahu et al., 2003; Ma et al., 2005). In the present study, the activities of amylase decreased with the increase of dietary PL, which indicated that dietary PL could benefit the maturation of the exocrine pancreas. Alkaline phosphatase (AKP) is a kind of metal enzyme which mainly exists in root and brush border of foregut epithelial cells and promotes nutrient uptake

**Table 6**
Fatty acid composition (% total fatty acids) of neutral lipid fraction in the whole body of *Larmichthys crocea* larvae fed the diets with graded phospholipid levels for 30 days (mean ± S.D., n = 3).[a]

| 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) | LP (49.9) |
|---|---|---|---|---|---|---|
| C16:0 | 15.87 ± 0.32[b] | 16.65 ± 0.31[b] | 17.02 ± 0.49[b] | 16.74 ± 1.90[b] | 15.97 ± 0.92[b] | 20.77 ± 0.90[a] |
| C18:0 | 4.02 ± 0.12[c] | 4.28 ± 0.29[bc] | 4.46 ± 0.28[bc] | 4.52 ± 0.26[bc] | 4.89 ± 0.24[b] | 7.60 ± 0.51[a] |
| C20:0 | 6.48 ± 0.12[a] | 6.31 ± 0.04[a] | 6.58 ± 0.06[a] | 6.15 ± 0.61[a] | 5.77 ± 0.28[a] | 2.12 ± 0.30[b] |
| ∑SFA[b] | 26.37 ± 0.20[b] | 27.23 ± 0.54[b] | 28.06 ± 0.75[ab] | 27.41 ± 1.03[b] | 26.63 ± 0.88[b] | 30.48 ± 1.71[a] |
| C14:1 | 4.61 ± 0.09[a] | 3.95 ± 0.24[ab] | 3.66 ± 0.11[bc] | 2.96 ± 0.64[c] | 1.76 ± 0.18[d] | 2.88 ± 0.19[c] |
| C16:1 | 6.74 ± 0.09[b] | 6.13 ± 0.23[bc] | 5.60 ± 0.11[bc] | 4.93 ± 0.73[cd] | 3.62 ± 0.29[d] | 11.46 ± 1.51[a] |
| C18:1n−9 | 14.32 ± 0.09[b] | 14.25 ± 0.13[b] | 14.55 ± 0.09[b] | 14.9 ± 0.57[ab] | 15.44 ± 0.33[a] | 11.24 ± 0.01[c] |
| C18:1n−7 | 4.37 ± 0.05[b] | 4.07 ± 0.07[bc] | 3.83 ± 0.10[bc] | 3.51 ± 0.10[cd] | 3.05 ± 0.21[d] | 5.91 ± 0.72[a] |
| ∑MUFA[c] | 30.05 ± 0.21[ab] | 28.4 ± 0.49[abc] | 27.64 ± 0.13[bc] | 26.3 ± 2.03[cd] | 23.88 ± 0.92[d] | 31.48 ± 2.03[a] |
| C18:2n−6 | 3.60 ± 0.16[e] | 8.54 ± 0.27[d] | 13.52 ± 0.19[c] | 19.46 ± 0.83[b] | 26.22 ± 0.18[a] | 4.92 ± 1.23[e] |
| C20:4n−6 | 1.28 ± 0.03[a] | 1.16 ± 0.11[ab] | 0.99 ± 0.08[bc] | 0.84 ± 0.10[cd] | 0.72 ± 0.04[d] | 1.24 ± 0.17[ab] |
| ∑n−6PUFA[d] | 4.88 ± 0.13[e] | 9.7 ± 0.16[d] | 14.51 ± 0.14[c] | 20.29 ± 0.73[b] | 26.94 ± 0.17[a] | 6.15 ± 1.06[e] |
| C18:3n−3 | 1.39 ± 0.02[d] | 1.53 ± 0.04[d] | 1.72 ± 0.07[c] | 2.03 ± 0.10[b] | 2.32 ± 0.02[a] | 0.94 ± 0.04[e] |
| C20:5n−3 | 5.72 ± 0.08[a] | 4.99 ± 0.24[b] | 4.27 ± 0.13[c] | 3.66 ± 0.18[cd] | 3.09 ± 0.11[d] | 4.12 ± 0.51[c] |
| C22:6n−3 | 13.07 ± 0.11[a] | 11.56 ± 0.97[ab] | 9.50 ± 0.44[bc] | 7.78 ± 1.73[c] | 6.92 ± 0.51[c] | 11.47 ± 1.63[ab] |
| ∑n−3PUFA[e] | 20.19 ± 0.07[a] | 18.09 ± 1.17[ab] | 15.49 ± 0.45[bcd] | 13.46 ± 1.8[cd] | 12.33 ± 0.62[d] | 16.53 ± 2.1[bc] |
| ∑PUFA | 25.06 ± 0.06[d] | 27.79 ± 1.01[c] | 30.00 ± 0.42[c] | 33.76 ± 1.09[b] | 39.28 ± 0.58[a] | 22.68 ± 1.04[e] |
| n−3HUFA[f] | 20.08 ± 0.11[a] | 17.72 ± 1.32[ab] | 14.76 ± 0.58[bc] | 12.28 ± 2.00[cd] | 10.73 ± 0.66[d] | 16.83 ± 2.31[ab] |
| C18:1n−9/n−3HUFA | 0.71 ± 0.01[c] | 0.81 ± 0.07[c] | 0.99 ± 0.04[bc] | 1.24 ± 0.27[ab] | 1.44 ± 0.11[a] | 0.67 ± 0.09[c] |
| n−3/n−6PUFA | 4.14 ± 0.13[a] | 1.87 ± 0.15[c] | 1.07 ± 0.04[d] | 0.67 ± 0.11[d] | 0.46 ± 0.02[d] | 2.76 ± 0.82[b] |

[a] Some fatty acids, of which the contents are minor, trace amount or not detected, such as C22:0, C24:0, C14:1, C20:1n−9, C22:1n−11, C20:2n−6, C20:3n−6, C22:5n−3, were not listed in the table.
[b] SFA: saturated fatty acids.
[c] MUFA: mono-unsaturated fatty acids.
[d] n−6 PUFA: n−6 poly-unsaturated fatty acids.
[e] n−3 PUFA: n−3 poly-unsaturated fatty acids.
[f] n−3 HUFA: n−3 highly-unsaturated fatty acids.

**Table 7**
Fatty acid composition (% total fatty acids) of polar lipid fraction in the whole body of *Larmichthys crocea* larvae fed the diets with graded phospholipid levels for 30 days (mean ± S.D., n = 3).[a]

| 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) | LP (49.9) |
|---|---|---|---|---|---|---|
| C16:0 | $21.64 \pm 1.54^{ab}$ | $22.25 \pm 0.62^{ab}$ | $21.03 \pm 0.93^{ab}$ | $21.8 \pm 1.39^{ab}$ | $20.32 \pm 0.15^{b}$ | $23.97 \pm 1.32^{a}$ |
| C18:0 | $6.47 \pm 0.18^{b}$ | $6.76 \pm 0.17^{b}$ | $6.59 \pm 0.58^{b}$ | $6.91 \pm 0.29^{b}$ | $7.11 \pm 0.21^{b}$ | $10.16 \pm 0.27^{a}$ |
| C20:0 | $2.89 \pm 0.12^{a}$ | 2.77 ± 0.09a | $2.69 \pm 0.16^{a}$ | $2.66 \pm 0.20^{a}$ | $2.54 \pm 0.10^{a}$ | $1.09 \pm 0.23^{b}$ |
| ∑SFA[b] | $30.99 \pm 1.73^{b}$ | $31.78 \pm 0.59^{ab}$ | $30.31 \pm 1.64^{b}$ | $31.37 \pm 1.56^{b}$ | $29.96 \pm 0.21^{b}$ | $35.22 \pm 1.28^{a}$ |
| C14:1 | $2.14 \pm 0.18^{a}$ | $1.85 \pm 0.08^{a}$ | $1.43 \pm 0.12^{b}$ | $1.23 \pm 0.15^{b}$ | $0.77 \pm 0.03^{c}$ | $1.33 \pm 0.11^{b}$ |
| C16:1 | $3.92 \pm 0.27^{b}$ | $3.45 \pm 0.08^{b}$ | $2.64 \pm 0.04^{c}$ | $2.46 \pm 0.22^{cd}$ | $1.89 \pm 0.16^{d}$ | $4.96 \pm 0.62^{a}$ |
| C18:1n−9 | $12.30 \pm 0.63^{a}$ | $12.02 \pm 0.08^{a}$ | $11.37 \pm 0.75^{ab}$ | $11.51 \pm 0.22^{ab}$ | $11.51 \pm 0.25^{ab}$ | $10.70 \pm 0.20^{b}$ |
| C18:1n−7 | $3.51 \pm 0.12^{b}$ | $3.21 \pm 0.05^{bc}$ | $2.86 \pm 0.16^{cd}$ | $2.81 \pm 0.10^{d}$ | $2.51 \pm 0.05^{d}$ | $3.93 \pm 0.31^{a}$ |
| ∑MUFA[c] | $21.87 \pm 1.20^{a}$ | $20.53 \pm 0.27^{ab}$ | $18.30 \pm 1.04^{bc}$ | $18.01 \pm 0.66^{c}$ | $16.68 \pm 0.45^{c}$ | $20.91 \pm 1.23^{a}$ |
| C18:2n−6 | $2.63 \pm 0.30^{e}$ | $6.37 \pm 0.09^{d}$ | $9.75 \pm 0.51^{c}$ | $14.75 \pm 0.34^{b}$ | $20.93 \pm 0.26^{a}$ | $3.03 \pm 0.49^{e}$ |
| C20:4n−6 | $2.20 \pm 0.01^{a}$ | $2.04 \pm 0.01^{a}$ | $1.74 \pm 0.09^{b}$ | $1.61 \pm 0.10^{b}$ | $1.38 \pm 0.05^{c}$ | $1.60 \pm 0.02^{b}$ |
| ∑n−6PUFA[d] | $4.82 \pm 0.32^{e}$ | $8.41 \pm 0.09^{d}$ | $11.49 \pm 0.59^{c}$ | $16.36 \pm 0.38^{b}$ | $22.31 \pm 0.29^{a}$ | $4.63 \pm 0.51^{e}$ |
| C18:3n−3 | $0.70 \pm 0.05^{d}$ | $0.81 \pm 0.01^{cd}$ | $0.85 \pm 0.06^{c}$ | $1.05 \pm 0.07^{b}$ | $1.28 \pm 0.01^{a}$ | $0.47 \pm 0.04^{e}$ |
| C20:5n−3 | $7.04 \pm 0.09^{a}$ | $6.85 \pm 0.06^{a}$ | $6.04 \pm 0.16^{b}$ | $5.76 \pm 0.46^{b}$ | $5.02 \pm 0.19^{c}$ | $4.92 \pm 0.22^{c}$ |
| C22:6n−3 | $21.36 \pm 0.67^{a}$ | $21.29 \pm 0.39^{a}$ | $19.02 \pm 0.69^{ab}$ | $17.78 \pm 1.90^{b}$ | $16.82 \pm 0.90^{b}$ | $21.28 \pm 2.00^{a}$ |
| ∑n−3PUFA[e] | $29.10 \pm 0.61^{a}$ | $28.95 \pm 0.37^{a}$ | $25.91 \pm 0.84^{ab}$ | $24.60 \pm 2.42^{b}$ | $23.11 \pm 1.0\ 9^{b}$ | $26.17 \pm 1.55^{ab}$ |
| ∑PUFA | $33.93 \pm 0.39^{cd}$ | $37.36 \pm 0.39^{bc}$ | $37.39 \pm 1.40^{bc}$ | $40.96 \pm 2.72^{ab}$ | $45.43 \pm 1.31^{a}$ | $30.80 \pm 2.05^{d}$ |
| n−3HUFA[f] | $30.60 \pm 0.60^{a}$ | $30.17 \pm 0.35^{a}$ | $26.80 \pm 0.87^{abc}$ | $25.15 \pm 2.46^{bc}$ | $23.22 \pm 1.13^{c}$ | $27.29 \pm 1.53^{c}$ |
| C18:1n−9/n−3HUFA | $0.40 \pm 0.03^{b}$ | $0.40 \pm 0.01^{b}$ | $0.42 \pm 0.01^{ab}$ | $0.46 \pm 0.05^{ab}$ | $0.50 \pm 0.03^{a}$ | $0.39 \pm 0.03^{b}$ |
| n−3/n−6PUFA | $6.06 \pm 0.53^{a}$ | $3.44 \pm 0.05^{b}$ | $2.26 \pm 0.06^{c}$ | $1.50 \pm 0.12^{d}$ | $1.04 \pm 0.04^{d}$ | $5.67 \pm 0.29^{a}$ |

[a] Some fatty acids, of which the contents are minor, trace amount or not detected, such as C22:0, C24:0, C14:1, C20:1n−9, C22:1n−11, C20:2n−6, C20:3n−6, C22:5n−3, were not listed in the table.
[b] SFA: saturated fatty acids.
[c] MUFA: mono-unsaturated fatty acids.
[d] n−6 PUFA: n−6 poly-unsaturated fatty acids.
[e] n−3 PUFA: n−3 poly-unsaturated fatty acids.
[f] n−3 HUFA: n−3 highly-unsaturated fatty acids.

by intestinal epithelial cell. The activity of AKP was positively correlated with the absorption of lipid, glucose, calcium and inorganic phosphorus (Tengjaroenkul et al., 2000). Thus, AKP can be used to evaluate the capacity of nutrient absorption by intestine of vertebrate larvae (Segner et al., 1989). During early larval stage, the intestinal development is not perfect and the chorionic villus brush border corresponding enzyme activity is low. The intestinal enzymes AKP and aminopeptidase N showed an abrupt increase from day 20 after hatchery. Activities of enzyme were significantly higher in brush border of midgut chorionic villus of large yellow croaker larvae 20 day post hatchery (Ma et al., 2005). In this study, AKP activity in brush border of chorionic villus increased significantly with the increase of dietary PL. Dietary PL could affect the development of intestine which could be indicated by the leucine aminopeptidase activity of brush border (Freund et al., 1990; Henning, 1987).

Results in the present study showed that dietary PL significantly affected the crude protein and lipid composition of fish larvae. Crude protein content was significantly higher in larvae fed diets with moderate dietary PL. Crude lipid content increased significantly with the increase of dietary PL and this was consistent with the findings of Geurden et al. (1999) who have found that PL could promote retention of lipid, especially the polar lipids in common carp. Studies have shown that PL could promote the maturation of digestive tracts and perform emulsifying properties, which resulted in nutrient retention (Salhi et al., 1994; Zambonino Infante and Cahu, 1999). Meanwhile, lecithin helps lipoprotein synthesis, enhances the efficiency of nutrient absorption from the epithelium of the digestive tract and subsequently improves nutrient transport to the body tissue, ultimately increasing fatty acid utilization and decreasing the EFA requirement (Hadas et al., 2003). The ratio of neutral to polar lipid decreased with the increase of dietary PL. This indicated that larval fish could deposit polar lipid efficiently. Furthermore, PL could increase the oxidation of neutral lipids. However, there is no evidence in this study and more investigation are needed in the following studies. Though phospholipid metabolism is poorly studied in fish, evidences showed that PL-derived mediators play similar roles in fish as they do in mammals (Tocher, 1995; Tocher et al., 2008).

In this study, fish larvae fed microdiets had significantly higher stress tolerance than counterparts fed live prey. This indicated that dietary PL could enhance stress resistance to a large extent. This was consistent with the findings of Kanazawa, (1997) and Liu et al. (2002) who found that lecithin was effective in increasing the stress tolerance for sea bream, marbled sole and gilthead sea bream larvae. Preliminary studies have shown that dietary PL could increase cell membrane fluidity by enhancing the content of unsaturated fatty acids fatty acid and thus increase the stress resistance to low temperature (Cao et al., 1997). Furthermore, PL could improve stress tolerance to high or low salinity by increasing the activities of $Na^{+}$, $K^{+}$-ATPase, which have been proven in *Penaeus vannamei* larvae (Coutteau et al., 1996) and *Plectorhynchus cinctus* juveniles (Chen et al., 2002).

In conclusion, 57.2 g kg$^{-1}$ dietary PL could increase survival, growth performance and stress tolerance of large yellow croaker larvae. Further

**Table 8**
Effect of different dietary phospholipids levels on stress resistance of *Larmichthys crocea* larvae (45 dph, mean ± S.D., n = 3).[a]

| 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) | LP (49.9) |
|---|---|---|---|---|---|---|
| Increasing water temperature (%) | 31.8 ± 6.4 | 34.3 ± 15.8 | 41.0 ± 4.9 | 42.9 ± 10.1 | 36.7 ± 4.7 | 38.9 ± 7.9 |
| Declining water temperature (%) | $56.3 \pm 8.8^{a}$ | $68.8 \pm 0.0^{a}$ | $68.8 \pm 0.0^{a}$ | $71.9 \pm 4.4^{a}$ | $78.1 \pm 13.3^{a}$ | $21.9 \pm 4.4^{b}$ |
| Increasing salinity (21 h) (%) | $45.7 \pm 15.4^{a}$ | $41.3 \pm 9.2^{a}$ | $45.7 \pm 15.4^{a}$ | $60.9 \pm 18.4^{a}$ | $63.0 \pm 9.2^{a}$ | $6.5 \pm 3.1^{b}$ |
| Declining salinity (60 h) (%) | $50.0 \pm 3.1^{bc}$ | $47.8 \pm 6.2^{bc}$ | $63 \pm 3.1^{ab}$ | $69.6 \pm 6.2^{a}$ | $43.5 \pm 3.1^{c}$ | $6.5 \pm 3.1^{d}$ |
| DO level when 50% larvae die(mg l$^{-1}$) | $2.5 \pm 0.2^{b}$ | $2.3 \pm 0.1^{b}$ | $2.0 \pm 0.1^{b}$ | $2.1 \pm 0.1^{b}$ | $2.2 \pm 0.2^{b}$ | $3.0 \pm 0.2^{a}$ |

[a] Data with the same superscript letter in the same row are not significantly different as determined by Tukey's test ($P > 0.05$).

studies are needed to investigate the specific mechanisms about how dietary PL exert the physiological effects.

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