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Effects of dietary protein levels on the growth, survival, amylase and trypsin activities in large yellow croaker, *Pseudosciaena Crocea* R., larvae

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Abstract

A feeding trial was conducted to investigate the effects of dietary graded protein levels on the growth, survival, amylase and trypsin activities of large yellow croaker (*Pseudosciaena crocea* R.) larvae from 12 to 42 days after hatching (DAH). Five approximately isoenergetic microbound diets (16.65 MJ/kg diet) were formulated to contain different protein (47.1%, 52.0%, 57.1%, 62.2% and 67.5%) levels. Frozen copepods, containing 54.5% crude protein (CP), 6.0% crude lipid, 27.2% ash and 6.7% glycogen, were used as a control. Each diet was randomly fed to triplicate groups of larvae with an initial mean body weight of 1.76 ± 0.09 mg (mean ± SD) in 180 L white plastic tanks, and each tank was stocked initially with 3500 larvae. Both the survival and the specific growth rate (SGR) of large yellow croaker larvae significantly increased with increasing dietary protein level up to 57.1%, and decreased thereafter. Frozen copepods resulted in intermediate survival and low SGR compared with the other diets. Whole-body moisture and protein of larvae were not significantly affected by the dietary protein level. In contrast, whole-body lipid of larvae fed diet with 47.1% CP was significantly higher ($P < 0.05$) than those from fish fed the diets containing more than 57.1% CP. Additionally, fish fed the frozen copepods had the lowest whole-body protein and lipid. The amylase-specific activity increased with increasing dietary carbohydrate level during the period of this experiment. However, trypsin activity was not significantly affected by the dietary protein content before 42 DAH, indicating a later onset of trypsin than amylase in the regulation of enzymatic synthesis induced by a dietary substrate.

Keywords: large yellow croaker larva, *Pseudosciaena crocea*, protein, growth, amylase, trypsin

Introduction

Until now, protein requirements for marine fish larvae were scarcely studied, and the dietary protein level is normally fixed between 50% and 70% based on data obtained from juveniles and live preys (Watanabe & Kiron 1994; Cahu & Zambonino Infante 2001). However, a detailed estimate of the optimal dietary protein concentration is essential for the development of nutritionally well-balanced and cost-effective formulated diets (Mazid, Tanaka, Rahman, Simpsom & Chichester 1979). An attempt to determine the optimal dietary protein level for marine fish larvae was made by Péres, Cahu, Zambonino Infante, Le Gall and Quazuguel (1996), who weaned 15-day-old sea bass (*Dicentrarchus labrax*) larvae using isoenergetic formulated diets with graded protein levels from 30% to 60%, and observed the best growth and survival with 50% dietary protein.

The development of successful microdiets requires knowledge of the digestive abilities of marine fish larvae during ontogenetic development (Kolkovski 2001; Zambonino Infante & Cahu 2001). These abilities can be evaluated by analysing the activities of digestive enzymes. Recent studies have shown that

in some larvae of marine fish species, pancreatic enzymes, such as amylase and trypsin, were detected even before mouth opening, and performed major digestive functions (Zambonino Infante & Cahu 1994a; Ribeiro, Zambonino Infante, Cahu & Dinis 1999; Buchet, Zambonino Infante & Cahu 2000). Moreover, these marine fish larvae are able to adjust the levels of their digestive enzymes to the dietary composition and the concentration of nutrients (Zambonino Infante & Cahu 1994a, b; Péres *et al.* 1996; Péres, Zambonino Infante & Cahu 1998; Buchet, Zambonino Infante & Cahu 2000; Tovar, Zambonino Infante, Cahu, Gatesoupe, Vázquez–Juárez & Lésel 2002).

As the basis for the development of artificial microdiets, previous studies have investigated the feeding habits, ontogenesis of digestive enzymes and developmental features of the digestive system of large yellow croaker larvae and juveniles (Yu, Mai, Duan, Ma, Cahu, Zambonino Infante, Liufu, Tan, Zhang & Xu 2003; Ma, Cahu, Zambonino Infante, Yu, Duan, Le Gall & Mai 2005; Mai, Yu, Ma, Duan, Gisbert, Zambonino Infante & Cahu 2005). However, information on the nutrient requirements of large yellow croaker larvae is lacking. The present study was designed to determine the effects of graded dietary protein level on the growth, survival and proximate composition of large yellow croaker larvae. The activities of pancreatic amylase and trypsin in response to graded dietary protein levels were also evaluated to provide a better understanding of the digestive processes.

Materials and methods

Experimental diets

The formulation and proximate composition of the formulated diets are presented in Table 1. Low-temperature-processed, white fish meal, mussel meal and squid meal, together with casein hydrolysate (A–2427, Sigma, St Louis, MO, USA), were used as the protein sources. Soy lecithin and cod liver oil were used as the lipid sources. Lycopene [Green Valley Bitotech Products (Wuxi), Wuxi, China] was supplemented in the formulated diets as a visual stimulant, and betaine and glycine were used as the chemical stimulants. Five isoenergetic practical microbound diets (Diet 1–Diet 5) were formulated to contain graded levels of protein (47.1%, 52.0%, 57.1%, 62.2% and 67.5%) with various levels of carbohydrate (26.1%, 19.9%, 13.6%, 7.4% and 1.1%). All formulated diets were maintained approximately isoenergetic by adjusting the α-starch, cod liver oil and soy lecithin of the ration in relation to the protein level and by keeping the lipid fractions identical. The particle sizes of the formulated diets ranged from 150 to 250 μm for the fish larvae between 12 and 25 days after hatching (DAH) and 250–425 μm for the fish larvae thereafter. The methodology used to prepare the microbound diets is patent pending. Frozen copepods (Diet 6, 300–950 μm in length), dominated (about 88%, in number) by the copepod *Calanus sinicus*, with the remaining 12% consisting of a mixture of *Euchaeta* larvae (6.5%), *Euchaeta concinna* (3.7%) and *Labidocera euchaeta* (1.8%), were used as a control diet as live ones could not be captured at sea. Most of the larval fish farms in China feed copepods from 12 DAH to large yellow croaker larvae. The assayed composition of frozen copepods is shown in Table 1. All formulated diets were packed in separate aluminium bags and stored at –20 °C until used.

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). A total of 63 000 larvae of the 12 DAH age, each weighing approximately 1.76 ± 0.09 mg, were used in this study in 18 white plastic tanks (73 × 53 × 60 cm, water volume 180 L), and each was stocked initially with 3500 individuals. All tanks were placed in an indoor concrete pond (800 × 400 × 160 cm). The larvae were reared in seawater that had been filtered through two-grade sand filter and a cloth filter. During the rearing period, water temperature (24 ± 1 °C), pH (8.0 ± 0.2) and salinity (27 ± 3‰) were regularly monitored and adjusted when needed. About 50–300% of the water volume was renewed daily and there was a light permanent agitation by air bubbling. The photoperiod was set to a 12L:12D cycle with a maximum light intensity of 1000 lx at the water surface. The water surface was skimmed constantly with a polyvinylchloride pipe and accumulations of feed and faeces on the tank bottoms were siphoned twice daily.

Larvae were fed with rotifers *Brachionus plicatilis* (0.5–1.5 × 10^4 individuals/L seawater) from 3 to 11 DAH. The rotifers used as feed had been enriched with a mixture of unicellular green algae (*Chlorella*), yeast (Mauri Yeast, Harbin, China) and 50DE–emulsion oil (Shandong Marine Fisheries Research Institute, Yantai, China) to increase the DHA and EPA contents, according to Zheng, Su, You and Weng (1996). The assayed composition of the rotifers was

Table 1 Formulation (g/100 g dry diet) and proximate composition of the experimental diets (% dry matter)

Diets (designated protein level)	Diet 1 (45)	Diet 2 (50)	Diet 3 (55)	Diet 4 (60)	Diet 5 (65)	Diet 6 (frozen copepods)
Ingredients						
White fish meal*	36.00	39.50	43.00	46.50	50.00	
Casein hydrolysate†	18.25	21.25	24.25	27.25	30.25	
Mussel meal	2.00	2.00	2.00	2.00	2.00	
Squid meal	5.00	5.00	5.00	5.00	5.00	
α-starch	26.10	19.85	13.60	7.35	1.10	
Sodium alginate	2.00	2.00	2.00	2.00	2.00	
Soy lecithin	5.00	4.95	4.90	4.85	4.80	
Cod liver oil	3.00	2.80	2.60	2.40	2.20	
Vitamin premix‡	1.00	1.00	1.00	1.00	1.00	
Mineral premix§	1.00	1.00	1.00	1.00	1.00	
Betaine	0.10	0.10	0.10	0.10	0.10	
Glycine	0.05	0.05	0.05	0.05	0.05	
Choline chloride	0.20	0.20	0.20	0.20	0.20	
Immune stimulants¶	0.25	0.25	0.25	0.25	0.25	
Antioxidant	0.05	0.05	0.05	0.05	0.05	
Proximate compositions						
Dry matter	93.1	93.2	92.1	94.7	94.4	21.3
Crude protein ($N \times 6.25$)	47.1	52.0	57.1	62.2	67.5	54.5
Starch/glycogen	26.1	19.9	13.6	7.4	1.1	6.7
Crude lipid	12.9	12.5	12.6	13.2	13.0	6.0
Ash	10.5	11.0	11.7	13.1	13.4	27.2
Gross energy (MJ kg^{-1})	16.79	16.65	16.66	16.60	16.53	12.36

*Contained 70.9% crude protein and 6.9% crude lipid.

†A–2427, Sigma.

‡Composition (IU or g kg^{-1} vitamin premix): 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.

§Composition (g kg^{-1} mineral premix): $Ca(H_2PO_4)_2 \cdot H_2O$, 675.0; $CoSO_4 \cdot 4H_2O$, 0.15; $CuSO_4 \cdot 5H_2O$, 5.0; $FeSO_4 \cdot 7H_2O$, 50.0; KCl, 50.0; KI, 0.1; $MgSO_4 \cdot 2H_2O$, 101.7; $MnSO_4 \cdot 4H_2O$, 18.0; NaCl, 80.0; $Na_2SeO_3 \cdot H_2O$, 0.05; $ZnSO_4 \cdot 7H_2O$, 20.0.

¶Provided by Qingdao Marine Science and Technology Bio-Tech, Qingdao, China.

61.9 ± 0.5% crude protein (CP), 13.7 ± 0.6% crude lipid and 13.2 ± 0.2% ash (DM, mean ± SD). *Chlorella* was continuously supplied at a concentration of 2–4 × 10^4 cells/mL in the rearing pond.

From 12 to 42 DAH, the larvae were weaned to the six dietary groups Diet 1 to Diet 6 (three tanks per group). The fish were manually fed in excess five times (06:00, 09:00, 12:00, 15:00, 18:00 hours) daily during daylight.

Sampling and dissection

To estimate the wet body weight (BW), 300 larvae at 12 DAH were randomly sampled from the base population and 22, 32 and 42 DAH, 30 individuals were randomly sampled from each tank in the early morning before first feeding. At the end of the experiment, fish survival was determined by counting the individuals remaining in each tank. The remaining fish from each treatment were collected at the end of the experiment and then freeze-dried for subsequent analysis.

Hundred individuals at 12 and 22 DAH, and 50 individuals at 32 and 42 DAH, were randomly collected from each tank in the early morning before first feeding for enzymatic assays. The samples were immediately frozen in liquid nitrogen and then stored at –80 °C until processed.

Whole-body homogenates were used for enzyme determination in fish at 12 DAH. Pancreatic segments (PS) of fish collected 22, 32 and 42 DAH were removed under a dissection microscope as described by Cahu and Zambonino Infante (1994) on a glass plate maintained at 0 °C.

Analytical methods

The samples of diets and fish were dried to a constant weight at 105 °C to determine the dry matter content.

Nitrogen (N), lipid and ash analyses were carried out as described by the Association of Official Analytical Chemists (AOAC) (1990). Protein content was estimated as N × 6.25. The gross energy content was determined using an automatic Parr 1281 oxygen bomb Calorimeter (Parr, Moline, IL, USA). The starch in the microbound diets and glycogen in frozen copepods contents were determined after enzymatic degradation as described by Hemre, Karlsen, Lehmann, Holm and Lie (1993).

For enzyme assays, samples were homogenized in 5 volumes (v/w) of ice-cold (0 °C) distilled water. Trypsin activity was assayed according to Tseng, Grendell and Rothman (1982) using *N*α–Benzoyl–DL–arginine–*p*–nitroanilide (B–4875, Sigma) as a substrate. Amylase activity was measured according to Métais and Bieth (1968) using an iodine solution to reveal non-hydrolysed starch (S–9765, Sigma). Enzyme activities were expressed as specific activities (mU/mg protein). Protein was determined according to Bradford (1976) using bovine serum albumin (BSA, A–2153, Sigma) as a standard.

Calculations and statistical methods

The following calculations were performed:

$$\text{Specific growth rate (SGR, \%/day)} = ((\text{Ln}\, W_\text{f} - \text{Ln}\, W_\text{i})\text{d}) \times 100$$

$$\text{Final biomass} = W_\text{f} \times N_\text{f}$$

where W_f is the final wet body weight, W_i is the initial wet body weight, N_f is the final number of fish and d is the experimental period in days.

Results are given as mean ± SD ($n = 3$). All percentage data were arcsine transformed before analysis. The data were compared for the same day using one-way analysis of variance and Tukey's honest significant difference test (Tukey HSD test) using the software program SPSS 11.5 for Windows. A significance level of 5% was used for all comparisons.

Results

Survival

At the end of the experiment, the survival was significantly affected ($P < 0.05$) by the dietary protein levels. Survival increased as dietary protein increased up to 57.1%, and decreased above this level. Fish fed diets containing 57.1% and 62.2% protein had significantly higher ($P < 0.05$) survival (37.6% and 35.1% respectively) than fish fed diets containing 47.1%, 52.0% and 67.5% protein (17.8–28.7%). Fish fed frozen copepods showed survival (32.9%) similar to fish fed the 62.2% protein diet (Table 2).

Growth

At 22 DAH, no significant difference ($P > 0.05$) was observed for the mean BW between the experimental diets, but the fish fed the copepod diet had a higher mean BW ($P < 0.05$). However, as fish grew older, the mean BW increased significantly ($P < 0.05$) with increasing dietary protein up to 57.1% and decreased beyond this level. In contrast, the larger mean BW of copepod fed larvae on 22 DAH was lost by 42 DAH. At the end of the experiment, the maximum mean BW of 37.30 ± 0.28 mg was reached with the 57.1% protein diet. The mean BW of fish fed the 47.1% protein diet was only 23.66 ± 0.33 mg. Moreover, the mean BW of fish fed frozen copepods reached 26.51 ± 0.40 mg, which was significantly lower ($P < 0.05$) than for fish fed the 52.0% protein diet, but higher than for fish fed the 47.1% protein diet. Similar trends were observed for SGR (Table 2).

The biomass significantly increased ($P < 0.05$) up to a dietary protein level of 57.1%, and decreased beyond this level. Fish fed frozen copepods reached a similar biomass as fish fed a 67.5% protein diet (Table 2).

Whole-body proximate composition

Whole-body moisture and protein were not significantly affected by the dietary protein level (Table 3). Fish fed the diet containing 47.1% protein level had the highest lipid level among all the dietary treatments. There was no significant difference in the whole-body lipid content of fish fed the diets containing 57.1%, 62.2% and 67.5% protein levels. Fish fed the frozen copepods exhibited the lowest ($P < 0.05$) whole-body protein and lipid.

Enzyme activities

From 22 DAH onwards, the specific activity of amylase in the PS increased with increasing dietary carbohydrate and decreasing dietary protein (Table 4). As fish grew older, the higher the carbohydrate content in the diet, the slower the specific amylase activity declined. Fish fed frozen copepods had an amylase activity similar to fish fed 62.2% and 67.5% protein diets.

No significant difference ($P > 0.05$) was observed in the specific trypsin activity among fish fed diets containing different protein levels and frozen copepods until 32 DAH (Table 5). However, the specific activity of trypsin increased with increasing dietary protein level at 42 DAH. At this time, fish fed diets containing 57.1% or more dietary protein, or frozen copepods, had significantly higher ($P < 0.05$) trypsin-specific activity than fish fed the lower protein diets. Moreover, 42-day-old fish fed diets containing 57.1% or more dietary protein, or frozen copepods, showed more increase in trypsin-specific activity than fish on 12, 22 and 32 DAH, while this effect was not obvious in fish fed diets containing 47.1% or 52.0% protein.

Discussion

In the present study, the survival and growth were significantly affected by dietary protein concentrations, indicating that dietary protein plays an important role in the survival and growth of large yellow croaker larvae. A similar trend was observed by Péres *et al.* (1996), who weaned 15-day-old sea bass larvae using isoenergetic formulated diets with graded protein levels from 30% to 60%, and observed the best growth and survival with 50% of dietary protein. Eguia, Kamarudin and Santiago (2000) also obtained higher survival and growth in river catfish (*Mystus nemurus*) larvae fed a formulated diet containing 60% protein compared with those in larvae fed the lower protein (45–55%) diets.

The survival increased as dietary protein increased up to 57.1%, and decreased thereafter. In China, copepods are often fed in most of the large yellow croaker larvae farms following the rotifer period. However, the presumed highest survival was not observed for the larvae fed copepods in the present study, but was similar to that of fish fed a 62.2% protein diet. The result indicated that the microparticles of the formulated diet were well ingested by the larvae. Based on the number of microcapsules counted in the gut of gilthead seabream (*Sparus aurata*) larvae, Yufera, Fernandez-Diaz and Pascual (1995) demonstrated that microcapsules were ingested at similar rates to living prey. Indeed, mortality was partially caused by a low ingestion rate of the microparticles at the onset of weaning. Meanwhile, handing stress such as fish transfer and water exchange also led to some mortality.

Fish growth increased with increasing dietary protein level up to 57.1%, but decreased at higher

Table 2 Growth and survival of large yellow croaker larvae fed the experimental diets with graded levels of protein at different DAH*

Diets	Diet 1 (47.1)	Diet 2 (52.0)	Diet 3 (57.1)	Diet 4 (62.2)	Diet 5 (67.5)	Diet 6 (frozen copepods)
Survival (%)	17.8 ± 0.57^{e}	25.4 ± 0.75^{d}	37.6 ± 1.49^{a}	35.1 ± 1.12^{ab}	28.7 ± 0.83^{c}	32.9 ± 0.57^{b}
Initial BW (mg, 12 DAH)	1.76 ± 0.09	1.76 ± 0.09	1.76 ± 0.09	1.76 ± 0.09	1.76 ± 0.09	1.76 ± 0.09
BW (mg) at 22 DAH	4.52 ± 0.22^{b}	4.57 ± 0.32^{b}	4.76 ± 0.19^{b}	4.72 ± 0.20^{b}	4.64 ± 0.11^{b}	5.25 ± 0.18^{a}
BW (mg) at 32 DAH	16.20 ± 0.36^{d}	17.27 ± 0.27^{c}	22.10 ± 0.31^{a}	21.66 ± 0.40^{ab}	20.86 ± 0.36^{b}	20.34 ± 0.29^{b}
Final BW (mg, 42 DAH)	23.66 ± 0.33^{f}	28.07 ± 0.66^{d}	37.30 ± 0.28^{a}	34.97 ± 0.34^{b}	30.33 ± 0.24^{c}	26.51 ± 0.40^{e}
SGR (% day^{-1})	8.7 ± 0.05^{f}	9.2 ± 0.08^{d}	10.2 ± 0.02^{a}	10.0 ± 0.03^{b}	9.5 ± 0.03^{c}	9.0 ± 0.05^{e}
Final biomass (g tank^{-1})	14.74 ± 0.20^{e}	24.99 ± 0.58^{d}	49.09 ± 0.36^{a}	42.90 ± 0.41^{b}	30.42 ± 0.24^{c}	30.46 ± 0.46^{c}

*Means ± SD ($n = 3$).
Same superscript letter in the same row are not significantly different determined by Tukey's test ($P > 0.05$).
BW, body weight; DAH, days after hatching.

Table 3 Whole-body proximate composition (% wet weight basis) of the 42-day-old large yellow croaker larvae fed the experimental diets with graded levels of protein*

Diets	Diet 1 (47.1)	Diet 2 (52.0)	Diet 3 (57.1)	Diet 4 (62.2)	Diet 5 (67.5)	Diet 6 (frozen copepods)
Moisture	82.7 ± 0.50	82.5 ± 0.47	82.1 ± 0.56	82.2 ± 0.30	82.3 ± 0.31	83.1 ± 0.30
Crude protein	11.4 ± 0.30^{ab}	11.5 ± 0.28^{ab}	12.0 ± 0.36^{a}	11.8 ± 0.24^{a}	11.7 ± 0.17^{ab}	11.0 ± 0.16^{b}
Crude lipid	1.9 ± 0.05^{a}	1.8 ± 0.03^{ab}	1.7 ± 0.06^{b}	1.7 ± 0.05^{b}	1.7 ± 0.05^{b}	1.4 ± 0.04^{c}

*Means ± SD ($n = 3$).
Same superscript letter in the same row are not significantly different determined using Tukey's test ($P > 0.05$).

Table 4 Amylase-specific activities (U mg^{-1} protein) in the pancreatic segments of large yellow croaker larvae fed the experimental diets with graded levels of protein at different DAH*

Diets	Diet 1 (47.1)	Diet 2 (52.0)	Diet 3 (57.1)	Diet 4 (62.2)	Diet 5 (67.5)	Diet 6 (frozen copepods)
12 DAH	1.26 ± 0.25	1.26 ± 0.25	1.26 ± 0.25	1.26 ± 0.25	1.26 ± 0.25	1.26 ± 0.25
22 DAH	1.68 ± 0.22^{a}	1.39 ± 0.35^{ab}	1.28 ± 0.68^{ab}	0.80 ± 0.44^{bc}	0.36 ± 0.26^{c}	0.67 ± 0.16^{bc}
32 DAH	1.42 ± 0.38^{a}	1.11 ± 0.29^{ab}	0.93 ± 0.17^{ab}	0.99 ± 0.33^{ab}	0.67 ± 0.28^{b}	0.84 ± 0.16^{b}
42 DAH	1.34 ± 0.24^{a}	1.03 ± 0.15^{ab}	0.93 ± 0.17^{b}	0.77 ± 0.19^{bc}	0.41 ± 0.19^{c}	0.53 ± 0.22^{c}

*Means ± SD ($n = 3$).
Same superscript letter in the same row are not significantly different determined using Tukey's test ($P > 0.05$).
DAH, days after hatching.

Table 5 Trypsin-specific activities (mU mg^{-1} protein) in the pancreatic segments of large yellow croaker larvae fed the experimental diets with graded levels of protein at different DAH*

Diets	Diet 1 (47.1)	Diet 2 (52.0)	Diet 3 (57.1)	Diet 4 (62.2)	Diet 5 (67.5)	Diet 6 (frozen copepods)
12 DAH	60.49 ± 13.36	60.49 ± 13.36	60.49 ± 13.36	60.49 ± 13.36	60.49 ± 13.36	60.49 ± 13.36
22 DAH	37.94 ± 23.85	31.92 ± 9.12	40.56 ± 6.83	33.78 ± 8.44	29.02 ± 4.22	50.83 ± 2.23
32 DAH	37.11 ± 12.78	30.38 ± 5.42	47.69 ± 9.68	36.52 ± 5.60	48.49 ± 10.20	38.91 ± 9.59
42 DAH	55.86 ± 10.41^{b}	66.63 ± 14.74^{b}	86.82 ± 5.26^{a}	87.57 ± 13.59^{a}	102.42 ± 10.56^{a}	96.37 ± 6.16^{a}

*Means ± SD ($n = 3$).
Same superscript letter in the same row are not significantly different determined using Tukey's test ($P > 0.05$).
DAH, days after hatching.

dietary protein levels. Similar trends were observed in other species for larvae (Péres *et al.* 1996) and juveniles (Fiogbé, Kestemont, Mélard & Micha 1996; Elangovan & Shim 1997). Although the formulated diets were isoenergetic (16.65 MJ kg^{-1} diet) in this study, the protein to energy ratios varied from 28.05 to 40.83 mg KJ^{-1} as dietary protein increased from 47.1% to 67.5%. The poor growth performance of larvae fed diets with either lower or higher protein levels had probably been affected by unsuitable dietary protein to energy ratios. In some cases, a reduction in fish growth could have been caused by the lack of non-protein energy in diets containing higher protein, and excesses of protein would be used as an energy source rather for tissue growth (Jauncey 1982; Wilson 1989). The dietary requirement for protein is, in fact, a requirement for the essential amino acids contained in the dietary protein (Tibbetts, Lall & Anderson 2000). When white fish meal and casein hydrolysate were used as the main protein sources and the dietary energy value was around 16.65 MJ kg^{-1} diet, the 57.1% dietary CP level appeared to meet the protein requirements of the large yellow croaker larvae in the present study. This result supported the speculation of Duan, Mai, Zhong, Si and Wang (2001), who suggested that early juveniles of large yellow croaker required more than 47% dietary protein. Indeed, fish larvae have higher protein requirements to break down into amino acids, which are then used for rapid growth and as an energy source compared with juveniles (Conceição, van der Meeren, Verreth, Evjen, Houlihan & Fyhn 1997; Rønnestad, Tonheim, Fyhn, Rojas-García, Kamisaka, Koven, Finn, Terjesen, Barr & Conceição 2003). In practice, commercial feeds of large yellow croaker larvae and early juveniles in China are usually formulated at a 50% level, which was lower than the optimal protein concentration obtained in this study.

Whole-body moisture was not correlated to the dietary protein level, which was consistent with many reports for other fish species (Ng, Soon & Hashim 2001; Kim, Wang & Bai 2002; Kim & Lee 2009). The dietary protein level did not significantly affect the whole-body protein of the fish. Similar trends have been reported by Ng, Soon and Hashim (2001) and Lee, Cho, Lee and Yang (2001). Although dietary lipid and energy levels were constant, fish fed the lower protein diets had higher whole-body lipid accumulation. Similar results have also been observed in other studies with juveniles of *Zacco barbata* (Shyong, Huang & Chen 1998) and silver perch (Yang, Liou & Liu 2002). In contrast, crude lipid of the river catfish after 16 days of feeding did not differ significantly, but body protein increased with increasing dietary pro-

tein level from 45% to 60% (Eguia, Kamarudin & Santiago 2000). A previous study (Duan *et al.* 2001) observed no significant difference in the carcass composition of early juvenile large yellow croaker cultured for 60 days as the dietary protein level increased from 34% to 47%.

Cahu and Zambonino Infanto (1994) found an increase in amylase-specific activity in response to 12% dietary starch in sea bass larvae. Péres *et al.* (1996) also observed that amylase-specific activity increased in 18-day-old and 35-day-old sea bass larvae with increasing dietary starch from 4.5% to 24.5%. A similar adaptation of amylase activity to the dietary starch content was also observed for large yellow croaker larvae in this study. The higher dietary starch level led to the higher amylase-specific activity, suggesting a modulation of amylase by the dietary starch content. Although the carbohydrate types differed in formulated diets and frozen copepods, frozen copepods resulted in amylase-specific activity similar to that of the formulated diets containing 62.2% protein, due to their similar carbohydrate content. Unlike amylase, trypsin-specific activity was not significantly affected by the dietary protein level until 42 DAH, which was similar to the results obtained by Péres *et al.* (1996). Moreover, frozen copepods did not exhibit significantly different trypsin-specific activity compared with formulated diets containing protein equal to or above 57.1%. The decline in the trypsin-specific activity at 22 and 32 DAH was probably attributed to protein deposition in fish tissues. The result was in agreement with the findings of Péres *et al.* (1996) and Péres, Zambonino Infante and Cahu (1998) for sea bass larvae.

Pre-hydrolysed protein has been used in artificial microdiets in order to enhance the growth and survival of larval fish. However, high levels of pre-hydrolysed protein resulted in growth depression (Zambonino Infante, Cahu & Péres 1997; Cahu, Zambonino Infante, Quazuguel & Le Gall 1999; Cahu & Zambonino Infante 2001; Tonheim, Espe, Hamre & Rønnestad 2005; Kotzamanis, Gisbert, Gatesoupe, Zambonino Infante & Cahu 2007; Kvale, Nordgreen, Tonheim & Hamre 2007; Hermannsdottir, Johannsdottir, Smaradottir, Sigurgisladottir, Gudmundsdottir & Bjornsdottir 2009). Tang, Wu, Zhao and Pan (2008) observed that levels of 10–15% dietary fish protein hydrolysate supplementation improved the growth and immunity of large yellow croaker juvenile. In the present study, the casein hydrolysate fraction ranged between 18.25% and 30.25% in the diet as partial protein sources, which probably affected the larval survival, growth and related enzymatic activities. Further research is required to evaluate the effect of peptide fractions of protein hydrolysates and determine their optimal inclusion levels in microdiets for large yellow croaker larvae.

In conclusion, this study showed that the regulation of amylase synthesis was more efficient than that related to trypsin in younger large yellow croaker larvae. As high dietary starch resulted in poor growth and survival, the efficient regulation of amylase during early developmental stages is not related to an essential need for dietary starch. The levels of 57.1% protein with 13.6% dietary carbohydrate were suitable for large yellow croaker larvae under the conditions of this experiment.

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