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Effects of dietary lipid levels on growth, survival and lipid metabolism during early ontogeny of *Pelteobagrus vachelli* larvae

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ABSTRACT

A feeding trial was conducted to investigate the effect of dietary lipid level on darkbarbel catfish (*Pelteobagrus vachelli*) larvae during ontogeny with regard to growth, survival and lipid utilization. Larvae were fed, from mouth opening to 20 days after hatching (DAH), with five isonitrogenous microdiets containing different lipid levels (58, 74, 111, 151 and 199 g kg^{-1} diet). Live prey (newly hatched *Artemia*, unenriched) was used as the control diet. The activities of lipoprotein lipase (LPL), hepatic lipase (HL), pancreatic lipase (PL) and LPL gene expression at 3 DAH (mouth opening), 6 DAH, 11 DAH and 20 DAH were examined. The results showed that dietary lipid significantly affected survival and growth of darkbarbel catfish larvae. At the end of the feeding trial, larvae fed diets containing 111 to 151 g lipid kg^{-1} had significantly higher survival. Specific growth rate (SGR) of larvae fed the diet containing the highest dietary lipid (199 g kg^{-1}) was significantly ($P<0.05$) lower while no significant differences were observed among other groups fed formulated diets. LPL mRNA level generally increased first with increasing dietary lipid levels and then reached a plateau at different sampling ages. A similar pattern was observed for LPL activity only at 6 DAH and 20 DAH. High dietary lipid increased HL activity at 20 DAH. At 6 DAH, highest PL activity was observed at 199 g lipid kg^{-1} diet. Higher dietary lipid resulted in earlier elevated activities of LPL, PL and HL. The specific activities of the above three enzymes and LPL mRNA expression were detected at mouth opening and were significantly influenced by age. The activities of these enzymes increased first and then decreased or reached a plateau during development. The results suggest that dietary lipid could modify lipid utilization during ontogeny of darkbarbel catfish larvae.

1. Introduction

Dietary lipids are the main source of energy for the development of fish larvae, and the supply of essential fatty acids in sufficient amounts is critical for fast growing larvae (Sargent et al., 2002). Fatty acid requirements have been extensively studied in fish larvae using live prey enriched with different oils. During the last two decades, the substitution of live food by artificial diets for the early stages of fish has been a major concern in aquaculture research in order to reduce costs and increase predictability of juvenile production. However, there is still a lack of information on optimal dietary lipid level in artificial diets for fish larvae.

The ability of larvae to assimilate the required nutrients is dependent on the composition of the diet and on their capacity of modulating their digestive enzymes and metabolic processes (Cahu and Zambonino Infante, 2001). Recently, increasing attention has been paid to the influence of diet formulation on enzyme expression and development, and studies have showed that dietary composition could affect the development of fish larvae (reviewed by Infante and Cahu, 2007). Better knowledge of the physiology and developmental mechanisms of fish larvae is critical to replace live food with formulated diets, and to allow identification of potential quality control indicators for fish larval rearing.

Lipoprotein lipase (LPL), hepatic lipase (HL) and pancreatic lipase (PL) are three members of the lipase gene family, as determined by amino acid sequence similarity and gene organization (Wong and Schotz, 2002). These lipases share a high degree of structural similarity with each other but play different roles in lipid metabolism. LPL hydrolyzes triacylglycerols present in plasma lipoproteins and supplies free fatty acids for storage in adipose tissue, or for oxidation in other tissues (Nilsson-Ehle et al., 1980). HL is primarily synthesized in liver and is involved in chylomicron-remnant and high density lipoprotein metabolism (Santamarina-Fojo et al., 1998). PL is synthesized by pancreatic acinar cells, where it is secreted into the intestinal lumen and aids in the intestinal hydrolysis of long-chain triglyceride fatty acids (Verger, 1984). Recently, the LPL cDNA has been cloned in several fish species including zebrafish (*Danio rerio*) (Arnault et al., 1996), rainbow trout (*Oncorhynchus mykiss*) (Lindberg

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and Olivecrona, 2002), gilthead sea bream (*Sparus aurata*) (Saera-Vila et al., 2005) and red sea bream (*Pagrus major*) (Oku et al., 2006). The nutritional regulation of LPL has been studied in several species. In red sea bream, the effects of dietary lipid level and dietary fatty acid supplementation were investigated (Liang et al., 2002a,b). In gilthead sea bream, the effects of season and dietary composition on LPL gene expression in different tissues were studied (Saera-Vila et al., 2005). Albalat et al. (2006, 2007) examined the nutritional and insulin regulation of LPL activity in gilthead sea bream and rainbow trout. These studies indicated that LPL was regulated in response to the physiological state of the fish. However, little is known about LPL activities and gene expression at the larval stage.

Pelteobagrus vachelli (darkbarbel catfish) is an important freshwater aquaculture species in China. Due to its high market value, the culture of this species has increased rapidly in recent years. But the larvae culture still relies on live food, which is a drawback for large-scale larval rearing. The purpose of this study was to investigate the effect of lipid level in a compound diet on growth and mortality of first-feeding larvae of *P. vachelli* and to understand the effects of dietary lipid level on LPL, HL, PL activities and LPL gene expression during larval development.

2. Materials and methods

2.1. Diets and animals

Five isonitrogenous diets were formulated to contain 58, 74, 111, 151 and 199 g lipid kg^{-1} diet using different levels of fish oil. Formulation and proximate composition of experimental diets are shown in Table 1.

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

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Ingredient					
White fish meal (USA) [a]	450	450	450	450	450
Casein hydrolysate[b]	100	100	100	100	100
Squid meal[c]	150	150	150	150	150
Fish oil[d]	0	45	90	135	180
A-starch[e]	50	50	50	50	50
Cellulose[f]	181	136	91	46	1
Soy lecithin[g]	10	10	10	10	10
Mineral premix[h]	50	50	50	50	50
Vitamin premix[i]	5	5	5	5	5
Choline chloride	1	1	1	1	1
Alginate sodium[j]	3	3	3	3	3
Chemical composition (g kg^{-1} dry matter)					
Dry matter	883	868	882	884	884
Crude protein	435	426	415	420	437
Crude lipid	58	74	111	151	199
Ash	117	117	128	127	123
Gross energy (kJ g^{-1})	17.8	18.1	18.9	19.3	21.5

[a] American Seafood, purchased from Coland Feed Co. Ltd. Wuhan, P. R. China. Chemical composition: moisture: 4.26%; crude protein: 68.93% of dry matter; crude lipid: 8.97%; ash: 12.15%; gross energy: 19.06 kJ g^{-1}.
[b] A-2427, Sigma, St Louis, MO, USA.
[c] Purchased from Coland Feed Co. Ltd. Wuhan, P. R. China.
[d] Anchovy oil from Peru, purchased from Coland Feed Co. Ltd. Wuhan, P. R. China.
[e] A-starch: corn starch, Wuhan Starch Processing Company, Wuhan, P. R. China.
[f] Cellulose: Ehua Phama Expipents, Liaocheng, Shandong, P. R. China.
[g] Soy lecithin: Taijin Bodi Chemical Holding Co., Ltd., Tianjin, P. R. China.
[h] Mineral premix (mg kg^{-1} diets, H440): NaCl, 500; $MgSO_4 \cdot 7H_2O$, 4575.0; $NaH_2PO_4 \cdot 2H_2O$, 12,500.0; KH_2PO_4, 16,000.0; $Ca(H_2PO_4)_2 \cdot H_2O$, 6850.0; $FeSO_4$, 1250.0; $C_6H_{10}CaO_6 \cdot 5H_2O$, 1750.0; $ZnSO_4 \cdot 7H_2O$, 111.0; $MnSO_4 \cdot 4H_2O$, 61.4; $CuSO_4 \cdot 5H_2O$, 15.5; $CoSO_4 \cdot 6H_2O$, 0.5; KI, 1.5; Starch, 6385.1.
[i] Vitamin premix (per kg diets): vitamin A, 5500 IU; vitamin D3, 1000 IU; vitamin E, 50 IU; vitamin K, 10 mg; niacin, 100 mg; riboflavin, 20 mg; pyridoxine, 20 mg; thiamin, 20 mg; biotin, 0.1 mg; D-calacium pantothenate 50 mg; folacin, 5 mg; B12, 20 mg; ascorbic acid, 100 mg; inositol, 100 mg.
[j] From Damao Chemicals Company, Tianjin, P. R. China.

The ingredients were finely ground (to less than 50 μm) and sent to a professional aquafeed company (Shanghai Yujiaao Co; Ltd. China), in which the commercial microbound diets were produced. The method used to prepare the microdiets resembled the method of particle-assisted rotational agglomeration (PARA) (Hardy and Barrows, 2002). The particle size of the formulated diets ranged from 200 to 250 μm for fish between 3 and 12 days after hatch (DAH) and 250–380 μm for the larvae thereafter. All formulated diets were stored at −18 °C until used. The proximate composition of diets was determined following the standard procedures of AOAC (1995). Gross energy was determined by combustion in a microbomb calorimeter (Phillipson microbomb calorimeter, Gentry Instruments Inc., Aiken, USA).

A detailed description of the experimental fish and their culture can be found in Wang et al. (2005). Briefly, 2-day-old yolk-sac larvae were obtained from the fish farm of the Fisheries College, Huazhong Agriculture University, Wuhan, Hubei, China and were distributed into 24 cages (26×24×20 cm) which were placed in 24 rectangular fiberglass tanks (water volume, 150 l) with 300 larvae per cage. The rectangular fiberglass tanks were connected to a recirculation system. System water was replaced 10% per day. The water flow into each rectangular tank was about 8 l min^{-1}. During the experiment, water temperature and pH were monitored daily and dissolved oxygen and ammonia-N measured weekly. Water temperature was maintained at 27±0.5 °C; ammonia-N was less than 0.5 mg l^{-1}; dissolved oxygen varied between 6 and 7 mg l^{-1}; pH varied between 6.4 and 6.6. Residual chlorine was determined weekly and was below 0.05 mg l^{-1}. Photoperiod was set at a 12 h light:12 h dark cycle (light period from 08:00 to 20:00 h) and light intensity was kept at 40 lx at the water surface.

Each test diet was randomly assigned to four replicate tanks and larvae were fed from 3 DAH. Live food (newly hatched *Artemia*, unenriched) was used as the control diet. Commercial *Artemia* cysts were bought from Xinjiang, China and newly hatched *Artemia nauplii* contained 52% protein and 25% lipid on a dry-weight basis (Zeng et al., 2001). During the trial, fish were hand fed the experimental diets or *Artemia* four times daily: at 8:00, 12:00, 16:00, and 20:00 h. Excess food was offered to ensure that fish were fed to satiation. Uneaten feed and faeces were removed by siphoning twice daily before feeding. Mortality of larvae was recorded daily.

2.2. Sampling

At the beginning of the feeding trial (3 DAH), triplicates of 200 larvae were sampled, batch weighed, immersed in liquid nitrogen and kept at −70 °C for further enzyme analysis. Triplicates of 20 larvae were randomly sampled for RNA extraction. The samples were submerged in RNASafer® Reagent (Omega Bio-tek, USA) at a ratio of about 1 volume tissue to 15 volumes of RNASafer® Reagent, frozen immediately in liquid nitrogen and kept at −70 °C until analyzed.

Three samples of each treatment (one sample from each of three tanks per sampling) were randomly collected at 08:00h on sampling day. At each sampling, three tanks were randomly selected and fifty larvae at 6 DAH, 40 larvae at 11 DAH or 30 larvae at the end of experiment (20 DAH) were collected from each tank and weighed to calculate growth, and then sampled for enzyme analysis. Ten larvae from each tank (three tanks were randomly selected at each sampling) at 6 DAH, 11 DAH and 20 DAH were sampled for RNA extraction. At the end of the feeding trial, all fish in each tank were counted and batch weighed.

2.3. Enzyme assays

Frozen samples were thawed and homogenized in ice-cold, 0.9% physiological saline with a conical glass homogenizer. The total body homogenate was centrifuged at 4 °C, 15,000×*g* for 15 min. The supernatant, free from the lipid layer, was used to determine LPL, HL and PL activity and soluble protein content. LPL and HL activities were

determined using the Lipoprotein Lipase (LPL)/Hepatic Lipase (HL) Detection Kit (cat#: A067) from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). PL activities were assayed using a commercial kit (Jiancheng Bioengineering Institute, Nanjing, People's Republic of China). Enzyme specific activity was expressed as units mg^{-1} soluble protein. Protein concentration of tissue homogenates was determined by the Bradford method (1976) using a protein assay kit (Nanjing Jiancheng Bioengineering Institute, China) with bovine serum albumin as a standard.

2.4. Total RNA extraction, reverse transcription and subcloning

Total RNA was isolated using Trizol reagent (Invitrogen, USA) and treated with RNase-free DNase (Takara, Japan). Total RNA was quantified spectrophotometrically by determination at OD260. The purified total RNA (2 μg) was then reverse transcribed. Reverse transcription was performed with oligo(dt)18 primer using a first strand cDNA synthesis kit (Toyobo, Japan).

Degenerate polymerase chain reaction (PCR) primers were designed based on highly conserved regions from LPL sequences of other fish available in Genbank and were synthesized by Sangon (Shanghai, China): Lipoprotein lipase F: 5′-ACA T(C/T)T ACC CCA A (C/T)G G(A/T)G G-3′; R: 5′-TGC GAA TGT GGA AAG TGT C-3′. Amplifications were performed using Pfu Taq DNA polymerase (MBI Fermentas, Lithuania). The PCR conditions were: 1 min at 94 °C; 30 cycles of 30 s at 94 °C, 30 s at 52 °C, 1 min at 72 °C; another 2 min at 72 °C. Amplification products were resolved on a 1.5% agarose gel with a 200-bp ladder as a marker and bands of the expected size were purified using a DNA extraction kit (MBI Fermentas, Lithuania) according to the user's manual. The purified DNA was checked on a 1.5% agarose gel. Twenty microliters of rehydrated purified DNA was again put in GeneAmp PCR System 9600 with 1 U Taq DNA polymerase (MBI Fermentas, Lithuania), 2.5 μl Taq DNA polymerase buffer, 1.5 μl $MgCl_2$ and 1 μl dNTP for 1 min at 94 °C, followed by 72 °C for 30 min. pGEM®-T vector system (Promega, USA) was used to ligate the PCR products into the pGEM-T vector overnight at 4 °C. Two microliters of each ligation reaction were transformed into competent DH5α *E. coli* (with a transformation efficiency of 1×106 cfu μg^{-1} DNA) and then plated on LB agar plates (containing ampicillin, IPTG and X-Gal, Sigma, USA) overnight at 37 °C. White colonies were selected and transferred to LB broth containing ampicillin, cultured at 37 °C for 4 h under shaking at 225 rpm. Specific primers for LPL and M13 were used to select the plasmid clones, which contained the insert. Only plasmid clones containing a target insert were sent to UnitedGene (Shanghai, China) for sequencing. Fig. 1 showed the partial nucleotide sequence and deduced amino acid sequence of *P. vachelli* LPL cDNA. The sequence (GenBank accession number: EU882966) revealed a fragment of 528 bp highly homologous to zebrafish (81%), European seabass (79%) and Atlantic salmon (78%) lipoprotein lipase.

2.5. Real-time PCR

Levels of LPL transcript were measured by real-time PCR (SYBR Green I), using β-actin as a housekeeping gene. Primers for real-time PCR (Table 2) were designed based on the cloned PCR fragments of LPL cDNA for 5′-RACE and 3′-RACE. Real-time RT-PCR was conducted by amplifying 1.0 μl of cDNA with the SYBR Green qPCR kit (Finnzymes, Finland) on a Chromo4 Real-Time Detection System (MJ Research, Cambridge, MA). Amplification conditions were: 10 min at 94 °C; 40 cycles of 20 s at 94 °C, 20 s at 60 °C, 25 s at 72 °C; another 5 min at 72 °C. Melting curve analysis of amplification products was performed at the end of each PCR reaction to confirm that a single PCR product was detected. Each sample was run in triplicate, and PCR reactions without the addition of the template were used as negative controls. After completion of the PCR amplification, data were analyzed with the Option Monitor software version 2.03 (MJ Research, Cambridge, MA). Relative expression ratio of LPL was calculated based on the PCR efficiency (E) and the Ct of a sample versus the control (3 DAH group), and expressed in comparison to the reference gene (β-actin), according to Pfaffl's mathematical model (Pfaffl, 2001).

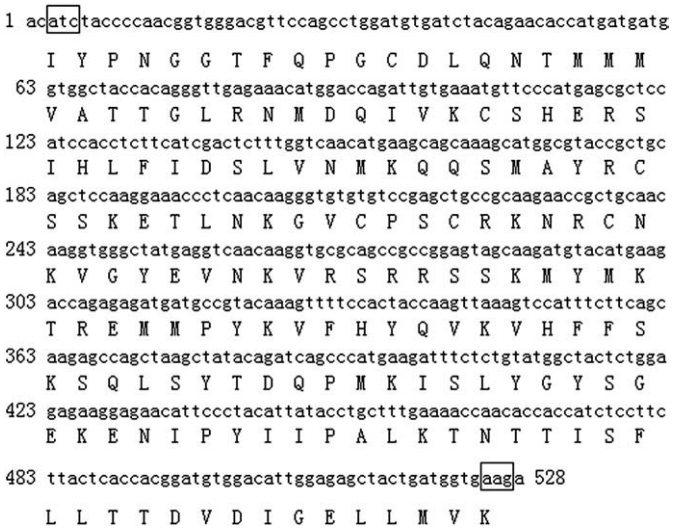

Fig. 1. The partial nucleotide sequence and deduced amino acid sequence of *Pelteobagrus vachelli* LPL cDNA.

$$Ratio = [(E_{LPL})^{\Delta Ct(control-sample)}] / [(E_{actin})^{\Delta Ct(control-sample)}]$$

2.6. Statistical analysis

SPSS 11.5 for Windows was used for statistical analysis. Data on survival and growth were compared by one-way ANOVA followed by Duncan's multiple range test when significant differences were found at the $P<0.05$ level. Enzyme activities and mRNA levels were compared by a two-way ANOVA followed by Duncan's multiple range test when significant differences were found at the $P<0.05$ level. The two independent factors were age and dietary lipid level. The homogeneity of variances was first checked using the Levene's test and arcsin transformed data were used for HL activities. When a significant interaction was found between the two factors, a one-way ANOVA was performed to analyze the results.

3. Result

3.1. Survival

Survival of fish larvae fed *Artemia* was about 95.5%, indicating that the rearing conditions were suitable for the fish larvae (Table 3).

Larvae survival significantly increased with increasing dietary lipid from 58 to 151 g kg^{-1} (Diets 1–4; $P<0.05$), and then decreased at 199 g lipid kg^{-1} (Diet 5) (Table 3). The survival of larvae fed the diets containing 111 and 151 g lipid kg^{-1} (Diets 3 and 4) was significantly

Table 2
Real-time PCR primers used in the experiment.

Primer	Sequence (5′–3′)	Size (bp)
Lipoprotein lipase F	TGGCTACCACAGGGTTGAGA	197
Lipoprotein lipase R	GACCTCATAGCCCACCTTG	
β-actin F	CACTGTGCCCATCTACGAG	200
β-actin R	CCATCTCCTGCTCGAAGTC	

higher than that of larvae fed other diets ($P<0.05$), but significantly lower than those fed *Artemia* ($P<0.05$). Fig. 2 showed that there was a mortality peak from 8 DAH to 12 DAH in larvae fed formulated diets, accounting for about 60% to 85% of the overall mortality throughout the growth experiment.

3.2. Growth

Table 3 shows that the larvae fed live prey had significantly better growth from the first sampling time compared to the larvae fed formulated diets ($P<0.05$). There were no significant differences in fish wet weight at 6 DAH between the treatments fed formulated diets ($P>0.05$). At 11 DAH, the larvae fed high dietary lipid (Diet 3, 4 and 5) showed significantly lower wet weight ($P<0.05$). At the end of the feeding trial (20 DAH), final wet weight and specific growth rate (SGR) of larvae fed Diet 5 containing highest dietary lipid (199 g kg^{-1}) was significantly lower ($P<0.05$) while no significant differences were observed among the other groups fed formulated diets ($P>0.05$).

Coefficient of variation (CV) values of final body weight of larvae fed the diets with 58 to 74 g lipid kg^{-1} were significantly higher than that of larvae fed the other diets ($P<0.05$). Overall larvae fed diets with 111 and 151 g lipid kg^{-1} exhibited the best growth performance among formulated diet group. Larvae fed live prey showed significantly higher final weight gain and lower CV of final body weight compared to larvae fed formulated diets ($P<0.05$).

3.3. LPL, HL, PL activities and LPL mRNA ratio

Table 4 showed the result of two-way ANOVA on LPL, HL, PL activities and mRNA ratio. Both dietary lipid and fish age affected LPL, HL, PL activities and mRNA ratio and there was an interaction between dietary lipid and fish age ($P<0.05$).

Table 5 showed that dietary lipid level did significantly affect LPL activities at 6 DAH and 20 DAH ($P<0.05$). At 6 DAH, dietary lipid increased LPL activity while the fish fed with *Artemia* showed the lowest value ($P<0.05$). There was no significant difference in LPL activities at 20 DAH in the fish fed with formulated diets ($P>0.05$). At 58 g lipid kg^{-1} diet, the highest LPL activity was observed at 11 DAH while higher dietary lipid resulted in earlier LPL activity increase at 6 DAH ($P<0.05$).

Table 6 showed that dietary lipid could increase HL activity at 20 DAH ($P<0.05$) while there was no significant difference in HL activities from 111 to 199 g lipid kg^{-1} diet ($P>0.05$). No significant effect of dietary lipid was observed on HL activities at 6 DAH or 11 DAH ($P>0.05$). Higher HL activities were observed at 6 DAH and then decreased at 20 DAH ($P<0.05$).

Table 7 showed that dietary lipid significantly affected fish PL activity at 6, 11 and 20 DAH ($P<0.05$). At 6 DAH, PL activity increased with dietary lipid ($P<0.05$). At 11 DAH, highest PL activity was observed at 74 g lipid kg^{-1} diet and decreased at 199 g lipid kg^{-1} diet ($P<0.05$). At 20 DAH, highest PL activity was observed at 74 and 111 g lipid kg^{-1} diet and decreased at 199 g lipid kg^{-1} diet ($P<0.05$). At 58–151 g lipid kg^{-1} diet, highest PL activity was observed at 11 DAH while highest dietary lipid (199 g kg^{-1}) showed earlier high PL activity at 6 DAH ($P<0.05$).

Table 8 showed that, after fish mouth opening (from 6 DAH), increased dietary lipid resulted in increased LPL mRNA ratio ($P<0.05$).

4. Discussion

Dietary lipid deficiency often leads to poor survival and growth in fish larvae (Rainuzzo et al., 1997) and fish larvae require optimal dietary lipid to maintain high survival and growth. In the present study, *P. vachelli* larvae fed the diet containing 111 g kg^{-1} lipid and 151 g kg^{-1} lipid showed significantly higher survival than larvae fed other formulated diets. Similar results were reported in large yellow croaker larvae which had significantly higher survival when fed a diet containing 164 g lipid kg^{-1} compared to those fed 83, 126 or 248 g lipid kg^{-1} (Ai et al., 2008). High dietary lipid resulted in lower survival and reduced growth in the present study and some previous reports including yellow croaker larvae (Ai et al., 2008) and juvenile cobia (Chou et al., 2001) and on-growing turbot (Regost et al., 2001). This could be due to increased malonaldehyde in oxidized lipid, which is toxic to fish (Baker and Davies, 1997). Decreased growth at high dietary lipid levels also might be a result of a decrease in the efficiency or activity of digestive enzymes, a reduction in absorption efficiency and/or a decrease in food and thus protein intake (Izquierdo et al., 2000; Morais et al., 2007). High dietary neutral lipid levels had been observed to result in growth reduction of fish larvae (Pousão-Ferreira et al., 1999; Izquierdo et al., 2000; Olsen et al., 2000; Gawlicka et al., 2002; Ai et al., 2008). High dietary neutral lipid could result in the accumulation of large lipid droplets in the enterocytes, which might reduce fatty acid absorption efficiency and ultimately larval growth (Morais et al., 2005).

It normally takes several weeks for *P. vachelli* larvae consuming live prey to be weaned onto formulated diets. Yuan et al. (2005) reported that *P. vachelli* larvae opened mouth and started exogenous feeding on 3–4 DAH, and the yolk sac was completely consumed during 6–7 DAH at 26 °C. The median lethal time (LD_{50}) for starved larvae of *P. vachelli* was 10 DAH. In the present study, the mortality peak occurred during 8 DAH to 12 DAH in larvae fed formulated diets. This mortality could be due to a failure to first feeding. The formulated diet feeding could also induce a delay in larvae development compared to live prey feeding. Co-feeding *P. vachelli* larvae with live prey and formulated diet might improve the nutritional condition of larvae and pre-condition them to accept the formulated diet when live prey was withdrawn (Rosenlund et al., 1997).

At the end of this growth trial in the present study, *P. vachelli* larvae fed the low lipid diets obtained higher mortalities and CV values of final body weight but similar weight gain. It could be due to the high mortality and most small fish died.

PL activity was detected at 3 DAH (first feeding) in *P. vachelli* larvae. Lipase specific activity was detectable at 3 DAH at high levels in

Table 3
Effect of dietary lipid levels on growth and survival of darkbarbel catfish larvae (mean ± SE).

Dietary lipid level (g kg^{-1})	58	74	111	151	199	*Artemia* (control)
Survival (%)	11.6 ± 4.3[a]	18.5 ± 6.3[a]	27.4 ± 3.7[b]	32.2 ± 3.8[b]	17.7 ± 4.4[a]	95.5 ± 0.3[c]
Wet weight (mg)						
6 DAH	4.6 ± 0.2[a]	4.6 ± 0.1[a]	4.5 ± 0.0[a]	4.7 ± 0.2[a]	4.7 ± 0.0[a]	8.1 ± 0.5[b]
11 DAH	5.8 ± 0.4[a]	5.6 ± 0.4[a]	4.9 ± 0.1[b]	4.8 ± 0.1[b]	4.8 ± 0.2[b]	18.3 ± 0.9[c]
20 DAH	12.8 ± 1.8[a]	12.5 ± 1.1[a]	10.5 ± 0.5[a]	10.6 ± 0.5[a]	7.8 ± 0.4[b]	53.42 ± 3.1[c]
SGR(%/day)	6.9 ± 1.0[a]	7.5 ± 0.7[a]	5.9 ± 0.3[a]	6.0 ± 0.3[a]	4.2 ± 0.3[b]	15.5 ± 0.4[c]
CV of FBW (%)	59.8 ± 6.9[a]	50.2 ± 4.9[a]	38.6 ± 2.5[b]	35.1 ± 2.2[b]	32.7 ± 2.9[b]	15.7 ± 0.3[c]

[a–c]Means in same row with the same superscripts are not significantly different ($P>0.05$).
SGR: Specific growth rate (%/day) = 100 × [ln (final wet body weight) − ln (initial wet body weight)]/days.
CV of FBW (%): coefficient of variation of final body weight = 100 * SD/mean, where mean is average final body weight, SD = standard deviation of final body weight.

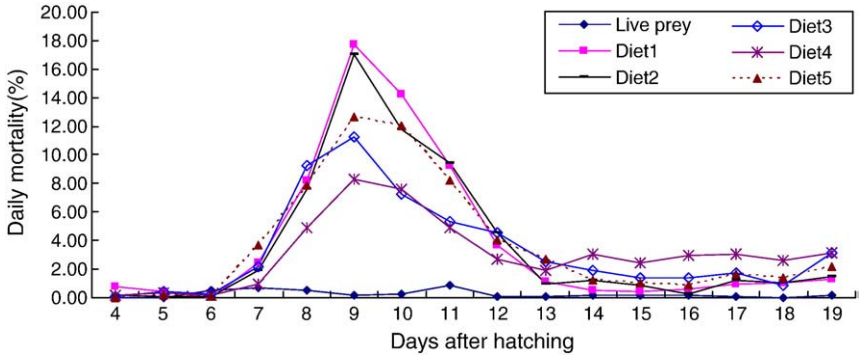

Fig. 2. Daily mortalities of darkbarbel catfish larvae (daily dead larvae/initial number of larvae, mean per treatment) during development.

Table 4
Two-way ANOVA tests of significance for the interaction of different diets and ages on LPL, HL, PL activities and LPL mRNA level.

Effect	LPL activity		LPL mRNA		HL activity		PL activity	
	df	*p*	*df*	*p*	*df*	*P*	*df*	*p*
Diet	5	0.000	5	0.000	5	0.000	5	0.000
Age	3	0.000	3	0.000	3	0.003	3	0.000
Diet*age	10	0.000	10	0.023	10	0.001	10	0.000
Pooled standard error	35[a]		38		35[a]		35[a]	

[a] One replicate sample was missing.

Table 5
Effect of dietary lipid levels on LPL activity of darkbarbel catfish larvae (means ± SE).

Dietary lipid level (g kg^{-1})	58	74	111	151	199	*Artemia* (the control)
3 DAH	0.10 ± 0.02^{A}	0.10 ± 0.02^{A}	0.10 ± 0.02^{A}	0.10 ± 0.02^{A}	0.10 ± 0.02^{A}	0.10 ± 0.02^{A}
6 DAH	0.43 ± 0.03^{Bab}	0.74 ± 0.11^{Babc}	0.79 ± 0.16^{Cbc}	0.83 ± 0.09^{Bc}	0.86 ± 0.11^{Bc}	0.40 ± 0.07^{Ba}
11 DAH	0.76 ± 0.01^{C}	0.76 ± 0.10^{B}	0.48 ± 0.09^{B}	0.69 ± 0.07^{B}	0.55 ± 0.07^{C}	0.12 ± 0.01^{A}
20 DAH	0.29 ± 0.03^{Dab}	0.28 ± 0.04^{Cab}	0.32 ± 0.02^{Bb}	0.30 ± 0.05^{Aab}	0.32 ± 0.05^{Db}	0.19 ± 0.02^{Aa}

Means with different capital superscripts are significantly different between sampling day ($P<0.05$) and means with different small superscripts are significantly different between dietary lipid ($P<0.05$).

Table 6
Effect of dietary lipid levels on HL activity of darkbarbel catfish larvae (means ± SE).

Dietary lipid level (g kg^{-1})	58	74	111	151	199	*Artemia* (the control)
3 DAH	0.10 ± 0.02^{A}	0.10 ± 0.02^{A}	0.10 ± 0.02^{A}	0.10 ± 0.02^{A}	0.10 ± 0.02^{A}	0.10 ± 0.02^{A}
6 DAH	0.43 ± 0.03^{Ba}	0.74 ± 0.11^{Ba}	0.79 ± 0.16^{Ba}	0.83 ± 0.09^{BCa}	0.86 ± 0.11^{Ba}	0.40 ± 0.07^{Ba}
11 DAH	0.76 ± 0.01^{B}	0.76 ± 0.10^{B}	0.48 ± 0.09^{AB}	0.69 ± 0.07^{C}	0.55 ± 0.07^{C}	0.12 ± 0.01^{A}
20 DAH	0.29 ± 0.03^{Aab}	0.28 ± 0.04^{Abc}	0.32 ± 0.02^{ABcd}	0.30 ± 0.05^{Bbc}	0.32 ± 0.05^{Cd}	0.19 ± 0.02^{Aa}

Means with different capital superscripts are significantly different between sampling day ($P<0.05$) and means with different small superscripts are significantly different between dietary lipid ($P<0.05$).

Table 7
Effect of dietary lipid levels on PL activity of darkbarbel catfish larvae (means ± SE).

Dietary lipid level (g kg^{-1})	58	74	111	151	199	*Artemia* (the control)
3 DAH	4.21 ± 0.33^{A}	4.21 ± 0.33^{A}	4.21 ± 0.33^{A}	4.21 ± 0.33^{A}	4.21 ± 0.33^{A}	4.21 ± 0.33^{A}
6 DAH	5.10 ± 0.39^{Aab}	7.89 ± 0.47^{Bbc}	7.26 ± 1.36^{Ab}	6.83 ± 0.39^{Bb}	10.07 ± 0.55^{Bc}	4.11 ± 0.15^{Aa}
11 DAH	12.43 ± 0.45^{Bb}	17.26 ± 0.89^{Cc}	11.39 ± 1.17^{Bb}	15.61 ± 1.22^{Cc}	5.59 ± 0.69^{Ca}	5.82 ± 0.77^{ABa}
20 DAH	9.38 ± 1.53^{Cabc}	11.22 ± 0.18^{Dbc}	11.74 ± 0.74^{Bc}	8.47 ± 0.14^{Bab}	8.48 ± 0.86^{Bab}	7.42 ± 0.84^{Ba}

Means with different capital superscripts are significantly different between sampling day ($P<0.05$) and means with different small superscripts are significantly different between dietary lipid ($P<0.05$).

Table 8
Effect of dietary lipid levels on LPL mRNA expression of darkbarbel catfish larvae expressed as a ratio to 3 DAH values (means ± SE).

Dietary lipid level (g kg^{-1})	58	74	111	151	199	*Artemia* (the control)
3 DAH	1.00 ± 0.00^{A}	1.00 ± 0.00^{A}	1.00 ± 0.00^{A}	1.00 ± 0.00^{A}	1.00 ± 0.00^{A}	1.00 ± 0.00^{A}
6 DAH	2.39 ± 0.37^{Bab}	3.67 ± 0.69^{Bbc}	2.78 ± 0.88^{Aab}	3.95 ± 0.16^{Bbc}	4.84 ± 0.20^{Cc}	1.66 ± 0.17^{Ba}
11 DAH	2.79 ± 0.32^{Bab}	3.50 ± 0.70^{Babc}	5.61 ± 0.95^{Bc}	5.51 ± 0.49^{Cc}	4.69 ± 0.86^{BCbc}	2.51 ± 0.17^{Ca}
20 DAH	2.13 ± 0.11^{Bab}	1.69 ± 0.17^{Aa}	2.68 ± 0.32^{Abc}	3.69 ± 0.36^{Bd}	3.26 ± 0.23^{Bcd}	2.47 ± 0.28^{Cabc}

Means with different capital superscripts are significantly different between sampling day ($P<0.05$) and means with different small superscripts are significantly different between dietary lipid ($P<0.05$).

red drum (Lazo et al., 2000), while at 2 DAH (first feeding) in the brush border and cytoplasm of enterocytes of *Senegalese sole* (Ribeiro et al., 1999). The presence of enzymatic capacity before first feeding suggests that these enzymes were induced by genetic control, not by food intake. There are strong indications that fish larvae are capable of efficiently digesting and absorbing lipids from the start of exogenous feeding (Morais et al., 2007). In the present study, both age and dietary lipid level significantly affect PL activity. PL activity showed an increasing trend from 3 DAH to 11 DAH, and then decreased or stayed at the same level until 20 DAH. The decline in enzyme activity was not due to a diminution in enzyme synthesis but a consequence of growth, development of new organs and tissues, and an increase in tissue proteins. This particular enzymatic profile is specific to early larval stages, and characterizes post-hatching changes in the activity of digestive enzymes in fish larvae (Wang et al., 2006).

The effects of dietary lipid on PL activity were different at different growth stages in the present experiment. At 6 DAH, an increase in lipid level induced PL activity. However, at 20 DAH and particularly at 11 DAH, PL activity decreased when larvae were fed the highest lipid level. In sea bass, pancreatic lipase activity increased with dietary triglyceride level ranging from 13% to 23%, showing a threshold, around 20% triglycerides, at which the maximum lipase activity and messenger RNA level were reached (Cahu et al., 2003). However, it has been suggested that the intestinal receptors for intraluminal stimulants require only a very low concentration for activation, meaning that the length of the intestine exposed to these products is the main determinant of pancreatic secretory response (Singer, 1987). If fish larvae showed similar response, the ingestion rate of the diet might also play a major role in determining lipase activity. This may explain what we observed at 11 and 20 DAH, larvae fed high dietary lipid with reduced food intake and lower PL activity. In accordance with this, the bile salt-dependent lipase content in turbot larvae was not significantly affected by the lipid level of the prey but appeared to be a function of the ingestion rate (Hoehne-Reitan et al., 2001). Morais et al. (2007) reviewed some recent studies and concluded that neutral lipase synthesis might not be a limiting factor for larval growth.

LPL and HL are two key regulatory enzymes in lipid uptake. Black and Skinner (1986) reported that HL was the dominating lipase in muscle and adipose tissue in rainbow trout, while Lindberg and Olivecrona (1995) only found HL activity in liver of rainbow trout and concluded that LPL was the main extrahepatic lipoprotein-metabolizing lipase in fish. To date there has been no information about LPL and HL activity and gene expression in fish larvae from mouth opening. In the present study, activity of both LPL and HL of *P. vachelli* could be detected at first feeding (3 DAH). From first feeding to 20 DAH, LPL and HL activity showed an increase initially and then a decrease in all groups though the timing of decrease was not exactly the same among treatment groups. Age also significantly influenced LPL mRNA level. The fast increase and decrease in LPL activity and mRNA level reflected the characteristic of different stages and indicated that LPL synthesis of *P. vachelli* larvae during ontogeny was regulated at least at the transcriptional level. But the observed changes in mRNA level might or might not result in physiologically relevant changes in enzyme concentration (Sánchez-Paz et al., 2003). Therefore, the changes of enzyme activity and its mRNA level are dependent on the translation fidelity and efficiency of mRNA.

Effects of dietary lipid on LPL activity and mRNA level was observed as early as 6 DAH in the present study and high dietary lipid induced both higher LPL activity and mRNA level. This suggested an increase in lipid utilization in *P. vachelli* larvae during development. At 11 DAH and 20 DAH, LPL mRNA level showed an increase and later reached a plateau with increasing dietary lipid level while LPL activity was not significantly influenced by dietary lipid. These results suggested that LPL activity of *P. vachelli* larvae was only partly regulated at the mRNA level. In mammals, regulation of LPL is complicated and can occur at different levels (transcriptional, translational and posttranscriptional). Changes in LPL activity have not always been accompanied by parallel changes in mRNA levels (Lee et al., 1998; Bergo et al., 1996). In gilthead sea bream, LPL activity changes were followed by an increase in LPL mRNA levels in the adipose tissue but the changes in LPL expression showed a time lag (Albalat et al., 2007).

Since whole larvae were pooled for the analysis of enzyme activity and gene expression in the present study, their specific tissue source could not be distinguished. More tissue-specific technical approaches such as histochemistry and in situ hybridization techniques might allow finer-resolution and more definitive examination of some of the ontogenetic questions.

In summary, LPL, HL and PL of *P. vachelli* could be detected at mouth opening, and age significantly influenced the activities of three enzymes and LPL mRNA level. Dietary lipid level could partly modify the enzyme activities and might thus affect lipid digestion and utilization. Lipolytic activity and lipid utilization of *P. vachelli* larvae increased with increased dietary lipid level to some certain degree while high dietary lipid level could inhibit lipid digestion.

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