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Optimum dietary lipid requirement of *Pangasianodon hypophthalmus* juveniles in relation to growth, fatty acid profile, body indices and digestive enzyme activity

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Abstract The present experimental trial was conducted to determine the optimal dietary lipid requirement of juveniles of *Pangasianodon hypophthalmus* (Sauvage, 1878) commonly referred to as the striped catfish using completely randomized design (CRD). Purified diets with five different dietary lipid levels (3, 6, 9, 12 and 15%; fish oil/sunflower oil (1:1) were used to feed triplicate groups of Pangasius with initial mean weight of 13.54–14.12 g twice a day for 8 weeks. The highest weight gain ($P < 0.05$) was observed in fish fed with 9 and 12% lipid diets, followed by diet with 6% lipid, then by 15% lipid diet, and the lowest weight gain was observed in fish fed with 3% lipid. Other parameters such as specific growth rate (SGR), feed efficiency (FE) and protein efficiency ratio (PER) showed the same trend of weight gain. The hepatosomatic index (HSI), viscerosomatic index (VSI) and intraperitoneal fat (IPF) values increased with the increase of dietary lipid level. The feed conversion ratio (FCR) ($P < 0.05$) showed significant effects ($P < 0.05$) with variations in dietary lipid levels. The minimum FCR (1.95) was observed at 9% lipid inclusion-fed fishes and maximum FCR (3.53) was noticed at 3% lipid inclusion fed fishes. Higher and lower digestive enzyme activities were respectively observed in 9 and 15% lipid diets. The muscle fatty acid profile also varied with different dietary lipid levels. The higher level of ω-3, ω-6 fatty acid contents was recorded in the muscle of fish fed with 9% lipid diet, and lower level of fatty acid content was recorded in fish fed with 3% lipid diet. However, using second-order polynomial regression analysis, the optimal dietary lipid requirement of *P. hypophthalmus* juveniles was found to be 10.1%.

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Keywords Diet · Lipid level · *Pangasianodon hypophthalmus* · Striped catfish · Fatty acid · Digestive enzyme

Introduction

Pangasianodon hypophthalmus, commonly referred to as the striped catfish, contributes to the major catch of Mekong river fishery and is considered the most important inland fisheries in the globe. Capture-based aquaculture of *P. hypophthalmus* was developed in Vietnam, Thailand and Cambodia due to the fact that it is a prolific spawner that can be easily harvested from the flowing river. This species has a large economic importance in most of the countries of Southeast Asia, including Malaysia. Striped catfish fillets have a stable market in most of the world countries. *P. hypophthalmus* is the fastest-growing food fish in the world. Currently, the expansion of the culture of this fish has been hampered because of the lack of good quality feeds. The farmers use supplementary feeds such as trash fish, vegetables and chicken viscera for this species. One of the major problems faced by the feed industry is the lack of knowledge about the exact nutrient requirements of *P. hypophthalmus*. Currently, *P. hypophthalmus* feeds are prepared based on the nutrient requirements of channel catfish (*Ictalurus punctatus*). It is now time to emphasize the importance of the ideal nutrient requirements for this species. This study will provide a clear idea of the optimum lipid requirement of *Pangasius*. Dietary protein is a major factor affecting growth performance of fish and, also, an important source of energy. It has a tremendous effect on the cost of feed (Miller et al. 2005) due to the fact that protein is more costly compared to lipids and carbohydrates (Lovell 1989). This leads to the idea of increasing the lipid levels in the fish diet in order to meet its energy requirements.

Optimizing protein and energy levels in a diet during feed formulation promotes growth, minimizes nitrogenous output and reduces the cost of feed. When the diet is prepared with excess protein levels, some of this excess protein will be utilized for energy production (Ruohonen et al. 1999; Jahan et al. 2002). Hillestad et al. (1998) supported that feed conversion ratio (FCR) and higher nitrogen and phosphorous retention were improved in fish when diets with higher lipid levels were ingested. Furthermore, a higher lipid concentration in feed pellets increases the water stability (Chaiyapechara et al. 2003). The non-protein sources such as lipid are used to replace the dietary protein that is not utilized from fish. Lipids must be included in fish diets in order to maximize the use of protein for growth. Dietary lipids are a dominant factor in order to attain high-quality fish meat, and it is also important that the required amount of lipid be incorporated in the fish diet.

An excessive amount of dietary lipid not only creates associated problems in feed manufacturing but also produces fatty fish. The use of lipid as a source of energy and essential fatty acids in fish feed and its importance in feed technology have been recognized. Because lipid content is approximately twice as many calories per gram protein and carbohydrate, it contributes greatly to the energy levels in the diet, even when present in a relatively low quantity (Sargent et al. 1989). Further, feeds with minimum dietary lipid concentration required for maximal protein sparing action for a particular species is effective at a relatively low cost. However, excess lipid in feed is not recommended because it could lead to a reduction in feed consumption by fish (Ling et al. 2006). Moreover, the excessive dietary energy can cause an abnormal high lipid deposition in the fish body, which is adverse to health and flesh quality of farmed fish. Therefore, it is necessary to optimize the dietary lipid

requirement of *P. hypophthalmus* for the development of a nutrient-balanced and cost-effective diet for commercial farming.

Materials and methods

Experimental fish and feeding

One hundred and fifty juveniles of *P. hypophthalmus* (average wt. 13.54–14.12 g) were distributed randomly in five distinct experimental groups, in triplicate, following a completely randomized design. The setup consisted of 15 plastic rectangular tanks (200-L capacity) covered with perforated lids to prevent the fish from jumping out. Five iso-nitrogenous (30%) purified diets with respectively 3-6-9-12 and 15% of lipid diets were prepared. Equal amounts of sunflower oil and cod liver oil were used as source of lipid. Vitamin-free casein and gelatin were used as protein sources, whereas starch and α-cellulose were used as source of carbohydrate and binder, respectively. Proximate analysis of the diets and carcass tissue were done by standard methods (AOAC 1995) given in Table 1. Fish were fed ad libitum twice a day, and the feeding rate was adjusted accordingly.

Table 1 Formulation, proximate composition and energy content (MJ kg^{-1}) of the experimental diets

Ingredient	Dietary lipid level (%)				
	L_3	L_6	L_9	L_{12}	L_{15}
Casein	29	29	29	29	29
Gelatin	7	7	7	7	7
Starch	25	25	25	25	25
Dextrin	17	17	17	17	17
Cellulose	15.38	12.38	9.38	6.38	3.38
CMC	1.5	1.5	1.5	1.5	1.5
Cod liver oil	1.5	3	4.5	6	7.5
Sun flower oil	1.5	3	4.5	6	7.5
Vitamin-mineral mix	2	2	2	2	2
BHT	0.02	0.02	0.02	0.02	0.02
Betaine	0.1	0.1	0.1	0.1	0.1
Proximate composition (%)					
Moisture	7.23	8.71	8.27	8.26	8.36
Dry matter	92.77	91.29	91.73	91.74	91.63
Ash	2.92	3.05	1.22	3.60	2.30
Protein	35.83	35.21	35.09	35.44	35.37
Ether extract	3.48	6.30	9.94	12.00	15.90
NFE	50.54	46.73	45.48	40.7	38.06
GE (MJ kg^{-1})	19.56	20.94	22.32	23.7	25.08

Growth performance and body indices

The following parameters were calculated based on the formulae mentioned below:

Growth parameters

$$\text{Weight gain } (\%) = (\text{Final weight} - \text{Initial weight}) \Big/ (\text{Initial weight}) \times 100$$

$$\text{Specific growth rate } (\text{SGR}) = (\ln\text{final weight} - \ln\text{initial weight}) \Big/ (\text{Number of days}) \times 100$$

$$\text{Feed conversion ratio } (\text{FCR}) = \text{Total feed given} \Big/ \text{Total weight gain}$$

$$\text{Feed efficiency ratio } (\text{FER}) = \text{Total wet weight gain } (\text{g}) \Big/ \text{Total feed given}$$

$$\text{Protein efficiency ratio } (\text{PER}) = \text{Total weight gain } (\text{g}) \Big/ \text{Total protein intake}$$

$$\text{Survival } (\%) = (\text{Total number of harvested animal}) \Big/ (\text{Total number of stocked}) \times 100$$

Body indices

$$\text{Hepatosomatic index } (\text{HSI}\%) = (\text{Liver weight } (\text{g})) \Big/ (\text{Weight of fish } (\text{g})) \times 100$$

$$\text{Viscerosomatic index } (\text{HSI}\%) = (\text{Viscera weight } (\text{g})) \Big/ (\text{Weight of fish } (\text{g})) \times 100$$

$$\text{Intraperitoneal fat } (\text{IPF}\%) = (\text{Intraperitoneal fat } (\text{g})) \Big/ (\text{Weight of fish } (\text{g})\ ) \times 100$$

Enzyme assays and protein estimation

Each treatment consisted of anaesthetizing six fish using clove oil at 50 ul/l of water. The fish were then dissected and the tissues including, liver, intestine and muscle, were immediately removed. A

5% tissue homogenate was prepared in chilled 0.25 M sucrose solution by Teflon-coated mechanical homogenizer (REMI Equipment, Mumbai, India). The whole procedure was performed in ice-cold conditions. Homogenized samples were centrifuged at 8000 rpm for 10 min at 4 °C. The supernatant was collected in glass vials and stored in deep freezer (−20 °C) for enzyme assay.

Casein digestion method (Drapean 1974) was followed in order to determine protease activity. The enzyme reaction mixtures consist of 1% casein in 0.05 M Tris PO_4 buffer (pH 7.8) and incubated for 5 min at 37 °C. Then, the tissue homogenate was added to the enzyme mixture. After 10 min, the reaction was stopped by adding 10% TCA and the whole content was filtered. Tissue homogenate was added to make the reagent blank just before stopping the reaction without incubation. The amount of enzyme needed to release acid-soluble fragments equivalent to Δ0.001A280 per minute at 37 °C and pH 7.8 was defined as 1 unit of enzyme activity.

The lipase activity was determined by the titrimetric method of Cherry and Crandell (1932), which is based on the measurement of fatty acids released by the enzymatic hydrolysis of triglycerides present in a stabilized emulsion of olive oil. The amount of a standard sodium hydroxide solution used to titrate the fatty acids released was taken as an index of lipase activity of the crude enzyme extract. The assay system consisted of 1.5 ml of stabilized lipase substrate and 1.5 ml of 0.1 M Tris-HCl buffer at PH 8.0, to which 1.0 ml of the crude enzyme extract was added. The assay mixture was incubated for 24 h. at 4 °C, after which the reaction was stopped by the addition of 3 ml 95% ethyl alcohol. The mixture was then titrated with 0.01 N NaOH using 0.9% (*w*/*v*) phenolphthalein as indicator.

The reducing sugars produced from the action of glucoamylase and amylase on carbohydrate was estimated, using the dinitro-salicylic acid (DNS) method (Rick and Stegbauer 1974). The reaction mixtures consisted of 1% (*w*/*v*) starch solution, phosphate buffer (pH 6.9) and tissue homogenate. The reaction mixtures were incubated at 37 °C for 30 min. DNS was added after incubation and kept in a boiling water bath for 5 min. After cooling, the reaction mixture was diluted with distilled water and the absorbance was measured at 540 nm. Maltose was used as the standard. Amylase activity was expressed as moles of maltose released from starch per min at 37 °C temperature.

Quantification of protein of the different tissues was carried out using the Bradford method (Bradford 1976). The Bradford assay relies on the binding of the dye Coomassie blue G250 to protein. Tissue homogenate (20 μl) was taken along with 180 μl distilled water and 250 μl 1 N NaOH added. After that, 5 ml Bradford reagent was added and kept for 5 min. A reading was taken at 595 nm against the blank. Protein content was expressed in mg/g wet tissue.

Analysis of fatty acid profile

Fatty acid profile of the muscle

The extraction of tissue's total lipids was carried out following the method described by Folch et al. (1957) with slight modifications. The extracted lipid was prepared by fatty acid methyl esters (FAME) and analysed in GC-MS for a fatty acid profile.

Extraction of lipids

A 5 g of fresh muscle sample was finely ground with a mixture of 20 ml chloroform and 10 ml methanol using a tissue homogenizer. Then the mixed solution was filtered through Whatman

No. 1 filter paper into a separating funnel. Then 0.85% NaCl was added to this, mixed properly and kept for separation into two layers. The lower chloroform layer with lipid was collected in a pre-weighed flat bottom flask and the solvent was evaporated, using a rotary evaporator. The weight of the flask with lipid was taken, and the weight of lipid was calculated from the difference in the weight of the flask with lipid and without lipid.

Preparation of fatty acid methyl esters

The lipid extract was esterified by following the method of AOAC (1995). Lipid extracted from samples were heated with methanolic NaOH and then with Boron trifluoride-methanol solution for esterification. Amount of 5 ml *n*-heptane was added to recover the methyl esters in the organic phase. Saturated sodium chloride solution was added to the mixture, and a separating funnel was used to separate the aqueous and organic layers. The upper *n*-heptane phase was isolated and stored in glass vials in a refrigerator for further analysis.

Gas chromatography-mass spectrometry

Shimadzu Qp2010 Quadrupole GC-MS instrument equipped with a carbowax (30 m × 0.25 mm ID; 0.25 μm film thickness) capillary column (Cromlab SA) was used for separating fatty acids. Carrier gas (helium) was used; temperature of injector and detector were set at 250 °C. Injection was performed in split mode (1:15). The column temperature was programmed initially at 50 °C for 2 min then to increase at a rate of 10 °C per min to a final temperature of 230 °C. The FAM esters were separated at constant pressure (23.1 kPa), and peaks were identified by comparing the mass spectra with the mass spectral database.

Statistical analysis

The differences among treatments were tested by one-way analysis of variance (ANOVA), and the comparison among the groups was carried out following the method described by Duncans' multiple range test at $P < 0.05$ by SPSS 16.0. Second-order polynomial regression analysis was used to determine the optimum dietary lipid level for maximum growth and nutrient utilization. All data presented in the text, figures and tables were means ± standard error (SE) and the statistical significance was set at $P < 0.05$.

Results and discussion

In the present study, the relationship between fish growth and dietary lipid level was best expressed statistically by a second-order curve (Fig. 1). The maximum of the curve was obtained at 10.10% lipid. Based on the results of higher weight gain, specific growth rate (SGR), protein efficiency ratio (PER) and FCR, the lipid requirement of *P. hypophthalmus* was found to be 9.0% (L_9) in the experimental conditions (Table 2). The weight gain in fish fed with medium dietary lipid levels (6–12%) was significantly better ($p < 0.05$) than the fish reared on higher and lower lipid diets tested in the present experiment. The average body weight of the fish fed with 9.0% lipid was observed to be distinctly higher than rest of the dietary treatments. However, after reaching the maximum at 9.0% lipid diet, the weight gain showed a decrease when fishes were fed at 12 and 15%.

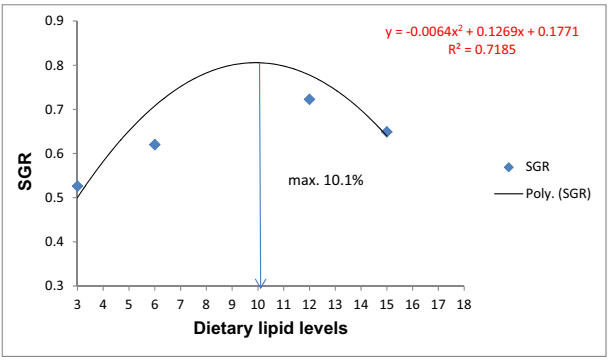

Fig. 1 Second-order regression of SGR on concentrations of dietary lipid indicates that the optimal lipid level for maximal growth of *P. hypophthalmus* is 10.1%

Similar observations of increasing weight gain with increase in dietary lipid up to an optimum requirement level and subsequently decrease with further increase in dietary lipid with similar observations were reported in juvenile grouper (Lin and Shaiu 2003), silver barb (Mohanta et al. 2008), and black catfish (Salhi et al. 2004). However, linear growth progression, with increase of dietary lipid concentration, was reported in various fish species, striped bass (Gaylord and Gatlin 2000) and Asian sea bass (Williams et al. 2003). In contrast, the linear decrease of growth rate with rise in dietary lipid was observed in threatened freshwater catfish (Raj et al. 2007) and Australian short-fin eel (De Silva et al. 2001).

Excess lipid not only suppresses de novo fatty acid synthesis but also reduces the ability of fish to digest and assimilate it, leading to a reduced growth rate (Sargent et al. 1989). The reduction in growth of fish at higher levels of dietary lipid as observed in this study (Table 2) may be attributed to reduced lipid assimilation or an imbalance in the protein/fat ratio. Berge and Storebakken (1991), De Silva et al. (1991) and Jafri et al. (1995) also reported no growth improvement in Atlantic halibut, red tilapia and mrigal respectively beyond a particular optimum protein/fat ratio in their diets. Addition of extra lipid, to conserve protein, appears to be of limited use beyond the 10.1% dietary lipid inclusion for *P. hypophthalmus.*

The dietary lipid requirement of 10.1% in the present study is similar to 8–10% of red bream (Marais and Kissils 1979), 9.0% of channel catfish (Winfree and Stickney 1984), 9.0% of catfish (Anwar and Jafri 1992), 7–8% of Indian major carps (Murthy 2002), 9.0% of juvenile grouper (Lin and Shaiu 2003 and 9.8% of juvenile marbled spinefoot fish (Ghanawi et al. 2011).

Table 2 Growth parameters of different treatment groups at the end of the experiment

Parameters	L_3	L_6	L_9	L_{12}	L_{15}
% wt. gain	$37.13^{d} \pm 0.89$	$45.08^{c} \pm 1.06$	$72.38^{a} \pm 1.10$	$54.30^{b} \pm 1.20$	$47.66^{c} \pm 2.47$
SGR	$0.52^{d} \pm 0.010$	$0.62^{c} \pm 0.012$	$0.90^{a} \pm 0.001$	$0.72^{b} \pm 0.012$	$0.64^{c} \pm 0.027$
FCR	$3.53^{a} \pm 0.08$	$2.96^{b} \pm 0.06$	$1.95^{d} \pm 0.02$	$2.51^{c} \pm 0.04$	$2.83^{b} \pm 0.12$
FER	$0.28^{d} \pm 0.006$	$0.33^{c} \pm 0.007$	$0.51^{a} \pm 0.006$	$0.39^{b} \pm 0.007$	$0.35^{c} \pm 0.016$
PER	$0.94^{d} \pm 0.02$	$1.12^{c} \pm 0.023$	$1.70^{a} \pm 0.02$	$1.32^{b} \pm 0.026$	$1.18^{c} \pm 0.054$

Mean values in the same row with different superscript differ significantly ($P < 0.05$), *SGR* specific growth rate, *FCR* feed conversion ratio, *PER* protein efficiency ratio, *FER* feed efficiency ratio. Data expressed as mean ± SE, $n = 3$

The FCR was significantly better in the diet consisting 9.0% lipid levels compared to the other 3.0, 6.0, 12.0 and 15.0 dietary lipid levels (Table 2). Similar to the present observation, the best FCR was observed in the medium range of 9.0% dietary lipid supplementation in juvenile grouper (Lin and Shaiu 2003). Satpathy et al. (2003) and Salhi et al. (2004) reported that an increase in dietary lipid improves the FCR for various fish species, including Indian major carps and common carp.

The SGR was significantly higher ($p < 0.05$) at a 9.0% dietary lipid level (Table 2) than in the other diets. The present results of increasing SGR up to 9.0% dietary lipid level are similar to several species as reported by Anwar and Jafri (1992, 1995) in catfish. El-Sayed and Garling Jr. (1988) reported that the SGR was found to be significantly higher at high dietary lipid levels (4.20–14.80%) than the lowest level of inclusion (1.70%) in the diet of *Tilapia zilli.* However, no variation in the SGR was reported with increase in dietary lipid in Nile tilapia (Hanely 1991), gilthead seabream (Santinha et al. 1999) and Atlantic salmon (Hemre and Sandnes 1999). Hemre and Sandnes (1999) reported significantly higher SGR during the spring season in Atlantic salmon at a higher level of dietary lipid incorporation (38.0–47.0%). The SGR improved with the increasing of dietary lipid as reported for red tilapia (De Silva et al. 1991), Japanese seabass (Ai et al. 2004) and black catfish (Salhi et al. 2004).

The PER in the present study increased linearly up to an optimum level of 9.0% in *P. hypophthalmus* and, subsequently, reduced with the further increase of dietary lipid levels (Table 2). The finding was similar to those of Jafri et al. (1995) for carp, Anwar and Jafri (1995) in air-breathing catfish. However, Satpathy et al. (2003) reported a significant increase in PER with an increase in dietary lipid (5.0–15.0%) when fed to rohu. Similar results were also obtained in striped bass (Millikin 1983) and Atlantic salmon (Hemre and Sandnes 1999). Lin and Shaiu (2003) reported no significant difference in PER with the feeding of 4.0–12.0% lipid in the diets to grouper. The lowest PER was recorded at the highest dietary lipid level of 16.0%.

The hepatosomatic index (HSI), viscerosomatic index (VSI) and intraperitoneal fat (IPF) increased more with feeding high lipid diets L9 and L15 than low lipid diets (3.0 and 6.0%) in the fish (Table 3). The increase in HSI values was directly proportional to the dietary lipids fed to fish. The finding on such linear increase is similar to the observation found in Pacific bluefin tuna juvenile (Biswas et al. 2009). A significant difference in HSI was found with the increase in dietary lipid level in striped bass (Millikin 1983), Atlantic salmon (Hemre and Sandnes 1999) and gilthead seabream (Santinha et al. 1999). Although there was a significant difference found in HSI with different dietary lipid levels in short-fin eel, the trend was not definite with regards to dietary lipid content (De Silva et al. 2001). The HSI was observed to be significantly lower in higher levels of dietary lipid than the lower level in growing European seabass (Ballestrazzi and Lanari 1996).

Table 3 Body indices (HSI, VSI, IPF) of different treatment groups at the end of the experiment

Treatment	HSI	VSI	IPF
L_3	$1.91^{c} \pm 0.01$	$7.21^{d} \pm 0.026$	$1.72^{d} \pm 0.029$
L_6	$2.03^{d} \pm 0.02$	$7.30^{c} \pm 0.014$	$2.04^{c} \pm 0.017$
L_9	$2.20^{c} \pm 0.01$	$7.46^{b} \pm 0.21$	$2.17^{b} \pm 0.13$
L_{12}	$2.27^{b} \pm 0.01$	$7.44^{b} \pm 0.017$	$2.22^{b} \pm 0.06$
L_{15}	$2.38^{a} \pm 0.02$	$7.51^{a} \pm 0.012$	$2.53^{a} \pm 0.01$

Data expressed as mean ± SE $n = 3$; mean values in the same column with *different superscripts* differ significantly ($P < 0.05$); *HSI* hepatosomatic index, *VSI* viscerosomatic index, *IPF* intraperitoneal fat

Table 4 Amylase, protease (U/mg protein) activity of different experimental groups fed different diet at end of the experiment

Treatment	Amylase Intestine	Protease	Lipase
L_3	$3.39^{b} \pm 0.25$	$16.09^{bc} \pm 0.29$	$43.21^{c} \pm 0.37$
L_6	$3.74^{ab} \pm 0.19$	$17.64^{b} \pm 0.95$	$54.87^{b} \pm 1.81$
L_9	$4.10^{ab} \pm 0.26$	$24.64^{a} \pm 1.16$	$78.07^{a} \pm 0.44$
L_{12}	$2.61^{b} \pm 0.40$	$14.81^{d} \pm 0.76$	$50.39^{b} \pm 2.59$
L_{15}	$2.80^{b} \pm 0.57$	$15.95^{bc} \pm 0.67$	$33.42^{d} \pm 1.15$

Activities are expressed as follows: protease as micromol of tyrosine released/min/mg protein; amylase as micromol of maltose released/min/mg protein; lipase as units/mg protein. *Different superscripts* in the same column signify statistical differences ($P < 0.05$) mean ± SE $n = 3$

Digestive enzyme activities and the utilization of nutrients are the most important factors for optimizing the feeding procedures, and the growth of fish is dependent on the digestive capacity. The type, source and amount of nutrients can alter the enzyme activity of digestive tract in fish (Debnath et al. 2007). Activity of the enzyme protease increased with increasing dietary lipid concentrations and the maximum value was achieved at 9.0% lipid level, beyond which it decreased (Table 4, Fig. 2). Similar results were observed by Mohanta et al. 2008, where the maximum protease activity was at the dietary level of 8.0% rather than the higher (12.0%) and lower (4.0%) lipid levels when the protein level in all the diets were maintained at 30.0% for silver barb. Gangadhara et al. (1997) also reported the maximum protease activity at the medium dietary lipid level of 6.0% rather than the higher (9.0%) and lower (4.0%) lipid levels when the protein level in all the diets were maintained at 30.0% for rohu. They also found that hepatopancreatic protease activity decreased with an increase in dietary lipid for the same species. Bazaz and Keshavanath (1993) observed that the highest level of dietary sardine oil inclusion (12.0%) increased the protease activity in mahseer, suggesting a higher protein turn over and active mobilization, which showed the protein-sparing of oil. An adaptive change in the activity of proteolytic enzymes in relation to the type of diet has been reported by Kawai and Ikeda (1972) and Scherbina et al. (1976) in carp. These amply justify the

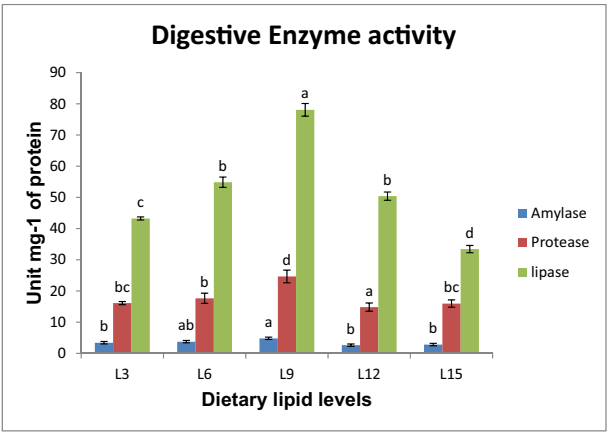

Fig. 2 Digestive enzyme activity in the intestine of different experimental groups at the end of the experiment

Table 5 Whole body proximate compositions of different experimental groups (% wet weight basis ± SE) at the end of experiment

Treatment	Moisture	Organic matter	Crude protein	Ether extract	Total ash
L_3	$74.31^{a} \pm 0.52$	$25.68^{b} \pm 0.52$	$11.44^{c} \pm 0.45$	$2.72^{e} \pm 0.25$	$8.96^{a} \pm 0.23$
L_6	$73.48^{a} \pm 0.42$	$26.51^{b} \pm 0.42$	$13.63^{b} \pm 0.11$	$4.74^{d} \pm 0.21$	$8.72^{a} \pm 0.22$
L_9	$72.91^{a} \pm 0.39$	$27.08^{b} \pm 0.39$	$14.44^{a} \pm 0.27$	$5.22^{c} \pm 0.91$	$7.36^{b} \pm 0.10$
L_{12}	$72.52^{a} \pm 0.46$	$27.47^{b} \pm 0.46$	$13.48^{b} \pm 0.14$	$6.47^{b} \pm 0.30$	$8.27^{a} \pm 0.16$
L_{15}	$70.39^{b} \pm 0.87$	$29.61^{a} \pm 0.87$	$13^{b} \pm 0.05$	$8.73^{a} \pm 0.06$	$8.58^{a} \pm 0.31$

Data expressed as mean ± SE, $n = 3$. Mean values in the same column with *different superscripts* differ significantly ($P < 0.05$)

maximum protease activity of 9.0% lipid group in correlation with fast growth of fish in the present study.

Lipase activity was found to be significantly higher at 9.0% lipid level in the diet (Table 4, Fig. 2). Mohanta et al. (2008) also reported higher intestinal lipase activity at 8.0%, while increasing dietary lipid from 4.0–12% for silver barb. Bazaz and Keshavanath (1993) found higher intestinal activity at 6.0% dietary inclusion of sardine oil rather than the higher (12.0%) and lower (3.0%) inclusion of the sardine oil for the mahseer. Gangadhara et al. (1997) also reported the increase in lipase activity, with increase in dietary lipid for rohu.

Because of the constant level of dietary carbohydrate in the present study, there was no significant ($p > 0.05$) increase in the amylase activity among different diets. This supports the results obtained by Mohanta et al. (2008) who also observed no significant difference in amylase activity, with a constant level of dietary carbohydrate for silver barb.

There was a significant ($p < 0.05$) increase in the carcass protein level among the different treatment groups, which reached its maximum at the medium dietary lipid concentration of 9.0% then decreased beyond this level (Table 5). Similar observations were found in air-breathing catfish (Anwar and Jafri 1992). Linear decrease in carcass protein with increased dietary lipid was reported in silver barb (Mohanta et al. 2008), which differs from the finding in tilapia by Hanely (1991), where there was no change in carcass protein with increase in dietary lipid fed to the fish.

Body lipid increased with increase in dietary lipid levels (Table 5). The result obtained is similar to that obtained in other fish species, such as silver barb (Mohanta et al. 2008), channel catfish (Garling and Wilson 1977) and Japanese seabass (Ai et al. 2004). The positive correlation between dietary and body lipid may indicate that when dietary lipid is supplied in excess, a proportion of the lipid is being deposited as body fat, which is in accordance to the earlier reports in other fishes (Mohanta et al. 2008).

Moisture content decreased with an increase in dietary lipid fed to fish (Table 5) as observed in channel catfish (Garling and Wilson 1976), common carp (Zeitler et al. 1984), mrigal (Jafri et al. 1995), catfish (Anwar and Jafri 1992), rohu (Satpathy et al. 2003) and silver barb (Mohanta et al. 2008). The present findings and earlier observations, as reported above, differ from the report of Hanely (1991), who did not observe any change in moisture content in tilapia when fish were fed with diets containing increased dietary lipids.

The ash content appeared to decrease until the optimum dietary lipid was at the level of 9.0% then increased with a further dietary lipid increase in the present experiment (Table 5). This was reported also by Anwar and Jafri (1992) in an air-breathing catfish. However, a linear decrease in carcass with increase in dietary lipid was reported in several other species (Zeitler et al. 1984; Jafri et al. 1995).

Table 6 Fatty acid profile of *Pangasianodon hypophthalmus*, flesh of the different experimental groups at the end of the experiment

Fatty acid	Initial	L_3	L_6	L_9	L_{12}	L_{15}
C12:0	5.37	5.12	3.42	5.37	5.05	3.83
C13:0	ND	11	11.12	2.86	ND	11.29
C14:0	11.09	ND	ND	ND	10.45	ND
C15:0	1.79	2.28	2.27	9.65	2.04	2.32
C16:0	8.99	6.71	17.7	2.32	6.69	8.29
C16:1 (n-7)	ND	ND	ND	8.34	5.71	8.49
C17:0	3.47	3.59	3.36	3.57	3.44	3.54
C18:0	ND	ND	12.39	ND	23.87	ND
C18:1 (n-9)	3.71	19	18.43	12.05	ND	2.67
C18:1 (n-7)	ND	ND	ND	9.69	11.48	11.97
C18:2 (n-6)	19.44	13.47	18.33	18.02	18.3	18.57
C18:3 (n-3)	ND	7.97	9.13	13.26	19.33	11.86
C20:1 (n-9)	8.07	8.33	9.69	7.72	7.67	ND
C20:2 (n-7)	8.38	ND	ND	3.81	3.94	8.21
C20:3 (n-7)	4.56	6.07	3.23	2.61	2.99	ND
C20:4 (n-6)	12.36	11.02	10.33	11.89	8.29	11.27
C20:5 (n-3)	11.74	6.45	10.41	11.75	13.1	10.47
C22:0	ND	ND	ND	11.66	ND	ND
C22:1 (n-7)	ND	ND	7.31	6.57	ND	5.63
C22:1 (n-9)	ND	7.37	ND	ND	6.78	ND
C22:6 (n-3)	21.38	9.64	16.26	20.61	20.54	11.67
Saturated	30.71	28.7	37.87	20.2	24.23	25.73
MUFA	11.78	34.7	33.05	44.37	31.64	28.76
n-3	33.12	24.06	35.8	45.62	52.97	51.09
n-6	31.8	24.49	28.66	29.91	26.59	29.84
n-3/n-6	1.041	0.982	1.249	1.525	1.992	1.712

MUFA monounsaturated fatty acid, *ND* not detected, $n = 3$

The FA profile of tissues is determined mainly by their dietary lipid levels, and freshwater fishes have a higher capacity than marine fishes for elongation and desaturation of fatty acids (Sargent et al. 1999, 2002; Bell and Sargent 2003). According to Mourente and Tocher (1993), freshwater fishes generally have a better capacity to desaturate and elongate 18:1 n-9, 18:2 n-6 and 18:3 n-3 fatty acids than marine fish. In the present study, ARA, EPA and DHA increased with increasing dietary lipid levels (3.0–9.0%) and then decreased with increasing dietary lipid levels beyond 12.0% (Table 6). From these findings, it could be inferred that diets containing the optimum lipid level induce greater deposition of essential fatty acids in fish muscle, which is in agreement with the results of other fish species, such as juvenile white seabass (Lopez et al. 2006) and large yellow croaker larvae (Ai et al. 2004). It has been established that the fatty acids composition of tissue lipids of fish are readily influenced by the dietary lipid levels (Cahu et al. 2000 and Gawlicka et al. 2002). However, further research will be needed to determine the dietary fatty acid requirements of Asian catfish.

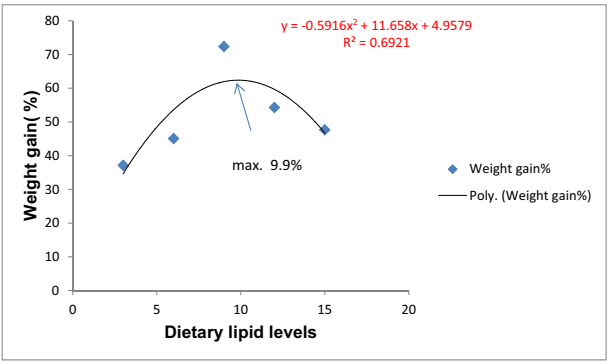

Fig. 3 Second-order regression of weight gain % on concentrations of dietary lipid indicates that the optimal lipid level for maximal growth of juvenile *P. hypophthalmus* is 9.9%

In conclusion, the optimal dietary lipid level for Asian catfish, *P. hypophthalmus* juvenile, was estimated to be 9.9 and 10.1%, based on percent weight gain and SGR used on second-order polynomial regression (Figs. 1 and 3).

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