Acta Ichthyologica et Piscatoria 51(3), 2021, 289–298 | DOI 10.3897/aiep.51.67079

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ACTA ICHTHYOLOGICA ET PISCATORIA

Effect of dietary lipid levels on growth, body composition, and enzyme activities of larvae of butter catfish, *Ompok bimaculatus* (Actinopterygii: Siluriformes: Siluridae)

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Academic editor: Jolanta Kiełpińska ♦ **Received** 14 October 2020 ♦ **Accepted** 6 March 2021 ♦ **Published** 13 September 2021

Citation: Paul BN, Chowdhury D, Das A, Mandal RN, Singh P, Adhikari S, Chakrabarti PP, Giri SS, Ghosh K (2021) Effect of dietary lipid levels on growth, body composition, and enzyme activities of larvae of butter catfish, *Ompok bimaculatus* (Actinopterygii: Siluriformes: Siluridae). Acta Ichthyologica et Piscatoria 51(3): 289–298. https://doi.org/10.3897/aiep.51.67079

Abstract

The Indian butter catfish, *Ompok bimaculatus* (Bloch, 1794), is a high-value catfish that has gained immense consumer preference in South-East Asia. However, information on the nutritional requirements of this species is scanty. Hence, an experiment was conducted to evaluate the effects of varying dietary lipid levels on growth, body composition, and activities of digestive and metabolic enzymes in larvae. Three isonitrogenous (40% crude protein) diets were formulated by supplementing fish and vegetable oil (1:1) at 4.5% (D1), 7% (D2), and 9.5% (D3) levels (containing crude lipid 5.7%, 8.0%, and 10.45%, respectively in diets D1–D3) to a fish meal- and oilcake-based formulated diet. Experimental diets were fed to butter catfish larvae (0.15 ± 0.01 g) in triplicate groups for a period of 42 days. Proximate compositions of the experimental diets, as well as fish carcass, were analyzed using standard procedures (AOAC 2005). Digestive and metabolic enzyme activities were analyzed at the completion of the experiment by standard methodology. Butter catfish larvae fed the diet D2 (8% crude lipid) resulted in the best performance in terms of weight gain (final weight 1.40 ± 0.07 g), net weight gain (1.31 ± 0.06 g), specific growth rate (5.50 ± 0.05% · day^{-1}), and protein efficiency ratio (2.39 ± 0.17). The highest lipid deposition (2.90 ± 0.12%) in the carcass was also recorded in fish reared on diet D2. The final weight, net weight gain, protein efficiency ratio, and specific growth rate were significantly ($P < 0.05$) higher in D2 having 8% lipid. Moisture and lipid contents of the whole body were significantly ($P < 0.05$) higher in larvae fed diet D2. Amylase activity in fish significantly ($P < 0.05$) decreased with increasing dietary lipid levels. The maximum alkaline protease, pepsin, and lipase activities were noticed in the larvae fed diet D2. Progressive decrease in liver glucose-6-phosphate dehydrogenase activities and significant increase ($P < 0.05$) in the activities of neoglucogenic enzymes (glucose-6-phosphatase and fructose-1,6-bis phosphatase) were noticed with an increase in dietary lipid levels. Significantly lower ($P < 0.05$) activities of LDH, ALT, and AST were recorded in the group fed diet D2. Results of the study indicated that 8% crude lipid in the diet could assure optimum growth and survival of butter catfish larvae during early development. An appraisal on growth, body composition, and digestive as well as metabolic function in the butter catfish larvae recorded in the study might provide some important information to consider application of formulated diets for the larviculture of *Ompok bimaculatus*.

Keywords

lipid, larvae, *Ompok bimaculatus*, growth, lipase, metabolic enzyme

Introduction

The Indian butter catfish, *Ompok bimaculatus* (Bloch, 1794), is indigenous to the South East Asian countries (Giri et al. 2019) and has recently gained immense importance because of its good taste, high lipoprotein, low fat, soft bony structure, and competitive prices (Rawat et al. 2018). It is an excellent source of ω-3 and ω-6 fatty acids, vitamins, minerals, protein, and fat (Paul et al. 2018). The wild population of *O. bimaculatus* has sharply declined due to ecological changes and indiscriminate fishing. Thus, the species has been categorized under the "near threatened" category by the IUCN Red List and faces a risk of extinction in nature (Lakra et al. 2010; IUCN 2014). Considering high demand, price, and IUCN status, the species has been prioritized for diversification of aquaculture as well as for conservation and restocking programs (Debnath et al. 2016). Although its aquaculture potential has been realized, the species has not yet received adequate attention due to insufficient information on larval rearing and culture technology.

The successful culture of any fish species largely depends on the accessibility of nutritionally balanced practical diets. Although species of the genus *Ompok* have been generally recognized as carnivorous to omnivorous, reports on nutritional requirements of the species are scanty (Chakrabarti et al. 2012). Therefore, no commercially formulated diet has yet been available for this species. Since captive breeding of *O. bimaculatus* has been established (Raizada et al. 2013), it is necessary to develop larval diets to ensure growth and survivability of the species during the stages of early development, which is essential for reliable and regular supply of the fish for widespread commercial production. A previous study conducted on *O. bimaculatus* larvae determined a required level of 40% crude protein in the diets for this species (Paul et al. 2020).

However, dietary protein requirements are known to be affected by the amount of non-protein energy sources in the diet (NRC 2011). When non-protein energy is insufficient, a part of dietary protein may be catabolized to supply energy affecting the growth of the organism. Therefore, supplementation of energy-yielding nutrients, mainly lipid has been suggested as a strategy to improve protein utilization in fish (Sankian et al. 2017). Supplementation of lipid rather than carbohydrate as a source of non-protein energy is generally more effective for enhancing dietary energy level as lipid is an energy-dense nutrient that is readily metabolized by fish, particularly the carnivorous one (NRC 1993). Further, all-round development and well being of fish are known to be greatly influenced by dietary lipids that are not only important as an energy source but also for the supply of essential fatty acids as well as carrier of fat-soluble vitamins (Glencross 2009). Moreover, the incorporation of a proper amount of lipid seems to be important as lipid level determines the palatability of the diet (Boonyaratpalin 1991). Therefore, the presently reported study was conducted to determine the effects of dietary lipid levels on the growth, survivability, body composition, and activities of digestive as well as metabolic enzymes in butter catfish larvae.

Vegetable and fish oils are rich in different fatty acids, which were recognized as effective for diverse freshwater fish species (Paul et al. 2011). Hence, in the presently reported study, a practical diet was fortified with a combination of vegetable and fish oils (1:1) to have the desired lipid levels in the diets. The nutrient utilization and digestive physiology in fish are indicated by the activity of digestive and metabolic enzymes that ultimately affect the growth and development of fish (Chen and Zhang 2004; Wei et al. 2010). Therefore, the presently reported study considered an appraisal of digestive enzymes and some key metabolic enzymes to evaluate the effects of formulated diets with varying lipid levels. The results of the study could be helpful to provide some important information for feed formulation of *O. bimaculatus* larvae.

Materials and methods

Experimental diets

Three experimental diets were formulated by incorporating equal proportions of fish oil (cod liver oil) and vegetable oil (sunflower oil) (1:1) at 4.5% (D1), 7% (D2), and 9.5% (D3) levels to a basal mixture of fish meal (**FM**), soybean meal (**SBM**), and groundnut oil cake (**GNOC**). After analysis of lipid content of the feed, it was noticed to contain 5.7%, 8.0%, and 10.5% crude lipids, respectively. The amount of lipid sources used was adjusted at the expense of wheat flour. A vitamin–mineral premix was added to the diets as per Paul et al. (1997). Dietary ingredients were finely powdered, sieved to obtain uniform particle size (<400 μm in diameter), mixed thoroughly, and fortified with a calculated amount of vitamin-mineral premix and oil sources. The prepared powered feeds were stored in a freezer at –20°C until use.

Experimental fish and feeding trial

The experiment was conducted at the Regional Research Centre of ICAR-Central Institute of Freshwater Aquaculture, Rahara, Kolkata. Farm-raised larvae of the butter catfish were collected from ICAR-Central Institute of Freshwater Aquaculture, Kalyani Field Station, and acclimatized to the laboratory condition for one week in fiber-reinforced plastic (**FRP**) tanks with the provision of continuous aeration. During this period the larvae were fed a basal formulated diet and natural food (mixed zooplankton and chopped tubifex). After acclimatization, the larvae (mean weight 0.15 ± 0.02 g; length 22.65 ± 1.70 mm; 14 days old) were randomly distributed in 9 FRP tanks at a stocking density of 50 fish per tank. Thus, there were three replicates for each dietary group. The experiment was conducted in 150 L FRP tanks, each

containing 50 L of water, with continuous aeration and water exchange at every 5 days interval. The powdered feed mixtures were made to soft dough with distilled water and the fish were fed ad libitum to apparent satiation twice daily, at 10.00 and 16.00 h, for 42 days. Feed consumption and mortality in each tank were recorded separately, and the survival rate was calculated. During the experimental period, water quality parameters were monitored on weekly basis following the standard methods of the American Public Health Association (APHA 2005) and noticed to vary within the acceptable range (temperature 28–30°C; pH 6.8–7.7, dissolved oxygen 6.8–7.4 mg · L^{-1}, total alkalinity 230–240 mg · L^{-1}, ammonia 0.26–0.64 mg · L^{-1}, nitrite 0.001–0.003 mg · L^{-1}, nitrate 0.002–0.074 mg · L^{-1}).

Proximate composition of experimental diets and fish carcass

Proximate compositions of the experimental diets, as well as fish carcass, were analyzed using standard procedures portrayed by the Association of Official Analytical Chemists (AOAC 2005). Moisture content was determined by oven drying (initially at 100 ± 5°C for 30 min, thereafter at 60°C); crude protein (Nitrogen × 6.25), by a semi-automatic digestion system together with micro Kjeldahl distillation Unit (KelPlus-Elite Ex, Pelican Equipments, Chennai, India); crude lipid (ether extract; petroleum ether, 60–80°C), by a Soxhlet apparatus (Socsplus, Pelican Equipments, Chennai, India); and ash, by combustion at 550°C in a muffle furnace. Nitrogen-free extract (**NFE**) was calculated by subtracting the sum of values for crude protein, crude lipid, ash, crude fiber, and moisture from 100 (Maynard et al. 1979). The gross energy of the diets was measured with a bomb calorimeter (Lab-X, Kolkata, India). Proximate analyses of the fish carcass (whole body) were done on wet weight basis.

Growth parameters

At the end of the feeding trial fish were collected from each tank, weighed, and analyzed for calculating the growth parameters. Net weight gain [%], specific growth rate (**SGR** [% · day^{-1}]), feed conversion ratio (**FCR**), protein efficiency ratio (**PER**), apparent net protein utilization (**ANPU**), and survivability [%] were calculated following standard methods described by Castell and Tiews (1980). The daily growth coefficient (**DGC**) was calculated as per Cowey (1992).

Estimation of digestive enzymes

Digestive enzymes (amylase, alkaline protease, pepsin, and lipase) of fish from each experimental set were estimated at the termination of the experiment. For each replicate, digestive tracts of 20 experimental fish from each tank were dissected out, washed thoroughly with chilled distilled water, taken on an ice-cooled Petri plate, and weighed. A 10% homogenate with chilled 0.1 (M) phosphate buffer (pH 7) was prepared and centrifuged at 10 000 rpm (10 min, 4°C). The ensuing supernatant was used as the enzyme extract to appraise the activities of the digestive enzymes. The protein content of the extract was estimated after Lowry et al. (1951) using bovine serum albumin (**BSA**) as standard. Amylase (α-amylase) activity was determined using dinitro salicylic acid (**DNSA**) reagent following Bernfeld (1955). Amylase activity (unit) was expressed as mg maltose liberated mg^{-1} protein h^{-1}. Alkaline protease activity was estimated using Hammerstein casein substrate according to Walter (1984). One unit of enzyme activity was defined as µg of tyrosine liberated mg^{-1} protein h^{-1}. Pepsin activity was resolved after Anson (1938) with minor modifications, using 2% hemoglobin as a substrate. The specific activity was expressed as µg of tyrosine liberated mg^{-1} protein min^{-1}. Lipase activity was determined with the olive oil substrate following Bier (1955). Lipase activity was expressed as µ mole of fatty acid liberated mg^{-1} protein h^{-1}.

Estimation of metabolic enzymes

Following the collection of the digestive tracts, hepatic tissues were removed, collected separately and a 10% homogenate was made in sucrose solution (0.25 M, pH 7.4). Remains of the cell along with nuclei were removed by centrifugation (1000 g, 30 min, 4°C), and the supernatants were further centrifuged (10 000 g, 15 min, 4°C) to get the mitochondrial pellets (Biswas et al. 2006). The resultant supernatant was again centrifuged (12 500 g, 1 h, 4°C) and the cytosolic fraction thus obtained was used as the crude enzyme extract for other metabolic enzyme assays. The mitochondrial pellet was treated with triton X-100 (0.1%), washed with PBS (0.1 M, pH 7.4) and the supernatant was used as crude extracts for mitochondrial metabolic enzyme assays. The tissue fractions were kept at –20°C until use. The soluble protein content of the crude enzyme extracts was determined following Lowry et al. (1951).

Hexokinase (HK) activity was measured by the reduction of NADP to produce NADPH according to Tranulis et al. (1996). Enzyme activity was expressed as µM of NADPH formed mg^{-1} protein h^{-1}. Pyruvate kinase (PK) activity was assayed after Driedzic and Almeida-Val (1996) with minor modification. Enzyme activity was presented as µmole of pyruvate converted to NADH mg^{-1} protein min^{-1}. Glucose-6-phosphatase (G6P) and fructose-1, 6-bis phosphatase (FBP) activities were measured by estimating the amount of phosphorus released from the substrates, glucose-6-phosphate (Marjorie 1964) and fructose-di-phosphate (Freeland and Harper 1959), respectively. Release of phosphorus by both the enzymes was estimated after Fiske and Subbarow (1925), and activities were expressed as µg of phosphorus released mg^{-1} protein min^{-1}.

Glucose-6-phosphate dehydrogenase (G6PD) activity was analyzed using glucose-6-phosphate (substrate) and NADP following Kornberg and Horecker (1955). Enzyme activity was expressed as µM of NADPH formed mg^{-1} protein h^{-1}. NADP-malic enzyme (NADP-ME) activity was determined using L-malic acid as substrate (Hsu and Lardy 1967, modified by Murphy and Walker 1974). Enzyme activity was presented as µM of NADPH formed mg^{-1} protein h^{-1}. Lipid peroxidation (LPO) activity was measured according to Okhawa et al. (1979). Enzyme activity was expressed as thiobarbituric acid reactive substance (TBARS) formed mg^{-1} protein min^{-1}.

Alanine transaminase (ALT) activity was determined using α-ketoglutarate and DL-Alanine as substrates (Reitman and Frankel 1957). ALT activity was expressed as µM of pyruvate formed mg^{-1} protein min^{-1}. Likewise, Aspartate transaminase (AST) activity was measured with the substrate solution containing α-ketoglutarate and DL-aspartic acid (Reitman and Frankel 1957). AST activity was expressed as µM of oxaloacetate formed mg^{-1} protein min^{-1}. Glutamate dehydrogenase (GDH) activity of the crude mitochondrial enzyme extract was measured using sodium glutamate and tetrazolium salt (Lee and Lardy 1965). Enzyme activity was expressed as µM of formazan formed mg^{-1} protein h^{-1}.

Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) as per Snedecor and Cochran (1994) to calculate the effect of dietary lipid level on growth performance and activities of the digestive as well as metabolic enzyme of fish and the least significance (LSD) was used for comparison of the mean values. Data are presented as treatment mean ± standard error of the mean (SE).

Results

The ingredients and proximate composition of the experimental diets are presented in Table 1. Experimental diets were isoproteinous (crude protein ≈ 40%). However, supplementation of fish oil and vegetable oil has led to varying crude lipid levels in the diets (D1–D3) as 5.7%, 8.0%, and 10.45%, respectively. All experimental diets were readily accepted by the *O. bimaculatus* larvae.

The growth performance of *O. bimaculatus* larvae fed varying levels of dietary lipid for 42 days is depicted in Table 2. The growth of the larvae was significantly ($P < 0.05$) affected by the dietary crude lipid levels. The net weight gain (%) of the larvae showed an increasing trend with increasing levels of the dietary lipid up to 8% and thereafter decreased. Butter catfish larvae fed diet D2 containing 8% crude lipid had the highest weight gain, which was significantly different ($P < 0.05$) from other dietary lipid levels. The highest values of PER and ANPU were recorded in fish fed diet D2. The value of FCR was the lowest for fish fed diet D2, however, didn't differ significantly between the diets D1 and D3. Butter catfish larvae in all treatment groups survived well (more than 80%) during the experimental period, and there were no significant differences among the groups.

Proximate carcass compositions of the butter catfish larvae fed experimental diets are presented in Table 3.

Table 1. Feed formulation and proximate composition (% DM Basis) of the experimental diets.

Parameter	Experimental diet		
	D1	D2	D3
Fish meal	53.00	53.0	53.00
Groundnut oil cake	15.00	15.0	15.00
Soybean meal	10.00	10.0	10.00
Wheat flour	10.50	8.0	5.50
Carboxy methyl cellulose	2.00	2.0	2.00
Fish:Veg. oil (1:1)	4.50	7.0	9.50
Vitamin-mineral mix*	5.00	5.0	5.00
Proximate composition [% DM basis]			
Dry matter	92.85 ± 0.06	92.37 ± 0.23	92.06 ± 0.05
Crude protein	40.46 ± 0.06	40.18 ± 0.49	40.61 ± 0.83
Crude lipid	5.70 ± 0.20	8.00 ± 0.25	10.45 ± 0.45
Total Ash	14.40 ± 0.30	15.40 ± 0.20	16.50 ± 0.30
Nitrogen free extracts	29.50 ± 0.37	27.61 ± 2.05	21.80 ± 0.28
Crude protein:crude fat	7:1	5:1	4:1
Energy [kJ g^{-1}]	13.85 ± 0.02	14.09 ± 0.08	14.39 ± 0.08

*Vitamin-mineral premix contains: Vitamin A (as acetate) 5000 I.U., cholecalciferol 1000 I.U., thiamine monocitrate 10 mg, riboflavin 10 mg, pyridoxine hydrochloride 5 mg, cyanocobalamine 15 µg, nicotinamide 75 mg, calcium pantothenate 10 mg, ascorbic acid 150 mg, α-tocopheryl acetate 25 mg, biotin 5 mg, folic acid 5 mg, menadione 100 mg, choline chloride 50 mg, PABA 5 mg, myoinositol 10 mg, calcium lactate 0.125, magnesium oxide 60 mg, dried ferrous sulphate 30 mg, manganese sulphate 2 mg, copper sulphate 2 mg, zinc sulphate 2 mg, sodium molybdate 0.25 mg, sodium bortate 0.80 mg, potassium iodate 20 mg, bicalcium phosphate 0.10 g, cobalt chloride 20 mg (Paul et al. 1997).

Table 2. Growth performance in *Ompok bimaculatus* larvae fed with graded levels of lipid.

Parameter	Experimental diet		
	D1	D2	D3
Initial weight [g]	0.15 ± 0.02	0.14 ± 0.01	0.15 ± 0.01
Final weight [g]	1.10 ± 0.12^{a}	1.40 ± 0.07^{b}	1.06 ± 0.03^{a}
Net weight gain	0.95 ± 0.12^{a}	1.31 ± 0.06^{b}	0.91 ± 0.03^{a}
Specific growth rate [%]	4.73 ± 0.35^{a}	5.50 ± 0.05^{b}	4.66 ± 0.22^{a}
Daily growth coefficient	0.73 ± 0.02^{a}	1.003 ± 0.05^{b}	0.76 ± 0.09^{a}
Survivability	83.85 ± 6.15	83.85 ± 6.15	79.55 ± 5.46
Number of dead fish	25	25	31
Feed conversion ratio	1.86 ± 0.10^{b}	1.39 ± 0.05^{a}	1.74 ± 0.07^{b}
Protein efficiency ratio	1.31 ± 0.09^{a}	2.39 ± 0.17^{b}	1.30 ± 0.08^{a}
Apparent net protein utilization	16.09 ± 0.92^{a}	23.19 ± 1.10^{b}	17.18 ± 0.82^{a}

Data are presented as mean ± standard error of the mean; mean values with different superscripts in a row differ significantly ($P < 0.05$).

Table 3. Carcass composition [g · 100 g^{-1}] of *O. bimaculatus* larvae fed different levels of lipid.

Constituent [g · 100 g^{-1}]	Experimental diet		
	D1	D2	D3
Moisture	79.37 ± 0.09^{a}	80.93 ± 0.22^{b}	79.80 ± 0.17^{a}
Crude protein	13.93 ± 0.09	14.40 ± 0.21	14.03 ± 0.08
Crude lipid	2.50 ± 0.06^{a}	2.90 ± 0.12^{b}	2.77 ± 0.07^{b}
Ash	1.70 ± 0.06	1.97 ± 0.09	1.80 ± 0.06

Data are presented as mean ± standard error of the mean; mean values with different superscripts in a row differ significantly ($P < 0.05$).

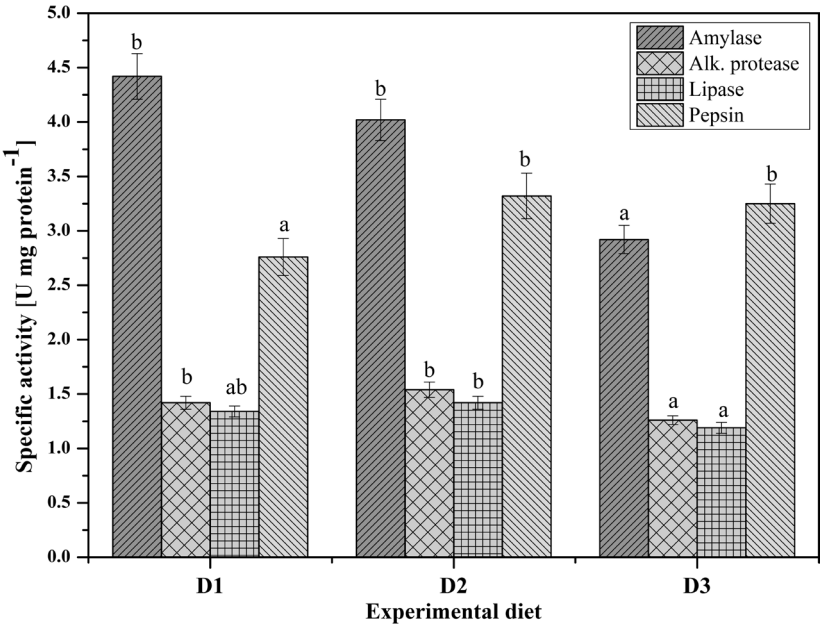

Figure 1. Specific activity of digestive enzymes of *Ompok bimaculatus* larvae fed varying levels of crude lipid. Mean values (± SE) with no common superscript letters are significantly different ($P < 0.05$).

Moisture and lipid contents of the whole body were significantly ($P < 0.05$) affected by the dietary lipid levels, being the highest in larvae fed diet D2 containing 8% crude lipid. However, varying dietary lipid had no significant effect on crude protein and ash contents of *O. bimaculatus* larvae at the tested lipid levels.

Digestive enzymes i.e., amylase, alkaline protease, lipase, and pepsin of the butter catfish larvae fed diets with varied lipid levels are given in Fig. 1. Overall, digestive enzymes were significantly ($P < 0.05$) affected by the dietary lipid levels. Amylase activity in *O. bimaculatus* larvae significantly ($P < 0.05$) decreased with increasing dietary lipid levels. The highest alkaline protease and lipase activities were noticed in the butter catfish larvae fed diet D2 consisting of 8% crude lipid, while it was not significantly ($P < 0.05$) different from the group fed diet D1. The highest pepsin activity was also documented in the fish fed diet D2, although it did not differ significantly from the larvae that received diet D3 with 10.45% dietary lipid.

Activities of the hepatic enzymes involved in the intermediary metabolism of carbohydrate, protein, and lipid are depicted in Table 4. Varying dietary lipid levels led to significant differences ($P < 0.05$) in the activities of PK, G6P, FBP, G6PD, LDH, ALT, and AST in *O. bimaculatus* larvae. While no significant differences were detected in the activities of HK, NADP-ME, GDH, and LPO, the activities of PK and two major neoglucogenic enzymes, G6P and FBP, significantly increased ($P < 0.05$) with the increase in the dietary lipid levels. The activity of G6PD, a key enzyme of lipogenesis, revealed a significant decrease ($P < 0.05$) with an increase in the dietary lipid level from 5.7% (D1) to 8% (D2). Further, significantly lower ($P < 0.05$) activities of LDH, ALT, and AST were recorded in *O. bimaculatus* larvae fed diet D2 with 8% dietary lipid.

Table 4. Specific activity [U mg protein^{-1}] of metabolic enzymes of *Ompok bimaculatus* larvae fed varying levels of lipid.

Enzyme	Experimental diet		
	D1	D2	D3
Hexokinase	9.82 ± 0.35	10.27 ± 0.47	10.54 ± 0.51
Pyruvate kinase	5.6 ± 0.24^{a}	6.2 ± 0.27^{b}	6.4 ± 0.29^{b}
Lactate dehydrogenase	0.845 ± 0.03^{c}	0.507 ± 0.02^{a}	0.690 ± 0.03^{b}
Malate dehydrogenase	2.35 ± 0.11	2.24 ± 0.09	2.20 ± 0.11
Glucose 6 phosphatase	4.05 ± 0.13^{a}	4.38 ± 0.17^{ab}	4.62 ± 0.22^{b}
Fructose 1,6 bis phosphatase	3.10 ± 0.11^{a}	3.42 ± 0.14^{ab}	3.72 ± 0.15^{b}
Alanine aminotransferase	3.88 ± 0.16^{b}	3.55 ± 0.09^{a}	3.78 ± 0.12^{b}
Aspartate aminotransferase	6.55 ± 0.17^{b}	6.15 ± 0.12^{a}	6.45 ± 0.14^{b}
Glutamate dehydrogenase	5.12 ± 0.20	5.20 ± 0.23	5.28 ± 0.27
Glucose-6-phosphate dehydrogenase	32.6 ± 0.81^{b}	27.5 ± 0.76^{a}	26.4 ± 0.72^{a}
Lipid peroxidation	0.92 ± 0.06	0.96 ± 0.004	1.02 ± 0.006

Data are presented as mean ± standard error of the mean; mean values with different superscripts in a row differ significantly ($P < 0.05$).

Discussion

During the experimental rearing of the *O. bimaculatus* larvae, water temperature varied within a narrow range (28–30°C) that was considered suitable since a temperature of around 30°C was suggested as optimum for the growth of catfish (Paul and Giri 2016). Other water quality parameters were also within the acceptable range as recommended elsewhere (Paul et al. 2000; Debnath et al. 2016). Apart from environmental factors, rearing of early larval stages under captive condition depends mostly on the availability of suitable diets that are readily acceptable and consists of nutrients at the required level to support growth and well being of the fish. Different larval stages of fish may have specific nutritional requirements (Malla and Banik 2015). Digestive systems of fish larvae are immature and therefore they depend on live food organisms to a great extent for the supply of exogenous

enzymes. Generally, fish larvae do not prefer artificial diets, even if larviculture with formulated diets is essential for large-scale production of any species. The limited success of the dry formulated diets in larval rearing might be attributed to insufficient feed intake, imbalanced protein (non-protein energy sources), impaired digestive, as well as metabolic functions (Lee et al. 2002).

In the presently reported study, formulated diets were readily accepted by the 14 day old *O. bimaculatus* larvae. The study suggests that 8% lipid in a diet with 40% crude protein might support the growth and survivability of the butter catfish larvae during early development. The required lipid level detected in the presently reported study was close to the suggested lipid levels documented for other catfishes. For example, 6.5% and 7% optimum dietary lipid requirements were reported for *Ompok pabda* (Hamilton, 1822) fry (Paul et al. 2011) and *Mystus montanus* (Jerdon, 1849) (see Raj et al. 2007), respectively. Among carps, 6.5% lipid in the diets of *Ctenopharyngodon idella* (Valenciennes, 1844) (see Jin et al. 2013) and 7% lipid for the juveniles of common carp, *Cyprinus carpio* Linnaeus, 1758 (see Choi et al. 2015) supported maximum growth. On the contrary, elevated lipid requirements have also been suggested. For example, lipid levels of 10% for larvae of magur, *Clarias batrachus* (Linnaeus, 1758) (see BIS 2014b) and 17% for far eastern catfish, *Silurus asotus* Linnaeus, 1758 (see Kim et al. 2012) were reported. Therefore, the majority of the preceding studies suggested varying lipid requirement levels between 6% and 10% in diverse fish species, with few exceptions. Hence, the presently reported study considered this narrow level of variation for evaluation of the lipid levels. The ability of the fish to use lipid as a source of energy was noticed to vary among diverse fish species (Jauncey (1982)). Thus, different fish species at different life stages might require different dietary lipid levels and it needs to be evaluated separately for individual fish species. Our results were in agreement with some of the previous reports depicting 8% lipid requirement as optimal for a minor carp, *Barbonymus gonionotus* (Bleeker, 1849) (see Paul et al. 2010) and fingerlings of rohu, *Labeo rohita* (see Mishra and Samantaray 2004). BIS (2014a) also suggested an 8% crude lipid requirement for carp spawn and fry.

The presently reported study revealed that an increase in the dietary lipid level from 5.7% to 8% was associated with maximum growth and increased SGR [% · day−1] of the butter catfish larvae. Similarly, the lowest FCR and the maximum PER and ANPU values were recorded in the larvae fed diets with 8% crude lipid (D2). Our result was in compliance with the preceding reports indicating that increase in the dietary lipid up to a certain level might aid in efficient protein utilization that results in improved growth of the fish (Jauncey 1982; Kim et al. 2012). Similar results were recorded for the stinging catfish, *Heteropneustes fossilis* (Bloch, 1794) (see Akand et al. 1991) and rohu, *L. rohita* (see Mishra and Samantaray 2004). In contrast to these observations, high dietary lipid might cause to reduce fish growth, as documented for gibel carp, *Carassius gibelio* (Bloch, 1782); and Chinese long snout catfish, *Leiocassis longirostris* Günther, 1864 (see Pei et al. 2004). In the presently reported study, the group of larvae fed diet D3 with 10.45% crude lipid was associated with poor growth, which was in agreement with Pei et al (2004). When the non-protein energy source in the diet becomes insufficient or inaccessible, the protein is used as a source of energy instead of growth (Mohanta et al. 2009). In the presently reported study, the groups reared with diets D1 (5.7% lipid) and D3 (10.45% lipid) portrayed relatively poor growth that might be indicative of poor utilization of the non-protein energy source (Wang et al. 2018). Further, in the presently reported study, around 80% survivability of the butter catfish larvae was achieved with the formulated diets during the feeding trial. Previously, 52.18% and 45.82% survivability of the *O. bimaculatus* larvae with egg custards and compound feed was documented by Malla and Banik (2015), which was relatively lower than the presently reported findings. Improved survivability accomplished in the presently reported study could be due to improved feed utilization by the larvae.

An increase in dietary lipid levels seems to be an important consideration for the food fishes as it might have a significant effect on the carcass quality (Cowey 1993). There might be a positive correlation between lipid levels in the diets and carcass lipid deposition (Cowey 1993), which was in harmony with the presently reported study. Similar observations have been recorded in several species, e.g., rockfish, *Sebastes schlegelii* Hilgendorf, 1880 (see Lee et al. 2002); Eurasian perch; *Perca fluviatilis* Linnaeus, 1758 (see Mathis et al. 2003); cobia, *Rachycentron canadum* (Linnaeus, 1766) (see Craig et al. 2006); and grouper, *Epinephelus malabaricus* (Bloch et Schneider, 1801) (see Williams 2007). On the contrary, Paul et al. (2011) could not find any difference in carcass lipid in another species of butter catfish, *O. pabda* by feeding different levels of lipid. In the presently reported study, carcass protein content was not significantly affected by the dietary lipid levels, which was consistent with previous reports on the juveniles of pike perch, *Sander lucioperca* (Linnaeus, 1758) (see Schulz et al. 2008) and cobia (Webb et al. 2010). Overall, the carcass composition of the *O. bimaculatus* larvae detected in the presently reported study was similar to the previous report by Debnath and Sahoo (2013).

Although the ontogeny of the digestive enzymes during the early development of the *O. bimaculatus* has been documented by some authors (Pradhan et al. 2013; Chowdhury et al. 2019), to the authors' knowledge, there is no information on the diet-related changes in the digestive enzymes in the butter catfish. Adaptations of the digestive system in different species exhibit close association with their diet (Fernandez et al. 2001). Thus, changes in digestive enzyme activity could be correlated with the biochemical composition of food and feeding behavior of fish (Kuzmina 1996). In the presently reported study, amylase activity in *O. bimaculatus* larvae was noticed to be significantly decreased with elevated dietary

lipid levels. Previously, amylase activity in gilthead sea bream, *Sparus aurata* Linnaeus, 1758, was noticed to be influenced by dietary lipid levels (Fountoulaki et al. 2005). While, maximum activities of the alkaline protease, pepsin, and lipase were recorded with the group that was fed 8% lipid (D2) and achieved the highest growth. Digestive enzymes might contribute towards efficient digestion of the dietary components, which could be reflected through the growth of the fishes. Thus, increased growth in fish (fed 8% dietary lipid) associated with enhanced digestive enzyme activities might be indicative of better nutrient utilization in fish as stated elsewhere (Mandal and Ghosh 2018).

The presently reported study appraised activities of some major metabolic enzymes to evaluate the effects of the varying dietary lipid levels. Activities of the amino acid catabolizing enzymes were influenced by the dietary lipid levels. The fish liver is the hotspot for transdeamination with ALT and AST as the major enzymes (Enes et al. 2006; Kumar et al. 2008). A decrease in the activities of ALT, AST, and LDH might suggest reduced protein catabolism in fish fed diet D2 with 8% lipid. LDH is the enzyme of the glycolytic pathway that mediates the bidirectional conversion of pyruvate to lactate. A hike in LDH activity could be noticed under stress (Chatterjee et al. 2006). Thus, reduced LDH activity in the fish reared on D2 might indicate no or negligible stress on the experimental fish. In the presently reported study, increased activities of the gluconeogenic enzymes (G6P and FBP) coincided with an increase in the dietary lipid levels. Gluconeogenesis is a major pathway for glucose homeostasis, where glucose is produced from non-carbohydrate precursors (e.g., amino acid, lactate, glycerols). Increased activity of the neoglucogenic enzymes associated with decreased activity of digestive amylase might be indicative of the production of glucose by gluconeogenesis to meet the energy demand in this carnivorous species. No significant variation was noticed in the activity of the major glycolytic enzyme, HK. G6PD is the key enzyme catalyzing the first step of the HMP-shunt (pentose phosphate pathway) that generates NADPH for lipogenesis and stress management (Pandolfi et al. 1995). In the presently reported study, the activity of the lipogenic enzyme (G6PD) was inhibited by an increase in the dietary lipid, which was similar to the observations recorded in juveniles of Senegalese sole, *Solea senegalensis* Kaup, 1858 (see Dias et al. 2004; Guerreiro et al. 2012). Further, NADP-ME, GDH, and LPO activities were more or less unaffected by the dietary lipid levels. NADP-ME is responsible for NADP-dependent oxidative decarboxylation (malate to pyruvate and carbon dioxide) with the generation of NADPH that may be utilized for lipid biosynthesis, while GDH had been considered as a sensitive indicator of stress (Susan et al. 2010). Therefore, the results of the presently reported study might suggest that increased dietary lipid levels are neither required to augment lipid biosynthesis by the fish nor to induced stress on the experimental fish.

Conclusion

Results of the presently reported study indicated that 8% crude lipid in the diet with 40% crude protein might assure optimum growth and survival of *Ompok bimaculatus* larvae during early development. An appraisal on growth, body composition, and digestive as well as metabolic function in the butter catfish larvae recorded in the study might provide some important information to consider the application of formulated diets for the larviculture of *Ompok bimaculatus*.

Acknowledgments

The authors greatly acknowledge the help and support of Dr. S.K. Swain, the Director, ICAR-Central Institute of Freshwater Aquaculture and Head, Department of Zoology, the University of Burdwan for providing the necessary facility to conduct the work.

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