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Optimization of dietary protein and lipid levels for butter catfish, (*Ompok bimaculatus*) (Bloch, 1794) fingerlings: An appraisal on growth, body composition, digestive enzymes, and metabolic function

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ABSTRACT

A completely randomized 3 × 3 factorial feeding trial was conducted to evaluate the effects of dietary protein and lipid levels on growth, feed utilization, body composition, and digestive as well as metabolic enzymes of the butter catfish (*Ompok bimaculatus*) fingerlings. Fish (average weight 0.66 ± 0.12 g; 30 d) were fed nine experimental diets containing three protein levels (35%, 40%, 45%) and three lipid levels (5%, 10%, 15%) in triplicate groups for a period of 90 days. The formulated diets were named P35L05, P35L10, P35L15, P40L05, P40L10, P40L15, P45L05, P45L10, and P45L15 (P = Protein; L = Lipid) respectively. The results indicated no protein-sparing effect of lipid in *O. bimaculatus* fingerlings beyond 35% dietary protein. Overall, diets P40L5 and P35L10 had significantly ($P < 0.05$) higher SGR (% day^{-1}) and lower FCR than other experimental groups. The highest weight gain (10.89 ± 0.07 g) and carcass protein (16.71 ± 0.2%) were recorded in fish fed diet P40L05. Increasing dietary lipid levels brought about an increase in carcass lipid in fish. Activities of α-amylase and acid-protease were decreased (except at 5% lipid), whereas alkaline-protease was increased with increasing dietary protein. An increase in pyruvate kinase (PK, glycolytic enzyme) activity and decrease in glucose-6-phosphate dehydrogenase (G6PD, lipogenic enzyme) activity were noticed with increasing dietary lipid content. A decrease in PK and increase in G6PD activities were observed with increasing dietary protein levels. Activities of the gluconeogenic enzymes (glucose-6-phosphatase, fructose-1,6-phosphatase) increased significantly ($P < 0.05$) with increasing dietary protein and lipid levels. Amino acid catabolizing enzymes, alanine transaminase and aspartate transaminase, were slightly increased with increasing dietary protein levels. Considering growth, body composition, metabolic function, and survivability (>95%), the diet containing 40% crude protein with 5% lipid might be suggested as optimum for *O. bimaculatus* fingerlings under the tested conditions.

KEYWORDS

Body composition; digestive enzymes; feed efficiency; metabolic enzymes; *Ompok bimaculatus*

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Introduction

The butter catfish, *Ompok bimaculatus* (Bloch, 1794), although indigenous to India, are also distributed in rivers, lakes, floodplains, and wetlands of other South East Asian countries, e.g., Bangladesh, Borneo, Java, Sumatra, Laos, Sri Lanka, Nepal, Malaya, Myanmar, Pakistan, Thailand, Cambodia, and Vietnam (Giri et al. 2019). The species has immense consumer preference and economic importance due to its excellent taste, balanced nutrient profile, soft bony structure, and high market price, especially in the east and north east of India as well as Bangladesh (Banik, Goswami, and Malla 2012; Paul et al. 2020a). It has been recognized as an excellent source of ω-3 and ω-6 fatty acids, lipoproteins, vitamins, and minerals together with easily digestible low fat content (Paul et al. 2020b). The wild population of *O. bimaculatus* has intensely declined due to anthropogenic activities like indiscriminate fishing and habitat degradation leading to ecological changes (Paul et al. 2020a). Considering restricted distribution and reduced abundance, the species has been categorized under near threatened category by the International Union for the Conservation of Nature and Natural Resources (IUCN) red list experiencing a risk of extinction in nature (IUCN 2014; Lakra et al. 2010). Because of high demand, competitive prices, and IUCN status, the species has been prioritized as a candidate for diversification of aquaculture and *in situ* conservation along with restocking through ranching programs (Debnath et al. 2016; Paul et al. 2020a). However, even though the aquaculture potential of *O. bimaculatus* has been recognized of late, the species has not yet attracted adequate attention for captive rearing, and thus information on the nutritional requirements of this species at different life stages is scarce (Biswas et al. 2020, 2019; Chakrabarti et al. 2012; Paul et al. 2021, 2020a).

Standardization of breeding protocol and feeding strategy accompanied by nutritionally balanced formulated diets are the prerequisites to establishing captive culture conditions for any fish species. Captive breeding of *O. bimaculatus* has already been established (Banik, Goswami, and Malla 2012; Raizada et al. 2013). Other studies conducted very recently indicated dietary requirements of 40% crude protein (Paul et al. 2020a) and 8% crude lipid (Paul et al. 2021) to assure optimum growth and survival of the *O. bimaculatus* larvae during early development. Requirement of around 35% dietary protein was suggested for the fingerlings of this species (Biswas et al. 2020). However, to the authors' knowledge, studies on dietary requirements of the major nutrients for the fingerlings of *O. bimaculatus* have not yet been reported. Thus, it was considered reasonable to appraise the nutritional requirements of *O. bimaculatus* fingerlings to ensure regular and reliable supply of the fingerlings for commercial production and conservation issues.

Protein is the most important and often an expensive dietary component; determination of its requirement levels is fundamental to formulating high-quality as well as cost-effective diets (Wang et al. 2013). Adequate levels of good-quality protein in fish feed is necessary to support high growth at the younger stages. If added in excess, the surplus protein would be converted to energy through catabolism of amino acids, and that should be avoided as it would increase the feed cost and nitrogenous waste output (National Research Council 2011; Wu and Gatlin 2014). Therefore, it is important to improve protein utilization for synthesis of body protein and important biomolecules rather than for energy purposes (Lee, Jeon, and Lee 2002). Dietary protein requirements in fish could be affected by the amount of nonprotein energy sources in the diet. Elevated lipid or carbohydrate levels in the diet could spare protein without negatively affecting growth and feed utilization efficiency in fish (De Silva, Gunasekera, and Shim 1991; Wang et al. 2013). Species under the genus *Ompok* have been generally recognized as carnivorous to omnivorous and prefer natural food items like zooplankton and tubifex (Malla and Banik 2015). Similarly, the carnivorous to omnivorous feeding aptitude of *O. bimaculatus* has been established (for review, see Gupta 2015). In carnivorous fish species, lipid is generally preferred as a nonprotein energy source since lipid is an energy-dense nutrient that is readily metabolized, and carbohydrate is less available in their natural food (Lee, Jeon, and Lee 2002). In addition, dietary lipids serve as the source of essential fatty acids and a carrier of fat-soluble vitamins. However, excessive lipid in the diet could disrupt the health and growth of fish because of abnormal lipid deposition in the body, and thus careful considerations must be given regarding protein-to-lipid ratios in formulated diets (Lee, Jeon, and Lee 2002; Shapawi et al. 2014; Wang et al. 2013). Thus, the present study was conducted to optimize dietary protein and lipid levels for formulation of practical diets for butter catfish fingerlings. Moreover, the present study evaluated the protein-sparing potential of lipid and appraised whether an interaction of protein and lipid existed for growth performance, body composition, and enzyme activities in *O. bimaculatus* fingerlings.

As requirement levels for protein and lipid may vary in different species and at different life stages, designing species-specific and age-specific diets can improve performance of the fish under commercial aquaculture. Optimization of the dietary protein:lipid ratio and their interaction has been studied in diverse fish species (Guerreiro et al. 2012; Kim and Lee 2009; Sagada et al. 2017; Wang et al. 2013). However, no commercial formulated diet has yet been available for the fingerlings of *O. bimaculatus*. Considering different protein and lipid levels studied for diverse fish species and reports on the nutrient requirements of the *O. bimaculatus* larvae, in the present study, practical diets were formulated with three protein (35%, 40%, 45%) and three lipid levels (5%, 10%, 15%) in a 3 × 3 factorial design to optimize the dietary protein and lipid

levels. Therefore, the present study considered an appraisal of digestive enzymes along with some key metabolic enzymes to evaluate the effects of formulated diets with varying protein and lipid levels. The results of the study might be helpful in making some important information available for formulation of diets for *O. bimaculatus* fingerlings.

Material and methods

Fish and culture conditions

Butter catfish (*O. bimaculatus*) fry and fingerlings were raised in the wet laboratory of the Department of Zoology, The University of Burdwan. Fish were handled and the experiment was performed following the approved guidelines of the Institutional Ethical Committee. Prior to the experiment, the fish were acclimated to the experimental conditions for one week in fiber-reinforced plastic (FRP) tanks (150 L) with continuous aeration; during this period fish were fed a formulated diet containing 40% crude protein and 8% lipid. After acclimatization, the fingerlings (average weight 0.66 ± 0.12 g; average length 4.8 ± 0.20 cm; 30 d old) were randomly distributed in 27 FRP tanks (nine experimental sets in triplicates) at a stocking density of 100 fish per tank. The fish were manually fed to apparent satiation twice a day at 10:00 and 16:00 hours, and it was ensured that no feed was leftover. Feed consumption and mortality in each tank were recorded separately, and survivability (%) was calculated. Seasoned groundwater was used for rearing of the experimental fish. The experiment was conducted for 90 days with continuous aeration and daily exchange of 25% water to ensure good water quality. During the experimental period, water quality parameters (temperature, pH, and dissolved oxygen) were monitored on a regular basis using a portable multiparameter analyzer (Orion Star™ A329) that utilizes the standard methods of American Public Health Association (American Public Health Association [APHA] 2012). Total alkalinity was determined by titration of the water sample with H_2SO_4 (0.1 N) and expressed as $CaCO_3$ equivalent (APHA 2012). Water quality parameters were noticed to vary within a narrow range: temperature 28°C–30°C, pH 6.8–7.7, dissolved oxygen 6.8–7.4 mg/L, and total alkalinity 230–240 mg/L.

Experimental design and diets

The experiment had a completely randomized 3 × 3 factorial design with triplicate groups. Nine experimental diets were formulated consisting of three protein levels (35%, 40%, 45%) and three lipid levels (5%, 10%, 15%). The formulated diets were named P35L05, P35L10, P35L15, P40L05, P40L10, P40L15, P45L05, P45L10, and P45L15 (P = Protein, L = Lipid) respectively.

Proximate compositions of the dietary ingredients were determined and diets were formulated using WinFeed software (Version 2.8). A blending of soybean meal (SBM), groundnut oil cake (GNOC), and casein along with fish meal (FM) at varied levels served as the protein sources; a combination of vegetable and fish oils (1:1) at different inclusion levels met the requirement of desired lipid levels in the diets. Crude protein (CP) and crude lipid (CL) levels of the major ingredients were: SBM (CP 45%, CL 2.5%), GNOC (CP 39%, CL 8%), and FM (CP 55%, CL 8%). All dry ingredients were finely powdered with a laboratory grinder, sieved to obtain uniform particle size (<400 μm, diameter), and mixed thoroughly with the required amount of vitamin-mineral premix (Supradyn, Piramal Enterprises Ltd., Mumbai, India) and oil sources. Carboxymethyl-cellulose (1%) was added as a binder, and the mixture was made to stiff dough with an appropriate amount of lukewarm water. The dough was pelleted (1 mm in diameter) with an electrically operated pelletizer and dried initially in the sun (6 h) and further in a hot air oven at 60°C (96 h). The dried pellets were crumbled, packed in airtight plastic bags, and stored in a refrigerator at 4°C until use. Formulation and proximate composition of the experimental diets are depicted in Table 1.

Proximate compositions of diets

Analyses of proximate compositions of the experimental diets (dry weight) were carried out following the procedures of the Association of Official Analytical Chemists (Association of Official Analytical Chemists [AOAC] 2005). Dry weight was determined by oven drying (initially at 100 ± 5°C for 30 min, thereafter at 60°C) until constant weight. Crude protein (N × 6.25) was determined by a semi-automatic Kjeldahl system (KjelTRON, Tulin Equipments, Chennai, India); crude lipid by ether extraction (petroleum ether, 60 to 80°C) using a Soxhlet apparatus (Socsplus, Pelican Equipments, Chennai, India); crude fiber as loss on ignition of dried fat-free residues after digestion with 1.25% H_2SO_4 and 1.25% NaOH using a Fibraplus system (Pelican Equipments, Chennai, India); and ash by combustion at 550°C in a muffle furnace. Nitrogen-free extract (NFE) was determined by subtracting the sum of values for crude protein, crude lipid, crude fiber, ash, and moisture from 100 (Maynard et al. 1979). Gross energy of the experimental diets was measured with a bomb calorimeter (Lab-X, Kolkata, India).

Growth parameters

After termination of the experiment, 20 fish were randomly picked from each tank, weighed, and analyzed to evaluate the growth parameters. Live weight gain (%), specific growth rate (SGR; % day^{-1}), protein efficiency ratio (PER), feed conversion ratio (FCR), apparent net protein utilization (ANPU %), and

Table 1. Ingredient composition and proximate composition of the experimental diets on a dry matter (%) basis.

	Diets								
Ingredients (g/100 g)	P35L05	P35L10	P35L15	P40L05	P40L10	P40L15	P45L05	P45L10	P45L15
Fishmeal	18	18	18	29	29	29	40	40	40
Wheat flour	37	32	27	26	22	17	16	11	6
Casein	10	10	10	10	10	10	10	10	10
Soybean meal	15	15	15	15	15	15	15	15	15
Ground nut oil cake	15	15	15	15	15	15	15	15	15
Fish oil + veg. oil (1:1)	2	7	12	2	6	11	1	6	11
Vitamin + mineral*	2	2	2	2	2	2	2	2	2
Carboxy methyl cellulose	1	1	1	1	1	1	1	1	1
Proximate composition (% dry matter)									
Dry matter	93.02 ± 1.51	92.87 ± 1.45	92.67 ± 1.46	93.12 ± 1.32	92.31 ± 1.40	92.05 ± 1.37	91.91 ± 1.38	91.72 ± 1.35	91.52 ± 1.47
Protein	35.42 ± 4.1	35.11 ± 4.0	34.9 ± 3.7	40.76 ± 3.8	40.22 ± 3.4	40.09 ± 3.1	45.58 ± 2.1	45.24 ± 2.7	44.82 ± 2.9
Lipid	5.15 ± 0.24	10.21 ± 0.17	15.40 ± 0.19	5.0.±0.25	9.87 ± 0.17	14.85 ± 0.12	5.19 ± 0.22	10.09 ± 0.16	14.92 ± 0.14
Ash	10.50 ± 0.74	10.15 ± 0.56	9.81 ± 0.62	10.02 ± 0.47	9.78 ± 0.68	9.56 ± 0.87	9.67 ± 0.54	9.45 ± 0.69	9.25 ± 0.78
Crude fiber	22.78 ± 2.14	21.54 ± 2.12	20.78 ± 2.14	20.52 ± 1.97	19.52 ± 2.15	18.98 ± 1.78	17.98 ± 1.69	17.74 ± 1.75	16.95 ± 1.85
NFE	26.15 ± 2.84	22.99 ± 2.69	19.12 ± 1.78	23.63 ± 1.67	20.61 ± 1.48	16.52 ± 1.47	21.58 ± 1.65	17.48 ± 1.47	14.06 ± 1.54
KJ/g	18.86	20.02	21.22	19.28	20.35	21.49	19.69	20.79	21.87
P:E ratio (mg/KJ)	18.78	17.54	16.45	21.14	19.76	18.66	23.15	21.76	20.49

*Vitamin+mineral contains Vitamin A I.P.(as acetate) 10,000 I.U, Cholecalciferol I.P.(Vitamin D3) 1000 I.U., Thiamine mononitrate I.P. 10 mg, Riboflavine I.P. 10 mg, Pyridoxine Hydrochloride I.P. 3 mg, Cyanocobalmin I.P. 15 mcg, Nicotinamide I.P. 100 mg, Calcium Pantothenate I.P. 16.30 mg, Ascorbic Acid I.P. 150 mg, α Tocopheryl Acetate I.P. 25 mg, Biotin U.S.P. 0.25 mg, Tribasic Calcium Phosphate I.P. 129 mg, Magnesium Oxide Light I.P. 60 mg, Dried Ferrous Sulfate I.P. 32.04 mg, Manganese Sulfate Monohydrate B.P. 2.03 mg, Total Phosphorus in the preparation 25.80 mg, Copper Sulfate Pentahydrate B.P. 3.39 mg, Zinc Sulfate I.P. 2.20 mg, Sodium Molybdate Dihydrate B.P. 0.25 mg, Sodium Borate B.P. 0.88 mg.

survivability (%) were determined following standard methods outlined by Steffens (1989), a detailed description of which was depicted in Ghosh and Mondal (2015).

Digestive enzymes

Activities of the digestive enzymes (α-amylase, alkaline protease, pepsin, and lipase) in fish were determined at the initiation and completion of the experiment. Digestive tracts of 20 fish from each experimental tank were taken out, cleaned properly with chilled distilled water, kept on an ice-cooled Petri plate, and weighed. Pooled samples collected from each tank were used for a replicate; thus there were three replicates for each experimental set. A 10% homogenate was prepared with chilled phosphate buffer (0.1 M, pH 7.4) and centrifuged at 10,000 rpm (10 min, 4°C). The resulting supernatant was used as the enzyme extract for estimation of digestive enzymes. Protein content of the supernatant was analyzed using bovine serum albumin as a standard (Lowry et al. 1951). Amylase activity was determined after Bernfeld (1955) using dinitro salicylic acid (DNSA) reagent, and unit activity (U) was expressed as mg maltose liberated mg^{-1} protein hr^{-1}. Alkaline protease activity was measured following Walter (1984), using Hammerstein casein substrate and presented as μg of tyrosine liberated mg^{-1} protein hr^{-1} (U). Acid protease (pepsin) activity was determined using hemoglobin (2%) substrate according to Anson (1938) with minor alterations as described in Worthington (1991) and expressed as μg of tyrosine liberated mg^{-1} protein min^{-1} (U). Lipase activity was measured after Bier (1955) using olive oil substrate and documented as μ mole of fatty acid liberated mg^{-1} protein hr^{-1} (U).

Metabolic enzymes

For determination of metabolic enzymes, hepatic tissues were collected and a 10% homogenate (in 0.25 M sucrose, pH 7.4) was prepared. Following removal of cellular debris by centrifugation (1,000 g, 30 min, 4°C), the supernatant was further centrifuged at 10,000 g (15 min, 4°C) to obtain mitochondrial pellets (Biswas et al. 2006). The supernatant was centrifuged at 12,500 g (1 h, 4°C), and the resultant fraction was used as the source of cytosolic metabolic enzymes. Although the mitochondrial pellet was further processed with triton X-100 (0.1%) and repeatedly washed (10,000 *g*, 30 min, 4°C) with PBS (0.1 M, pH 7.4), the supernatant thus obtained was used as the source of mitochondrial metabolic enzymes. The tissue fractions were stored in −20°C until use.

Among carbohydrate metabolizing enzymes, hexokinase (HK) was determined after Tranulis et al. (1996) through the reduction of NADP to generate NADPH. HK activity was presented as μM of NADPH formed mg^{-1} protein

h^{-1} (U). Pyruvate kinase (PK) was measured following Driedzic and Almeida-Val (1996) with minor alteration. PK activity was expressed as μmole of pyruvate converted to NADH mg^{-1} protein min^{-1} (U). Gluconeogenic enzymes, glucose-6-phosphatase (G6P), and fructose-1,6-bis phosphatase (FBP) were determined through the amount of phosphorus (Pi) released from glucose-6-phosphate (Marjorie 1964) or fructose-di-phosphate (Freeland and Harper 1959) as substrates respectively. Release of Pi was measured following Fiske and Subbarow (1925). G6P and FBP activities were expressed as μg of phosphorus released mg^{-1} protein min^{-1} (U).

Among lipogenic enzymes, glucose-6-phosphate dehydrogenase (G6PD) was determined using glucose-6-phosphate as the substrate and NADP (Kornberg and Horecker 1955). G6PD activity was expressed as μM of NADPH formed mg^{-1} protein hr^{-1} (U). NADP-malic enzyme (NADP-ME) activity was measured using L-malic acid as the substrate following Hsu and Lardy (1967) with alterations proposed by Murphy and Walker (1974). NADP-ME activity was expressed as μM of NADPH formed mg^{-1} protein h^{-1} (U).

Amino acid catabolizing enzymes, alanine transaminase (ALT) and aspartate transaminase (AST), were measured using α-ketoglutarate and DL-Alanine or DL-Aspartic acid substrates respectively (Reitman and Frankel 1957). ALT activity was presented as μM of pyruvate formed mg^{-1} protein min^{-1} (U); AST activity was expressed as μM of oxaloacetate formed mg^{-1} protein min^{-1} (U). Mitochondrial glutamate dehydrogenase (GDH) activity was determined after Lee and Lardy (1965) using sodium glutamate and tetrazolium salt. GDH activity was expressed as μM of formazan formed mg^{-1} protein h^{-1} (U).

Compositions of fish carcass

Analyses of proximate compositions of the fish carcass (wet weight) were carried out by standard procedures of the Association of Official Analytical Chemists (AOAC 2005) as described in a previous section.

Statistical analysis

Data were presented as mean ± standard error (SE). Data were analyzed by one-way (dietary treatments) and two-way (dietary protein and lipid levels) analysis of variance (ANOVA) following Zar (2010). Multiple comparisons were made by Tukey's post hoc HSD test to analyze significant differences ($P < 0.05$) between the means of experimental groups.

Results

Data pertaining to growth performance, feed utilization, and survivability of *O. bimaculatus* fingerlings fed diets with different protein and lipid levels for 90 days are presented in Table 2. Results of the one-way ANOVA indicated that net weight gain, SGR (% per day), FCR, and protein utilization were significantly ($P < 0.05$) influenced by varying levels of dietary protein and lipid. The highest weight gain was recorded in fish fed diet P40L5. Overall, diets with 45% crude protein produced fish with lower SGR, PER, and ANPU. Increasing lipid levels from 5% to 15% resulted in a decrease in growth and protein utilization in fish fed diets with 40% or 45% crude protein. Survivability rates in butter catfish fingerlings were >90% except the groups fed diets P45L10 and P45L15. Analysis by two-way ANOVA revealed that SGR, FCR, PER, ANPU, and survivability were significantly affected by the dietary protein and lipid levels ($P < 0.05$). Further, except for survivability, there were significant interaction between the dietary protein and lipid levels on the growth and feed utilization parameters (e.g., SGR, FCR, PER, and ANPU) of *O. bimaculatus* fingerlings.

Activities of the digestive enzymes are depicted in Table 3. Overall, one-way ANOVA indicated that activities of α-amylase and acid protease were decreased (except at 5% lipid, up to 40% CP), whereas alkaline protease was increased with increasing levels of dietary protein. The maximum α-amylase and acid protease activities were noticed in the fish fed diet P35L10, though it was not significantly different from the groups fed diets P35L15 and P40L05. Significantly ($P < 0.05$) higher alkaline protease activity was recorded with the fish fed diets with 45% crude protein irrespective of tested lipid levels, when compared to other protein levels. Lipase activity increased significantly with increasing dietary lipid from 5 to 10 at the 35% CP level. Although the maximum lipase activity was noticed in the fish fed diet P45L05, that didn't differ significantly ($P < 0.05$) with the other groups receiving 40% or 45% crude protein in the diets. Thus, at higher CP levels, lipase activity didn't differ significantly with increasing lipid levels. According to two-way ANOVA, activities of the tested digestive enzymes were significantly affected ($P < 0.05$) by dietary protein and lipid levels and their interaction.

Some of the key hepatic enzymes concerned with intermediary metabolism of protein, lipid, and carbohydrate are portrayed in Table 4. One-way ANOVA showed that varying dietary protein and lipid levels led to significant differences in the activities of PK, G6P, FBP, G6PD, ALT, and AST in *O. bimaculatus* fingerlings; differences were not significant for the activities of HK, ME, and GDH. Activity of the glycolytic enzyme PK significantly decreased with increasing dietary protein levels but increased by increasing lipid levels at the same protein level. Activities of two major neoglucogenic enzymes, G6P and FBP, significantly increased with increases in the dietary

Table 2. Growth performance and feed utilization efficiency of *Ompok bimaculatus* fingerlings fed varying dietary protein and lipid levels for 90 days.

Experimental diets	Initial (g)	Final Weight (g)	Live weight gain	FCR$^{\alpha}$	SGR$^{\beta}$	PER$^{\gamma}$	ANPU$^{\Sigma}$	Survivability
P35/L05	0.652 ± 0.12	10.05 ± 0.06^{ab}	9.40 ± 0.08^{ab}	1.46 ± 0.01^{b}	2.77 ± 0.02^{b}	1.95 ± 0.02 ^{f}	32.06 ± 0.39^{c}	96 ± 0.57^{b}
P35/L10	0.661 ± 0.11	11.08 ± 0.06^{e}	10.43 ± 0.10^{e}	1.37 ± 0.01^{a}	3.00 ± 0.04 ^{cd}	2.09 ± 0.01 ^{g}	35.00 ± 0.6^{d}	93 ± 1.52^{b}
P35/L15	0.656 ± 0.12	10.43 ± 0.07^{bcd}	9.78 ± 0.09^{bcd}	1.57 ± 0.01^{c}	2.75 ± 0.02^{b}	1.82 ± 0.02^{d}	30.26 ± 0.48^{c}	92 ± 1.15^{b}
P40/L05	0.657 ± 0.13	11.54 ± 0.08^{f}	10.89 ± 0.07 ^{f}	1.34 ± 0.01^{a}	3.08 ± 0.04^{d}	1.86 ± 0.02^{e}	31.44 ± 0.20^{c}	95 ± 1.15^{b}
P40/L10	0.672 ± 0.16	10.86 ± 0.07^{e}	10.21 ± 0.09^{e}	1.51 ± 0.01^{bc}	2.91 ± 0.03^{c}	1.65 ± 0.01^{c}	27.66 ± 0.40^{b}	93 ± 0.57^{b}
P40/L15	0.681 ± 0.19	10.42 ± 0.10^{bcd}	9.77 ± 0.06^{bcd}	1.63 ± 0.02 ^{cd}	2.71 ± 0.03^{b}	1.53 ± 0.01^{b}	25.40 ± 0.44^{b}	90 ± 2.30^{ab}
P45/L05	0.654 ± 0.12	9.99 ± 0.06^{a}	9.34 ± 0.06^{a}	1.67 ± 0.02^{d}	2.41 ± 0.02^{a}	1.33 ± 0.02^{a}	21.56 ± 0.52^{a}	91 ± 2.08^{ab}
P45/L10	0.665 ± 0.14	10.08 ± 0.08^{ac}	9.43 ± 0.07^{ac}	1.71 ± 0.03^{de}	2.37 ± 0.02^{a}	1.30 ± 0.02^{a}	20.92 ± 0.57^{a}	88 ± 1.52^{ab}
P45/L15	0.654 ± 0.11	10.28 ± 0.10^{ad}	9.63 ± 0.06^{ad}	1.76 ± 0.02^{e}	2.3 ± 0.02^{a}	1.27 ± 0.02^{a}	20.14 ± 0.37^{a}	83 ± 3.00^{a}
Means of main effect								
P35	–	10.52	9.87	1.46^{A}	2.84^{B}	1.94^{B}	32.44^{B}	93.66
P40	–	10.94	10.29	1.49^{A}	2.90^{B}	1.82^{B}	28.16^{B}	92.66
P45	–	10.12	9.46	1.71^{B}	2.36^{A}	1.29^{A}	20.87^{A}	87.33
L5	–	10.53	9.88	1.49	2.75	1.84	28.35	94.00
L10	–	10.67	10.02	1.53	2.76	1.67	27.86	91.33
L15	–	10.38	9.72	1.65	2.59	1.54	25.26	88.33
Two-way ANOVA (P value)								
Protein	–	0.001	0.001	P < 0.001	0.005	P < 0.001	P < 0.001	0.003
Lipid	–	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Interaction	–	P < 0.001	P < 0.001	P < 0.001	0.032	P < 0.001	P < 0.001	0.744

Note. Data are presented as Mean ± SE of three determinations (n = 3). Means with different superscripts in a column differ significantly (P < 0.05).
$^{\alpha}$Feed conversion ratio, $^{\beta}$Specific growth rate, $^{\gamma}$Protein efficiency ratio, $^{\Sigma}$Apparent net protein utilization.

Table 3. Activities of digestive enzymes in *Ompok bimaculatus* fingerlings fed varying dietary protein and lipid levels for 90 days.

Experimental diets	Amylase	Alkaline protease	Lipase	Pepsin
P35/L05	10.86 ± 0.08^{c}	1.63 ± 0.03^{a}	1.37 ± 0.02^{a}	3.35 ± 0.06^{b}
P35/L10	12.25 ± 0.10^{e}	1.68 ± 0.01^{ab}	1.61 ± 0.03^{bc}	3.65 ± 0.07^{b}
P35/L15	11.96 ± 0.12^{de}	1.74 ± 0.03^{ac}	1.56 ± 0.03^{b}	3.42 ± 0.06^{b}
P40/L05	11.86 ± 0.11^{de}	1.82 ± 0.02^{bcd}	1.72 ± 0.03^{bd}	3.55 ± 0.07^{b}
P40/L10	11.5 ± 0.12^{d}	1.94 ± 0.04^{de}	1.81 ± 0.04^{d}	2.89 ± 0.05^{a}
P40/L15	9.65 ± 0.12^{b}	1.96 ± 0.03^{df}	1.78 ± 0.03^{d}	2.92 ± 0.05^{a}
P45/L05	10.52 ± 0.07^{c}	2.12 ± 0.04^{fg}	1.86 ± 0.04^{d}	2.75 ± 0.05^{a}
P45/L10	9.95 ± 0.15^{b}	2.16 ± 0.04^{g}	1.78 ± 0.04^{cd}	2.64 ± 0.06^{a}
P45/L15	8.77 ± 0.11^{a}	2.06 ± 0.04^{efg}	1.71 ± 0.03^{bd}	2.61 ± 0.05^{a}
Means of main effect				
P35	11.69	1.68^{A}	1.51	3.47^{B}
P40	11.00	1.91^{B}	1.77	3.36^{AB}
P45	9.74	2.11^{C}	1.78	3.13^{A}
L05	11.08	1.86	1.65	3.45
L10	11.23	1.93	1.73	3.37
L15	10.12	1.92	1.68	3.14
Two-way ANOVA (P value)				
Protein	$P < 0.001$	0.024	0.019	$P < 0.001$
Lipid	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Interaction	$P < 0.001$	0.027	$P < 0.001$	$P < 0.001$

Note. Data are presented as Mean ± SE of three determinations ($n = 3$). Means with different superscripts in a column differ significantly ($P < 0.05$).

protein and lipid levels. Activity of the lipogenic enzyme, G6PD, revealed a significant decrease with an increase in the dietary lipid levels and increased with an increase in the dietary protein levels. Further, activities of amino acid catabolizing enzymes, ALT and AST, revealed a marginal increase with an increase in the dietary protein levels for all of the tested lipid levels.

Based on one-way ANOVA, whole body lipid and protein were significantly affected by varying levels of dietary proteins and lipids, although moisture and ash contents remained statistically neutral (Table 5). The highest crude protein in the carcass was recorded in the group P40L05, although it did not differ significantly ($P < 0.05$) with the groups P35L10 and P40L10. Further, an increase in dietary lipid levels at both 40% and 45% crude protein resulted in a decrease in carcass protein deposition in fish. The maximum crude lipid in the carcass was noticed in fish fed diet P40L15, which was not significantly different ($P < 0.05$) to the fish fed diets P35L10, P40L05, and P40L10. An increase in dietary lipid levels brought about an increase in carcass lipid contents in fish at the same protein level, although the difference was significant with only 35% crude protein. The two-way ANOVA indicated that dietary protein and lipid levels and their interaction had a significant influence on carcass protein and lipid of *O. bimaculatus* fingerlings. However, there was no significant interaction between dietary protein and lipid levels on carcass ash and moisture contents.

Table 4. Hepatic metabolic enzymes of *Ompok bimaculatus* fingerlings fed varying dietary protein and lipid levels for 90 days.

	Glycolytic enzyme		Gluconeogenic enzyme	
Experimental Diets	Hexokinase	Pyruvate Kinase	Glucose 6 Phosphatase	Fructose 1,6 Phosphatase
P35/L05	10.43 ± 0.27	7.25 ± 0.06^{d}	4.15 ± 0.03^{a}	2.87 ± 0.05^{a}
P35/L10	10.56 ± 0.27	8.67 ± 0.08 ^{f}	4.27 ± 0.05^{ab}	3.15 ± 0.06^{ab}
P35/L15	10.72 ± 0.27	9.89 ± 0.12 ^{g}	4.38 ± 0.03^{bc}	3.35 ± 0.06^{bc}
P40/L05	11.23 ± 0.26	6.01 ± 0.06^{b}	4.42 ± 0.04^{bcd}	3.67 ± 0.07 ^{cd}
P40/L10	11.52 ± 0.27	6.82 ± 0.06^{c}	4.57 ± 0.06^{cde}	3.75 ± 0.07^{de}
P40/L15	10.89 ± 0.25	7.65 ± 0.05^{e}	4.61 ± 0.05^{def}	3.96 ± 0.07^{de}
P45/L05	11.67 ± 0.28	5.35 ± 0.05^{a}	4.82 ± 0.05 ^{f}	4.03 ± 0.07^{ef}
P45/L10	10.96 ± 0.29	6.21 ± 0.05^{bd}	5.21 ± 0.04 ^{g}	4.27 ± 0.08 ^{fg}
P45/L15	10.76 ± 0.27	6.96 ± 0.06 ^{cd}	5.37 ± 0.04 ^{g}	4.42 ± 0.07 ^{g}
Means of main effect				
P35	10.57	8.6	4.27^{A}	3.12^{A}
P40	11.21	6.83	4.53^{A}	3.79^{B}
P45	11.13	6.17	5.13^{B}	4.24^{B}
L05	11.11	6.2	4.46	3.52
L10	11.01	7.23	4.68	3.72
L15	10.79	8.17	4.78	3.91
Two-way ANOVA (P value)				
Protein	0.871	P < 0.001	P < 0.001	P < 0.001
Lipid	0.368	P < 0.001	P < 0.001	P < 0.001
Interaction	0.494	P < 0.001	0.005	0.555

(*Continued*)

Table 4. (Continued).

	Lipogenic enzymes		Amino acid catabolizing enzymes		
Experimental diets	Glucose 6 phosphate dehydrogenase	Malic enzyme	Alanine aminotransferase	Aspartate aminotransferase	Glutamate dehydrogenase
P35/L05	29.72 ± 0.26^{b}	2.52 ± 0.14	3.65 ± 0.06^{ab}	6.10 ± 0.06^{b}	5.60 ± 0.10
P35/L10	26.42 ± 0.41^{a}	2.47 ± 0.15	3.54 ± 0.04^{a}	5.96 ± 0.06^{ab}	5.55 ± 0.06
P35/L15	24.34 ± 0.55^{a}	2.38 ± 0.17	3.56 ± 0.05^{a}	5.62 ± 0.07^{a}	5.53 ± 0.08
P40/L05	34.54 ± 0.48^{d}	2.75 ± 015	3.80 ± 0.09^{ad}	6.55 ± 0.09^{b}	5.65 ± 0.08
P40/L10	33.17 ± 0.34^{cd}	2.68 ± 0.13	3.65 ± 0.06^{a}	6.25 ± 0.07^{ab}	5.62 ± 0.09
P40/L15	31.65 ± 0.34^{bc}	2.62 ± 0.14	3.71 ± 0.03^{ac}	6.17 ± 0.10^{ab}	5.56 ± 0.08
P45/L05	39.67 ± 0.80^{e}	2.95 ± 0.13	4.06 ± 0.05^{de}	6.97 ± 0.06^{c}	5.70 ± 0.05
P45/L10	37.43 ± 0.82^{e}	2.81 ± 0.10	3.92 ± 0.08^{bcde}	6.80 ± 0.10^{c}	5.65 ± 0.07
P45/L15	34.27 ± 0.49^{cd}	2.75 ± 0.12	3.78 ± 0.04^{ae}	6.83 ± 0.08^{c}	5.62 ± 0.05
Means of main effect					
P35	26.82^{A}	2.46^{A}	3.58^{A}	5.89^{A}	5.56
P40	33.12^{B}	2.68^{B}	3.72^{AB}	6.32^{A}	5.61
P45	37.12^{B}	2.84^{B}	3.92^{B}	6.87^{B}	5.65
L05	34.64	2.74	3.84	6.54	5.65
L10	32.34	2.65	3.7	6.34	5.6
L15	30.08	2.58	3.71	6.21	5.57
Two-way ANOVA (P value)					
Protein	$P < 0.001$	0.395	0.011	$P < 0.001$	0.473
Lipid	$P < 0.001$	0.011	$P < 0.001$	$P < 0.001$	0.342
Interaction	0.112	0.997	0.395	0.115	0.999

Note. Data are presented as Mean ± SE of three determinations ($n = 3$). Means with different superscripts in a column differ significantly ($P < 0.05$).

Table 5. Whole body carcass composition (%) of *Ompok bimaculatus* fingerlings fed varying dietary protein and lipid levels for 90 days.

Experimental diets	Moisture	Crude protein	Crude lipid	Crude ash
P35/L05	79.75 ± 2.19	16.21 ± 0.05^{b}	2.5 ± 0.02^{a}	1.78 ± 0.02
P35/L10	79.54 ± 1.25	16.61 ± 0.04^{d}	2.71 ± 0.02^{b}	1.81 ± 0.02
P35/L15	78.67 ± 1.57	16.42 ± 0.03^{c}	2.89 ± 0.03^{e}	1.83 ± 0.01
P40/L05	78.82 ± 2.54	16.71 ± 0.02^{d}	2.82 ± 0.02 ^{cd}	1.86 ± 0.03
P40/L10	79.27 ± 2.28	16.52 ± 0.03 ^{cd}	2.85 ± 0.04^{de}	1.76 ± 0.01
P40/L15	79.06 ± 1.42	16.40 ± 0.04^{c}	2.91 ± 0.01^{e}	1.74 ± 0.02
P45/L05	79.98 ± 2.25	16.04 ± 0.02^{ab}	2.74 ± 0.01^{bc}	1.84 ± 0.01
P45/L10	78.57 ± 2.05	15.95 ± 0.03^{a}	2.76 ± 0.01^{bd}	1.76 ± 0.01
P45/L15	78.86 ± 2.78	15.87 ± 0.04^{a}	2.82 ± 0.02 ^{cd}	1.76 ± 0.03
Means of main effect				
P35	79.32	16.41^{B}	2.70	1.80
P40	79.05	16.54^{B}	2.86	1.78
P45	79.13	15.95^{A}	2.77	1.78
L05	79.51	16.32	2.68	1.82
L10	79.12	16.36	2.77	1.77
L15	78.86	16.23	2.87	1.77
Two-way ANOVA (P value)				
Protein	0.897	$P < 0.001$	$P < 0.001$	0.388
Lipid	0.913	$P < 0.001$	$P < 0.001$	0.387
Interaction	0.951	$P < 0.001$	$P < 0.001$	0.434

Note. Data are presented as Mean ± SE of three determinations ($n = 3$). Means with different superscripts in a column differ significantly ($P < 0.05$).

Discussion

Nutritional requirements are the reflection of growth and physiology in fish, as dietary nutrients are utilized for energy metabolism, tissue restoration, and growth (Wang et al. 2013). The present study aimed at determining the optimal combination of dietary protein and lipid levels to support growth, feed utilization, and metabolic functions in *Ompok bimaculatus* fingerlings under captive conditions. The use of lipid in the diets has to be critically evaluated as excessive lipid may not only interfere with pellet quality and shelf life of the diets but also growth and feed utilization in fish (Li et al. 2010). Thus, an appropriate lipid level in fish diets is of great importance. On the other hand, dietary protein is the major factor affecting growth of fish along with feed cost (Lovell 1989). In general, increasing the dietary protein level could be linked with improved fish production, especially for carnivorous fish (Lee, Jeon, and Lee 2002). Dietary lipid levels might have considerable influence on the effect of protein on the growth and normal well-being of fish. To the best of our knowledge, this is the first report on the effects of varying dietary protein and lipid levels on growth, feed utilization, body composition, and metabolic enzymes of *O. bimaculatus* fingerlings. In the presently reported study, the maximum weight gain and SGR were noticed in *O. bimaculatus* fingerlings fed the diet P40L05 with 40% crude protein and 5% lipid. However, SGR and FCR did not vary significantly from the fish fed diet with 35% crude protein and 10% lipid (P35L10). Therefore, improved growth and feed utilization efficiency associated with increasing lipid content of the diet from 5% to 10% at

the 35% dietary protein level might indicate the protein-sparing effect of dietary lipid in *O. bimaculatus* fingerlings, as suggested elsewhere (Sagada et al. 2017). Such protein-sparing effect was also noticed in several fish species, including bagrid catfish (*Pseudobagrus fulvidraco*) (Kim and Lee 2005), blunt snout bream (*Megalobrama amblycephala*) (Li et al. 2010), Asian catfish (*Pangasius hypophthalmus*) (Liu et al. 2011), juvenile northern snakehead fish (*Channa argus*) (Sagada et al. 2017), and Juvenile Yellow Drum (*Nibea albiflora*) (Wang et al. 2018).

In the present report, an increase of crude protein from 35% (with 10% lipid) to 45% did not cause significant improvement in fish growth and feed utilization efficiency. This could be an indication that the minimal amino acid requirement was met at 35% protein and beyond which excess protein might be extravagantly converted to energy and nitrogenous excreta. Similar observations were documented in previous reports on diverse fish species (Sagada et al. 2017; Tu et al. 2015), including fingerlings of *O. bimaculatus* (Biswas et al. 2020). Further, SGR tended to decrease and FCR tended to increase with increasing the lipid levels from 5% to 15% in the diets with higher levels of protein (40% and 45%). This might indicate that elevating the dietary lipid levels from 5% to 15% did not induce protein-sparing action in *O. bimaculatus* fingerlings fed diets with ≥ 40% dietary protein. In accordance to our report, Wang et al. (2013) indicated that golden pompano (*Trachinotus ovatus*) reared in net pens attained higher weight gain (%) at the dietary lipid level of 6.5% than at 12.5% when fed with varying dietary protein levels (33%–50%). Similarly, juveniles of ayu (*Plecoglossus altivelis*) (Lee, Jeon, and Lee 2002) and flounder (*Paralichthy solivaceus*) (Lee and Kim 2005) exhibited faster growth at the dietary crude lipid levels of 6.0%–6.5% than at 13%–19%. Thus, as in the presently reported study, previous works also confirmed that protein-sparing action could not be sustained beyond certain levels of dietary protein and lipid (Sagada et al. 2017). Furthermore, the present study recorded a marginal improvement in weight gain (%) with increasing the protein level from 35% (diet P35L10, 10% lipid) to 40% (diet P40L05, 5% lipid). Incorporation of 40% crude protein for *O. bimaculatus* was in agreement with the protein requirement of catfish, as reported previously (BIS 2014).

Growth is extremely influenced by digestive and absorptive physiology, which affects utilization of the ingested nutrients in an organism (Sagada et al. 2017). The capability of fish to efficiently utilize a given diet could be apprehended by the activity of digestive enzymes and their responsiveness toward diverse dietary compositions (Pérez-Jiménez et al. 2009). Thus, adaptations of the digestive enzymes to feeding and related metabolic alterations might be used as a clue for formulation of nutritionally effective diets (Lundstedt, Melo, and Moraes 2004). However, to the

authors' knowledge, diet-related changes in the activity of digestive enzymes in the butter catfish fingerlings appears to be mostly unexplored and warrants more insight for better understanding of the relationship between digestive as well as metabolic enzymes and growth performance. In the presently reported study, increasing dietary protein and lipid levels were inversely proportional to dietary carbohydrate content and associated with a decrease in α-amylase activity. Our study was in accordance with the results obtained in hybrid catfish (*Clarias batrachus* × *C. gariepinus*) fed diets with high protein levels (Giri et al. 2003) and contradicted with African catfish *C. gariepinus* (Ali and Jauncey 2004). The present study noticed to enhance alkaline protease activity with increasing dietary protein levels, which was in agreement with the preceding reports on hybrid catfish (*Clarias batrachus* × *C. gariepinus*) (Giri et al. 2003), spotted sorubim (*Pseudoplatystoma corruscans*) (Lundstedt, Melo, and Moraes 2004), silver barb (*Puntius gonionotus*) (Mohanta et al. 2008), and rice field eel (*Monopterus albus*) (Ma et al. 2014). Thus, it could be suggested that dietary protein levels might ascertain protease secretion and augment proteolytic activities in the digestive tract (Huang, Zhao, and Luo 2013). Although results on the acid protease activity recorded in the present study did not comply with this hypothesis, it was similar to the observation of Santos et al. (2020) depicting an increase in the CP level from 36% to 42% associated with a decrease in acid protease activity. On the contrary, protease activity was not significantly affected by dietary protein levels in gibel carp (*Carassius auratus gibelio*) (Ye et al. 2015) and juvenile northern snakehead fish (*C. argus*) (Sagada et al. 2017). Several studies reported a positive correlation between intestinal lipase activity and dietary lipid levels (Sagada et al. 2017; Wang et al. 2018). However, in the present study, lipase activity didn't differ significantly with increasing lipid contents at higher CP levels, supporting our observation that there was no protein-sparing effect of lipid in juvenile *O. bimaculatus* at ≥ 40% dietary protein level.

The present study evaluated activities of some key metabolic enzymes to appraise consequences of the varying dietary protein-lipid levels in juvenile *O. bimaculatus*. Neoglucogenesis is an important pathway for glucose homeostasis, where glucose is synthesized from noncarbohydrate precursors (e.g., amino acid, glycerol, lactate). The present study recorded increased activities of the neoglucogenic enzymes (G6P and FBP) with an increase in both dietary protein and lipid levels. Enhanced activity of the neoglucogenic enzymes noticed in this study linked with reduced activity of digestive amylase might indicate production of glucose by neoglucogenesis so as to accomplish the energy demand in *O. bimaculatus*, which is supposed to be a carnivorous fish (Paul et al. 2021). The major glycolytic enzyme, HK, was not significantly affected by the varying dietary protein and lipid levels in the present study.

However, activity of another glycolytic enzyme PK decreased and neoglucogenic enzyme FBP increased with an increase in the dietary protein, which was in accordance with the observation made by Wang et al. (2018) in brown trout (*Salmo trutta fario*).

The lipogenic enzyme, G6PD, catalyzes the first step of the HMP-shunt (pentose phosphate pathway) generating NADPH for lipid biosynthesis and stress management (Pandolfi et al. 1995). The present study noticed around 10 times higher activity of G6PD than that of ME with an increase in dietary protein levels, suggesting that NADPH production for lipogenesis is mainly obtained from the pentose-phosphate pathway (Sá, Pousão-Ferreira, and Oliva-Teles 2007). Further, activity of the G6PD was inhibited in the present study by an increase in the dietary lipid, which was similar to the observations recorded in brown trout (*Salmo trutta fario*) (Wang et al. 2018) and Senegalese sole (*Solea senegalensis*) (Guerreiro et al. 2012). In contradiction, Sá, Pousão-Ferreira, and Oliva-Teles (2006) couldn't detect a significant effect of dietary protein or lipid levels on G6PD activity in White sea bream (*Diplodus sargus*). ME is associated with NADP-dependent oxidative decarboxylation (malate to pyruvate and carbon dioxide) generating NADPH that is likely to be utilized for lipid biosynthesis; GDH is considered a sensitive stress indicator (Paul et al. 2021; Susan et al. 2010). ME and GDH activities were more or less unaffected by the dietary protein and lipid levels, which was consistent with the findings of Wang et al. (2018) in brown trout. Consequently, results of the present study might suggest that increasing dietary lipid at the same protein level could neither improve lipogenesis nor induce stress in *O. bimaculatus* fingerlings.

ALT and AST are the main transaminases in fish liver (Sá, Pousão-Ferreira, and Oliva-Teles 2007). In the present study, activities of the amino acid-catabolizing (ALT and AST) enzymes were influenced by the dietary protein and lipid levels. The activities of ALT and AST were proportional to the increasing dietary protein levels, which was similar to the observations made by Sá, Pousão-Ferreira, and Oliva-Teles (2007) in White Sea bream and Wang et al. (2018) in brown trout. Increased ALT and AST activities might be indicative of increased protein catabolism at high dietary protein levels corroborated with the increased activities of alkaline proteases. Further, increased dietary lipid at the same protein level was associated with a decrease in the activities of both ALT and AST, suggesting use of lipid as an energy source along with reduced catabolism of protein. Similar observations were reported in other species, e.g., *Sparus aurata* (Fernández et al. 2007) and *Salmo trutta fario* (Wang et al. 2018).

Increasing dietary lipid levels might have a significant effect on the carcass quality of fish (Cowey 1993). The presently reported study noticed an increased accumulation of body lipid with an increase in the dietary

lipid contents at each protein level. Similar effects were also detected in numerous species, e.g., rockfish (*Sebastes schlegelii*) (Lee, Jeon, and Lee 2002), Eurasian perch (*Perca fluviatilis*) (Mathis, Feidt, and Brun-Bellut 2003), bagrid catfish (*P. fulvidraco*) (Kim and Lee 2005), cobia (*Rachycentron canadum*) (Craig, Schwarz, and McLean 2006), Malabar grouper (*Epinephelus malabaricus*) (Williams 2007), brown-marbled grouper (*Epinephelus fuscoguttatus*) (Shapawi et al. 2014), and juveniles of snakehead fish (*Channa argus*) (Sagada et al. 2017). In contrast, Paul et al. (2011) could not detect any difference in carcass lipid in another species of butter catfish (*O. pabda*) by feeding different levels of lipid. In the present study, the diets containing the maximum protein level were not associated with the highest whole body protein in *O. bimaculatus* fingerlings, which was comparable with the other findings (Rahimnejad et al. 2015; Tuan and Williams 2007). On the contrary, several studies suggested a significant increase in the body protein content with the increase in the dietary protein level (Chen et al. 2010; Wang et al. 2016). Further, whole body moisture and ash contents of *O. bimaculatus* fry in the present study were not affected by the dietary treatments. In accordance, varying dietary protein and lipid levels did not have any effect on whole body ash content of the grouper (*E. fuscoguttatus*) (Shapawi et al. 2014). Although numerous studies indicated a decrease in whole body moisture content with increasing dietary protein (Sagada et al. 2017; Wang et al. 2016) and lipid levels (Lim et al. 2009; Luo et al. 2005; Sagada et al. 2017).

Conclusion

Considering the highest weight gain (%) and SGR (% per day), the lowest FCR, and 95% survivability achieved with the diet P40L05 together with the carnivorous nature of the species, it may be concluded that 40% crude protein with 5% lipid was optimum for the growth and feed utilization of *O. bimaculatus* fingerlings. In addition, since no protein-sparing effect of lipid in juvenile *O. bimaculatus* was noticed beyond the 35% dietary protein level, excessive dietary lipid should be avoided for economic diet formulation as well as health concerns (Craig, Schwarz, and McLean 2006). Variations in some key intermediary metabolic enzymes owing to alterations in the dietary components recorded in the present study might suggest high metabolic adaptability of this species to the varying dietary protein and lipid levels. Moreover, information generated on growth, body composition, and digestive as well as metabolic function of the butter catfish fingerlings might aid in the formulation of practical diets for captive rearing of the highly valued catfish *O. bimaculatus*.

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