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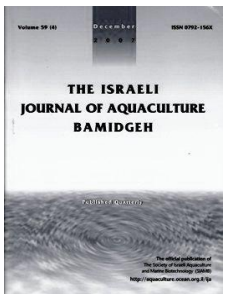

Effect of Dietary Protein and Lipid Levels on Growth and Nutrient Utilization of Freshwater Angelfish *Pterophyllum scalare*

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Abstract

Nine semi-purified diets containing three levels of protein (300, 350, 400 g/kg) and three levels of lipid (60, 80, 100 g/kg) were fed *ad libitum* to juvenile freshwater angelfish *Pterophyllum scalare* (1.64±0.01 g) to determine the optimum dietary protein and lipid levels. Fish were stocked in 27 flow-through fiber-reinforced plastic tanks with 100 l water (10 fish/replicate) and fed 60 days. Fish were batch-weighed every 15 days to determine growth. The dietary protein level had a significant effect on protein efficiency rate (PER) but not on weight gain, feed conversion ratio (FCR), or specific growth rate (SGR). In contrast, the dietary lipid level had a significant effect on weight gain, FCR, and SGR but not on PER. The interaction of dietary protein and lipid had a significant effect on weight gain, FCR, SGR, and PER. No protein-sparing effect of the lipid was observed. Fish fed the diet containing 300 g protein and 60 g lipid per kg diet had a significantly better weight gain, SGR, PER, and FCR.

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Introduction

Feed constitutes 50-60% of the total production cost in aquaculture. Protein is the single most expensive component of fish feeds. As protein is the most essential and expensive ingredient in prepared feeds, it should be carefully incorporated within diets to meet the nutritional needs of the cultured organism and reduce feed costs. Optimally, dietary protein should be utilized for growth rather than energy (Yang et al., 2003; Mohanta et al., 2008).

The end product of protein metabolism in fish is ammonia, which is a nitrogenous waste product in aquaculture. Accumulation of nitrogenous excretory products can cause deterioration of water quality. Dietary lipid used for energy can spare proteins and reduce nitrogenous waste production (Ghanawi et al., 2011). One of the challenges that face fish nutritionists is to substitute expensive dietary protein with inexpensive non-protein energy sources such as lipid. Lipid is an important source of energy while protein supports mainly growth. In lipid-deficient diets, expensive protein is used for energy and life support rather than for growth. Therefore, optimization of dietary protein and lipid levels is very important to formulate cost-effective practical diets for fish.

Ornamental fish are reared in a very limited water environment where the chances of water quality deterioration due to nitrogen and organic loads derived from unused feed and excreta are greater than in aquaculture of food fish. In the present study, we evaluated the effect of dietary protein and lipid levels on growth and nutrient utilization in freshwater angelfish *Pterophyllum scalare*, a popular high-valued freshwater ornamental fish of the Cichlidae family.

Materials and Methods

Experimental diets. Nine semi-purified diets with three levels of protein (30%, 35%, 40%) and three levels of lipid (6%, 8%, 10%) were prepared with different protein/lipid ratios (Table 1). The dietary ingredients (except gelatin) were weighed and put into nine aluminum trays, one for each diet. Water was heated in a 1-l beaker to 80°C using an electric heater and the gelatin was dissolved in it with slow stirring. The dextrin and vitamin/mineral mixture were thoroughly mixed with oil, added to the gelatin, blended in a mixer, and added to the trays. Finally, carboxymethyl cellulose was added to make a dough. The dough was steam cooked for 5 min in a pressure cooker and passed through a hand pelletizer to obtain 2-mm diameter pellets. The pellets were oven-dried at 60°C

Table 1. Ingredients and chemical composition of experimental diets for angelfish.

	Dietary treatment (%protein/%lipid)								
	30/6	*30/8*	*30/10*	*35/6*	*35/8*	*35/10*	*40/6*	*40/8*	*40/10*
Casein[1]	27.5	27.5	27.5	32.0	32.0	32.0	36.5	36.5	36.5
Corn starch[1]	28.0	24.9	21.6	23.3	20.0	16.8	18.5	15.3	12.0
α-Cellulose[1]	12.6	15.1	17.7	13.7	16.4	19.0	15.0	17.6	20.2
Dextrin[1]	12.0	10.6	9.3	10.0	8.6	7.2	7.9	6.5	5.2
Gelatin[1]	6.9	6.9	6.9	8.0	8.0	8.0	9.1	9.1	9.1
Sunflower[2]/cod liver oil[3] (1:1)	6.0	8.0	10.0	6.0	8.0	10.0	6.0	8.0	10.0
Vitamin/mineral mix[4]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Carboxymethyl cellulose[1]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Chemical composition (%, dry matter basis)									
Crude protein	29.75	30.62	30.18	35.00	35.87	35.43	39.37	40.25	39.81
Ether extract	6.4	7.8	10.4	6.2	8.2	9.6	6.4	8.4	10.1
Ash	4.6	4.2	3.9	4.5	4.3	3.7	4.4	4.2	3.8
Nitrogen free extract (NFE)	59.25	53.78	55.52	54.30	51.63	51.27	49.83	47.15	46.29
Gross energy (MJ/kg)	17.30	17.43	17.94	17.14	17.47	17.85	17.05	17.51	17.93

[1] Himedia, Mumbai, India
[2] Marico Industries Ltd., Mumbai, India
[3] Universal Medicare Private Ltd., Mumbai, India
[4] EMix Plus, Mumbai, India, per kg: vitamin A 22,00,000 IU; vitamin D3 440,000 IU; vitamin B2 800 mg; vitamin E 300 mg; vitamin K 400 mg; vitamin B6 400 mg; vitamin B12 2.4 mg; calcium pantothenate 1,000 mg; nicotinamide 4 g; choline chloride 60 g; Mn 10,800 mg; I 400 mg; Fe 3000 mg; Zn 2000 mg; Cu 800 mg; Co 180 mg; Ca 200 g; P 120 g; L-lysine 4 g; DL-methionine 4 g; selenium 20 ppm

and stored in a refrigerator at 4°C until use. Fresh feed was prepared in every 15 days using the same lot of ingredients to prevent any change in the nutrient composition of the experimental feeds.

Experimental design and fish maintenance. One thousand uniformly-sized freshwater angelfish fingerlings (average wt 1.4 g) were procured from the local market (Panaji, Goa, India) and acclimatized to laboratory conditions in the Fisheries Wet Laboratory of the ICAR Research Complex for Goa, in Goa, India, in four 500-l flow-through fiber-reinforced plastic tanks with continuous aeration. During acclimatization, the fish were fed a semi-purified diet (30% protein; 16.7 MJ/kg gross energy). After 15 days of acclimatization, the fish were stocked at 10 fish per tank in twenty-seven 300-l indoor flow-through fiber-reinforced plastic tanks containing 100 l water. The water flow was maintained at 0.5 l/min using seasoned ground water and the natural light cycle was 12 h light/12 h darkness. Fish were fed *ad libitum* four times a day (08:00, 11:00, 14:00, 17:00) for 60 days and batch-weighed every 15 days to determine growth and examine them for disease. The final weight of each replicate was recorded at the end of experiment on day 60.

Proximate chemical analysis of feed. The proximate compositions of the diets were analyzed in triplicate (AOAC, 1990). Dry matter was estimated by oven drying the samples at 105°C until a constant weight was reached. Crude protein (%) was calculated by estimating the nitrogen content by the micro-Kjeldahl method and multiplying by 6.25. Ether extract was determined by solvent extraction with petroleum ether, boiling point 40-60°C, for 10-12 h. Total ash content was determined by incinerating the sample at 650°C for 6 h and crude fiber by acid digestion (1.25%) followed by alkali digestion (1.25%). Gross energy in diets, fecal samples, and fish bodies was calculated using a bomb calorimeter (model 1341, Parr Instrument Co., Moline, Illinois, USA).

Water analyses. Water quality was analyzed every 15 days (APHA, 1989). Temperature ranged 26.5-28.7°C, pH 7.4-7.8, dissolved oxygen 6.7-7.2 mg/l, total alkalinity 112.2-117.4 mg $CaCO_3$/l, total hardness 105.1-108.9 mg $CaCO_3$/l, ammonia nitrogen (NH_3-N) 0.06-0.14 mg/l, nitrate nitrogen (NO_3^{-1}-N) 12.4-17.9 mg/l, nitrite nitrogen (NO_2^{-1}-N) 0.08-0.11 mg/l, and phosphate (P_2O_5) 0.03-0.05 mg/l.

Statistical analysis. Data are expressed as means±standard error. Two-way analysis of variance (ANOVA) was used to determine differences between protein and lipid concentrations (3 × 3 factorial) using SPSS 11.5 statistical software. Tukey's HSD multiple comparison test was used to determine significant differences between treatment means. Differences were considered significant when $p>0.05$.

Results

There were no mortality, abnormality, or disease incidents in any of the treatment groups. Dietary protein levels significantly affected PER but not weight gain, SGR, or FCR (Table 2). Dietary lipid levels significantly affected weight gain, SGR, and FCR but not PER. The interaction of dietary protein and lipid significantly affected weight gain, SGR, FCR, and PER. Irrespective of dietary protein level, fish fed 6% lipid had a significantly higher weight gain, SGR, and PER and lower FCR than the other dietary lipid groups.

Table 2. Effect of diets with different protein and lipid levels on growth and nutrient utilization of juvenile angelfish (*Pterophyllum scalare*), means±SE, n=3.

Treatment	*Initial wt (g)*	*Final wt (g)*	*Wt gain (g)*	*SGR*	*FCR*	*PER*
Protein (%/diet)						
30	1.63±0.01	3.30±0.08	1.67±0.08	2.34±0.07	1.54±0.03	2.16±0.05^{c}
35	1.62±0.01	3.25±0.11	1.63±0.10	2.30±0.10	1.57±0.03	1.80±0.04^{b}
40	1.66±0.01	3.26±0.10	1.60±0.09	2.24±0.09	1.61±0.03	1.57±0.04^{a}
p value	0.09	0.93	0.88	0.73	0.29	0.01
Lipid (%/diet)						
6	1.65±0.01	3.61±0.03^{c}	1.96±0.03^{c}	2.61±0.03^{c}	1.47±0.01^{a}	1.99±0.09
8	1.65±0.01	3.19±0.05^{b}	1.54±0.04^{b}	2.20±0.04^{b}	1.57±0.02^{b}	1.82±0.09
100	1.62±0.01	3.02±0.04^{a}	1.40±0.04^{a}	2.07±0.05^{a}	1.68±0.02^{c}	1.72±0.08
p value	0.23	0.01	0.01	0.01	0.01	0.11

Table 2 (cont.).

Protein x lipid (%/diet)						
30 x 6	1.65±0.03	3.60±0.04^{b}	1.95±0.04^{b}	2.60±0.06^{b}	1.44±0.02^{a}	2.33±0.04^{f}
30 x 8	1.64±0.02	3.21±0.06^{a}	1.57±0.06^{a}	2.24±0.06^{a}	1.53±0.03^{b}	2.13±0.04^{e}
30 x 10	1.61±0.01	3.09±0.07^{a}	1.48±0.07^{a}	2.17±0.08^{a}	1.64±0.03^{cd}	2.01±0.03^{d}
35 x 6	1.63±0.01	3.61±0.04^{b}	1.99±0.04^{b}	2.66±0.04^{b}	1.47±0.02^{ab}	1.94±0.03^{d}
35 x 8	1.64±0.03	3.18±0.11^{a}	1.54±0.08^{a}	2.21±0.06^{a}	1.56±0.03^{bc}	1.78±0.04^{c}
35 x 10	1.60±0.01	2.96±0.11^{a}	1.36±0.10^{a}	2.04±0.12^{a}	1.68±0.03^{d}	1.68±0.02^{b}
40 x 6	1.67±0.03	3.61±0.10^{b}	1.94±0.07^{b}	2.57±0.05^{b}	1.50±0.02^{ab}	1.69±0.02^{bc}
40 x 8	1.66±0.03	3.17±0.11^{a}	1.50±0.10^{a}	2.14±0.10^{a}	1.63±0.03^{cd}	1.53±0.03^{a}
40 x 10	1.65±0.02	3.01±0.02^{a}	1.36±0.03^{a}	2.00±0.06^{a}	1.71±0.03^{d}	1.47±0.02^{a}
p value	0.44	0.01	0.01	0.01	0.01	0.01

[1] Specific growth rate = 100(ln final wt - ln initial wt)/days
[2] Feed conversion ratio = total dry wt of feed intake/total live wt gain
[3] Protein efficiency ratio = total live wt gain/total protein intake

Discussion

The protein and lipid requirements of freshwater ornamental fish range 29-50% and 6-10%, respectively (Lochmann and Phillips, 1994; Elangovan and Shim, 1997; Chong et al., 2000, 2004; Kruger et al., 2001; Martino et al., 2002; Royes et al., 2005, 2006; Ling et al., 2006; Singh et al., 2007; Ribeiro et al., 2007; Ng et al., 2008; Ergiin et al., 2010). We observed that angelfish require 30% protein and 6% lipid for best growth, within the range for other freshwater ornamental fish. Further, our results show that dietary lipid individually and in combination with protein significantly improve weight gain and SGR.

In our study, the FCR was unaffected by the dietary protein level but significantly affected by the lipid level, alone and in combination with protein. Since FCR rose with the increase in dietary lipid, we suggest that angelfish have a limited capacity to utilize dietary lipids.

Wide variations in PER were reported for Malaysian mahseer (1.32-1.50; Ng et al., 2008), juvenile swordtail (0.55-0.89; Kruger et al., 2001), and silver dollar (1.08-3.08; Singh et al., 2007). The PER obtained in the present study (1.47-2.33) is higher or within the above ranges. In our study, dietary lipid alone had no significant effect on PER. In contrast, the dietary protein level alone and in combination with lipid significantly affected PER. PER significantly dropped as dietary protein increased for every lipid level, probably due to utilization of more dietary protein as energy when high protein diets are fed to fish. Similarly, PER values dropped as dietary lipid increase for every protein level.

Protein utilization for growth may be improved by partially replacing dietary protein with lipid and/or carbohydrate to produce a protein-sparing effect. The protein-sparing effect associated with increased dietary lipid was reported for several species of fish. In the present study, the concomitant decrease of dietary protein from 40% to 30% and increase of dietary lipid from 6% to 10% resulted in significantly poorer growth and FCR. Similarly, growth and FCR were significantly poorer when dietary protein decreased from 35% to 30% and dietary lipid increased from 6% to 10%. This implies a lack of protein-sparing effect of lipid on freshwater angelfish. Thus, we conclude that *P. scalare* fingerlings have a limited capacity to utilize dietary lipid. Based on these results, the optimum dietary protein and lipid levels for juveniles *P. scalare* are 30% and 6%, respectively. This information may be helpful in formulating cost-effective nutritionally-balanced practical diets for juvenile angelfish.

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