Optimal Dietary Protein and Lipid Levels for Growth of Long-nosed Barbel, *Hemibarbus longirostris*

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A 10-week feeding trial with four dietary protein levels (22%, 32%, 42% and 52%) and two dietary lipid levels (8% and 17%) was conducted to investigate the optimum dietary protein and lipid levels for growth of long-nosed barbel fingerlings. Survival rate of fish was not affected by either the dietary protein or the dietary lipid level. Weight gain and feed efficiency were affected by the dietary protein level ($P<0.01$), but not by the lipid level, and increased with the dietary protein level at the both lipid levels. Weight gain and feed efficiency of fish fed the 52% protein diets with 8-17% dietary lipids were not significantly different from those of fish fed the 42% protein diets with 8-17% dietary lipids and 32% protein diet with 17% dietary lipid. Daily feed intake of fish was not affected by either dietary protein or dietary lipid level. Protein efficiency ratio and protein retention rate of fish fed the 32% protein diet with 17% dietary lipid were significantly higher than those of fish fed the 52% protein diets with 8-17% dietary lipids. Moisture content of fish fed the diets containing 8% lipid were higher than those of fish fed the diets containing 17% dietary lipid at each protein level. Crude lipid content of fish fed the diets containing 17% dietary lipid were higher than that of fish the fed the diet containing 8% dietary lipid at each protein level. The results of this study indicated that 32% protein and 17% lipid could be the optimum dietary level for growth of juvenile long-nosed barbel.

Key words: Dietary protein, Dietary lipid, Long-nosed barbel, *Hemibarbus longirostris*

Introduction

In the nutrition studies of fish, determining the dietary protein requirement for the growth of fish is a primary consideration because protein is not only the major constituent of fish body, but also it has critical functions as enzyme and hormone. Generally, increasing protein level in diets up to optimal level can lead to improve fish production, especially for carnivorous fish (NRC, 1993). Protein utilization for animal growth may be improved by partially replacing dietary protein with lipid and/or carbohydrate to produce a protein-sparing effect. However, excessive energy in diets can lead to increase body lipid deposition and growth reduction of fish because of lacking of necessary nutrient for growth resulted from reduction of feed consumption (Daniels and Robinson, 1986). On the contrary, insufficient non-

protein energy in diets causes protein waste as the proportion of dietary protein used for energy increase, and ammonia excreted after amino acids are metabolized can reduce water quality (Phillips, 1972; Shyong et al., 1998). Therefore, it is important to improve dietary protein utilization for body protein synthesis rather than for energy purposes. A protein-sparing effect associated with increasing dietary energy level has been reported for several species of fish (Cho and Kaushik, 1990). Higher energy levels generally come from increased dietary lipid as lipid is an energy-dense nutrient and readily metabolized by fish (NRC, 1993).

The long-nosed barbel, *Hemibarbus longirostris* belonging to the *Cyprinina* family, is widely distributed in Korea, Japan and China (Kim and Park, 2007). However, population of this species is currently decreasing by overexploitation, environmental pollution and other causes. Consequently, in

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order to increase the population resources, it is essential to develop aquaculture techniques such as for artificial larval mass production and development of feed quality for growth. Development of nutriationally well-balanced and cost-effective feeds is essential to increase the production of the fish. However, no information on the essential nutrients requirements of *H. longirostris* is available. Therefore, this study was conducted to determine the optimal dietary protein and lipid levels for growth of long-nosed barbel.

Materials and Methods

Fish and feeding trial

Juveniles *H. longirostris* were produced at Cheong Buk Inland Fisheries Research Institute (Korea). They were acclimated to laboratory conditions for one month by feeding a commercial diet containing 50% protein and 7% lipid. After the conditioning period, fish (average weight, 2.3±0.1 g) were randomly distributed into 100 L plastic tanks (60 L of water each) of a flow-through aquarium system at a

density of 30 juvenile per tank. Three replicate groups of fish were hand-fed to visual satiety three times a day (6 days a week) for 10 weeks. Satiation feeding was determined by the point of cessation of voluntary feeding activity by fish. Fish were carefully fed by the same person during the feeding trial. Water was continuously supplied to each aquarium at a flow rate of 2 L/min. At the initiation and the end of the feeding trial, fish in each tank was collectively weighed by an electric balance after being starved for 24 h. The water temperature was maintained at 23.0± 1.0°C, and photoperiod was fixed at a natural condition during the feeding period.

Design and preparation of experimental diets

Ingredients and proximate compositions of the experimental diets are presented in Table 1. A 4 (crude protein of 22%, 32%, 42% and 52%)×2 (crude lipid of 8% and 17%) factorial design with triplicates was applied. Eight experimental diets were formulated. Fish meal was used as major protein source in diet. Squid liver oil and soybean oil were added as lipid sources, and dextrin and wheat flour as car-

Table 1. Ingredients and proximate composition of the experimental diets

	Dietary protein levels (%)							
	22		32		42		52	
	Dietary lipid levels (%)							
	8	17	8	17	8	17	8	17
Ingredients (%)								
Herring meal	20	20	35	35	50	50	65	65
Wheat flour	40	40	30	30	20	20	10	10
Dextrin	18	10	18	10	18	10	18	10
Squid liver oil	4	4	3	3	2	2	1	1
Soybean oil	2	10	2	10	2	10	2	10
Vitamin premix[1]	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Mineral premix[2]	2	2	2	2	2	2	2	2
α-Cellulose[3]	12	12	8	8	4	4	-	-
Choline salt[3]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Proximate composition (%, DM basis)								
Crude protein	22.0	21.5	31.0	32.3	41.9	41.7	51.7	51.1
Crude lipid	7.9	16.8	7.9	17.5	8.1	17.2	7.9	16.5
Ash	3.7	3.8	5.6	5.7	7.6	7.4	9.4	9.7
Crude fiber[4]	13.4	13.4	9.3	9.3	5.1	5.1	1.0	1.0
Gross energy (kcal/g)[4]	4.7	5.2	4.8	5.3	4.9	5.4	4.9	5.4
GE/P ratio	21.4	24.1	15.4	16.4	11.6	12.9	9.5	10.5

[1]Vitamin premix contained the following vitamins diluted in cellulose (g/kg premix): ascorbic acid, 92.7; α-tocopheryl acetate, 14.5; thiamin, 2.1; riboflavin, 7.0; pyridoxine, 1.4; niacin, 27.8; Ca-D-pantothenate, 9.7; myo-inositol, 139.1; D-biotin, 4.2; folic acid, 0.5; p-amino benzoic acid, 13.9; K_3, 1.4; A, 0.6; D_3, 0.002; cyanocobalamin, 0.003.
[2]Mineral premix contained the following minerals (g/kg premix): $MgSO_4 \cdot 7H_2O$, 80; $NaH_2PO_4 \cdot 2H_2O$, 370; KCl, 130; Ferric citrate, 40; $ZnSO_4 \cdot 7H_2O$, 20; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_3 \cdot 6H_2O$, 0.15; KI, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4 \cdot H_2O$, 2.0; $CoCl_2 \cdot 6H_2O$, 1.0.
[3]Sigma, St. Louis, MO, USA.
[4]Calculated based on analyzed data of dietary ingredients.

bohydrate sources. All ingredients were thoroughly mixed with distilled water and pellets were prepared using a pelleting machine. The pellets were dried at room temperature overnight and stored at -30°C until used.

Sample collection and chemical analyses

Fifty fish at the initiation and all surviving fish at the end of the feeding trial were sampled after 36 h starvation and stored at -75°C for proximate composition analysis. Proximate composition of the experimental diets and whole body were analyzed according to standard methods (AOAC, 1995). Crude protein content (N×6.25) was determined by Kjeldahl method using an Auto Kjeldahl System (Buchi, Flawil, Switzerland). Moisture content was measured after oven drying at 105°C for 6 h. Crude lipid was determined by ether-extraction method (SER 148, Milano, Italy) and ash content was determined by a muffle furnace at 550°C for 4 h.

Statistical analysis

The data were subjected to one- and two-way ANOVA to test the effects of dietary protein and lipid. Where significant ($P<0.05$) differences were detected in the one-way ANOVA test, Duncan's multiple range test (Duncan, 1955) was used to rank the groups. The data are presented as mean±SE of three replicate groups. All statistical analyses were carried out using the SPSS Version 11.0 (SPSS Inc., Michigan Avenue, Chicago, Illinois, USA).

Results

Survival of fish was above 97% at the end of the feeding trial in all groups and was not affected by either dietary protein level or dietary lipid level. Weight gain, feed efficiency and protein efficiency ratio of long-nosed barbel fingerlings fed the experimental diets are given in Table 2. Weight gain and feed efficiency were not affected by dietary lipid level, but significantly ($P<0.001$) improved with dietary protein level. The best body weight gain of fish fed the 52% protein diets with 8-17% lipid was not significantly different from those of fish fed the 42% protein diets with 8-17% lipid and 32% protein diet with 17% lipid. Feed efficiency was improved as increasing of dietary protein level up to 42% and 32% protein with 8% and 17% lipid, respectively ($P<0.05$). Protein efficiency ratio was influenced by dietary protein level ($P<0.02$), but not by dietary lipid level. Protein efficiency ratio of fish fed 32% protein diet with 17% lipid level was significantly higher than that of fish fed the 52% protein diets ($P<0.05$).

Feed intake and nutrient retention are presented in Table 3. Although daily feed intake was not affected by dietary protein and lipid levels, daily protein and lipid intake were affected by dietary protein and lipid levels, respectively. Protein retention was influenced by dietary protein level ($P<0.04$), but not by dietary lipid level. Protein retention of fish fed the 32% protein diet with 17% lipid level was significantly

Table 2. Growth and feed efficiency of juvenile Long-nosed barbel fed the diets containing various protein and lipid levels for 10 weeks[1]

Dietary protein (%)	Dietary lipid (%)	Initial mean weight (g)	Weight gain (%)[2]	Feed efficiency (%)[3]	Protein efficiency ratio[4]
22	8	2.34 ± 0.08	38.7 ± 2.08^a	22.2 ± 1.84^a	1.00 ± 0.084^{ab}
	17	2.25 ± 0.07	40.5 ± 5.99^a	22.5 ± 3.34^a	1.05 ± 0.154^{ab}
32	8	2.38 ± 0.11	54.1 ± 7.46^{ab}	30.4 ± 3.16^{ab}	0.98 ± 0.102^{ab}
	17	2.33 ± 0.09	66.8 ± 4.83^{bc}	36.9 ± 0.91^{bc}	1.15 ± 0.027^b
42	8	2.37 ± 0.11	64.3 ± 5.18^{bc}	36.4 ± 1.06^{bc}	0.87 ± 0.026^{ab}
	17	2.34 ± 0.06	72.2 ± 11.21^{bc}	43.3 ± 4.38^c	1.03 ± 0.105^{ab}
52	8	2.31 ± 0.08	74.3 ± 4.41^c	39.6 ± 2.41^c	0.77 ± 0.049^a
	17	2.29 ± 0.06	74.6 ± 1.13^c	41.1 ± 2.19^c	0.80 ± 0.042^a
Two-way ANOVA					
Dietary protein			$P<0.001$	$P<0.001$	$P<0.02$
Dietary lipid			$P<0.2$	$P<0.06$	$P<0.1$
Interaction			$P<0.7$	$P<0.5$	$P<0.8$

[1]Values (mean±S.E. of three replications) in the same column not sharing a common superscript are significantly different ($P<0.05$).
[2]Weight gain (%)=(final body weight - initial body weight)×100 / initial body weight.
[3]Fish wet weight gain×100/feed intake (dry matter).
[4]Fish wet weight gain/protein intake.

Table 3. Feed intake and nutrient retention of juvenile Long-nosed barbel fed the diets containing various protein and lipid levels for 10 weeks[1]

Dietary protein (%)	Dietary lipid (%)	Daily feed intake (%)[2]	Daily protein intake (%)	Daily lipid intake (%)	Protein retention (%)[3]	Lipid retention (%)[3]
22	8	2.28 ± 0.074[ns]	0.50 ± 0.015[a]	0.18 ± 0.006[a]	15.4 ± 1.26[ab]	13.5 ± 1.77[ab]
	17	2.27 ± 0.175	0.49 ± 0.037[a]	0.38 ± 0.049[b]	15.6 ± 2.16[ab]	10.8 ± 2.33[a]
32	8	2.18 ± 0.123	0.68 ± 0.039[b]	0.17 ± 0.010[a]	15.7 ± 2.28[ab]	18.2 ± 2.50[b]
	17	2.12 ± 0.168	0.69 ± 0.054[b]	0.37 ± 0.184[b]	17.6 ± 0.58[b]	14.8 ± 2.13[ab]
42	8	2.08 ± 0.115	0.87 ± 0.047[c]	0.17 ± 0.012[a]	13.5 ± 0.43[ab]	15.8 ± 3.37[ab]
	17	1.90 ± 0.024	0.79 ± 0.012[bc]	0.33 ± 0.003[b]	16.0 ± 1.64[ab]	14.7 ± 1.24[ab]
52	8	2.14 ± 0.061	1.11 ± 0.029[d]	0.17 ± 0.006[a]	12.1 ± 0.78[a]	18.8 ± 1.97[b]
	17	2.08 ± 0.132	1.01 ± 0.067[d]	0.34 ± 0.023[b]	12.3 ± 1.11[a]	13.6 ± 0.72[ab]
Two-way ANOVA						
Dietary protein level		$P<0.4$	$P<0.001$	$P<0.3$	$P<0.04$	$P<0.2$
Dietary lipid level		$P<0.2$	$P<0.3$	$P<0.001$	$P<0.3$	$P<0.06$
Interaction		$P<0.9$	$P<0.7$	$P<0.5$	$P<0.8$	$P<0.8$

[1] Values (mean ± S.E. of three replications) in the same column not sharing a common superscript are significantly different ($P<0.05$).
[2] Feed intake (dry matter) × 100 / [(initial fish wt.+ final fish wt.+ dead fish wt.) / 2 × days fed].
[3] Fish protein (or lipid) deposited × 100 / protein (or lipid) intake.

Table 4. Proximate composition of whole body of Long-nosed barbel fed the diets containing various protein and lipid levels for 10 weeks[1]

Dietary protein (%)	Dietary lipid (%)	Moisture (%)	Crude protein (%)	Crude lipid (%)	Ash (%)
Initial		79.9	14.0	2.7	3.3
22	8	75.6 ± 0.07[cde]	15.3 ± 0.16	4.8 ± 0.68[ab]	3.2 ± 0.08
	17	73.4 ± 0.14[a]	14.9 ± 0.20	7.9 ± 0.61[d]	3.1 ± 0.01
32	8	75.4 ± 0.25[cd]	15.8 ± 0.73	4.7 ± 0.19[ab]	3.2 ± 0.07
	17	73.9 ± 0.82[ab]	15.4 ± 0.21	7.1 ± 1.11[cd]	3.0 ± 0.09
42	8	76.3 ± 0.30[de]	15.5 ± 0.10	3.5 ± 0.75[a]	3.3 ± 0.11
	17	74.6 ± 0.35[bc]	15.6 ± 0.16	5.9 ± 0.43[bc]	3.1 ± 0.08
52	8	76.7 ± 0.17[e]	15.7 ± 0.16	3.7 ± 0.22[a]	3.1 ± 0.17
	17	75.0 ± 0.38[bc]	15.7 ± 0.07	5.5 ± 0.62[abc]	3.1 ± 0.08
Two-way ANOVA					
Dietary protein		$P<0.01$	$P<0.3$	$P<0.05$	$P<0.9$
Dietary lipid		$P<0.001$	$P<0.4$	$P<0.001$	$P<0.2$
Interaction		$P<0.9$	$P<0.8$	$P<0.8$	$P<0.4$

[1] Values (mean ± SEM of three replications) in the same column not sharing a common superscript are significantly different ($P<0.05$).

higher than that of fish fed the 52% protein diets ($P<0.05$). Lipid retention of fish was influenced by dietary protein and lipid level, but the value tended to decrease with increasing dietary lipid level.

The proximate composition of whole body of long-nosed barbel fed the diets containing various protein and lipid levels are shown in Table 4. Moisture content of whole body of whole body was influenced by dietary protein ($P<0.01$) and lipid levels ($P< 0.001$). Crude lipid content of whole body were influenced by both dietary protein ($P<0.05$) and lipid levels ($P<0.001$). The body moisture content of fish tended to increase with increasing dietary protein at same lipid level, but body lipid content tended to decrease with increasing dietary protein. The body moisture content of fish fed the 8% lipid diets was significantly higher than that of fish fed the 17% lipid diets ($P<0.05$) at same dietary protein level. On the other hand, whole body lipid content of fish fed the 8% lipid diets was lower than that of fish fed the 17% lipid diets ($P<0.05$) at same protein level, except for the 52% protein diet. Crude protein and ash contents of whole body were not affected by dietary protein and lipid levels.

Discussion

Many studies have been carried out to determine

the protein requirements of fish, and the estimated protein requirements ranged from 30% to 55% in the diets according to fish species, fish size, dietary protein source, environmental conditions, and so on (NRC, 1993). Improved growth and feed efficiency ratio with increasing dietary protein levels are well known in carnivorous fish. Weight gain and feed efficiency ratio in this study also improved with increasing dietary protein level, whereas these values of fish fed the 32% protein diet with 17% lipid were not significantly different compared to those of the 42-52% protein diets with 8% and 17% lipid levels. This indicates that the optimum dietary protein level is about 32-42% for growth of long-nosed barbel. The optimum dietary protein level for bagrid catfish fingerlings determined in this study is lower compared with the dietary protein requirements reported for carnivorous fish, such as Malaysian catfish, rockfish, trout and salmon (Delong et al., 1958; De Silva and Perera, 1985; Khan et al., 1993; Lee et al., 2002; Lee et al., 2001), but was similar to protein requirements estimated for carp, tilapia and channel catfish (De Silva and Perera, 1985; Garling and Wilson, 1976; Ogino and Saito, 1970). Generally, protein and lipid requirements of herbivorous and omnivorous fish species which requires high dietary carbohydrate level are lower than those of carnivores (Lie et al., 1988; Ruohonen et al., 2003). Considering protein utilization of this species, long-nosed barbel may have omnivorous feeding characteristics.

Weight gain and feed efficiency in this study were lower compared to other studies for fresh water fish spices (De Silva and Perera, 1985; Garling and Wilson, 1976; Khan et al., 1993; Lee et al., 2001). Although, the exact reason of the low growth performance is not known, but it was thought to relate to natural characteristics of this species.

Improvement in growth rate, feed efficiency and protein utilization for long-nosed barbel in the 32% protein diet with 17% lipid, compared to the 32% protein diet with 8% lipid and to that with 42-52% protein diets indicated protein-sparing effect of lipid agreeing with other studies (Page and Andrews, 1973; De Silva et al., 1991; Vergara et al., 1996; Harpaz et al., 1999; Lee et al., 2002). These trends indicate that increasing the dietary energy level by lipid will provide a more efficient utilization of dietary protein for the growth of fish. However, in the 42-52% protein diets, protein-sparing effect was not observed in this study maybe due to sufficient protein levels. Also, no beneficial effects of excessive dietary lipid on performance in other species of fish were observed (Davis and Arnold, 1997; Jover et al., 1999;

Lee et al., 2003). Thus it is important to provide an adequate level and ratio of dietary protein and non-protein energy in order to reduce catabolism of protein for energy.

The P/E ratios for optimum growth of several fish species ranged from 81 to 112 mg/kcal (NRC, 1993). The optimum weight gain was obtained from the diets containing 32% protein with 17% lipid and 42-52% protein with 8-17% lipid in this study, corresponding to a P/E ratio of 76-130 mg/kcal. This interval of P/E ratio in this study is somewhat wide. A positive correlation was found between dietary P/E ratio and growth rate at both lipids, indicating that adequate levels of protein and energy in the diets must be carefully considered when estimating optimum P/E ratio as described by Garling and Wilson (1976).

It has been suggested that feed intake is regulated by the dietary available energy (Lee and Putnam, 1973; Jobling and Wandsvik, 1983), probably because the fish eat to satisfy their energy requirements. The lower feed intake of high energy diets has been shown in other species of fish (Lee and Putnam, 1973; Page and Andrews, 1973). However, daily feed intake in this study was not influenced by dietary energy. The difference in feed intake between studies appear to be due to different fish species, fish size, rearing conditions, or dietary energy and/or protein levels used in the studies.

The lipid contents of fish fed the high lipid diet were significantly higher than that of fish fed the low lipid diet at same protein level. These trends seem to be closely related to dietary energy level. The lipid contents of fish in this study were positively correlated with dietary energy ($r=0.74$; $P<0.006$). This is an agreement with other studies showing that lipid content of fish fed high energy diets is higher than that of fish fed low energy diets (Hillestad and Johnsen, 1994; Catacutan and Coloso, 1995; Lie et al., 1988).

The results of this study indicate that an increase of dietary lipid level from 8 to 17% can improve growth performance, feed efficiency ratio and protein utilization of long-nosed barbell when proper protein is provided. On the basis of this study, 32% protein and 17% lipid is recommended for practical feed of juvenile long-nosed barbel.

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