Effects of dietary protein concentration on growth and muscle composition of juvenile *Zacco barbata*

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Abstract

The effects of dietary protein concentration on growth and muscle composition of juvenile *Zacco barbata* were investigated using white fish meal as the major protein source. Six experimental moist diets containing 20.5 to 45.3% protein were fed to triplicate groups of 16 fishes (0.338 ± 0.002 g) for 10 weeks. There was no mortality among test fishes throughout the experiment. Specific growth rates and feed conversion ratios for fish fed diets containing 30.7, 35.2, 40.9, and 45.3% protein were better ($P < 0.05$) than those fed diets containing 20.5 and 25.8% protein. Fish fed 35.2, 40.9, and 45.3% protein diets excreted more ($P < 0.05$) ammonia than fish fed diets containing 20.5 and 25.8% protein. Fish on the lowest protein diet (20.5%) showed the highest protein efficiency ratio. Fish fed high-protein diets had higher protein and ash content in their muscle. Whereas, fish fed high-protein diets had lower lipid content in muscle than those fed low-protein diets. Analyzed by a broken-line model with final body weight as the indicator, the optimal dietary protein level for *Z. barbata* is approximately $32.0 \pm 0.1\%$. © 1998 Published by Elsevier Science B.V. All rights reserved.

Keywords: Dietary protein concentration; Muscle composition; *Zacco barbata*; Optimal protein requirement; Ammonia excretion

1. Introduction

Zacco barbata is an endemic freshwater fish that is widely distributed in the mountain streams of western Taiwan (Shen et al., 1993). In recent years, the culture of

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this fish species has been expanding due to an increasing demand from consumers, especially tourists from urban areas. Studies on *Z. barbata* have concentrated on reproductive biology and breeding behavior (Chuang and Lin, 1995; Yan et al., 1995). Little nutritional information is available for this species. Fish farmers have been using various commercial feeds to raise this fish. Among them, commercial eel feeds with protein content of 45% or higher are the most widely used feeds for culture of *Z. barbata* in fish farms.

It is well known that the protein content of diets should be maintained at a suitable level to minimize feed costs. Besides, when dietary protein level exceeds the requirement, the fish excretes more ammonia nitrogen into the surrounding environment (Cai and Summerfelt, 1992; Buttle et al., 1995; Cai et al., 1996; Tidwell et al., 1996), thus reducing the quality of the culture water. Growth of the fish also might be affected since extra energy is required to deaminate the excess amino acids absorbed (Jauncey, 1982). On the contrary, a protein-deficient diet results in reduced growth of the fish (Brecka et al., 1995; Shiau and Lan, 1996; Huang and Shyong, 1998).

The objective of this study was to investigate the growth performance, muscle composition, and ammonia excretion of *Z. barbata* fed different levels of dietary protein. The optimal dietary protein level for this fish species also was estimated.

2. Materials and methods

Six isocaloric test diets were prepared to contain 3810 kcal metabolizable energy (ME)/kg and levels of protein ranging from 20.5 (diet A) to 45.3% (diet F) by increments of 5% (Table 1). The ME values were calculated using the physiological fuel values of 4 kcal/g for carbohydrate and protein and 9 kcal/g for lipid (Maynard and Loosli, 1969). This level of ME was selected because the energy of 3700 kcal/kg was good for the growth of another fish species grown in the same habitat (Huang and Shyong, 1998). The major protein source was white fish meal obtained from Fleshier Fishing, New Zealand. Commercial aquacultural premixes of vitamins and minerals were purchased from Taiwan Cyanamid.

For the sake of good palatability and protein quality, white fish meal was used as the protein source in this study. The feed ingredients were crushed by a hammer mill, passed through a 35-mesh screen, and mixed in a KitchenAid heavy duty mixer. Menhaden fish oil were added to the feed mixtures at the final mixing stage. The resulting powdered diets were stored at -40°C in a laboratory freezer until the time of feeding.

Juvenile *Z. barbata*, hatched in our laboratory, were raised in a 500-l fiber glass tank prior to the experiment. A commercial feed (Tung Bao, Tainan, Taiwan) was used to acclimate the fish to the laboratory conditions 2 weeks before the experiment. Proximate composition of the commercial diet was as follows: moisture, 11.5%; crude protein, 40.8%; crude lipid, 3.7%; crude fiber, 2.7%; ash, 15.3%. After the acclimation, fish were selected and randomly assigned into 18 glass aquaria (45 L $\times$ 30 W $\times$ 30 H cm) at a stocking density of 16 fish each. Three aquaria, randomly arranged, were assigned to each of the test diets. Mean initial body weight of fishes was 0.338 ± 0.002 g for all

Table 1

Diet formula and proximate analysis of the experimental diets (%)

	A	B	C	D	E	F
<i>Ingredient</i>						
Fish meal	24	33	42	51	60	69
Corn meal	48.4	39.4	31.4	22.4	15.4	6.4
Menhaden fish oil	7	7	6	6	5	5
α -starch	20	20	20	20	20	20
Calcium phosphate	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin premix ^a	0.2	0.2	0.2	0.2	0.2	0.2
Mineral premix ^b	0.2	0.2	0.2	0.2	0.2	0.2
<i>Analyzed nutrient content</i>						
Moisture	7.1	7.5	7.0	6.5	7.3	6.9
Crude protein	20.5	25.8	30.7	35.2	40.9	45.3
Crude lipids	7.6	7.7	8.8	8.3	9.5	9.9
Crude fiber	1.5	1.2	1.2	0.9	0.8	0.5
Ash	4.3	5.5	6.9	7.9	9.2	10.1
NFE ^c	59.0	52.3	46.4	42.2	33.5	28.3
ME (kcal/kg diet)	3860	3820	3840	3800	3780	3800

^aOne-kg vitamin premix contained 2,000,000 IU retinol, 400,000 IU vitamin D₃, 20 g α -tocopherol, 2 g vitamin K₃, 2 g thiamin, 5 g riboflavin, 2 g vitamin B₆, 0.01 g vitamin B₁₂, 10 g pantothenic acid, 15 g niacin, 0.5 g folic acid, 50 g choline chloride, 50 g ascorbic acid, and 20 g inositol.

^bOne-kg mineral premix contained 70 g manganese, 10 g copper, 100 g iron, 80 g zinc, 1 g iodine, and 50 g choline chloride.

^cNFE was calculated by difference.

treatments. Each aquarium containing 30 l of freshwater was continuously aerated by an air stone. All aquaria were cleaned daily in the afternoon by siphoning off accumulated waste materials. Approximately 10 l of water in each aquarium was replaced with aerated freshwater daily. Water temperature, dissolved oxygen, pH, and total hardness of the water were monitored every week. Water temperature was kept between 24 and 26°C throughout the experiment; dissolved oxygen ranged from 6.0 to 7.6 mg/l; pH values ranged from 8.19 to 8.56; total hardness ranged from 153 to 160 mg/kg.

The experimental fish were fed 6% of their body weight daily. This amount was close to the maximum daily ration consumed by juvenile *Z. barbata* during the acclimation period. Immediately before feeding, the powdered feeds were mixed with equal weights of water to form ball shape moist paste diets. Moist test diets were fed to fish once daily at 9 AM for 10 weeks. All fish in the aquaria were individually weighed every 2 weeks. The amounts of feeds supplied to test fish were adjusted every 2 weeks according to the results of body weight measurement.

At 24 h after the last feeding, two fish from each aquarium were taken for ammonia excretion determination. Each fish was placed in a 500-ml BOD bottle and kept in a dark room for 4 h at 26°C. The ammonia content in the bottle was measured at the beginning and the end of the 4-h period using the indophenol blue method (Solorzano, 1969). The measurement of ammonia excretion was made 24 h after the last feeding because the variation of values was stable for fish on the same diet group at this stage

(Shu, 1995). This measurement also served as a good indicator for dietary protein adequacy for *Varicorhinus barbatulus* (Huang and Shyong, 1998).

At the end of the experiment, eight fish were sampled randomly from each aquarium. The muscles were carefully scratched off the fish, pooled, and ground. Diets and fish muscles were analyzed in triplicate for their proximate composition. Moisture, crude protein, crude fiber, and ash were determined following methods of the Association of Official Analytical Chemists (A.O.A.C., 1984). Crude protein was determined by Kjeldhal procedure using Kjeltac System from Tecator, Sweden. Crude lipid was extracted using the method of Folch et al. (1957).

All data were analyzed statistically by analysis of variance (Steele and Torrie, 1960) using SigmaStat statistical software from SPSS. Tukey's multiple comparison test was used to evaluate the mean difference among individual diets at the 0.05 significance level. The broken-line regression model was used to estimate the dietary protein requirement of juvenile *Z. barbata* (Robbins et al., 1979).

3. Results

Specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) of the test fish could be divided into two groups according to the statistical significance, fish with dietary protein equal to or less than 25.8% and those fed dietary protein equal to or more than 30.7% (Table 2). Fish fed diets containing 20.5 and 25.8% protein had significantly lower SGR than those fed high dietary protein diets ($P < 0.05$). The values of FCR for fish fed diets containing protein equal to or greater than 30.7% were significantly lower than fish fed 20.5 and 25.8% protein diets ($P < 0.05$). Data of PER for test fish could not be easily separated into two groups as SGR and FCR did. However, clearly PER decreased when dietary protein level increased.

Muscle moisture contents were the same for fish fed diets containing 20.5 to 45.3% dietary protein ($P > 0.05$, Table 3). However, the muscle protein, lipid, and ash were influenced by the dietary protein level. Muscle protein contents increased significantly when dietary protein levels increased. Whereas, the muscle lipid content was low for

Table 2

Percentage weight gain, SGR, FCR and PER of *Z. barbata* fed experimental diets for 10 weeks¹

	Dietary protein (%)					
	20.5	25.8	30.7	35.2	40.9	45.3
SGR ²	1.55 ± 0.04 ^a	1.74 ± 0.04 ^a	2.06 ± 0.02 ^b	2.11 ± 0.05 ^b	2.11 ± 0.06 ^b	2.13 ± 0.10 ^b
FCR ³	3.37 ± 0.08 ^a	2.97 ± 0.08 ^b	2.44 ± 0.02 ^c	2.46 ± 0.07 ^c	2.40 ± 0.05 ^c	2.53 ± 0.13 ^c
PER ⁴	1.34 ± 0.04 ^a	1.21 ± 0.03 ^{ab}	1.24 ± 0.01 ^{ab}	1.08 ± 0.03 ^{bc}	0.95 ± 0.02 ^c	0.83 ± 0.05 ^c

¹ Within each row, means (± S.E.) with different superscript letters are significantly different ($P < 0.05$).

² SGR(%/day) = 100[ln(final body weight) – ln(initial body weight)]/feeding days.

³ FCR = dry feed intake/wet weight gain.

⁴ PER = wet weight gain/protein intake.

Table 3

Composition of muscle (%) from *Z. barbata* fed experimental diets for 10 weeks^{1,2}

	Dietary protein (%)					
	20.5	25.8	30.7	35.2	40.9	45.3
Moisture	74.0 ± 0.1 ^a	73.2 ± 0.4 ^a	74.4 ± 0.1 ^a	73.8 ± 0.2 ^a	74.0 ± 0.1 ^a	73.7 ± 0.5 ^a
Crude protein	19.8 ± 0.5 ^b	20.3 ± 0.3 ^{ab}	20.2 ± 0.3 ^{ab}	21.0 ± 0.2 ^{ab}	21.7 ± 0.6 ^a	21.9 ± 0.4 ^a
Crude lipid	4.4 ± 0.1 ^{ab}	4.7 ± 0.1 ^a	4.6 ± 0.4 ^a	4.0 ± 0.2 ^{ab}	4.0 ± 0.1 ^{ab}	3.5 ± 0.1 ^b
Ash	2.1 ± 0.03 ^{cd}	2.2 ± 0.07 ^{bcd}	1.9 ± 0.02 ^d	2.3 ± 0.07 ^{bc}	2.5 ± 0.08 ^{ab}	2.7 ± 0.05 ^a

¹Initial fish muscle contained 74.0% moisture, 20.1% protein, 4.3% lipids, and 2.1% ash.²Within each row, means (± S.E.) with different superscript letters are significantly different ($P < 0.05$).

fish fed high-protein diets. Fish fed the diet containing 45.3% protein had highest ash content in muscle.

Protein levels in diets significantly influenced the amount of ammonia excreted from test fish. The amounts of ammonia excreted by fishes fed 35.2, 40.9 and 45.3% protein diets were 30.1, 30.1, and 35.2 mg kg⁻¹ h⁻¹, respectively. These values were significantly ($P < 0.05$) higher than those from fish fed 20.5 and 25.8% protein diets. They excreted 23.1 and 23.4 mg kg⁻¹ h⁻¹ ammonia.

Final mean body weights were 1.00, 1.14, 1.43, 1.48, 1.49, and 1.51 g for fish fed 20.5, 25.8, 30.7, 35.2, 40.9, and 45.3% dietary protein, respectively. Using final body

Fig. 1. The effect of dietary protein level on growth of *Z. barbata*. Each point represents the means of three groups of fish, with 16 fishes per group. The dietary protein requirement for *Z. barbata* is approximately $32.0 \pm 0.1\%$ when analyzed by a broken-line model.

weights as the indicator, and analyzed by a broken-line model, to estimate the optimal dietary protein level for juvenile *Z. barbata* as shown in Fig. 1, the regression equations were $Y = 0.10955 + 0.04254X$ and $Y = 1.3755 + 0.002912X$. The dietary protein requirement of juvenile *Z. barbata* was estimated to be $32.0 \pm 0.1\%$ since this was the break point that gave the least mean square error.

4. Discussion

From the results of this experiment, we observed that the indicators of growth performance such as SGR and FCR of juvenile *Z. barbata* improved when dietary protein concentration increased. However, the values of these indicators leveled off when dietary protein concentration reached 30%. This means that the optimum dietary protein level is about 30%. Above this level of protein, growth was similar and without significant difference among diets containing 30.7 to 45.3% protein. The FCR values in Table 2 were likely to be higher than the true FCR because we did not take into account the feed lost in the water. The test fish were fed moist paste diets in this study because of better palatability. Although the diets were consumed within 15 min, the loss of feed into the surrounding water was unavoidable. In this study, we assumed that the loss of feed was proportionally the same for all test diets.

Opposite to the above indicators, fish fed low-protein diets had higher PER than those fed high-protein diets. The results of high PER values at low dietary protein level has also been observed in carp (Ogino and Saito, 1970), grass carp (Dabrowski, 1977), silver carp (Singh, 1990), *Cirrhinus mrigala* fry (Das and Ray, 1991), gilthead sea bream (Santinha et al., 1996), and *V. barbatulus* (Huang and Shyong, 1998). This observation has been suggested to be due to high utilization of dietary protein as an energy source, especially when high-protein diets were fed to the fish (Kim et al., 1991; Santinha et al., 1996). In the present study, with 20.5 and 25.8% protein diets, fish did not have enough protein and showed significantly lower growth than the other fish. On the other hand, with diets containing 30.7 to 45.3% protein, fish had enough protein for their maximum growth. Meanwhile, with high availability of protein, the fish used a high percentage of protein to obtain energy in relation to fish fed less dietary protein. As a result, the PER of test fish decreased when dietary protein increased. This also resulted in the high ammonia excretion for fish on high-protein diets.

The concentration of dietary protein had profound effect on muscle composition of fish. There was a significant increase in muscle protein content with increasing dietary protein level. Similarly, fish fed high-protein diets tended to have lower muscle lipid contents and higher ash contents. Similar results of dietary protein on carcass composition has been observed in other studies with common carp (Zeitter et al., 1984), plaice (Cowey et al., 1972), and snakehead fry (Mohanty and Samantaray, 1996). Nose and Arai (1972), however, found that carcass lipid content in Japanese eel increased when dietary protein level increased.

Ammonia excretion has been suggested as an indicator of dietary protein adequacy (Cai et al., 1996). Fish consuming dietary protein in excess to their requirements are expected to excrete significantly more ammonia than those consuming inadequate

protein. Our data on ammonia excretion suggests that dietary protein levels of 20.5 and 25.8% were inadequate for juvenile *Z. barbata*. This was in accordance with the growth performance data. Based on the results of ammonia excretion and growth performance data, the minimum dietary protein level to achieve maximum growth for juvenile *Z. barbata* was about 30% under our experimental conditions. The estimated dietary protein requirement was $32.0 \pm 0.1\%$ for *Z. barbata* when analyzed by the broken-line model using final body weight as the indicator. This was quite close to the dietary protein requirement of *V. barbatulus*, a fish species grown in the same habitat as *Z. barbata*. The dietary protein requirement for *V. barbatulus* ranged from 29 to 35%.

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