Protein Requirement of Fingerling Wuchan Fish, *Megalobrama amblycephala*

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Abstract: The dietary protein requirement of wuchan fish, *Megalobrama amblycephala,* was examined by feeding test diets containing vitamin-free casein and corn oil as protein and lipid sources, respectively. With an increase of dietary protein level from 25 to 35%, weight gain and feed efficiency increased remarkably, but no further increase was observed at higher protein levels. Increasing the dietary lipid level from 4 to 8% did not increase growth rate but did increase the feed efficiency slightly. The carcass protein content tended to increase, whereas carcass fat decreased with increasing dietary protein levels. The carcass fat was higher at the high lipid level. The optimum level of dietary protein for growth was estimated to be about 33% on dry matter basis. The daily protein requirement was estimated to be 9 g/kg fish/day. The lack of influence of the dietary lipid on the protein requirement may be related to the herbivorous food habit of this fish.

Introduction

Wuchan fish, *Megalobrama amblycephala* (Family: Cyprindae), is a herbivorous bream that occur in some lakes in the mid-lower Yangtze River basin in China. They are regarded as an excellent food fish. They are cultured throughout China because of their favorable features for aquaculture such as rapid growth, herbivorous food habit, deliciousness and strong resistance to bacterial diseases. Although there are some report on the reproductive biology and culture techniques,[1,2] information on their nutritional requirements, especially protein requirement, is sparse.

Since the work of DELONG *et al.*[3] on the minimum amount of dietary protein producing optimum weight gain in chinook salmon, many fish nutritionists have used various semipurified and purified test diets to estimate the protein requirements of various fish species.[4-21] Those studies using mainly carnivorous or omnivorous fishes indicate that higher percentages of protein are required by fishes than by birds and omnivorous mammals. As wuchan fish mainly feed on aquatic plants and algae in nature, their protein requirement is assumed to be lower than in the other fishes.

This paper deals with the protein requirement of wuchan fish. The effect of dietary lipid levels on the protein requirement was also investigated.

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Materials and Methods

Test diets Vitamin-free casein was used as the protein source. Based on the results of a preliminary experiment, corn oil and the mixture of alpha-potato starch and dextrin were used as the lipid and carbohydrate sources, respectively. The composition and ingredients of the test diets are shown in Table 1. Two lipid levels were designed by adding 5 or 10% corn oil into the diets. In each lipid level, 6 protein levels from 25% to 53% were achieved by adding appropriate amounts of casein. The differences in protein levels were adjusted by carbohydrate mixture and cellulose to produce isoenergetic diets. The composition of the mineral and vitamin mixtures were as described by OGINO *et al.*[22] and HALVER[23]. To promote the feeding activity, a 1% amino acid mixture (alanine, glycine and proline) was also added to every diet.

Table 1. Percentage composition and nutrient contents of the test diets

Ingredient & nutrient	Diet No.											
	1	2	3	4	5	6	7	8	9	10	11	12
Vitamin-free casein	25	30	35	40	45	55	25	30	35	40	45	55
Corn oil	5	5	5	5	5	5	10	10	10	10	10	10
Carbohydrate mix[a]	58	51	45	38	32	19	56	50	44	38	31	18
Mineral mix[b]	5	5	5	5	5	5	5	5	5	5	5	5
Vitamin mix[c]	3	3	3	3	3	3	3	3	3	3	3	3
Amino acid mix[d]	1	1	1	1	1	1	1	1	1	1	1	1
Cellulose	3	5	6	8	9	12	0	1	2	3	5	8
Protein[e]	25.0	29.7	34.0	38.7	43.3	52.5	25.0	29.2	34.8	38.6	44.2	52.9
Lipid[e]	3.5	3.6	3.8	3.6	3.6	4.2	7.4	7.5	7.8	8.1	8.4	8.8
Carbohydrate[e]	57.4	50.8	44.6	37.9	31.6	20.0	55.8	50.2	43.9	37.6	30.8	17.1
Ash[e]	4.1	4.0	4.2	4.3	4.4	4.7	4.1	3.9	4.1	4.0	4.3	4.0
DE (kcal/g diet)[f]	3.43	3.42	3.41	3.38	3.36	3.42	3.61	3.71	3.77	3.74	3.78	3.73

a α-Potato starch: dextrin = 1 : 1.
b OGINO *et al.*[22]
c HALVER[23].
d Ala: Gly: Pro=28.5 : 50.8 : 21.7.
e % on dry weight basis.
f Digestible energy calculated using the following values: protein, 4.5; fat, 8.5; carbohydrate, 3.5 kcal/g (WANG *et al.*[21]).

The ingredients were mixed thoroughly with addition of about 30% water, and pelleted into 1 mm moist pellets using a laboratory mill. The pellets were stored at −20℃ until used. The proximate analyses of the test diets were conducted as described below. The caloric contents of the diets were calculated using the following values: 4.5, 3.5 and 8.5 kcal/g for protein, carbohydrate and lipid, respectively.[21] The calculated available energy values of Diets 1-6 were 3.4 kcal/g, and those of Diets 7-12 were 3.7 kcal/g (Table 1).

Fish and feeding procedures Fingerlings of wuchan fish, *Megalobrama amblycephala*, weighing about 8 g, were obtained from Oriental Yeast Co., Tokyo, Japan. They were maintained on a commercial diet for carp in freshwater aquaria at 25-27℃ for one week prior to the experiment. Then they were divided into 12 groups of 18 fish each. Each group was kept in an aquarium (60×35×30 cm) with a circulating water system. The experiments were conducted during August to September, 1986. Fish were fed on these test diets twice daily at about 3 % of their total body weight for 30 days. Fish were weighed at 10-day intervals and the amount of diets fed was adjusted accordingly.

Measurement and determination At the end of the experiment, 3 fish were randomly selected from each group, and the blood was taken from the caudal vessels in heparinized syringe. All the fish were killed immediately by a knife and stored at −20℃ until analyzed. Hematocrit value was determined by the microcentrifugation method, and hemoglobin concentration by cyanmethemoglobin method. Red blood cells were counted using the hematocytometer. The moisture of the diet and fish was analyzed by desiccation at 105 ℃. Crude protein was analyzed by semimicro-KJELDAHL method, crude fat by extraction with ether and crude ash by ignition at about 550℃. The digestible carbohydrate of the diets was determined by the phenol sulfuric acid method.

Statistical analysis Differences among the groups were tested for significance ($p < 0.05$) by DUNCAN's new multiple range test. The protein requirement was estimated by the broken line analysis.[9]

Results

Fig. 1 shows the growth curves of the 12 groups of fish. All the fish grew normally during the experimental period, but a slight growth retardations were found in groups 1, 2, 7 and 8, which were fed on the lower protein diets. As shown in Table 2, no significant difference was recognized in the daily feeding rates of the 12 groups. At the end of the experiment, the average body weight of groups 1, 2, 7 and 8 appeared lower than those of the other groups, but these differences were not significant. No significant difference was seen in the hematological characteristics among these groups at the end of the experiment (data not shown).

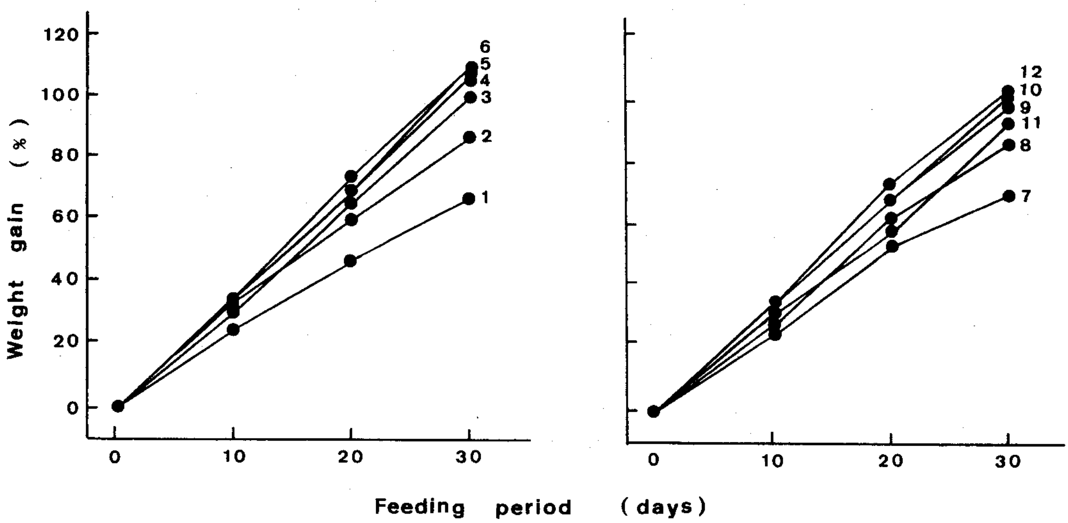

Fig. 1. Percent weight gains of the fish fed on test diets 1-12.

Fig. 2 shows the relation between growth rates and dietary protein levels. In both low and high lipid levels, the growth rates increased linearly as the dietary protein level increased up to 34 %, and reached a plateau thereafter. Increase in the dietary lipid level from 4 to 8 % did not promote the growth rates. Based on broken line analysis, the regression equations are as follows:

At 4 % dietary lipid level, $Y = -41.26 + 4.42X$

$Y = 106.3$

break point $R = 33.4$ (MSE = 2.00)

At 8 % dietary lipid level, $Y = -22.56 + 3.84X$

$Y = 102.6$

break point $R = 32.6$ (MSE = 1.93)

Thus, the optimum dietary protein level was evaluated to be 33.4% at 4 % lipid level, and 32.6% at 8 % lipid level (Fig. 2).

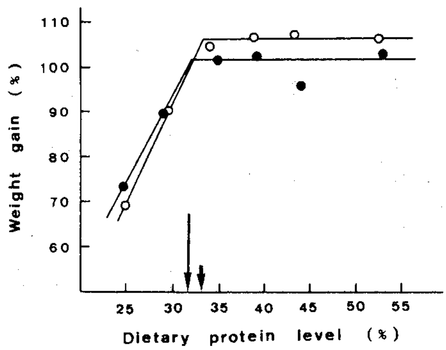

Fig. 2. Relationship between dietary protein levels and percent weight gain of fish. The open and closed circles indicate those obtained from dietary lipid level of 4 and 8 %, respectively. Short arrow indicates the optimum dietary protein level evaluated by broken line analysis at 4 % dietary lipid level; long arrow indicates that at 8 % dietary lipid level.

The protein intake was calculated according to the quantity of diet consumption, and was proportional to the dietary protein level (Table 2). The minimum amount of daily protein intake producing maximum growth was estimated to be 9.2 and 8.9 g/kg fish/day when the dietary lipid level was 4 and 8 %, respectively.

Table 2. Growth,feeding rate and feed efficiency of the fish fed test diets for 30 days

Diet No.	Average body weight (g)[a] Initial	Final	Average weight gain (g)	Percent weight gain	Daily feeding rate (%)	Feed efficiency[b] (%)	Daily protein intake (g/kg/day)	Protein efficiency ratio
1	8.18	13.84[c]	5.66	69.2	2.74	62.4	6.94	2.53
2	8.03	15.27[c]	7.24	90.0	2.76	75.0	8.19	2.48
3	8.11	16.55[c]	8.44	104.0	2.71	84.2	9.21	2.27
4	8.16	16.87[c]	8.70	106.0	2.64	87.7	10.2	1.98
5	8.11	16.77[c]	8.66	106.9	2.71	85.6	11.8	1.71
6	8.14	16.38[c]	8.44	107.8	2.64	89.7	13.7	1.67
7	8.11	14.06[c]	5.95	73.4	2.74	65.3	6.85	2.61
8	8.14	15.47[c]	7.28	89.5	2.74	75.1	8.01	2.57
9	8.17	16.42[c]	8.25	100.9	2.62	85.4	9.11	2.45
10	8.12	16.46[c]	8.33	102.5	2.51	90.2	9.67	2.34
11	8.06	15.27[c]	7.65	94.9	2.43	88.4	10.7	2.00
12	8.09	16.53[c]	8.44	104.3	2.49	91.8	13.2	1.73

a Means of 18 fish. b % on dry matter basis. c Values within a given column followed by the same letter are not significantly different ($P<0.05$).

The trend of feed efficiency was very similar to that of growth rate in both lipid levels (Fig. 3).

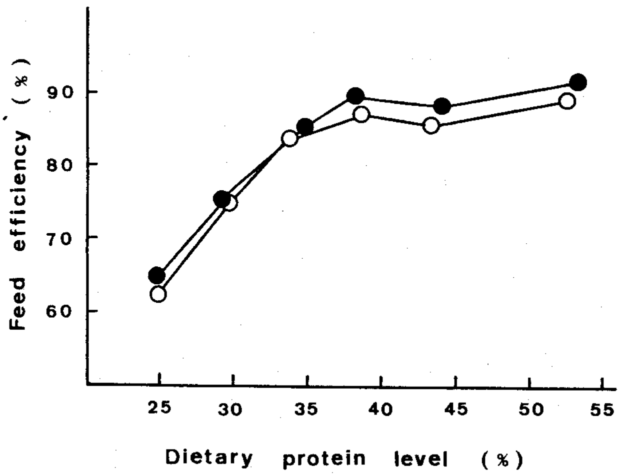

Fig. 3. Effect of ditary protein level on the feed efficiency.
The symbols are the same as those in Fig. 2.

At both dietary lipid levels, the protein efficiency ratio declined as the protein level increased from 25 to 53%. Increment of lipid level in the diets elevated the protein efficiency ratio slightly (Table 2). At either dietary lipid level, the percent protein retention (100 x body protein gain/dietary protein intake) was poor at low protein levels, and increased with increasing dietary protein levels, reaching a peak at 34 or 38% and then dropped quickly at higher protein levels (Fig. 4).

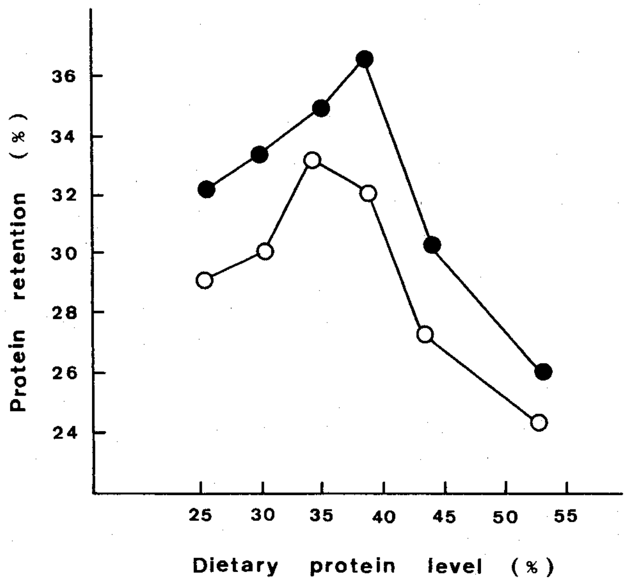

Fig. 4. Effect of dietary protein level on protein retention of fish.
The symbols are the same as those in Fig. 2.

Table 3 shows the proximate composition of the fish at the end of the experiment. There was a tendency of decline in carcass fat and increase in carcass protein contents when the dietary protein level increased from 25 to 53 %. The ash content was not influenced by the dietary protein level. High dietary lipid level resulted in higher carcass fat contents.

Table 3. Proximate composition of fish carcass at the end of the experiment

Diet No.	Crude Protein (%)	Crude fat (%)	Crude ash (%)	Moisture (%)
1	13.2 (52.4)[a]	8.5 (30.0)	3.1 (11.1)	71.9
2	13.5 (56.0)	6.9 (27.2)	3.2 (12.6)	74.7
3	14.2 (56.7)	5.9 (25.3)	3.0 (12.9)	76.6
4	14.5 (57.4)	5.3 (22.6)	3.4 (14.7)	76.6
5	14.4 (61.1)	5.1 (21.9)	3.4 (14.4)	77.5
6	14.6 (65.4)	4.1 (18.2)	3.0 (13.4)	77.5
7	13.8 (49.5)	9.1 (32.5)	3.3 (11.8)	72.2
8	14.0 (52.2)	7.9 (29.8)	3.5 (13.2)	73.3
9	14.6 (56.0)	7.3 (27.7)	3.5 (13.4)	73.6
10	15.3 (58.1)	6.9 (26.3)	3.3 (12.4)	73.7
11	15.0 (56.9)	7.1 (26.8)	3.1 (11.7)	73.6
12	15.0 (60.3)	5.4 (21.8)	3.3 (13.1)	75.1

a Values in parentheses refer to % on dry weight basis.

Discussion

The protein requirement of fingerling wuchan fish, expressed as a percentage of the diet, was 33 % on dry matter basis when casein was used as the sole dietary protein source. This value was similar or slightly lower than those of common carp[4,5,16], channel catfish[13], rainbow trout[5,9,10], ayu[20] and tilapias[14,17,21], but much lower than those of the Japanese eel[7], chinook salmon[3], coho salmon[8], chum salmon[18], plaice[6], milkfish[15], smallmouth bass[19], largemouth bass[19], red sea bream[12] and yellowtail[11].

Values expressed as a percentage of the diet, however, do not provide detailed informations on the efficiency of protein utilization. A low value may mean that either the fish utilize the protein more efficiently or consume more feed. OGINO[5] has reported that when the feeding rate was changed from 2.5 to 3.5 %, the dietary protein level for maximum protein retention in carp decreased from 50 to 35 %.

In the present study, the amount of protein required for maximum growth for fingerling wuchan fish was evaluated to be about 9 g/kg fish/day, which is considerably lower than those of carp[4,5], rainbow trout[5,10], and ayu[20]. However, a similar value (8.8 g) was reported for another herbivorous fish, *Tilapia nilotic* a[21], suggesting that the protein requirement of the herbivorous fish is lower than those of the carnivorous or some omnivorous species.

It is well known that the nonprotein energy contents in diet, or the dietary lipid and carbohydrate contents affect the protein requirements of fish. AKIYAMA *et al.*[18] reported that the protein requirement of the carnivorous chum salmon decreased from 43 to 38%, when dietary lipid level was elevated from 5 to 10%. In the present study, the dietary lipid levels had little effect on the protein requirement of wuchan fish. The results suggest that unlike carnivorous fish, herbivorous wuchan fish perhaps are unable to utilize efficiently a large amount of lipid.

It is generally considered that fish, like the other vertebrates, do not have an absolute protein requirement but require a well-balanced mixture of essential and dispensable amino acids. Therefore, the amount of dietary protein required by fish is directly influenced by the amino acid composition of the dietary protein.[24] Since the quantitative requirements of individual essential amino acids of wuchan fish are unknown, it is difficult to know whether the contents of the individual amino acids in the diets were sufficient in the present experiments. However, levels of certain amino acids in casein seem to be insufficient for some fishes. It has been also found in salmon that a casein diet supplemented with amino acids simulating the essential amino acid pattern of fish body protein resulted in better growth than a diet containing casein alone.[25,26] Thus further experiments are desirable to confirm the dietary protein requirement of wuchan fish by feeding the casein diets supplemented with certain amino acids to simulate the essential amino acid pattern of the whole body protein of this fish.

As is evident from this study, when casein was used as a dietary protein source, the protein requirement of fingerling wuchan fish for maximum growth was about 9 g/kg fish/day. When expressed as a percentage of the diet, the protein requirement was about 33% at a feeding rate of 2.5−2.8% and dietary lipid level of 4−8%. Since increased dietary lipid levels had little effect on the protein requirement, such a large amount of dietary lipid may not be utilized efficiently by this herbivorous fish.

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Literature Cited

1) TSAO, W. S.: *Acta Hydrobiologica Sinica,* **2**, 115-122 (1960).

2) KO, H. W.: *Acta Hydrobiologica Sinica,* **5**, 293-312 (1975).

3) DELONG, D. C., HALVER, J. E. and MERTZ, E. T.: *J. Nutr.*, **65**, 589-599 (1958).

4) OGINO, C., TAKEUCHI, L., TAKEDA, H. and WATANABE, T.: *Nippon Suisan Gakkaishi,* **45**, 1527-1532 (1979).

5) OGINO, C.: *Nippon Suisan Gakkaishi,* **46**, 385-388 (1980).

6) COWEY, C. B., POPE, J. A., ADRON, J. W. and BLAIR, A.: *Br. J. Nutr.*, **28**, 447-456 (1972).

7) NOSE, T. and ARAI, S.: *Bull. Freshwater Fish. Res. Lab.*, **22**, 145-155 (1972).

8) ZEITOUN, I. H., ULLREY, D. E., HALVER, J. E., TACK, P. I. and MAGEE, W. T.: *J. Fish.*

Res. Board Can., **31**, 1145-1148 (1974).

9) ZEITOUN, I. H., ULLREY, D. E. and MAGEE, W. T.: *J. Fish. Res. Board Can.*, **33**, 167-172 (1976).

10) SATIA, B. P.: *Prog. Fish-Cult.*, **36**, 80-85 (1974).

11) TAKEDA, M., SHIMENO, S., HOSOKAWA, H., KAJIYAMA, H. and KAISYO, T.: *Nippon Suisan Gakkaishi*, **41**, 443-447 (1975).

12) YONE, Y.: In "Proceedings of the First International Conference on Aquaculture Nutrition" ed. by PRICE, K. S., SHAW, W. N. and DANBERG, K. S., p. 39-64, Univ. of Delaware, Delaware (1976).

13) GARLING, D. L. Jr. and WILSON, R. P.: *J. Nutr.*, **106**, 1368-1375 (1976).

14) MAZID, M., TANAKA, Y., KATAYAMA, T., RAHMAN, M. A., SIMPSON, K. L. and CHICHESTER, C. O.: *Aquaculture*, **18**, 115-122 (1979).

15) LIM, C., SUKHAWONGS, S. and PASCUAL, F. D.: *Aquaculture*, **17**, 195-201 (1979).

16) TAKEUCHI, T., WATANABE, T. and OGINO, C.: *Nippon Suisan Gakkaishi*, **45**, 983-987 (1979).

17) WINFREE, R. A. and STICKNEY, R. R.: *J. Nutr.*, **111**, 1001-1012 (1981).

18) AKIYAMA, T., YAGISAWA, I. and NOSE, T.: *Bull. Natl. Res. Inst. Aquaculture*, No. 2, 35-42 (1981).

19) ANDERSON, R. J., KIENHOLZ, E. W. and FLICKINGER, S. A.: *J. Nutr.*, **111**, 1085-1097 (1981).

20) ARAI, S. and NOSE, T.: *Bull. Natl. Res. Inst. Aquaculture*, No. 4, 99-105 (1983).

21) WANG, K. W., TAKEUCHI, T. and WATANABE, T.: *Nippon Suisan Gakkaishi*, **51**, 133-140 (1985).

22) OGINO, C., TAKEUCHI, L., TAKEDA, H. and WATANABE, T.: *Nippon Suisan Gakkaishi*, **45**, 1527-1532 (1979).

23) HALVER, J. E.: *J. Nutr.*, **62**, 225-243 (1957).

24) WILSON, R. P.: In "Nutrition and Feeding in Fish" ed. by COWEY, C. B., MACKIE, A. M. and BELL, J. G., p. 1-16, Academic Press, London (1985).

25) ARAI, S.: *Nippon Suisan Gakkaishi*, **47**, 547-550 (1981).

26) OGATA, H., ARAI, S. and NOSE, T.: *Nippon Suisan Gakkaishi*, **49**, 1381-1385 (1983).

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