Snakehead (Channa sp.) 幼魚の飼料中タンパク質の要求量

誌名	日本水産學會誌
ISSN	00215392
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発行元	日本水産學會
巻/号	48巻10号
掲載ページ	p. 1463-1468
発行年月	1982年10月

Bulletin of the Japanese Society of Scientific Fisheries 48(10), 1463–1468 (1982)

A Preliminary Study on the Dietary Protein Requirement of Juvenile Snakehead

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(Received March 13, 1982)

The dietary protein requirement of juvenile snakehead *Channa micropeltes* was investigated by feeding eight semipurified diets containing varying levels of dietary protein within a range of 25.5 to 56.5% by weight over a 56-day feeding period at 28°C.

On the basis of daily weight gain and daily protein deposition these results indicate that the requirement by juvenile snakehead for dietary protein was about 52% of protein in the diet when fish meal is used as the sole protein source. The relationship between dietary protein level and food conversion ratio, protein efficiency ratio, apparent net protein utilisation, apparent protein digestibility, apparent dry matter digestibility, hepatosomatic index, and gross carcass composition were determined and discussed in relation to other fish species.

The snakehead *Channa* sp. is a tropical freshwater fish species which is progressively gaining importance as a farmed fish in South East Asia. At present the snakehead farming industry utilises trash fish, alone or in combination with agricultural by-products, as feed and this represents up to 70% of the total operating costs involved.[1] Despite the use of these currently successful feeding strategies, there is no information available on the nutritional requirements of snakeheads; other than physiological studies on growth and feed utilisation in relation to temperature,[2] starvation,[3] ration size,[4] body weight,[5] water depth[6] and partial pressure of oxygen in water.[7]

In view of the extremely carnivorous habit of the snakehead,[1] and the high cost of good quality dietary protein sources for fish,[8] it is essential that nutrient requirements be determined, and so enable the formulation of well balanced and cost effective feeds.

The following study was conducted to investigate the effect of feeding semi-purified diets containing varying dietary levels of protein, ranging from 25 to 60% by weight, on the growth performance, feed efficiency, protein utilisation and carcass composition of juvenile *Channa micropeltes* held under laboratory conditions.

Materials and Methods

The composition of the experimental diets is shown in Table 1. Eight semi-purified diets were formulated. Within all diets herring meal (brown fish meal) was used as the sole source of dietary protein, and the level adjusted so as to provide dietary crude protein concentrations ranging from 25% (diet 1) to 60% (diet 8) of the dry diet by dietary increments of 5%. All diets were formulated to an estimated gross energy content of approximately 450 kcal/100 g and were prepared as described previously.[9]

Juvenile snakehead (ca. 130 g weight) were obtained from the London Catfish Centre, and randomly distributed between eight 220 litre glass aquaria at a stocking density of seven fish per tank. Experimental tanks were contained within a single recirculation system and each tank was continuously supplied with freshwater at a rate of 2 litres/min. The water temperature was maintained at 28±1°C, and a 12 h light cycle provided by fluorescent lighting. Dissolved oxygen, total ammonia and pH was monitored over the course of the experiment and varied between 87–92% saturation, 0.4–1.0 mg/*l* and 5.8–6.3 respectively.

At the start of the experiment five fish were sacrificed, killed by a sharp blow on the head, and stored at −20°C for subsequent carcass analysis. Experimental fish were fed twice daily, seven days a week, at a fixed feeding level of 2% body weight per day (previous studies within the laboratory using dry pelleted trout feeds indicated that a maximum feeding level of 2.12% per day for juvenile snakehead at 28°C). Experimental fish were weighed individually at fortnightly intervals as described previously,[9] and feeding levels adjusted accordingly. The total duration of the experiment was

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Table 1. Composition of experimental diets (%)

Component	Diet No.							
	1	2	3	4	5	6	7	8
Herring meal	35.0	42.0	49.0	56.0	63.0	70.0	77.0	84.0
Dextrin	17.6	15.5	13.4	11.2	8.8	6.5	4.2	1.8
Corn starch, raw	35.2	31.0	26.8	22.3	17.7	13.0	8.3	3.7
Herring oil	1.7	1.0	0.3	0	0	0	0	0
Corn oil	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Cellulose binder	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin premix*1	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Mineral premix*2	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Chromic oxide	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
				Nutrient content				
Moisture	5.4	6.6	6.4	5.2	5.7	5.2	4.7	5.6
Crude protein	25.5	28.9	34.5	39.0	43.6	47.8	53.9	56.5
Crude lipid	7.7	8.6	8.8	9.3	9.9	10.0	11.5	12.4
Ash	7.7	8.7	9.5	11.0	12.0	13.2	14.3	15.6
Available carbohydrate	52.8	46.5	40.2	33.5	26.5	19.5	12.5	5.5
Gross energy (kcal/100 g)*3	430	432	440	445	449	446	466	462
Gross protein energy (%)	33.1	38.9	44.5	50.0	55.4	61.2	65.8	69.7
Estimated digestible energy (kcal/100 g)*4	303	315	332	346	360	368	398	405

*1 To supply/100 g diet; Thiamine HCl 6 mg; Riboflavin 15.2 mg; Pyridoxine HCl 3.6 mg; Calcium pantothenate 40 mg; Inositol 142 mg; Biotin 2 mg; Folic acid 1.5 mg; para Aminobenzoic acid 30 mg; Choline chloride 6448 mg; Nicotinic acid 53.2 mg; Cyanocobalamin 0.01 mg; Retinol palmitate 200 IU; Alpha tocopherol acetate 30 mg; Ascorbic acid 200 mg; Menadione 4 mg.

*2 To supply/100 g diet; $Ca(H_2PO_4)_2 \cdot H_2O$ 1.37 g; $CaCO_3$ 0.11 g; $MgCO_3$ 0.48 g; $FeSO_4 \cdot 7H_2O$ 0.06 g; KCl 0.10 g; NaCl 0.16 g; $AlSO_4 \cdot 6H_2O$ 0.4 mg; $ZnSO_4 \cdot 7H_2O$ 8.0 mg; $CuSO_4 \cdot 5H_2O$ 2.0 mg; $MnSO_4 \cdot 4H_2O$ 5.4 mg; $CaIO_3 \cdot 6H_2O$ 0.5 mg; $CoSO_4 \cdot 4H_2O$ 2.0 mg.

*3 Calculated on an estimated 5.7 kcal/g protein; 9.5 kcal/g lipid; 4.0 kcal/g carbohydrate.

*4 Calculated on an estimated 5.0 kcal/g protein; 9.0 kcal/g lipid; 2.0 kcal/g carbohydrate.

eight weeks. During the final week of the experiment faecal samples were collected from individual fish by hand stripping, pooled, dried at 105°C for 24 h, and stored for subsequent chemical analysis. On the final day of the experiment five fish from each treatment were sacrificed, liver weight determined and thin slices of liver and muscle removed for histological examination. The individual fish carcasses (including liver) were then subsequently taken for gross chemical analysis. Moisture, crude protein, lipid and ash were performed on the whole fish carcass, faeces and diets as described previously.[9] For apparent digestibility measurement chromic oxide was determined in the faeces and diets by the method of FURAKAWA and TSUKAHARA.[10] For histological examination tissues were fixed in 10% buffered formalin, embedded in paraffin wax, sectioned at 5 mμ, and stained with haematoxylin and eosin.

Differences in body weight, liver somatic index and carcass composition between treatments were tested for significance ($P<0.05$) by DUNCAN's multiple range test.[11]

Results and Discussion

All fish soon became accustomed to the experimental diets and fed aggressively for the duration of the experiment. The growth response is shown is Figs. 1 and 2, and Table 2. Despite the differences in initial fish weights at the start of the experiment, percentage weight gain and daily specific growth rate increased proportionally and food conversion efficiency (food fed/weight gain) decreased proportionally with increasing dietary protein concentration. A similar dose-response for dietary protein has also been shown in carp,[12] plaice[13] and gilthead bream.[14] This is in contrast to the apparent growth depressing effects of high protein diets observed in milkfish,[15] grouper,[16] plaice[17] and puffer fish.[18] It should be emphasised however, that the highest level of dietary protein fed during the present study was only 56.5% by weight, as compared with dietary levels equal or in excess of 70% in the majority of the other fish species studied.

On the basis of daily tissue protein deposition

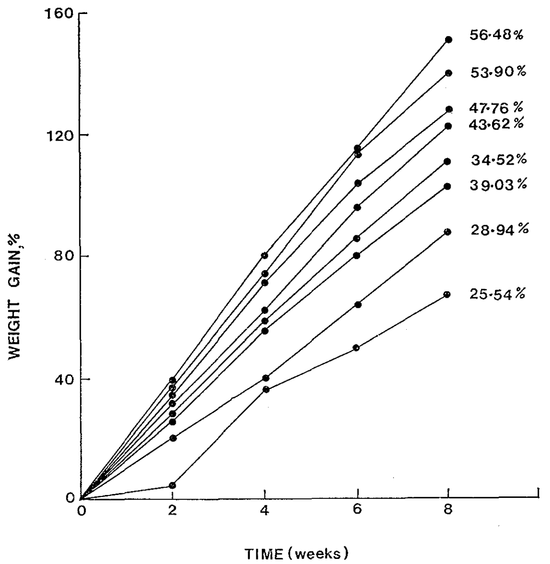

Fig. 1. Effect of protein levels on the weight gain of snakehead. Percentage indicates the dietary protein level.

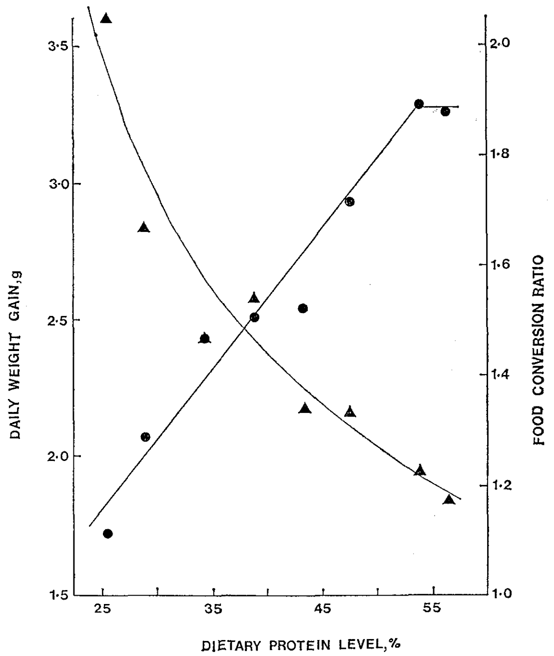

Fig. 2. Relationship between daily weight gain (●) and food conversion ratio (▲) and dietary protein level.

(Fig. 3) there was a progressive increase in protein deposition to a maximum level of about 52% dietary protein, beyond which protein deposition remained constant. A similar trend has also been observed in the common carp[12] and grass carp,[19] maximum tissue protein deposition occurring at dietary protein concentrations of 38 and 45.6% respectively.

The relationship between protein efficiency ratio (PER) and apparent net protein utilisation (NPU) and the estimated percentage of protein calories present in each diet is shown in Fig. 4. PER ranged from 1.51 to 2.08, the highest value occurring in fish fed the diet containing 38.9% protein calories (28.9% dietary protein), and thereafter decreasing progressively as the percentage of protein calories increased. The dose-response was similar to that observed with plaice[17] and grouper,[16] maximum values of PER occurring at dietary protein levels of 40% in both instances. By contrast, PER was found to be highest at the lowest dietary protein level tested and fell almost linearly as dietary protein increased in carp,[12] grouper,[16] puffer fish,[18] grass carp[19] and gilthead bream.[14]

Apparent NPU decreased from 50.5 to 37.2 with increasing dietary protein calories. A similar decrease has also been observed in carp,[12] plaice[17] and grass carp.[19] The correlation between apparent NPU and percentage protein calories observed during the present study can be given by the regression equation $NPU = 64.0 - 0.39\,P$, where P is the percent of protein calories in the diet.

The effect of dietary protein level on apparent protein and dry matter digestibility is shown in

Table 2. Growth and percentage liver weight of experimental fish after 56 days

Mean values	Diet No. 1	2	3	4	5	6	7	8	±SE*1
Initial weight (g)	144.27[a]	132.27[a]	122.27[a]	136.68[a]	115.74[a]	128.04[a]	130.60[a]	120.85[a]	8.827
Final weight (g)	241.30[a]	249.02[a]	259.25[a]	276.97[a]	257.70[a]	292.16[a]	314.50[a]	303.60[a]	16.92
Specific growth rate (%)*2	0.918	1.129	1.336	1.260	1.430	1.473	1.570	1.646	
Liver wt./100 g body wt.	1.91[bc]	2.31[c]	2.17[c]	1.92[bc]	2.03[bc]	1.59[ab]	1.58[ab]	1.15[a]	0.178

*1 Standard error, calculated from residual mean square in the analysis of variance.
*2 Specific growth rate = $\log_e$ final body weight − $\log_e$ initial body weight/time (days), ×100. abc: Mean values for components with the same superscripts are not significantly ($P<0.05$) different.

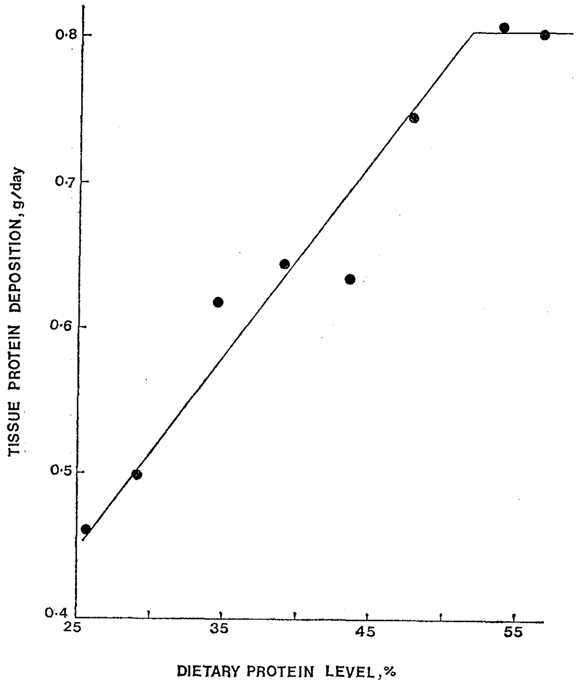

Fig. 3. Relationship between daily tissue protein deposition per fish and dietary protein level.

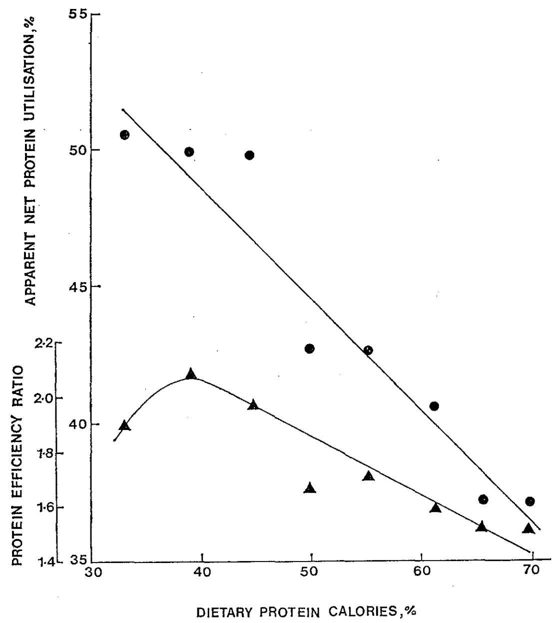

Fig. 4. Relationship between apparent net protein utilisation (●), protein efficiency ratio (▲) and dietary protein calories.

Fig. 5. Surprisingly, despite the high apparent NPU observed at the three lowest protein levels tested, apparent protein digestibility increased from 80 to 91% between dietary protein levels of 25.5% and 39.0%, and thereafter remained relatively constant at 91%. By contrast, apparent dry matter digestibility remained constant at 52% between dietary protein levels of 25.5% and 34.5%, sharply rose to 71% at a dietary protein level of 39.0%, and subsequently increased to a level of 79% with progressive protein substitution. A similar increase in apparent protein digestibility with increasing dietary protein level has also been observed in channel catfish[20] and rainbow trout,[21] although in the latter case when corrections were made for endogenous nitrogen losses, true protein digestibility remained constant. During the present study it is believed that the low apparent protein and dry matter digestibility observed within fish fed the three lowest dietary protein levels was due to the high carbohydrate content of these diets. The studies of SHIMENO *et al.*[22] on carbohydrate metabolism in the yellowtail (a marine carnivorous fish) have shown that high dietary levels of purified carbohydrate (potato starch) have a deleterious effect on growth, feed efficiency, and result in reduced protein and carbohydrate digestibility. Similarly, the research of SINGH and NOSE[23] and NAKAMURA *et al.*[24] with rainbow trout has shown that the digestibility of uncooked corn starch and dextrin varied with their dietary inclusion level, digestibility being considerably lower at high inclusion levels.

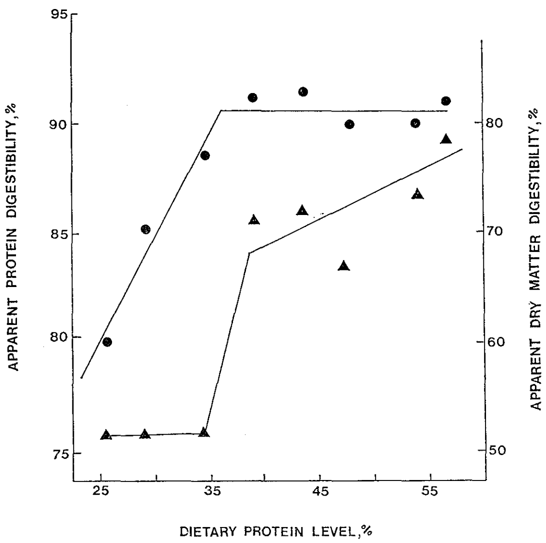

Fig. 5. Relationship between apparent protein digestibility (●), apparent dry matter digestibility (▲) and dietary protein level.

Dietary protein level also had a profound effect

Table 3. Proximate composition of fish at the start and end of the 56 day feeding trial (Values are expressed as % by weight, wet basis)

Constituent		After 56 days								±SE
	Initial	1	2	3	4	5	6	7	8	
Moisture	69.26[c]	62.96[a]	63.90[a b]	63.92[a b]	63.15[a]	63.26[a]	64.09[a b]	64.84[b]	65.00[b]	0.366
Crude protein	18.54[a]	21.77[b]	21.08[b]	22.13[b]	22.20[b]	22.14[b]	22.43[b]	22.08[b]	22.19[b]	0.438
Lipid	4.51[a]	7.06[b]	7.17[b]	6.87[b]	7.11[b]	6.97[b]	6.52[b]	6.16[b]	5.99[b]	0.421
Ash	5.40[a]	6.57[b]	6.55[b]	6.38[b]	6.40[b]	6.49[b]	6.46[b]	6.07[b]	6.21[b]	0.216

on fish carcass composition (Table 3). There was a significant increase ($P<0.05$) in carcass moisture content and a decrease in carcass lipid content with progressive dietary protein substitution. A similar decrease in carcass lipid content has also been observed in plaice,[17] grouper[16] and eel[25] with increasing dietary protein concentration. However, in contrast to the studies with plaice[17] and gilthead bream,[14] there was no increase in carcass protein content with increasing protein substitution during the present study.

There was a significant decrease ($P<0.05$) in the liver weight: body weight ratio with increasing dietary protein level (Table 2). In addition, histological investigation revealed proportionately less fatty infiltration and glycogen deposition within the liver and to a lesser extent the muscle of fish fed the high dietary protein inclusion levels. Increased liver somatic index and elevated glycogen and lipid liver deposition has been observed within fish fed diets containing high dietary concentrations of carbohydrate (where carbohydrate replaced dietary protein).[22,25]

Before any conclusion can be drawn from this study it should be pointed out that interpretation of the data presented is subject to a prior knowledge of the utilisation of dietary protein, lipid and carbohydrate as energy sources in relation to varying dietary energy levels.[26] At the outset of this experiment it was assumed that relatively high dietary levels of digestible carbohydrate would be tolerated and assimilated by snakehead. However, the low dry matter digestibility and reduced apparent protein digestibility of certain rations suggest that high dietary concentrations of purified carbohydrate are not well tolerated and utilised by snakehead. Consequently, although the gross energy content of all rations was relatively constant, on the basis of estimated digestible energy content, there was a progressive increase in digestible energy with increasing protein substitution, ranging from 303 kcal/100 g to 405 kcal/100 g (Table 1). In view of the poor digestibility of high dietary concentrations of carbohydrate therefore, it may be more advisable within such studies to use dietary lipid as a means of replacing or sparing dietary protein. On the basis of the present study, the optimum protein level for juvenile snakehead on the basis of daily weight gain and tissue protein deposition was estimated to be about 52% in the diet when herring meal was used as a protein source. The estimated protein requirement of snakehead is similar to that obtained for other carnivorous fish species; 50% for plaice,[17] grouper[16] and puffer fish[18] respectively.

Acknowledgements

The authors would like to acknowledge the assistance of the Overseas Development Administration and the support of Professor R. ROBERTS throughout this work.

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