STUDIES ON THE PROTEIN REQUIREMENT OF POSTLARVAE OF THE PENAEID PRAWN *PENAEUS INDICUS* H. MILNE EDWARDS USING PURIFIED DIETS

T. I. CHARLES JOHN BHASKAR AND SYED AHAMED ALI
Central Marine Fisheries Research Institute, Cochin 682 018.

ABSTRACT

Six purified diets were formulated and prepared using casein, starch, fish-and-groundnut oil mixture (1 : 1 ratio), vitamins, minerals and other additives, with the protein content varying from 20 to 70%, to study the protein requirement of the postlarvae (PL 1 to PL 42) of the prawn *Penaeus indicus*.

The results of the feeding experiments conducted separately on group I (PL1 to PL10), group II (PL11 to PL25) and group III (PL27 to PL42) for a duration of 10, 15 and 15 days, respectively, showed that the growth of group I postlarvae increased with the increasing protein content of the diet up to 40% and declined thereafter. The growth obtained by the diet with 40% protein was highly significant ($P < 0.01$). In group II postlarvae, the protein requirement was between 30 and 50%. In group III postlarvae, maximum growth was obtained with the diet with 30% protein and it was significantly different ($P < 0.05$) from the performances of the other diets. It is concluded that the dietary protein requirement of postlarvae decreases from 40% in the initial stages to 30% as they grow to advanced stages. Similarly, the rate of growth was found to be relatively faster in the young postlarvae.

INTRODUCTION

In the recent past there has been considerable advancement in the techniques for hatchery production of prawn seed. The techniques involve rearing of early larval stages in the hatchery, providing suitable environement and food, and further culturing of the postlarvae in nursery until they attain stockable size. Continuous research is being carried out with varying results in many parts of the world for the development of appropriate compounded feed required for different phases of prawn culture. In this context, a knowledge on the nutritional requirement of species for its different developmental stages of life forms an essential prerequisite for the preparation of nutritionally balanced feed. Indiscriminate use of nutrients in the diet may not only enhance the cost but also be deterimental to the animal. Protein constitutes the most important nutrient in the diet, contributing to the growth performance of the species, and forms one of the major items determining the cost factor of the diet.

Most of the protein-requirement studies were carried out only in juveniles or adult prawns. Practically no information is available in the literature on the protein requirement of the postlarvae of prawn and how it varies with their age. The present study was therefore aimed at the evaluation of protein requirement in the diet for postlarvae of the Indian white prawn, *Penaeus indicus*, and its variation with their age, using compounded purified diets.

Material and Methods

Formulation and preparation of purified diets

To study the protein requirements of the postlarvae of *P. indicus*, six purified diets, designated as 1, 2, 3, 4, 5 and 6, were formulated using casein (fat-free), starch, a mixture of sardine oil and groundnut oil (in the ratio 1 : 1), cholesterol, L-Arginine, vitamins, minerals and cellulose. All the diets were isocaloric, but the protein content varied from 20 to 70% by varying the casein proportion in the diet. Starch was used as the binder as well as a source of the carbohydrate.

The dry ingredients, starch, casein and cellulose were powdered and sifted separately with a 100-micron sieve. The sifted out powders, along with cholesterol and lipid, all weighed separately, were mixed together and homogenised in an electrical blender. Vitamins, minerals and L-Arginine were dissolved in 60 ml of distilled water (for 100 g of diet) and added to the mixture. The mixture was

Table 1. *Percentage composition of purified diets* 1 *to* 6.

Ingredients	Diets					
% age	1	2	3	4	5	6
Casein (fat free)	20.00	30.00	40.00	50.00	60.00	70.00
Starch	54.45	44.45	34.45	24.45	14.45	4.45
Lipid (Groundnut oil: Sardine oil 1: 1)	12.00	12.00	12.00	12.00	12.00	12.00
Cholesterol	0.50	0.50	0.50	0.50	0.50	0.50
L-Arginine. Hcl	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin and mineral mixture *	8.00	8.00	8.00	8.00	8.00	8.00
Cellulose	5.00	5.00	5.00	5.00	5.00	5.00
Total	100.00	100.00	100.00	100.00	100.00	100.00

* Each 100 g of diet contained vitamins: Thiamin mononitrate 40 mg, Riboflavin 8 mg, Nicotinamid 40 mg, Pyridoxine Hcl 12 mg, Calcium pantothenate 40 mg, Cyanocobala-4 mcg, Ascorbic acid 200 mg, Calciferol 1600 IU, Vitamin E (as dl-Alpha Tecopherol Acetate) 6 mg, Biotin 0.2 mg, Vitamin A (as acetate) 16000 IU; and Minerals: Calcium carbonate, 3.3 mg, Calcium phosphate 0.832 g, Potassium dihydrogen phosphate 2.4 g, Magnesium phosphate (dibasic) 192 mg, Ferrous sulphate 42.5 mg.

again thoroughly kneaded into a dough and steamed for 15 minutes. The dough was then cut into small blocks and dried in an electrical oven at 60 ± 2°C for 12 h. The dried blocks were converted into granules of three different sizes, 300, 400 and 500 microns. The granules were stored in polythene bags in air-tight plastic containers until use. The composition of the thus granulated diets is given in table 1.

Feeding experiments

Hatchery-raised postlarvae (belonging to the same brood) of the prawn, *P. indicus,* were used in the experiments. The postlarvae PL1 to PL10 were reckoned as group I, PL11 to PL25 as group II and PL27 to PL42 as group III. The animals were randomly selected and stocked 5-l-capacity circular glass containers containing 4 litres of sea water filtered through bolting-silk cloth (No. 30). The postlarvae of group I, with an average size and dry weight, taken at random of 30 animals, of 5.05 mm and 0.057 mg, respectively, were stocked at a rate of 20 per container. The postlarvae of group II and group III, with an average size and dry weight of 9.96 mm and 0.89 mg, and 13.43 mm and 0.46 mg, respectively, were stocked at the rates of 10 per container. Three replicates were kept for each treatment. The left-over food was removed and complete water was changed everyday. Intermittent aeration was provided in all the containers. At the end of each experiment, the animals, after having their average size recorded, were dried at 60 ± 2°Cfor 12 h for measuring the dry weight and dry weights were measured for individual treatments.

The group I animals were fed with the prepared diet of 300-micron particle size, at the rate of 100 mg per container per day, in two divided doses, at 1000 and 1700 hours. The experiment was continued for 10 days. The feeding experiment on group II animals were conducted with the diet having 400-micron particle size, at the rate of 150 mg per day per container in two similar doses, for 15 days. In the case of group III postlarvae, the diets used were of 500-micron particle size. The animals were fed at the rate of 200 mg per day per container as indicated in the I and II groups. The experiment was continued for a period of 15 days.

The hydrogrological characters of the rearing media, in respect of each experiment, are presented in table 2.

TABLE 2. *Salinity temperature and* pH *of the rearing media.*

	(PL1-PL10)	(PL11-PL25)	(PL27-PL42)
	Set I	Set II	Set III
Salinity ‰	32.0 ± 1.382	20.82 ± 1.386	20.44 ± 0.812
Temperature °C	31.25 ± 0.12	29.72 ± 0.620	27.63 ± 1.037
pH	7.84 ± 0.03	7.67 ± 0.161	7.59 ± 0.·95

TABLE 3. *Results of feeding experiment conducted with Group I postlarvae* (PL1 to PL10).

	Diets					
	1	2	3	4	5	6
No. of animals stocked	20	20	20	20	20	20
Initial average total length (mm)	0.05	5.05	5.05	0.05	0.05	0.05
Initial average dry weight (mg)	0.057	0.057	0.057	0.057	0.057	0.057
Final average total length (mm)	6.31	6.46	7.36	6.80	6.56	6.70
Final average dry weight (mg)	0.325	0.206	0.36	0.20	0.11	0.165
Growth in length (%)	24.95a	27.90a	45.74	34.60b	29.90ab	32.0b
Growth in weight (%)	470.18a	262.57abc	531.58d	250.88bc	92.98bc	189.47c
Survival (%)	22.50	46.67	22.50	25.00	27.50	26.23

Values with the same superscripts in any row are not statistically different from each other ($P < 0.05$).

RESULTS

In group I postlarvae, length and dry weight increased with increase in protein level up to 40% in the diet (table 3) and declined thereafter. The diet 3, with 40% protein, gave the highest growth, 45.74% and 531.58% respectively of the initial length and dry weight. The treatments were found to be significantly different among themselves ($P < 0.05$). The growth obtained by the diet with 40% protein was highly significant ($P < 0.01$). The growth curve (fig. 1, curve A) of the postlarvae has shown a clear peak at 40% protein level indicating the protein required for maximum growth.

The results of the feeding experiment conducted on group II postlarvae are given in table 4. In this group the maximum growth increment was obtained by the diets with 30% and 50% protein. The growth curve (fig. 2, curve B) showed the peak at 30% protein, declined slightly at 40% level and again rose at 50% approximately to the level at 30% protein. The curve drawn, using standard deviation (dotted line), however showed gradual increase in the growth up to 30% level; it was steady between 30 and 50% and declined thereafter. Analysis of variance showed that the treatments were significantly different among themselves ($P < 0.05$). However, comparison of means has shown that treatments 2, 3 and 4 were not significantly different from each other, but they were significantly different from treatments 1, 5 and 6. From these results it was presumed that the protein requirement for group II postlarvae lies between 30 and 50%.

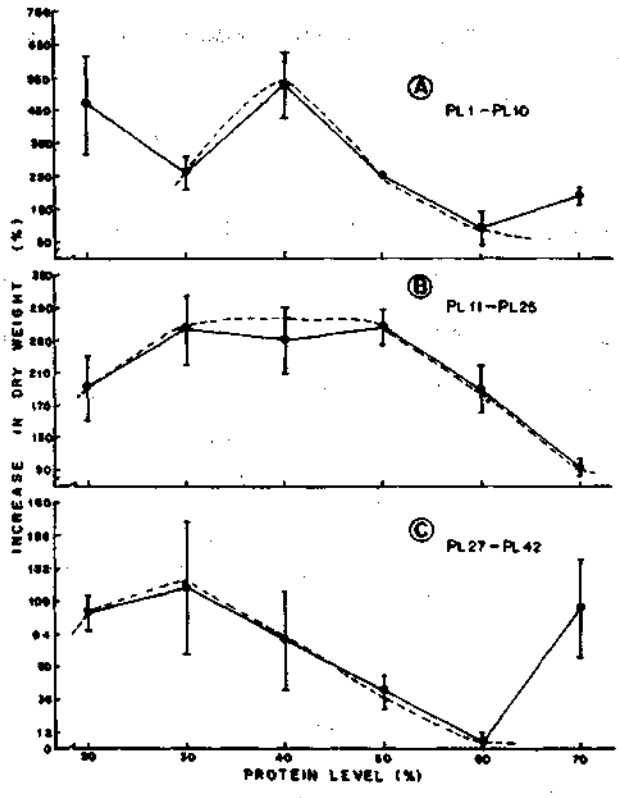

FIG. 1. Growth curves of postlarvae of *P. indicus*, showing the relationship between dietary protein level and growth. Curve A: Group I postlarvae (PL1 to PL10); Curve B: Group II postlarvae (PL11 to PL25); Curve C: Group III postlarvae (PL27 to PL42).

TABLE 4. *Results of the feeding experiment with Group II postlarvae* (PL11 to PL25).

	Diets					
	1	2	3	4	5	6
No. of animals stocked	10	10	10	10	10	10
Initial average total length (mm)	9.96	9.96	9.96	9.96	9.96	9.96
Initial average dry weight (mg)	0.89	0.89	0.89	0.89	0.89	0.89
Final average total length (mm)	12.45	14.40	13.29	13.73	12.59	12.16
Final average dry weight (mg)	2.606	3.240	3.103	3.257	2.580	1.725
Growth in length (%)	25.00a	44.58b	33.43c	37.85c	26.41a	22.09a
Growth in dry weight (%)	192.88a	264.04b	248.69b	265.92b	189.89	93.82
Survival (%)	90.00	100.00	86.67	86.67	74.20	85.00

Values with the same superscripts in any row are not statistically different from each other ($P < 0.05$).

TABLE 5. *Results of the feeding experiment conducted with Group III postlarvae* (PL25-PL42).

	Diets					
	1	2	3	4	5	6
No. of animals stocked	10	10	10	10	10	10
Final average total length (mm)	13.14	13.14	13.14	13.14	13.14	13.14
Final average dry weight (mg)	3.46	3.46	3.46	3.46	3.46	3.46
Initial average total length (mm)	17.17	18.53	17.43	16.58	16.56	17.51
Initial average dry weight (mg)	6.923	7.557	6.200	4.913	3.616	7.035
Growth in length (%)	35.24ab	41.02a	32.65b	26.18c	26.03c	33.26ab
Growth in dry weight (%)	100.09ab	118.41a	79.28bc	42.00cd	4.49d	103.32ab
Survival (%)	90.00	73.00	77.00	63.00	75.00	70.00

Values with the same superscripts in any row are not statistically different from each other ($P < 0.05$).

The data obtained on group III postlarvae are summarised in table 5. The diet with 30% protein recorded the highest growth in length (41.02%) and weight (118.4%) in this group. The growth curve (fig. 1, curve C) has shown a peak at 30% protein level, declined thereafter until 60% protein level and risen at 70% protein level, though not as much as at 30% protein level. Analysis of variance has shown that the treatments were significantly different among themselves ($P < 0.05$) and the growth obtained by 30% protein diet was significantly different ($P < 0.05$) from that of the diets 3, 4 and 5. However, no significant difference was found among the diets 1, 2 and 6. The protein requirement for group III postlarvae was therefore indicated at 30% level. It can be seen from the results that the young postlarvae (group I) grew faster (weight increase 531.58%), followed by group II (weight increase 364.04%) and group III (weight increase 118.4%) postlarvae. Thus the rate of growth of postlarvae declined with their age during the experiment.

DISCUSSION

The results of the present study have shown that the group I postlarvae of *P. indicus* require 40% protein in diet. The group II postlarvae have not shown the requirement precisely, but it appears to lie between 30 and 50%, while group III postlarvae have shown a requirement at 30% protein level. The protein requirement in the diet thus decreased as the age of the postlarvae progressed. Colvin (1976) and Ahamad Ali (1982a) have indicated that the protein require-

ment in the diet for juvenile *P. indicus* was about 40%. The former author used prawn meat and the latter used a mixture of mantis shrimp protein and groundnut cake as protein source. The protein requirement shown by group I and II postlarvae in the present study is in agreement with these findings. However, the protein requirement shown by group III postlarvae is comparatively low.

Such lower protein requirements were reported for the juvenile also of other penaeid species: by Shewbart et al (1973) in *P. aztecus* (between 22 and 30%), Andrews et al (1972) in *P. setiferus* (30%), Sick and Andrews (1973) in *P. duorarum* (between 28 and 30%) and Sedgewick (1979) in *P. merguiensis* (34 to 42%). The levels of carbohydrate and lipid present in the diet may also influence the dietary protein requirement. If the diet contains adequate levels of energy components the growth may be higher even at lower protein levels. Carbohydrate in diet is reported to have protein sparing action (Andrews et al 1972, Sick and Andrews 1973 and Ahamad Ali 1982b) up to 40%. The diets 2 and 3 used in the present study, with 30 and 40% protein, contained 44.45% and 34.45% of starch, respectively, in addition to 12% lipid. These factors might have also contributed to some extent for the lower protein requirement.

While comparing the growth obtained by the use of diets with 0, 30, 40 and 50% proteins, Khannapa (1979) observed that the diet with 30% protein gave highest growth increment in the postlarvae of *P. monodon* over a period of 30 days. But, over a period of 16 weeks, the growth increment was the highest in the case of the diet with 40% protein. The size group of the postlarvae used in these experiments is however not clear. Colvin and Brand (1977) have reported that the protein requirement for early postlarvae of penaeid prawns exceeds 40% but decreases to less than 30% as the size increases. The findings in the present study are in agreement with the above observations. However, Bages and Sloane (1981) observed that the growth of postlarvae of *P. monodon* (14 days old) was proportional to the protein level in the diet (25 to 55%).

In the present experiments the growth increment at optimum protein level was highest in the case of group I postlarvae (531.68%). In the case of group II and III postlarvae it was 264.04% and 118.4%, respectively. These results indicate that the younger postlarvae grow faster and, therefore may require higher levels of protein in the diet, but afterwords this level can safety be brought down.

ACKNOWLEDGEMENT

The authors are extremely grateful to Dr. E. G. Silas, Director, CMFRI, for the constant encouragement during this investigation. We are also thankful to Sri. K. H. Mohamed, Officer-in-Charge, Prawn Culture Laboratory, Narakkal, for the guidance.

REFERENCES

AHAMAD ALI, S. 1982 a. Relative efficiencies of pelletized feeds compounded with different animal proteins and the effect of protein level on the growth of the prawn *Penaeus indicus*. *Proc. Symp. Coastal Aquaculture*, 1: 321-328.

AHAMAD ALI, S. 1982 b. Effect of carbohydrate (starch) level in purified diets on the growth of *Penaeus indicus*. *Indian J. Fish.*, 29 (1 & 2): 201-208.

ANDREWS, J. W., L. V. SICK AND G. J. BAPTIST. 1972. The influence of dietary protein and energy levels on growth and survival of penaeid shrimp. *Aquaculture*, 1: 341-347.

BAGES, M. AND L. SLOANE. 1981. Effects of dietary protein and starch levels on growth and survival of *Penaeus monodon* (Fabricus) postlarvae. *Aquaculture*, 25: 117-128.

COLVIN, P. M. 1976. Nutritional studies on penaeid prawns: Protein requirement in compounded diets for juvenile *Penaeus indicus*. *Aquaculture*, 7: 315-326.

COLVIN, P. M. AND C. W. BRAND. 1977. The protein requirement of penaeid shrimp at various lifecycle stages in controlled environment systems. *Proc. World, Maricult.* Soc., 8: 821-840.

KHANNAPA, A. 1979. The effects of various protein levels on the growth and survival of *Penaeus monodon*. *Thai. Fish. Gazette*, 31: 51-60.

SEDGEWICK, R. W. 1979. Influence of dietary protein and energy on growth, food consumption and food conversion efficiency in *Penaeus merguiensis*. *Aquaculture*, 16: 7-30.

SHEWBART, K. L., W. L. MIES AND P. D. LUDWIG. 1973 Nutritional requirements of the brown shrimp, *Penaeus aztecus*. *U. S. Dep. Comm. Rep. No. Com.* 73-11794. 52 pp.

SICK, L. V. AND J. W. ANDREWS. 1973. Effect of selected dietary lipids, carbohydrates and proteins on the growth, survival and body composition of *Penaeus duorarum*. *Proc. World Maricult. Soc.*, 4: 263-276.