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Protein requirement of juvenile *Penaeus indicus*. 1. Food consumption and growth

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Abstract. Juveniles of *Penaeus indicus* were fed with different concentrations of protein diet (0–60% of dry weight of diet) with purified lipid free casein as protein source. During the experimental period of 30 days, survival was only 27% in the group fed with protein free diet. Survival (75%) and protein efficiency ratio (3·7) were maximum at 40 and 30% protein level, respectively; specific food consumption (4·3–6·7%) and food conversion ratio (1·3–1·4) were lowest at 30 and 40% protein level, respectively. Another experiment conducted to precisely quantify the near optimum protein requirement for maximum growth of *Penaeus indicus* revealed significantly higher growth and protein efficiency ratio in the groups receiving 35–37·5% protein than the other groups.

Keywords. *Penaeus indicus*; juvenile; protein requirement; food consumption; growth; protein efficiency.

1. Introduction

Protein requirement of prawns has been well studied and reviewed from time to time by a number of workers (New 1976; Maguire 1980; Pandian 1989). It is known to range from 22–60% of dry weight of the diet (Shewbart *et al* 1972; Deshimaru and Yone 1978). The wide range of values on protein requirement is mainly due to difference in the size of the experimental prawns, physiological status and other intrinsic and extrinsic factors (Abdel Rahman *et al* 1979; Maguire 1980). However, available studies on post-larval or juvenile *Penaeus indicus* suggests that the optimum protein requirement ranges from 40–43% of dry weight of the diet (Colvin 1976; Ali 1982; Bhaskar and Ali 1984). Though indicative of near precise quantification of protein requirement of *P. indicus*, these studies were conducted by offering qualitatively none too superior diet to the post-larvae juveniles. These studies were conducted by using semi-purified or compounded diets and only limited studies are available on the protein requirement of prawns by using purified diets (Bhasker and Ali 1984). As purified diets considerably reduce extraeneous nutritional factors and allow precise quantification of any particular nutrient requirement (D'Abramo *et al* 1982), more information is required on the protein requirement of prawns by using purified diets. In the present study, the following two experiments were conducted on the juveniles of *P. indicus* by using casein (lipid free), which is the only protein source available in highly purified state: (i) the effect of different protein levels (0–60% of dry weight of diet) on growth and food consumption, and (ii) near optimal quantification of protein in the juveniles by using test diets ranging from 32·5–47·5% of protein.

2. Materials and methods

2.1 *Experimental animals*

Juveniles of *P. indicus* belonging to the same brood stock were transported from Narrakal Prawn Hatchery of CMFRI, Cochin to the laboratory. The post-larvae were reared under laboratory conditions for 15–20 days and fed with compounded diets until they reached the required size (length: 20 ± 5 mm; live weight: 20 ± 8 mg). Healthy juveniles of uniform size were blotted in the folds of filter paper, weighed to the nearest mg and transferred to the experimental aquaria. In each aquarium, 20 juveniles were reared and each treatment had 3 replicates. Prior to the commencement of the experiment, the experimental animals were starved for 24 h and allowed to recover from handling stress. To determine the initial dry weight, a few prawns were initially weighed and sacrificed by immersing in boiling water for a brief period (Clifford and Bricks 1983) and left for drying at 40°C for 48 h in an oven. The dried samples were reweighed. Similar procedure was adopted at the end of the experiment to determine the final dry weight of the prawns. Growth of the prawns was calculated and represented as percentage increase on wet or dry weight basis.

2.2 *Experimental aquaria*

The experiments were conducted in plastic aquaria (54 cm dia.; 24 cm depth), mounted on vertical steel racks. In each aquarium 40 l of filtered, irradiated and dilute seawater (salinity: 20 ± 2·5‰) (Colvin 1976) was used. The water was continuously aerated and was replaced on alternate days.

2.3 *Test diets*

Isocalorically adjusted graded levels of protein test diets from 0–60% with an interval of 10% protein for the first experiment and from 32·5–47·5% with an interval of 2·5% protein for the second experiment were formulated following earlier studies (Kanazawa *et al* 1970, 1977; Adelung and Ponat 1977; Conklin *et al* 1978). Finely powdered, preweighed ingredients except cellulose, starch, lipids (oils) and vitamins were mixed in Warring blender. Gelatin was dissolved in cold water and later boiled with cellulose and starch. After gelatinization, corn oil and cod-liver oil containing fat soluble vitamins were added and heated in water bath for 10 min. The ingredients were mixed thoroughly and then steamed at 15 lb pressure for 5 min. The steamed feed was allowed to cool to room temperature and then the vitamin mixture was added and mixed thoroughly. The pH of the diet was adjusted to 6·8 (Kanazawa *et al* 1977) using 0·1 N NaOH and the feed was stored in polyethylene bags in a freezer. The moisture content in the feed was adjusted to 30%. Before feeding the prawns, the feed was thawed to room temperature and made into small balls and weighed. The protein content in the diets were accordingly adjusted without changing the calorie content. The composition of the formulated diets for each experiment is as shown in table 1.

Table 1. Composition of experimental diets.

Ingredient g/100g	Experiment 1							Experiment 2						
	0	10	20	30	40	50	60	32·5	35	37·5	40	42·5	45	47·5
Casein (lipid-free)	—	11	21	31	41	51	61	34	36	39	41	44	46	49
Egg albumin	—	1	1	1	1	1	1	1	1	1	1	1	1	1
Gelatin	—	1	1	1	1	1	1	1	1	1	1	1	1	1
Glucosamine-HCl	—	1	1	1	1	1	1	1	1	1	1	1	1	1
Sucrose	20·11	19·9	15·43	11·68	7·24	4·68	1·18	10·25	9·65	8·28	7·24	6·86	6·65	5·45
Glucose	10·19	7·19	6·53	4·96	3·68	2·32	0·9	4·61	4·41	4·20	3·68	3·33	2·75	2·5
Starch	43·45	32·59	27·39	22·61	18·51	12·98	7·65	21·65	20·58	18·89	18·51	15·61	14·92	13·66
Cod-liver oil	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Corn oil	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Cholesterol	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5
Sodium succinate	0·3	0·3	0·3	0·3	0·3	0·3	0·3	0·3	0·3	0·3	0·3	0·3	0·3	0·3
Sodium citrate	0·3	0·3	0·3	0·3	0·3	0·3	0·3	0·3	0·3	0·3	0·3	0·3	0·3	0·3
Mineral mixture*	7·41	7·41	7·41	7·41	7·41	7·41	7·41	7·41	7·41	7·41	7·41	7·41	7·41	7·41
Vitamin mixture**	3·24	3·24	3·24	3·24	3·24	3·24	3·24	3·24	3·24	3·24	3·24	3·24	3·24	3·24
Agar-agar	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Cellulose	4	4	4	4	4	4	4	4	4	4	4	4	4	4

*$CaHPO_4 \cdot 2H_2O$ 2·5; $MgSO_4 \cdot 7H_2O$ 2·0; KH_2PO_4 1·5; $NaH_2PO_4 \cdot 2H_2O$ 1·0; $MnSO_4 \cdot H_2O$ 0·14; $FeSO_4$ 0·1; $ZnSO_4 \cdot 7H_2O$ 0·1; $C_6H_5O_7Fe \cdot 5H_2O$ 0·05; $Co(NO_3)_3$ 0·01; $CuSO_4 \cdot 5H_2O$ 0·01.

**B-Carotene 0·014; calciferol 0·002; α-tocopherol acetate 0·032; menadione 0·032; ascorbic acid 2·424; thiamine hydrochloride 0·01; riboflavin 0·008; nicotinic acid 0·032; pyridoxine hydrochloride 0·016; calcium pantothenate 0·06; folic acid 0·001; p-aminobenzoic acid 0·014; choline chloride 0·3; inositol 0·3; biotin 0·004; cyanocobalamin 0·001.

2.4 *Estimation of food consumption*

The prawns were fed with the test diets twice a day at the rate of 10% (dry weight feed) of live body weight/day. The uneaten food was collected every day by siphoning the water through bolting silk; the residue was washed in distilled water to remove the adhering salts and transferred to preweighed aluminium foils, dried and weighed at 70°C for 48 h. The total dry feed consumed by the prawns in each group was determined every day by subtracting the dry weight of uneaten food from the dry weight of food offered. Specific food consumption (SFC) (Bordner and Conklin 1981; Gopal 1986), food conversion ratio (FCR) and protein efficiency ratio (PER) were calculated as follows:

$$\text{SFC (\%)} = \frac{\text{Total initial dry wt. of food offered} - \text{Total final dry wt. of food uneaten}}{\text{Number of animals surviving at the end of experiment} \times \text{experimental period (days)} \times \text{mean animal wet wt.(g)}} \times 100$$

$$\text{FCR} = \frac{\text{Total dry wt. of food offered} - \text{Total dry wt. of uneaten food}}{\text{Final wet wt. of prawns} + \text{Wet wt. of dead prawns} - \text{Initial wet wt. of prawns}}$$

$$\text{PER} = \frac{\text{Final wet wt. of prawns} - \text{Initial wet wt. of prawns}}{\text{Total protein intake}}$$

For each of these parameters, the mean values of the replicates per treatment was considered for the statistical calculation.

2.5 *Experimental conditions*

The mean water temperature, pH and ammonia levels in the aquaria for both experiments were 27·7 ± 1·9/27·6 ± 2·27°C; 8·4 ± 1·0/8·02 ± 0·5 and 0·04 ± 0·01/0·02 ± 0·003 NH_4-N mg/l/day, respectively. Samples of each diet were analysed for crude protein, lipids and moisture following the methods suggested by AOAC (1975). Water samples were analysed using standard methods (Strickland and Parsons 1972; Spotte 1979).

2.6 *Statistical analysis*

All the parameters (SFC, FCR, PER) estimated are apparent and no correction factor was introduced for the exuviae and dead prawns eaten by the cohabitors during the experimental study. The data collected were statistically analysed using the test of significance and variance (ANOVA) and the means of the treatments were compared by the least significance difference method (LSD) (Snedecor and Cochran 1973).

3. Results and discussion

3.1 *Survival*

The protein concentration in the diet significantly ($P < 0.05$) influenced the survival of *P. indicus*. During the experimental study of 30 days, only 27% of prawns receiving 0% protein level survived and the percentage of survival was 75% in the group receiving 40% protein (figure 1). As more than 50% of prawns survived at 10% protein level, 10% protein in the diet may be considered as the critical protein level for the survival of the juveniles. Beyond 40% protein, the survival rate declined, indicating the adverse effect of excess dietary protein on the prawns. Weekly survival rate at different protein levels shows that the mortality of prawns fed with protein free diet was higher from the second week onwards. It was observed that most of the prawns receiving protein free diet succumbed immediately after molting. They were also not active and the cohabitors resorted to cannibalism. Cannibalism was evident in all the groups, more so in the groups receiving excess protein (>40%).

3.2 *Specific food consumption*

The SFC of *P. indicus* was influenced by the protein content in the diet. It was 33·7% for the groups receiving protein free diet as against amongst the groups receiving 10–60% protein, it was minimum (4·3%) at 40% protein (figure 2). Thus, prawns fed on protein free diet consumed 8 times more than the juveniles fed with protein in the diet. Inspite of the fact that the prawns fed with protein free diet consumed more than the other prawns, even then there was large scale mortality suggesting that protein forms the basic nutrient in the diet of the prawns. Further it was observed that as the protein concentration increases, the SFC falls sharply up to 40% protein level. Thus, it appears that juveniles of prawns when exposed to

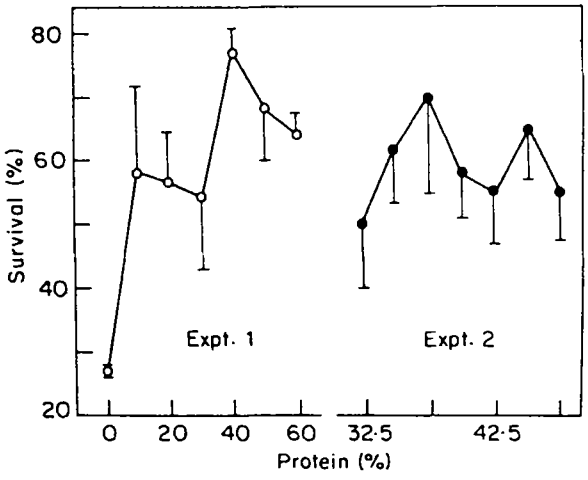

Figure 1. Per cent survival of juvenile *P. indicus* fed with different levels of protein in the diet (0–60%/32·5–47·5%).

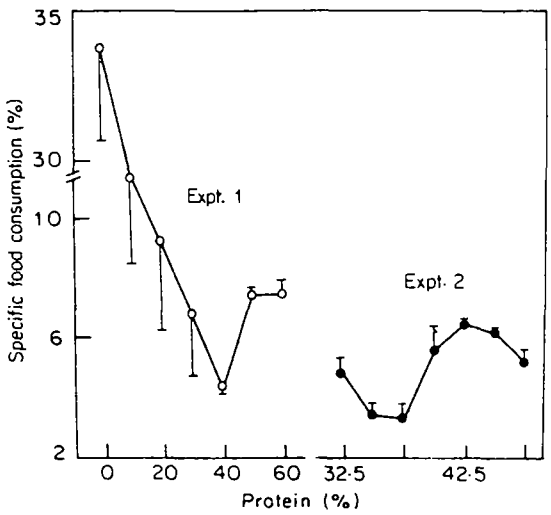

Figure 2. Specific food consumption in juvenile *P. indicus* fed with different levels of protein in the diet (0–60%/32·5–47·5%).

optimum level do not load the stomach with excess energy but prefer to consume required quantum of protein based energy. However, exposure to very high protein levels (50 and 60%) induces the prawns to increase the food consumption, thereby loading the stomach with very high protein energy.

3.3 *FCR and PER*

The FCR and PER (figure 3) were also significantly influenced by the protein content in the diet. The FCR sharply declined from $4{\cdot}6 \pm 0{\cdot}4$ in the prawns fed with protein-free diet to $1{\cdot}3 \pm 0{\cdot}9$ at 30% protein; these values were statistically significant ($t = 16{\cdot}9$; $P < 0{\cdot}05$). Similarly, the prawns fed with 30% protein had significantly higher PER than the PER of other groups.

3.4 *Growth*

The mean gain in dry weight of the prawns increased from 125% in the group exposed to protein-free diet to 522% in the group exposed to 40% protein and thereafter declined to 430% at 60% protein (figure 4). The sharp increase in growth of the group receiving 30% protein than the earlier group (20% protein) and the maximum growth at 40% protein in terms of dry as well as wet weight suggest that 30–40% of protein may be the optimum level for the maximum growth of the juvenile prawn. The difference in mean gain in dry weight between 30 and 40% protein levels was not statistically significant (dry wt.; $t = 0{\cdot}25$, $P > 0{\cdot}05$). The decline in growth at high protein levels (50 and 60%) reveals the deleterious effect of excess protein in the diet (Venkatramiah *et al* 1975; Bages and Sloane 1981; Vernberg 1987). Thus, it was observed that when juvenile prawns are fed with optimum protein diets, the growth and protein efficiency tends to be maximum and the

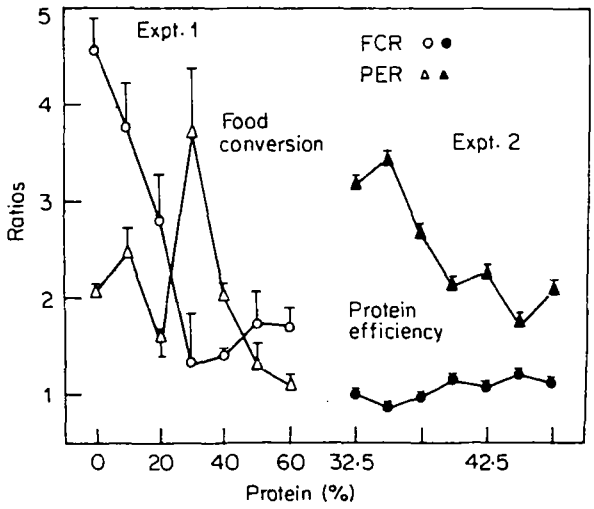

Figure 3. Food conversion ratio and protein efficiency ratio in juvenile *P. indicus* fed with different levels of protein in the diet (0–60%/32·5–47·5%).

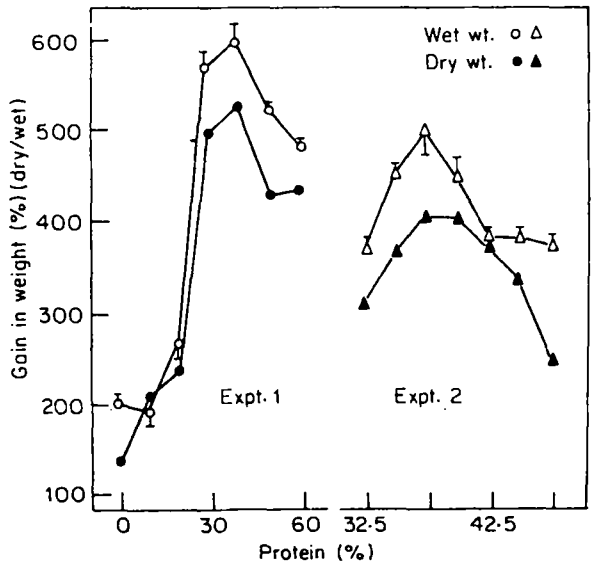

Figure 4. Per cent gain in weight by juvenile *P. indicus* fed with different levels of protein in the diet (0–60%/32·5–47·5%).

specific food consumption and food conversion ratio are the lowest thereby suggesting the importance of protein based energy diets which are efficiently utilized by the animals.

3.5 *Near optimal protein requirement*

The second experiment on the juveniles of *P. indicus* was conducted based on the

results of the first experiment to precisely quantify the optimal protein requirement. It was observed that there was no significant difference in the survival percentage in the test groups (32·5% vs 47·5%; $t = 1{\cdot}35$; $P > 0{\cdot}05$) since the diets offered were near optimal requirement. However, comparing other parameters it was observed that the SFC was significantly low at 35% (3·4%) and 37·5% (3·3%) protein levels compared to the SFC of other protein levels (figure 2). For instance, the SFC of juveniles feeding on 37·5% protein diet (3·3 ± 0·6%) was significantly ($t = 20{\cdot}42$; $P < 0{\cdot}05$) lower than the SFC of juveniles feeding on 40% protein diet (5·6 ± 0·7%). Similarly, the FCR (0·8) was the lowest and the PER (3·5) was the highest at 35% protein level. Thus, all these parameters substantially augment to the maximum mean gain in weight obtained at 37·5% protein level thereby indicating the optimal level around 35 and 37·5% protein level (figure 4). The growth of the juveniles declined beyond 40% protein, confirming the growth pattern obtained in experiment 1 (figure 4).

A comparison of food consumption and growth of the prawns of the experiments 1 and 2 revealed a few differences between these two experiments. The growth, especially, was slower in experiments 1 and 2. The difference may be due to genetic variations in the brood stock, as the juvenile prawns used for the experiments were from two different stocks. Nevertheless, a definite trend was obtained in experiment 2 which has facilitated identification of near optimal protein requirement of the juvenile prawns.

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