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Requirements of linoleic and linolenic acid in the diet of Indian white prawn *Penaeus indicus* (H Milne Edwards)

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Feeding trials using purified diets were carried out for determining the requirements of linoleic and lenolenic acid in the diet of Indian white prawn *Penaeus indicus*. Nine isonitrogenous and isocaloric diets were formulated containing palmitic acid as basal lipid source. Various levels of either individual or in mixture of linoleic and linolenic acids were used in these diets. When fed on diet without linoleic or linolenic acid (diet 1) the prawns exhibited poor growth, food conversion ratio, protein efficiency ratio and protein utilisation thus indicating that these fatty acids are essential for growth of *P. indicus*. Diet containing 2% linolenic acid showed higher growth rate than that contained 1%. Diet containing 1% linoleic acid supported significantly higher growth. Among the four diets, compounded with mixture of linoleic and linolenic acid in different percentage and proportion. Diet with a mixture of linoleic and linolenic acid incorporated at 1 % level in the ratio of 0.5 : 0.5 produced superior growth compared to remaining diets with higher levels of these fatty acids.

Although the essential fatty acid (EFA) content of crustaceans is very high, they are unable to synthesize these from other saturated fatty acids[1]. Earlier nutritional studies have shown that crayfish *Astacus astacus*[2], the lobster *Homarus gammarus*[3] and prawn, *Penaeus japonicus*[4] require EFAs such as linoleic acid (18:2 n-6), linolenic acid (18:3 n-3), eicosapentaenoic acid (20:5 n-3) and docosahexaenoic acid (20:6 n-3) in their diet for normal survival and growth. Kanazawa & coworkers[5,6] reported the absence of *de novo* synthesis of these EFAs from acetate or palmitic acid in *P.japonicus*[5], *P. monodon* and *P.merguiensis*[6]. Feeding experiments[4,7-9] have shown that the juveniles of *P. japonicus* have a higher weight gain with diets containing linoleic acid (18:2n-6) or linolenic acid (18:3n-3), or eicosapentaenoic acid (20:5n-3) or docosahexaenoic acid (22:6n-3) than (18:1n-9). The absence of lenolenic acid (18:3n-3) in diets resulted in poor weight gain in the prawn *P.aztecus*[10]. Similar observations were found for the prawn *P. stylirostris*[11]. The necessity of 18:3n-3 and 18:2n-6, for the growth and survival of larval stages of *P.japonicus* has been reported[12]. Above information suggest that linoleic acid and linolenic acid are essential fatty acid in the diet for survival and growth of prawns as they lack the ability of *de novo* synthesis of these fatty acids. Normally purified ingredients are used to study the dietary requirement of fatty acids, but earlier reports[13-15] on fatty acid requirements of *P.indicus* are by using compounded feed containing natural ingredients which also contain other nutrients than linolenic acid or linoleic acid, therefore, it was essential to study whether these fatty acids alone or in mixture are required for *P.indicus*. Therefore, the present experiments were conducted by using all the purified ingredients including linoleic acid and linolenic acid to understand their requirement in diets of juvenile prawn *P.indicus*.

Materials and Methods

Feeding experiments of 30 days duration were conducted in the laboratory. About 40 litres of seawater of salinity $20 \pm 2 \times 10^{-3}$ was used in each experimental container, which was aerated continuously. Water was siphoned and filtered through a biological filter and used for subsequent

Table 1—Composition of basal diets (%)

Ingredients g/100 g diet	
Casein	31.0
Egg albumin	7.5
Amino acids mixture[1]	5.0
Glucosamine	0.8
Sodium citrate	0.3
Sodium succinate	0.3
Starch	12.0
Glucose	4.9
Sucrose	11.0
Cholesterol	0.5
Lipids[2]	12.0
Vitamin mixture[3]	3.2
Mineral mixture[4]	8.5
Cellulose powder	3.0
Total	100.00
Agar-agar	5.00
Distilled water	100-120 ml.

[1] Amino acids mixture (g/100 g diet) arginine-1, methionine-0.5, glycine-2, taurine-0.5, glutamic acid-1.

[2] Lipid mixture-codliver oil:Soyabean oil:lecithin in the ratio of 56 : 28 : 16

[3] Vitamin mixture (mg/100 g diet) thiamine HCL (B1)-4.9, riboflavin (B2)-8, para.amino benzoic acid-10, inositol-400, niacin-40, calcium pantothenate-60, pyridoxine HCL-12, menadione-4, beta-carotene-9.6, alpha-tocopherol (vitamin E)-20, calciferol- 1.2, cynacobalamin (B 12)-0.8, sodium ascorbate-2000, folic acid-0.8, choline chloride-600.

[4] Mineral mixture (g/100 g diet) -K_2HPO_4-2, $Ca(PO_4)_2$ - 2.72, $MgSO_4$ ($7H_2O$) - 3.04, NaH_2PO_4 ($2H_2O$) - 0.79, $MnSO_4$ ($5H_2O$) - 0.004, $FeSO_4$ ($7H_2O$) - 0.015.

Table 2—Composition of dietary lipids / fatty acids in the test diets used for juveniles of *Penaeus indicus*.

Diet no.	Dietary lipids / fatty acids used for juveniles
1	10 % Palmitic acid
2	9 % Palmitic acid + 1 % linolenic acid
3	8 % Palmitic acid + 2 % linolenic acid
4	9 % Palmitic acid + 1 % linoleic acid
5	8 % Palmitic acid + 2 % linoleic acid
6	9 % Palmitic acid + 0.5 % linoleic acid + 0.5% linolenic acid
7	8 % Palmitic acid + 1 % linoleic acid + 1 % linolenic acid
8	7 % Palmitic acid + 2 % linolenic acid + 1 % linoleic acid
9	7 % Palmitic acid + 2 % linoleic acid + 1 % linolenic acid
10	5.6 % Codliver oil + 2.8 % soyabean oil + 1.6 % lecithin

two days. On the fourth day, the entire water was replaced with fresh seawater of same salinity. The environmental factors maintained for experiments are : salinity ($20 \pm 2 \times 10^{-3}$), temperature (26° - 29.7°C), dissolved oxygen (4.9 - 6.4 mg/l), *p*H (7.9-8.3) and total ammonia-N (0.03 - 0.1 ppm) and were within the tolarable limits.

Nine isonitrogenous and isocaloric purified diets were formulated and prepared with slight modifications[4, 14, 16, 17]. Composition of the basal diet and dietary lipids is given in the Table 1 and 2 respectively. Palmitic acid as source of basal lipid and linolenic acid (18:3n-3) and linoleic acid (18:2n-6) as source of EFAs were used in the diets 1 to 9. A mixture of codliver oil, soyabean oil and lecithin was used as lipid source in diet 10.

Juveniles of *P. indicus* ranging from 20 to 24 mm in length, 49 to 53 mg in average wet weight and 11.42 mg.in initial dry weight from the same brood reared in the prawn culture laboratory of CIBA, Narakkal, were randomly selected for the experiments. Stocking was done at the rate of ten juveniles per container. Feeding rate was 10-20 % of the total body weight, which was given in two doses viz. 0.25 in the morning at 9 h and 0.75 in the evening at 18 h. Leftover feed as well as faecal strands were separately collected daily by siphoning, dried in an electric oven and dry weight recorded for calculation of food conversion ratio (FCR) and protein efficiency ratio (PER). Proteins, lipids, carbohydrate, cholesterol, dry weight and wet weight of the juveniles, diets and faecal matter were determined by standard methods[18 - 22]. The results on survival, gain in length and weight, FCR, PER and post - experimental proximate composition of the test species were subjected to statistical analysis.

Results

Survival - Diet containing palmitic acid alone (diet 1) resulted in significantly ($P < 0.05$) low survival (Table 3). Inclusion of linoleic acid in diets 4 and 5 or linolenic acid in diets 2 and 3 produced significantly better survival (Table 3). During first two weeks survival was around 90 % in all the treatments (diets 2 - 9). Mortality started at the end of third week and survival declined to 40 to 55 % in all the treatments (Table 3). However, survival of prawn with the diet 1 having no linoleic or linolenic acid was comparatively less (70 %) during first two week and further decreased to 25 % in fourth week. Survival was uniformly very good with diet 10.

Growth performance - Gain in length, wet weight, dry weight, FCR and PER (will be referred

as "growth performance" in the text) of prawn was significantly ($P < 0.05$) influenced by the diet fed to them (Fig.1 A-L). Among all diets, diet 1 containing only palmitic acid as lipid source produced lowest growth performance. Of the two diets (diets 2 and 3) containing linolenic acid diet 3 with 2 % linolenic acid produced significantly ($P<0.05$) higher growth performance than diet 2 with 1% linolenic acid. However inclusion of 1% linoleic acid in the diet (diet 4) supported significantly ($P < 0.05$) higher growth than diet 5 with 2 % linoleic acid. Inclusion of linolenic acid in the diets significantly ($P < 0.05$) enhanced

Table 3—)Weekly survival of juvenile prawns *P.indicus* fed on the various diets containing fatty acids.

Diet no.	Survival of Juveniles (%)				
	1st Week	2nd Week	3rd Week	4th Week	Final
1	100	70	50	27	25
2	100	90	49	43	40
3	100	90	51	45	45
4	100	90	63	55	55
5	100	90	73	55	55
6	100	90	66	45	45
7	100	90	80	55	55
8	100	90	73	43	40
9	100	90	76	43	35
10	100	100	100	100	90

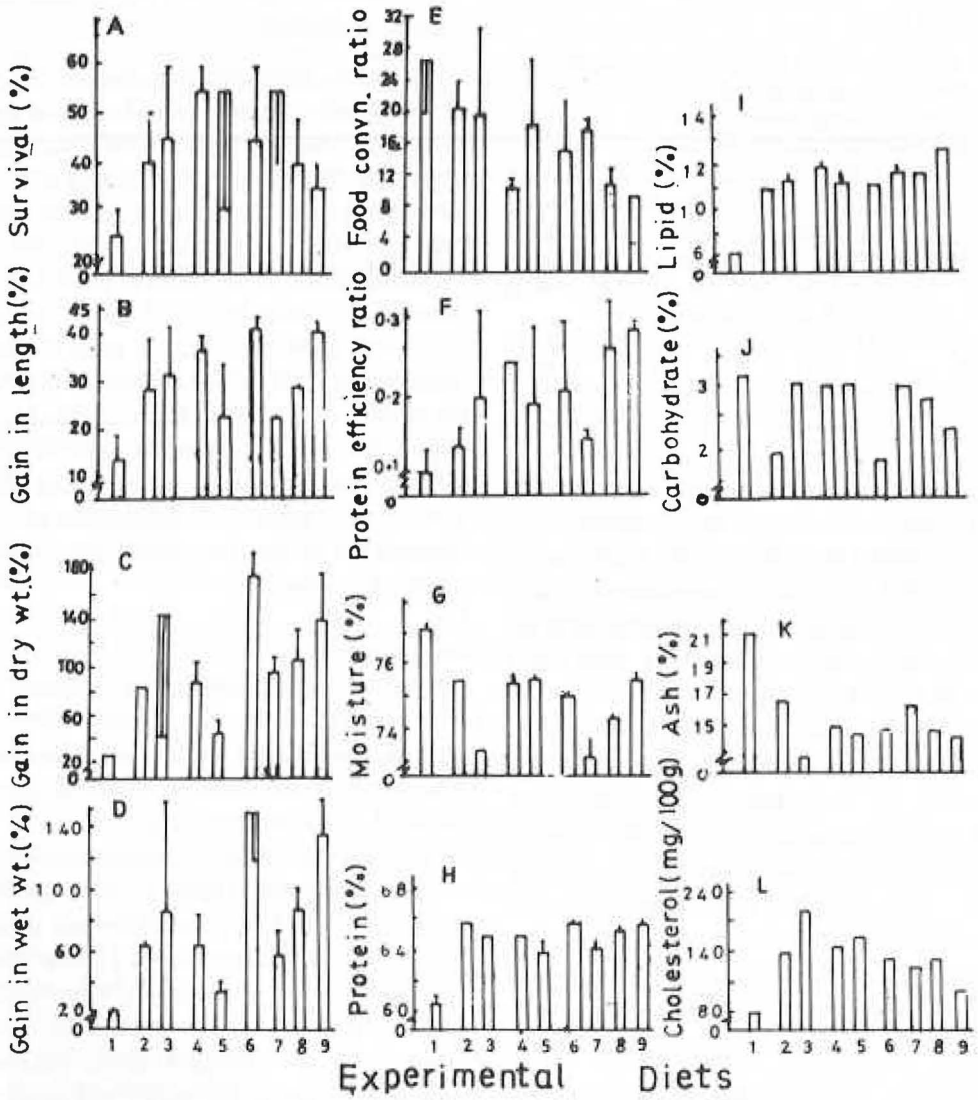

Fig.1—Results of the experiment in juvenile *P.indicus* fed on diets 1-10 containing different levels of fatty acids. A - % survival, B - % gain in length, C - % gain in wet weight, D - % gain in dry weight, E - Food Conversion ratio, F - Protein efficiency ratio, G - % moisture, H - % protein, I - % lipid, J - % carbohydrate, K - % cholesterol, L - % ash.

growth performance when compared to linoleic acid. Among the four diets compounded with mixture of linoleic and linolenic acid (diets 6-9), diet 6 in which 1 % level of these fatty acids in the ratio of 0.5 : 0.5 supported superior growth performance. Growth performance with diet 10 (control) compared better than the other diets.

Proximate composition - Juvenile prawns fed on the diet deficient in unsaturated fatty acid had relatively high moisture (Fig.1-G) and ash content (Fig.1-K) but low protein (Fig.1-H), lipid (Fig.1-I) and cholesterol contents (Fig.1-L). Inclusion of linoleic and linolenic acid in the diets also relatively improved the protein and lipid retention in prawns in most of treatments. No significant differences in the lipid content of prawns was found when fed on diets 2 to 9, containing various levels of linolenic and linoleic acid. Protein and lipid contents of prawns were significantly higher and moisture and ash content significantly lower in prawns fed on diet 10 (control).

Discussion

The poor growth performance of the prawns fed on diet 1 without either linolenic acid (18:3n-3) or linoleic acid (18:2n-6) indicates that the essentiality of these fatty acids for the prawn *P. indicus*. However, the superior growth performance of the prawns fed on diets (diets 2-9) containing either linolenic acid (18:3n-3) or linoleic acid (18:2n-6) or mixture of 18:3n-3 and 18:2n-6 indicate that these fatty acids are indispensable for *P. indicus*. The results suggest that the prawn *P.indicus* requirs dietary sources of both linoleic and linolenic acids for normal growth, although activity of linoleic acid as required fatty acid appears to be inferior than that of linolenic acid. It has also been reported by feeding experiment that *P. japonicus* also have higher weight gain with diet containing linoleic acid (18:2n-6) and linolenic acid (18:3n-3) than with that of palmitic acid (18:0)[4,7-9], and nutritive value of 18:3n-3 was superior than 18:2n-6.

Experimental results further indicate that level of 2 % linolenic acid (diet 3), 1 % linoleic acid (diet 4) and 1 % mixture of lenolenic and linoleic acid in the ratio of 0.5 : 0.5 (diet 6) are optimum levels required for promoting better growth in juvenile *P. indicus*. Among all the diets, diet 6 with 1 % mixture of linolenic acid and linoleic acid showed superior growth performance as compared to all other diets. Data on FCR and PER and nutrient deposition in the body of *P.indicus* clearly indicates that mixture of linoleic and linolenic fatty acids are essential for the efficient utilization of the food and protein. This indicates that a mixture of linoleic acid (18:2n-6) and linolenic acid (18:3n-3) could be more useful in the diet for promoting better growth in prawn *P. indicus* than individual of these EFAs. New[23] also suggested that the ratio of linolenic acid : linoleic acid (n-3 : n-6) is important in the diet of prawn indicating the importance of linoleic (n-6) along with linolenic (n-3) fatty acid. Importance of linoliec acid (18:2n-6) along with linolenic acid (18:3n-3) was also reported by Colvin[13] for *P. indicus* and Deshimaru *et al*[24] for *P. japonicus*.

Thus these results confirm and suggest that the *P. indicus* requires dietary sources of both linolenic and linoleic fatty acid for normal growth and mixture of sources containing linolenic and linoleic fatty acid could be more useful for formulating superior diet for promoting better growth in juvenile prawn *P. indicus*.

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