DIETARY LIPID REQUIREMENTS OF THE JUVENILES OF INDIAN WHITE PRAWN PENAEUS INDICUS H. MILNE EDWARDS

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ABSTRACT

Seven isonitrogenous and isocaloric diets containing graded lipid levels (0–18% lipid content) were formulated and prepared using chemically purified ingredients. A mixture of codliver oil, soyabean oil and lecithin was used as the lipid source. Each diet was fed to triplicate groups of juvenile *Penaeus indicus* for 30 days. Data were collected to assess the survival rate, growth rate, feed conversion ratio (FCR), protein efficiency ratio (PER) and body composition. The growth, survival and PER increased with the increase in dietary lipid level from zero to 12%. No significant improvement in growth occurred by inclusion of lipid at levels above 12%. A decline in the protein content of the prawns was observed above 12% lipid. There was also no significant difference in FCR for lipid level above 9% in diet.

Key words: Dietary lipid requirements, Penaeus indicus H. Milne Edwards.

Lipids are indispensable nutrients for growth and survival of shrimps and prawns (Kanazawa, 1985). However, information regarding quantitative lipid requirement of prawns is limited, though most researchers included lipid in their dietary formulations for prawns. Lipids derived from plant products, animal products and mixture of plant and animal products have been used in the diets of prawns according to their availability (Kanazawa, 1985). The level of lipid used in the diets also varied according to their convenience, without considering the dietary requirement of the animal concerned.

The most comprehensive studies on lipid requirements of prawns have been those of Kanazawa *et al.* (1970, 1977b) and Deshimaru *et al.* (1979) and Kanazawa (1985) who have investigated the quantitative lipid requirements of the juveniles of the Kuruma prawn, *Penaeus japonicus.*

Earlier studies on lipid nutrition of *P. indicus* are those of Colvin (1976) who studied the effect of selected seed oils on growth and fatty acid composition of juvenile *P. indicus* and Read (1981) who reported the response of juvenile

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P. indicus to various plant and animal oils. Colvin (1976) supplemented a constant level of 5% plant oil in the experimental diets, in addition to the lipid present in other ingredients (fish meal) and reported that a diet containing 9.8% lipid gives better growth in juvenile *P. indicus*. Read (1981) supplemented lipids at 3 and 4.5% levels in various diets containing selected lipid sources and found that a diet with a 3% mixture of fish oil and sunflower oil in the ratio of 2:1 gives better survival and growth in juvenile *P. indicus*. Despite these preceding reports, so far, no information exists on the effects of graded levels of lipids on any stage of *P. indicus*. In the present study the effect of graded levels of lipids (in the diet) on growth, FCR, PER and body composition of juveniles of *P. indicus* in a brackish water medium was examined.

MATERIALS AND METHODS

Feeding experiments were conducted in laboratory aquaria with three replicates for each of the treatments at the Centre of Advanced Studies in Mariculture, Central Marine Fisheries Research Institute, Kochi. Plastic tubs of about sixty litres capacity were chosen as experimental containers since they do not have any harmful effect on animals.

About 40 litres of brackish water of salinity $20 \pm 2\%$ was used in each container and continuous aeration was provided. Dissolved oxygen level (4 to 6 mg/l) and ammonia level (0.05 to 0.11 PPM) in the experimental medium were within admissible limits. The temperature and pH of water ranged between 27° C to 30° C and 7.9 to 8.2 respectively during experiment.

About 3/4 of the water was changed daily after removing the faeces and left-over feed and every fourth day complete water was changed after cleaning the tubes.

Formulation and preparation of feeds

Seven isonitrogenous and approximately isocaloric diets were formulated and prepared using purified ingredients following the formulae and guidelines provided by Kanazawa *et al.* (1970, 1977b) with little modification. Composition of the basal diet is given in Table 1.

The lipid levels ranged from zero to eighteen per cent in the diets (Table 2). The level of glucose, sucrose and cellulose was adjusted to maintain approximately isocaloric levels in each of the diets (Table 2). Earlier observations (Deshimaru *et al.*, 1979) indicate that a mixture of marine and plant lipids produce maximum growth in *P. japonicus* and that lecithin is essential for juvenile *P. japonicus* (Kanazawa, 1985). So a mixture of codliver oil, soyabean oil and lecithin in the ratio 58 : 26 : 16 was used as the lipid source in the diets, as this mixture of lipids provides the required fatty acids in the diet (Table 3).

Ingredients were procured from authorised dealers of respective manufacturing companies (Sigma USA, BDH England, SRL and HIMEDIA India and Table 1. Ingredient composition (%) of the basal diets used for juveniles of Penaeus indicus

Ingredients	Diet for juveniles gm/100 gm	
Casein .	31.00	
Egg albumin	7.50	
Amino acids mixture ¹	5.00	
Glucosamine	0.80	
Sodium citrate	0.30	
Sodium succinate	0.30	
Starch	12.00	
Glucose ²	3.50	
Sucrose ²	19.40	
Cholesterol	0.50	
Lipids ²	0.00	
Vitamin mixture ³	3.20	
Mineral mixture ⁴	8.50	
Cellulose powder	2.00	
Total	100.00	
	5.00	
Distilled water	100–120 ml	

¹Amino acids mixture (g/100 g diet): Arginine—1.00, Methionine—0.50, Glycine—2.00, Taurine— 0.50, Glutamic acid—1.00.

²Lipid mixture: as given in Table 2.

³Vitamin mixture (mg/100 g diet): Thiamine HCL (B₁)—4.9, Riboflavin (B₂)—8.0, Para-amino benzoic acid—10.90, pyridoxine HCL—12.00, Menadione—4.00, β Carotene—9.60, α Tocopherol (Vitamin E)—20.00, Calciferol—1.20, Cynacobalamin (B₁₂)—0.08, Sodium ascorbate—2000.00, Folic acid—0.80, Choline chloride—600.00. Inositol—400.00, Niacin—40.00 Calcium-Pantothenate=60.00.

⁴Mineral mixture (g/100 g diet): K₂HPO₄—2.00, Ca(PO₄)₂—2.72, MgSO₄ (7H₂O)—3.02, NaH₂PO₄-2H₂O—0.790, MnSO₄-5H₂O—0.004, FeSO₄ · 7H₂O-0.025.

Ingredients* Experimental diets, number 1 2 3 4 5 7 6 Codliver oil 0.0 1.68 3.36 5.04 6.72 8.40 9.98 Soyabean oil 0.0 0.84 1.68 2.52 3.36 4.20 4.99 Lecithin 0.0 0.48 0.96 1.44 1.92 2.40 3.03 (Phospholipid) Glucose 9.50 8.50 7.50 6.00 5.00 4.00 2.40 Sucrose 20.00 17.00 14.50 12.50 10.50 8.50 5.50 Cellulose powder 1.40 2.40 3.40 3.40 3.40 3.40 5.00

Table 2. Ingredient composition (%) of diets used in the experiment to determine the lipid requirement of juveniles (*P. indicus*)

*Casein, egg albumin, aminoacid mixture, glucosamine, sodium citrate, sodium succinate, starch, cholesterol, mineral mixture, vitamin mixture, carrageenan used in these diets is as given in the Table 1.

Fatty acid	P. indicus						
-	Marine	Estuarine	Estuarine	Estuarine			
14:0	2.4	3.5	1.26	1.13			
14:1	0.613	0.813	0.89	_			
15:0	1.6	1.5	0.89	-			
16.0	15.4	20.4	14.14	15.48			
16:1	8.3	12.9	7.22	7.53			
17:0	3.0	2.7	1.92	2.24			
17:1	1.0	0.94	_	0.94			
18:0	7.4	7.4	7.28	8.19			
18:1w9	13.0	13.5	9.95	12.81			
18:2w6	2.5	4.9	2.26	4.26			
18:3w3	1.1	1.6	0.99	1.03			
18:4w3	1.9	1.5	1.46	_			
20:1w9	-	-	2.52	1.39			
20:4w6	6.1	4.6	6.50	8.68			
20:5w3	9.5	9.1	11.17	11.24			
24:0	2.4	0.8	1.54				
24:1	0.9		3.23	1.21			
22:5w3	2.1	1.1	-	1.88			
22:6w3	11.9	5.2	9.30	11.00			
Total saturated	29.1	36.0	27.03	27.04			
Monounsaturated	23.83	28.7	23.81	24.97			
Total w6	8.6	9.6	8.76	12.97			
Total w3	25.6	18.5	22.92	23.27			
HUFA w3	22.6	15.4	20.47	22.48			
HUFA w6	6.1	4.6	6.50	8.68			
Author	Read (1	977)	Colvin (1976)	Chandge (1987)			

Table 3. Fatty acid composition (%) of tipid (from whole body of prawn from estuarine and marine *P. indicus*)

E-Merk Germany). Codliver oil was purchased from Universal Generic Pvt Bombay an associate of British Codliver Oil Ltd. HUL; lecithin (L α phosphatidyl-choline) from Sigma USA; and cholesterol was purchased from BDH England. Casein and egg albumin were the main source of protein. Arginine 1% level was included in all diets so as to improve the amino-acid balance of the diets to that of *P. indicus* tail muscle (Colvin, 1976). Methionine (0.5%) was included in the diets as Kitabayashi *et al.* (1971) found that diet supplemented with 0.53 methionine gave better growth rate in the case of *P. japonicus.* Glutamic acid (1%), glycine (2%) and taurine (0.5%) were used in the diets since the free aminoacids play a role in palatability. Glucosamine was added in the diets as it is known to promote growth in prawn (Kanazawa *et al.*, 1970 and 1977a; Kitabayashi *et al.*, 1971) as well as serves as the precursor for chitin synthesis. 10% NaOH. The lipid mixture and vitamins were added during final mixing. The diets were steamed for 10 minutes, cooled and passed through a household mincer having 2 mm diameter aperture. The feed strands were dried in an oven at 50°C to contain about 15% moisture.

Feeding experiment

Juveniles of *P. indicus* (initial length 25–30 mm, live weight 0.136 to 0.142 g and dry mean weight 0.029-0.0709 g) were used. In each of the tubs, ten juveniles were stocked. For initial dry weight determination 40 juveniles of same length and weight group were sacrificed and kept in an oven at 60°C for 48 hours and initial mean dry weight was recorded.

The feeding experiment was carried out for a period of 30 days. Feeding was done at a rate of 15% of live body weight, twice a day, 1/4 in morning and 3/4 in the evening for the first four days, and thereafter food was offered only in the evening as prawns were observed to feed more actively in the evening and night (Ahamad Ali, 1982). Feed introduced in the petri dishes kept at the bottom in the middle of the tubs. Similar feeding methods were also adopted by Ahamad Ali (1982) and reported better growth in prawn *P. indicus*.

Everyday in the morning left-over feed as well as fecal strands were separately collected by siphoning, washed with fresh water to remove adhering salts, dried and weighed. Dry weight and moisture content of prawns were determined by drying the prawns in an electrical oven at 50°C for 48 hours to get constant dry weight. The protein content of prawn and diets was determined by Lowry's method (Lowry *et al.*, 1951), carbohydrate by phenolsulfuric acid method (Dubois *et al.*, 1956), lipids by Bligh and Dyer (1959), cholesterol by Hestrin (1949) and ash content by AOAC (1975). Fatty acid profile of each of the lipid sources (Table 4) used in this experiment was determined adopting procedures of Morrison and Smith (1964). Gas liquid chromatography of fatty acid methyl ester was carried out using the Hewlett-Packard Microprocessor controlled gas liquid chromatography (Model 5840 A) with a flame ionization detector.

Analysis of data

Mean survival, gain in length, wet weight, dry weight, FCR, PER and percentage of moisture, protein, lipid, carbohydrate, ash and cholesterol content in each of the replicate group of prawns was calculated and recorded. Analysis of variance (ANOVA) was done on the means of each parameter to find out if the dietary treatments hold any significant influence on observed parameters.

Fatty acid	Soyabean oil	Lecithin (phospholipid)	Codliver oil	Mixture of codliver oil, soyabean oil, lecithin	Essential fatty acids
12:0	-	-	0.27	0.20	
14:0	0.373	-	5.43	5.78	
14:1	_	_	_	_	
15:0		-	0.32	0.10	
16:0	11.77	20.20	10.03	10.52	
10:1w7	-	-	11.35	6.88	
17:0	_	_	0.80	0.45	
17:1	_	-	-	0.04	
18.0	3.664	-	1.79	2.59	
18:1w9	22.98	8.8	23.61	21.59	
18:2w6	51.80	60.1	3.17	18.10	18.10
18:3w3	7.38	7.3	0.50	3.11	3.11
20:0	-	-	-	_	
20:1w9	-	-	11.26	6.25	
20:4w6		8.0		2.89	
20:5w3		-	10.41	4.03	4.03
22:5w3		-	2 <u>—</u> 21	0.95	
22:6w3	1.601	_	12.50	7.50	7.0
Total saturated	15.80	20.20	18.35	19.64	31.88
Total monounsaturated	22.98	8.8	46.76	34.76	28.128
Total w6	51.8	60.1	9.743	20.99	21.0
Total w3	7.38	7.3	23.42	17.59	14.0
Total HuFA	1.601	8.0	24.28	14.8	
Total PuFA	60.78	75.4	26.58	36.58	13.72
Ratio of w6:w3					3:2

Table 4. Fatty acid composition (%) of lipid sources used in the diets of Penaeus indicus

When significant influence was observed the data was processed to find out if the observed difference between treatments was significant or not by least significant difference test with the help of a Hewlett-Packard master computer.

To clarify the dietary lipid requirements of *P. indicus* second sets of experiments was conducted using only two diets (Diet 9 containing 10% lipid and Diet 10 containing 12% lipid), adopting same material method explained earlier, except initial length and weights of experimental animals (Tables 5 and 6).

RESULTS

The results of the feeding experiments are shown in Figs. 1A to 1F and 2A to 2F and in Tables 3, 4, 6, 7 and 8.

Ing	gredients*	Experimental diets				
	-	Diet 9	Diet 10			
	Casein	31.00	31.00			
	Egg albumin	7.50	7.50			
	Amino acid mixture ¹	5.00	5.00			
	Glucosamine	0.80	0.80			
	Sodium citrate	0.30	0.30			
	Sodium succinate	0.30	0.30			
	Starch	12.00	12.00			
	Glucose	5.00	5.00			
	Sucrose	12.50	10.50			
	Cholesterol	0.50	0.50			
	Lipids ²	10.00	12.00			
	Vitamin mixture ³	3.20	3.20			
	Mineral mixture ⁴	8.50	8.50			
	Cellulose powder	3.40	3.40			
	Total	100.00	100.00			
	Agar agar	5.00	5.00			
	Distilled water	100-120 ml	100–120 ml			

Table 5. Ingredients composition (%) of the experimental diets used in the second experiment to determine lipid requirement of juvenile *Penaeus indicus*

*Percentage of:

¹Amino acid mixture

²Lipid mixture

³Vitamin mixture

⁴Mineral mixture used in these diets are as given in Table 1.

Table 6. Growth performance obtained in the second experiment of *Penaeus indicus* fed on diets containing only two lipid levels

1	Determination	Experimental diets			
		Diet 9	Diet 10		
1)	Initial stocking	10.00	10.00		
'2)	Final survival (%)	90.00 (± 0.0)	90.00 (± 10.0)		
3)	Initial length (mm)	20.90	21.34		
4)	Final length (mm)	43.34	47.55		
5)	Increase in length (mm)	22.59 (± 0.06)	26.21 (± 0.37)		
6)	Gain in length (%)	108.86 (± 0.025)	122.92 (± 2.89)		
7)	Initial wet weight (g)	0.0516	0.0513		
8)	Final wet weight (g)	0.357	0.469		
9)	Gain in wet weight (g)	0.3055 (± 17.57)	0.4177 (± 16.61)		
10)	Gain in wet weight (%)	592.05 (± 27.35)	814.23 (± 41.084)		
11)	Initial dry weight (g)	0.01142	0.01142		
12)	Final dry weight (g)	0.07945	0.1206		
13)	Gain in dry weight (g)	0.06803 (± 4.345)	0.10918 (± 4.99)		
14)	Gain in dry weight (%)	595.70 (± 38.045)	956.04 (± 43.694)		

The survival rate of juvenile prawns ranged from 43 to 85% in the various treatments (Fig. 1A) and it was significantly (P < 0.05) influenced by the dietary lipid level. The survival was low in groups of juveniles fed the lipid-free diet (Diet 1). Addition of 3% lipid significantly (P < 0.05) improved the survival (70%) (Fig. 1A). Diets containing lipid levels of 9, 12, 15 and 18% produced relatively high survival rates (70% to 85%) (Fig. 1A).

The data for growth rate of juvenile prawns expressed as percentage of the mean gains in length, live weight and dry weight are shown in Figs. 1B to 1D and absolute weight gain in Table 7. The lipid free diet (Diet 1) produced lowest growth rate and inclusion of lipid in the diets (Diet 2 to 7) significantly promoted growth in juvenile prawns (Figs. 1B to 1D). The growth of prawns on a diet containing 12% lipid (Diet 5 and Diet 10) was significantly higher (P < 0.05) than the prawns fed a diet with lower levels of lipids (Diet 1 to 4). The slight increase in growth observed at 15% lipid level (Diet 6) was not statistically significant.

The diet containing 12% lipid (Diet 5 and Diet 10) provided the best FCR (2:164) and PER (1:42) among all the diets (Figs. 1E and F). Deletion of lipid from the diet of prawn (Diet 1) resulted in significantly high FCR and low PER. Proximate composition of post-experimental prawn are shown in Figs. 2A to 2F. The prawns fed the lipid-free diet (Diet 1) had significantly (P < 0.01) lower protein, lipid and cholesterol contents, but significantly higher (P < 0.01) moisture, carbohydrate and ash contents than those fed on the diets containing lipids. While the protein lipid and cholesterol levels in prawn increased the moisture carbohydrate and ash contents decreased with the increase in dietary lipid level up to 12% (Figs. 2A to 2F). However, dietary lipid level above 12% had no significant effect on the chemical composition of experimental prawns.

Results of the second set of experiments confirm the observations of the first experiment (Table 6). Diet 10 containing 12% lipid produced significantly better growth performance than Diet 9 containing 10% lipid (Table 6).

The experimental growth observed with 12% lipid diet is comparable with the natural growth calculated according to formula provided by Prabhakarrao (1967) (Table 8).

A compounded feed (Diet 8) development by Ahamed Ali and Mohammed (1982) reportedly provided better growth than fresh clam meat in *Penaeus indicus* and was used as additional control in addition to 0% lipid diet (Diet 1). The growth performance of prawn *P. indicus* fed on Diet 5 was comparatively better than that of diet 8 containing compounded feed (Table 7).

DISCUSSION

The present study indicates the essentiality of lipid in the diet and also reveals the effect of different levels of lipids on survival and growth performance of *P. indicus.*

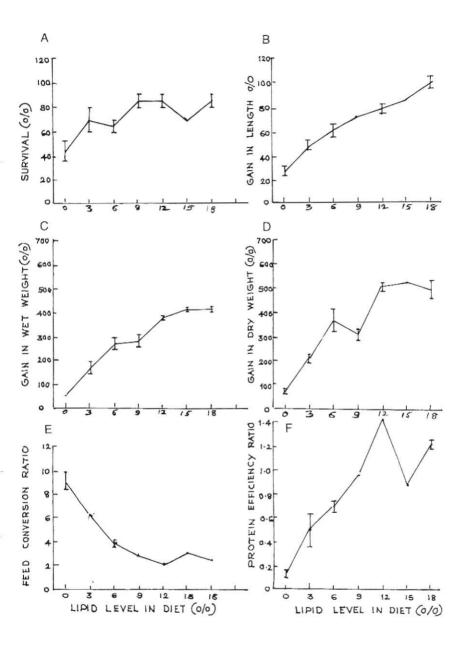


Fig. 1. Survival rate and growth of *P. indicus* fed on diets containing graded levels of lipid.
(A) Survival (%). (B) Gain in length (%). (C) Gain in wet weight (%).
(D) Gain in dry weight (%). (E) Feed Conversion Ratio. (F) Protein Efficiency Ratio.

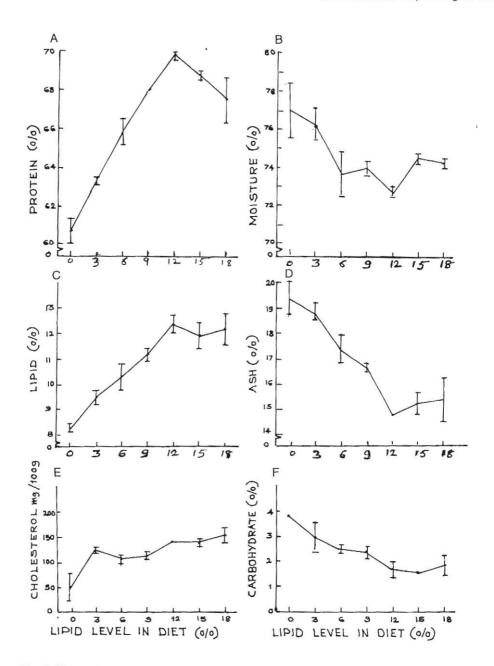


Fig. 2. Biochemical composition of *P. indicus* fed on diets containing graded levels of lipid. (A) Protein (%). (B) Lipid (%). (C) Carbohydrate (%). (D) Ash (%). (E) Cholesterol (%). (F) Carbohydrate (%).

Sr. No.	Determination/ Description	Experimental diets Lipid level is given in the bracket							
		1 (0.0%)	2 (3%)	3 (6%)	4 (9%)	5 (12%)	6 (15%)	7 (18%)	8 Com- pounded feed
1)	Average initial weight (g)	0.137	0.137	0.137	0.137	0.137	0.137	0.137	0.137
2)	Average final weight (g)	0.220	0.372	0.519	0.528	0.650	0.704	0.701	0.568
3)	Net gain in weight (g)	0.083	0.233	0.382	0.390	0.513	0.567	0.564	0.431
4)	Percentage gain in weight	60.58	170.07	279.07	285.29	374.63	413.86	411.78	315.1
5)	Average initial length (mm)	29.8	29.8	29.8	29.8	29.8	29.8	29.8	29.8
6)	Average final length (mm)	37.91	44.00	48.00	51.56	53.00	55.50	59.50	51.46
7)	Net gain in length (mm)	8.05	14.25	18.2	21.76	23.20	25.70	29.70	21.63
8)	Percentage gain in length	27.21	47.65	61.07	73.00	77.84	86.20	99.66	72.59

Table 7. Gain in absolute wet weight and length in prawns Penaeus indicus fed on diets containing graded levels of lipids and a compounded feed (Ahamad Ali and Mohammed, 1982)

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Experimental diet no.		Lipid level (%)	Experimental length of prawns (male and female combine) (mm)	Corresponding natural weight in male prawns (g)	Corresponding natural weight in female prawns (g)	Corresponding weight of experimental prawns (male and female combined) (g)
	1	2	3	4	5	6
A)	First expe	eriment				
	1	0	37.91	0.2984	0.3241	0.220
	2	3	44.00	0.3589	0.5127	0.372
	3	6	48.00	0.4820	0.6701	0.519
	4	9	51.76	0.6170	0.8350	0.528
	5	12	53.00	0.6780	0.9090	0.650
	6	15	55.50	0.7960	1.0476	0.704
	7	18	59.50	0.7960	1.2979	0.701
	8 (Con	trol)	51.46	0.6130	0.8302	0.568
B)	Second e	xperiment				
	9	10	43.34	0.3380	0.4894	0.3571
	10	12	47.55	0.3376	0.6510	0.4695

Table 8. Comparison between absolute weight achieved by the experimental prawns and natural weight calculated at respective length as per Prabhakarrao (1967)

Lipids play an important role in the energy production processes of crustacean tissues and as a source of essential fatty acids, phospholipids and as carrier of fat soluable vitamins (Teshima and Kanazawa, 1980a and b). The phospholipids are important in the transport of fatty acids and other lipids and also as a component of biomembranes in the cellular and subcellular organelles, provides the structural integrity to these membranes and flexibility for iontransport (Teshima and Kanazawa, 1980a; Lehninger, 1984). Thus the essentiality of lipids at 12% level in the diet can be ascribed to the diverse functions these molecules perform in prawns.

Moulting is an indispensable phenomenon in Crustacea and the involvement.⁴ of lipid during moulting has been well established (Read, 1977). The process of ecdysis requires large amount of energy amounting to 25.6% of the total energy gained during intermoult period (Read and Caulton, 1980). This is a substantial loss of stored energy (lipid). Considering the increased frequency of moulting in juvenile stages of prawns and lipid being the principal energy supplier a level of 9 to 12% lipid seems to be necessary in the diet to meet the metabolic and growth requirements in juvenile stages of *Penaeus indicus*. Earlier reports on nutrition of lobster by Boghen and Castell (1980) and Conklin *et al.* (1980) also reported maximum growth in juvenile stages of lobster when fed on diets containing 16.5% lipid (Boghen and Castell, 1980) and 15.7% lipid (Conklin *et al.*, 1980). These reports suggest that lipid level required for crurtaceans, may be as high as 15% and prawns may not be an exception. Growi

performance of prawn (*P. indicus*) on lipid-free diet was very poor and was improved significantly by inclusion of lipids in diets at a level of about 6%. Probably this may be the minimum level required for *P. indicus* for general performance. However, for optimum performance in growth, a dietary lipid level of 12% is required for *P. indicus* reared in brackish water medium (Figs. 1B to 1F and Table 6). Inclusion of more than 12% lipid in the diet has no corresponding significant improvement in growth performance of prawns though prawns could tolerate higher dietary lipid level as high as 18% without any deleterious effect on the growth performance.

The steady increase in growth performance and protein content of prawn with the increase in dietary level of lipid from 3 to 12% (Figs. 2B to 2F) can be ascribed to the protein sparing action of dietary lipids. The increased level of lipid (12%) in Diet 5 and Diet 10 might have provided the energy and essential fatty acids (Table 4) required for metabolic activities of the animal, including that required for moulting thereby more and more protein had been spared for growth. This is also clearly evident from the better food and protein conversion values (Figs. 1E and 1F) when the dietary level of lipid was increased (12%). Diet with lower levels of lipid (3 to 6%) produced poor growth (Figs. 1B to 1D) as well as poor utilization of food (Fig. 1E) and protein (Fig. 1F) in prawns, as the animals could be deriving the metabolic energy partly from protein. It is thus clear from the present study that lipid at adequate levels can significantly spare protein for growth as has been established for fish by Watanabe (1982). Addition of lipids with EFA as an energy source to a diet help in effective utilization of dietary protein in fish (Watanabe, 1982).

Reports are available on quantitative lipid requirement of juvenile prawn (Kanazawa et al., 1977b and Deshimaru et al., 1979). Kanazawa et al. (1977b) reported poor growth with lipid-free diet, maximum growth with 12% lipid and reduced growth at 16% when powdered pollack residual oil was included in diet of P. japonicus indicating 12% lipid is optimum level which agrees with the present observation in P. indicus. However, with the same species, Deshimaru et al. (1979) used a mixture of pollack liver oil and soyabean oil in the ratio of 1:1 and 3:1 as the lipid sources reported highest growth and reed efficiency at 6% lipid level in the diet. Thus for the same species (P. ajaponicus) from the same country (Japan) two groups of workers reported two different values of lipid required for optimum growth of prawn. The significant differences observed by these authors may be because of the contents of other nutrients in the diets. For instance the protein and cholesterol contents of the diets used by Kanazawa et al. (1977b) were 50% and 0.5% respectively whereas Deshimaru et al. (1979) used 60% protein and 2% cholesterol in their diets. In the present experiment with P. indicus relatively higher level of lipid (12%) was able to produce more growth than lower levels of lipid when protein level was comparatively lower than Deshimaru et al. (1979) and constant (37.5%). Besides, P. indicus being an omnivore, requires relatively lower level of protein (about 37.5%, Gopal, 1986) and perhaps lipid is utilized as an effieient energy source by P. indicus, thus sparing protein for growth. Growth of

animals depends upon the proper utilization of the ingested food and proteins. In the present experiments, food and protein utilization were significantly influenced by the dietary lipid levels. Deletion of lipid from the diet resulted in significantly high FCR and low PER (Figs. 1E and 1F) indicating inefficient utilization of food and protein by the prawns. Inclusion of lipid in the diet significantly improved the FCR and PER up to 12% lipid and above this level lipid had no beneficial effect on the food and protein utilization of prawns. There was not much loss in protein percentage in left-over feed, indicating that loss of protein due to leaching was minimum. Protein utilization was found to be better when enough fat and carbohydrates were provided in the diet. Similar observation was also reported in the case of *Macrobrachium rosenbergii* (Clifford and Brick, 1978). Apparently *P. indicus* juveniles require 12% lipid for proper utilization of food and protein as FCR and PER were also found to be better with diets containing 12% lipid.

The efficacy of dietary lipids in promoting growth depends mainly upon its composition. Growth of prawn also depends upon the types and content of fatty acids in the dietary lipids (Kanazawa, 1985), rather than the total quantity of lipids used in the diet. The better growth obtained in the present trial may be because of the use of codliver oil and soyabean oil alongwith lecithin in required levels (10% codliver oil + soyabean oil and 2% lecithin). This mixture of lipids provided all essential fatty acids (Table 4) in the required proportion 18:2n-6 (18.1%), 18:3n-3 (3.11%), 20:5n-3 (4.03%), 22:6n-3 (7%) of total lipids). The mixture of lipids also provides required levels of n-6 (21%) and n-3 (14%) (Table 4). As the ratio of n-6:n-3 also dominate the nutritive value of lipids for promoting growth in prawns (New, 1976). The mixture used in dietary lipids provides 3:2, n-6:n-3 ratio (Table 4) which appears to be sufficient to provide better growth in P. indicus. Besides the essential fatty acids, adequate levels of phospholipid, cholesterol, and antioxidants should be available in the dietary lipid source for effective utilization of diets as these components of diet are also equally important for promoting growth in prawns (Kanazawa, 1985). The diet used in the present trial also contained 2% phospholipids, 0.5% cholesterol and 0.02% a Tocopherol (a natural antioxidant) to meet the specific requirement of juvenile prawns. The inclusion of 2% phospholipid (lecithin) in the diet seems to have significantly promoted utilization of lipid and protein and thus PER in P. indicus as also reported in P. japonicus by Kanazawa et al. (1985).

The chemical composition of prawns is also significantly influenced by the dietary lipid level. The data clearly indicate that for efficient synthesis of protein, lipid should be present in adequate level. This is evident from the low level of protein in the tissue of juveniles fed the lipid-free diet and higher level of protein in those fed the diet with 12% lipid.

The level (9 to 12%) recommended above would be necessary in compounded diet to be used in intensive culture practices when there is no endogenous food supply in pond. In a practical situation, the recommended level mentioned above, however, may be altered with reference to pellatibility problem as well as nutritive value of natural food produced in the pond.

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REFERENCES

- Ahamad Ali, Syed, and Mohammed, K.H. (1982). Utilization of prawn and Mantis shrimp for compounding feeds for the culture of *Penaeus indicus*. Proceedings of the symposium on Harvest and Post Harvest Technology of Fish. Nov. 24–27, 1982. pp. 615–618.
- Ahamad Ali, S. (1982). Relative efficiencies of pelletised feeds compounded with different animal proteins and the effect of protein level on the growth of the prawn *Penaeus indicus*. Proc. Symp. Coastal Aquaculture 1982, 1: 321–328.
- AOAC, (1975), Official Methods of Analysis of the Association of official Agricultural Chemists, AOAC. Washington. D.C. 13th Edn., 1094 P.
- Bligh, E.G. and Dyer, W.J. (1959). A rapid Method of total lipid extraction and purification. Can. J. Biochem. Physical., 37(8): 911-917.
- Boghen, A. and Castell, J.D. (1980). Consideration of the lecithin and protein requirements of juvenile lobster *Homarus americanus*. *In:* Proceedings of the Juvenile Lobster Nutrition Workshop, University of Maine. *Publ. Marine Sea Grant Walpole ME*, USA. pp. 21–28.
- Chandge, M.S. (1987). Studies on lipid nutrition in larvae and juveniles of the Indian white prawn *Penaeus indicus* H. Milne Edwards, Ph.D. thesis, Cochin University of Science and Technology. 194 pp.
- Clifford, H.C. and Brick, R.W. (1978). Protein utilization in the fresh water shrimp *Macrobrachium* rosenbergii, Proc. World Maricult. Soc. 9: 195–208.
- Colvin, P.M. (1976). The effect of selected seed oils on the fatty acid composition and growth of *Penaeus indicus. Aquaculture* 8: 81-89.
- Conklin, D.E., D'Abramo L.R., Bordner, C.E. and Baum, N.A. (1980). A successful purified diet for culture of juvenile lobsters, the effect of lecithin. *Aquaculture* **21**: 243–249.
- Deshimaru, Kuroki, O.K. and Yone, Y. (1979). The composition and level of dietary lipid appropriate for growth of prawn. *Bull. Jap. Soc. Sci. Fish.* **45**(5): 519–594.
- Dubios, M., Guilles, K.A., Hamilton, J.K., Regers, P.A. and Smith, I. (1956). Calorimetric method for determination of sugars and related substances. *Analyst. Chem.* 28: 350–356.
- Forster, J.R.M. and Beard, T.A. (1973). Growth experiment with *Palaemon serratus* Pennant, fed with fresh and compounded foods. *Fishery Invest. London, Ser.* II, **27**(7): 16.
- Gopal, C. (1986). Nutritional studies in juvenile *Penaeus indicus* with reference to protein and vitamin requirements. Ph.D. Thesis. Cochin University of Science and Technology. 306 pp.
- Hestrin, S. (1949). Cholesterol estimation for tissue. J. Biol. Chem. 180: 249.
- Kanazawa, A. (1985). Nutrition of penaeid prawns and shrimps. Proc. of the First Intl. Conf. on the culture of penaeid prawns/shrimps. Illoilo city, Philippines, 1984. 123–130.
- Kanazawa, A., Shimaya, M., Kawasaki, M. and Kashiwada, K. 1970. Nutritional requirements of prawn-I. Feeding on artificial diet. Bull. Jan. Soc. Fish. 36: 949–954.
- Kanazawa, A., Teshima, S. and Tokiwa, S. (1977a). Nutritional requirements of prawn-V. Requirements of choline and inositol. *Men. Fac. Fish. Kagoshima Univ.* **25**(1): 47–51.
- Kanazawa, A., Teshima, S. and Tokiwa, S. (1977b). Nutritional requirement of prawn-VIII. Effect of dietary lipids on growth. Bull. Jap. Soc. Sci. Fish. 43(7): 849-856.

Kanazawa, A., Teshima, S. and Sakamoto, M. (1985). Effect of dietary lipids, fatty acids and phospholipids on growth and survival of prawn *Penaeus japonicus* larvae. Aquaculture 50: 39–49.

Kitabayashi, K., Kurata, H., Shudo, K., Nakmura, K. and Ishikawa, S. (1971). Studies on formula feed for Kuruma Prawn-III on the growth promoting effects of both arginine and methionine *Bull. Tokai Reg. Fish. Res. Lab.* pp. 119–127.

Lehninger, A.L. (1964). Biochemistry. 4th ed. Kalyani Publishers. Ludhiana, India. 1104 pp.

- Lowry. O.H. Rosebrough, N.J. Farr. A.L. and Randall, R.J. (1951). Protein measurement with the Folin-phenol reagent. J. Biol. Chem. 193: 175–275.
- Morrison, W.R. and Smith, L.M. (1964). Preparation of fatty acid methyl esters and dimethylacetous from lipid with boron Fluoride methanol. J. lipid. Res. 5: 600–648.

New, M.B. 1976. A review of dietary studies with shrimp and prawns. Aquaculture 9: 101-144.

- Prabhakarrao, A.V. (1967). Some observations on the biology of *Penaeus indicus* H. Milne Edwards and *Penaeus monodon* Fabricus from the Chilka lake. *Ind. Jourl. Fish.* 14 (1&2): 251–270.
- Read, G.H.L. (1977). Aspects of lipid metabolism in *Penaeus indicus* Milne Edwards. M.Sc. Thesis, University of Natal. 106 pp.
- Read, G.H.L. (1981). Response of *Penaeus indicus* (Crustacea, Penaeidea) to purified and compounded diets of varying fatty acid composition. *Aquaculture* 24: 245–256.
- Read, G.H.L. and Caulton, M.S. (1980). Chances in mass and chemical composition during the moult cycle and ovarian development in immature and mature *Penaeus indicus* Milne Edwards, *Comp. Biochem. Physiol.* 66a: 431–437.
- Teshima, S. and Kanazawa, A. (1980a). Transport of dietary lipids and role of serum lipoproteins in the prawn. *Bull. Jap. Soc. Sci. Fish.* **46**: 55–57.
- Teshima, S. and Kanazawa, A. (1980 b). Lipid constituents of serum lipoproteins in the prawn. *Bull. Jap. Soc. Sci. Fish.* **46:** 57–62.
- Watanabe, T. (1982). Lipid nutrition in fish Comp. Biochem. Physiol. 73B(1): 3-15.

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