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RELATIVE EFFICIENCIES OF DIFFERENT LIPIDS AND LIPID LEVELS IN THE DIET OF THE PRAWN *PENAEUS INDICUS*

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ABSTRACT

Among the four lipids, cod liver oil, prawn head oil, sardine oil and soybean lecithin, tested at 6% level in a purified diet, for the prawn *Penaeus indicus*, the diets having prawn head oil and a mixed lipid consisting of all the four lipids in equal proportions, showed significantly ($P<0.01$) higher growth and the best food conversion ratio (FCR). These were followed by the diets having soybean lecithin, sardine oil and cod liver oil in the decreasing order. Investigations on the effect of lipid level in the diet on the growth, FCR and survival of the prawn have shown that the diet having 6% lipid produced significantly higher growth ($P<0.01$), best FCR and high survival rate. While the growth, FCR and survival of the prawns fed with the diets having less than 6% lipid, were poor, the animals fed with the diets having higher lipid levels did not show any improvement.

Using a mixed lipid and carbohydrate (starch) in the ratio 1:7, the calorific value of the diet was varied from 271.68 Kcal/100 g to 462. 43 Kcal/100 g, keeping the protein constant. Feeding experiments conducted with these diets have shown that the growth of prawns increased and the FCR improved with increase in the dietary energy. The diet having 414.72 Kcal/100 g recorded the highest growth and the best FCR, while further increase in the calorific value of the diet had no beneficial effect on the growth and FCR of the prawns. The significance of qualitative and quantitative lipid requirements of prawns in relation to the calorific value of the diet is discussed.

INTRODUCTION

Besides being a good source of energy in the diet, lipid is an essential nutrient for prawns. Many-a- physiologically important biochemicals are synthesised in the body from the fatty acids derived from the dietary lipid. It is in this context the quality of the dietary lipid is more significant than its quantity. The importance of lipid in prawn and shrimp nutrition was reviewed by New (1976). The requirement of different classes of lipids, fatty acids, phospholipids and steroids for different species of penaeid prawns were studied and the results were recently reviewed by Kanazawa (1984). In the present study, four different lipids were identified and their relative efficiencies in the diet of the Indian white prawn *Penaeus indicus* were evaluated. The effect of lipid level and the calorific value in the diet on the growth, food conversion ratio and survival was investigated and the results discussed.

MATERIAL AND METHODS

Cod liver oil, prawn head oil, sardine oil and soybean lecithin were the four lipids evaluated for the prawn *Penaeus indicus*. The source of these lipids are as given below.

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(i) Cod liver oil : A commercial prodoiluct sold under the brand name "Seven Seas" cod liver oil, manufactured by Universal Generics Pvt. Ltd., Apollo Street, Bombay - 23.

(ii) Prawn head oil : Prepared in the laboratory.Prawn head waste in fresh condition was obtained from a local processing unit and finely ground by adding water (1:1) in an electrical wet grinder. It was then extracted with a mixture of chloroform and methanol (2:1) at room temperature. The solvent was removed by vacuum evaporation; the residue dried at 60°C was used as prawn head oil.

(iii) Sardine oil : A commercial product prepared and sold by a local fish processing company.

(iv) Soybean lecithin : Obtained from 'SIGMA' Chemical Company, P.O., Box 14508, St. Louis MO 63178 U. S. A.

Cod liver oil and soybean lecithin already contained the preservative, while 100 pp m of butylated hydroxy anisole (BHA) was added as preservative (antioxidant) to prawn head oil and sardine oil.

Formulation and preparation of diets

For evaluating the identified lipid sources, a basal purified diet, made up of casein (fat free), glucose, sucrose, vitamin mixture, mineral mixture, cellulose and other additives such as cholesterol, glucosamine, sodium succinate and sodium citrate were used. The diets were prepared as dry pellets of 3 mm diameter using polyvinyl alcohol as the binder.

Experiment - 1: In the first experiment, six different isocaloric diets were formulated by adding separately cod liver oil, sardine oil, prawn head oil, soybean lecithin and a mixture of all the four lipids in equal proportion, at 6% level to the basal diet and resultant diets were designated as P_1, P_2, P_3, P_4 and P_5 respectively. The diet P_0 was formulated containing no lipid in it. The composition of the diets is shown in Table 1.

Experiment - 2 : In the first experiment, the mixed lipid consisting of all the four lipids in equal proportion, gave the best results. Using this mixed lipid, six different diets were formulated by adding 1, 3, 6, 9, 12 and 18% of it to the basal diet, to study the effect of quantitative lipid level in the diet on the growth, food conversion and survival of the prawn. These diets were designated as P_6, P_7, P_8, P_9, P_{10} and P_{11} and their composition is shown in Table 2.

Experiment - 3 : The results of the second experiment indicated that increasing the lipid level in the diet beyond certain level had no beneficial effect, even though the calorific value of the diet had been raised. This prompted to investigate the influence of dietary energy level on growth, food conversion ratio, and survival of the prawn. For this purpose, five diets were formulated consisting of 40% protein (casein), and a mixture of lipid (mixed lipid) and carbohydrate (starch) in the ratio 1:7, at different levels to raise the calorific value of the diets. The resultant diets had a minimum digestible energy of 271.68 Kcal/100 g, and a maximum of 462.43 Kcal/

TABLE 1. *Ingredient composition of the diets P_0 to P_5*

Ingredients	Diet No.					
	P_0	P_1	P_2	P_3	P_4	P_5
Casein (fat free)	45.0	45.0	45.0	45.0	45.00	45.0
Sucrose	16.5	10.5	10.5	10.5	10.5	10.5
Starch	22.8	14.8	14.8	14.8	14.8	14.8
Cod liver oil	—	6.0	—	—	—	1.5
Sardine oil	—	—	6.0	—	—	1.5
Prawn head oil	—	—	—	6.0	—	1.5
Soybean lecithin	—	—	—	—	6.0	1.5
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5
Glucos amine Hcl	0.8	0.8	0.8	0.8	0.8	0.8
Sodium citrate	0.3	0.3	0.3	0.3	0.3	0.3
Sodium succinate	0.3	0.3	0.3	0.3	0.3	0.3
Vitamin and mineral mixture (*)	5.8	5.8	5.8	5.8	5.8	5.8
Cellulose	5.0	13.0	13.0	13.0	13.0	13.0
Polyvinyl alcohol	3.0	3.0	3.0	3.0	3.0	3.0

(*) 100 g of diet contained the following vitamins and minerals: vitamin A (acetate) 10,000 I. U., thiaminemononitrate 25 mg, riboflavin 5 mg, nicotinamide 25 mg, pyridoxine hydrochloride 7.5 mg, calcium pantothenate 25 mg, cynocobalamine 5 mcg, ascorbic acid 125 mg, calciferol 1000 I. U., tocopherol acetate 3.75 mg, biotin 0.125 mg, magnesium phosphate 120 mg, manganese phosphate 1.5 mg, ferrous sulphate 16.5 mg, calcium phosphate 0.52 g, calcium lactate 1.0 g, Potassium hydrogen phosphate 0.5 g.

100 g, which was determined by Gallenkamph bomb calorimeter. The diets were designated as E, E, E, E, and E, and the composition is given Table 3.

Preparation of diet: The dry ingredients of the diet were finely ground first and mixed according to the formula. To this, the lipid emulsified with 0.5 g of glycerol mono—oleate, was added and thoroughly mixed. The binder was melted in water (100 ml/100 g diet) at 70°C and the diet mixture was added to it. After homogenising, the diet was steamed for 10 minutes and cut into small cubes. It was dried in an electric oven at 60°C for 12 hours. The dry diet was prepared into small particles of 3 to 5 mm size and stored in desicator until use.

Biological evaluation : Hatchery reared, early juveniles of the Indian white prawn *Penaeus indicus* (H. Milne Edwards) having an average length of 26 mm and weight of 0.075 g were used in the feeding experiments.

Six animals were kept in circular perspex tanks containing 10 litres of filtered (through No. 30 bolting cloth) water for feeding the diets. There were three replicates for each treatment arranged randomly. The diet was offered to the animals in petridish kept at the bottom of each tank. The animals

TABLE 2. *Ingredient composition of the diets P_6 to P_{11}*

Ingredients	Diet No.					
	P_6	P_7	P_8	P_9	P_{10}	P_{11}
Casein(fat free)	45.0	45.0	45.0	45.0	45.0	45.0
Lipid mixture (*)	1.0	3.0	6.0	9.0	12.0	18.0
Sucrose	13.0	12.0	10.5	9.0	7.5	4.5
Starch	17.3	16.3	14.8	13.3	11.8	8.8
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5
Glucosamine Hcl	0.8	0.8	0.8	0.8	0.8	0.8
Sodium citrate	0.3	0.3	0.3	0.3	0.3	0.3
Sodium succinate	0.3	0.3	0.3	0.3	0.3	0.3
Vitamin and mineral mixture (*) (*)	5.8	5.8	5.8	5.8	5.8	5.8
Cellulose	13.0	13.0	13.0	13.0	13.0	13.0
Sodium alginate	3.0	3.0	3.0	3.0	3.0	3.0

(*) Lipid mixture : A mixture of cod liver oil, prawn head oil, sardine oil and soybean lecithin in 1: 1: 1: 1 ratio.
(*) (*) Vitamin and mineral mixture was same as used in the diets of the experiment 1 (Table 1).

were fed at the rate of 20% of their body weight every day. It was regulated according to the diet remained uneaten the next day. Daily sediments were siphoned out, left-over diet recovered and 90% of the water was replaced by fresh batch of water. Aeration was provided with the help of air blower using air stones. The salinity, oxygen, temperature and p^H of water used were maintained at 15.6 ± 1‰, 3.8 ml^{-1}, 28°C and 8.0 ± 0.1 respectively. Separate sets of feeding experiments were conducted with the three groups of diets and the duration of each feeding experiment was 30 days. The growth of the animals was calculated as follows.

$$\text{Growth \%} = \frac{\text{Final measurement} - \text{Initial measurement} \times 100}{\text{Initial measurement}}$$

Food conversion ratio was determined as follows.

$$\text{Foodconversion ratio} = \frac{\text{Average weightof dry diet consumed}}{\text{Average increase in live-weight}}$$

At the end of the feeding experiments, the animals fed with different groups of diets were analysed for lipid content by the method of Bligh and Dyer using chloroform-methanol mixture (2:1). The data obtained on growth and food conversion ratio were subjected to Analysis of Variance, following the method of Snedecor and Cochran (1973).

RESULTS

The results of the experiments conducted with diets P_0 to P_5 are shown in Table 4. The diet P_0 with zero lipid resulted in poor survival, low growth of the animals and showed high food conversion ratio (FCR).

TABLE 3. *Ingredient composition of the diets* E_1 to E_5

Ingredients	Diet No.				
	E_1	E_2	E_3	E_4	E_5
Casein (fat free)	40.00	40.00	40.00	40.00	40.00
Lipid mixture (*)	1.25	2.5	3.75	5.0	6.25
Starch	8.75	17.50	26.25	35.00	43.75
Cholesterol	0.50	0.50	0.50	0.50	0.50
Glucosamine Hcl	0.80	0.80	0.80	0.80	0.80
Sodium citrate	0.30	0.30	0.30	0.30	0.30
Sodium succinate	0.30	0.30	0.30	0.30	0.30
Vitamin and mineral mixture (*) (*)	5.80	5.80	5.80	5.80	5.80
Cellulose	42.00	32.00	22.00	12.00	2.00
Agar agar	2.00	2.00	2.00	2.00	2.00
Calorific value K cal/ 100 g	271.68	319.37	367.06	414.75	462.43
Protein : Caloric ratio	1 : 5.88	1 : 7.98	1 : 9.17	1 : 10.36	1 : 11.56

(*) Lipid mixture : Same as used in experiment 2 (Table 2).
(*) (*) Vitamin and mineral mixture : Same as used in the diets P_0 to P_5 (Table 1).

TABLE 4. *Results of the feeding experiment on juvenile* P. indicus *fed with diets* P_0 *to* P_5 *for 30 days*

Particulars	Diet No.					
	P_0	P_1	P_2	P_3	P_4	P_5
Growth in length %	20.9bc	19.1bc	22.5bc	42.9a	29.7ab	57.1a
Growth in weight %	98.7ef	103.7cd	112.0bc	257.8b	162.3c	305.4a
Food conversion ratio	11.6c	5.8b	7.9cd	3.7a	6.9c	4.1a
Survival	33.0	75.0	75.0	66.5	83.0	58.4
Body lipid of animals after the feeding experiment % (on dry basis)	14.2	21.7	18.9	16.4	19.5	16.9

Note : Values with different superscripts differ significantly among themselves. Growth in length and weight and FCR significant at 1% level ($P<0.01$).

Among the four individual lipid sources tested, the mixed lipid source having all the four lipids in equal proportion (diet P_5) produced significantly (P<0.01), the highest growth in length. This was followed by the diet (P_3) having prawn head oil, soybean lecithin, sardine oil and cod liver oil in the decreasing order. The diet with prawn head oil recorded the lowest FCR (3.7) compared to the other diets. However, the FCR recorded by the mixed lipid diet was not significantly (P<0.01) different from that of the diet with prawn head oil. The diet with sardine oil showed high FCR (7.9) followed by the diets with soybean lecithin (6.9) and cod liver oil (5.8). The survival of the animals fed with the diet having prawn head oil was the highest (83%) and that of the diet with mixed lipid was the lowest (58.4%). The prawns fed with zero lipid diet showed low body lipid and the body lipid of the animals fed with different diets showed slight variation, though the differences were not significant.

From the results of the feeding experiments with diets P_6 to P_{11} (Table 5), it could be seen that the growth of prawns gradually increased and the FCR improved as the lipid level in the diet increased from 1 to 6%. Further increase in the lipid level in the diet did not result in higher growth, but showed marginal decrease in it. The diet (P_8) with 6% lipid produced significantly (P<0.01) higher growth and the best FCR (P<0.05), compared to all the other diets. The survival of the prawns fed with this diet was also higher. The body lipid of the animals fed with diets having different lipid levels did not show any significant variation.

The results of the feeding experiment with diets E_1 to E_5, are given in Table 6. The diet E_4 having 414.75 Kcal/100 g had recorded the highest growth in length (P<0.05) and weight (P<0.05) and lowest food conversion ratio (P<0.01). The survival of the prawns fed with this diet was also high. The effect of dietary energy on the growth and FCR is shown in Fig. 1. The growth of the prawns gradually increased (Fig. 1a) with the increase in dietary energy level and recorded a Peak at 414.72 Kcal/100 g. The growth curve descended as the dietary energy further raised to 462.43 Kcal/100 g. The FCR gradually improved (Fig.1b) with the increase in the dietary energy and reached the lowest value at 414.72 Kcal/100 g and started raising at 462.43 Kcal/100 g.

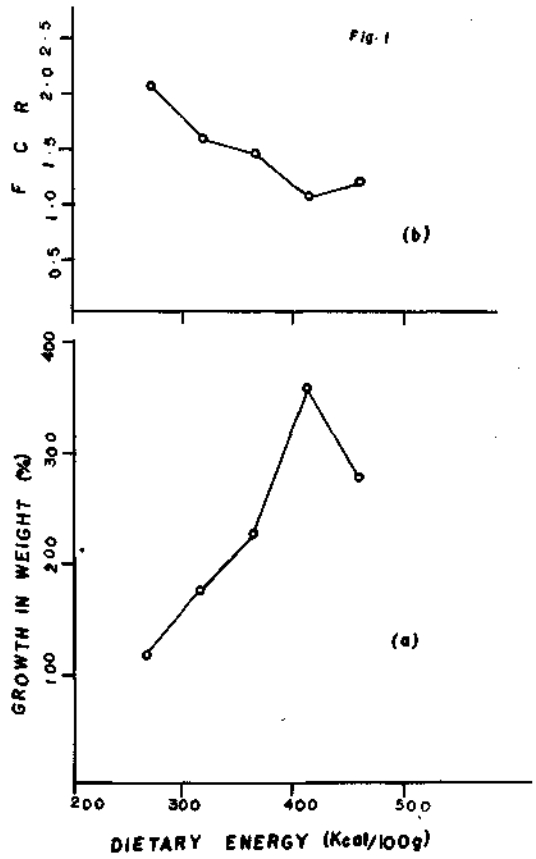

FIG. 1. Effect of dietary energy level on (a) growth and (b) food ratio in juvenile *P. indicus*.

TABLE 5. *Results of the feeding experiment on juvenile* P. indicus *fed with diets* P_6 *to* P_{11} *for 30 days*

Particulars	Diet No.					
	P_6	P_7	P_8	P_9	P_{10}	P_{11}
Growth in length %	43.1de	56.6b	73.5a	55.6bc	44.3cd	68.4a
Growth in weight %	305.4de	391.3b	586.2a	524.5bc	464.8cd	401.8cd
Food conversion ratio	8.266	3.42a	2.78a	3.10a	3.45a	3.38a
Survival	61.0	55.5	83.3	61.1	77.7	77.7
Body lipid of animals after the feeding experiment % (on dry basis)	19.3	18.0	18.2	16.9	17.7	18.6

Note : The Values with different superscripts differ significantly among themselves. Growth in length and weight significant at 1% ($P < 0.01$) and food conversion ration at 5% ($P < 0.05$).

TABLE 6. *Results of the feeding experiment on juvenile* P. indicus *fed with the diets* E_1 *to* E_5 *for 30 days*

Particulars	Diet No.				
	E_1	E_2	E_3	E_4	E_5
Growth in length %	40.1cd	50.8bc	62.5ab	77.4a	65.1ab
Growth in weight %	119.3cd	177.1bc	229.6bc	363.1a	283.5ab
Food conversion ratio	2.08c	1.61b	1.47b	1.11a	1.22a
Survival %	53.0	40.0	55.0	80.0	67.0

Note : The values with different superscripts differ significantly among themselves. While growth in length was significant at 5% ($P<0.05$), growth in weight and food conversion ratio were significant at 1% ($P<0.01$).

DISCUSSION

The results of the experiments conducted in the present study clearly demonstrated that lipid is essential in the diet of prawn for growth and survival and also emphasised that the source of the lipid influences the results. Kanazawa and Teshima (1977) and Kanazawa *et al.* (1979b) demonstrated that penaeid prawns are not capable of synthesising polyunsaturated fatty acids (PUFA) such as linolenic acid (18 : 2ω6), linolenic acid (18 : 3ω3), eicosa Pentaenoic acid (20 : 5ω3) and docosahexaenoic acid (22 : 6ω3). They concluded that these fatty acids are essential for prawns and should be supplied through their diet. As the different lipid sources vary in their fatty acid composition, their nutritive value for prawns depends upon the quality and the quantity of PUFA.

Colvin (1976) tested sunflower oil,

ground nut oil, linseed oil and soybean oil at 5% level in the diet of *P. indicus* and did not find any difference in the growth of prawns fed with the diets having these different oils. But in the present study, the mixed lipid source (having cod liver oil, sardine oil, lecithin and prawn head oil in equal proportion) resulted in superior growth and food conversion ratio (FCR) in the same prawn, compared to the individual lipid sources. Among the individual lipid sources tested, prawn head oil recorded superior growth and FCR. Joseph and Meyers (1975) and Joseph and Williams (1975) reported that prawn head oil is rich in PUFA which are essential for prawns and described it as a potential additive in the feeds of marine animals. The results obtained by prawn head oil in *P. indicus* in the present study corroborated these observations.

Kanazawa *et al.* (1979 c) and Teshima *et al.* (1982) demonstrated that phospholipids are essential in the diet of prawns. Later Kanazawa (1982, 1983) found that the diet when fortified with 1% soybean lecithin, improved the growth and survival of the prawn *Penaeus japonicus*. These observations are similar to the results shown by *P. indicus* with soybean lecithin diet in the present study. Guary *et al.* (1976) had shown that sardine oil had high nutritive value in the diet of *P. japonicus* and AQUACOP (1978) reported that cod liver oil as the best source of lipid in the diet of *P. merguiensis*. But both the lipids were found to be less superior to prawn head oil and soybean lecithin for *P. indicus*. Based on the results obtained in the present study, it is recommended to use a mixed lipid in the diet of *P. indicus* as it may provide the balanced PUFA in the diet required by this prawn. Alternatively, prawn head oil is also a potential source of lipid in the diet of *P. indicus*. It is estimated that 2,200 tonnes of prawn head is available (Wood and Coulter, 1988) per annum in the country. Part of this can be utilized for extraction of oil which can have high utility in prawn feeds.

Andrews *et al.* (1972) observed that addition of 10% lipid (a mixture of beef tallow, menhaden oil, and corn oil in equal parts) to the diets of *Penaeus setiferus*, had an adverse effect on growth and survival. The negative effect was more significant at dietary protein levels between 28 and 40%. Forster and Beard (1973) reported that addition of 7.5% corn oil or cod liver oil in the diet of *Palaemon serratus* had no advantage and when the lipid level was increased to 15% in the diet, the growth was significantly reduced. In the present study, *P. indicus* had shown a quantitative lipid requirement of 6 to 9% in the diet and higher levels had no beneficial effect. These findings are in agreement with the earlier results. However, Sick and Andrews (1973) reported that *Penaeus duorarum* fed with the diet having 10% fat and 1% cholesterol had shown faster growth and high survival.

The results obtained in the present study are significant in indicating that the quality of the lipid source in the diet is more important than its quantity. It should also be noted that enhancing the lipid level in the diet indiscriminately can have adverse effect on growth. Recent studies (Kanazawa, 1984; Chandge, 1987) on the lipid for prawns have been devoted to the investigations on the requirement of fatty acids, phospholipids and steroids. It is now fairly clear that penacid prawns require ω3 fatty acids in their diet. Significant among them are eicosapentaenoic acid (20 : 5ω3) and docosahexaenoic acid (22 : 6ω3). The optimum requirement of these two fatty acids was found to be 1% each in the diet of *P. japonicus* (Kanazawa *et al.*, 1979a). In

addition to this the penacid prawns require 0.5 to 1.0% cholesterol and 1% of phospholipid, especially, lecithin, having either choline or inositol in its molecule. Among the different lipid sources available, lipids derived from marine animals such as sardine oil, cod liver oil, pollack liver oil, shrimp head oil and clam oil are rich in polyunsaturated fatty acids (PUFA) and also in highly unsaturated fatty acids (HUFA). On the other hand, vegetable oils are relatively deficient in HUFA. With the detailed information now available regarding relative efficiencies, it is possible to select and use most suitable lipid source at required levels in prawn diets.

Experiments on the dietary energy level yielded interesting results. For the highest growth and the best FCR, the prawns showed a requirement of 414 Kcal/100 g in the diet. The results also indicated that a mixture of lipid and carbohydrate is more appropriate source of energy in the diet apart from the protein. The diet which showed the best performance contained 40% protein,5% lipid and 35% carbohydrate. The protein requirement in the diet of most of the penaeid prawns has been reported to be in the range of 40% (Kanazawa, 1984). However, availability of adequate energy in the diet through lipid and carbohydrate, spares the protein (Andrews *et al.*, 1972; Sick and Andrews, 1973; Ahamad Ali; 1982 b). It has been advocated that high energy low protein diets give better performance (Hysmith *et al.*, 1972). The results obtained in the present study not only conformed the observations made above, but also emphasised the need for quantifying the dietary energy for the best performance.

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