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Nutritive Value of Natural Lipid Sources for Indian White Prawn, *Penaeus indicus* H. Milne Edwards

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ABSTRACT

Experiments were conducted to ascertain the nutritive value of natural lipid sources for juveniles of *P. indicus*. Sixteen isonitrogenous and isocaloric diets were prepared using twelve naturally available oils, either individually or in combinations. The diets containing marine animal lipid sources produced significantly better grwoth, FCR, PER and protein retention in prawns than the diets containing only plant oils. Among the individual marine animal lipids, prawn head oil appeared to be a suitable lipid source. The diets containing a mixture of plant and animal lipids produced superior growth than individual plant or animal lipids (except prawn head oil). Among the mixtures of lipid sources, a combination of cod liver oil, soybean oil and lecithin proved to be superior.

INTRODUCTION

The need for formulated feeds in prawn culture is well recognised. In India, commercial prawn feeds are being manufactured by Mysore Snack Foods, Tata Oil Mills Company Ltd., Hindustan Lever Ltd., etc. However, prawn nutrition continues to be an important research area. Importance of lipids in prawn nutrition needs no emphasis. Nutritional response of prawns to dietary lipids from natural sources has been studied by several workers (Sick and Andrews, 1973; Colvin, 1976; Sandifer and Joseph, 1976; Guary *et al.*, 1976; Kanazawa *et al.*, 1970, 1977; Aquacop, 1978; Read, 1981). Similarly, essentiality of certain fatty acids in the diet has been demonstrated by Kanazawa (1985) in a number of species of penaeid prawns and by Read (1981) in *Penaeus indicus*. Nevertheless, concentrated efforts are needed to identify suitable natural lipids, both from plant and animal sources for incorporating into formulated feeds. It has been confirmed that the type, content and proportion of dietary fatty acids are important in prawn nutrition (Kanazawa *et al.*, 1977). Also, instead of plant lipids or marine lipids alone, a combination of the two is more effective in promoting prawn growth as indicated by recent investigations (Deshimaru and Kuroki, 1974; Deshimaru *et al.*, 1979; Colvin, 1976; Read, 1981).

Penaeus indicus being a commercially important prawn along the Indian coast, was selected for the present study. An attempt was made to identify suitable lipid sources (plant or animal or their combinations) for incorporation in feeds of juvenile *P. indicus* based on the dietary lipid requirement.

MATERIAL AND METHODS

Experimental set up: Feeding experiments of 35 days duration were conducted in triplicate in the laboratory at the Central Marine Fisheries Research Institute, Cochin (CMFRI). Fifty litre capacity, round bottom plastic nonreactive tubs were used as experimental containers. About 40 litres of sea water of salinity 20 ± 2‰ was used in each container and continuous aeration was provided. Water was siphoned and filtered through a biological filter and used for subsequent two days. On the fourth day, the entire water was replaced with fresh sea water of same salinity.

Formulation and preparation of diet: Purified diets were formulated employing the methods of Kanazawa *et al.* (1970, 1977, 1982) and Read (1981) with slight modifications. Composition of the basal diet is given in Table 1, 1A and 1B. Sixteen isonitrogenous and isocaloric diets were prepared using twelve naturally occuring oils and lecithin, either individually or in combination (Table 2). Prawn head oil was extracted from prawn head waste, while the remaining oils were purchased from the local market. Earlier studies (Chandge, 1987) have indicated that 12% lipid level consisting of cod liver oil, soyabean oil and lecithin produced maximum growth, FCR (food conversion ratio), PER (protein efficiency ratio) and protein retention in juvenile *P. indicus* as compared to higher or lower levels of lipid; therefore 12% lipid level was maintained in all the diets. Carrageenan was used as the binder.

Feeding experiments: Juveniles of *P. indicus* ranging from 20 to 25 mm total length and 42 to 48 mg

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Table 1. Ingredient composition of the basal diet used for *P. indicus*

Ingredients	g/100 g diet
Casein	31.00
Egg albumin	7.50
Amino acid mixture*	5.00
Glucosamine	0.80
Sodium citrate	0.30
Sodium succinate	0.30
Starch	12.00
Clucose	4.90
Sucrose	11.00
Cholesterol	0.50
Lipids**	12.00
Vitamin mixture	3.20
Mineral mixture	8.50
Cellulose powder	3.00
Total	100.00
Carrageenan	5.00
Distilled water	100-120 ml

* Amino acid mixture (g/100 g diet), Arginine 1.0, Methionine 0.5, Glycine 2.0, Taurine 0.5, Glutamic acid 1.0

** See Table 2.

Table 1 A. Vitamin mixture used in the diet

Vitamins	mg/100g diet)
Thiamine HCL (B_1)	4.90
Riboflavin (B_2)	8.00
Para-amino benzoic acid	10.00
Inositol	400.00
Biotin	0.40
Niacin	40.00
Calcium pantothenate	60.00
Pyridoxin HCL	12.00
Menadione	4.00
β -Carotene	9.60
α -Tocopherol (Vitamin E)	20.00
Calcipherol	1.20
Cynocobalamine (B_{12})	0.08
Sodium ascorbate (vitamin C)	2000.00
Folic acid	0.08
Choline HCL	630.00
Total	3200.00

Table 1 B. Mineral mixture used in the diet

Mineral	(g/100 gm diet)
K_2HPO_4	2.00
$Ca_3(PO_4)_2$	2.72
$MgSO_4.7H_2O$	3.02
$NaH_2PO_4.2H_2O$	0.79
$MnSO_4.5H_2O$	0.004
$FeSO_4.7H_2O$	0.015
Total	8.549

average wet weight from the same brood, reared in the prawn culture laboratory of CMFRI, Narakkal, were randomly selected for the experiments. Stocking was done @ ten juveniles per tub. Feeding rate was 10-20% of the total body weight, which was given in two doses *viz.*, 1/4 in the morning at 9 hr and 3/4 in the evening at 18 hr. The left over feed as well as faecal strands were separately collected daily by siphoning, dried in an electric oven and their dry weight recorded for calculation of FCR and PER.

Table 2. Apparent digestibility coefficient of food (dry matter) for the juvenile prawns fed on diets containing natural lipid sources

Diet No.	Lipid sources	Apparent digestibility coefficient	Food conversion ratio (FCR)	Protein efficiency ratio (PER)
1	Mustard oil	19.13 ± 1.11	9.83 ± 0.62	0.27 ± 0.01
2	Cotton seed oil	23.46 ± 2.54	9.50 ± 0.40	0.28 ± 0.01
3	Soybean oil	24.10 ± 3.04	7.86 ± 0.63	0.33 ± 0.03
4	Safflower oil	24.90 ± 3.18	8.50 ± 0.40	0.31 ± 0.01
5	Groundnut oil	41.67 ± 6.74	6.16 ± 0.77	0.38 ± 0.10
6	Sunflower oil	47.63 ± 0.00	5.06 ± 1.02	0.55 ± 0.08
7	Linseed oil	39.60 ± 5.53	6.48 ± 0.64	0.39 ± 0.04
8	Corn oil	40.90 ± 3.21	6.45 ± 0.32	0.41 ± 0.02
9	Sardine oil	55.34 ± 2.45	4.41 ± 0.15	0.60 ± 0.02
10	Cod liver oil	61.23 ± 1.59	4.07 ± 0.31	0.76 ± 0.05
11	Shark liver oil	59.70 ± 3.47	3.98 ± 0.59	0.69 ± 0.10
12	Sardine oil + Sunflower oil (50:50)	60.50 ± 5.37	8.50 ± 0.49	0.85 ± 0.18
13	Sardine oil + Groundnut oil (50:50)	62.4 ± 1.42	3.26 ±0.09	0.82 ± 0.02
14	Prawn head oil + Soyabean oil (50:50)	87.67 ± 3.77	2.06 ± 0.04	1.29 ± 0.30
15	Prawn head oil	76.60 ± 8.56	2.53 ± 0.36	1.07 ± 0.15
16	Cod liver oil + Soyabean oil + Lecithin (56:28:16)	90.76 ± 4.26	2.0 ± 0.31	1.38 ± 0.23

The promixate composition of juveniles, diets and faecal matter was determined using methods of Lowry *et al.* (1951) for protein, Bligh and Dyer (1959) for lipids, Dubois *et al.* (1956) for carbohydrate, Hestrin (1949) for cholesterol and AOAC (1965) for dry weight and ash content. The fatty acid profile of each lipid source used in the experiment and that of post experimental juveniles was determined adopting the procedure of Morrison and Smith (1964). Gas liquid chromatography of fatty acid methyl esters was carried out using a Hewlett Packard microprocessor controlled gas-liquid chromatograph (model 5840) with a flame ionisation detector. Quantitative identification of fatty acid esters was obtained by comparison with relative retention time of known standards. The identified peaks were quantified using an integrator.

The results on survival, gain in length and weight, food conversion ratio (FCR), protein efficiency ratio (PER), and post-experimental proximate composition of the test

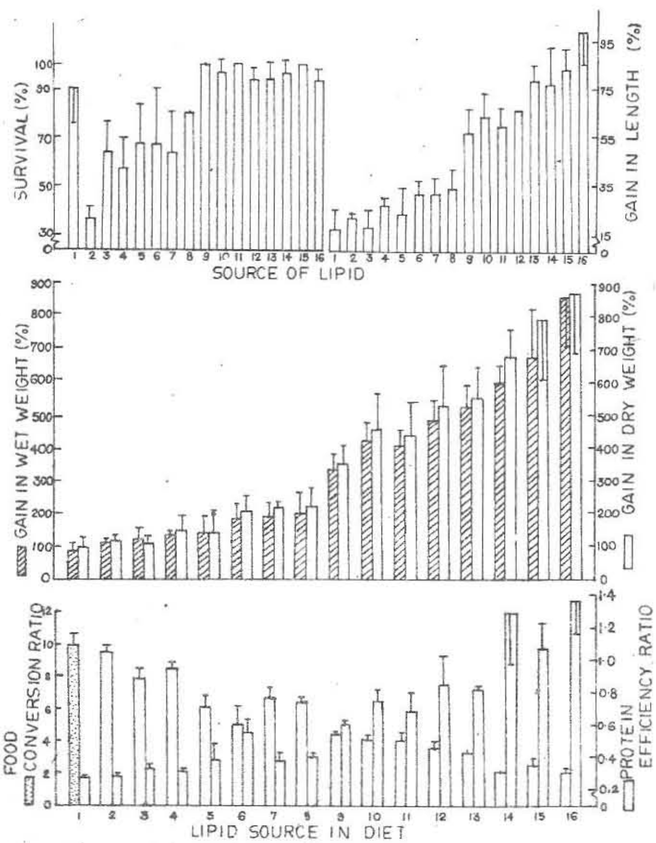

Fig 1. Histogram showing survival rate, growth, FCR and PER of juvenile *Penaeus indicus* fed on diets containing natural lipid sources

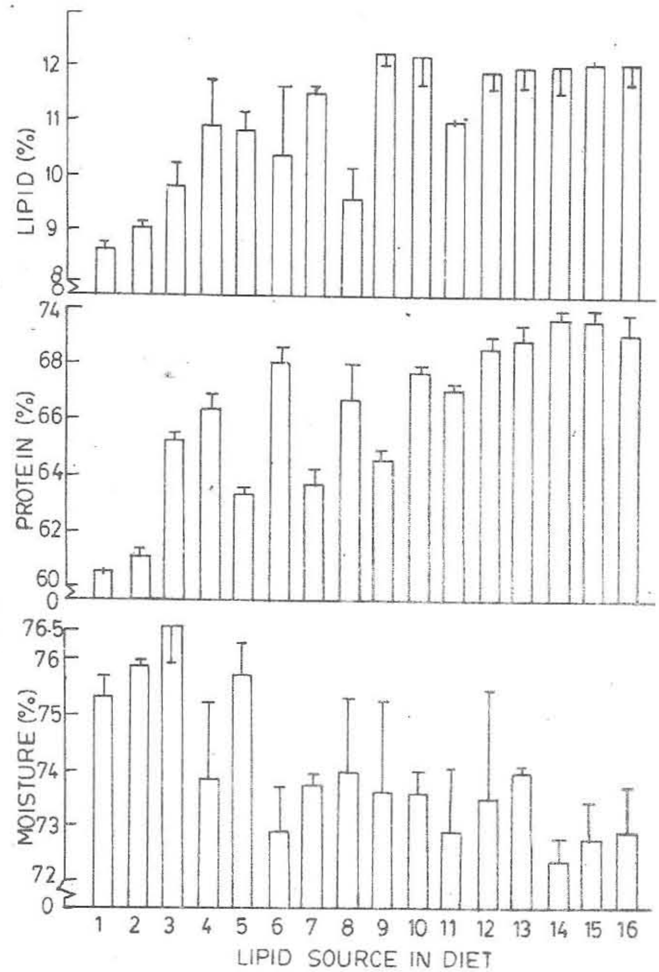

Fig 2. Histogram showing per cent moisture, protein and lipid content of juvenile *Penaeus indicus* fed on diets containing natural lipid sources

species were subjected to statistical analysis (Analysis of variance and least significant difference test) using Hewlett Packard Master Computer.

Results and Discussion

Results of the experiments are summarised in Table 3 and depicted in Figs. 1, 2 and 3.

Oxygen level was in the range of 4.8 to 6.4 mg/litre, while ammonia content was 0.03 to 0.11 ppm in sea water. The temperature of water ranged between 26° and 29.7°C and pH from 7.9 to 8.3 during the experiment.

Survival: Diet containing animal (marine) lipids or mixture of plant and animal lipids (diets 9 to 16) produced significantly higher ($P<0.05$) rate of survival than that of only plant oil-based diets (diets 1 to 8)with the exception of mustard oil diet, which produced a survival rate of 90%.

Growth performance: Amongst the plant lipid diets, those with linseed oil (diet 7) and corn oil (diet 8) produced relatively better growth than the remaining ones (diets 1 to 5). Compared to plant lipid diets, animal lipid diets produced better growth performance. Amongst individual animal oils, prawn head oil (diet 15) produced significantly higher ($P<0.05$) growth. However, it was the diets with a mixture of plant and animal oils (except diet 15 with prawn head oil) which showed significantly greater ($P<0.05$)growth performance, the best growth being obtained with diet 16, having a mixture of codliver oil, soybean oil and lecithin. Similar observations have been reported in the case of *P. japonicus* by Deshimaru *et al.* (1979), wherin feed efficiency and growth were better with a mixture of animal and plant lipids like pollack liver oil and soybean oil.

Proximate composition: The proximate composition of juveniles was influenced significantly ($P<0.05$) by dietary lipids (Figs. 2 and 3). Diets with animal lipids or mixture of animal and plant lipids resulted in relatively higher protein and lipid contents, but less moisture, ash and carbohydrate than those with plant lipids alone.

Digestibility: Apparent digestibility coefficient of diets was also influenced by the source of dietary lipid. The digestibility observed in the present experiments

Table 3. Fatty acid composition (%) of natural lipids used in diet and

Diet No.		1		2		3		4		5		6		7		8	
Fatty acids	Wild prawn standard	Mustard oil	Prawn lipid	Cotton seed	Prawn lipid	Soyabean oil	Prawn lipid	Safflower oil	Prawn lipid	Groundnut oil	Prawn lipid	Sun flower oil	Prawn lipid	Linseed oil	Prawn lipid	Corn oil	Prawn lipid
12.0	-	0.027	11.276	0.474	10.142	-	16.170	0.262	13.919	0.313	2.752	-	14.595	0.012	5.167	-	5.203
14.0	1.13	0.068	10.437	1.278	10.407	0.373	11.599	0.929	9.149	0.216	4.994	0.044	13.33	0.075	4.355	0.054	5.203
14.1	-	-	-	0.170	-	-	-	0.040	0.322	-	-	-	-	-	0.233	-	-
15.0	-	-	0.465	0.145	0.609	-	0.138	-	0.253	-	0.611	-	-	-	0.822	-	0.485
16.0	15.48	2.633	11.323	20.619	12.897	11.772	16.135	7.213	14.219	13.441	18.28	6.358	12.607	9.565	14.904	15.166	14.332
16.1w7	7.53	9.478	1.118	1.581	0.609	-	0.519	0.161	0.461	-	-	-	1.626	-	1.776	-	2.006
17.0	2.24	-	-	0.182	-	-	-	0.282	1.590	-	1.664	-	-	0.0376	1.908	-	1.212
17.1	0.94	-	-	0.573	-	-	-	-	-	-	-	1.241	-	-	-	-	-
18.0	8.19	-	3.727	3.077	4.793	3.664	5.020	2.188	4.747	1.958	6.523	-	4.337	3.744	7.923	2.004	4.52
18.1w9	12.81	26.298	17.008	18.320	17.240	22.984	18.14	15.524	13.758	50.943	26.774	34.84	14.595	23.067	19.361	29.831	21.30
18.2w6	4.29	16.711	14.957	52.224	18.326	51.803	20.74	71.896	25.004	33.074	24.668	57.467	18.435	22.288	19.514	50.021	23.086
18.3w3	1.03	3.47	5.917	0.681	5.985	7.380	4.639	1.118	4.217	3.636	6.353	0.35	2.620	41.059	09.574	2.837	9.005
20.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20.1w9	1.39	-	0.698	-	1.244	-	1.138	-	0.829	-	0.946	-	0.812	-	-	-	1.124
20.4w6	8.68	-	2.132	-	8.050	-	0.796	-	0.783	-	0.165	-	1.536	-	1.451	-	-
20.5w3	11.24	0.2196	2.480	-	0.40	-	1.492	-	0.783	-	2.307	-	3.298	-	6.183	-	2.026
22.5w3	1.88	-	-	-	-	-	1.591	-	0.783	-	-	-	-	-	-	-	2.756
22.4w6	-	-	2.795	-	-	-	-	-	6.222	-	-	-	4.732	-	-	-	-
22.6w3	11.00	1.688	0.093	-	0.316	1.601	0.412	-	1.451	-	0.303	-	2.65	-	3.096	-	3.352
Total Saturated	27.04	2.723	37.228	25.775	38.848	15.809	49.65	10.874	43.877	15.92	34.824	6.40	44.60	13.421	35.414	17.22	30.955
Mono unsaturated	22.67	35.776	18.824	20.644	19.093	22.984	19.787	15.599	15.047	50.943	27.72	36.08	17.033	23.067	21.37	28.88	24.43
18:2w6	4.29	16..711	14.957	52.224	18.326	51.803	20.74	71.896	25.004	33.074	24.668	57.46	18.435	22.288	19.574	50.021	23.086
18:3w3	1.03	3.47	5.917	0.681	5.985	7.380	4.639	1.118	4.217	3.636	6.353	0.35	2.620	41.059	9.503	2.831	9.005
Total w6	12.97	16.711	19.884	52.224	26.376	51.803	21.674	71.896	32.606	33.074	24.836	57.467	24.70	22.288	20.954	50.021	23.080
Total w3	25.97	5.378	8.49	0.681	6.723	7.380	8.189	1.118	7.668	3.636	9.367	0.350	10.366	41.059	18.853	2.031	17.138
Total PUFA>20C	32.08	1.90	7.50	-	8.788w6	-	5.914	-	9.144	-	3.482	-	8.566	-	-	-	8.133
Total 20:5w3+ 22:6w3	12.88	1.90	2.573	-	0.716	-	3.493	-	2.238	-	2.66	-	5.948	-	9.279	-	5.378

followed the trend of FCR and PER (Table 3). Digestibility was relatively low in diets with plant oils, but improved with the addition of animal lipids. Diet with a mixture of cod liver oil, soyabean oil and lecithin was found to be the best with 90.76% digestibility. The apparent digestibility of diets containing plant lipids appears to be poor as compared to that of animal lipids which contain W3-HUFA having low melting points. The digestibility of lipids is reported to decrease with increase in melting point (Takeuchi *et al.*, 1979) Thus, the increase in digestibility of diet resulted in better food conversion ratio and protein efficiency ratio with diets containing animal lipid and *vice versa* with plant lipid diets.

Fatty acid analysis: Results of the fatty acid analysis of dietary lipids, experimental juvenile prawns and wild juvenile prawns (used as reference) are given in Table 3. Diets containing plant oil induced relatively greater deposition of linoleic acid (18:2W6) and linolenic acid (18:3W3) than those with animal oils, which induced greater deposition of 20:5W3 and 22:6W3 fatty acids. The diet with a mixture of cod liver oil, soyabean oil and lecithin and that having a mixture of prawn head oil and soyabean oil produced fatty acid pattern almost similar to the dietary lipids, suggesting that fatty acid pattern of the test prawns, to a greater extent, depended upon the fatty acid profile of dietary lipids.

Nutritive value of dietary lipids in crustaceans, particularly prawns, depends on the content and levels of essential fatty acids available in the diet (Deshimaru and Kuroki, 1974; Deshimaru *et al.*, 1979; Kanazawa, 1985). In the present study on *P. indicus,* poor response to diet containing only plant lipids is mainly due to deficiency of HUFA of W3 series such as 20:5W3 and 22:6W3 (Table 3). The marine animal oils had high levels of HUFA of W3 series (20:5W3 and 22:6W3) which seems to have induced superior response, as they presumably satisfied the essential fatty acid needs to a great extent. However, the best response obtained with a mixture of lipids of plant and animal, particularly the diet containing a mixture of cod liver oil, soyabean oil and lecithin, lipid from the whole body of post-experimental juvenile *Penaeus indicus*

9		10		11		12		13		14		15		16	
Sardine oil	Prawn lipid	Cod liver oil	Prawn lipid	Shark liver oil	Prawn lipid	Sardine oil + Sunflower oil	Prawn lipid	Groundnut oil + Sardine oil	Prawn lipid	Prawn head oil + Soya-bean oil	Prawn lipid	Prawn head oil	Prawn lipid	Cod liver oil+Soya-been oil+ Lecithin	Prawn lipid
0.0677	2.870	0.276	5.263	-	6.728	0.335	12.57	0.19	5.20	0.008	0.532	0.016	5.22	4.804	5.105
4.945	4.583	5.436	4.501	0.813	6.606	2.47	11.672	2.57	11.87	2.113	9.74	3.833	0.163	5.78	4.832
0.158	-	-	-	-	-	0.079	-	0.079	0.263	0.165	-	0.33	-	-	0.085
0.398	2.99	0.322	-	-	-	0.199	-	0.199	-	0.60	2.064	1.36	-	0.10	0.459
13.693	5.99	10.036	18.619	14.983	15.431	10.084	19.10	11.851	19.11	17.313	14.91	22.854	20.06	10.52	16.473
7.136	5.405	11.356	6.069	1.510	2.597	3.068	1.908	3.768	2.57	5.269	2.319	10.539	7.34	6.383	2.55
0.745	7.507	0.806	0.674	0.278	0.950	0.372	0.487	0.372	-	0.78	0.23	1.56	1.549	0.45	-
0.933	-	-	-	-	-	0.466	-	0.460	-	0.642	3.456	1.294	-	0.04	1.254
12.797	2.535	1.796	4.212	4.380	6.094	7.716	7.693	8.348	6.37	6.29	5.38	8.929	7.117	2.591	5.360
19.648	17.625	23.6117	27.193	39.22	25.986	31.149	21.558	35.29	26.91	16.80	15.56	12.638	25.20	21.59	18.889
1.528	9.494	3.178	4.449	3.2055	5.577	24.619	13.134	17.73	10.62	27.20	18.51	2.92	9.135	18.10	19.026
0.5	1.6	0.506	5.675	1.274	0.243	4.68	0.182	4.50	2.50	5.33	4.54	1.161	2.365	3.116	3.982
1.219	0.060	-	-	-	-	0.604	0.142	0.609	-	0.304	-	-	0.815	6.25	0.748
0.993	9.616	11.264	0.218	1.445	3.266	0.496	0.466	0.496	0.260	0.248	3.503	4.597	0.57	-	0.595
0.082	0.936	-	-	-	0.877	0.041	0.77	0.041	-	0.655	-	-	-	2.89	0.54
8.250	7.065	10.412	6.02	3.36	9.64	9.50	6.712	9.48	6.508	3.50	8.06	9.163	6.8	4.03	4.5
7.046	1.895	-	-	-	-	-	-	-	-	2.98	3.82	0.597	1.059	-	-
0.542	-	-	-	12.0	0.414	0.271	-	0.271	-	-	-	-	-	-	-
10.734	8.18	12.508	13.726	10.674	10.970	3.523	11.08	3.523	5.60	7.417	4.45	14.837	7.662	6.95	7.00
33.864	26.49	18.35	34.90	20.48	35.859	21.78	50.625	24.139	42.55	27.214	32.856	38.525	34.82	30.49	32.977
28.868	32.646	46.76	33.44	42.175	31.84	35.259	23.99	40.099	30.003	23.124	24.838	29.39	33.11	28.128	23.373
1.528	9.494	3.178	4.449	3.2055	05.577	24.619	13.134	17.73	10.62	27.20	18.51	2.92	9.135	18.10	19.026
0.05	1.646	0.506	5.695	1.274	0.243	4.68	0.182	4.50	2.50	5.33	4.54	1.161	2.365	3.116	3.982
2.152	10.43	3.178	4.449	3.2055	16.454	24.9318	13.904	18.042	10.62	27.84	18.00	2.92	9.135	21.00	19.57
26.58	18.74	23.426	25.421	15.31	20.875	17.695	17.894	17.51	14.618	19.221	20.83	25.75	15.685	14.09	14.793
26.654	15.942	24.286	19.74	37.365	21.901	13.327	9.957	13.322	12.36	4.08	19.83	29.19	15.439	13.87	12.64
18.98	18.076	22.92	19.74	14.03	20.61	13.27	17.79	13.003	12.10	10.91	12.51	24.03	14.46	10.98	11.50

demonstrates that prawns have dietary requirement for a blend of lipids containing adequate levels of 18:2W6, 18:3W3, 20:5W3, 22:6W3 and 20:4W6 (arachidonic acid). This is clearly evident from the fatty acid profile of lipids used (Table 4). Although this diet had a good proportion of W6 fatty acids, it is not reflected proportionately in the tissue fatty acid profile. Perhaps W6 fatty acids are utilised for the production of energy and W3 fatty acids are preferentially retained for body building as indicated by the high percentage of W3 fatty acids in the tissues. Joseph and William (1975) and Sandifer and Joseph (1976) have also made similar observations in *Macrobrachium rosenbergii.*

It is generally assumed that the fatty acid needs of a species to a great extent reflect the fatty acid pattern of animals. In general, the total percentage of W3-HUFA present in dietary lipids and percentage of W3-HUFA deposited in the body of *P. indicus* showed some similarity (Table 3), suggesting the influence of dietary lipids. Similar observations have been made in *P. indicus* by Colvin (1976) and in *P. japonicus* by Kanazawa *et al.* (1978). The response of prawns fed with prawn head oil also be attributed to its fatty acid pattern. Besides, the concentration of phospholipids in prawn head oil might be a factor contributing to better performance of the animals as these phospholipids are essential for prawns (Kanazawa *et al.*, 1979, 1985). The growth promoting effect of prawn head oil in prawns has also been reported by Sandifer and Joseph (1976) in *M. rosenbergii.* The poor response produced by mustard oil, cotton seed oil and shark liver oil might be due to the presence of large amount of erucic acid (22:0) in mustard oil, cyclopropenoid acid and malvalic acid in cotton seed oil and squalene in shark liver oil. Erucic acid is known to have growth inhibiting effect in prawns while cyclopropenoid and malvalic acids produce undesirable biological activity (Lee and Sinnhuber, 1972) and reduce the growth rate in trout (Sinnhuber *et*

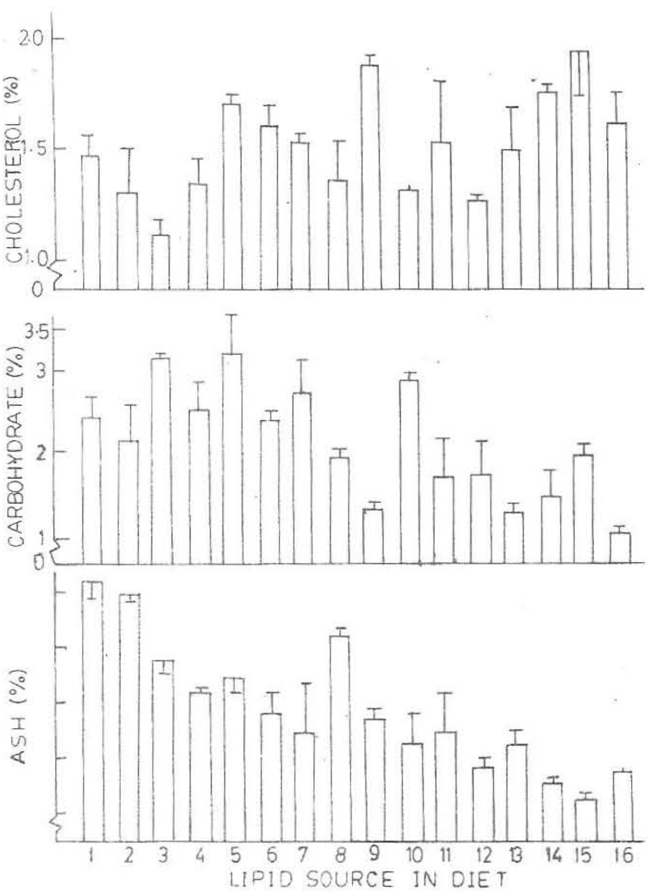

Fig 3. Histogram showing percent carbohydrate, ash and cholesterol content of juvenile *Penaeus indicus* fed on diets containing natural lipid sources

al., 1968). Squalene also has growth inhibiting activity as reported in *P. merguiensis* (Aquacop, 1978).

From the foregoing account, it can be summarised that the nutritive value of lipids in prawns depends upon essential fatty acid content of the dietary lipid source as observed by Watanabe (1982). Besides, phospholipid in the diet also promotes growth, perhaps by enhancing absorption and inter organ transport of dietary lipids and cholesterol (Kanazawa *et al.*, 1985). The present study indicates that *P. indicus* requires lipid sources which can provide W6 and W3 PUFA and phosholipids in adequate levels and optimum proportions.

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