STUDIES ON LIPID NUTRITION IN LARVAE AND JUVENILES OF THE INDIAN WHITE PRAWN PENAEUS INDICUS H. MILNE EDWARDS

THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY OF THE COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY

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CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON LIPID NUTRITION IN LARVAE AND JUVENILES OF THE INDIAN WHITE PRAWN, PENAEUS INDICUS H. MILNE EDWARDS"** is the bonafide record of the work carried out by Shri. MANOHAR S. CHANDGE under my guidance and supervision and that no part thereof has been presented for any other Degree.

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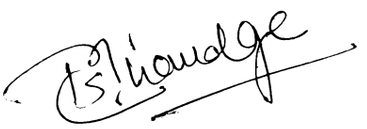
DECLARATION

I hereby declare that this thesis entitled **"STUDIES ON LIPID NUTRITION IN LARVAE AND JUVENILES OF THE INDIAN WHITE PRAWN, PENAEUS INDICUS H. MILNE EDWARDS"** has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.



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C O N T E N T S

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P R E F A C E

India has acquired the premier place among prawn producing countries of the world with a total prawn production of 2.04 lakh tonnes in 1984-85, constituting about 12% of the total world production of shrimps and prawns. Prawns also form an important group in the marine fisheries of India, contributing to about 12.65% of the total marine fish production of 1.615 million tonnes in 1984-85 (Anon, 1986). Besides, prawns form the major component in the marine products exported from India. Of the 86,178 tonnes of marine products exported from the country during the financial year 1984-85, prawns accounted for 64% by quantity (55,000 tonnes) and 86% by value (Rs.330 crores) indicating the significance of prawns in India's marine products export (MPEDA Statistics,1986). India also continued to be the major exporter of prawns to Japan and the USA.

In practice, scope for appreciable increase in landing of prawns from capture fishery is limited due to many problems involving resource management, environmental conservation, and operational cost. Statistics of prawns landings from India showed that there has been steady increase in the catch of prawns from 1968, with a peak(2.2 lakh tonnes) in 1975 (Silas et al., 1984) and the prawn production has declined to 2.04 lakh tonnes in 1984-85(Anon,1986). In spite of increase in fishing

efforts our prawn landings remained stable and declined (Silas et al., 1984). Thus the capture fishery for prawn is fairly well developed and known fishing grounds are well exploited, with the result increase in production from this source is quite unlikely. However the demand for prawn supplies from all over the world is increasing every year. Increase in production can be expected only from (1) new grounds that may be discovered in course of time and (ii) generating new resources by way of culture. About three to four fold increase in prawn production is possible in India by way of culture.

As a result of research conducted so far, significant advancements have been made in the culture of commercially important penaeid prawns in India. Indigeneous techniques of breeding and rearing of larvae to stocking size under controlled conditions have been developed. Concurrently, researches for finding suitable feed for the larvae, juveniles and adults were taken up. However, a great deal of research is yet to be carried out on priority basis, to develop nutritionally efficient feed formulations for larvae, post-larvae, juveniles and adults of prawns, since it has been well established that commercially viable culture technologies for penaeid prawns could be developed only by judicious use of operational inputs, particularly feed, which accounts for the major share often exceeding 50% of the total operational costs in intensive

prawn culture operations. Practical feeds for prawns should contain adequate levels of nutrients such as proteins, lipids, carbohydrates, minerals and vitamins to promote growth under normal condition, and thus knowledge of nutritional requirements of the cultured species is a prerequisite for formulating feeds. However, with the exception of P. japonicus nutritional studies on prawns and shrimps are very few and fragmentary (Kanazawa, 1985).

In India, although significant advances have been made in the culture of a variety of fish and shellfish species, studies on nutrition and its relevance to prawn culture have been very few untill 1980(CMFRI News Letter, 29-30, 1985). During the past few years research has been intensified in this area at the CMFRI through the UNDP/FAO/ICAR subproject "Centre of Advanced Studies in Mariculture". The present investigation in lipid nutrition in larvae and juveniles of the Indian white prawn, P. indicus is one among the series of investigations carried out on the nutritional requirements of prawns.

Recent studies with prawns indicate that their moulting growth, and maturation are affected by the type of lipids supplied in the diets (Kanazawa et al., 1970, 1977a, 1979a, 1985; Colvin, 1976a; Read, 1977). Unlike higher vertebrates which are known to require linoleic acid (18:2w6) as an essential fatty acid, aquatic organisms, such as prawns, have been found to require linolenic acid (18:3w3)

eicosapentaenoic acid (20:5w3) and docosahexaenic acid (22:6:w3) in addition to 18:2w6 as essential fatty acids (Kanazawa and Teshima, 1977; Read 1977, Kanazawa et al., 1979b). There has also been significant differences between species in their EFA requirements. Recent studies indicated that deficiency of EFAs in the diet leads to poor growth, mortality and severe pathological syndromes. The fecundity, fertilization rate and hatchability of eggs also have been found to be very much reduced (Watanabe, 1982). Similarly, cholesterol is an essential nutrient for crustaceans, deficiency of which results in the crustaceans inability to synthesize hormones essential for moulting and gonadial maturation. Despite the recognition of the importance of lipids in the diet of prawns, studies are lacking to determine the dietary requirement of lipids by Indian penaeid prawns. Therefore, experimental studies were conducted to determine the lipid requirements of larvae, post-larvae and juveniles of one of the most important cultivated species of Indian penaeid prawns, Penaeus indicus. It is hoped that this investigation would help understand the role of lipids in the nutrition of P. indicus, as well as to formulate diets for larval and juvenile P. indicus.

About 24 laboratory experiments were conducted with larvae, post-larvae and juveniles of P. indicus to determine the essentiality and nutritional requirement of total lipids,

phospholipids, fatty acids, cholesterol and to ascertain the nutritional value of natural lipid sources for *P. indicus*. Based on the results of these experiments the essentiality and quantitative dietary requirements of lipids, phospholipids, cholesterol and EFA in the diet of larvae, post-larvae and juveniles of *P. indicus* have been worked out. With a view to identifying suitable plant and animal lipid sources for formulating practical diets for larvae, post-larvae and juvenile prawns, the nutritive value of 15 naturally occurring lipids and their mixtures were evaluated, and it was found that a mixture of plant and animal lipids is the best source for promoting growth, feed utilization in larvae post-larvae and juveniles of *P. indicus*. Fatty acids profile of the lipid sources and post-experimental juveniles were obtained to know the effect of dietary lipids on fatty acid composition of the prawn.

The thesis embodying the details of the investigation has been organised into five Chapters each with an introduction, material and methods, results and discussion sections. While a general introduction and a general material and methods section preceeds the chapters, the summary and reference sections follows the five chapters.

In the first chapter, details of four sets of experiments conducted to determine the dietary requirement of total lipids in the diet and to elucidate the effect of various dietary

levels of lipids on the larvae, post-larvae and juveniles of *P. indicus*, are presented. Chapter II deals with the studies on the essentiality and dietary requirements of phospholipids (lecithin) for larvae, post-larvae and juveniles of *P. indicus*. In Chapter III the effects of selected levels of dietary linoleic and linolenic acids on the prawns have been presented. The results have been compared to that of a diet containing a mixture of lipid sources, which provide a blend of fatty acids essential for prawns.

The nutritional value of various plant and marine lipid sources and their mixtures for prawns have been studied with reference to the response obtained in the animals as well as the fatty acids patterns of the lipids and presented in Chapter IV. In the fifth Chapter the essentiality and dietary requirement of sterol (cholesterol) for larvae, post-larvae and juveniles *P. indicus* are dealt.

The omega (w) classification of fatty acids is used extensively in the thesis. Three numbers are specified for a unsaturated fatty acid. For instance linolenic acid is expressed as 18:3w3. The first number indicates the number of carbon atoms in the chain, the second the number of double bonds and the third inclusive number of carbon atoms from methyl terminal to carbon atoms of first double bond. The last number is the omega (w) number. Thus the linolenic acid

has the structure

$CH_3-CH_2-CH = CH_2-CH = CH_2-CH = CH-CH_2 = CH(CH_2)_7-Co_2H$

and is designated as 18:3w3. When fatty acid is saturated then double bond is not present so designated or expressed as 12:0 for lauric acid, 16:0 for palmitic acid and so on.

Though great care has been taken in planning and conducting the experiments, certain disadvantages were observed in the feeding experiments with larvae, particularly in providing an adequate particle size for protozoeal stagesthough earlier methods suggested by Kanazawa et al. (1982b)were adopted. Further detailed studies in the lipid requirement of the larvae is suggested as soon as a desired food particle is developed.

As stated by Kanazawa (1985) the types and contents of essential fatty acids dominate the nutritive value of dietary lipids. So the nutritive value of dietary lipids is discussed with reference to essential fatty acid contents of the lipids concerned, although other lipid components such as phospholipids, sterols and fat soluble vitamins may influence the dietary value of lipids for the prawn P. indicus. Since the levels of latter biomolecules have not been monitored in the lipids, they have been mostly excluded from the discussion sections.

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Manohar S. Chandge

L I S T O F T A B L E S

L I S T O F F I G U R E S

Figure

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CHAPTER II

CHAPTER III

CHAPTER IV

CHAPTER V

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Nutrition is the process of providing nourishment to the living organism for its healthy upkeep, growth and reproduction. Nutrient substances for this purpose are provided by food. An individuals nutritional status is dependent on the provision of sufficient nutrient substances, and good utilisation of these nutrients. Poor status of nutrition may be caused by eating food that is inadequate in amount or kind or due to failure in assimilation and utilization of nutrients from the ingested food. The chief function of food is to supply nutrient material to meet the physiological needs of the organisms, such as to supply energy, to build and maintain the cells and tissues, and to regulate body processes.

In general, there are two types of nutrients - energy nutrients (proteins, lipids, and carbohydrates) and non-energy nutrients (vitamins and minerals). Among the energy nutrients, carbohydrates and lipids form chief sources of energy, but protein, primarily, is utilized for growth. In formulated feeds both energy nutrients and non-energy nutrients should be found in adequate levels and in balanced proportions.

Most of the aquatic organisms, including prawns, in their natural environment satisfy their nutritional needs from the live-food they consume. However in culture systems, where higher population stocking densities are maintained the available natural food may not be adequate to promote fast

growth and in such systems supplementary feeding may be essential. Besides, production of prawns in intensive systems depends upon the supply of nutritionally balanced formulated, complete feeds.

An important aspect of prawn nutrition is that the food requirement varies according to their size. The early larval stages are filter feeders and require microparticulate feeds; whereas the juveniles and adults are predominently bottom feeders and require macroparticulate feeds. The early larvae and post-larvae prefer live-food organisms, such as phytoplankton, brine shrimp nauplii, rotifer, cladocerans etc. The production of these live-food organisms on shrimp farm is generally nature-dependent and involves higher inputs and manpower, and thus live-food production process enhances the cost of prawn seed production. This situation calls for urgent need for the development of appropriate compounded artificial feeds for prawn larvae and post-larvae.

Thus feed is one of the essential operational inputs for the development of semi-intensive and intensive prawn culture techniques. It is also generally recognised that feed accounts for the largest single item of running expenditure in intensive prawn culture operations, sometimes involving as much as 50% of the cost of production of marketable size prawns. The quality, quantity and cost of feed are of paramount importance to the

success of intensive prawn culture operations. In order to formulate nutritionally adequate, least-cost feeds, information on the nutritional requirements of the different growth stages of prawn species is a pre-requisite.

During the past one and half decade numerous studies have been made to determine the nutritional needs of a variety of crustaceans, including prawns. These studies have brought to light a number of inherent problems in nutritional studies with crustaceans. According to Hanson and Goodwin (1977) prawn nutrition is a multivariate phenomenon of imposing dimension with eleven major variables interacting with one another. These variables are (1) stage of growth (2) species (3) water quality and temperature (4) feed stability (5) preservation (6) percentage and derivatives of lipids (7) percentage and amino acid composition of protein (8) percentage and derivatives of carbohydrate (9) health of the species (10) effect of feeds which occur naturally in rearing environment and (11) feeding rate. Thus basic knowledge of nutritional requirements of a cultivable species is very essential for development of practical diets.

In general, prawns require both energy nutrients and non-energy nutrients for proper survival and growth. Practical feeds should be formulated to contain all these nutrients in adequate levels and in right proportions, so as to attain maximum growth with optimum quantity of food, without much

wastage. Indiscriminate use of nutrients in diets will not only enhance the cost of feed formulations but may also be detrimental to animals due to pollution of the culture medium. Besides information on the nutrients requirement of prawn species, information is also required on the intrinsic and extrinsic factors which alter nutritional requirements, nutritional value of feed ingredients, attractability, palatability, and water stability of feeds, so as to develop least-cost, highly efficacious diets.

Information on prawn nutrition is relatively less as compared to those available on fish nutrition. Reviews on the subject of feeding and nutrition, digestion and metabolism, and vitamins in crustaceans, including certain cultivable species, were made by Marshall and Orr (1960), Vonk (1960) and Fisher (1960) respectively. Forster (1976) and Provasoli (1975) also reviewed the nutritional studies in crustaceans. Subsequently, New (1976) offered an excellent review of the literature available at the time on dietary studies with prawns and shrimps. This was followed by reviews by Kinne (1977), Biddle (1977), Conklin (1980b), Castell et al. (1981) and Dall and Moriarity (1983). Apart from these, the books published by Imai (1977), Hanson and Goodwin (1977) and Stickney (1979) treat some aspects of nutrition of crustaceans. Recently, New (1980) provided a bibliography of prawn and shrimp nutrition.

According to Dall and Moriarity (1983) crustaceans appear to have all the dietary nutrient requirements usually associated with higher vertebrates. Yet knowledge of nutritional requirements of prawn is still fragmentary and meager; and most efforts have been devoted to the development of compounded diets suitable for aquaculture. Except for the addition of few chemically pure substances such as vitamins and lipids, most of these diets were comprised of crude constituents (Dall and Moriarity, 1983).

Several studies have been carried out to understand the nutritional requirement of prawns (New,1976; 1980) with diets of different protein, lipid, carbohydrate, mineral and vitamin composition. While the results of these studies have considerably contributed to the knowledge of nutritional demand of these animals, there is wide differences in the observations of the various workers on the requirements of the optimum dietary levels of both major and minor nutrients, which provide optimum growth and highest survival rate in a given species. As reviewed by New (1976) and Forster (1976) there are many difficulties in adopting the results of various studies reported earlier, as these authors used various natural sources of nutrients for compounding the diets and these trials were mostly restricted to juveniles. The natural sources contain different levels of protein, lipids, vitamins, minerals and carbohydrates. Many authors have not also given the chemical composition of the diets they used. Animals of widely

differing initial lengths and weights, and genetical origin, have been used with variable stocking densities and duration of experimental trials. Besides, many authors have not supported their results with data on feed efficiency, protein efficiency ratio, tissue composition analysis etc. (New 1976).

New (1976) stressed the need to have basic nutritional studies on prawns and shrimps to achieve real progress. Dall and Moriarity (1983) also agrees with the views suggested by New (1976) that most of the efforts have been devoted to empirical development of diets suitable for aquaculture, but it is necessary to develop purified diets to understand the optimum dietary requirements, along with good and bad effects of excesses and deficiencies of nutrients on the animal concerned. Kanazawa et al. (1970) developed a purified diet with chemically pure ingredients, for the first time, to study the nutritional requirements of Penaeus japonicus. Subsequently Kanazawa et al. (1977b) modified the ingredients composition of the above diet and reported it as an effective purified diet for nutritional studies with prawns and other crustaceans.

More recently, Kanazawa (1985) reviewed the nutrition of penaeid prawns and shrimps and reported the essentiality of adequate levels of proteins, lipids, carbohydrates, vitamins and minerals by P. japonicus for proper growth and survival bringing out the deficiency diseases, when reared on diets lacking some of these nutrients. On the basis of

this knowledge compounded artificial diets are used practically for commercial production of P. japonicus, as substitute for traditional live-food in Japan.

The development of artificial diets for larval penaeids is one of the most important research areas in prawn culture (Kanazawa, 1985). Villegas and Kanazawa (1980) prepared microparticulate diets for larval penaeids, both as substitute for live-food and for nutritional studies. The nutritional requirements of prawn larvae were studied by using microparticulate diet as a substitute for live-food, such as diatoms and Artemia, from zoea I stage to post-larvae in seed production of P. japonicus (Villegas and Kanazawa, 1980; Kanazawa and Teshima, 1983; Kanazawa et al., 1985). In India, Mohamed et al. (1983) prepared a compounded microparticulate diet for larval rearing of P. indicus by using local ingredients and reported 12.5% survival in laboratory and 66% survival in out-door plastic pools.

Proteins are indispensable nutrients for growth and maintenance of animals and greatly influence the cost of feed. Therefore, determination of optimum dietary levels of proteins for various prawn species has been the subject of several studies (Table 1). Although protein level as investigated in these studies ranges from 15 to 60% in the diet, it is generally opined that a protein level of 27-35% is optimum requirement for the juvenile penaeids (New, 1976).

TABLE - 1 OPTIMUM PROTEIN LEVELS SUGGESTED FOR MAXIMUM GROWTH FOR VARIOUS PRAWN SPECIES

S.No.	Name of prawn	Recommended protein level in diet (%)	Test protein source	Author(s)
1.	Penaeus japonicus	50.00	Soyabean protein	Kanazawa et al. (1970)
2.	"	60.00	Squid meal, white fish meal, mysid meal, Sludge and yeast	Deshimaru and Shigueno (1972)
3.	"	40.00	Soyabean, fishmeal and Shrimp meal	Balazs et al. 1973)
4.	"	52.00 to 57.00	Casein and egg albumin	Deshimaru and Yone(1978c)
5.	Penaeus indicus	43.00	Prawn and Fish meals	Colvin (1976a)
6.	P. indicus, Post-larvae 25-42	30.00 to 50.00	Casein	Charles and Ahamed Ali (1984)
7.	Penaeus monodon	45.80	Casein and defatted Fish meal	Lee (1971)
8.	Penaeus merguiensis	43.00 to.55.00	Casein	AQUACOP (1978)
9.	Penaeus setiferus	28.00 to 32.00	Menhaden meal	Andrews et al. (1972)
10.	Penaeus duorarum	28.00 to 30.00	Soyabean meal	Sick and Andrews(1973)
11.	Penaeus aztecus	23.00 to 31.00	-	Shewbart et al. (1973)
12.	"	51.50	Soya flour	Zein-Eldin and Corliss (1976)
13.	"	40.00	Fish protein	Venkataramiah et al. (1975)
14.	Penaeus stylirostris	35.00	-	Colvin and Brand(1977)
15.	Penaeus californiensis	30.00 to 35.00	-	Colvin and Brand(1977)
16.	Metapenaeus monoceros	55.00	Casein	Kanazawa et al. (1981)
17.	Palemon serratus	30.00 to 40.00	Cod fish meal and shrimp meal	Forster and Beard (1973)
18.	Macrobrachium rosenbergii	35.00	Soyabean, tuna, shrimp	Balazs and Ross (1976)

However the dietary protein requirement reported for juvenile P. indicus is around 43% (Colvin, 1976). And the protein requirement of post-larval P. indicus (PL 1-10) is 40% and PL 11-25 and PL 25-42 is in the range of 30 to 50% in the diet (Charles and Ahamed Ali, 1984). Mohamed et al. (1983) used 36.8% protein in a compounded diet for larval P. indicus and found 66.5% survival in out door plastic pool experiment. The protein requirement of larval P. japonicus appears to be in the range of 45 to 55% or more when dietary carbohydrate level decreased from 25 to 5% (Teshima and Kanazawa, 1984).

The variations in protein requirement of prawn species have been attributed to the species type, physiological conditions, feeding habits, age and size of animals etc. Besides, the amino acids profile of the proteins used are also suspected to significantly influence the protein requirements. However the specific quantitative amino-acid requirements have not been fully established for any of the prawn species, so far. The essential dietary amino acids for shrimps are qualitatively similar to those for other animals. All studies of essentiality on amino acids have so far been made using radio labelled precursor technique because diets in which intact proteins were replaced by purified amino acids, and fed to prawns resulted in poor response (Deshimaru and Kuroki, 1974c; 1975 a and b). Based on the isotopic precursor technique; arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine were found to be essential

amino acids for the euryhaline prawn, Palaemon sp. (Cowey and Forster, 1971) the crab Cancer sp. (Lasser and Allen, 1976) the lobster Homarus sp. (Gallagher and Brown, 1975), marine shrimp Penaeus sp. (Shewbart et al., 1972; Kanazawa and Teshima, 1981) and freshwater prawn Macrobrachium sp. (Miyajima et al., 1976). The quantitative amino acid requirements are not known for any species of crustacean.

The nutritional importance of carbohydrate for prawn was studied by Tyagi and Prakash (1967), Cowey and Forster (1971), Forster and Gabbott (1971), Kitabayashi et al.(1971b), Andrews et al. (1972), Sick and Andrews (1973), Deshimaru and Yone (1978 b), Abdel-Rahman et al. (1979) Ahamad Ali (1982) and Pascual et al. (1983). Generally, glucose is found to be poorly assimilated than starch or glycogen (Forster and Gabbott, 1971). Inclusion of more than 10% glucose to diets generally retarded growth of prawn as in P. aztecus, (Andrews et al., 1972) P. duorarum (Sick and Andrews, 1973) and P. japonicus (Deshimaru and Yone, 1978b; Abdel-Rahman et al. 1979). Abdel-Rehman et al.(1979) have shown that P. japonicus juveniles had a better weight gain on diets containing disaccharides (sucrose & maltose) and polysaccharides (dextrin and starch) than on diets containing monosaccharides (glucose and galactose). Aquacop (1978) suggested that starch appears to be more suitable than glucose. Pascual et al. (1983) have demonstrated that sucrose and dextrin are better than other carbohydrates. The carhohydrate in the diet

is found to have protein sparing action up to 40% level (Andrews et al., 1972; Sick and Andrews, 1973; Ahamed Ali, 1982b). Ahamed Ali (1982b) studying the effect of carbohydrate in purified diets observed that the growth of P. indicus juveniles enhanced with the increase in dietary carbohydrate level up to 40%.

Minerals and trace metals are important structural components of organs, tissues and exoskeleton. Besides, they are also important for acid-base regulation and some of the mineral elements function as inorganic cofactors in enzyme catalysed reactions in the animal body. In spite of their functional role, very few studies exist on mineral requirement of prawns. Prawns and shrimp are suspected to absorb some minerals from the water, to some extent. But they may require a dietary source of some minerals for growth, because of repeated loss of certain minerals during molting. Deshimaru et al. (1978) and Deshimaru and Yone (1978a) have shown uptake of calcium in P. japonicus from sea water and stated that the prawn does not require calcium, magnesium and iron. But Kanazawa et al. (1984) are of the opinion that addition of calcium to diets may be necessary to maintain the ratio of calcium and phosphorus (1:1) in the diets. Kitabayashi et al. (1971a) have also pointed out the importance of the Ca/P ratio indicating an optimum ratio of 1:1 for P. japonicus. Hunter and Colvin (1977) have shown that Ca/P ratio of 2.2:1 to be optimum for growth of juvenile P. californiensis. The

necessity of phosphorus in the diet of *P. japonicus* has been reported by Kitabayashi *et al.* (1971a) Deshimaru *et al.* (1978) and Kanazawa *et al.* (1984). Deshimaru *et al.* (1978) have reported that *P. japonicus* require phosphorus (2.0%) potassium(1.0%) and trace metals (0.2%). Kanazawa *et al.* (1984) have shown that this species require calcium (1.0%) phosphorus (1.0%) magnesium(0.3%) Potassium (0.9%) and copper (0.6%) in dry diets.

Fisher (1960) reviewed the requirement of vitamins in crustaceans. In general, most of the B Group vitamins, vitamin C and E are found to be essential; whereas, vitamin A is not essential. Kitabayashi *et al.* (197c) found accelerated growth of *P. japonicus* with vitamin C in the diet. Several workers(Kanazawa *et al.*, 1976b; Guary *et al.*, 1976b; Deshimaru and Kuroki,1976;1979 have shown that *P. japonicus* juveniles require about 300 to 1000mg of ascorbic acid, 120 mg of choline, 200-400 mg of inositol, 6-12 mg of thiamine and 12 mg of pyridoxine per 100 g of diet, respectively. Black death in *P. stylirostris* has been caused by ascorbic acid deficiency (Magarelli *et al.*, 1979). A dietary intake of 0.1% was found to be sufficient to prevent nutrition related deaths among the shrimp (Lightner *et al.*, 1979). Recently, Kanazawa (1985) examined the requirement of larval *P. japonicus* for various vitamins using microparticulate diet, and reported that the prawn larvae require vitamin E nicotinic acid, choline, pyridoxine, biotin, folic acid ascorbic

acid, cyanocobalamine, vitamin D, inositol, riboflavin, thiamine, and β-carotene. The shortage of any one of these vitamins resulted in retardation of metamorphosis and high mortality during larval development.

Lipids, the water insoluble organic biomolecules have several important biological functions, such as, as a source of energy and essential fatty acids (EFA), carrier of fat soluble vitamins, emulsifier of lipids, transport of absorbed lipids, synthesis of steroid hormones and synthesis of prostaglandins. Lipids also serve as a reserve energy source which, can be used during starvation and moulting. The phospholipids are emulsifying agents and on combination with protein and carbohydrate they are more effective as emulsifying agents (West et al.,1974). Some phospholipids such as phosphotidylcholine also enhance the sterol solubilisation, when associated with N-(N dodecanosarcosyl) taurine (DST) in crustaceans and this helps in digestion and assimilation of food (Lester et al., 1975). The phospholipids also play important roles in the transport of fatty acids and other lipids (Teshima and Kanazawa, 1979); as being a component of the biomembranes in cellular and subcellular organelles provides the structural integrity and flexibility for selective ion transport (Lehninger 1984; Gilbert and O'Connor, 1970). Sterols have also predominent role as the precursor for the biosynthesis of vitamin D(New,1976) and hypodermis formation (Guary and Kanazawa, 1973).

Historically, lipids have always featured prominently in the Chemistry of aquatic species especially the marine animals. Marine lipids are characteristically different from the fats of terrestrial animals in that they are rich in w3 PUFA and fat soluble vitamins (Sargent, 1976). The presence of relatively high levels of lipids in aquatic organisms means lipid rather than carbohydrate is favoured as energy reserve in aquatic species including crustaceans (Sargent, 1976).

The hepatopancreas and ovaries are regarded as major lipid storage organs in Crustacea (O'Connor and Gilbert, 1969). Renaud (1949) implicated both glycogen and fatty acids in key roles during the moult cycle of Cancer magister. Changes in lipid content of hepatopancreas in prawn clearly demonstrate its similarity to other crustaceans. The use of lipid in moulting indicate that it is an economical source of energy in both metabolic and storage terms as well as aiding in bouyancy (Read, 1977). Lipids are also useful in providing metabolic water on oxidation along with energy; this water is useful in the maintenance of osmoregulation. Lipids also provide bouyancy to animals (Sargent, 1976). Data available have shown that crustaceans accumulate lipids in hepatopancreas at premoult period and the stored lipids are postulated to play an important role during the period late-premoult to ecdysis (Renaud, 1949; Patroles et al., 1978). The process of ecdysis consumed 25.6% of the overall energy gain during intermoult period. This is

a heavy price paid for growth in an animal with an exoskeleton for growth (Read and Caulton, 1980).

Recent studies on prawns (Read, 1977; 1981; New, 1976; Kanazawa, 1985) indicate that their growth, metamorphosis, maturation and moulting are influenced by the type and level of lipids used in their diet. The most important groups of lipids from the point of view of nutrition of prawns are 1) fatty acids (2) phospholipids and (3) steroids.

Despite the recognition of the importante of lipids in the diet of prawns, there is no detailed information on the qualitative and quantitative lipid requirements of P. indicus. Therefore, experimental studies were conducted to determine the dietary requirements for total lipids, phospholipids, two essential fatty acids and natural lipid sources by larvae, post-larvae and juveniles of one of the most suitable cultivated species of penaeids, the Indian white prawn, Penaeus indicus. The details of the work done are presented in the five chapters following the general material and methods section.

GENERAL MATERIALS AND METHODS

GENERAL MATERIALS AND METHODS

Experimental set up

All experiments were conducted at the wet laboratory attached to the Centre of Advanced Studies in Mariculture of the CMFRI, Cochin. All the experiments were conducted by using three replicates for each treatment. The experimental aquaria were arranged on steel racks. Glass beakers were used for larval rearing experiments with one litre of sea water of salinity ranging from 33 to 35‰ and pH 7.9 to 8.2, with arrangements to supply air vigorously. Besides supply of oxygen, aeration helped in dispersion and floating of the microparticulate diet in the water. A glass tube devise fitted in the beakers helped to keep the water in motion along with the food materials. A constant water temperature of 30 ± 1 °C was maintained by temperature control systems. Photo-period of 12 hours light and 12 hours dark was maintained with the help of electrical tubes in order to maintain the natural light conditions for all experiments.

Plastic tubs were chosen as experimental containers for post-larvae and juveniles since they do not have any harmful effect on animals so held (Berhhard and Battera, 1970). Round bottomed blue coloured plastic basins were used for post-larval experiments. Five litres of seawater of salinity 32‰ ± 2‰ was filled into each of the basins for post-larval 1 to 10

stage, and about 7 litres of seawater of salinity 20 ± 2‰ was used as the rearing medium for post-larvae 11-25. For the rearing experiments with juvenile prawns fifty litres capacity round bottomed plastic basins with about 40 litres of seawater of salinity 20 ± 2‰ were used. All the containers were provided with continuous air supply through aerators, plastic tubing and diffusor stones in order to supply oxygen.

Seawater was collected from the sea off-Cochin beyond 40 meters depth. It was transported to the laboratory in plastic jerry-cans,pooled into plastic pools and filtered with 60 micron mesh bolting silk cloth. The required water of various salinities were prepared by adding dechlorinated tap water to the seawater. The water was aerated well using aerators and used for the experiments. Salinity, dissolved oxygen, pH and ammonia levels were regularly monitored and data are presented in each of the chapters.

In the experiments with larvae and post-larvae 1 to 10 the rearing medium was changed daily with fresh sea water of the same salinity. The rearing medium of post-larvae 11-25, was changed completely every alternate day with fresh seawater of same salinity level. Metabolites were removed daily and the water was siphoned, filtered with 60 µ mesh bolting silk and used for one more day before effecting complete change every alternate day. Similarly, the rearing

medium of juveniles was changed every fourth day with fresh sea water of required salinity. Metabolites were removed daily and the water was siphoned, filtered in a biological filter facility and used for two subsequent days, and on the fourth day complete water was siphoned and changed with fresh seawater of same salinity.

Experimental animals

The larvae, post-larvae and juveniles of P. indicus were regularly obtained from the Prawn Hatchery of the Central Marine Fisheries Research Institute at Narakkal near Cochin. All the animals used for any one particular experiment were obtained from the same brood to avoid genetics based variations. Same procedure was followed for all the nutritional experiments.

Protozoeae-1 were used for each of the larval experiment which were reared up to the post-larval-1 stage. Post-larvae-1 (first post-larvae) were used for marine phase of post-larval nutritional studies and were reared up to Pl-10 stage (10 day old). Post-larvae-11 (11 days old post-larvae) were used for nutritional studies till they reached advanced stage (PL-25). This is a brackish water phase in the life-cycle of prawn P. indicus. Juvenile prawns of mean length around 25 mm to 30 mm were used for all the nutritional experiments on juvenile P. indicus.

The larvae, post-larvae and juveniles of *P. indicus* required for different nutritional experiments were transported in polyethylene seed transportation bags of 5 litres and 10 litres capacity. These bags were half-filled with seawater of required salinity. Experimental animals were stocked at the rate of 1000 larvae, 500 post-larvae 1, 250 post-larvae 10 and 50 juveniles of *P. indicus* per litre in the transportation bags. After introducing the animals, the transportation bags were filled with oxygen or compressed air and transported to the experimental laboratory of the Central Marine Fisheries Research Institute at Cochin within 2 hours.

In the laboratory the animals were introduced into large plastic tubs containing seawater of required salinity and acclimatized. Larvae were acclimatised only for 3 to 4 hours, post-larvae were acclimatized for one day and juveniles were acclimatized for a week or till they reached the desired size before the beginning of experiment.

Formulation and preparation of feeds:

All the diets, for each of the experiment, were formulated to contain same level of protein and approximately isocaloric gross energy levels. The diets for juvenile prawns were formulated to contain 37.5%, ± 1 protein in all the experiments. Considering the fact that the larvae may require relatively greater percentage of protein in the diet

a protein level of 47.5% ± 1% was maintained for the experiments with larvae. Energy content was maintained by adjusting the level of glucose, sucrose and cellulose, while varying the lipid levels in the diet. However the starch level was kept at 12% in all the diets as starch is also a binding agent. For most of the experiments carrageenan was used as the binder. Agar agar (5%) was used only for experiments on lipid and cholesterol requirements with juveniles.

Chemically purified ingredients were used for preparation of diets. The diets were formulated following the formulae provided by Kanazawa et al. (1970, and, 1977b), Read (1981) Deshimaru and Shigueno (1972) Deshimaru et al. (1979) and Kanazawa et al. (1982) with little modifications. The composition of the basal diets for both larval and juvenile prawns are given in the Table 2.

Purified feed ingredients were procured from authorised dealers of respective manufacturing companies (ICN Biochemicals, USA; Sigma USA, BDH, SRL, HIMEDIA, MERCK etc.) Codliver oil was purchased from Universal Genereric Pvt. Bombay (an associate of British codliver oil Ltd., HULL); purified fatty acids and lecithin (L α phosphatidyl choline) from Sigma USA; cholesterol from BDH England; fatty acids standard from Applied Science Laboratories, USA and Suppleco, Switzerland. Casein was selected as main source of protein in the diet as it is

the only protein available in highly purified form to avoid extraneous factors. Besides, it contains fairly good balance of essential amino acids required for prawns and it was also used in nutritional studies by several workers. Egg-albumin was included in casein basal diets, to improve the amino acids balance in the diets. Comparing the amino acids profile of casein with that of P. indicus muscle (Colvin, 1976a) it was found that the arginine content was slightly low in casein; where as the arginine content was quite high in prawn muscle. Therefore, arginine was supplemented at 1% level in all the diets. Kitabayashi et al. (1971 b) found that diet supplemented with 0.53% methionine and 0.83% of arginine gave better growth rate in the case of P. japonicus. So 0.5% of methionine was used in addition to arginine. Glutamic acid (1%) glycine (2%) and taurine (0.5%) were used as attractants. Free aminoacids play a role in the palatability of fresh diet; a tactile response is induced by glutamic acid (Takei and Ai, 1971).

Penaeid prawns are known to utilize polysaccharides very efficiently; so 12% starch was included along with 7.5% sucrose and small percentage (3.5%) of glucose as source of carbohydrate. Starch when geletinised also had a binding capacity. The diets were adjusted with glucose, sucrose and cellulose powder to maintain isocaloric levels. Glucosamine was added in the diets as it is known to promote growth in prawn (Kanazawa et al., 1970 and 1977b; Kittabayashi et al.,

1971a), as well as serves as the precussor for chitin synthesis. Vitamins mixture and mineral mixture were included in levels suggested by Kanazawa et al. (1970,1977b, 1982b) for Kuruma prawn P. japonicus. A mixture of soyabean oil, codliver oil and lecithin, and cholesterol were used as sources of lipids for most of the experiments. New (1976) suggested that a binder should be used in the diet of prawn to avoid loss of nutrients through leaching and to maintain texture in saline water. Accordingly, agar-agar or carrageenan was included in the diet as binder in the experimental diets.

Basal dietary formulae used for the experiments with larvae, post-larvae and juveniles are given in Table 2. In general, the basal ingredient composition remained unchanged for all the experiments, except the levels of lipids, glucose, sucrose and cellulose powder. The specific changes made in the ingredients as required for specific experiments, are given in material and methods section of respective chapters.

Diets were prepared following the method of Kanazawa et al. (1970, 1977 and 1982 b) with slight modifications. All the ingredients were powdered in a grinder and seived through 60 µ mesh. The ingredients for each of the diet were weighed on a Mettler Electronic Balance and kept separately Casein, egg albumin, sucrose, glucose, glucosamine, sodium citrate, sodium succinate, cholesterol, amino acid mixture, vitamin mixture were mixed thoroughly in a mortar and pestle.

TABLE - 2 BASAL INGREDIENT COMPOSITION (%) OF REFERENCE DIETS

INGREDIENTS	Diet for Larvae and Post-larvae 1-10	Diet for Post-larvae 11-25	Diet for juveniles
Casein	37.00	37.00	31.00
Egg Albumin	9.00	9.00	7.50
Amino Acids Mixture[1]	5.00	5.00	5.00
Glucosamine	0.80	0.80	0.80
Sodium citrate	0.30	0.30	0.30
Sodium succinate	0.30	0.30	0.30
Starch	12.00	12.00	12.00
Glucose	3.50	3.00	4.90
Sucrose	7.9	6.4	11.00
Cholesterol	0.5	0.5	0.5
Lipids[2]	10.00	12.00	12.00
Vitamin Mixture[3]	3.20	3.20	3.20
Mineral mixture[4]	8.50	8.50	8.50
Cellulose powder	2.00	2.00	3.00
Total	100.00	100.00	100.00
Carrageenan/Agar-agar	5.00	5.00	5.00
Distilled water	100-120 ml	100-120 ml	100-120 ml

1. Amino acids mixture (g/100 g diet) Arginine 1.00, Methionine 0.50, Glycine 2.00, Taurine 0.50, Glutamic acid 1.00
2. Lipid Mixture - Codliver oil: Soyabean Oil: Lecithin in the ratio of 56.00 : 28.00 : 16.00
3. Vitamin mixture (mg/100 g diet) Thiamine HCL (B_1) 4.9, Riboflavin (B_2) - 8.0, Para-Amino Benzoic acid - 10.90, Inositol - 400.00, Niacin 40.00, Calcium Pantothenate 60.00, Pyridoxine HCL 12.00, Menadione 4.00, β -Carotene 9.60, α Tocopherol (Vitamin E) - 20,000, Calciferol 1.20, Cynacobalamin (B_{12}) 0.08, Sodium Ascorbate - 2000.00, Folic acid 0.80, Choline chloride 600.00,
4. Mineral Mixture (g 100 g diet) - K_2HPO_4 - 2.00, $Ca(PO_4)_2$ - 2.72 $MgSO_4$ $7H_2O$ - 3.02, NaH_2PO_4. 2H20 - 0.790, $MnSO_4$. $5H_2O$ - 0.004, $FeSO_4.7H_2O$ - 0.015

Water soluble vitamins and minerals were dissolved in water separately. Required lipids were weighed separately and mixed with the diet. Required quantity of distilled water was taken in a beaker; boiled on a water bath and starch was added and gelatinized. To this carageenan/agar-agar was added along with cellulose and boiled till this mixture was fully cooked and mixed thoroughly. Then the mixture of dietary ingredients were added along with mineral mixture, vitamin mixture and mixed thoroughly in the beaker. The pH was adjusted to 6.8 - 7 by 10% NaOH. This mixed diet was steamed for 10 minutes, cooled and passed through a house hold pelletizer having 2 mm diameter aperture. The wet feed strands were freeze dried at -20°C or oven dried at 50°C. Those diets required for the larval experiments and for fatty acid requirements experiment of post-larvae and juveniles were freeze dried at -20°C and diets required for remaining experiments were dried in electrical oven at 50°C. Diets required for each experiment was prepared just 2 days before the beginning of the experiment and stored in polyethelene containers in freezer at -20°C. Pelleted diets for juveniles were dried to contain about 20 to 25% moisture and pellets for post-larvae 11-25 to contain 15 to 20% moisture. However moisture content was less than 15% in the diet for larvae and post-larvae 1-10.

Freeze dried diets were powdered and seived to obtain particle sizes 100 μ, 75 μ and 37 μ through the required

mesh seives. Preliminary experiments showed that larvae ingest and show better growth with particles less than 37 μ. Therefore, all the experiments on nutritional requirements on larval prawns were conducted by using the microparticulate diet particles of size less than 37 μ. Diets for post-larvae were dried, powdered in a grinder and seived to obtain particles of 300 μ and 1000 μ size. Particles of 300 μ size were used for post-larvae 1 to 10 stage; whereas, particles of 1000 μ size were used for post-larvae 11-25 stage. Pellets of 5-6 mm size were fed to juveniles.

Stocking of animals

The number of animals subjected to each treatments were 150 larvae at the protozoea-1 stage 60 post-larvae at post-larvae stage 1, 45 post-larvae at post-larvae-11 and 30 juveniles in the respective experiments. Thus each of the replicates had either 50 larvae or 20 post-larvae of Pl 1 stage, or 15 post-larvae of PL 11 stage or 10 juveniles of *P. indicus*.

The total length of animals (from tip of rostrum to tip of telson) was measured near to 0.5 mm. In order to determine the wet weight of animals excess water was removed using filter paper and thereafter weighed in a mettler electronic balance/chemical balance. For the dry weight determination 40 animals of same length and approximately same weight were sacrificed and kept in anoven at 60°C $\pm$ 2 for 12 hours and

the dry weight was recorded. Thus initial average dry weight of post-larvae and juveniles was determined for all the experiments.

Feeding rates and schedules

Using microparticulate diets villegas and Kanazawa (1980) and Kanazawa et al. (1982a) found better growth rate in larval P. japonicus at a feeding rate of 0.16 mg/larva/day, when compared to lower or higher rates. So 0.16 mg/larva / day was used in the present study for all the larval nutritional experiments. Food was distributed five times a day in equal doses at an interval of about 4-5 hours.

Feeding rate for post-larval prawn PL 1-10 was about 30 to 40% of the total body weight, which was distributed into three doses, 1/4 dose in the morning, 1/4 dose in the afternoon, and 1/2 dose in the evening.

Feeding rate for post-larval prawn, PL 11-25 was about 30 to 40% of the total body weight which was divided into two doses, 1/4 in the morning and 3/4 in evening at 17.30 hrs, for 4 days and thereafter food was offered only in the evening at 18 hrs. Juveniles were fed at the rate of about 20% of the biomass, twice a day. Initially, 1/4 dose in the morning and 3/4 dose in the evening were administered; but subsequently, after 4 days, food was offered only once a day in the evening at 18 hrs, as prawns are more active during evening and night.

Every day in the morning left-over food as well as fecal strands were separately collected by siphoning in the experiments on post-larval PL 11-25 and juvenile prawns. The left-over food and fecal matter were washed with fresh-water to remove adhering salts, dried and stored in aluminium foils. The dry weight of the left-over food and fecal matter was recorded. Dry weight of left-over food and fecal matter was considered for calculation of FCR, PER and for calculating the apparent digestibility.

Hydrological parameters:

Hydrological parameters such as salinity, dissolved oxygen, ammonia, pH and water temperature were monitored regularly. Food consumption and growth are shown to be markedly influenced by environmental parameters such as temperature, feeding rate, photoperiod (Bordner and Conklin, 1981). Long photoperiod caused reduced growth and food consumption of juvenile lobster (Bordner and Conklin, 1981); so the photoperiod was adjusted by keeping darkness from 18.00 hrs. to 06.00 hrs. and natural light was available during day time between 06.00 and 18.00 hrs.

Experimental duration:

Larval rearing experiments were conducted for periods ranging from 8 to 15 days, till larvae reached the post-larvae 1 stage. Survival rates at the various larval stages were recorded. Post-larval experiments on PL 1 to PL 10 stage were conducted for 10 days and on PL 11-25 stage were

conducted for 15 days. Juvenile experiments were conducted for a period of one month (30 days).

Collection of biological data

Survival of larvae, post-larvae and juveniles were recorded at the beginning and at the end of each experiment. Besides survival of larvae at various stages, survival during metamorphosis was also recorded. Mean survival in the three replicates was taken for statistical calculation for further finalisation of results.

Total length from the tip of rostrum to the tip of telson in mm was recorded. Wet weight of each prawn was taken by removing water adhered to the appendages and gills of prawns by blotting paper on a mettler electronic balance. Dry weight of prawn (post-larvae and juveniles) was recorded by drying all the prawns in an electrical oven at 50°C for 48 hours or by drying the prawns in a freeze dryer at -20°C for 48 hours.

The survival and dry weight gains of post-larvae 1-10 were considered as more important parameters than gain in wet weight and length because the animals are so small that the water adhered to the appendages will lead to some error in the results.

After taking final length, wet weight and dry weight the prawns were stored in plastic bags or in polylthelene containers and kept in desiccators for biochemical analysis.

Biochemical analysis

The biochemical composition of juveniles and post-larvae 11-25 were determined after the experimental trials. Chemical analysis of prawns, diets and fecal matter was done by using standard methods as reviewed by Giese (1967) and AOAC (1965). Protein, lipid, carbohydrate, ash and cholesterol contents were expressed in percentage dry weight basis. Dry weight was determined by drying the prawns in electrical oven at 50°C for 24 to 48 hours to get constant dry weight. Along with dry weight, moisture content of prawns was also determined. The prawns from fatty acid requirement experiments and experiments on nutritional evaluation of natural lipid sources were freeze dried at -20°C.

The protein content of prawns and diet was determined by Lowry's Method (Lowry *et al.*, 1951) carbohydrate by phenol sulfuric acid method (Dubois *et al.*, 1956) lipids by Bligh and Dyer (1959), cholesterol by Hestrin (1949) and ash content by AOAC (1975).

Analysis of data

Final length, wet weight and dry weight of individual experimental animals were recorded and mean survival, gain in length, wet weight and dry weight for each replicate was calculated. Similarly percentage of moisture, protein, lipid carbohydrate, ash and cholesterol contents of each replicate groups of prawns was calculated and recorded. Data on food

conversion ratios and protein efficiency ratios were determined for post-larvae and juveniles and apparent digestibility of food was determined only in the case of juvenile experiments on nutritive value of natural lipid sources.

Food conversion ratio was calculated by estimating food consumed per unit time divided by growth per unit time.

$$\text{Food conversion ratio} = \frac{F}{(W_2 - W_1) + D} = \frac{\text{Weight of Food consumed}}{\text{Live weight gain of prawns}}$$

F = Total amount of food consumed in g

W1 = Mean initial weight in g

W2 = Mean final weight in g

D = Weight of dead animal in g.

Protein efficiency ratios (PER) were computed as

$$\text{PER} = \frac{\text{Live weight gain}}{\text{Protein intake}}$$

Digestibility coefficient was calculated only for the experiment on nutritive value of various natural sources of lipids for juveniles.

$$\text{Digestibility coefficient} = \frac{In - Fn}{In} \times 100$$

In = Food intake (food consumed)

Fn = Weight of dry fecal strands

Average of three replicates were calculated for finalization of results and for drawing the figure. Data obtained

were subjected to statistical analysis. Analysis of variance (ANOVA) was done on the means of each parameter to find out if the dietary treatments hold any significant influence on the observed parameters. When significant influence was observed the data were processed to find out if the differences observed between the treatments were significant or not by least significant difference test with the help of a Hewlett Packard Master computer.

CHAPTER - I

TOTAL LIPID REQUIREMENTS

I N T R O D U C T I O N

A recent review on nutrition of shrimps and prawns (Kanazawa, 1985) indicates that lipids are indispensable nutrients for growth and survival of these animals. However, information regarding optimal lipid requirement of prawns is limited, though most researchers included lipids in their dietary formulations for prawns. Several authors (Lee, 1970; Shudo et al., 1971; Forster, 1972; Andrews et al., 1972; Sick and Andrews, 1973; Sick et al., 1973; Zein Eldin and Meyers, 1973; Guary et al., 1976a; Sandifer and Joseph, 1976; D'Abramo et al., 1980; Ponat and Adelung, 1980) have used lipids, derived from plant products, animal products and mixture of plant and animal products, in the diets of crustaceans according to availability in local areas. The level of lipid used in their diets also varied according to their convenience, without considering the dietary requirement of the animal concerned. Deshimaru and Shigueno (1972) included 8.8% crude fat in their best diet for P. japonicus. Shudo et al. (1971) reported that the addition of 4% squid liver oil in the diet improved the growth of P. japonicus. Forster and Beard (1973) and Andrews et al. (1972) reported inhibition of growth in the prawns, Palaemon serratus and Penaeus setiferus at lipid levels of 15% and 10% supplementation, respectively. Sick and Andrews (1973) found that 10% lipid promotes better growth in the prawn P. duorarum than a lipid free diet. Deshimaru and

Kurcki (1974a) found that a diet containing 6% lipid promoted better growth in P. japonicus than a lipid free-diet or a diet with 12% lipid. While reviewing the nutritional requirements, Forster (1976) reported that penaeid prawns do not require high levels of dietary lipids and suggested that optimum level may fall between 5 and 10% in the various species.

The most comprehensive studies on lipid requirements of prawns have been those of Kanazawa et al. (1970, 1977b) and Deshimaru et al. (1979) who have investigated the quantitative lipid requirements of the Kuruma prawn, Penaeus japonicus. Kanazawa et al. (1970) reported better growth of P. japonicus, when fed a purified diet with 8% soyabean oil as the lipid source. Subsequently, Kanazawa et al.(1977b) conducted experiments to determine the optimum dietary lipid level by using graded lipid levels in the diets for P. japonicus, and obtained maximum growth with 12% powdered pollack residual oil. Similarly, 7% codliver oil was found to give better growth in P. merguiensis (Aquacop, 1978). Guary et al. (1976a) used 4% sardine oil or 4% clam oil in purified diets and reported better growth in P. japonicus than with 4% peanut oil.

The optimum lipid level required in the diet of P. indicus has not been studied so far. Earlier studies on lipid nutrition of P. indicus are those of Colvin (1976b) who studied the effect of selected seed oils on growth and fatty acid composition of juvenile P. indicus and Read (1981) who studied the response of juvenile P. indicus to various plant and animal oils. Colvin (1976b) 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 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 3% mixture of fish oil and sunflower oil in the ratio of 2:1 gives better survival and growth in juvenile P. indicus. Few authors from India prepared diets for various stages of P. indicus using lipids as a component in their diets of P. indicus. Ahamed Ali (1982) used 6% lipid in the diet of juveniles; Charles and Ahamed Ali (1984) used 10-12% lipid in the diet of post-larval prawn. Mohamed et al. (1983) used 10.1% lipid in the diet of larval P. indicus and reported good growth. Despite all these preceeding reports, so far, no information exists on the effects of graded levels of lipids on any stage of P. indicus. So the present investigation was carried out to find out the quantitative requirement of lipids in the diet of larvae, post-larvae and juveniles of P. indicus.

MATERIALS AND METHODS

Four sets of experiments were conducted in the laboratory to study the level of lipid required for optimum growth, survival, better utilization of food and protein, and for maximum protein deposition in the larvae, post-larvae and juveniles. In general, the experimental design, aquaria used, seawater, salinity, experimental animals, stocking density, method of formulations and preparation of diets, details about feeding

and rearing techniques, duration of experiments, collection of data on survival, growth, FCR, PER proximate composition and method of analysis of data are same as described in the general materials and methods section (pp 15-29). The basal ingredient composition of the diets is same as given in Table 2, for all the stages of P. indicus.

Eight diets for larvae and post-larvae and seven diets for juveniles were prepared containing isonitrogenous and isocaloric levels. The lipid levels ranged from 0 to 14% in larval and post-larval experimental diets and from 0 to 18% in juvenile diets. The levels of glucose, sucrose and cellulose powder were adjusted to maintain approximately isocaloric levels in each of the diets (Table 3 and 4). Phytoplankton was used as a control diet for larval experiment and a reference diet NPCL 017, a compounded diet from CMFRI Cochin was used as a control for post-larval and juvenile experiments.

Dietary lipid source used in all the experimental diets comprised of codliver oil, soyabean oil and lecithin in the ratio of 56:28:16. Earlier observations (Deshimaru and Kuroki, 1974a, Deshimaru et al.,1979) indicate that a mixture of marine and plant lipids produce maximum growth in juvenile P. japonicus and lecithin appears to be essential for larval and juvenile P. japonicus (Kanazawa, 1985). So a mixture of codliver oil, soyabean oil and lecithin was used as source of lipid in the diets to determine the quantitative dietary lipid requirements for larvae, post-larvae and juvenile prawns.

TABLE - 3 INGREDIENT COMPOSITION (%) OF DIETS USED IN THE EXPERIMENTS TO DETERMINE THE LIPID REQUIREMENT OF LARVAE, POST-LARVAE 1-10 AND POST-LARVAE 11-25.

Ingredients*	Experimental diets							
	1	2	3	4	5	6	7	8
Codliver oil	0.0	1.12	2.24	3.36	4.48	5.60	6.72	7.84
Soyabean oil	0.0	0.56	1.12	1.68	2.24	2.80	3.36	3.92
Lecithin (phospholipid)	0.0	0.32	0.64	0.96	1.28	1.60	1.92	2.24
Glucose	6.5	5.50	5.50	4.50	3.50	2.50	2.00	1.50
Sucrose	13.9	12.90	10.90	9.00	7.50	6.50	5.00	3.5
Cellulose powder	1.6	3.0	3.00	3.90	4.40	4.40	4.40	4.40

* The percentages of casein, egg albumin, aminoacid mixture, glucosamine, sodium-citrate, sodium succinate, starch, cholesterol, mineral mixture, vitamin mixture, carrageenan/ Agar-agar used in these diets are same as given in Table 2.

TABLE - 4 INGREDIENT COMPOSITION (%) OF DIETS USED IN THE EXPERIMENT TO DETERMINE THE LIPID REQUIREMENT OF JUVENILES

Ingredients *	Experimental diets						
	1	2	3	4	5	6	7
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 (Phospholipid)	0.0	0.48	0.96	1.44	1.92	2.40	3.03
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

* The percentages of casein, egg albumin, aminoacid mixture, glucosamine, sodium citrate, sodium succinate, starch, cholesterol, mineral mixture, vitamin mixture, Agar-agar used in these diets is same as given in the Table No.2.

TABLE - 5 ENVIRONMENTAL FACTORS, STOCKING DENSITY PER TREATMENT, MEAN INITIAL LENGTH AND WEIGHTS OF ANIMALS, AND FEEDING LEVEL FOR THE EXPERIMENT ON LIPID REQUIREMENT

Parameters	Stages of the prawn			
	Larvae	Post-larvae 1-10	Post-larvae 11-25	Juveniles
Salinity (‰)	34.0 ± 2	32.0 ± 2	20.0 ± 2	20.0 ± 2
Temperature (°C)	29-31	26-30	26.4-29.2	27.0-30.5
pH	8.0-8.3	8.00-8.2	7.9-8.3	7.8-8.2
Dissolved oxygen in water (mg/l)	4.2-6.2	4.2-6.3	3.9-6.2	3.8-6.0
Total Ammonia -N in seawater (ppm)	0.02-0.06	0.04-0.09	0.04-0.09	0.05-0.11
Initial number of prawn/diet	150	60	45	30
Average initial length (mm)	-	5.05	9.5	29.00 to 31.0
Average initial wet weight (mg)	-	0.239	2.92	136 to 142
Average initial dry weight (mg)	-	0.067	0.806	29.70
Feeding level % of biomass	100	30-40	30-40	20-30

Water quality parameters such as salinity, temperature, dissolved oxygen content, pH and total ammonia concentration were monitored regularly, and found that mean levels of these environmental parameters were more or less similar among all the treatments in each of the experiments (Table 5). Initial stocking, mean length, wet weights and dry weights of the experimental animals are given in Table 5. The differences in initial mean lengths as well as in weights of prawns found among the treatments of each experiment were statistically insignificant.

R E S U L T S

LARVAE

Feeding trials were conducted in larvae to examine the nutritive value of purified diets with or without lipids, and the results were compared with those obtained with the control groups fed live-food (phytoplankton). The results are shown in Table 6A and 6B. All the larvae died at the protozoea-1 stage within two days without metamorphosis, when no food was supplied (Table 6A Treatment 10) indicating that larvae having exhausted the yolk nutrients reserve in nauplius stage, required an exogenous source of nutrients through food. In the control group (Treatment 9) where live phytoplankton culture was fed the development of larvae followed a normal sequence showing the highest survival rate. In this treatment

36.67% of the protozoeae reached the post-larval 1 stage, within 8 days of the experiment. Of the remaining eight treat treatments only one (Diet-6), in which 10% lipid was included in the diet, produced 20.67% post-larvae 1 after 10 days. The growth and survival rates of larvae obtained in this treatment were significantly ($P < 0.05$) lower than those of the control group receiving live-food (phytoplankton). Exclusion of lipid from the diet (Diet 1) resulted in total mortality of larvae at protozoea-II stage itself on the 3rd day of the experiment, thus proving the essentiality of lipid as a component in the diet. In treatments 2 and 3, in which lipid level of 2% and 4% were included in the diets, none of the larvae reached the post-larval 1 stage. Total mortality of larvae occurred at mysis stage on 5th day in treatment 2 (2% lipid) and on 8th day in treatment 3 (4% lipid). In all other treatments (4 to 8), few larvae reached the post-larval stage (Table 6A) and there were no significant differences between them in the survival rate, with the exception of diet 6 (10% lipid) which produced significantly higher survival rates than diet 1 to 8 containing lipid level from zero to 14%.

The trends in larval mortality at different larval stages can be seen from Table 6B. With the exception of the control group fed with phytoplankton, larval mortality was more during protozoeal stages than during mysis stages in almost all the treatments. Mortality of larvae was

TABLE - 6A GROWTH AND SURVIVAL OF P. INDICUS LARVAE FED ON DIETS CONTAINING GRADED LEVELS OF LIPID.

Diet No.	Lipid Level (%)	Survival rates (%) at various developmental stages of prawn larvae							Feeding Period Days
		P1	P2	P3	M1	M2	M3	PL1	
1	0	100	69.34	-	-	-	-	-	2
2	2	100	60.00	26.67	11.33	4.67	-	-	6
3	4	100	40.00	12.00	6.00	4.00	4.00	-	8
4	6	100	60.00	31.34	24.67	20.00	6.67	3.34	12
5	8	100	78.00	46.67	36.00	26.67	24.67	14.00	10
6	10	100	77.34	46.00	36.67	28.67	26.00	21.67	10
7	12	100	61.34	48.00	35.34	24.67	20.67	13.34	10
8	14	100	62.00	46.00	33.34	27.34	21.34	9.34	10
9	Phyto-plankton	100	89.34	80.00	54.67	44.67	38.67	36.67	8
10	No food	100	0.00	0.00	-	-	-	-	-

P1, P2, P3 = Protozoeal stages of larvae

M1, M2, M3 = Mysis stages of larvae

PL1 = Post-larvae 1 stage

TABLE - 6B SURVIVAL RATE (%) OF LARVAE AT VARIOUS DEVELOPMENTAL STAGES DURING METAMORPHOSIS

Diet No.	Lipid level (%)	Survival rate (%) of larvae at various developmental stages				
		P1	From P1 to P3	From P3 to M1	From M1 to M3	From M3 to P11
1	0.0	100	-	-	-	-
2	2.0	100	26.67	42.50		
3	4.0	100	12.33	50.00	66.67	
4	6.0	100	31.33	78.72	27.02	90.00
5	8.0	100	46.67	77.14	68.51	56.75
6	10.0	100	46.00	79.90	70.90	79.48
7	12.0	100	48.00	73.60	58.49	64.51
8	14.0	100	46.00	72.46	64.00	43.75
9	Phytoplankton	100	80.00	68.34	70.73	94.82
10	No food	100	-	-	-	-

relatively less in all the treatments from protozoea-III(P-III) to mysis-1 (M-1) stage and was found to be less than 30% in treatments 4 to 8 and the control. Larval mortality was less than 40% in all the treatments from 3 to 9 during mysis 1 to mysis III stages, except for treatment 4 containing 6% lipid. Larval mortality decreased during metamorphosis from mysis III to post-larva 1 stage, being less than 50% in all the treatments from 4 to 7 and the control. In general, mortality was more during the protozoeal stages (P1 to PIII stage), compared to that recorded during the metamorphosis from P III to PL I stage. In treatments 1, 2 and 3, where the larvae were fed on diets containing less than 6% lipid, complete mortality of larvae occurred before reaching the post-larval stage 1.

Thus, the minimal dietary lipid level required for normal growth and metamorphosis of larval P. indicus is about 10% in the diet under the present experimental conditions.

POST-LARVAE 1-10

The results of the feeding experiment conducted to determine the total lipid requirements of post-larvae 1-10 are plotted in Fig. 1. Analysis of variance of the data showed that the survival and growth rates of post-larvae are significantly ($P<0.05$) influenced by the dietary lipid levels.

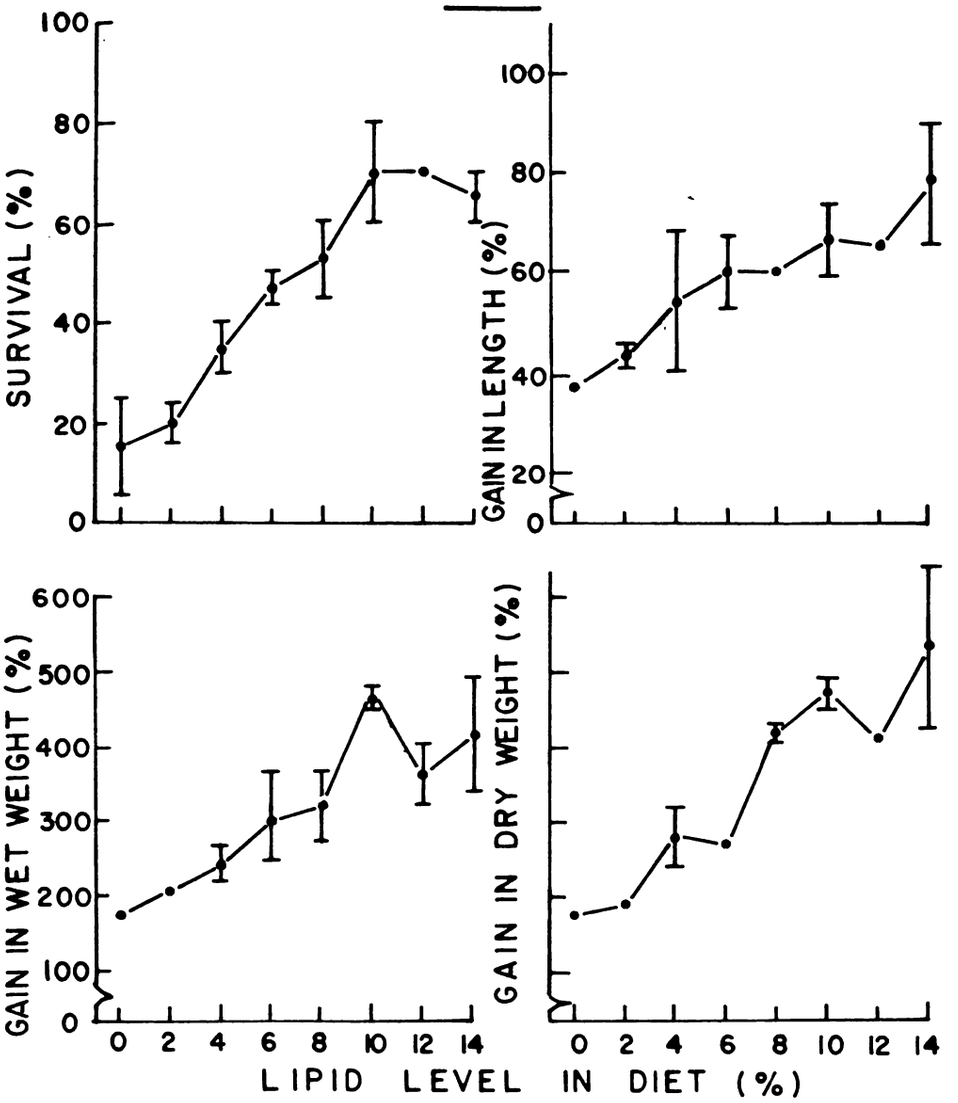
The survival rate (Fig. 1) of post-larvae ranged from 15 to 70% in the various dietary treatments. It was

significantly low (15%) in post-larval groups fed the lipid free diet (Diet-1). Diets 5 to 8, in which dietary lipid level formed 8 to 14%, produced higher survival rates than diets 1 to 3, in which dietary lipid level was less than 8%. Although there was no statistically significant differences in survival between diets with 8, 10, 12 and 14% lipid, the observed value (Fig. 1) clearly indicate relatively higher survival (70%) in the groups fed diets containing 10 and 12% lipid.

Growth (the mean percent gain in length, wet weight and dry weight) of post-larvae (Fig.1) was significantly ($P<0.05$) influenced by the dietary lipid levels. Deletion of lipid from the diet (Diet-1) resulted in significantly ($P<0.05$) lower growth rates of post-larvae. Growth of post-larvae fed on diets with lipid ranging from 10 to 14% was found to be significantly ($P<0.05$) higher than that recorded with diets containing 4% or less of lipid. Besides, the mean percent gain in dry weight produced by the diets containing 10 to 14% lipid was significantly ($P<0.05$) higher than that produced by diets containing 6% to 8% lipid. There was also no significant difference in the wet weight and dry weight of post-larvae between diets containing 10, 12 or 14% lipid. These results indicate that 10% lipid in the diet is effective for promoting growth in post-larvae 1-10. By increasing the lipid level to 12 or 14% in the diet no significant improvement in growth was attained.

Fig. 1 Survival rate and growth of post-larvae 1-10 fed on diets containing graded levels of lipids

FIG. 1.


SURVIVAL (%)
GAIN IN LENGTH (%)
GAIN IN WET WEIGHT (%)
GAIN IN DRY WEIGHT (%)
LIPID LEVEL IN DIET (%)

It can be observed from Fig. 1, the growth of post-larvae increased with the dietary lipid level and was found to be optimum when dietary lipid level was 10%. Although the gains in length and dry weight were higher in the post-larvae fed the diet with 14% lipid than the diet with 10% lipid, the observed differences were statistically insignificant.

POST-LARVAE 11-25

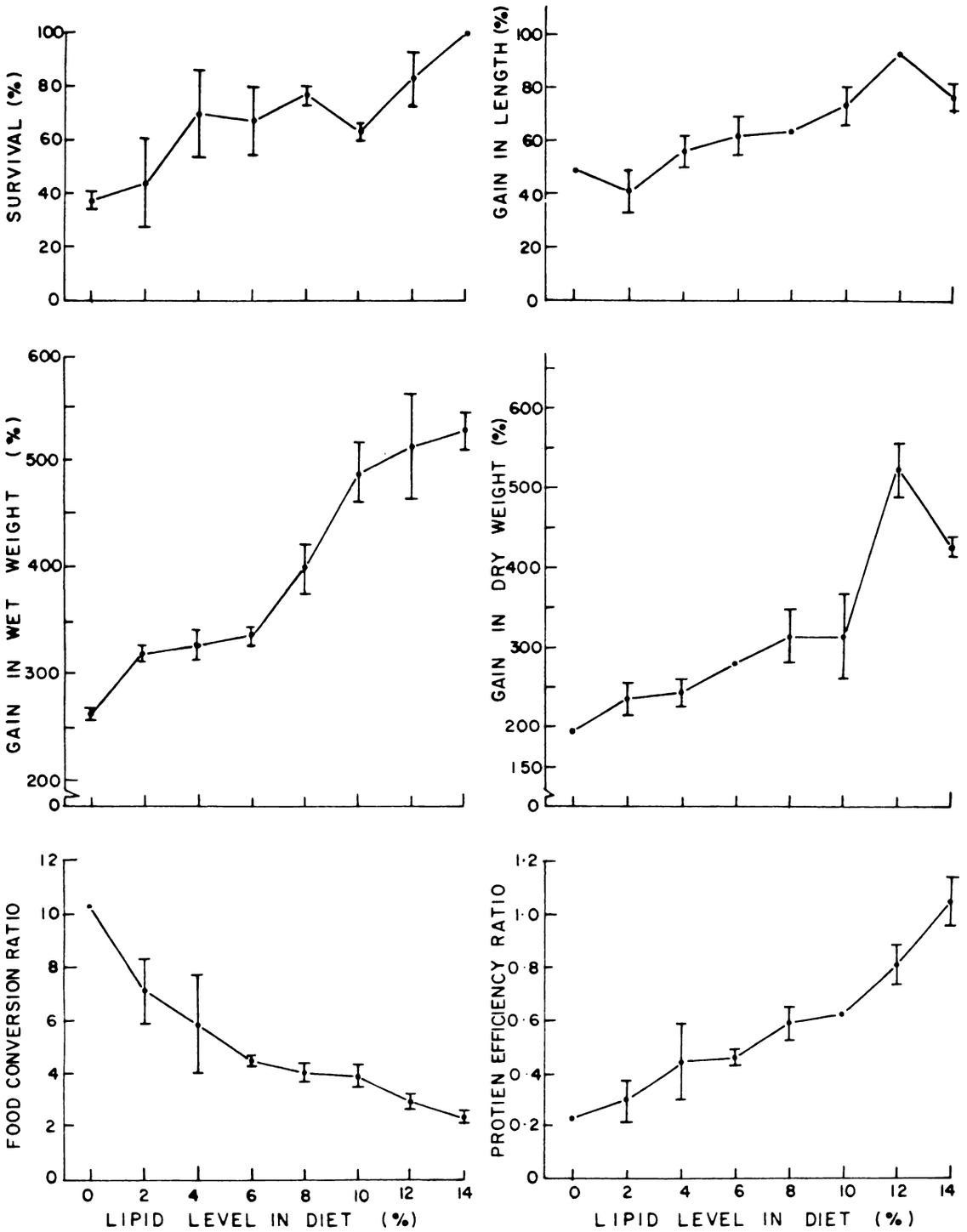
The results of the feeding experiments conducted in post-larval P. indicus with 8 diets containing various levels of lipids, viz. 0.0, 2, 4, 6, 8, 10, 12 and 14% are shown in Table 7 and Fig. 2. The survival rates of post-larvae recorded from the various treatments ranged from 36 to 100% (Fig.2), and were significantly influenced by the dietary lipid level. The lipid-free diet produced significantly ($P<0.05$) lower survival (30%) than other diets. The survival rate significantly improved on addition of 4% lipid in the diets. However, addition of 2% lipid did not significantly improve survival under the feeding regimes. Diet 8 containing 14% lipid produced the highest (100%) survival rate, which was followed by Diet 7(12% lipid) which produced 83% survival. These results indicate that post-larvae 11-25 of P. indicus require a dietary level of 12 to 14% lipid in the diet for optimum survival.

The growth data for post-larval prawns expressed as percentages of mean gains in length, wet weight and dry weight are shown in Fig.2. The growth of post-larvae was also significantly ($P<0.05$) influenced by the dietary lipid levels. The lipid-free diet (Diet 1) produced the lowest percent gain in length and wet weight. The significantly ($P<0.05$) low growth of post-larvae fed on the diet without lipid indicates the essentiality of dietary lipid for post-larvae 11-25. Incorporation of 2% lipid in the diet significantly ($P<0.05$) improved growth. Growth of post-larvae fed on diets (Diet 7 and 8) containing lipid levels of 12% and 14% was significantly ($P<0.05$) higher than those fed on the diets containing 10% or less percent of lipid.

Significantly the highest gain in length, wet weight and dry weight were observed in the post-larvae fed on the 12% lipid diet. Incorporation of 14% lipid in the diet did not significantly improve growth over that of 12% lipid. In fact, the dry weight gain was significantly lower in post-larvae fed on 14% lipid diet than with 12% lipid. The mean percent wet weight gain was significantly ($P<0.05$) higher in the post-larvae fed on diets containing 10, 12 and 14% lipid than those fed diets containing less than 10% lipid (Diets 1 to 5). Although slight differences were observed in the growth of post-larvae among diets 1, 2, 3 and 4, the differences were not significant. In general, the growth of the post-larvae increased steadily with the dietary lipid level from 2% to 12%.

Fig. 2 Survival rate, growth, FCR and PER of post-larvae 11-25 fed on diets containing graded levels of lipids

FIG. 2


SURVIVAL (%)
GAIN IN LENGTH (%)
GAIN IN WET WEIGHT (%)
GAIN IN DRY WEIGHT (%)
FOOD CONVERSION RATIO
PROTIEN EFFICIENCY RATIO
LIPID LEVEL IN DIET (%)
LIPID LEVEL IN DIET (%)

Food conversion ratios (FCR) and protein efficiency ratios (PER) for various diets are shown in Fig. 2. The FCR and PER were significantly ($P<0.05$) affected by the dietary level of lipid. Deletion of lipid from the diet (Diet-1) significantly affected the utilization of food and protein. Incorporation of lipids in the diets improved the utilisation of food and protein by the post-larvae. There was a steady decrease in the FCR and increase in the PER with increasing dietary levels of lipid. The FCR and PER were found to be significantly ($P<0.05$) better for diets containing 12% (Diet 7) and 14% (Diet 8) lipid than all other diets. There was no significant difference in FCR and PER between diets 7 and 8, with 12% and 14% lipid.

The influence of dietary levels of lipid upon the moisture, protein, lipid, carbohydrate and ash contents of the post-larvae is shown in Table 7. The dietary lipid level had significant ($P<0.05$) effect on the composition of post-larvae. Post-larvae fed the lipid-free diet (Diet 1) and those fed diet with 2% lipid had relatively lower protein and lipid contents, but higher moisture, ash and carbohydrate contents than those fed other diets. While the protein and lipid contents were relatively higher, the carbohydrate and ash contents were relatively lower in the post-larvae fed diets 7 and 8 containing 12 and 14% lipid respectively than that of post-larvae from other treatments. The protein and lipid contents of post-larvae increased with the dietary level

TABLE - 7 EFFECTS OF DIETARY LIPID LEVELS ON THE BIOCHEMICAL COMPOSITION OF THE POST-LARVAE 11-25.

Diet No.	Lipid Level in the diet (%)	Moisture (%)	Percentage on dry weight basis			
			Protein	Lipid	Carbo-hydrate	Ash
1	0.00	77.52	60.50	8.90	3.86	19.85
		±0.60	±0.50	±0.70	±0.06	±0.35
2	2.00	79.72	63.25	9.48	2.95	19.80
		±1.06	±0.25	±0.25	±0.65	±0.10
3	4.00	78.29	65.80	10.20	3.02	18.85
		±1.65	±0.30	±0.50	±0.32	±0.35
4	6.00	77.41	67.00	10.65	2.75	17.61
		±0.61	±0.10	±0.55	±0.05	±0.59
5	8.00	77.06	66.85	11.53	2.37	16.65
		±0.63	±0.65	±0.51	±0.23	±0.15
6	10.00	75.74	68.03	11.90	2.67	16.23
		±0.74	±0.03	±0.50	±0.07	±0.26
7	12.00	76.34	69.80	12.85	1.67	15.50
		±0.90	±0.21	±0.35	±0.32	±1.30
8	14.00	77.14	68.75	12.14	1.49	15.65
		±0.29	±0.25	±0.64	±0.03	±1.15

of lipid, except for treatment 5; where as the ash and carbohydrate levels decreased as the dietary lipid level increased, with minor variations. The post-larvae fed the diet containing 12% lipid had the highest protein and lipid contents.

JUVENILES

The results of the feeding experiments conducted in juvenile P. indicus with diets containing graded levels of lipid are shown in Fig. 3 and 4.

The survival rate of juvenile prawns ranged from 43 to 85% in the various treatments (Fig. 3) and it was significantly ($P<0.05$) influenced by the dietary lipid level. The survival was significantly ($P<0.05$) low in groups of juveniles fed the lipid-free diet (Diet 1). Addition of 3% lipid significantly ($P<0.05$) improved the survival (70%). Diets containing lipid levels of 9, 12, 15 and 18% produced relatively high survival rates (70% to 85%). The survival rate of prawns was not significantly improved by inclusion of lipid levels greater than 9% in the diets.

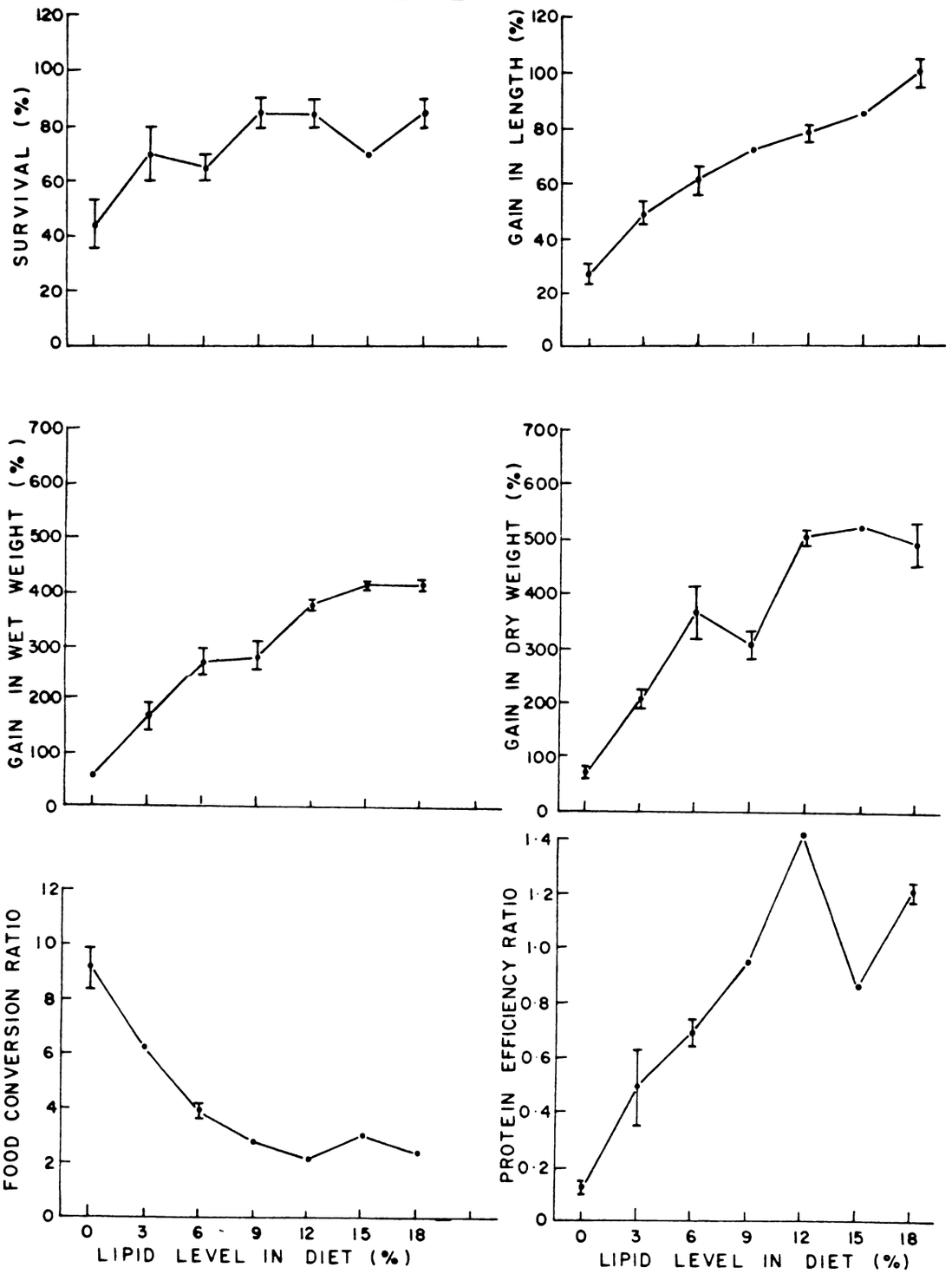
The data for growth of juvenile prawns expressed as percentages of the mean gains in length, wet weight and dry weight are shown in Fig. 3. The growth of juvenile prawns was also significantly ($P<0.05$) influenced by the dietary lipid levels. It is evident from the Fig. 3 that the lipid-free diet (Diet 1) produced significantly ($P<0.05$) the lowest growth rate and

that the inclusion of lipid in the diets (Diets 2 to 7) significantly promoted growth in juvenile prawns. Growth of prawns steadily increased with the dietary level of lipid from 3 to 18%, with the exception of percent dry weight gain which showed a peak at 15% lipid. The growth of prawns on a diet containing 12% lipid was significantly higher than the prawns fed a diet with lower levels of lipids. Although diets with 15% and 18% lipid levels produced greater growth rates than the diet with 12% lipid, the increase in growth was not statistically significant.

Food conversion ratios and protein efficiency ratios obtained for various diets are shown in Fig. 3. Analysis of variance of the data showed that the dietary lipid level significantly ($P < 0.05$) influence the FCR and PER. The diet 5 containing 12% lipid provided the best FCR (2.164) and PER (1.42). The PER recorded for diet 5 containing 12% lipid was significantly greater ($P \leq 0.05$) than all other diets, with the exception of diet 7 containing 18% lipid. There was also no significant difference in the PER between diets 5 and 7 Deletion of lipid from the diet of prawn (Diet 1) resulted in significantly high FCR and low PER indicating the poor utilization of food and protein. Inclusion of increasing levels of lipids in diets 2, 3 and 4 significantly improved FCR. However inclusion of lipids at levels of 15 or 18% did not significantly improve the FCR over that recorded at 12% lipid level. The PER increased with the dietary concentration of

Fig. 3 Survival rate, growth, FCR and PER of juvenile prawns fed on diets containing graded levels of lipids.

FIG. 3

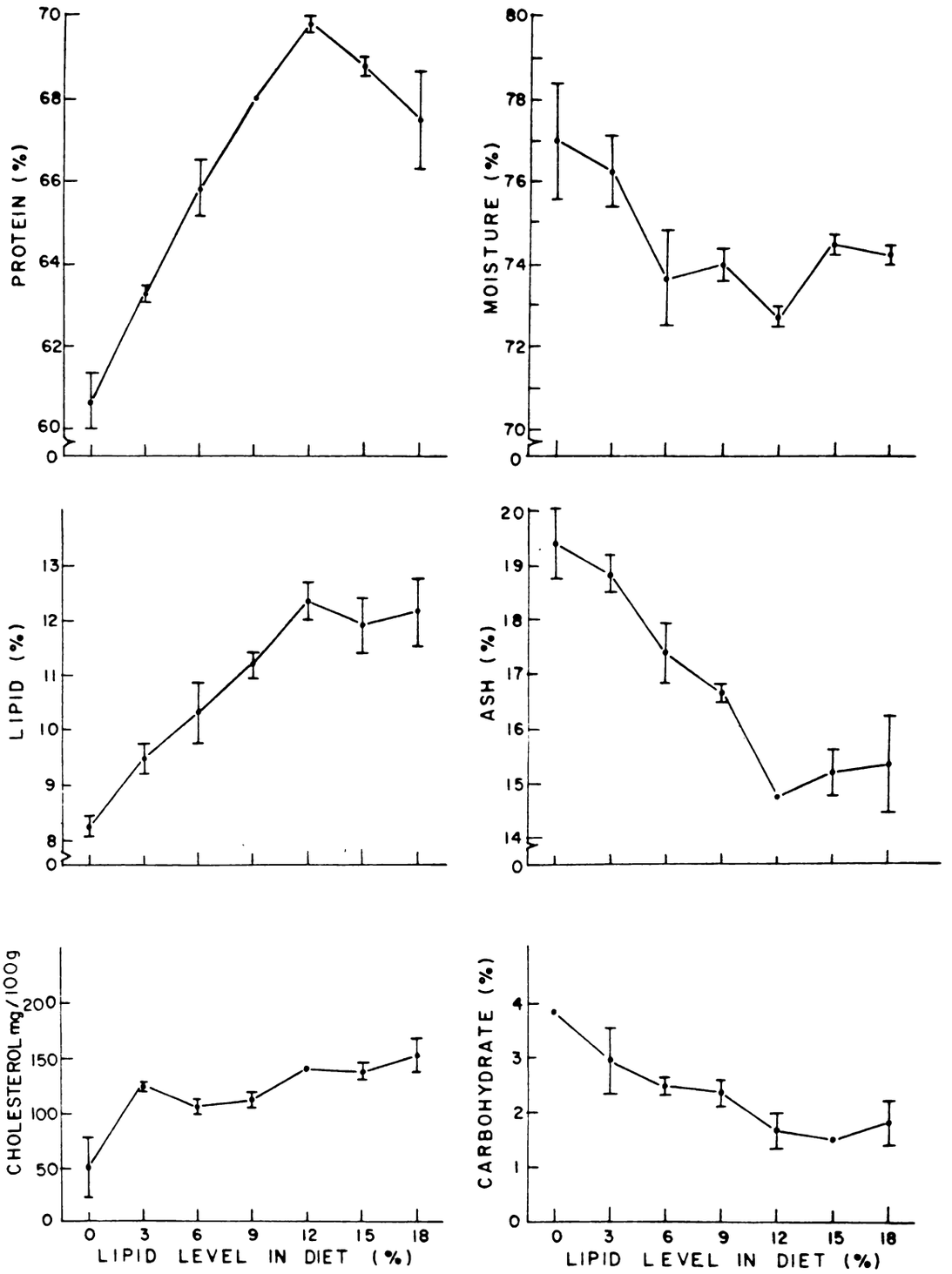

SURVIVAL (%)
GAIN IN LENGTH (%)
GAIN IN WET WEIGHT (%)
GAIN IN DRY WEIGHT (%)
FOOD CONVERSION RATIO
PROTEIN EFFICIENCY RATIO
LIPID LEVEL IN DIET (%)
LIPID LEVEL IN DIET (%)

lipids up to 12% lipids in the diet. But inclusion of lipids at 15 and 18% resulted in low PERs when compared to 12% lipid diet.

The influence of dietary lipid level upon the moisture, protein, lipid cholesterol, carbohydrate and ash content in the body of prawns is shown in Fig. 4. The chemical composition of prawn was also significantly influenced by the dietary lipid level. The protein, lipid and cholesterol contents were significantly ($P < 0.05$) lower, but the moisture, carbohydrate and ash contents were significantly ($P < 0.05$) higher in the prawns fed the lipid free-diet (Diet 1) than those prawns fed on the diets containing various levels of lipids. While the protein, lipid and cholesterol contents in prawns increased, the moisture, carbohydrate and ash contents decreased, when the dietary lipid was increased from zero to 12%. However, there was slight increase in the moisture and ash levels in prawns fed the 15% lipid diet, in which the protein and lipid contents were relatively low. Inclusion of 18% lipid in the diet did not significantly alter the composition of experimental prawns from that of 15% lipid diet fed prawns. These results indicate that dietary lipid level above 12% has no significant effect on the chemical composition of experimental prawns. The prawn's fed on diets containing more than 9% lipid had significantly higher protein and lipid contents but significantly lower ash contents than those fed on diets containing less than 6% lipid.

Fig. 4 Biochemical composition of juvenile prawns fed on diets containing graded levels of lipids.

FIG. 4


PROTEIN (%)
70
68
66
64
62
60
0
MOISTURE (%)
80
78
76
74
72
70
0
LIPID (%)
13
12
11
10
9
8
0
ASH (%)
20
19
18
17
16
15
14
0
CHOLESTEROL mg/100g
200
150
100
50
0
0 3 6 9 12 15 18
LIPID LEVEL IN DIET (%)
CARBOHYDRATE (%)
4
3
2
1
0
0 3 6 9 12 15 18
LIPID LEVEL IN DIET (%)

To clarify the actual dietary lipid requirement of juvenile prawns one more experiment was conducted by using two diets containing 10% and 12% lipid levels and it was found that the growth, protein deposition and, food and protein utilisation were significantly improved by feeding the 12% lipid diet, when compared to 10% lipid diets (Fig. 12, 13, 14, 15). These results thus confirm the observations made in the first experiment, that 12% lipid is optimum for juvenile prawns, when a mixture of plant and animal lipid sources are used in the diet.

Thus these results indicate that juvenile prawns require lipid as an essential nutrient for proper survival, growth promotion and for better utilization of food and protein. These results also indicate that juvenile prawns require a dietary lipid level of about 12% for proper growth and utilization of the food and protein. Further, it is also clear that there is no beneficial effect by inclusion of lipids at 15 or 18% levels in the diets, though juvenile P. indicus could utilize lipid levels as high as 15% without significantly affecting growth and feed efficiency.

D I S C U S S I O N

The present study clearly indicates the essentiality of lipid for proper survival, growth, conversion of food and protein, and retention of protein and lipid in the body of

Penaeus indicus. Deficiency of lipid in diets induced heavy mortalities in larvae and post-larvae, besides severely affecting growth and metamorphosis. Sub-optimal levels of lipid in diets also affected the survival and growth of larvae and post-larvae. The highest growth as well as survival in groups of larvae and post-larvae fed diets containing 10% lipid suggest that this may be the optimum for these stages of *P. indicus*. Studies with *P. japonicus* also revealed the essentiality of lipid for proper survival and growth (Kanazawa *et al.*, 1970; Kanazawa, 1985). Villegas and Kanazawa (1980) reported good survival (34.2%) of larval *P. japonicus* on a diet containing 8% lipid. Mohamed *et al.*, (1983) reported a survival of 12.5% for larval *P. indicus* when fed a compounded diet containing 10.1% lipids in the laboratory experiments, but the same diet gave survival as high as 66.67% in out door plastic pools. These observations suggest that to some extent prawn larvae accept artificial diets.

Mortality trends in larvae indicate that protozoeae are unable to ingest and utilize the feed effectively, probably due to the non-availability of preferred dietary particle size. However, the decrease in mortality during the mysis stage suggests that the feed particles were quite adequate for the mysis stages as well as up to their metamorphosis to post-larva 1 stage. Despite this, the effects of the dietary lipid was clearly evident in the various

dietary treatments. The complete mortality of larvae in groups which were fed on the diet containing less than 6% lipid indicate that larval P. indicus require a minimum of 6% lipid in the diet for maintenance, and a dietary lipid level of 10% is required for normal metamorphosis and growth. It is also evident from the study that there is no beneficial effect if lipid level is increased to more than 10% in the diets. The relatively good survival and growth rate of larvae in the control groups (larvae fed with phytoplankton) indicate that the environmental conditions (Table 5) were normal in the aquaria.

Although survival and growth of post-larvae 1-10, 11-25 and juveniles were very poor when fed on the lipid free diet, survival and growth were significantly improved by inclusion of lipids in the diets at a level of about 6%. Probably this may be the minimum level required for these stages for maintenance. However for optimum performance normal growth, efficient conversion of food and protein, and for protein synthesis, a dietary level of about 8-10% for post-larvae 1-10 and 9 to 12% for post-larvae 11-25 and juveniles are required. Besides, it is clear that inclusion of more than 12% lipid in the diet has no beneficial effect though the post-larvae and juveniles could tolerate dietary lipid levels as high as 14 to 18% without any deleterious effect on growth, but without any corresponding improvement in performance. Despite these variations the proximate composition of post-larvae 11-25 and juvenile prawns were

not significantly altered by diets containing more than 8% lipid.

A reference dietary treatment was also kept along with the test dietary treatments, with similar environmental parameters. The reference diet used in this experiment was procured from NPCL of CMFRI Cochin. The growth and survival of larvae, post-larvae and juveniles fed on the reference diet was normal and were relatively better than most of the other dietary treatments, used in respective experiments, thus showing that the quality of water (Table 5) was quite normal, for the survival and growth of animals. This diet is used regularly and found successful for normal growth and survival of prawn at the prawn culture Laboratory of CMFRI, Cochin. (CMFRI News Letter Number 29 & 30 July, December 1985). The ingredients used in this diet are - squilla meal, prawn meal, fish meal, ground nut cake and tapioca powder. This diet contained a lipid mixture of plant and animal origin. But all the ingredients are of natural origin supplying various levels of protein, carbohydrates, vitamins and minerals. The better growth observed in prawns fed this diet is a combined effect of natural ingredients and does not reflect the effect of a single nutrient such as lipids, so the results of the present study are not compared with this reference diet.

Lipid plays important role in the energy production processes of crustacean tissues and as a source of essential fatty acids, sterols, phospholipids and as carrier of fat soluble vitamins (Teshima and Kanazawa, 1980 a and b). The phospholipids play an important role in the transport of fatty acids and other lipids, and also as a component of the biomembranes in the cellular and subcellular organelles, provide the structural integrity to these membranes and flexibility for ion transport (Lenhinger, 1984; Teshima and Kanazawa, 1980a). Thus the essentiality of the lipids in the diet can be well ascribed to the diverse kinds of functions these biomolecules perform in prawns.

Moulting is an indispensable and very important phenomena in Crustacea. The involvement of lipid during moulting has been well established (Forster, 1976; Read, 1977), P. indicus is not an exception (Read, 1977). Most of the studies (Read, 1977; Renaud, 1949) indicate the profound changes taking place in lipid, both quantitatively and qualitatively, during moulting. Crustaceans accumulate large quantity of lipid in the hepatopancreas from intermoult to premoult stage, (O' Connor and Gilbert, 1969; Teshima et al., 1977) and the stored lipids are utilised for energy during late pre-moult and ecdysis (Renaud, 1949; Patrols et al.,1978). The process of ecdysis require 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 (lipids) and clearly demonstrate the high price paid for growth by the animal (Read and Caulton, 1980). Thus lipid is found to be very essential for growth and survival by all stages of prawn since its involvement in ecdysis as the primary energy supplier and its deficiency seems to induce severe mortality.

The steady increase in growth of prawn with the increase in dietary level of lipid from 2 to 14% in post-larvae and from 3 to 18% in juvenile prawns can be ascribed to the protein sparing action of dietary lipids. The increased level of lipid in the diet might have provided the large quantum of energy required for metabolic activities of the animal, besides reducing the cost of energy towards 'Specific Dynamic Action', while more and more protein had been spared for growth. This is also clearly evident from the better food and protein conversion values, when dietary level of lipid was increased. Diets with lower levels of lipid produced poor growth as well as poor utilization of food and protein in the prawns, because the animals might be deriving the metabolic energy partly from protein.

It is thus clear from the study that lipid at adequate levels can significantly spare protein for growth. Similar effect of dietary lipid in sparing protein has been reported for fish by Watanabe (1982). According to him addition of lipids with essential fatty acids as an energy source to a diet helps in the effective utilization of dietary protein in

fish. The main protein sparing effect of dietary lipids is to replace protein which could otherwise have been catabolised and used for energy production. The sparing of dietary protein by lipid has also been established for various species of fish (Lee and Putnam, 1973; Page and Andrews, 1973; Takeuchi et al., 1978 a,b,c; Shimano et al., 1980; Bromley, 1980). By using various levels of lipid from 5 to 25% at a constant level of protein (35%) Takeuchi et al. (1978c) have observed that with an increase in amount of dietary lipids, both the value of protein efficiency ratio and net protein utilization increased giving maximum protein retention and best weight gain in fish when fed a diet containing 18% lipid. Takeda et al. (1975) demonstrated that the protein requirement in yellow tail diet was reduced from 77% to 55% without retardation in growth when high level of pollack liver oil was used in the diet.

The efficacy of dietary lipids in promoting growth depends mainly upon its composition. Besides the essential fatty acids, adequate levels of phospholipids, cholesterol and antioxidants should be available in the lipid source for effective utilization of diets. This has clearly been demonstrated for crustaceans. According to Kanazawa (1985) the type and content of essential fatty acids dominate the nutritive value of lipids. However other lipid components, such as phospholipid and sterol are equally important (Kanazawa, 1985). Presence of antioxidants like α-tocopherol in the diet prevent the oxidation and thus found to be important for maintenance of

quality of PUFA in the diet (Watanabe, 1982). Thus better growth obtained in P. indicus may be because of the use of codliver oil, soyabean oil and lecithin at an adequate level (9 to 12%) which could supply all necessary fatty acids and phospholipids. Besides, the diets also contained 0.02% ∝-tocopherol and 0.5% cholesterol in addition to the total lipids.

Reports on quantitative lipid requirements of larvae and post-larvae, by using graded levels of lipid in the diets, are not available in literature. But few reports are available on quantitative lipid requirement of juvenile prawns by using graded levels of lipid in the experimental diets (Kanawaza et al., 1977 b; and Deshimaru et al.,1979). For the first time Kanazawa et al. (1970) reported very good growth in P. japonicus when fed with a purified diet containing lipid level of 8%. Kanazawa et al. (1977b) reported poor growth with the lipid free diet, and the maximum growth when dietary lipid level was 12% powdered pollack residual oil but weight gain was reduced when lipid level was 16% indicating 12% lipid is optimum level which agrees with the present observation on P. indicus. However, with the same species Deshimaru et al. (1979) conducted an experiment by using mixture of pollack liver oil and soyabean oil in the ratio of 1:1 and 3:1 and reported highest growth and feed efficiency at 6% lipid level in the diet, which contained 20 to 30% w6 and 10 to 17% w3 fatty acids. When Kanazawa

et al. (1977b) conducted a experiment by using diet containing mixture of powdered pollack residual oil and soyabean oil in the ratio of 6:4 at 10% level reported equivalent growth to that of the prawn fed on a diet containing 12% powdered pollack residual oil.

Thus for the same species (P. japonicus) 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. The protein and cholesterol contents of the diets used by Kanazawa et al. (1977b) were 50% and 0.5% respectively. Where as, Deshimaru et al. (1979) used 60% protein and 2% cholesterol in their diets. These observations further suggest the protein sparing effect of dietary lipids. 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 constant (37.5%). Besides, P. indicus being a omnivore, requires relatively lower level of protein (about 37% - Gopal, personal communication) and perhaps lipid is utilized as a more efficient energy source by P. indicus, thus sparing protein for growth. In other words, protein utilization in prawn appears to be improved by the inclusion of proper lipid levels in the diet. Protein utilization was found to be better when enough amount of fat and carbohydrate were provided in the diet of Macrobrachium rosenbergii (Clifford and Brick

(1978). Apparently P. indicus post-larvae and juveniles require 10 to 12% lipid for proper utilisation of protein as FCR and PER were also found to be better with diets containing 10 to 12% lipid.

One more important reason may be the dietary lipid source of P. indicus consisted of 10% liquid lipid containing codliver oil and soyabean oil in the ratio of 2:1 and 2% phospholipid (lecithin). Deshimaru et al. (1979) also reported better growth in P. japonicus with pollack liver oil and soyabean oil when used in the ratio of 2:1. But they did not include phospholipid (lecithin) in the diet of P. japonicus. Phospholipids are essential for the solubilization of cholesterol as well as play important role in the transport of lipids. The inclusion of 2% phospholipid in the diet seems to have significantly influenced utilization of lipid, and protein in P. indicus. P. japonicus being a predominantly carnivorous species may have more requirement for protein as compared to the omnivorous, P. indicus which may require more energy and less protein in the diet. However, Forster (1976) suggested that prawns are not nutritionally homogenous group; therefore considerable interspecific differences in dietary requirements may occur.

The quantitative dietary lipid requirement of prawns is also dependent on the quality of lipid used in the diet as lipids vary in their composition, especially in the fatty acids content (Table 31). According to Colvin (1976b) P. indicus have specific nutritional requirement for PUFA of w3 and w6 series fatty acids.

Studies carried out on fatty acid requirements during the present investigation and presented in Chapter III also confirms the above observation. Thus it is clear that even in the presence of PUFA of w3 and w6 fatty acids in the lipids, P. indicus require optimal dietary lipid levels (9 to 12%) in the diet for optimum performance.

Growth of animals depends upon the proper utilisation 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 indicating inefficient utilization of food and protein by the prawns. Inclusion of lipid in the diet significantly improved the FCR and PER upto 12% lipid and above this level lipid had no beneficial effect on the food and protein utilization of prawns.

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 tissues of post-larvae 11-25 and juveniles fed the lipid-free diet and higher level of protein in those fed the diet with 12% lipid. Besides, a steady increase in protein content of prawns was evident as the lipid level in the diet increased. Although lipid

deposition increased with the level of dietary lipid, the differences in lipid deposition in various groups of prawn when fed above 6% lipid in the diet were not significant. Very few reports are available on influence of dietary lipid level on the chemical composition of crustaceans. Sick and Andrews (1973) observed increase in lipid content (7.2%, 7.28% and 8.58%) of prawn P. aztecus on feeding diets containing 10% of lipids namely, beef-tallow, corn oil and linseed oil, when compared to that of lipid-free diet. Colvin (1976b) also observed only 71% protein and 3.94% lipid in the pre-experimental prawn P. indicus, which increased to 72.3% protein and 5.06% lipid respectively, on feeding diet containing 9.8% lipid.

CHAPTER - II

PHOSPHOLIPID (LECITHIN) REQUIREMENTS

INTRODUCTION

Broadly lipids can be grouped into neutral and polar lipids. While the neutral group includes hydrocarbons, cholesteryl esters, triglycerides, cholesterol and free fatty acids, the polar lipids are primarily composed of the phospholipids. The two fractions have entirely different functions. The neutral lipid usually serves as an energy reserve and consequently varies widely in content. Whereas the polar lipid has a transport and structural function and is more stable (O' Connor and Gilbert, 1968). Each of these groups contain fatty acids, but of various chain lengths and degrees of saturation. Phospholipids tend to be more unsaturated than neutral lipids due to their high content of polyunsaturated fatty acids. The number and positions of the double bond in the hydrocarbon chain have importance in both physical and nutritional characteristics.

In view of their importance in the transport of lipids and as structural component of biomembranes, many studies have been carried out on the phospholipid content and its composition in crustaceans. Gopakumar and Nair (1975) reported that phospholipid constitutes 62% of the total lipids in Penaeus indicus. Subsequent studies by Read (1977) also showed that phospholipids formed 60% of the total lipids in the same species. Several other reports have also shown that phospholipids are the major lipids of crustaceans, such as the lobster Homarus

americanus (Bligh and Scott, 1966) the crab Carcinus maenas and the prawn P. japonicus (Teshima and Kanazawa, 1978 a)

Variations in the fatty acids profile has also been observed between neutral and polar lipids. While the neutral lipid fatty acids pattern, to a large extent, conforms to that of dietary lipids, the phospholipid fatty acids mirror the biosynthetic pathway operating in animals (Ackman, 1967). Sargent (1976) who reviewed the phospholipids of marine organisms is of the opinion that the gross composition of biomembranes in terms of their major phospholipid classes will be the same in all life forms. The majority of biomembranes conform to the same basic structure, whether this be regarded as the classical tripartite structure of lipid sandwiched between two layers of protein, or the more modern idea of "protein floating in the sea of lipid" (Sargent, 1976).

The presence of lipoprotein has been reported in several crustaceans, such as the blue crab, Callinectes sapidus (Lee and Puppione, 1978), the lobster, H. americanus (Barlow and Ridgway, 1969) and the crab C. maenas (Ceccaldi and Martin, 1969). In most of the crustaceans phospholipids seem to be present as lipoproteins in the serum and play important function in lipid transport (Teshima and Kanazawa, 1980a). Teshima and Kanazawa (1980a) who studied the lipid component of lipoprotein from P. japonicus serum reported that the lipo-

protein of prawn serum contained an abundance of phospholipids forming 69-87% of lipids. The properties of the prawn serum lipoproteins obviously differ from those of human serum lipoprotein (Hatch and Lees, 1968). The protein and lipid ratio of lipoprotein of prawns is approximately 1:1 with the lipid composed of 75% phosphotidylcholine and 10% phosphatidylethanolamine (Teshima and Kanazawa, 1980a). Studies by several investigators revealed that phospholipids, particularly phosphatidylcholine and phosphatidylethanolamine, to be the principal circulating lipids in crustacean hemolymph (Gilbert and O' Connor, 1970; Allen 1972; Lee and Puppione, 1978). In common with other life forms the major phospholipids in crustaceans are phosphatidylcholine and phosphatidylethanolamine, which are important from nutritional point of view (Sargent, 1976).

Phosphatidylcholine (lecithin) is an important nutrient for crustacean growth and metabolism. Van Den Oord et al. (1964) and Teshima and Kanazawa (1978a and b) suggested that crustacean phospholipids probably play important role in emulsification, absorption and interorgan transport of lipids. Lester et al. (1975) observed that lecithin enhanced cholesterol solubilization when associated with N-(N-dodecanosarcosyl) taurine (DST) a model of the type of detergents synthesized by crustaceans. Kanazawa et al. (1979e) found that inclusion of lecithin from the short-necked clam at 1% level in the purified diet of Penaeus japonicus had a growth promoting

effect. Conklin et al. (1980) found that the inclusion of soy lecithin into purified diets fed to juvenile lobsters eliminated mortality associated with a "moult death syndrome." D'Abramo et al. (1981a) showed that the active ingredient of soy lecithin was phosphatidylcholine and suggested that the lecithin molecule was associated with lipoprotein that efficiently transported cholesterol from hepatopancreas to the various parts of the body through hemolymph. The relation between dietary phosphatidylcholine and serum cholesterol uptake and transport in tissues of the lobster Homarus sp. was investigated by D'Abramo et al. (1982). The absence of soya phosphatidylcholine in the purified diet fed to juvenile lobster caused a significant decrease in the concentration of total cholesterol and phospholipids in the serum (D'Abramo et al., 1982).

Dietary phospholipids other than soya lecithin also reduced the levels of cholesterol and phospholipids significantly, thus inducing moult-death syndrome (D'Abramo et al., 1981a). These observations revealed that the survival of juvenile lobsters is dependent upon the quantity and quality of phosphatidylcholine containing ingredients. Sources of phosphatidylcholine with PUFA were more effective at lower levels, suggesting that effective cholesterol transport also depends upon constituent fatty acids of the phosphatidylcholine molecules (D'Abramo et al., 1981a).

Several studies have also shown that phospholipids especially lecithin, when included in the diets, promoted growth in crustaceans (Kanazawa et al., 1979c; Conklin, 1980, a & b; Conklin et al., 1980). Kanazawa et al., (1979c) indicated that the addition of 1% Tapes phospholipid, especially, lecithin fraction to the diet with 7% Pollock liver oil resulted in increased weight gain in prawn. Since the fatty acid fractions had no such growth promoting effect, Kanazawa et al. (1979c) suggested that the high nutritive value of Tapes lipids is not only due to the high content of w3 highly unsaturated fatty acids (w3 HUFA), but due to certain effects of phospholipid molecules themselves. Lecithin fraction of Tapes lipid had the highest growth promoting effect among the phospholipids tested. In juvenile lobster addition of soya lecithin fraction to purified diets, prevented mortalities (Conklin et al., 1980a) and the optimum level of soy lecithin in the diet of lobster was approximately 8%. A purified diet containing soy lecithin fed to juvenile lobsters (Homarus americanus) produced excellent survival (Conklin et al., 1980c).

A preliminary study was conducted by Boghen and Castell (1980) to compare different diets with and without lecithin and the results of this study clearly indicated that all the diets with the exception of Conklin's lecithin supplemented diet (Conklin et al., 1980a) were unsatisfactory. Thus there seems to be distinct advantage in incorporating lecithin in the artificial diets for crustaceans. In a

subsequent study, Tridel and Castell (1980) showed that survival of juvenile lobsters increased with increasing lecithin level in a casein based diet up to 4-6%, after which it remained constant upto 10% level. Thus it was proved that crude soya lecithin has a factor necessary for good survival and growth of juvenile lobster (Tridel and Castell, 1980).

Soyabean phospholipids have also been reported to be essential for good growth and survival of P. japonicus larvae and 3% soyabean lecithin, along with 6% pollock liver oil as lipid source in artificial diet appears to be optimum level (Teshima et al., 1983; Kanazawa, 1985). The effects of phospholipids on growth, and survival of larvae of the prawn P. japonicus, were examined by Kanazawa et al., (1985) by using purified diets containing various levels of various phospholipids. P. japonicus larvae did not metamorphose to post-larvae, and died in 7 days when fed the diets containing no phospholipid (Teshima et al., 1982b), Growth and survival rate of prawn larvae were improved by adding soyabean phosphatidylcholine (PC) to the diets. These results suggested that P. japonicus larvae require dietary sources of phospholipid for growth and survival (Kanazawa et al., 1985). The efficacy of phospholipids in improving growth and survival varied with kinds and sources of phospholipids. Among the phospholipids tested, soyabean phosphatidylcholine, soyabean phosphatidylinositol (PI) and Bonito-egg phosphatidylcholine had high

efficacy as compared with other phospholipids (Kanazawa *et al.*, 1985).

The optimum level of soyabean phosphatidylcholine for *P. japonicus* larvae varied with the kinds of coexistent dietary lipids (Kanazawa *et al.*, 1985). The best growth and survival were attained on diets containing 6.0% soyabean phosphatidylcholine when 6% 18:1 w9 and 1% HUFA were used as basal lipids. But the inclusion of 3.5% soyabean phosphatidylcholine was enough to attain optimum growth and survival when 8% pollack liver oil was used as the lipid source.

As mentioned above the inclusion of some phospholipids is probably indispensable for growth and survival of prawn larvae and lobster juveniles. However, it is not known why such crustaceans as *P. japonicus* and *H. americanus* require dietary sources of phospholipids. Kanazawa *et al.* (1985) assumes that the prawn larvae may have a limited ability for phospholipid biosynthesis from fatty acids and/or diglycerides.

Thus the foregoing literature review reveals the importance of phospholipids especially phosphatidylcholine in the diet of crustaceans. However no information is available on the phospholipid requirement of *Penaeus indicus* till date. Considering the importance of phospholipids in moulting, survival, and growth or prawns, experiments were conducted in the laboratory to determine the effects of

selected levels of phospholipids (lecithin) on the larvae, post-larvae and juveniles of *P. indicus*.

MATERIALS AND METHODS

Among the various phospholipids tested by Kanazawa *et al.* (1985) soyabean lecithin (phosphatidylcholine) turned out to be the best for survival and growth of larval *P. japonicus*. Therefore, I have selected soyabean phosphatidylcholine (lecithin) as the phospholipid source to understand the dietary phospholipid requirement by the larvae, post-larvae and juveniles of the prawn *P. indicus*. The basal lipid source used was a mixture of codliver oil and soyabean oil in the ratio 2:1. The basal lipid level maintained in the diets were 10% for larvae and post-larvae 1-10, and 12% for post-larvae 11-25 and juveniles. Lecithin (phosphatidylcholine) was obtained from Sigma Chemicals, U.S.A.

Five sets of laboratory experiments were conducted to determine the essentiality and dietary phospholipid requirements of the larvae, post-larvae and juveniles of *P. indicus*. The composition of the basal diet for larvae, post-larvae and for juveniles is same as in Table 2. Minor changes have been made in the composition of the basal diet. The level of amino acids mixture was decreased from 5% to 4% by removing 1% of glutamic acid. Cholesterol level, was increased from 0.5 to 1% in the diet as lecithin promotes the utilization

TABLE - 8 COMPOSITION OF LIPIDS (%) IN THE DIETS FOR LARVAE, POST-LARVAE AND JUVENILE PRAWNS FOR LECITHIN REQUIREMENT EXPERIMENT

Ingredients	1	2	3	4	5	6	7	8	9
EXPERIMENT I - Experimental diets for larval prawn									
Lecithin	0.00	1.00	2.00	3.00	4.00	4.00	No food	phyto-plankton	-
Codliver oil	6.67	6.00	5.34	4.67	4.00	6.00	-	-	-
Soyabean Oil	3.33	3.00	2.66	2.33	2.00	0.00	-	-	-
EXPERIMENT II - Experimental diets for post-larvae 1-10									
Lecithin	0.00	2.00	4.00	6.00	8.00	10.00	2.00	4.00	6.00
Codliver Oil	6.67	5.34	4.00	2.67	1.34	0.00	0.00	5.34	4.00
Soyabean Oil	3.33	2.66	2.00	1.33	0.66	0.00	0.00	2.66	2.00
EXPERIMENT IIIA - Experimental diets for post-larvae 11-25									
Lecithin	0.00	0.25	0.50	0.75	1.00	1.25	1.50	1.75	-
Codliver oil	8.00	7.84	7.67	7.50	7.34	7.17	7.00	6.84	-
Soyabean Oil	4.00	3.91	3.83	3.75	3.66	3.58	3.50	3.41	-
EXPERIMENT IIIB - Experimental diets for post-larvae 11-25									
Lecithin	0.00	2.00	4.00	6.00	8.00	10.00	-	-	-
Codliver Oil	8.00	6.67	5.34	4.00	2.67	1.34	-	-	-
Soyabean Oil	4.00	3.33	2.66	2.00	1.33	0.66	-	-	-
EXPERIMENT IV - Experimental diets for juvenile prawns									
Lecithin	0.00	1.00	2.00	3.00	4.00	5.00	6.00	-	-
Codliver Oil	8.00	7.34	6.67	6.00	5.34	4.67	4.00	-	-
Soyabean Oil	4.00	3.66	3.33	3.00	2.66	2.33	2.00	-	-

TABLE - 9 ENVIRONMENTAL FACTORS, STOCKING DENSITY PER TREATMENT, MEAN INITIAL LENGTH AND WEIGHTS OF ANIMALS, AND FEEDING LEVEL FOR EXPERIMENT ON LECITHIN REQUIREMENT.

Parameters	Stages of the prawn				
	Larvae	Post-larvae 1-10	Post-larvae 11-25 EXP I	Post-larvae 11-25 EXP II	Juveniles
Salinity (‰)	34 ± 2	32 ± 2	20 ± 2	20 ± 2	20 ± 2
Temperature (°C)	29 to 31	27 to 30	26.5 to 28.5	26.5 to 28.5	26.5 to 29.5
pH	8.0-8.4	7.9-8.3	7.7-8.4	7.7-8.4	7.9-8.2
Dissolved oxygen (mg/l)	4.6 to 6.9	4.7 to 6.2	4.2 to 6.2	4.7 to 6.1	4.2 to 6.1
Total ammonia -N in seawater (ppm)	0.020-0.04	0.04-0.09	0.03-0.07	0.02-0.09	0.03-0.090
Initial number total of replicates	150	60	45	45	30
Initial length average (mm)	-	5.05	11.30	9.5	18.00 to 21.00
Initial wet weight average (mg)	-	0.239	5.667	2.92	30.00 to 38.00
Initial dry weight average (mg)	-	0.067	1.42	0.806	8.505
Feeding level % of the biomass	100	30-40	30-40	30-40	20-30

of cholesterol so little more cholesterol (1%) was included in the diet in order to get the benefit of increased level of lecithin for utilization of cholesterol. The vitamin level was increased from 3.2% to 3.6% by increasing choline chloride level from 0.6% to 1% of the diet because it plays important role in the phospholipid metabolism (Halver, 1962). Dietary lipid composition for larvae, post-larvae 1-10, post-larvae 11-25 and juveniles is shown in Table 8.

Control with phytoplankton was kept in larval experiment and reference diet NPCL 017 was kept for post-larvae and juvenile experiments.

Ingredients used, preparation of diet and methodology used in these experiments were similar as described in section on general material and methods (pp15-29). Hydrobiological conditions maintained during the experiment are shown in the Table 9.

R E S U L T S

LARVAE

Table 10A shows the results of the experiment on the lecithin requirements of larvae. The survival of the larvae were markedly affected by the dietary level of lecithin. All the survived larvae in the various treatments metamorphosed into post-larvae 1 within 8 days from protozoea-1.

All the larvae in treatment 7 in which food was not given died at protozoea-II stage. In the control, where phytoplankton was fed (Treatment-8), the development of the larvae followed a normal sequence and produced the highest survival of 34% at post-larvae-1 stage, which they attained within 8th day of the experiment. Among the various test diets (1 to 5) diet 3 containing 2% lecithin produced a survival rate of 22% at the post-larval stage. As such, there was no significant improvement in the survival rate of larvae by increasing the lecithin level above 2% in the diet. Incorporation of lecithin at higher level (above 2%) in diet 4 and 5 resulted in decreased survival rate. Deletion of lecithin from the diet resulted in relatively low survival rate. Although relatively low survival (11%) was recorded in groups of larvae fed the diet containing 4% lecithin (Diet 5) with a basal lipid constituting codliver oil + soyabean oil at 6% level, the survival of larvae increased to 23% on the diet (Diet 6) containing lecithin 4% and the basal lipid source was only 6% codliver oil.

Survival of larvae (Table 10A and 10B) from protozoea 1 (P1) to protozoea III (P III) was around 60% for diets 1 to 5 and 77.34% in the control and 72% for diet 6. Survival rate increased when larvae metamorphosed from P III to M1 (Mysis 1) stage with 88% for diets 2, 3 and 4 and around 80% for diets 5, 6 and 8. Survival rate however decreased and was minimum when larvae metamorphosed from mysis M1 to M3 stage. Survival of larvae from M_3 to post-larvae 1 stage was more than 67% for all the

TABLE - 10A GROWTH AND SURVIVAL OF P. INDICUS LARVAE FED ON DIETS CONTAINING GRADED LEVELS OF LECITHIN (PHOSPHOLIPIDS)

Diet No.	Lecithin Levels %	Survival rates % of various developmental stages of Prawn larvae							
		P1	P2	P3	M1	M2	M3	PL1	Feeding Period days
1	0.0	100	93.34	60.67	41.34	20.67	10.34	3.0	8
2	1	100	93.34	60.0	53.34	30.67	20.00	15.0	8
3	2	100	94.00	61.33	54.67	40.67	30.67	22.0	8
4	3	100	92.67	62.00	54.67	41.34	26.67	18.0	8
5	4	100	87.33	56.00	44.67	16.67	14.00	11.0	8
6	4	100	97-34	72.00	58.67	44.00	34.00	23.0	8
7	No food	100	Nil	-	-	-	-	-	-
8	Control	100	96.67	77.34	60.00	50.00	38.60	34.0	8

P1, P2, P3 = Protozoeal stages of larvae

M1, M2, M3 = Mysis stages of larvae

PL1 = Post-larva 1

TABLE - 10B SURVIVAL RATE (%) OF LARVAE AT VARIOUS DEVELOPMENTAL STAGES DURING METAMORPHOSIS

Diet No.	Lecithin Level %	Survival rate (%) of various developmental stages of prawn larvae				
		P1	From P1 to P3	From P3 to M1	From M1 to M3	From M3 to PL1
1	0.0	100	60.67	68.13	37.00	21.73
2	1.0	100	60.00	88.80	37.50	75.00
3	2.0	100	61.33	89.10	56.00	71.73
4	3.0	100	62.00	88.17	48.70	67.50
5	4.0	100	56.00	79.76	31.34	78.57
6	4.0	100	72.00	81.48	58.00	67.64
7	No Food	100	-	-	-	-
8	Control	100	77.34	77.58	64.44	84.48

dietary treatments (diets containing various levels of lecithin) and appears to be more than the stage M_1 to M_3.

POST-LARVAE 1-10

The results of the feeding experiment to determine the lecithin requirements of post-larvae 1-10 of P. indicus are plotted in Fig. 5. The survival rates of post-larvae fed the control diet (without lecithin) and test diets containing various levels of lecithin are presented in Fig. 5. Analysis of variance of the data showed that the survival rates of post-larvae were not significantly influenced by the dietary lecithin level. However, distinct variation was observed between the lecithin-free and test diets. The survival rate was low (68.34%) in the lecithin excluded diet. But inclusion of 2% lecithin in the diet considerably improved the survival rate and the highest survival rate (86.67%) was recorded in this treatment. Incorporation of lecithin in the diets at 6, 8 and 10% levels resulted in lowered survival rates, with the lowest at 10% lecithin in the diet. Though the survival rate was relatively low (63.34%) at 4% lecithin on a diet with total 10% lipid, it was higher (84.56%) at the same level of lecithin for a diet with total 12% lipid (Table. 11).

The growth rates of post-larvae 1-10 fed the control and test diets and expressed as percentage mean gains in length, wet weight and dry weight are shown in Fig. 5. Growth of post-larvae (Fig. 5) was also significantly ($P < 0.05$)

Fig. 5 Survival rate and growth of post-larvae 1-10 fed on diets containing graded levels of lecithin.

FIG.5

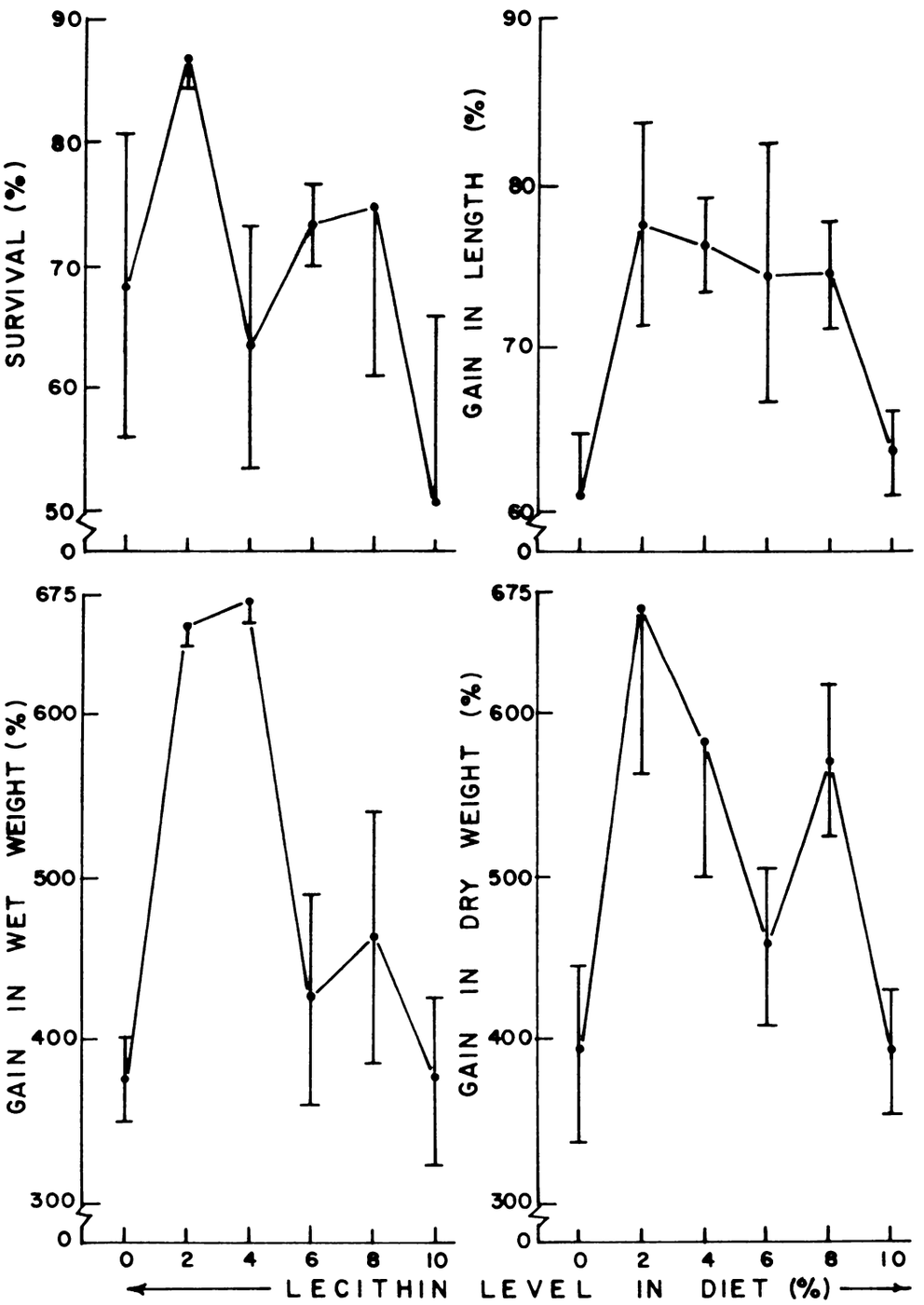

SURVIVAL (%)
GAIN IN LENGTH (%)
GAIN IN WET WEIGHT (%)
GAIN IN DRY WEIGHT (%)
90
80
70
60
50
0
675
600
500
400
300
0 2 4 6 8 10
LECITHIN LEVEL IN DIET (%)

TABLE 11 GROWTH AND SURVIVAL OF POST-LARVAE 1-10 FED ON DIETS CONTAINING GRADED LEVELS OF LECITHIN*

Treatment No.	7	8	9
Lecithin level in diet(%)	2.00	4.00	6.00
Survival (%)	80.00 ± 4.08	83.60 ±11.40	75.00 ± 7.07
Gain in length (%)	71.56 ± 6.10	84.56 ± 5.52	69.63 ± 0.56
Gain in wet weight (%)	428.59 ±31.00	596.65 ±31.38	583.39 ±33.70
Gain in dry weight (%)	516.91 ±30.66	616.42 ±44.77	611.44 ±37.23

* Total lipid level is 12% of the diet

influenced by the dietary lecithin level. Growth was significantly low ($P < 0.05$) when post-larvae were fed on the control diet without lecithin (diet1)and with 10% lecithin (Diet 6). But the diets containing 2% and 4% lecithin produced significantly ($P < 0.05$) higher growth than diets with 10% lecithin. However, addition of 4% lecithin did not significantly improve the growth of post-larvae, over that of 2% lecithin. The post-larval growth was greatly retarded when lecithin level in the diet was increased to 10% at a lipid level of 10%.

At 12% lipid level there was significant difference in the pattern of growth. In contrast to the high growth rate at 2% lecithin with 10% lipid diet, at 12% lipid level significantly higher growth was recorded at 4% lecithin level. However, increasing the dietary lecithin to 6% did not promote growth over that of 4% lipid (Table 11).

These results indicate that 2% lecithin in the diet is sufficient to promote growth in postlarvae 1-10 of *P. indicus*, at a lipid level of 10%. However, the post-larvae seems to require about 4% lecithin for fast growth at 12% lipid level.

POST-LARVAE 11-25

Experiment-1

Two sets of experiments were conducted to determine the optimal level of lecithin required in the diet. The results of the first experiment, in which post-larvae of *P. indicus* were fed, diets containing various levels of lecithin (0.25, 0.50, 0.75, 1.0, 1.25, 1.50, 1.75 g/100 g. of diet)

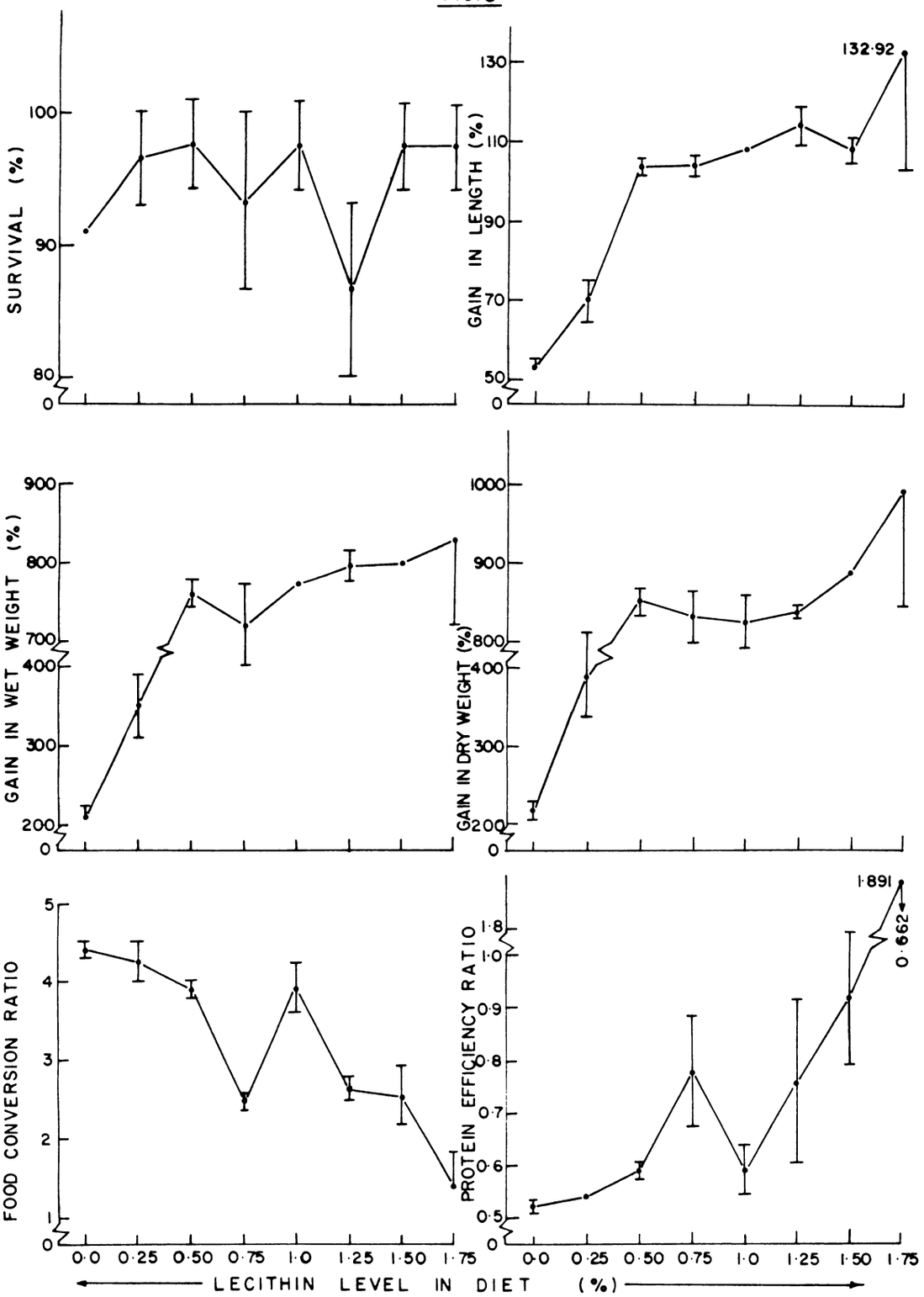
and the control diet without lecithin are given in Table 12 and shown in Fig. 6.

The survival rates of post-larvae was fairly high in all the treatments, and ranged from 86.7% to 100%. Thus the lecithin level in the diet did not significantly affect the survival rates of post-larvae 11-25. However, the growth (gain in length, wet weight and dry weight) was influenced significantly ($P < 0.05$) by the dietary level of lecithin (Fig. 6). Deletion of lecithin from the diet (Diet 1) resulted in lowest gains in length, wet weight and dry weight of post-larvae. But incorporation of 0.25% of lecithin significantly promoted growth. Inclusion of 0.5% lecithin in the diet (Diet 3) significantly enhanced growth over the diet 2 and the growth was more than two times to that recorded with diet 2 containing 0.25% lecithin. The growth of post-larvae increased with the level of lecithin from 0.25 to 1.75% in the diet.

Dietary lecithin level had significant ($P < 0.05$) effect on the food conversion and protein efficiency ratios. The highest FCR and lowest PER were recorded in the diet which had no lecithin; inclusion of lecithin at a level of 0.25% did not improve the FCR or PER significantly. But the food conversion and protein efficiency ratios were improved significantly ($P < 0.05$) by the inclusion of lecithin at a level of 0.5% in the diet. Food conversion ratio and protein efficiency ratios further improved significantly ($P < 0.05$) as the dietary

Fig. 6 Survival rate, growth, FCR and PER of post-larvae 11-25 fed on diets containing graded levels of lecithin (Experiment I)

FIG.6


SURVIVAL (%)
100
90
80
0
GAIN IN LENGTH (%)
132·92
130
110
90
70
50
0
GAIN IN WET WEIGHT (%)
900
800
700
400
300
200
0
GAIN IN DRY WEIGHT (%)
1000
900
800
400
300
200
0
FOOD CONVERSION RATIO
5
4
3
2
1
0
PROTEIN EFFICIENCY RATIO
1·891
0·662
1·8
1·0
0·9
0·8
0·7
0·6
0·5
0
0·0 0·25 0·50 0·75 1·0 1·25 1·50 1·75
LECITHIN LEVEL IN DIET (%)

lecithin was increased to 1.75%. The best food conversion and protein efficiency ratios were observed when dietary lecithin was 1.75% in the diet.

Results of proximate analysis of post-larvae are presented in Table 12. Though slight differences were observed among the eight diets in the moisture content, the diets containing the various concentrations of lecithin did not induce any significant change in the moisture content. The highest and lowest moisture contents of 74.3 and 69.6% were recorded in post-larvae fed on the control diet, and diet containing 1.75% lecithin respectively. The protein, lipid, carbohydrate and ash contents of post-larvae were significantly ($P < 0.05$) influenced by the dietary level of lecithin. The protein content was significantly ($P < 0.05$) the highest in the post-larvae fed the diet containing 1.75% of lecithin. Conversely, the post-larvae fed on the diet without lecithin had the lowest protein content. There was a steady increase in the protein content as the lecithin level in the diet increased. These results indicated that protein deposition in post-larvae is significantly influenced by the dietary level of lecithin.

The lipid content was significantly ($P < 0.05$) lower in post-larvae fed diets 1, 2 and 3 than that of diets 4, 5, 6, 7 and 8. The observed differences in lipid content of post-larvae among diets 1, 2, 3 and also among diets 5, 6, 7 and 8 were statistically insignificant. Although lipid content

was significantly influenced by dietary lecithin level, the lipid deposition did not significantly increase with the increasing concentrations of lecithin above 0.75% in the diet. From Table 12, it is clear that inclusion of 0.25% lecithin in the diet significantly enhanced lipid deposition over that of the control. The lipid content in post-larvae ranged from 9.15% to 12.9% with the lowest and highest levels in the lecithin free diet and 1.75% lecithin diet respectively.

The ash content in post-larvae did not show any specific trend in relation to the increasing dietary concentrations of lecithin. However, ash content was significantly ($P < 0.05$) low in post-larvae fed diets 2 and 3 when compared to other diets. The observed differences in the ash contents of post-larvae between diets 4, 5, 6 and 7 were statistically insignificant. The carbohydrate content was significantly ($P \leq 0.05$) high in post-larvae fed on the lecithin-free diet. But there were no significant differences among diets 2 to 8 in carbohydrate content of post-larvae.

The results of this experiment indicated that lecithin level in the diet significantly ($P \leq 0.05$) affect the gain in length, wet weight, dry weight, food conversion, protein efficiency ratio and protein retention in the body of post-larvae. It was also observed that the highest level of lecithin in the diet (1.75%) supported maximum growth, provided

TABLE - 12 EFFECTS OF DIETARY LECITHIN (PHOSPHATIDYLCHOLINE) LEVELS ON BIOCHEMICAL COMPOSITION OF THE POST-LARVAE 11-25.

Diet No.	Lecithin Level in the diet (%)	Moisture (%)	Percentage on dry weight basis			
			Protein	Lipid	Carbohydrate	Ash
1	0.00	74.30 ±0.05	61.00 ±1.00	9.15 ±0.15	4.05 ±0.15	18.95 ±0.00
2	0.25	72.76 ±0.51	61.33 ±0.30	11.45 ±0.05	2.65 ±0.55	16.50 ±0.50
3	0.50	71.74 ±1.50	62.50 ±0.50	11.20 ±0.20	3.30 ±0.10	16.55 ±0.45
4	0.75	71.45 ±0.88	62.10 ±0.10	12.30 ±0.10	3.25 ±0.15	18.85 ±0.01
5	1.00	73.46 ±1.02	64.05 ±0.05	12.05 ±0.15	3.00 ±0.10	18.25 ±0.25
6	1.25	72.97 ±1.55	65.50 +_0.50	12.15 ±0.05	2.55 ±0.35	19.00 ±0.00
7	1.50	71.23 ±1.31	67.75 ±0.25	12.35 ±0.95	2.25 ±0.05	18.05 ±0.05
8	1.75	69.59 ±0.11	69.85 ±0.01	12.90 ±0.00	1.65 ±0.00	17.00 ±0.00

the highest protein efficiency ratio and protein retention and better food conversion ratio. This has prompted to conduct another experiment with relatively higher concentrations of lecithin in the diet.

Experiment-II

The results of the second set of experiments to determine the phospholipid (lecithin) requirement of post-larvae 11-25 are given in Table 13 and shown in Fig. 7. Statistical analysis of the data from this experiment showed that the dietary lecithin level significantly influence the survival, growth, FCR, PER and contents of protein, lipid and cholesterol in the post-larvae.

There were no significant differences in the survival rates between diets 1 to 5. The survival rate was significantly ($P < 0.05$) low in the treatment with 10% lecithin (Diet 6). Diets 2 and 3 containing 2 and 4% lecithin, respectively produced relatively higher survival rates of 93.3% and 95.5% respectively.

The mean percent gains in length, wet weight and dry weight were significantly ($P < 0.05$) the highest in the post-larvae fed on the diet with 2% lecithin, among the dietary treatments. Deletion of lecithin from the diet produced relatively poor growth when compared to inclusion of 2% lecithin in the diet. Inclusion of lecithin at 4% and above

depressed growth. The highest growth (gains in length, wet weight and dry weight) recorded in post-larvae, when fed a diet with 2% lecithin indicate that the minimal lecithin level for maximum growth of post-larvae is about 2%. Statistical analysis of the data showed the significant influence of the diets on growth.

Conversion efficiency of food and protein is significantly influenced by the diets. Exclusion of lecithin from the diet resulted in significantly ($P<0.05$) low PER and high FCR. Inclusion of 2% lecithin in the diet significantly ($P<0.05$) improved the PER and FCR. However increasing the lecithin level in the diet above 2% resulted in relatively poor food and protein conversion ratios.

The lecithin-free diet fed post-larvae had significantly ($P<0.05$) lower protein, lipid and cholesterol contents, but significantly higher ($P<0.05$) ash and carbohydrate contents. The highest protein and lipid contents were found in post-larvae fed diets containing 2% and 4% lecithin respectively. The ash content of post-larvae was significantly ($P<0.05$) higher in diet 1 (lecithin-free diet) and diet 6 (containing 10% lecithin) than the other dietary treatments. The cholesterol content of post-larvae was significantly lower ($P<0.05$) in dietary treatment 1 (lecithin free diet) than the other treatments. But there were no significant differences between diets 2 to 6 in the cholesterol content of post-larvae.

Fig. 7 Survival rate, growth, FCR and PER of post-larvae 11-25 fed on diets containing graded levels of lecithin (Experiment-II).

Fig. 7

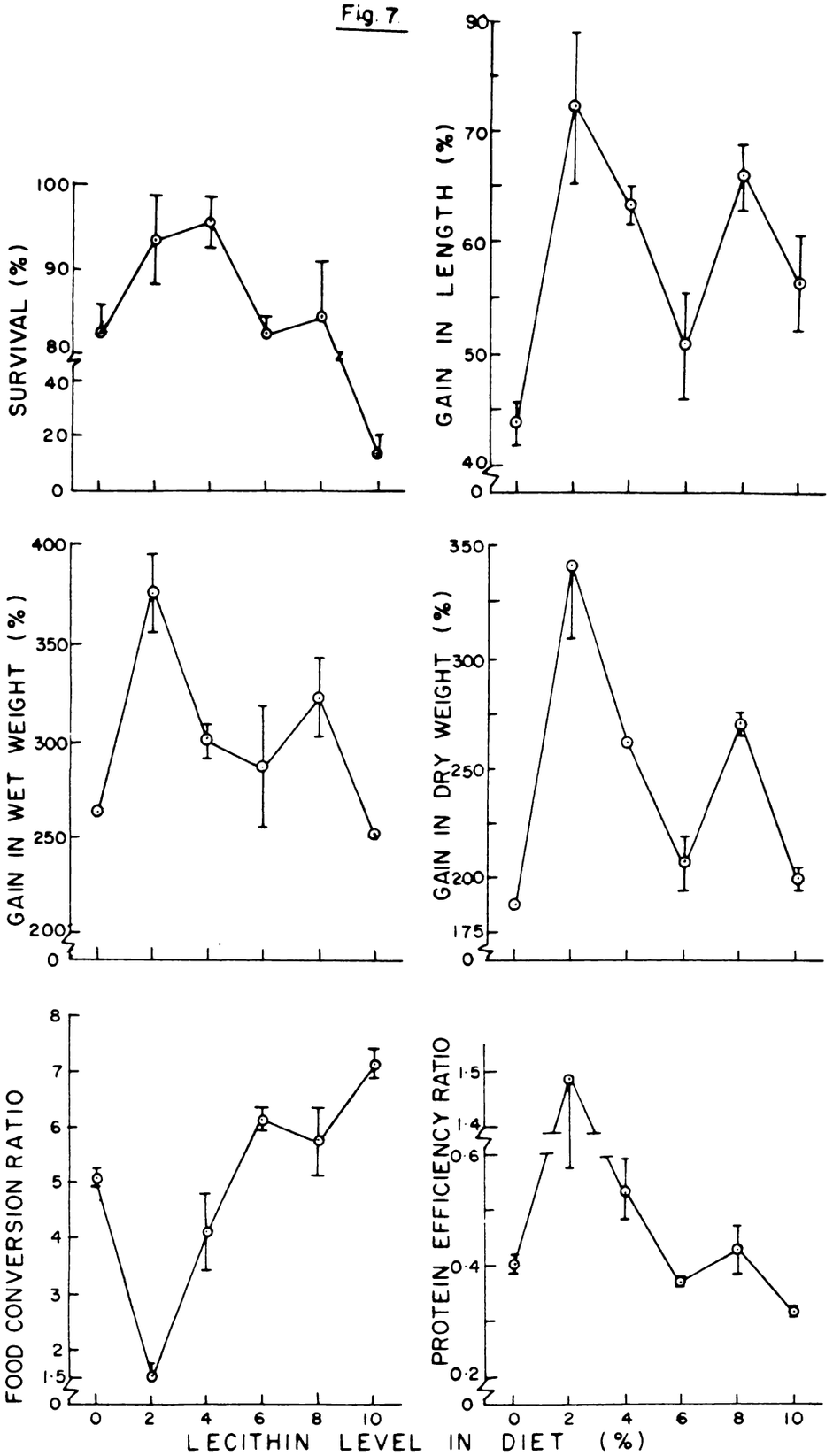

SURVIVAL (%)
100
90
80
40
20
0
GAIN IN LENGTH (%)
90
70
60
50
40
0
GAIN IN WET WEIGHT (%)
400
350
300
250
200
0
GAIN IN DRY WEIGHT (%)
350
300
250
200
175
0
FOOD CONVERSION RATIO
8
7
6
5
4
3
2
1·5
0
PROTEIN EFFICIENCY RATIO
1·5
1·4
0·6
0·4
0·2
0
0 2 4 6 8 10
LECITHIN LEVEL IN DIET (%)

TABLE - 13 EFFECTS OF DIETARY LECITHIN (PHOSPHATIDYLCHOLINE) ON THE BIOCHEMICAL COMPOSITION OF THE POST-LARVAE 11-25

Diet No.	Lecithin Level in the diet (%)	Moisture (%)	Percentage on dry weight basis				
			Protein	Lipid	Carbohydrate	Ash	Cholesterol mg/100 g
1	0.00	75.980	63.900	7.733	3.566	19.950	90.10
		±1.827	±1.900	±0.684	±0.471	±0.041	±8.20
2	2.00	74.680	69.720	11.980	1.553	16.636	173.40
		±0.696	±0.080	±0.596	±0.408	±0.228	±26.2
3	4.00	74.970	67.934	13.713	2.330	14.933	206.10
		±0.61	±0.654	±0.860	±0.466	±0.188	±60.10
4	6.00	76.770	67.440	11.136	2.080	15.067	193.30
		±1.304	±0.847	±0.167	±0.628	±0.590	±4.70
5	8.00	76.110	66.395	11.220	2.250	15.967	193.30
		±0.855	±1.195	±0.716	±0.318	±0.543	±12.40
6	10.00	77.690	64.730	11.570	3.167	18.400	200.00
		±1.770	±0.500	±0.488	±0.524	±1.232	±8.10

The post-larvae fed the lecithin-free diet had higher moisture and carbohydrate contents than those fed on diets containing various levels of lecithin. The moisture and carbohydrate contents were relatively low in post-larvae fed the diet with 2% lecithin. The observed variations in the moisture and carbohydrate contents of the post-larvae from various dietary treatments were not statistically significant. The post-larvae 11-25 were unable to utilize the ingested food and protein efficiently when lecithin deficient diet was fed thereby resulted in poor growth rate and protein retention in their body. The ingested food and protein were efficiently converted into tissues when post-larvae were fed with 2% lecithin in the diet thereby improved growth and protein retention followed. Since there was no significant improvement in the growth rate as well as in the food and protein utilization efficiency in the post-larvae above 2% lecithin in the diet it appears that the optimum lecithin requirements of the post-larval P. indicus is about 2% in the diet.

JUVENILES

Experiments with post-larvae 11-25 demonstrated the essentiality of lecithin in the diet. Besides the performance of the diet also did not significantly improve by the inclusion of lecithin levels greater than 2 to 4%. Based on these results, test diets were formulated to contain lecithin levels of 1, 2, 3, 4, 5 and 6% for juveniles. A diet without lecithin was also formulated. Each of the

experimental diets was fed to triplicate groups of juvenile prawns. The results are presented in Fig. 8.

Analysis of variance of the data showed that the dietary levels of lecithin significantly ($P<0.05$) affect the growth, FCR, PER and chemical composition of juvenile prawns. However survival rate of prawns was not significantly influenced by the test diets. Fairly high survival rates ranging from 93.34% to 100% were recorded (Fig. 8) from the various treatments. The reference diet (Diet 8) also produced high survival.

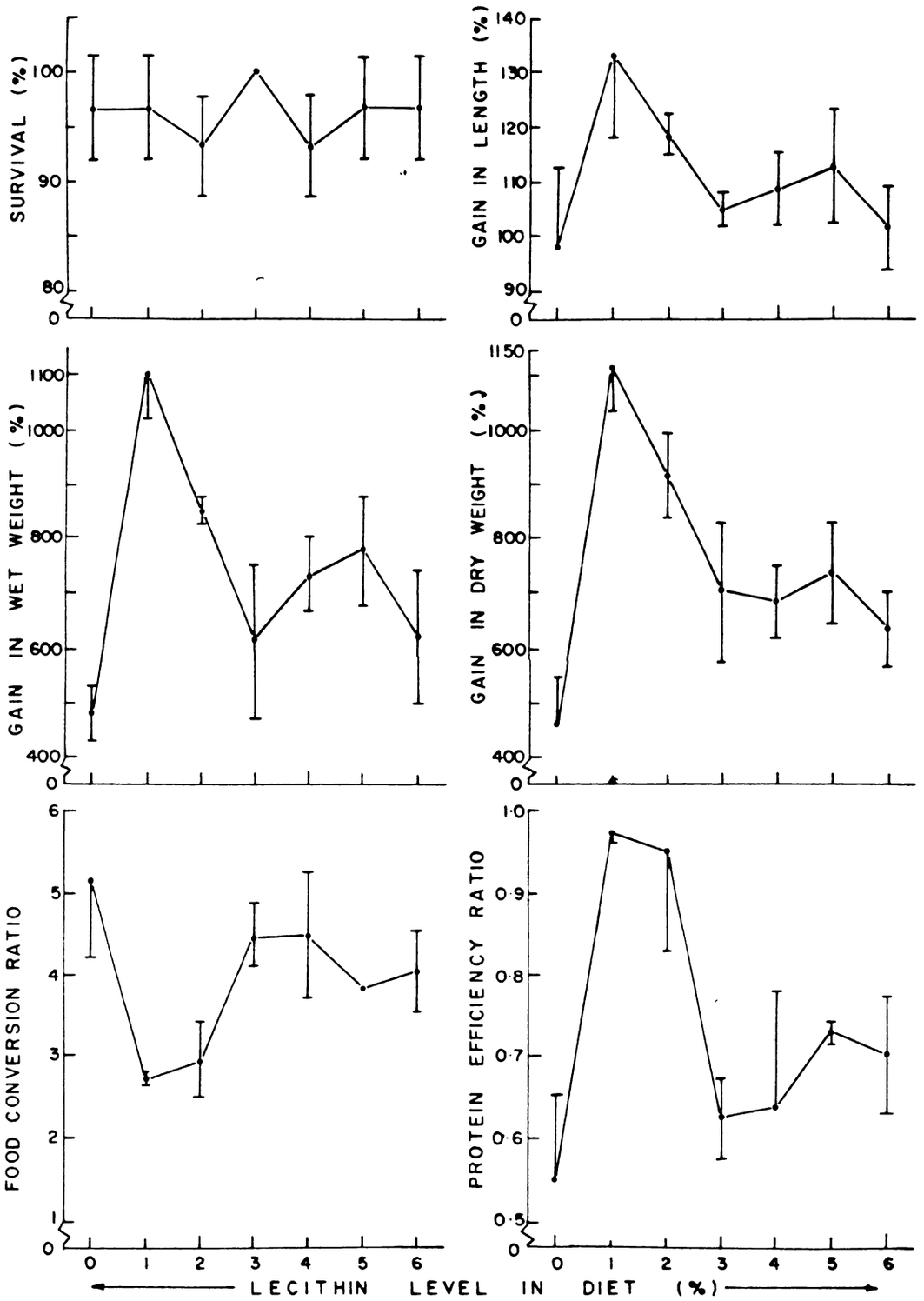
It is evident from the Fig.8 that the gains in length, wet weight and dry weight of juvenile prawns were significantly lower ($P<0.05$) for diet 1 (lecithin free diet) than for diet 2 (1% lecithin). Inclusion of 1% lecithin in the diet markedly enhanced the gain in length, wet weight and dry weight of juvenile prawns; and the highest growth was attained by the juvenile prawns fed on the diet 2, with 1% lecithin. The growth of juvenile prawns from this treatment was significantly ($P<0.05$) greater than that of all other dietary treatments. Incorporation of higher levels of lecithin (2% and above) in the diets resulted in significant growth reduction in juvenile prawns. There were no significant differences between diets 3 to 7 in prawn's growth. Thus it was clear that more than 2% lecithin has no significant effect on the gain in length, wet weight and dry weights of the prawn, P. indicus.

The food conversion ratios (FCR) and protein efficiency ratios (PER) for various diets are shown in Fig. 8. Deletion of lecithin from the diet (Diet 1) significantly ($P<0.05$) affected the utilization of food and protein by the prawn since the highest FCR and lowest PER were recorded for the lecithin-free diet. Diet 2 containing 1% lecithin provided the lowest FCR and highest PER in this feeding trial. The FCR and PER were not significantly improved by incorporation of 2% lecithin. However, inclusion of increasing levels of lecithin in diets 4 to 6 significantly ($P<0.05$) affected the utilization of food and protein. Thus there was no significant improvement in the utilization of food and protein by the prawn, when fed diets with more than 2% lecithin. These results indicate that 1% lecithin in the diet is sufficient enough to promote the food and protein utilization significantly in juvenile prawns.

The influence of dietary lecithin levels on the proximate composition (moisture, protein, lipid, carbohydrate, ash, and cholesterol) of prawns are shown in Fig. 9. While the protein, lipid and cholesterol contents were significantly lower ($P<0.05$), the moisture, carbohydrate and ash contents were significantly higher ($P<0.05$) in prawns fed the lecithin free-diet (Diet 1) than those fed diets containing lecithin (Diets 2-7). Addition of 1% lecithin significantly improved the level of protein, lipid and cholesterol in the prawn, but

Fig. 8 Survival rate, growth, FCR and PER of juvenile prawns fed on diets containing graded levels of lecithin.

FIG. 8.


SURVIVAL (%)
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90
80
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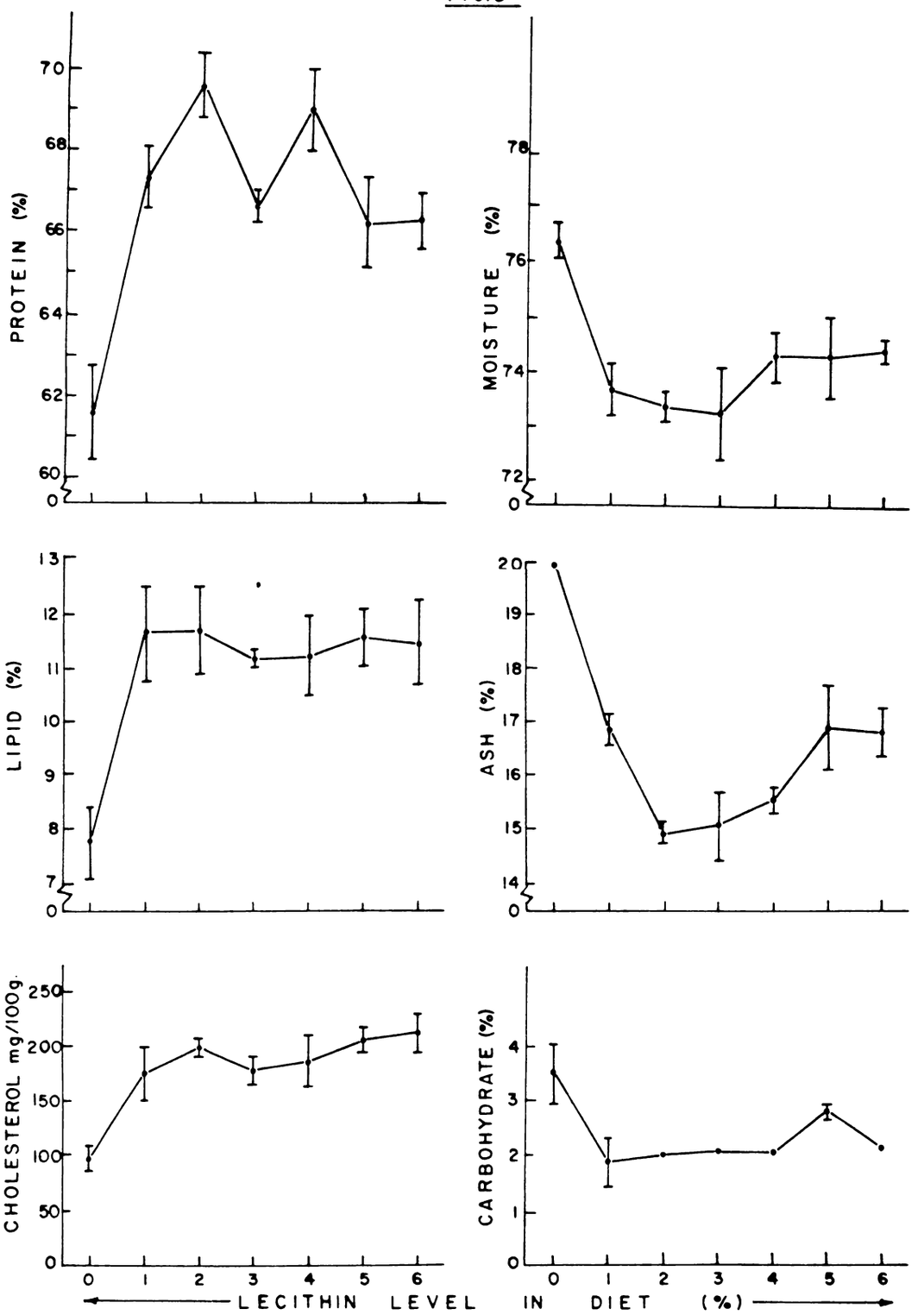
GAIN IN LENGTH (%)
140
130
120
110
100
90
0
GAIN IN WET WEIGHT (%)
1100
1000
800
600
400
0
GAIN IN DRY WEIGHT (%)
1150
1000
800
600
400
0
FOOD CONVERSION RATIO
6
5
4
3
2
1
0
PROTEIN EFFICIENCY RATIO
1·0
0·9
0·8
0·7
0·6
0·5
0
0 1 2 3 4 5 6
LECITHIN LEVEL IN DIET (%)

Fig. 9 Biochemical composition of juvenile prawns fed on diets containing graded levels of lecithin.

FIG.9


PROTEIN (%)
70
68
66
64
62
60
0
MOISTURE (%)
78
76
74
72
0
LIPID (%)
13
12
11
10
9
8
7
0
ASH (%)
20
19
18
17
16
15
14
0
CHOLESTEROL mg/100g
250
200
150
100
50
0
CARBOHYDRATE (%)
4
3
2
1
0
0 1 2 3 4 5 6
LECITHIN LEVEL IN DIET (%)

decreased the level of moisture, ash and carbohydrate. Although, variations in moisture, lipid and cholesterol contents of prawns were observed, when they were fed on diets 2 to 7 with various levels of lecithin, in most cases the observed variations were not statistically significant ($P < 0.05$). Only the prawns fed the diet 3 had significantly higher ($P < 0.05$) protein content than that of diet 2. Thus, it was evident that more than 1% lecithin level in the diet has no beneficial effect in promoting nutrient deposition in the prawns, with the exception of protein. Diets 3, 4 and 5 produced significantly low ash content in prawns, when compared to other diets.

D I S C U S S I O N

The results of the present study clearly demonstrate that the phospholipid, lecithin is an indispensable dietary nutrient for larvae, post-larvae and juveniles of *P. indicus*. The growth, survival and metamorphosis of larvae, and growth FCR and PER of post-larvae and juveniles seem to be greatly affected by lecithin deficiency in the diet. Besides, it is evident that for promoting high survival and growth, larvae and post-larvae require a dietary level of 2% lecithin. Similarly, for juvenile prawns 1% lecithin in the diet is found to be optimum for normal growth. Further the results show that inclusion of more than 2% lecithin in the diet has

no beneficial effect on survival and growth of larvae, post-larvae and juveniles of *P. indicus*.

Earlier studies on crustaceans also revealed that phospholipids are essential in their diet for survival and growth. Conklin *et al.* (1980) and D'Abramo *et al.* (1981a) have shown that inclusion of phosphatidylcholine in the diet is necessary for the survival and growth of the juvenile lobster, *Homarus americanus*. The essentiality of phospholipid (lecithin) in the diet has also been reported for *P. japonicus* larvae by Teshima *et al.* (1982b) and Kanazawa *et al.* (1985). These authors reported that larvae maintained on a diet without lecithin suffered 100% mortality before reaching the mysis stage. I have also observed relatively low survival in *P. indicus* larvae when fed a lecithin deficient diet. Kanazawa *et al.* (1985) observed that survival of the prawn larvae improved by a supplement of 1% soyabean lecithin to the diet and the maximum growth and survival were attained at 3.5% lecithin level in the diet. Similarly, Teshima *et al.* (1982b) reported that 3% lecithin in the diet promoted growth and survival in the same species. Thus *P. indicus* larvae seems to require relatively lower percentage of lecithin in the diet. However the findings demonstrate that penaeid prawn larvae, in general, may have a dietary requirement for phospholipids.

Although good survival was achieved with 2% lecithin diet and more than 2% lecithin diet had no beneficial effect

on the growth and survival of P. indicus larvae, the diet containing only marine lipid (codliver oil 6%) as basal lipid source and 4% lecithin, produced better survival as in 2% lecithin diet with cod liver oil and soyabean oil as lipid source. This observation indicates that use of higher level of lecithin in the diet appears to be useful when basal lipid source is only a marine lipid (codliver oil). It may be assumed that larval prawn probably require marine lipid (source of HUFA w3 series) or able to utilize marine lipid in a better way in the presence of higher levels (4%) of lecithin. Kanazawa et al. (1985) also made similar observations with larval P. japonicus in which the maximum survival and growth occurred, when fed on a diet containing 3.5% soyabean phosphatidylcholine along with 8% pollack liver oil as basal lipid source.

Small percentage of larvae and fairly good percentage of post-larvae and juveniles also survived on feeding the lecithin deficient diet. It is suspected that the phospholipids, including lecithin present in the basal lipid source used (soyabean oil and codliver oil) in the diet might have sustained the survival rate in larvae, post-larvae and juvenile prawn. However the quantity of phospholipids appears to be inadequate for augmenting the larval survival and metamorphosis. Yet, the essentiality of lecithin is clearly evident from the data on growth, FCR, PER and protein retention as the lecithin deficient diet

produced significantly low rate of growth, poor FCR, PER and protein retention in the post-larvae and juvenile prawns. Besides, the data for juveniles indicate that the diet containing 1% lecithin promote significantly better growth, FCR, PER and protein deposition. However, there seems to be relatively higher level of lecithin (2%) required for producing significantly better growth, FCR, PER and protein deposition in post-larval prawns. These results suggest that the larvae, post-larvae and juveniles of P. indicus require phospholipids in progressively decreasing levels, thus demonstrating the size related variation in quantitative phospholipid requirements. Besides the superior growth, FCR, PER and protein deposition in post-larvae at 2% and juvenile at 1% lecithin in the diets show that, these may be optimum for these sizes of P. indicus. Kanazawa et al. (1979e) also reported that 1% lecithin diet promoted very good growth in juvenile P. japonicus. Further they have pointed out that the growth promoting effects of dietary Tapes lipid observed in juvenile P. japonicus was not because of the presence of HUFA of w3 series in the dietary lipids but because of the good level of phospholipid present in the dietary lipids.

Some reports on juvenile lobster (Conklin et al., 1980, Boghen and Castell, 1980; Trider and Castell, 1980) suggested that the mortality in juvenile lobsters was prevented by the inclusion of soya lecithin in their diets and survival of

juvenile lobsters increased with increase in dietary level of lecithin, with an optimum range of 4 to 6%. In the present study with P. indicus the growth of larvae and post-larvae and juvenile prawns increased when dietary lecithin level was 2% but more than 2% lecithin had no beneficial effect on growth of various stages of P. indicus. These results indicate that more than optimum level of lecithin (1% for juvenile, 2% for post-larvae and larvae) has no beneficial effect on survival and growth).

Although reports on lecithin requirements of post-larvae are not available in the literature, Teshima et al. (1982b)&Kanazawa (1982, 1983) have reported that around 3% lecithin diet promoted very good survival and growth in larval P. japonicus. Similarly, Kanazawa et al. (1985) reported 3.5% lecithin produced better survival in P.japonicus larvae. All these findings clearly demonstrate the variations exhibited by crustaceans in their dietary phospholipid requirements.

Little is known about why dietary sources of phospho-lipids are effective in enhancing growth in prawns. Kanazawa (1985) and Kanazawa et al. (1985) while discussing the role of phospholipids in prawns assume that (1) prawns may have a limited ability for phospholipid biosynthesis at an adequate level from fatty acids and diglycerides (2) phospholipids take part in the emulsification of dietary lipids such as

triglycerides and cholesterol (3) some types of phospholipids may be necessary as constituents of lipoproteins, which play an important role in the transport of lipids. Similar conclusions can be drawn based on the findings during the present study with P. indicus.

It is also relevant to indicate that the phospholipids have some functional role during moulting. Conklin et al. (1980c) reported the role played by soyalecithin while eliminating mortality in lobster which was associated with 'moult death syndrome'. This syndrome is characterised by the inability of the lobster to extricate itself successfully from its skeleton during ecdysis (Boser and Rosemark, 1981). Prawn, like lobsters, cannot grow without moulting. So dietary phospholipids might supply the required phospholipid for moulting which result in enhancement in growth of prawn. In fact the larvae and post-larvae moult almost every alternate day so they may require more lecithin (phospholipid) than juveniles which moults at a slow rate. Since during moulting considerable physiological changes occur in the cells, tissues and organs through mobilisation of organic and inorganic metabolites and water in order to maintain homaeostasis the phospholipid requirements may be greater, particularly during moulting phase. Besides significant amount of energy is required during the moulting process which is primarily derived from reserve lipids, the transport and mobilisation of which

is through phospholipids. Thus since the early stages moult at greater frequencies there seems to be greater demand for dietary phospholipids than juvenile prawns. Thus dietary requirement of lecithin appears to be more for larvae and post-larvae than for the juvenile P. indicus. Although there is no harmful effect of higher levels (4%) of lecithin on the larvae post-larvae and juvenile prawns more than 4% lecithin retard the growth in various stages of P. indicus. These results further indicate that soya lecithin has a factor which helps in moulting and thereby promotes growth, when optimum levels are included in the diets.

It is well known that the phospholipids contribute to the structural and functional aspects of the cells. The mitochondria contain 25% lipid of which 95% is phospholipid (West et al.,1970). Phospholipids are also present in large quantity in biomembranes, thus it forms a part of dynamic system of anabolism and catabolism of animals (West et al., 1970). Several reports have shown that phospholipids were major lipid class (65 to 85% lipid) in the hemolymph lipid of crustaceans such as in lobster Homarus americanus (Bligh and Scott, 1966), and the prawn, P. japonicus (Teshima and Kanazawa, 1978a). Similarly Teshima and Kanazawa (1978 a and b) reported that the hemolymphlipid of prawn P. japonicus contain about 63% phospholipids and Teshima and Kanazawa (1979) suggested further that principal lipid transport is operated as form of phospholipid (lipoprotein). Thus

phospholipids appears to be essential for general metabolism of crustaceans and perhaps that is why diets containing lecithin were able to promote growth in the prawn, P. indicus. Importance of phospholipids, in the general metabolism of crustaceans has also been suggested by D'Abramo et al. (1982). They have shown that lobsters fed diets without soyalecithin had significantly reduced concentration of serum cholesterol and serum phospholipids. Thus lack of phospholipid in the diet apparently results in phosphatidylcholine deficiency in the hemolymph thereby affecting the effective transport of lipids.

It is assumed that the poor growth and FCR observed with lecithin free diet may be because of absence of adequate levels of lecithin in diet of post-larvae and juvenile P. indicus. It has been suggested that in crustaceans phospholipids probably play an important role in emulsification, digestion, absorption and interorgan transport of lipids (Van Den Oord, 1964; Lester et al., 1975; Teshima and Kanazawa, 1978a, b and Kanazawa et al., 1979). Lester et al. (1975) observed that lecithin enhanced cholesterol solubilization when associated with N-N dodecanosarcosyl taurine (DST) a model detergent synthesized by crustaceans. It is assumed that the lecithin provided in the diet of P. indicus might have influenced the digestion of lipids resulting in better food conversion ratio and protein efficiency ratio.

Among the biochemical constituents of prawn, protein assumes greater significance and the response of the animals to the diets are reflected in the efficiency of utilization of dietary protein and protein synthesis in the body. Lecithin when included in the diet may provide choline, which acts as a methyl donor during trans-methylation reactions thereby sparing the sulphur amino acid, methionine (another methyl donor) for enhancement of protein synthesis. Also choline on oxidation produce betaine which then serves as methylating agent. Betaine is acted by specific transmethylases which catalyses the transfer of one of the methyl group which is utilized for the conversion of homocysteine to methionine. Thus lecithin seems to be useful for the synthesis of methionine and thus for synthesis of protein which enhance the growth of prawn and produce better PER. Thus poor growth and low PER observed in the prawns on feeding lecithin free diet may be because the required methyl groups might have been drawn from methionine as a result of catabolism of protein, thus leading to reduced PER and growth of the animal. This appears to be one of the reasons why growth, PER and protein content of prawn P. indicus appears to be more on feeding the diet containing sufficient level of lecithin.

CHAPTER - III

FATTY ACIDS REQUIREMENT

I N T R O D U C T I O N

Until 1930, lipids were considered merely as energy nutrients for animals. However the work of Burr and Burr (1930) radically changed this concept. They reported that one of the fatty acids (linoleic acid) is essential for animals and its deficiency in diets results in poor growth and cause severe pathological syndromes. Subsequent researches have shown that aquatic organisms too need essential fatty acids (Kanazawa et al., 1979b). Observations made during the present investigation also clearly demonstrated the distinct variations in the response of Penaeus indicus larvae, postlarvae and juveniles to natural sources of lipids (Chapter 4). Since these variations are brought about by fatty acids profile of lipids, it is necessary to elucidate the fatty acid requirements of prawns.

Fatty acids occur in very large amounts as building block components of saponifiable lipids and only traces occur in free form in cells and tissues. About 100 different kinds of fatty acids have been isolated from lipids of various animals and plants. All possess a long hydrocarbon chain and a terminal carboxyl group. The hydrocarbon chain may be saturated without any double bond as in palmitic acid or it may have one double bond as in oleic acid then it is called as

monounsaturated or monoenic fatty acid. When two or more double bonds are present in the hydrocarbon chain, it is known as polyunsaturated fatty acid (PUFA) such as linoleic acid (18:2w6) and linolenic acid (18:3w3). Sometimes unsaturated hydrocarbon chain may have 20 or more carbon atoms then it is called as highly unsaturated fatty acid (HUFA) such as eicosapentaenoic acid (20:5w3) and docosahexaenoic acid (22:6w3). Unsaturated fatty acids have lower melting points than saturated fatty acids of the same chain. So they are abundant in marine animals and plants (Sargent, 1976).

Studies have shown that saturated and monounsaturated fatty acids can be biosynthesized de novo by all forms of animals so far examined; but polyunsaturated fatty acids are not biosynthesised denovo at an adequate level in majority of marine animals (Sargent, 1976). Certain fatty acids have specific nutritional importance which are not biosynthesised de novo are called as 'Essential Fatty Acids'(EFA). These fatty acids have to be included in the diets for normal survival, growth, maintenance and proper functioning of physiological processes (Burr and Burr, 1930; Alfin-slater and Aftergood, 1968). One important function of EFAs is in the biosynthesis of group of fatty acid derivatives called prostaglandins, which are hormone like compounds and in trace amounts have profound effect on a number of important physiological activities in animals (Lehninger, 1984).

The major concern with the polyunsatured fatty acids (PUFA) is due to the fact that they are essential dietary factors for all animals so far studied, including terrestrial and aquatic species (Sargent, 1976). A deficiency of w3 PUFA causes definite symptoms including cessation of growth (Castell et al., 1972a) and fin and skin erosion and shock syndromes in fishes (Sinnhuber, 1969; Castell et al., 1972a). Land mammals have high concentrations of w6 PUFA particularly linoleic acid (18:2w6) and arachidonic acid (20:4w6); where as high concentrations of w3 PUFA, such as linolenic (18:3w3) eicosapentaenoic (20:5w3) and docosahexaenoic fatty acids (22:6w3) are found in fish (Sargent, 1976) and in crustaceans (Kanazawa, 1985). Phospholipids of biomembranes are particularl rich in polyunsaturated fatty acids (Sargent, 1976). Polyunsaturated fatty acid deficiency in terrestrial mammals is characterized at the biochemical level by a fragility of biomembranes (Guarneri and Johnson, 1970).

In freshwater fish, the w3 acids predominate, although substantial amounts of w6 acids are also present. In marine fish, however, the level of w6 PUFA are significantly low, so that the ratio w3/w6 is substantially higher in marine fish than in freshwater fish (Ackman, 1967). ω3 PUFA such as 20:5w3 and 22:6w3 are predominant fatty acids in the prawn, P. japonicus (Kanazawa et al., 1977b). Similar pattern is also present in most of the other marine penaeid prawns

(Gopakumar and Nair, 1975; Guary et al., 1976a; Read, 1977; Bottino et al., 1980; Clark and Wickins, 1980). Estuarine prawns also have small percentage of w6 type fatty acids in addition to w3 fatty acids, as observed in P. indicus(Colvin, 1976b; Read, 1977). The main reason attributed to the presence of high concentrations of w3 fatty acids in marine animals as compared to w6 fatty acids, is relating to fluidity of lipids at low temperature, which inturn is related to the degree of unsaturation of fatty acids. The presence of w3 fatty acids may ensure biomembranes to retain their fluidity and normal physiological functions at low temperature. Hilditch and Williams (1964) reported that a decrease in environmental temperature is accompanied by an increase in degree of unsaturation of fish lipids. Besides, PUFA present in biomembranes of marine organisms play important role in osmoregulation in the marine environment (Sargent, 1976).

Prawn lipids have both saturated and unsaturated fatty acids, particularly greater percentage of w3 HUFA such as 20:5w3 and 22:6w3 (Gopakumar and Nair, 1975; Guary et al., 1976; Colvin, 1976b and Sargent, 1976). Although, essential fatty acids content of crustaceans is very high, they are unable to synthesize these fatty acids from other saturated fatty acids (Kanazawa, 1985). Nutritional studies have demonstrated that crustaceans require essential fatty acids in their diets for normal survival and growth (Kanazawa et al.,

1979b, 1979d, 1979f). Kanazawa and coworkers through radioactive tracer experiments reported the absence of *de novo* synthesis of linoleic (18:2w6) linolenic (18:3w3), eicosapentanoic (20:5w3) and docosahexaenoic (22:6w3) acids from acetate or palmitic acid, in *P. japonicus* (Kanazawa and Teshima, 1977) *P. monodon* and *P. merguiensis* (Kanazawa *et al.*, 1979c). Similarly, essentiality of PUFA in the diets is also shown for other crustaceans such as crayfish, *Astacus astacus* (Zandee, 1966b) and the lobster, *Homarus gammarus* (Zandee, 1967) All these results of tracer experiments indicate that 18:2w6, 18:3w3, 20:5w3 and 22:6w3 are essential fatty acids for crustaceans, especially the penaeid prawns (Kanazawa *et al.*, 1979 b,c). Several other reports also highlight the requirement of some of these essential fatty acids (18:2w6, 18:3w3, 20:5w3 and 22:6w3) for prawns and lobster (Shewbart and Mies, 1973; Provasoli, 1975; Colvin, 1976b; Guary *et al.*, 1976a; Bottino *et al.*, 1980; D'Abramo *et al.*, 1980; Read, 1981; Petrilla *et al.*, 1984).

Some reports indicate the synthesis of 18:2w6, 18:3w3, 20:5w3 and 22:6w3 from the radioactive acetate-^{14}C in the body of the prawns *P. monodon* and *P. merguiensis* (Kanazawa *et al.*, 1979c), in *P. japonicus* (Kanazawa *et al.*, 1979b) and in the mysid *Gnathophausia* sp. (Morris and Sargent, 1973) at a very slow rate. Infact, Kanazawa *et al.* (1977b, 1978, 1979d, 1979f) have shown by feeding experiments that juveniles of *P. japonic*

have a higher weight gain with diets containing 18:2w6,18:3w3, 20:5w3 or 20:6w3 than 18:1w9. Besides, 20:5w3 and 22:6w3 are more essential than 18:2w6 and 18:3w3 for P. japonicus (Kanazawa et al., 1979a). Absence of 18:3w3 in the diet also resulted in poor weight gain in P. aztecus (Shewbart and Mies, 1973) and in P. stylirostris (Fenucci et al., 1981). Bottino et al. (1980) reported that P. styliferus, P. aztecus and P. duorarum were unable to biosynthesize C 20 and C 22 PUFA from C 18 fatty acid precursors at adequate levels. These results indicate the essentiality of 20:5w3 and 22:6w3 fatty acids for prawns. Jones et al. (1979b) and Teshima and Kanazawa (1984) pointed out the necessity of w3 HUFA for growth and survival of larval stages of P. japonicus.

The foregoing informations suggest that penaeid prawns lack the ability for de novo synthesis of 18:2w6, 18:3w3, 20:5w3 and 22:6w3 at an adequate level and thus these fatty acids are found to be essential in their diet. Although experimental evidence by tracer techniques using radioactive acetate are not available for P. indicus, Colvin (1976b) suggested limited capacity for biosynthetic interconversion of EFA to longer chain polyunsaturated fatty acids of same type series and suggested that optimum ratio of w3/w6 fatty acids may be necessary for normal lipid metabolism in juvenile P. indicus.

Many reports are available on quantitative essential fatty acid requirements of fish (Watanabe, 1982). However

very few reports are available on quantitative dietary requirement of essential fatty acids for prawns and other crustaceans. So far, dietary requirements of fatty acids have been reported for P. japonicus (Kanazawa et al., 1979a), P. aztecus (Shewbart and Mies, 1973), P. stylirostris (Femucci et al., 1981), and to a limited extent for P. indicus (Read, 1981). Read (1981) reported the fatty acid requirement of juvenile P. indicus, using a compounded diet containing natural ingredients and he used a basic lipid level of 5% in the diet, without understanding the total lipid level required for optimum growth and survival of P. indicus. Besides, there is no report on the fatty acid requirement of larvae and post-larvae of P. indicus. Also Read (1981) and Colvin (1976b) have not used graded levels of purified fatty acids in their experimental diets to understand the fatty acid requirements. It has been observed in the present study that juvenile P. indicus require about 9 to 12% lipid level and the larvae and post-larvae require about 8-10% lipid level in the diet for optimum survival and growth. With this background information, the present study was carried out to determine the essential fatty acid requirements of larval, post-larval and juvenile P. indicus by using all purified ingredients.

MATERIALS AND METHODS

Four sets of laboratory experiments were carried out to study the effect of selected levels of fatty acids in diets on the larvae, post-larvae and juveniles of P. indicus. The first three sets of experiments were conducted to study the effect of selected levels of linolenic acid in the diets and to determine the linolenic acid requirement of larvae post-larvae 1-10 and 11-25. The fourth sets of experiments were conducted with juveniles of P. indicus by using eleven diets (Table 16) containing selected levels of linolenic and linoleic acids as individual fatty acids, and their combinations. A control diet containing a mixture of codliver oil, soyabean oil and lecithin, which has shown the best response in earlier experiments and had PUFA of w3 and w6 series, was used in all the experiments.

Palmitic acid was used as basal lipid source in all the experimental diets. Purified linolenic and linoleic acids obtained from Sigma Chemical Co., USA were used in all the experiments. Dietary composition for larvae, post-larvae 1-10 and 11-25, and juveniles is given in Tables 14, 15 and 16. A control diet containing a mixture of codliver oil, soyabean oil and lecithin was used in all the experiments with larvae post-larvae 1-10 and 11-25, and juveniles. Lipid was used at a level of 10% for larvae, post-larvae and juvenile experiments. In addition a diet with 12% lipid level was

TABLE - 14 INGREDIENTS COMPOSITION (%) OF THE BASAL DIETS USED FOR LARVAE, POST-LARVAE AND JUVENILES IN FATTY ACID REQUIREMENTS EXPERIMENTS

Ingredients	Diet for larvae post-larvae 1-10 post-larvae 11-25	Diet for juveniles
Casein	37.00	31.00
Egg albumin	9.00	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	3.50	5.00
Sucrose	7.00	12.00
Cholesterol	0.50	0.50
Lipids[2]	10.00	10.00
Vitamin mixture[3]	3.20	3.20
Mineral mixture[4]	8.50	8.50
Cellulose powder	2.00	3.00
Total	100.00	100.00
Carrageenan	5.00	5.00
Distilled water	120-130 ml	120-130 ml

Percentages of

1) Amino acid mixture (3) Vitamin mixture and (4) Mineral mixture used in this diet are as given in Table 2.

2) Lipids - Percentages of lipid as given in the Table No. 15 and Table No. 16.

TABLE - 15 COMPOSITION OF DIETARY LIPIDS/FATTY ACIDS IN THE TEST DIETS FOR LARVAE, POST-LARVAE 1-10 AND POST-LARVAE 11-2

Diet No.	Dietary lipids/fatty acids for larvae, post-larvae 1-10 and Post-larvae 11-25
1	10% palmitic acid
2	9% Palmitic acid + 1% linolenic acid
3	8% Palmitic acid + 2% linolenic acid
4	7% Palmitic acid + 3 % linolenic acid
5	6% Palmitic acid + 4% linolenic acid
6	5 % Palmitic acid + 5% linolenic acid
7	4% Palmitic acid + 6% linolenic acid
8 control	5.60% Codliver Oil+ 2.8% soyabean oil + 1.6% lecithin

TABLE - 16 COMPOSITION OF DIETARY LIPIDS/FATTY ACIDS IN THE TEST DIETS USED FOR JUVENILES

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	5.60% codliver oil + 2.8% soyabean oil + 1.6% lecithin
11	6.67% codliver oil + 3.33% soyabean oil + 2% lecithin

TABLE - 17 ENVIRONMENTAL FACTORS, STOCKING DENSITY PER TREATMENT, MEAN INITIAL LENGTH AND WEIGHTS OF ANIMALS AND FEEDING LEVEL FOR EXPERIMENT ON ESSENTIAL FATTY ACID REQUIREMENT.

Parameters	Stages of the prawn			
	Larvae	Post-larvae 1-10	Post-larvae 11-25	Juveniles
Salinity (‰)	34.0 ± 2	32.0 ± 2	20.0 ± 2	20.0 ± 2
Temperature (°C)	29.0 to 31.0	26.0 to 30.0	26.0 to 30.1	26.4 to 31.0
pH	8.0 to 8.3	8.0 to 8.2	7.8 to 8.2	7.6 to 8.2
Dissolved oxygen in water mg/l	4.6 to 6.2	4.6 to 6.4	5.00 to 6.2	3.60 to 5.7
Total ammonia -N in seawater (ppm)	0.02 to 0.06	0.04 to 0.09	0.03 to 0.10	0.03 to 0.11
Initial number	150	60	45	30
Average Initial length (mm)	-	6.0	12.50	20.0 to 24.0
Average initial wet weight (mg)	-	0.475	7.236	49.00 to 53.0
Average initial dry weight (Mg)	-	0.110	1.60	11.42
Feeding level % of biomass	100	30-40	30-40	20-30

used in experiments with juveniles.

Basal ingredients used for preparation of diets in this experiment are given in Table 14. The environmental factors maintained in the aquaria, the data on initial lengths, wet weights, dry weights of prawns and feeding levels are presented in Table 17. General procedures of feed preparations, experimental study and data collection and statistical processing are similar to that presented in general material and methods section of the thesis (pp15-29).

R E S U L T S

LARVAE

Results of the experiment conducted to determine the fatty acids requirement of the larvae are given in Table 18.

All the larvae fed on the test diets containing purified fatty acids died at protozoea I or II stage, before third day of the experiment. However, the larvae fed on the control diet (phytoplankton) metamorphosed into post-larvae 1 within 8 days with a survival of 36.0%, indicating environmental parameters were within the normal range of tolerance by larvae (Table 18A). Similarly, the diet containing a mixture of codliver oil and soyabean oil and lecithin produced fairly

TABLE - 18A GROWTH AND SURVIVAL RATE OF P. INDICUS LARVAE FED ON DIETS CONTAINING VARIOUS LEVELS OF FATTY ACIDS (Linolenic acid)

Diet No.	Linolenic acid level %	Survival rates (%) of various developmental stages of prawn larvae							
		P1	P2	P3	M1	M2	M3	PL1	Feeding period days
1	0.0	100	0.0	-	-	-	-	-	2
2	1.0	100	0.0	-	-	-	-	-	2
3	2.0	100	0.0	-	-	-	-	-	2
4	3.0	100	0.0	-	-	-	-	-	2
5	4.0	100	0.0	-	-	-	-	-	2
6	5.0	100	0.0	-	-	-	-	-	2
7	6.0	100	0.0	-	-	-	-	-	2
8.	Codliver oil + Soyabeen oil + Lecithin	100	77.34	46.00	36.67	27.00	26.00	21.67	8
9	Control (Phyto plankton)	100	89.00	78.00	54.67	42.50	38.67	36.67	8
10	No food	100	-	-	-	-	-	-	1

P1, P2, P3 = Protozoeal stage of larvae; M1, M2, M3 = Mysis stages of larvae; PL1 = Post-larva 1 stage

TABLE - 18B SURVIVAL RATE (%) OF LARVAE AT VARIOUS DEVELOPMENTAL STAGES DURING METAMORPHOSIS

Diet No.	Linolenic acid level (%)	Survival rate (%) of larvae at various developmental stages				
		P1	From P1 to P3	From P3 to M1	From M1 to M3	From M3 to PL1
1 to 7	0 to 6%	100	0.0	0.0	0.0	0.0
8	Codliver oil + Soyabean oil + Lecithin	100	46.0	79.90	70.9	79.48
9	Phytoplankton	100	80.0	68.34	70.73	94.82

good survival (26-70%) and the larvae attained the post-larval stage within 8 days. The survival of larvae in the latter two diets (phytoplankton and diet with natural lipid sources) was relatively less during protozoeal stages P I to P III but increased from mysis 1 to post-larvae 1 stage (Table 18 B).

POST-LARVAE 1-10

The results of the feeding experiment conducted in post-larvae 1-10 of P. indicus with diets containing graded levels of linolenic acid (18:3w3) ranging from zero to six percent and the control diet with natural lipid source are shown in Fig. 10. Survival of post-larvae 1-10 ranged from 56.00 to 94.00% (Fig. 10). Analysis of variance of data showed that dietary linolenic acid levels has significant influence on the survival of post-larvae 1-10. Deletion of linolenic acid from the diet (Diet-1) and addition of linolenic acid in the diet at relatively higher concentrations (more than 3%) produced significantly lower rates of survival (56.67 to 65%). Whereas, the diet with 1% linolenic acid provided significantly ($P<0.05$) higher rate of survival (83.84%) than the remaining diets. The control diet containing codliver oil, soyabean oil and soya lecithin produced significantly ($P<0.05$) the highest survival rate (93.34%).

The mean percent gains in length, wet weight and dry weight of post-larvae 1-10 (Fig. 10) were also significantly

($P < 0.05$) influenced by the dietary linolenic acid levels. Deletion of linolenic acid from the diet significantly affected growth. Inclusion of 1% linolenic acid in the diet significantly ($P < 0.05$) improved growth over that of linolenic acid-free diet. Inclusion of linolenic acid at levels greater than 1% did not significantly improve growth. However inclusion of 5.0 and 6.0% linolenic acid significantly ($P < 0.05$) retarded post-larval growth. Of all the diets, the control diet produced significantly ($P < 0.05$) superior growth. Although the growth of post-larvae 1-10 increased with the dietary level of linolenic acid from 1% to 3%, the increase in growth was not significantly higher than the growth produced by the diet containing linolenic acid at 1% level.

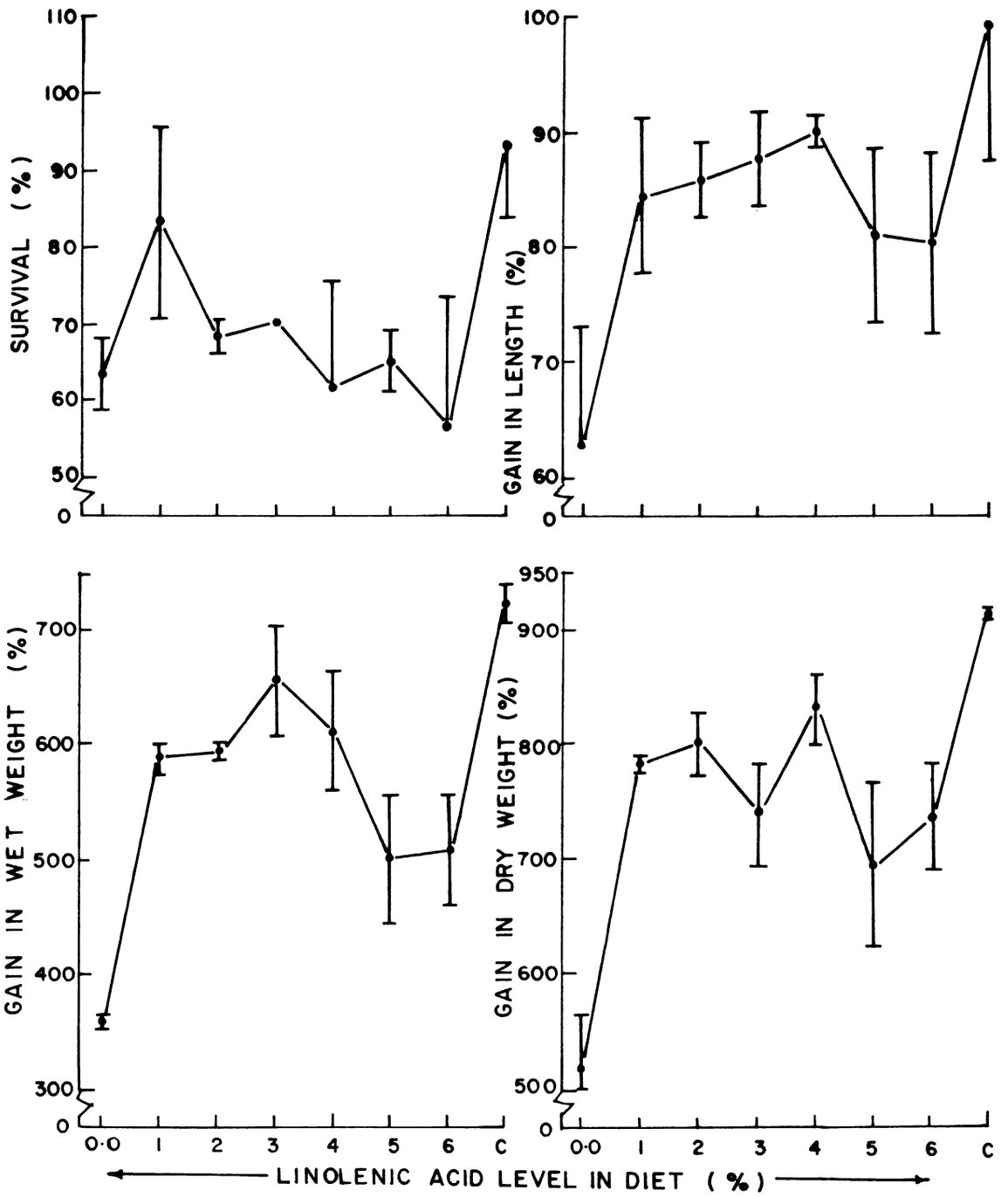
The poor gains in length, wet weight and dry weight of post-larvae with the linolenic acid deficient diet indicate the essentiality of linolenic acid in the diet of post-larvae 1-10, and 1% linolenic acid appears to be optimum for post-larvae 1-10. The significantly ($P < 0.05$) high growth of post-larvae fed on the control diet (Diet 8) in which codliver oil, soyabean oil and lecithin were incorporated indicate the importance of natural lipid sources containing PUFA of w3 and w6 series.

POST-LARVAE 11-25

A feeding experiment was conducted with post-larvae 11-25 of *P. indicus* by using seven test diets incorporating

Fig. 10 Survival rate and growth of post-larvae 1-10 fed on diets containing graded levels of linolenic acid (18: 3w3).

FIG.10.


SURVIVAL (%)
110
100
90
80
70
60
50
0
GAIN IN LENGTH (%)
100
90
80
70
60
0
GAIN IN WET WEIGHT (%)
700
600
500
400
300
0
GAIN IN DRY WEIGHT (%)
950
900
800
700
600
500
0
0·0 1 2 3 4 5 6 C
LINOLENIC ACID LEVEL IN DIET (%)

graded levels of linolenic acid ranging from zero to six per cent, and one control (Diet 8) with natural lipid sources.

Data for survival, growth, FCR, PER and chemical composition of post-larvae 11-25 are shown in Fig. 11 and Table 19.

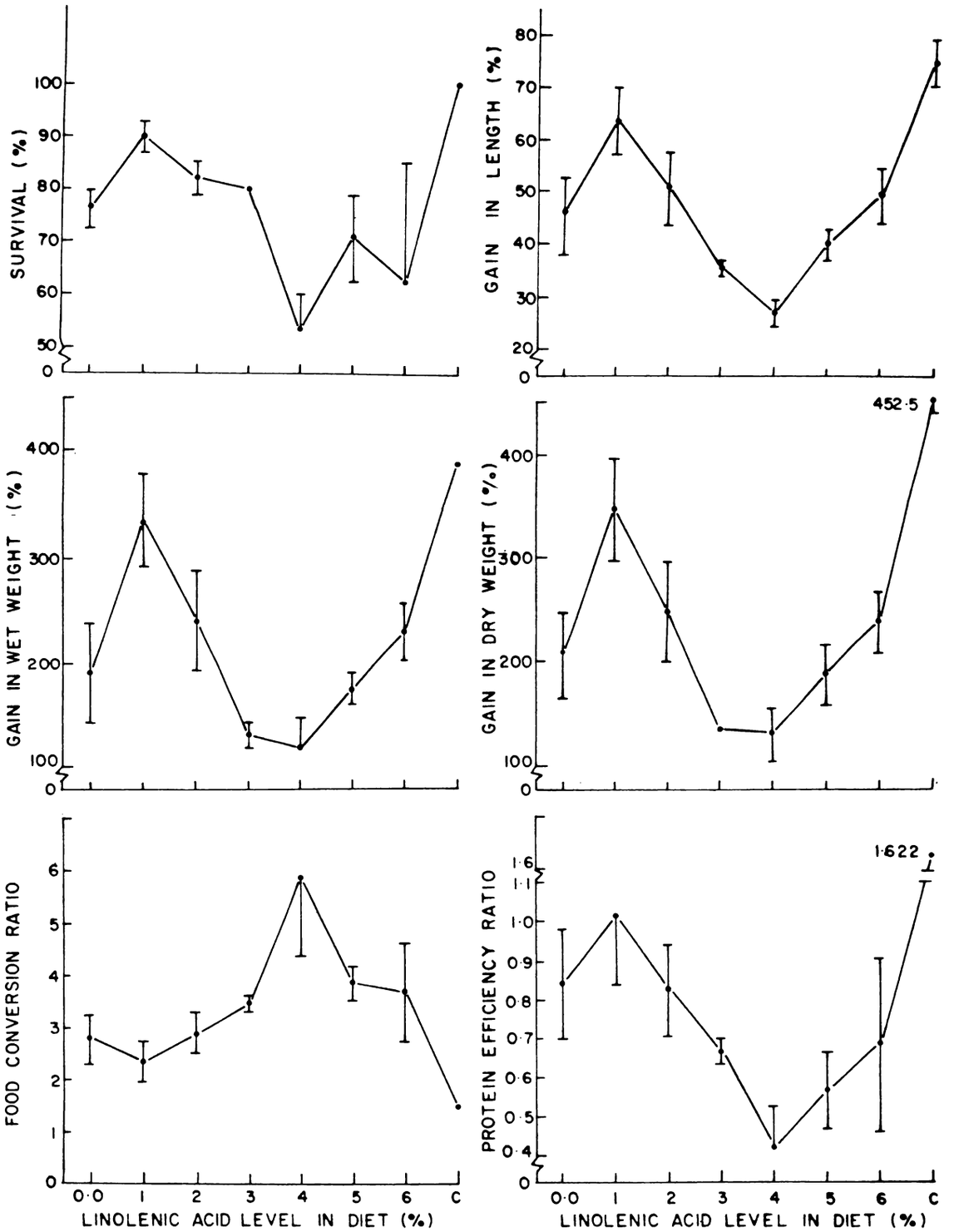
Survival rates of post-larvae ranged from 53 to 90% (Fig. 11) in the treatments 1 to 7 (Fig. 11) and 100% in the control, showing the significant ($P < 0.05$) effect of dietary levels of linolenic acid. Diet 2 containing 1% linolenic acid and the control diet produced significantly ($P < 0.05$) higher survival rates than diet 5 to 7. But inclusion of linolenic acid above 1% level resulted in significantly ($P < 0.05$) low survival rates.

The mean percent gain in length, wet weight and dry weight of post-larvae (Fig.11) was significantly low for treatment-1 (linolenic acid free-diet). But the growth of post-larvae was significantly ($P < 0.05$) improved by the inclusion of linolenic acid at a level of 1% in the diet. However, increasing the linolenic acid level in the diet beyond 1%, depressed growth. The control diet produced the highest gains in length, wet weight and dry weight of post-larvae.

Food conversion ratio (FCR) was significantly ($P < 0.05$) higher and protein efficiency ratio (PER) significantly lower

Fig. 11 Survival rate, growth FCR and PER of post-larvae 11-25 fed on diets containing graded levels of linolenic acid (18:3w3)

FIG. 11.


SURVIVAL (%)
GAIN IN LENGTH (%)
GAIN IN WET WEIGHT (%)
GAIN IN DRY WEIGHT (%)
452·5
FOOD CONVERSION RATIO
PROTEIN EFFICIENCY RATIO
1·622
0·0 1 2 3 4 5 6 C
LINOLENIC ACID LEVEL IN DIET (%)

($P<0.05$) for the diet without linolenic acid (Diet 1). The FCR and PER, were significantly ($P<0.05$) improved by the inclusion of 1% linolenic acid in the diet (Diet 2). However, addition of linolenic acid in the diet at levels above 1% did not improve the FCR or PER (Fig.11). The FCR and PER were significantly ($P<0.05$) superior for the control diet when compared to all other diets. Although 1% linolenic acid diet produced significantly better growth as well as food and protein utilisation as compared to the diets containing other levels of linolenic acid, the control diet containing natural lipid sources such as codliver oil, soyabean oil and lecithin, which contain polyunsaturated and highly unsaturated fatty acids of w3 and w6 series, provided significantly higher growth rate and better rates of food and protein utilization in post-larvae 11-25.

The moisture, protein, lipid and ash contents of post-larvae from various dietary treatments are given in Table 19. Analysis of variance of the data indicated that the proximate composition of post-larvae 11-25 was significantly ($P<0.05$) affected by the dietary level of linolenic acid. While the protein and lipid contents were significantly lower ($P<0.05$), the ash and carbohydrate contents were significantly higher ($P<0.05$) in post-larvae fed on the linolenic acid-deficient diet (Diet 1). But the protein and lipid contents of post-

TABLE - 19 EFFECTS OF DIETARY LINOLENIC ACID LEVELS ON THE BIOCHEMICAL COMPOSITION OF THE POST-LARVAE 11-25

Diet No.	Fatty acid Level in the diet (%)	Moisture (%)	Percentage on dry weight basis			
			Protein	Lipid	Carbohydrate	Ash
1	0.0	77.080	60.450	8.110	3.61	19.95
		±0.030	±0.250	±0.100	±0.11	±0.05
2	1.0	77.345	65.550	11.900	2.21	17.95
		±0.365	±0.350	±0.10	±0.11	±0.40
3	2.0	77.500	65.826	11.510	2.83	16.51
		±0.492	±0.783	±1.134	±0.12	±0.08
4	3.0	78.260	62.850	11.705	2.95	18.24
		±0.340	±0.150	±0.695	±0.05	±0.33
5	4.0	76.990	62.900	12.890	2.80	17.21
		±0.390	±0.100	±0.890	±0.10	±0.41
6	5.0	77.570	62.430	12.510	3.26	16.49
		±0.518	±0.684	±0.695	±0.094	±0.17
7	6.0	77.400	62.366	12.900	3.30	16.23
		±0.569	±0.590	±1.270	±0.10	±0.12
8	Control	74.970	68.550	13.50	1.25	14.13
		±0.707	±0.450	±1.400	±0.05	±0.06

larvae were significantly ($P<0.05$) higher and carbohydrate contents significantly ($P<0.05$) lower in post-larvae fed on a diet with 1% linolenic acid than that of diet 1. The protein retention was significantly ($P<0.05$) improved by the inclusion of 1 and 2% linolenic acid in diets 2 and 3 respectively. But protein retention was the highest in the post-larvae fed on the control diet, and it did not vary significantly between diets 4 to 7. Inclusion of 1.0% linolenic acid in the diet also significantly enhanced the lipid content of post-larvae. However, inclusion of increasing levels of linolenic acid in diets 3 to 7 did not significantly improve the lipid content of post-larvae. Though the post-larvae fed on the contrl diet had relatively higher lipid content than that of other diets, the observed differences were not significant. Ash content of post-larvae was significantly higher ($P<0.05$) in treatment 1 (diet 1 without linolenic acid) and significantly lower ($P<0.05$) in the control group (diet containing w3 and w6 fatty acids) than other treatments. There were no significant differences in the ash content of post-larvae in between treatments 2 to 7 (diets containing linolenic acid levels ranging from 1 to 6%).

JUVENILES

Results of the feeding experiments conducted in juvenile *P. indicus* with 11 diets, containihg selected levels of linolenic acid and linoleic acids, their combinations and the control diets

are shown in Table 20 and Fig. 12, 13, 14, 15 and 16.

Survival of juvenile prawns was significantly ($P < 0.05$) influenced by the dietary fatty acids. Deletion of both linoleic and linolenic acids from the diet, results in significantly ($P < 0.05$) low survival. Inclusion of linoleic acid (18:2w6) at 1% and 2% levels in the diets produced relatively better survival than inclusion of linolenic (18:3w3) acid at the same levels. Among the four diets with a mixture of linolenic acid (18:3w3) and linoleic acid (18:2w6), diet 7 containing 1% 18:3w3 and 1% 18:2w6 produced relatively higher survival. In general, survival was poor in all the treatments, except the controls (Diet 10 and 11). During the first fifteen days the survival was around 90% with all the diets, except for diet 1 (70%) containing only palmitic acid as lipid source. Mortality rate increased thereafter with an abrupt decline in prawn numbers in the 4th week of the experiment, with the exception of the control diet fed prawns. (Table 20).

The data for growth of juvenile prawns expressed as percentages of mean gains in length, wet weight and dry weight are shown in Fig. 12 and 13. The growth of prawns was significantly ($P < 0.05$) influenced by the diets fed to them. Among the diets, diet 1 containing only palmitic acid as the lipid source produced the lowest gains in length and weight. Of the two diets (Diet 2 and 3) containing linolenic acid, diet 3

TABLE - 20 WEEKLY SURVIVAL OF JUVENILE PRAWNS FED ON THE VARIOUS DIETS CONTAINING FATTY ACIDS

Diet No.	I WEEK %	II WEEK %	III WEEK %	IV 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
11	100	100	100	100	90

with 2% linolenic acid produced significantly higher growth than diet 2 with 1% linolenic acid. However, inclusion of 1% linoleic acid in the diet (Diet 4) supported higher growth than diet 5 with 2% linoleic acid. Inclusion of 2% linoleic acid significantly ($P < 0.05$) retarded growth. Inclusion of linolenic acid in the diets significantly ($P \leq 0.05$) enhanced growth when compared to linoleic acid.

Among the four diets compounded with mixtures of linoleic and linolenic acids (Diets 6, 7, 8 and 9), diet 6, in which linolenic acid and linoleic acid were incorporated at 1% level in the ratio of 0.5:0.5 supported superior growth. Though diet 9, containing 1% 18:3w3 and 2% 18:2w6 supported significantly ($P < 0.05$) higher growth than diets 7 and 8, it produced low survival rate (35%).

Of the two control diets containing the mixture of codliver oil, soyabean oil and soya lecithin, diet 11 containing 12% lipid produced the highest growth in this feeding trial. The mean percent gains in length, wet weight and dry weight recorded were 122.79%, 815% and 956%, respectively. Diet 10 containing 10% lipid also produced very high growth when compared to diets 1 to 9, which had purified fatty acids as a source of lipid.

Food conversion ratios and protein efficiency ratios obtained for various diets are shown in Fig. 14. The two

Fig. 13 Percent gain in wet weight and dry weight of juvenile prawns fed on diets containing different levels of fatty acids

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% linolenic acid + 1% linoleic acid
8	7% Palmitic acid + 2% linolenic acid + 1% linoleic acid
9	7% Palmitic acid + 2% linoleic acid + 1% linolenic acid
10	5.60% Codliver Oil + 2.8% soyabean oil + 1.6% lecithin
11	6.67% Codliver oil + 3.33% soyabean oil + 2% lecithin

Fig. 13.

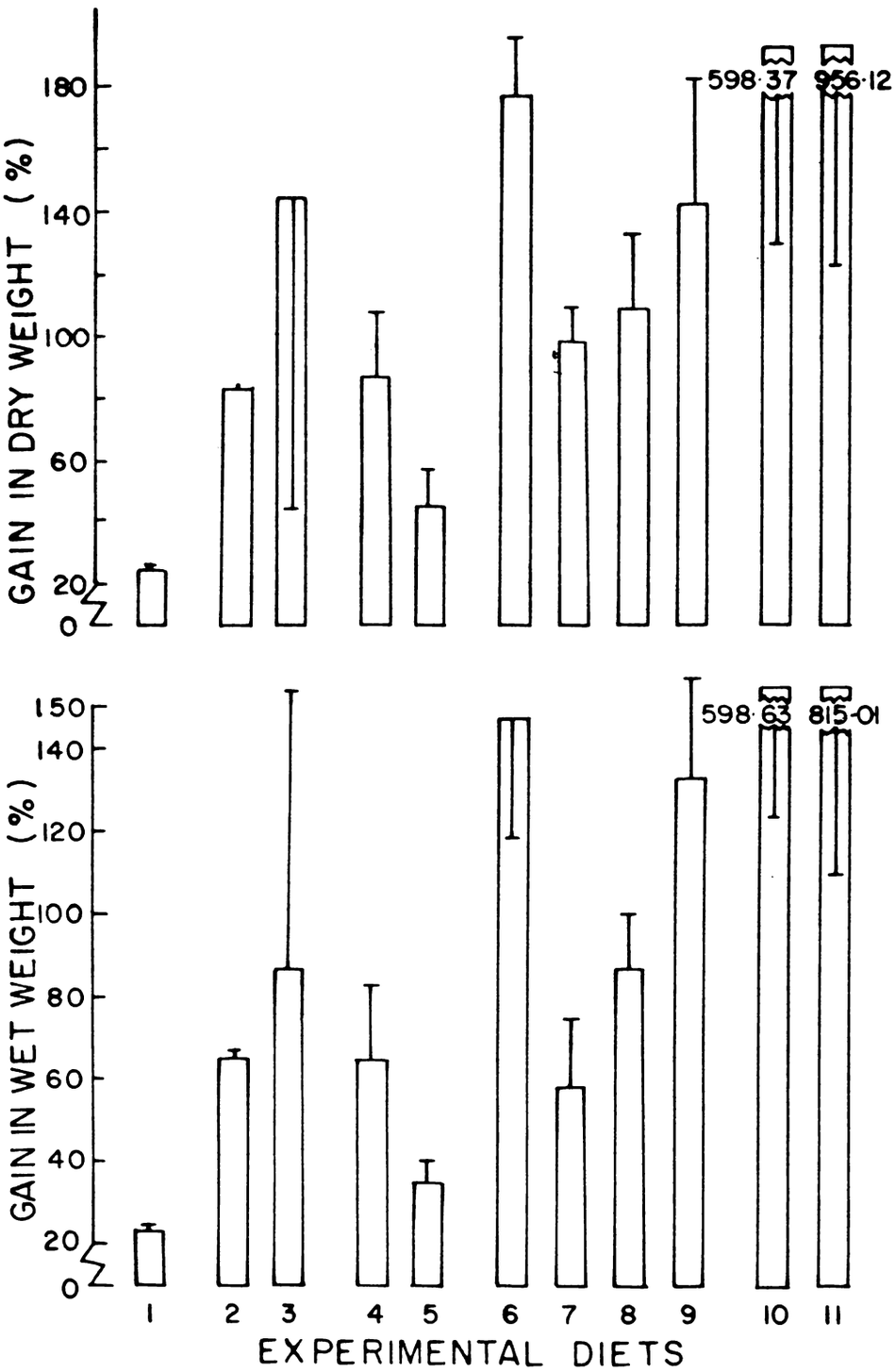

GAIN IN DRY WEIGHT (%)
180
140
100
60
20
0
598-37
956-12
GAIN IN WET WEIGHT (%)
150
140
120
100
80
60
40
20
0
598-63
815-01
1
2
3
4
5
6
7
8
9
10
11
EXPERIMENTAL DIETS

Fig. 13 Percent gain in wet weight and dry weight of juvenile prawns fed on diets containing different levels of fatty acids

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% linolenic acid + 1% linoleic acid
8	7% Palmitic acid + 2% linolenic acid + 1% linoleic acid
9	7% Palmitic acid + 2% linoleic acid + 1% linolenic acid
10	5.60% Codliver Oil + 2.8% soyabean oil + 1.6% lecithin
11	6.67% Codliver oil + 3.33% soyabean oil + 2% lecithin

control diets (Diet 10 and 11) producing the greatest growth and survival also provided the best FCR (Diet 10-3.40; Diet 11-2.754) and PER (Diet 10-0.804; Diet 11-1.012). The FCR and PER recorded for Diet 11 containing 12% lipids was significantly higher than all other diets including Diet 10 containing 10% lipid. Deletion of unsaturated fatty acids from the diet (Diet 1) resulted in significantly ($P < 0.05$) high FCR (26.54) and low PER (0.10).

Of the two diets containing only linolenic acid (Diet 2 and 3), diet 3 provided slightly higher PER; but there were no significant differences in the FCR between these two diets. Of the two diets containing only linoleic acid (Diet 4 and 5) diet 4 containing 1% linoleic acid provided significantly higher PER and lower FCR than diet 5. Among the four diets containing mixtures of 18:3w3 and 18:2w6, diet 9, provided significantly low FCR and high PER.

The influence of dietary lipids upon the moisture, protein, lipid, cholesterol, carbohydrate and ash content of prawns is shown in Fig. 15 and 16. The juvenile prawns fed on the diet deficient in unsaturated fatty acids had relatively high moisture and ash contents, but low protein, lipid and cholesterol contents.

The protein content was significantly ($P < 0.05$) higher in prawns fed on the control diets (Diet 10 and 11) than those

Fig. 14 FCR and PER of juvenile prawns fed on diets containing different levels of fatty acids.

Diet No.	Dietary lipids/fatty acid acid 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	5.60% Codliver oil + 2.8% soyabean oil + 1.6% lecithin
11	6.67% Codliver oil + 3.33% soyabean oil + 2% lecithin

Fig. 12 Percent survival and gain in length of juvenile prawns fed on diets containing different levels of fatty acids.

Diet No.	Dietary lipids/fatty acid 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	5.60% Codliver oil + 2.8% soyabean oil + 1.6% lecithin
11	6.67% Codliver oil + 3.33% soyabean oil + 2% lecithin

Fig. 12.

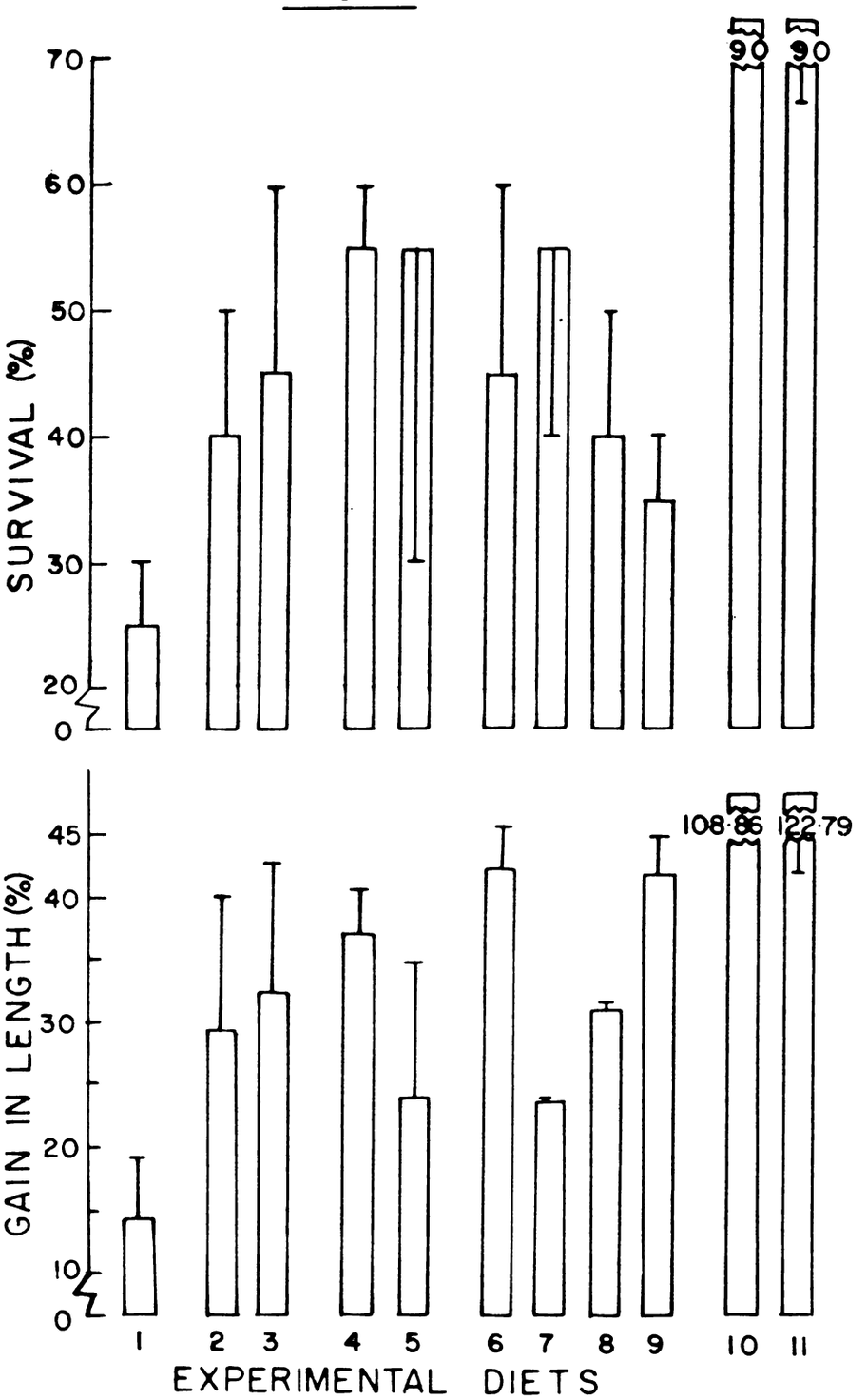

SURVIVAL (%)
70
60
50
40
30
20
0
90
90
GAIN IN LENGTH (%)
45
40
30
20
10
0
108·86
122·79
1
2
3
4
5
6
7
8
9
10
11
EXPERIMENTAL DIETS

Fig. 15 Percent moisture, protein and lipid composition of juvenile prawns fed on diets containing different levels of fatty acids

Diet No.	Dietary lipids/fatty acid 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% lenolenic 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	5.60% Codliver oil + 2.8% soyabean oil + 1.6% lecithin
11	6.67% Codliver oil + 3.33% soyabean oil + 2% lecithin

fed on other diets. Inclusion of linoleic and linolenic acid in the diets also relatively improved the protein and lipid retention in prawns in most of the treatments. Similarly, the ash content was significantly lowered in the control diet and by incorporation of unsaturated fatty acids in diets.

Lipid content was significantly higher in the groups of prawns fed the control diets (Diet 10 and 11) and Diet 9 having a mixture of 2% linoleic and 1% linolenic acid than those prawns fed other diets (Diet 1 to 8). However there was no significant difference in the lipid content of prawns on diets 1 to 8. Cholesterol content was significantly lower in prawns fed the diets without unsaturated fatty acids than all the remaining groups of prawns which were fed the diets with unsaturated fatty acids. Cholesterol accumulation was more in the prawn group fed on diet containing either 2% linolenic or linoleic acid (Diet 3 and 5). Cholesterol content was significantly low ($P < 0.05$) in prawns fed on the control diets containing PUFA of w3 and w6 series (treatment 10 and 11) and the prawns fed on a mixture of linolenic acid and linoleic acid in the ratio 1:2.

D I S C U S S I O N

The present experiments clearly indicate the essentiality of a blend of unsaturated fatty acids of the w3 and w6 series fo

Fig. 14

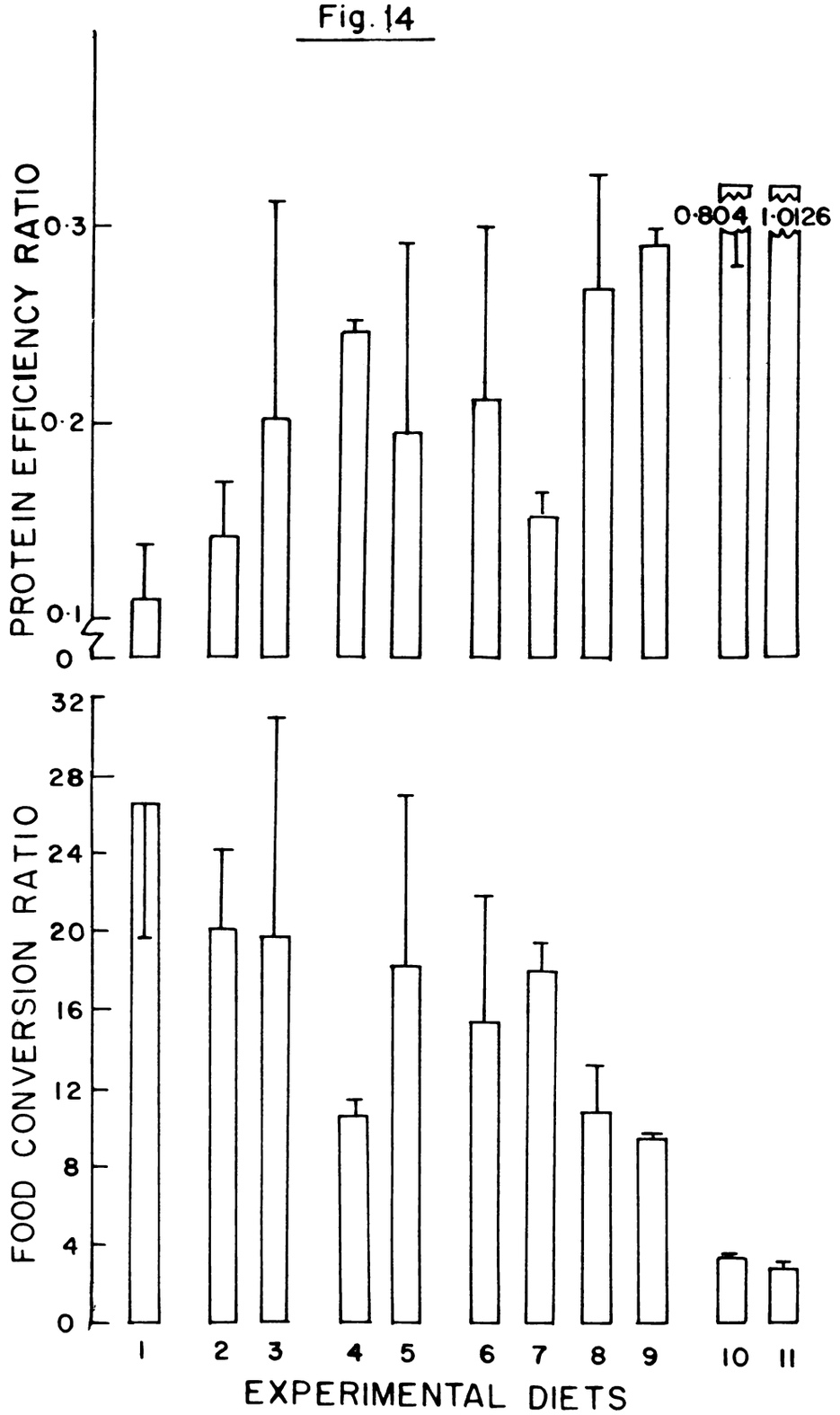

PROTEIN EFFICIENCY RATIO
0·3
0·2
0·1
0
0·804
1·0126
FOOD CONVERSION RATIO
32
28
24
20
16
12
8
4
0
1
2
3
4
5
6
7
8
9
10
11
EXPERIMENTAL DIETS

Fig. 15.

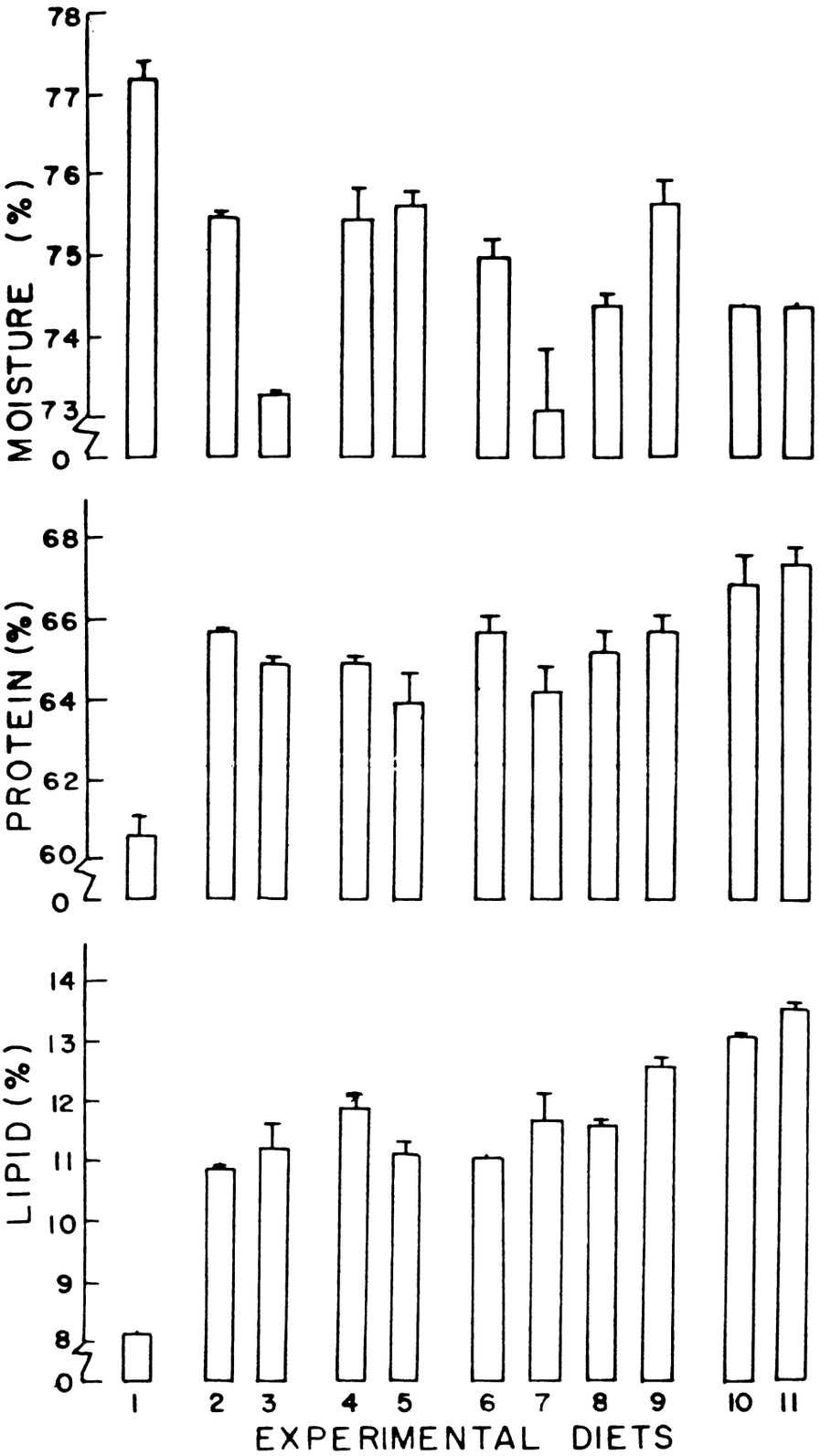
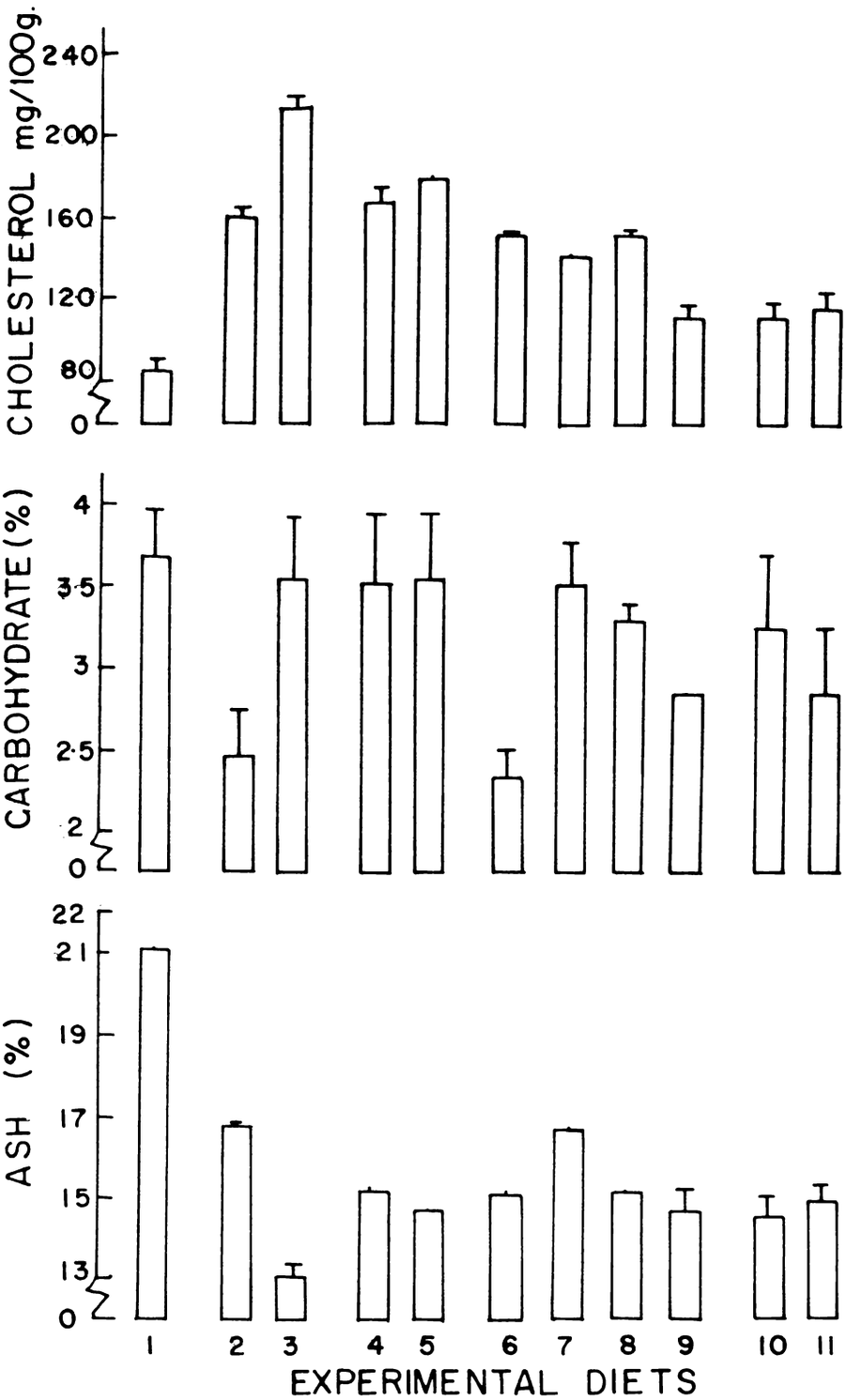

MOISTURE (%)
78
77
76
75
74
73
0
PROTEIN (%)
68
66
64
62
60
0
LIPID (%)
14
13
12
11
10
9
8
0
1
2
3
4
5
6
7
8
9
10
11
EXPERIMENTAL DIETS

Fig. 16 Percent cholesterol, carbohydrate and ash composition of juvenile prawns fed on diets containing different levels of fatty acids

Diet No.	Dietary lipids/fatty acids used for juvenile
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	5.60% Codliver oil + 2.8% soyabean oil + 1.6% lecithin
11	6.67% Codliver oil + 3.33% soyabean oil + 2% lecithin

Fig. 16.


CHOLESTEROL mg/100g.
240
200
160
120
80
0
CARBOHYDRATE (%)
4
3·5
3
2·5
2
0
ASH (%)
22
21
19
17
15
13
0
1 2 3 4 5 6 7 8 9 10 11
EXPERIMENTAL DIETS

proper survival, growth, FCR, PER and retention of protein and lipid in prawns. Diets containing purified fatty acids (linoleic and linolenic acid) were poorly accepted by the prawn larvae. Inclusion of these fatty acids caused completed mortality of larvae. But larval survival, growth and metamorphosis were significantly improved by the inclusion of mixture of codliver oil, soyabean oil and lecithin, which contain highly unsaturated fatty acids in the diets (Table 18A). Besides, when phytoplankton was fed to the larvae, they metamorphosed and grew to post-larvae 1, within 9 days. Similar observations were reported in P. japonicus in which all larvae died without metamorphosis to post-larvae 1, (Kanazawa et al., 1985) when fed diets with 1% level of EFA such as 18:2w6 and 18:3w3, individually, or a mixture of 18:2w6 and 18:3w3 (1:1) to the diet containing 7% 18:1w9 as lipid source. But diet containing 8% pollack liver oil + 3.5% soyabean phosphotidylcholine provided 86% survival, and they grew upto post-larvae 1 within 6 days. Jones et al. (1979b) and Teshima and Kanazawa (1984) pointed out the necessity of 20:5w3 and 22:6w3(w3 HUFA) for survival and growth of larval stages of P. japonicus. From the present study it is also evident that P. indicus larvae require lipids containing HUFA for proper growth and survival.

The poor rate of growth, food and protein utilization by the post-larvae and juvenile prawns when fed on a diet without either 18:3w3 or 18:2w6 indicates the essentiality of

18:3w3 and 18:2w6 for post-larvae and juvenile prawns. However the superior survival, growth, FCR, PER and deposition of lipids and protein in prawns fed on control diets containing highly unsaturated fatty acids indicate that these fatty acids are indispensable for post-larvae and juveniles of P. indicus also. The control diets had codliver oil, which is a rich source of 20:5w3 (eicosapentaenoic acid) and 22:6w3 (docosahexaenoic acid), which have been found to be essential for Penaeus japonicus (Kanazawa and Teshima, 1977; Kanazawa et al., 1979a). Besides, cod-liver oil contains 18:3w3, 18:2w6 and 20:4w6 at relatively low levels. Soyabean oil is a rich source of linoleic acid (18:2w6) and it also has low levels of 18:3w3. The incorporation of lecithin (phosphatidylcholine) a phospholipid rich in PUFA perhaps further help to improve survival significantly in P. indicus as observed for P. japonicus (Kanazawa, 1985) and lobsters (Conklin et al., 1980; and D'Abramo et al., 1981a). Since all these three lipid sources were incorporated in the control diets it appears that these diets provide a blend of fatty acids required for promoting growth and supporting good survival in P. indicus.

From the improvements in growth and survival brought about by the inclusion of linoleic and linolenic acids, it is evident that the post-larvae as well as juveniles of P. indicus may have limited capabilities to convert 18:3w3 and and 18:2w6 into the essential highly unsaturated fatty acids.

Probably the prawn is unable to convert these fatty acids into highly unsaturated fatty acids more efficiently due to low activity of the enzyme systems involved in chain elongation and desaturation as demonstrated in Penaeus japonicus (Kanazawa et al., 1977a, 1979d, 1979g and Teshima, 1978). Thus these results suggest the need for inclusion of lipids containing highly unsaturated fatty acids in adequate levels in the diet of P. indicus.

The control diet had fatty acid levels of 31.88% saturated; 28.8% monounsaturated; 18.1% 18:2w6 (linolenic), 3.116% 18:3w3 (linolenic) and 11.9% HUFA of w3 series (Table33). Thus this diet provides a blend of polyunsaturated fatty acids essential for larvae, post-larvae and juveniles, thereby produced the highest survival, growth and better efficiencies of conversion of food and protein, and deposition of nutrients.

Though 1% linoleic as well as 1% linolenic acid diets improved the growth over that of the diets deficient in these fatty acids, more than 1% (except 2% linolenic acid) level of these fatty acids has no beneficial effect on post-larvae or juvenile prawns. In fact, fatty acid levels above 3% seems to be detrimental to the animals. The retarded growth of post-larvae 1-10 and 11-25 on diets containing higher (more than 3%) levels of linolenic acid indicates that the prawn P. indicus is unable to tolerate high levels of linolenic acid in the diets. In addition to 18:3w3 it also require HUFA of

w3 series and 18:2w6 as essential fatty acids. Similar observations were made while evaluating the nutritive value of natural lipid sources for P. indicus (Chapter 4). Linseed oil containing high levels (41.05%) of 18:3w3 was unable to produce maximum growth in post-larvae and juveniles of P. indicus. This evidence further supports the present findings that higher levels of linolenic is unable to provide better growth in P. indicus. Besides, linoleic acid is found to be inferior to that of linolenic in efficacy. This observation agrees with that of Kanazawa et al. (1977a, and 1979d) in P. japonicus, in which also the dietary requirement of linolenic or linoleic acids was 1% of the diet and the effect of linolenic acid was found to be superior to linoleic acid. The requirement of linoleic (18:2w6, linolenic (18:3w3), eicosapentaenoic(20:5w3) and docosahaxaenoic (22:6w3) acids was shown by Kanazawa and Teshima (1977) and Kanazawa et al. (1979b) on P. japonicus and and by Kanazawa et al. (1979c) in P. Monodan and P. merguiensis. Shewbart and Mies (1973) also revealed that the growth of P. aztecus was improved by the addition of 1% 18:3w3 to the diet. Ferucci et al. (1981) reported the requirement of linoleic and linolenic acids for P. stylirostris, and they found a correlation between rate of growth and the percentage of 18:3w3 in the diet of juvenile P. stylirostris and in this species a ratio of 1.18 = (w3/w6) is favourable.

Recently Read (1981) studied the requirement of linolenic and linoleic acids for P. indicus and reported 2% 18:3w3 or 18:2w6 gives significantly better growth than 5% lauric acid, when he used a lipid level of 5% in the diet of P. indicus without considering the total lipid requirement of the species. Only 5% of total basal lipid in the diet may not be enough to supply the required energy for animals. In the present experiment, 8 to 9% basal lipid was used which could fulfil the demand of energy in the form of lipids required by the animal thereby linoleic and/or linolenic acid could be available for body building/growth. Thus 1 to 2% linolenic and 1% linoleic acid appears to be sufficient enough to promote growth in P. indicus postlarvae and juvenile prawn. Another important difference is that I have used all purified ingredients for lipid, protein and carbohydrates; but Read (1981) used natural ingredients which might have influenced the animals response to 18:3w3 and18:2w6.

In the present study with juvenile prawns the mixture of 18:2w6 and 18:3w3 when used in the ratio 0.5:0.5 at 1% level supported good growth. It appears that a mixture of these two acids provide a balanced proportion of these fatty acids. But when this mixture was used at 2% level in the ratio of 1:1 the growth of prawn decreased. Similar observations in juvenile P. indicus was reported by Read (1981), when he used mixture

of linoleic and linolenic acid in the ratio 0.5:0.5 along with 2% PUFA and 1% lauric acid in the diet he found better growth than the diet containing 1% linolenic acid. But when he used a mixture of linolenic and linoleic acid in the ratio 1:1 at 2% level growth decreased when compared to either of 2% linoleic or 2% linolenic acid.

Earlier reports on crustacean nutrition indicate, that the unsaturated fatty acids of the linolenic group (w3 series of fatty acids) are essential (Kanazawa et al., 1970a; Shewbart and Mies, 1073; Guary et al., 1976a; Castell and Covey, 1976; Sandifer and Joseph, 1976; Kanazawa and Teshima, 1977; Kanazawa et al., 1977b, 1979c; Jones et al., 1979b; D'Abramo et al., 1980; Bottino et al., 1980; Dall and Moriarity, 1983; Petrilla et al., 1984). Although, some prawns require more quantity of w3 fatty acids, particularly P. japonicus for growth promotion, P. indicus require both w3 as well as w6 series of fatty acids (Read, 1981), which is also confirmed in the present study. The control diets containing codliver oil and soyabean oil and lecithin produced maximum growth as this lipid provide a blend of fatty acids (w3 and w6) required for promoting growth and supporting good survival in P. indicus New (1976) suggested that linolenic and other w3 fatty acids are found to be essential for prawns and that the ratio of w3:w6 is important; high w3:w6 diets are beneficial for prawns, indicating the importance of w6 fatty acids along

with w3 fatty acids as is observed in the present study with P. indicus.

Kanazawa (1985) who reviewed the fatty acid requirement in prawns stated that prawns require 18:2w6, 18:3w3, 20:5w3, 22:6w3 fatty acids as essential nutrients as they are not biosynthesized. Kanazawa et al. (1977a, 1978, 1979d, 1979f) have shown by feeding experiments that juveniles of P. japonicus have a higher weight gain with diets containing 18:2w6, 18:3w3, 20:5w3, or 22:6w3 than with other acids like 18:1w9, 18:0 indicating the necessity of w3 fatty acids, especially w3 HUFA. There are many reports available on the w3 HUFA requirement of prawns and lobsters(Page 1[illegible]). But some reports are also available in which the importance of w6 fatty acids, alone with w3 fatty acids was indicated. Deshimaru et al. (1979)reported very good growth in the prawn P. japonicus when they fed a diet containing a mixture of pollack liver oil and soyabean oil in the ratio ranging from 3:1 to 1:1 containing 20 to 30% of w6 and 10 to 20% of w3 fatty acids and also suggested these levels of fatty acids to be optimum for growth of prawn. Deshimaru et al.(1979) and Golvin (1976b)reported plant oils rich in 18:2w6 along with fish oil to produce better growth in P. japonicus and P. indicus respectively.

Although many reports on nutritional requirements of various kinds of lipids for prawns are available (Sick et al., 1972; Shewbert and Mies, 1973; Sick and Andrews, 1973,

Forster and Beard, 1973; Balaz et al., 1973; New, 1976; Kanazawa et al., 1970, 1977b, Kanazawa 1985) most of these did not support the results with food conversion efficiencies and protein efficiency ratios. Data on the FCR, PER and nutrients deposition in body of P. indicus recorded during the present study also clearly indicate that a mixture of w6 and w3 polyunsaturated fatty acids are essential for the efficient utilization of the ingested food and protein. This is clear from the FCR and PER and percentages of lipid and protein deposited in the body of prawnsfed the control diets with a mixture of codliver oil, soyabean oil and lecithin. Besides, exclusion of unsaturated fatty acids from the diets (both w6 and w3) of prawn severely affected the utilization of ingested food and protein, and protein deposition in the body. Colvin (1976b) also reported better food and protein utilization, along with more protein and lipid deposition in the body by the juveniles of P. indicus when fed diets containing both plant and animal lipids which contain 18:2w6 and HUFA of w3 series. Read (1981) also reported better food conversion when juveniles of P. indicus were fed a diet containing a mixture of fish and sunflower oil. Thus the present findings agrees with the observations of the above authors that the post-larvae and juveniles of the prawn P.indicus require a mixture of plant and animal lipids which can provide high levels of HUFA of w3 and w6 series for better utilization of food and protein, and for more protein and lipid deposition

in the body. More protein deposition in the body may augment growth. Thus the higher growth in prawns fed on diets containing EFAs may be due to the protein sparing action of essential fatty acids. Castell et al. (1972a) and Watanabe (1982) also assume that essential fatty acids in diets may have protein sparing action in fish.

The essential fatty acids requirement of fin fish differ considerably from species to species. Rainbow trout require fatty acids of the linolenic family (w3) as EFA;where as the carp, eel and chum salmon require not only linolenic but also linoleic acids for good growth. On the other hand, these fatty acids were found to be ineffective for the marine fishes, red sea bream, plaice and yellow tail. The latter three species were found to require w3 HUFA such as 20:5w3 and 22:6w3 as essential fatty acids (Watanabe,1982). These observations indicate the significant influence of habitat on fatty acid requirements. As the post-larvae, juvenile and immature adults of P. indicus are generally found in low saline waters (Menon 1955, Menon and Raman 1962, Mohamed and Rao 1971, Paul Raj, 1976) they may have a requirement for HUFA of w3 series as well as for 18:2w6 and 18:3w3. However, the larvae of the species, as they are marine may have a requirement for enhanced levels of HUFA especially w3 series. But this needs further elucidation. The fatty acid composition of P. indicus

lipids shows more percent of 16:1, 18:2w6, 18:3w3 fatty acids during its estuarine phase of life. W3 PUFA like 20:5w3 and 22:6w3 are relatively less (8.4) in estuarine phase when compared to marine phase (21%). The ratio of w3/w6 was also less during estuarine phase and more in marine phase (Table 21) From the fatty acid content of prawn lipids and from the results of the present experimental studies it can be assumed that the post-larvae and juveniles of prawn (estuarine phase) may have a dietary requirement for 18:2w6, 18:3w3 along with 20:5w3 and 22:6w3 (w3 HUFA). Thus the superior growth, PER, protein retention and FCR observed in post-larval and juveniles when fed diets containing codliver oil, soyabean oil and lecithin can be due to the concentration of w3 and w6 fatty acids as these stages were reared in low-saline waters.

TABLE - 21 FATTY ACID COMPOSITION (%) OF LIPID (FROM WHOLE BODY OF PRAWN FROM ESTUARINE AND MARINE P. INDICUS AND MARINE AND PRESHWATER FISH

Fatty acid	P. indicus				Fish	
	Marine	Estuarine	Estuarine	Estuarine	Marine	Fresh
14:0	2.4	3.5	1.26	1.13	5.00	3.10
14:1	0.613	0.813	0.89	-	0.39	0.80
15:0	1.6	1.5	0.89	-	0.36	0.53
16:0	15.4	20.4	14.14	15.48	10.89	13.20
16:1	8.3	12.9	7.22	7.53	12.00	16.20
17:0	3.0	2.7	1.92	2.24	0.21	0.52
17:1	1.0	0.94	-	0.94	0.12	0.62
18:0	7.4	7.4	7.28	8.19	1.16	2.75
18:1w9	13.0	13.5	9.95	12.81	12.60	29.00
18:2w6	2.5	4.9	2.26	4.26	0.74	2.18
18:3w3	1.1	1.6	0.99	1.03	0.28	1.93
18:4w3	1.9	1.5	1.46	-	1.53	1.27
20:1w9	-	-	2.52	1.39	-	-
29:4w6	6.1	4.6	6.50	8.68	0.80	3.48
20:5w3	9.5	9.1	11.17	11.24	7.90	5.50
24:0	2.4	0.8	1.54	-	-	-
24:1	0.9	-	3.23	1.21	0.36	0.32
22:5w3	2.1	1.1	-	1.88	3.31	1.54
22:6w3	11.9	5.2	9.30	11.00	7.84	3.87
Total saturated	29.1	36.0	27.03	27.04	17.41	20.10
Mono unsaturated	23.83	28.7	23.81	24.97	25.47	46.94
Total w6	8-6	9.6	8.76	12.97	1.54	5.56
Total w3	25.6	18.5	22.92	23.27	20.42	14.11
HUFA w3	22.6	15.4	20.47	22.48	19.05	10.91
HUFA w6	6.1	4.6	6.50	8.68	0.8	3.48
Author	Read(1977)		Colvin(1976) Chandge		ACKMAN(1967)	

CHAPTER - IV

NUTRITIVE VALUE OF NATURAL LIPID SOURCES

INTRODUCTION

It is well recognised that the lipids derived from different sources significantly influence the response of the recipient animals, due to their composition. Since the fatty acids profile of dietary lipids have been found to significantly affect the response of prawns, it becomes essential to identify suitable natural lipid sources or their combinations for incorporating into practical feed formulations. Also, the fatty acids in dietary lipids have important metabolic significance. Certain fatty acids are essential for growth, maintenance and proper functioning of many physiological processes in animals (Alfin-Slater and Aftergood, 1968). An absolute essentiality of certain fatty acids in the diet has been demonstrated for many species of prawns (Kanazawa, 1985). Studies carried out in P. indicus during the present investigation (Chapter 3) also indicated that all the stages of P. indicus require lipids which can supply adequate levels of poly unsaturated fatty acids (HUFA) such as, 18:2w6, 18:3 w3, 20:5 w3 and 22:6 w3 which are essential for better growth and survival.

Earlier studies have revealed that the nutritive value of natural lipids for prawns and shrimps depend upon

the types and contents of essential fatty acids (EFA) in the dietary lipid source. High nutritive value of lipids, such as menhadden oil for P. duorarum has been attributed to the high contents of polyunsaturated fatty acids in the oil(Sick and Andrews, 1973). Shrimp-head oil has been found to be a good lipid source in the diet of M. rosenbergii (Sandifer and Joseph, 1976). Kanazawa et al. (1977 b) have also pointed out that the superior dietary value of marine lipids such as pollack liver oil and short-necked clam oil is due to their high contents of w3-HUFA, where as the inferior dietary value of soyabean oil is due to the shortage of w3-HUFA such as 20:5 w3 and 22:6 w3. Guary et al. (1976a) also showed a higher nutritive value for sardine oil and short-necked clam oil than for linseed oil and soyabean oil for P. japonicus. Aquacop (1978) reported that codliver oil promoted growth and survival of P. merguiensis as the best source of lipid.

The foregoing informations suggest that lipid sources rich in w3 HUFA are better for high survival and growth promotion in prawns. Recent investigations further indicate that mixtures of plant and marine lipids are more effective than only animal or plant lipids for promoting growth in prawns. Deshimaru and Kuroki (1974b) and Deshimaru et al. (1979) have shown that good lipid source for P. japonicus diet was

a mixture of soyabean oil and pollack liver oil. Colvin (1976b) reported that a mixture of wheat-germ oil, peanut oil and fish meal residual oil was a good lipid source in the diet for optimal growth in P. indicus. Read (1981) also reported that a mixture of sunflower oil and fish oil in the ratio 2:1 in the diet give maximum growth, compared to those diets containing only fish or plant oils. These informations suggest that prawns in general, may require a mixture of lipids from marine animals and plant oils in the diet for optimum growth and survival.

According to Alfin-Slater and Aftergood (1968) an animal's fatty acid requirement can be gauged from its tissue fatty acids composition. Inspection of P. indicus fatty acid pattern (Colvin, 1976b; Read, 1981) presented in Table 21 showed a preponderance of short and long chain w6 and w3 fatty acids similar to the marine fish lipids (Ackman, 1967). This indicates that P. indicus may need both w6 and w3 HUFA for growth and survival as reported for other prawns (Deshimaru et al., 1979). Although, Colvin (1976b) and Read (1981) reported that juvenile P. indicus require a mixture of plant and animal lipid source in the diet as P. indicus is an omnivore, they have used only 3% (Read, 1981) or 5% (Colvin, 1976b) lipid level in the diet along with natural ingredients for proteins and carbohydrates,

without understanding the actual lipid level required in the diet. Besides, there is no information on the effects of natural lipid sources on larvae and post-larvae of P. indicus. Therefore, as a part of the present study experiments were conducted to identify suitable plant and animal lipid sources for formulation of practical diets for larvae, post-larvae and juveniles of P. indicus.

M A T E R I A L A N D M E T H O D S

A total of six experiments were conducted; of these three experiments were conducted with larvae and one each with post-larvae 1-10, post-larvae 11-25 and juveniles. Fifteen naturally occurring oils were used for formulating the diets, either individually or in combinations of 2 or three oils. The basal dietary composition used for larvae, post-larvae 1-10, post-larvae 11-25 and juvenile P. indicus is same as given in Table 2. Lipid sources used in the experimental diets for larvae are given in Table 22, for post-larvae 1-10 in Table 23, for post-larvae 11-25 in Table 24 and for juveniles in Table 25.

All lipid sources were obtained from the local market; lecithin was obtained from Sigma Chemical Co., USA. All

TABLE - 22 LIPID SOURCES USED IN THE DIETSOF LARVAL PRAWNS

Experiment No.	Diet No.	Lipid source used
I	1	Phytoplankton (Control)
	2	Codliver oil
	3	Soyabean oil
	4	Codliver oil + Soyabean oil (5:5)
	5	Codliver oil + Soyabean Oil + Lecithin (4:2:4)
	6	Codliver Oil + Soyabean Oil + Lecithin (3.3 : 3.3 : 3.3)
	7	Codliver Oil + Soy lecithin (6:4)
	8	No Food
II	1	Shark-liver oil
	2	Sardine oil
	3	Prawnhead oil
	4	Codliver oil
	5	Phytoplankton (Control)
	6	Groundnut oil
	7	Sunflower oil
	8	Soyabean oil
	9	No Food

(Contd...)

TABLE - 22 LIPID SOURCES USED IN THE DIETSOF LARVAL PRAWNS (Contd.....)

Experimetnt No.	Diet No.	Lipid source used
III	1	Sardine oil + Ground nut oil (1:1)
	2	Sardine Oil + Soyabean Oil (1:1)
	3	Sardine Oil + Sunflower oil (1:1)
	4	Codliver oil + Sunflower oil (1:1)
	5	Shark-liver oil + Sunflower oil (1:1)
	6	Prawn head oil + Sunflower oil (1:1)
	7	Phytoplankton (Control)
	8	No Food

TABLE - 23 LIPID SOURCES USED IN THE DIETS OF POST-LARVAE 1-10

Diet No.	Lipid source used in the diets
1	Coconut oil
2	Mustard oil
3	Cotton seed oil
4	Safflower oil
5	Rapeseed oil
6	Groundnut oil
7	Gingely oil
8	Sunflower oil
9	Corn oil
10	Sharkliver oil
11	Linseed oil
12	Soyabean oil
13	Codliver oil
14	Sardine oil + Sunflower oil
15	Sardine oil
16	Prawnhead oil
17	Prawnhead oil + Soyabean oil
18	Codliver oil + Soyabean oil + Lecithin

TABLE - 24 LIPID SOURCES USED IN THE DIETS OF POST-LARVAE 11-25

Diet No.	Name of lipid source used
1	Coconut oil
2	Groundnut oil
3	Safflower oil
4	Mustard Oil
5	Soyabean oil
6	Rapseed oil
7	Linseed oil
8	Cotton seed oil
9	Gingely oil
10	Sunflower oil
11	Shark liver oil
12	Sardine oil
13	Corn oil
14	Codliver oil
15	Sardine + Sunflower oil
16	Prawn Head Oil + Soyabean oil
17	Prawn Head oil
18	Codliver oil + Soyabean Oil + Lecithin

TABLE - 25 LIPID SOURCES USED IN THE DIETS OF JUVENILE *P. INDICUS*

Diet No.	Lipid source used in the diet
1	Mustard oil
2	Cotton seed oil
3	Soyabean oil
4	Safflower oil
5	Groundnut oil
6	Sunflower oil
7	Linseed oil
8	Corn oil
9	Sardine oil
10	Codliver oil
11	Sharkliver oil
12	Sardine oil + Sunflower oil
13	Sardine oil + Groundnut oil
14	Prawnhead oil + soyabean oil
15	Prawnhead oil
16	Codliver oil + Soyabean Oil + Lecithin

TABLE 26 ENVIRONMENTAL FACTORS, STOCKING DENSITY PER TREATMENT, MEAN INITIAL LENGTH AND WEIGHTS OF ANIMALS, AND FEEDING LEVEL FOR EXPERIMENT ON NUTRITIONAL VALUE OF NATURAL LIPID SOURCES

Parameters	Stages of the prawn			
	Larvae	Post-larvae 1-10	Post-larvae 11-25	Juveniles
Salinity (‰)	34.0 ± 2	32.0 ± 2	20.0 ± 2	20.0 ± 2
Temperature (°C)	29.5 to 31.0	26.0 to 29.5	29.0 to 31.5	26.4 to 29.7
pH	8.0 to 8.2	7.9 to 8.3	8.0 to 8.3	7.9 to 8.3
Dissolved oxygen in water (mg/l)	4.9 to 6.9	4.8 to 6.8	5.0 to 7.9	4.8 to 6.4
Total ammonia -N in seawater (ppm)	0.03 to 0.07	0.04 to 0.09	0.03 to 0.10	0.03 to 0.11
Initial number of prawn/diet	150	60	45	30
Average initial length (mm)	—	6.00	12.00	20.00 to 25.0
Average initial wet weight (mg)	—	0.475	6.20	42.00 to 48.00
Average initial dry weight (mg)	—	0.110	2.10	11.42
Feeding level % of the biomass	100	30-40	30-40	20-30

other details about experimental set up and animals, formulation and preparation of diets, stocking of animals, experimental duration, data collection, composition analysis of experimental animals, statistical analysis of data are same as given in the general materials and methods section (pp 15-20).

Determination of the fatty acids profile

The fatty acids profile of each of the lipid sources used in the experiments and post-experimental juvenile prawns was determined adopting the procedures of Morrison and Smith(1964).

Immediately after measuring the final lengths and weights, the juvenile prawns were freeze dried in a Chemlab freeze dryer at -20°C. Lipid was extracted from the powdered freeze dried prawns.

Two methods frequently used for routine lipid extractions are those of Folch et al. (1957) and Bligh and Dyer (1959). Both use chloroform-methanol as reagents. Both the methods are efficient in extraction of total lipids. Although the yield appears higher using the Folch et al. (1957) method, the extract is not entirely free of non-lipid contaminants (Bligh and Dyer, 1959). For this reason, the Bligh and Dyer(1959) method was chosen as the standard technique for lipid extraction in which choloroform-methanol water in the ratio of 2:2:1.8 was used. To 500 mg of homogenised dry tissue, 10 ml choloroform

(electronic grade), 20 ml methanol and 8 ml distilled water were added, and blended in a homogenizer for 15 minutes; 10 ml choloroform was then added and blended for 10 minutes. This was followed by 10 ml of water and blending was continued for further 10 minutes to ensure complete lipid extraction. The homogenate was filtered through a whatman No.1 filter paper on Buchner funnel under slight suction and the filtrate transferred to separating funnel. The chloroform fraction was removed in a pre-weighed flask and dried to a constant weight under nitrogen. Samples were kept in petroleum ether in screw cap centrifuge tubes under nitrogen medium. Similar procedure was adopted for the oils used in the experiment.

After drying, the lipid was used for saponification. To 50 mg of lipid 5 ml of 10% KOH-methanol was added and the mixture was refluxed at 70 to 80°C on a water bath for 5-10 minutes under nitrogen. To this mixture added 4 ml of double distilled water and washed twice with 12 ml petroleum ether. The aqueous layer was separated and concentrated to remove the methanol. Then added dilute HCl to adjust the pH between 2-3. The acidified aqueous layer was extracted with three volumes of petroleum ether; filtered through sodium sulfate(anhydrous). Petroleum ether was evaporated under nitrogen to obtain free fatty acids.

To the fatty acids, added boron trifluoride methanol (2.5 ml to 5 ml) in a tube and passed nitrogen and then closed the tube with tephlon screw cap. The tubes were heated in a boiling water bath for two minutes, cooled and opened. The fatty acid methyl esters were extracted by adding 2 volumes of petroleum ether and one volume of water, centrifuged at 5000 rpm for 5 minutes in a refrigerated centrifuge. Petroleum ether from the upper layer was evaporated to obtain the methyl esters.

Gas liquid chromatography of fatty acids methyl esters

Gas liquid chromatography of the fatty acid methyl esters was carried out using a Hewlett-Packard Microprocessor controlled gas-liquid-chromatograph (Model 5840A) with a flame ionisation detector. Nitrogen (Indian oxygen Ltd., IOLAR Gases) of ultrapure quality was used as the carrier gas. The methyl esters were dissolved in a convenient volume of hexane or chloroform (electronic grade) and applied (1 µl) to Gas chromatographic column with a Hamilton microsyringe. Gas-liquid-chromatography was performed under the following conditions. The injection port was set at 200°C, the flame ionisation detector at 230°C and the column temperature at 180°C. Chart speed was 0.5 cm/minute. Flow rate of the nitrogen was 30 ml/minute. The range was set at 10^3 and attenuation 16. A 1/8" diameter and 3 m length spiral

stainless steel column packed with 15% diethylene glycol succinate (D.E.G.S) - stationary phase with a solid support of 100-120 mesh solid chromosorb W was used. Quantitative identification of the fatty acid esters was obtained by comparison with the relative retention time of known standards (Applied Science Laboratories, U.S.A. and Suppleco, Switzerland). The identified peaks were quantified using a integrator.

R E S U L T S

LARVAE

Three experiments were conducted with the larvae of Penaeus indicus to determine the best source(s) of lipids required for promoting survival and growth of larvae.

Experiment-I

The first experiment was conducted to examine the dietary value of codliver oil and soyabean oil individually as well as in combinations in various diets for larval P. indicus. The results of this experiment was compared with that of the control diet (phytoplankton) and given in Table 27 A.

All the larvae at protozoea 1 stage died within 2 days without metamorphosis when food was not supplied

(Treatment 8). In the control, where phytoplankton was fed (Treatment 1), the development of larvae followed a normal sequence with the highest survival of 32% at the post-larval 1 stage, which they attained within 8 days of the experiment. There was also significant difference ($P < 0.05$) in the survival of larvae among the test diets (Diet 2 to 6). Survival was found to be significantly ($P < 0.05$) high (22%) when larvae were fed either the diet containing codliver oil and lecithin in the ratio 6:4 (Diet 7), or codliver oil + soyabean oil + lecithin in the ratio 4:2:4 (Diet 5). But larval survival was significantly ($P < 0.05$) low (8%) when fed the diet containing soyabean oil as the sole lipid source. These results indicate that the mixture of codliver oil + soyabean oil + lecithin in the ratio of 4:2:4 or a mixture of codliver oil : lecithin (6:4) in the diet would be better sources of lipids in the diets of larval P. indicus.

Survival of larvae in all the treatments except soyabean oil diet, was more than 70% from protozoea 1 to protozoea 3 stage and remained more or less constant from protozoea 3 to mysis 1 stage with slight decrease in treatments 3, 4 and 5(61 to 66%); survival rates further declined during mysis 1 to mysis 3 stage (less than 52%) in all the treatments. However survival rates of larvae from mysis 3 to post-larvae 1 stage were relatively high, being 100%, 65% and 68% in the

TABLE - 27A GROWTH AND SURVIVAL OF P. INDICUS LARVAE FED ON VARIOUS LEVELS OF LIPID SOURCES. EXPERIMENT I

Diet No.	Name of lipid source used in diet and percentage	Survival rate (%) of various development stages of prawn larvae							
		P1	P2	P3	M1	M2	M3	PL1	Feeding pd.days
1.	Phytoplankton	100	90.00	90.0	82.0	51.33	38.67	27.33	8
2	Codliver oil 10	100	80.67	72.0	64.0	44.67	24.67	10.67	8
3	Soyabean oil 10	100	71.34	42.0	34.0	21.34	8.0	2.0	10
4	Codliver oil + Soyabean oil 5+5	100	74.67	61.34	51.34	28.0	10.67	2.0	10
5	Codliver oil + Soyabean oil + Lecithin, 4+2+4	100	77.34	64.00	54.0	33.34	14.67	6.0	10
6	Codliver oil + Soyabean oil + lecithin 3.3 + 3.3 + 3.3	100	76.00	66.67	55.34	42.67	28.0	15.34	10
7	Codliver oil + lecithin 6 + 4	100	87.34	80.67	72.00	61.34	33.34	18.67	9
8	No food	100	-	-	-	-	-	-	-

P1, P2, P3 = Protozoeal stage of larvae

M1, M2, M3 = Mysis stages of larvae

PL1 = Post-larvae 1.

TABLE - 27B SURVIVAL RATE (%) OF LARVAE AT VARIOUS DEVELOPMENTAL STAGES DURING METAMORPHOSIS

Diet No.	Name of lipid source used in diet and percentage	Survival rate (%) of larvae from P1 to P3, from P3 to M1 from M1 to M3 and from M3 to PL1				
		P1	from P1 to P3	from p3 to M1	from M1 to M3	from M3 to PL 1
1	Phytoplankton	100	83.33	77.6	64.9	76.19
2	Codliver oil 10.0%	100	70.0	84.76	51.68	56.52
3	Soyabean oil 10	100	60.0	62.22	39.28	54.54
4	Codliver oil + Soyabean oil5+5	100	70.66	66.03	51.42	40.84
5	Codliver oil + Soyabean oil + lecithin 4+2+4	100	75.36	61.06	47.82	100
6	Codliver oil + Soyabean oil + Lecithin 3.3 + 3.3 + 3.3	100	70.66	74.52	44.30	65.7
7.	Codliver oil + Soya lecithin 6+4	100	79.33	76.47	51.64	68.08
8-	No food	100	-	-	-	-

treatments 5, 6 and 7 respectively. In all other treatments survival rate was less than 60%. The survival of larvae in the control also followed a similar trend to that of treatment 5, 6 and 7.

Experiment 2

In this experiment seven oils were used in the diets of larvae. Results of these experiments are given in Table 28A. The results were compared with that of the control diet of phytoplankton (Treatment 5). All the larvae died with in 2 days at protozoea 1 stage without metamorphosis, when food was not supplied (Table 28 A,Treatment 9). In the control, where phytoplankton (Diet 5) was fed 38.0% of the protozoea-1 reached the post-larval 1 stage, within 8 days of the experiment. Survival rate of larvae was in the range 5.34 16.67% in all the treatments (Diet 6, 7 and 8), when diets with plant oils alone were fed, but the survival was relatively high (from 18 to 26.67%) on diets containing marine animal lipid. The duration of metamorphosis of larvae from protozoea 1 to post-larvae 1 was 8 to 10 days, when fed on the diet containing marine animal lipids, but 10 to 11 days were required for the larvae fed on diet containing plant lipids (Diet 6, 7 and 8). Survival was highest in the larvae fed with phytoplankton (control diet-5) and there was no significant difference between survival rates of larvae fed

TABLE - 28A GROWTH AND SURVIVAL OF P. INDICUS LARVAE FED ON DIETS CONTAINING VARIOUS LIPID SOURCES - EXPERIMENT II

Diet No.	Lipid Source	Survival rates (%) of various developmental stages of prawn larvae							
		P1	P2	P3	M1	M2	M3	PL1	Feeding Period days
1.	Shark liver oil	100	66.67	56.67	50.67	56.67	22.66	15.67	10
2	Sardine oil	100	92.67	68.67	62.00	48.0	38.00	26.67	8
3	Prawn-head oil	100	62.67	54.00	48.00	28.67	21.34	20.66	10
4	Codliver oil	100	60.00	49.34	44.00	28.67	20.67	18.67	8
5	Phytoplankton	100	90.67	90.67	82.00	50.67	38.00	38.00	8
6	Ground nut oil	100	56.67	44.67	30.00	25.34	18.00	10.67	11
7	Sunflower Oil	100	58.67	50.76	38.67	38.00	33.34	16.00	11
8	Soyabean oil	100	42.67	38.67	32.00	24.67	12.00	5.34	11
9	No food	100	-	-	-	-	-	-	-

P1, P2, P3 = Protozoeal stages of larvae

M1, M2, M3 = Mysis stages of larvae

PL1 = Post-larva 1.

on animal lipid Diets 1, 2 and 3 containing shark liver oil, sardine oil or prawn head oil. But survival of larvae was significantly lower in treatments 6, 7 and 8 (containing plant oils) than that of the control (Diet-5). Among all the dietary lipid sources tested diet 2 with sardine oil gave the highest survival (26.67%). Among the diets with plant oils diet 7 with sunflower oil produced relatively higher survival than that of diets containing ground-nut oil and soyabean oil as lipid sources.

Trends in mortality at different stages of the larvae are shown in Table 28 B. Larval mortality was more during the protozoeal stages than during the mysis stages in treatments 1, 6 and 7 (Diets 1, 6 and 7) and mortality was found to be more during the mysis stages than during protozoeal stages of larvae in treatments 2, 3 and 5 (Diets 2, 3 and 5), which contained sardine oil, prawnhead oil and phytoplankton respectively. There was no difference between survival rates of larvae at protozoeal and mysis stages in treatment 4 (cod liver oil) and 8 (soyabean oil). Survival of larvae from mysis 1 to post-larvae 1 was relatively higher in all the treatments from 1 to 5 and 8(which contained shark liver oil, sardine oil, prawn head oil, codliver oil, phytoplankton, and soyabean oil respectively) but was less in treatment 6 (ground nut oil diet) and 7(sunflower diet)

TABLE - 28 B SURVIVAL RATE OF LARVAE AT VARIOUS DEVELOPMENTAL STAGES DURING METAMORPHOSIS-EXPERIMENT II

Diet No.	Name of the lipid source used in diets	Survival rate (%) of larvae at various developmental stages				
		P1	From P1 to P3	From P3 to M1	From M1 to M3	From M3 to PL1
1	Shark liver oil	100	56.66	89.4	64.4	69.38
2	Sardine oil	100	68.66	90.29	61.29	70.17
3	Prawn head oil	100	54.0○	88.88	44.40	96.8
4	Codliver oil	100	49.33	89.18	47.0○	90.3
5	Phytoplankton	100	90.67	90.44	46.34	100
6	Groundnut oil	100	44.67	67.16	60.0○	59.25
7	Sunflower oil	100	50.67	76.31	86.2○	48.0
8	Soyabean oil	100	38.67	82.75	37.5○	44.4
9	No Food	100	-	-	-	-

Experiment 3

Results of the experiment conducted to determine the dietary value of mixtures of plant and marine animal lipid sources for larvae are shown in Table 29A. The results of this experiment was compared with that of the control diet (phytoplankton). As in the previous experiments, all the larvae died with in 2 days at protozoea 1 stage without metamorphosis, when food was not supplied (Treatment 9). In the control, where phytoplankton was fed (Treatment 8) the highest survival of 32% at the post-larval 1 stage was attained on the 8th day of the experiment.

Survival rate of larvae was significantly higher ($P < 0.05$) in dietary treatment 4 containing the mixture of codliver oil and sunflower oil, and dietary treatment 6 containing prawn head oil + sunflower oil than that of larvae fed on diet containing mixtures of sardine and groundnut oils (Diet 1), sardine and soyabean oils (Diet 2), and sardine and sunflower oils (Diet 3). Significant differences were not observed in survival rates between diets when the larvae were fed on diets containing mixtures of sharkliver and sunflower oils (Diet 5), codliver and sunflower oils (Diet 4), and prawn head oil and sunflower oil (Diet 6). The larvae fed on the diet containing a mixture of prawn-head oil and sunflower oil (Diet 6), and a mixture of codliver

TABLE - 29 A GROWTH AND SURVIVAL OF P. INDICUS LARVAE FED ON DIETS CONTAINING VARIOUS LIPID SOURCES USED IN MIXTURE. EXPERIMENT III.

Diet No.	Name of Lipid source used in diet	Survival rates (%) of various developmental stages of prawn larvae							
		P1	P2	P3	M1	M2	M3	PL1	Feeding Period
1.	Sardine Oil + Groundnut oil	100	74.67	54	46.67	26.0	13.34	9.33	8
2	Sardine Oil + Soyabean oil	100	91.34	47.34	47.33	24.67	14.0	8.667	8
3	Sardine Oil + Sunflower oil	100	83.34	59.33	47.33	32.0	21.34	14.0	8
4	Codliver oil + Sunflower oil	100	92.67	73.34	52.67	33.34	30.0	20.0	8
5	Shark liver oil + Sunflower oil	100	94.67	75.34	60.0	38.67	26.0	18.0	8
6	Prawnhead oil + Sunflower oil	100	94.00	85.34	65.34	33.34	27.34	21.34	8
7	Phytoplankton	100	96.67	77.34	60.00	50.0	38.67	32.67	-
8.	No Food	100	-	-	-	-	-	-	-

P1, P2, P3 = Protozoeal stages of larvae

M1, M2, M3 = Mysis stages of larvae

P1 1 = Post-larvae 1

TABLE - 29B SURVIVAL RATE (%) OF LARVAE AT VARIOUS DEVELOPMENTAL STAGES DURING METAMORPHOSIS. EXPERIMENT III.

Diet No.	Name of lipid source used in diet	Survival rate (%) of various developmental stages of prawn				
		P1	From P1 to P3	From P3 to M1	From M1 to M3	From M3 to PL 1
1	Sardine Oil + Ground nut oil	100	54.00	86.41	28.57	70.00
2	Sardine Oil + Soyabean Oil	100	47.30	100.00	29.57	61.90
3	Sardine Oil + sunflower oil	100	59.33	79.77	45.00	65.62
4	Codliver oil + Sunflower oil	100	73.30	71.80	56.96	66.67
5	Sharkliver oil + Sunflower oil	100	75.33	61.94	55.71	69.23
6	Prawnhead oil + Sunflower oil	100	75.33	69.00	52.64	78.00
7	Phytoplankton	100	77.34	77.50	64.50	84.48
8	No Food	100	-	—	-	-

oil and sunflower oil (Diet 4) produced significantly ($P < 0.05$) higher survival (21.34% and 20 %) among the dietary treatments 1 to 6, in this experiment.

Survival rate of larvae (Table 29B) in various treatments was more during protozoeal stage, i.e. P1 to P3, than during mysis stages M1 to M3 and M3 to PL1 stage in all treatments. Thus results showed more mortality of larvae occurred during mysis stage than protozoeal stage.

POST-LARVAE PL1-10

The results of the experiment to study the nutritive value of natural lipid sources for post-larvae 1-10 are shown in Fig.17 and 18. The results are treated into three groups, on the basis of fatty acids content of the dietary lipids (Table 32).

Survival (Fig.17) of post-larvae was more than 75% in all the treatments. The lowest survival of about 75% was recorded with the diets containing sunflower and coconut oils. Survival was more than 87% in all the remaining treatments, and was not significantly influenced by the dietary lipid sources used in this experiment.

Data for growth of post-larval prawn expressed as percentages of mean gains in length, wet weight and dry weight

are shown is Fig. 17 and 18. The growth of post-larvae 1-10 was significantly ($P < 0.05$) influenced by the dietary source of lipid. The growth was significantly ($P < 0.05$) high in post-larvae fed the diet 18, in which a mixture of codliver oil, soyabean oil and lecithin was used as source of lipid. Post-larval growth was relatively low in treatments 1 and 2, in which coconut oil and mustard oil containing feeds were used. Among the plant oils containing diets, the diet containing soyabean oil (treatment-12) produced the highest growth. Among the diets containing fish lipids (Table 23), diet with shark liver oil produced poor growth; whereas sardine oil produced better growth. In general, the fish lipids were found to be better dietary sources of lipid than plant oils (except soyabean and gingely oil) for promoting growth in post-larvae 1-10 (Fig.17 and 18).

However the growth of post-larvae was relatively more when a mixture of plant and animal lipids were used as dietary source of lipid than the individual plant or animal lipids. Among the mixtures of plant and animal lipids used in this experiment, a mixture of codliver oil, soyabean oil and lecithin (Treatment 18) produced the highest growth. Analysis of variance of the data and least significant difference test indicate that the growth was significantly ($P < 0.05$) higher in post-larvae in treatment 18 than all other treatments. Besides, the growth of post-larvae was

Fig. 17 Percent survival and gain in length of post-larvae 1-10 fed on diets containing natural lipid sources.

Diet No.	Name of lipid source used in diet
1	Coconut oil
2	Mustard oil
3	Cotton seed oil
4	Safflower oil
5	Rapeseed oil
6	Ground nut oil
7	Gingely oil
8	Sunflower oil
9	Corn oil
10	Shark liver oil
11	Linseed oil
12	Soyabean oil
13	Codliver oil
14	Sardine oil + Sunflower oil
15	Sardine oil
16	Prawn head oil
17	Prawn head oil + Soyabean oil
18	Codliver oil + Soyabean oil + Lecithin

FIG. 17.

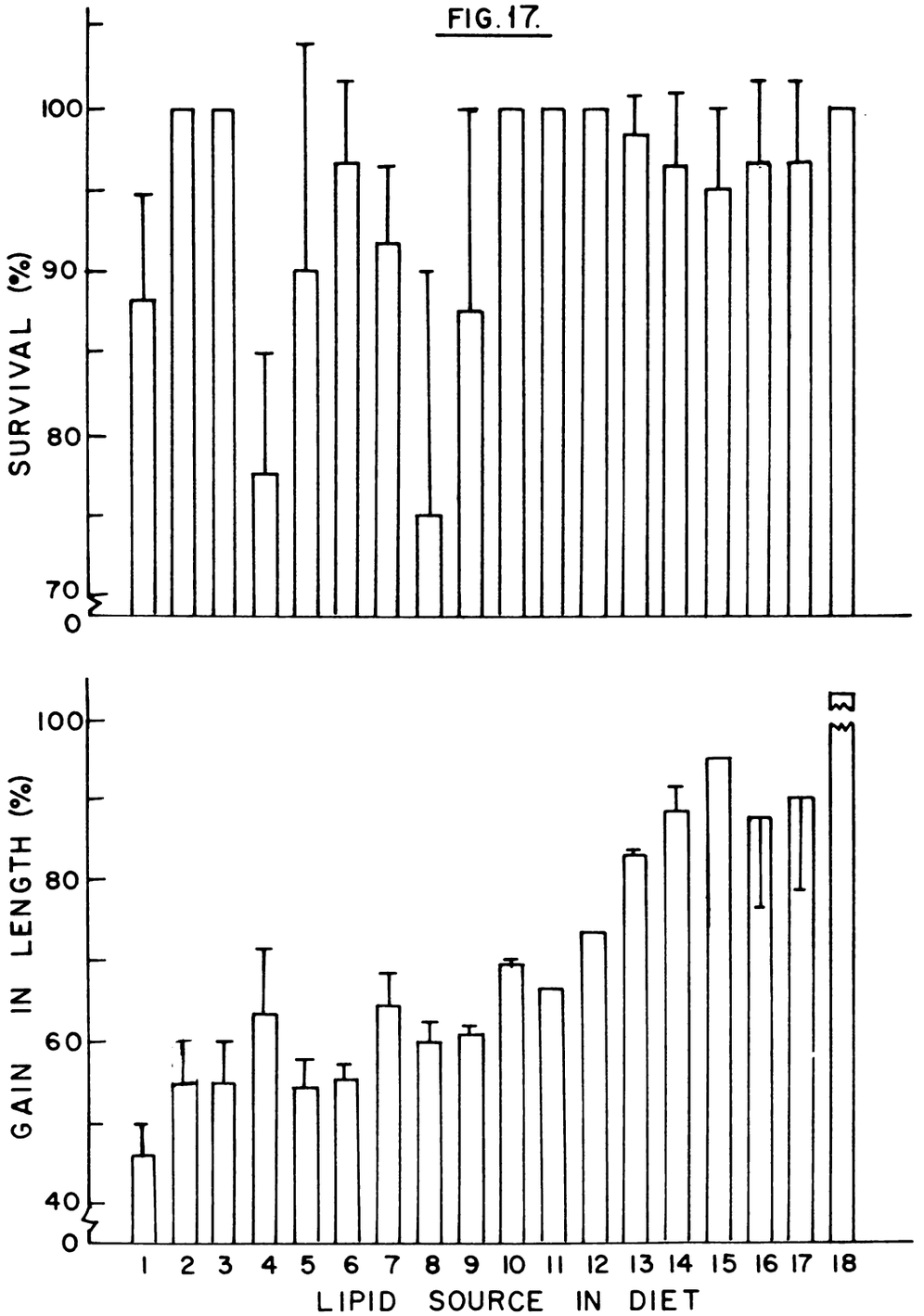
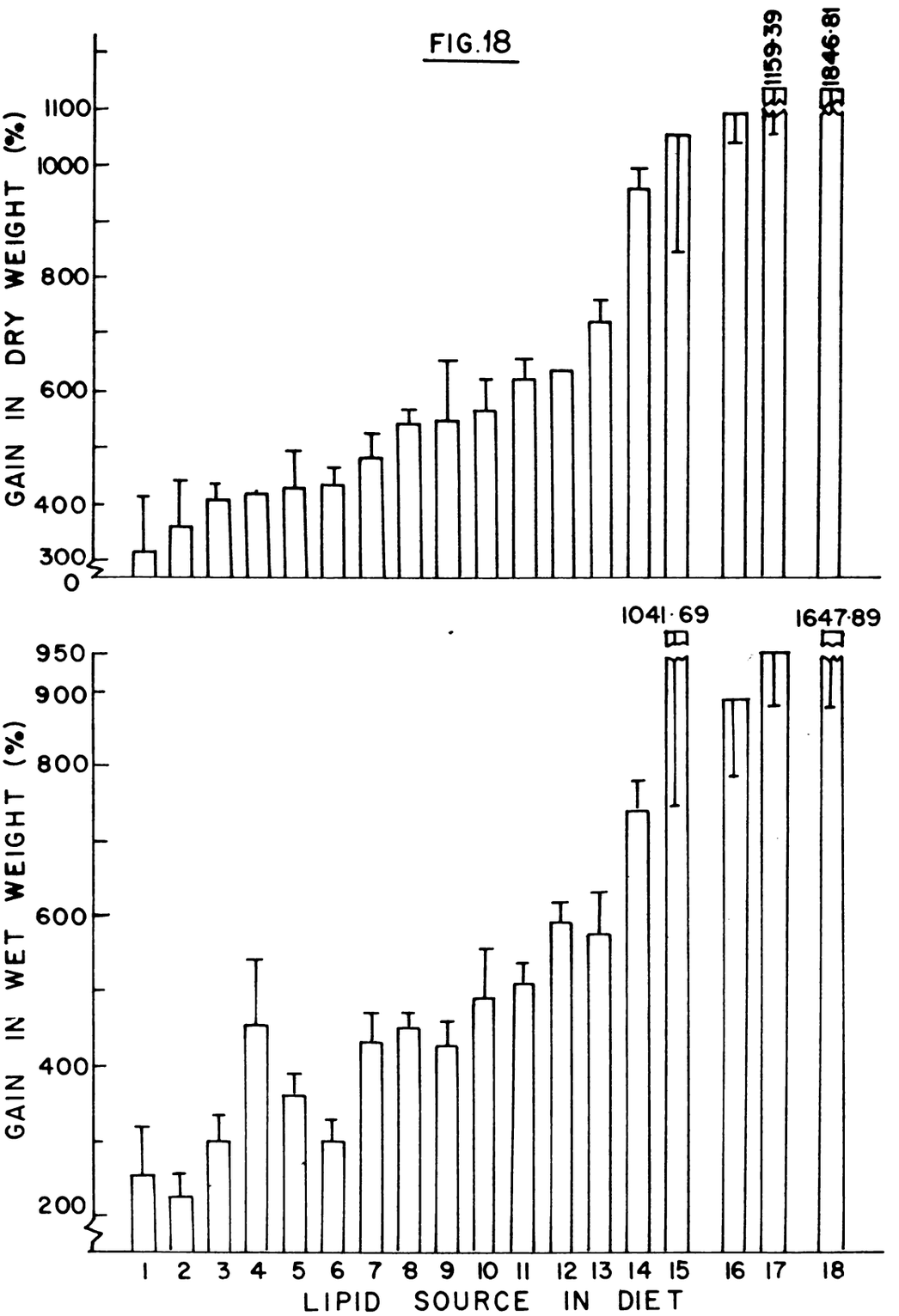

SURVIVAL (%)
100
90
80
70
0
GAIN IN LENGTH (%)
100
80
60
40
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
LIPID SOURCE IN DIET

Fig. 18 Percent gain in wet weight and dry weight of post-larvae 1-10 fed on diets containing natural lipid sources

Diet No.	Name of lipid source used in diet
1	Coconut oil
2	Mustard oil
3	Cotton seed oil
4	Safflower oil
5	Rapeseed oil
6	Groundnut oil
7	Gingely oil
8	Sunflower oil
9	Coron oil
10	Sharkliver oil
11	Linseed oil
12	Soyabean oil
13	Codliver oil
14	Sardine oil + Sunflower oil
15	Sardine oil
16	Prawn head oil
17	Prawn head oil + Soyabean oil
18	Codliver oil + Soyabean oil + Lecithin

FIG.18


GAIN IN DRY WEIGHT (%)
1100
1000
800
600
400
300
0
1159·39
1846·81
GAIN IN WET WEIGHT (%)
950
900
800
600
400
200
1041·69
1647·89
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
LIPID SOURCE IN DIET

significantly higher ($P < 0.05$) in all the treatments from 14 to 18, in which either animal lipids alone or mixture of plant and animal lipids were used as sources of lipid, than that of treatments from 1 to 9 and from 11 to 12 containing plant oil as lipid sources.

Among the plant oils soyabean oil was found to be relatively a better dietary lipid source, but mustard oil was found to be a poor source of dietary lipid for post-larvae 1-10 in promoting growth. Among the fish lipids sardine and prawn head oils were found to be relatively superior and shark liver oil inferior to all other fish oils. Among all the lipid sources used in this experiment the diets with mixture of fish and plant lipids were found to be very effective in promoting growth and a mixture of codliver oil, soyabean oil and lecithin in the ratio 5.34:2.66:2 found to be highly effective in promoting growth in post-larvae 1-10.

POST-LARVAE PL11-25

Results of the feeding experiment in post-larvae 11-25 of *P. indicus* with 18 diets containing various sources of lipids are given in Table 30 and shown in Fig. 19, 20 and 21. Survival of post-larvae in all the treatments was more than 84% (Fig.19) except for treatment 11 (sharkliver oil) and treatment 4(mustard oil) in which survival was relatively low, being 73.34 and 64.44% respectively.

Fig. 19 Percent survival and gain in length of post-larvae 11-25 fed on the diets containing natural lipid sources

Diet No.	Name of lipid source used in diets
1	Coconut oil
2	Groundnut oil
3	Safflower oil
4	Mustard oil
5	Soyabean oil
6	Rapeseed oil
7	Linseed oil
8	Cotton seed oil
9	Gingely oil
10	Sunflower oil
11	Shark liver oil
12	Sardine oil
13	Corn oil
14	Codliver oil
15	Sardine + Sunflower oil
16	Prawn head oil + Soyabean oil
17	Prawn head oil
18	Codliver oil + Soyabean oil + Lecithin

FIG.19.

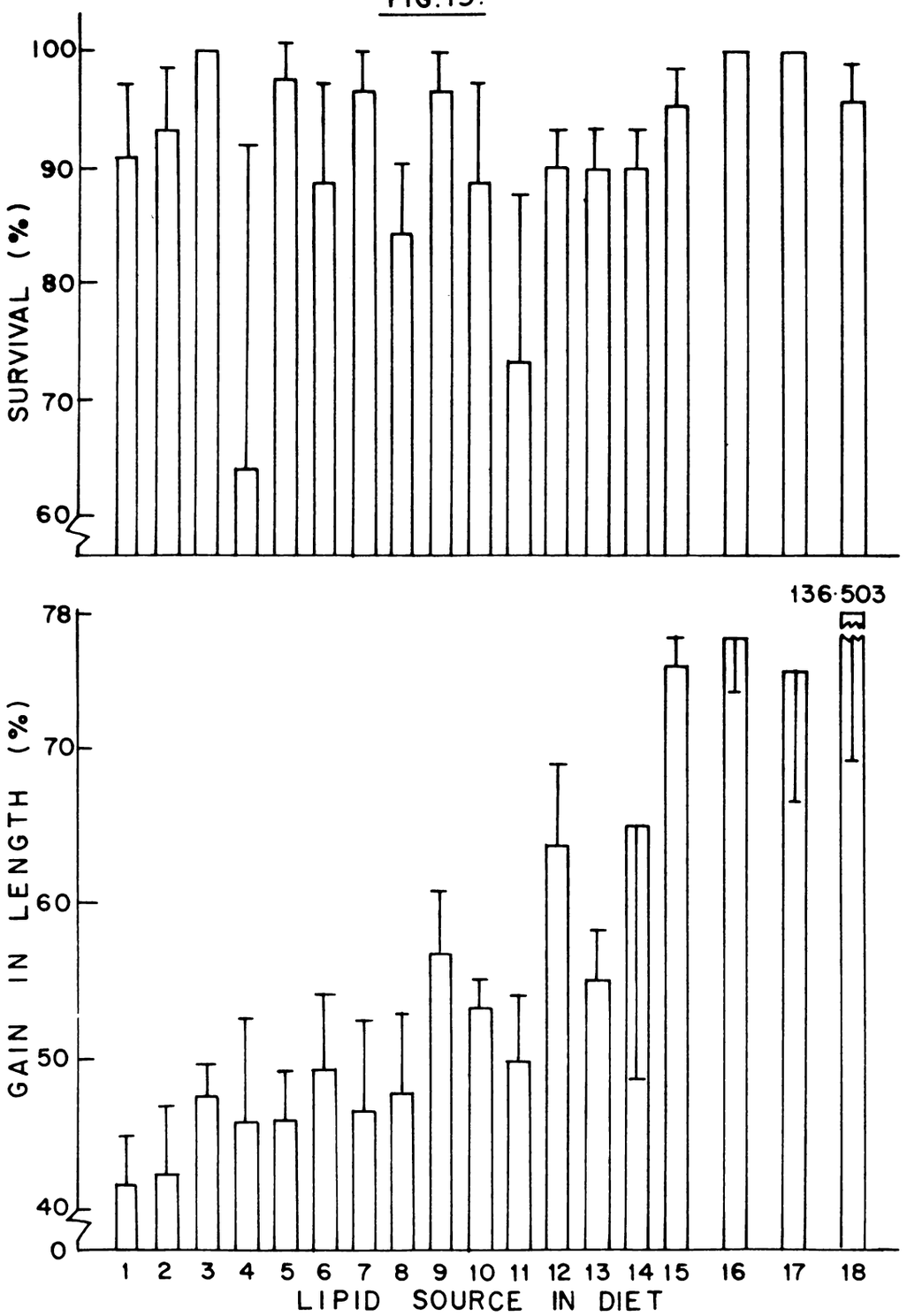
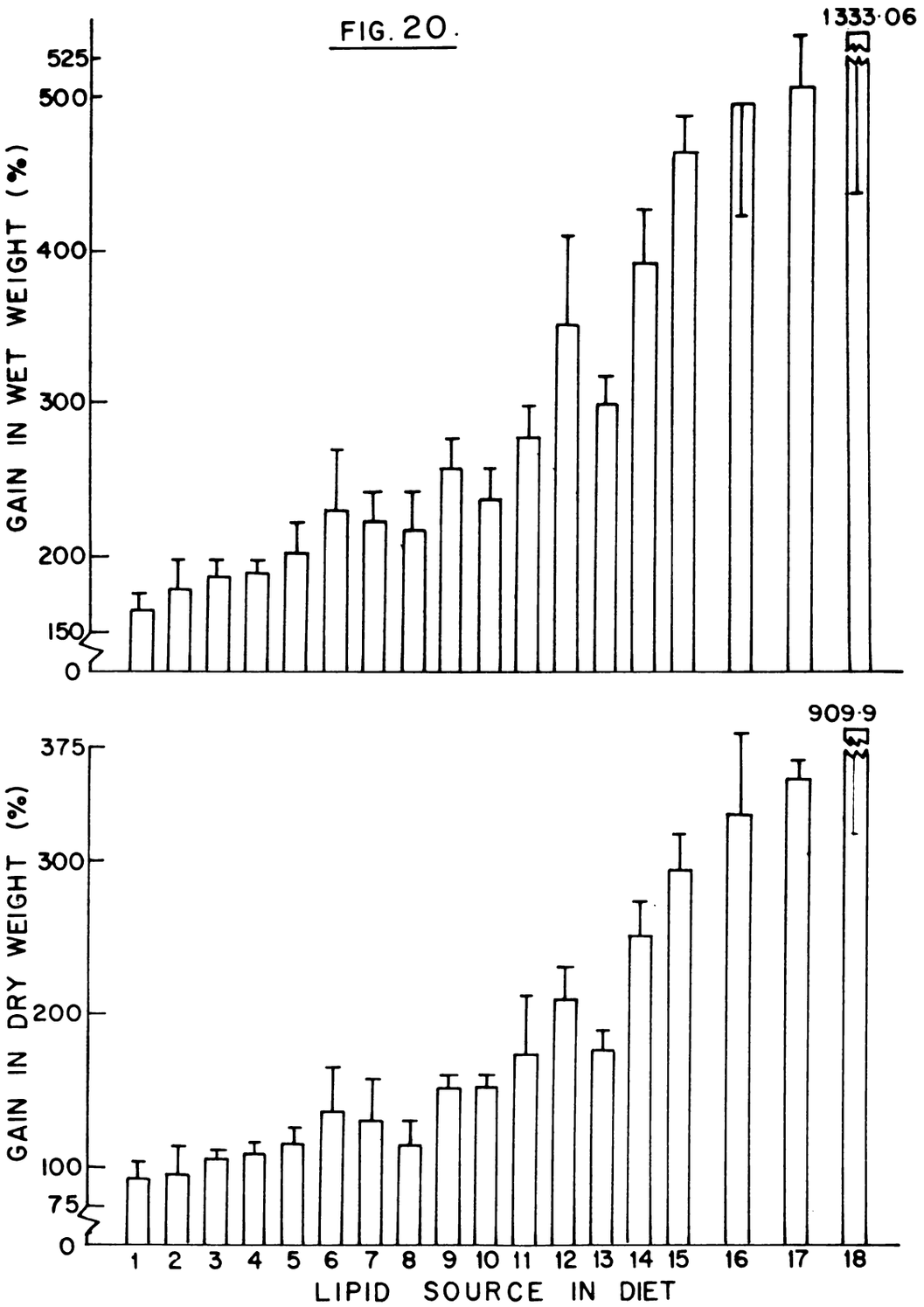

SURVIVAL (%)
100
90
80
70
60
GAIN IN LENGTH (%)
136·503
78
70
60
50
40
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
LIPID SOURCE IN DIET

Fig. 20 Percent gain in wet weight and dry weight of post-larvae 11-25 fed on diets containing natural lipid sources.

Diet No.	Name of lipid source used in diet
1	Coconut oil
2	Groundnut oil
3	Safflower oil
4	Mustard oil
5	Soyabean oil
6	Rapeseed oil
7	Linseed oil
8	Cotton seed oil
9	Gingely oil
10	Sunflower oil
11	Shark liver oil
12	Sardine oil
13	Corn oil
14	Codliver oil
15	Sardine oil + Sunflower oil
16	Prawn head oil + Soyabean oil
17	Prawn head oil
18	Codliver oil + Soyabean oil + Lecithin

FIG. 20.


1333·06
525
500
400
300
200
150
0
GAIN IN WET WEIGHT (%)
909·9
375
300
200
100
75
0
GAIN IN DRY WEIGHT (%)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
LIPID SOURCE IN DIET

Growth

The data for growth of post-larvae 11-25 expressed as percentage mean gains in length, wet weight and dry weight are shown in Fig. 19 and 20. The growth was significantly ($P < 0.05$) influenced by the dietary lipid sources. Growth was significantly higher ($P < 0.05$) in treatments 14 to 18 than the treatments 1 to 11 & 13. The growth of post-larvae, was significantly ($P < 0.05$) higher when fed a diet with a mixture of codliver oil + soyabean oil and lecithin (Diet 18) than that of diets with various other lipid sources.

Although statistically significant differences were not observed in the growth of prawns between diets with various plant oils (Diet 1 to 10 and 13), there were considerable variation in the observed growth values (Fig.19 and 20). Among the plant oils containing diets, diet 10 and 13 containing sunflower oil and corn oil respectively produced relatively better growth, and diet 1, containing coconut oil produced relatively poor growth.

Among the diets with individual marine animal oils, diet 17 with prawn head oil and diet 14 with codliver oil produced significantly ($P < 0.05$) higher growth than the diets with sardine oil (Diet - 12) and shark liver oil (Diet 11) in the post-larval prawn. In general, the diets with marine animal oils produced superior growth in post-larvae when

compared to that of the diets with plant oils, the only exception being the diet with corn oil (Diet 13), which produced relatively higher growth in post-larvae than the diet with shark liver oil. The diets with a mixture of plant and animal oils (Diet 15, 16 and 18) produced significantly ($P < 0.05$) greater growth than the post-larvae fed on the diets with either individual plant lipids or animal lipids with the exception of prawn-head oil. The diet containing a mixture of codliver oil, soyabean oil and lecithin (Diet 18) produced significantly better growth in post-larval prawn. But a mixture of sardine oil and sunflower oil (Diet 15) unexpectedly produced relatively less growth in post-larval prawn.

Food conversion ratio (FCR) and protein efficiency ratio(PER)

FCR and PER obtained for various diets are shown in Fig. 21. FCR and PER were also significantly influenced by the dietary lipid source. The diet 18 containing a mixture of codliver oil, soyabean oil and lecithin, which provided the best survival and growth in post-larvae, also provided the best FCR (1.005) and PER (2.256). Food conversion and protein efficiency ratios were also found to be significantly better ($P < 0.05$) with diets containing sardine oil (Diet 12), codliver oil (Diet 14), prawn head oil (Diet 17), or diets

containing mixture of plant and animal lipids.

Variations observed in the FCR and PER between diets 1 to 10 containing plant oils and diet 13 were not statistically significant although the diet 13, with corn oil provided relatively better FCR (2.57) and PER (0.896). But diet 1, with coconut oil provided very poor FCR (4.06) and PER (0.572). Although significant differences were not observed in the FCR and PER between diets 14, 15, 16, 17 and 18 the observed variations were very prominent (Fig. 21). Among the diets with marine animal lipids used in this experiment, diet 17 with prawn head oil and diet 14 with codliver oil provided better FCR and PER than those provided by Diet 11 containing shark liver oil.

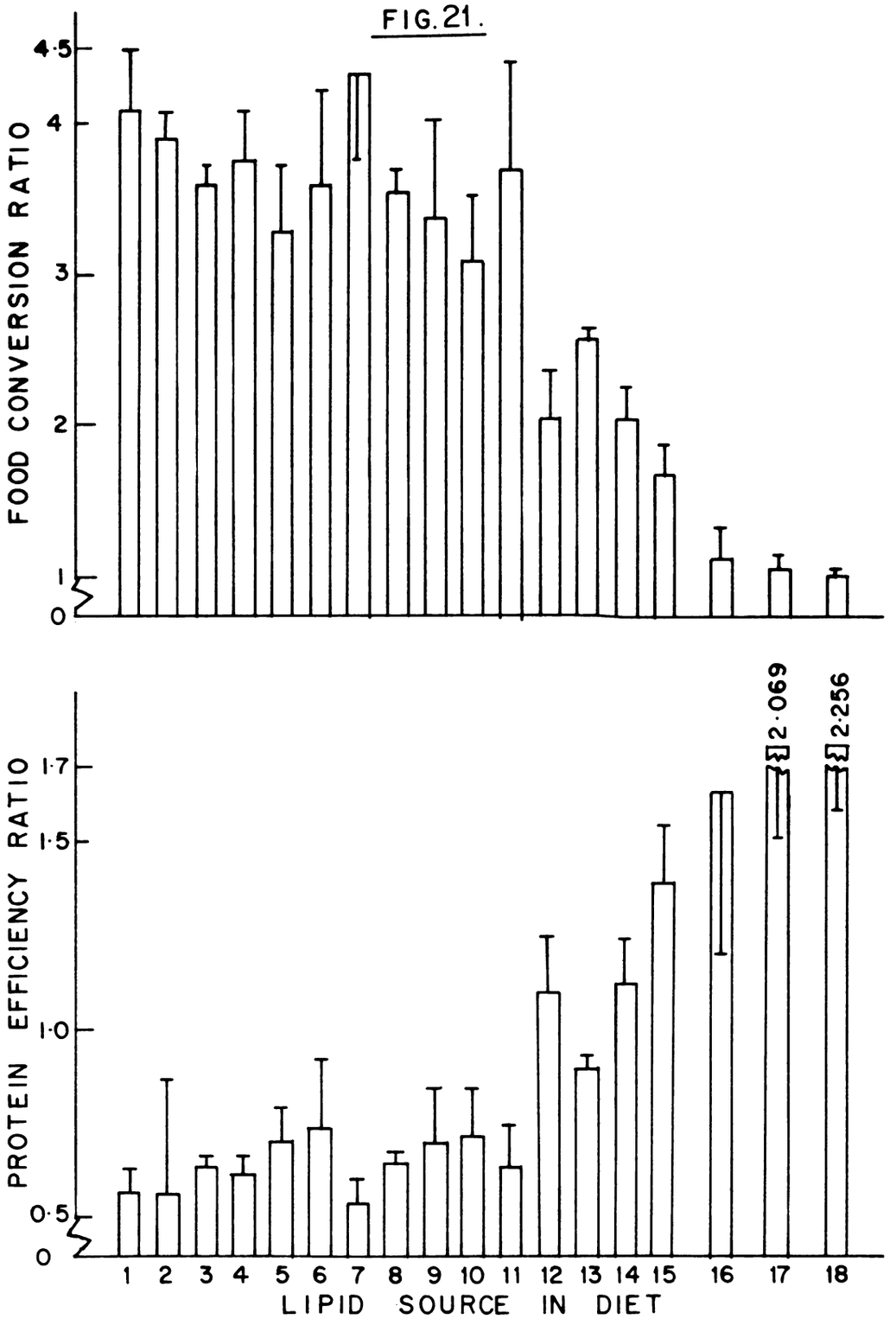
Diet 15, 16 and 18 containing a mixture of plant and animal lipids provided significantly better FCR and PER than those diets containing other lipid sources, with the exception of prawn head oil (Diet 17). Diet 18 in which a mixture of codliver oil + soyabean oil + lecithin was used, provided significantly the best PER and FCR among all the diets. Diet 15 containing a mixture of sardine oil + sunflower oil and diet 16 with prawn head oil + soyabean oil also provided relatively better FCR and PER than most of the other diets.

Thus the food and protein utilization in prawn was significantly influenced by the dietary lipid source and a

Fig. 21 FCR and PER of post-larvae 11-25 fed on diets containing natural lipid sources.

Diet No.	Name of lipid source used in diet
1	Coconut oil
2	Groundnut oil
3	Safflower oil
4	Mustard oil
5	Soyabean oil
6	Rapeseed oil
7	Linseed oil
8	Cotton seed oil
9	Gingely oil
10	Sunflower oil
11.	Sharkliver oil
12	Sardine oil
13	Corn oil
14	Codliver oil
15	Sardine oil + Sunflower oil
16	Prawn head oil + Soyabean oil
17	Prawn head oil
18	Cod liver oil + Soyabean oil + Lecithin

FIG. 21.


FOOD CONVERSION RATIO
4·5
4
3
2
1
0
PROTEIN EFFICIENCY RATIO
1·7
1·5
1·0
0·5
0
2·069
2·256
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
LIPID SOURCE IN DIET

mixture of plant and animal lipids seems to be the best source of lipid for promoting food and protein utilisation in post-larval prawn.

Biochemical composition of post-larvae 11-25

Chemical constituents of post-larvae were also influenced by the dietary lipid source. The effects of dietary lipid sources on moisture, protein lipid, carbohydrate and ash content of the post-larvae are shown in Table 30. The moisture content of post-larvae was found to be in the range of 73.8 to 77.10% in the various treatments. The observed differences in the moisture content of the post-larvae in between the treatments were statistically insignificant. However, post-larvae fed on the diets containing plant lipids (Diet 1 to 10 and 13) had relatively greater moisture contents than those fed on diets containing animal or mixture of animal and plant lipids.

Post-larvae fed on diets containing plant lipids had relatively less protein and lipids than those fed diets containing animal lipids or a mixture of plant and animal lipid as dietary lipid source (Table 30). But analysis of variance of the data did not show any significant difference between diets in the protein contents of post-larvae. However the lipid content of the post-larvae was significantly ($P<0.05$)

influenced by the lipid source used in the diet.

The protein content was within the range of 61.96 to 65.8% in the post-larvae fed on the diets containing plant lipids, except for the post-larvae fed on the diets containing the gingely oil, sunflower oil and corn oil, which had more than 68% protein. The protein content was in the range of 68.3 to 69.93% in the post-larvae fed on diets containing either animal lipid alone or a mixture of animal and plant lipids, with the exception of shark liver oil (66.58%).

Lipid content was significantly ($P<0.05$) low and found to be in the range of 8.6 to 10.5% in the post-larvae fed on diets containing plant lipids (Diets 1 to 10 and 13). But the lipid content was found to be significantly ($P<0.05$) high (10.23 to 11.83) in post-larvae fed on diets containing animal lipid or a mixture of animal and plant lipids.

Ash and carbohydrate contents in the body of post-larvae were also influenced significantly by the dietary lipid source. Ash and carbohydrate contents were relatively higher (Table 30) in the post-larvae fed on the diets containing plant lipids than those fed on diets containing animal or a mixture of animal and plant lipids. Thus, the diets containing a mixture of plant and animal lipids produced high survival, growth, protein and lipid deposition with

TABLE - 30 EFFECTS OF THE DIETS CONTAINING NATURAL LIPID SOURCES ON THE BIOCHEMICAL COMPOSITION OF THE POST-LARVAE 11-25

	Experimental diet numbers and dietary lipid sources used in the diet																
	Coconut oil 1	Ground-nut oil 2	Safflower oil 3	Mustard oil 4	Soyabean oil 5	Rapeseed oil 6	Linseed oil 7	Cotton seed oil 8	Gingely oil 9	Sunflower oil 10	Sharkliver oil 11	Sardine oil 12	Corn Oil 13	Codliver oil 14	Sardine + Sunflower oil 15	Prawn head oil + Soyabean oil 16	Prawn head oil 17	Codliver oil + Soyabean oil + Lecithin 18
1. MOISTURE(%)	76.696	76.373	75.620	75.590	75.820	76.376	77.100	76.747	76.440	74.260	75.480	74.845	76.560	75.790	76.460	75.480	75.683	75.660
	±0.859	±0.514	±0.390	±0.106	±0.376	±0.351	±0.350	±0.620	±0.420	±1.682	±2.158	±1.525	±0.090	±0.140	±0.402	±0.364	±0.004	±0.273
2. PERCENTAGE ON DRY WEIGHT BASIS																		
a) Protein	62.220	64.710	65.800	63.553	64.440	64.840	64.400	61.960	68.080	68.510	68.820	66.580	68.380	68.720	68.980	69.933	69.500	69.900
	±0.240	±0.473	±1.275	±0.564	±0.416	±0.674	±1.400	±0.141	±0.160	±0.217	±0.231	±0.020	±1.400	±0.500	±0.315	±1.329	±0.408	±0.536
b) Lipid	8.700	9.800	9.810	8.600	9.400	9.253	10.150	9.196	10.500	9.533	1.833	11 250	9.150	11.605	11.533	10.230	11.466	10.733
	±0.163	±0.480	±0.705	±0.100	±0.648	±0.506	±0.150	±0.433	±0.400	±0.402	±0.449	±1.055	±0.050	±1.100	±0.402	±0.124	±0.449	±0.339
c) Carbo-hydrate	2.983	2.673	3.800	1.960	2.813	2.563	2.150	2.890	1.175	2.210	1.250	3.770	1.350	1.320	1.920	1.673	1.736	1.160
	±0.084	±0.327	±0.108	±0.114	±0.077	±0.216	±0.135	±0.134	±0.095	±0.075	±0.163	±0.175	±0.110	±0.060	±0.081	±0.247	±0.163	±0.065
d) Ash	19.970	18.976	18.500	18.800	17.210	17.530	16.955	18.960	16.120	15.240	16.320	15.290	17.290	13.700	15.300	14.840	15.190	15.980
	±0.679	±0.565	±0.163	±0.804	±0.313	±0.618	±1.945	±0.066	±0.580	±0.811	±0.393	±0.010	±0.990	±0.750	±0.618	±0.104	±0.599	±0.?

better food and protein utilization. The mixture of codliver oil, soyabean oil and lecithin (Diet 18) proved to be the best source of lipid for post-larval growth.

JUVENILES

Results of the experiment with juveniles of P. indicus are given in Table 31, 32 and 33 and plotted in Fig. 22, 23 and 24. The results have been treated in three parts on the basis of the fatty acids profile of natural lipid sources and their effect on growth of juvenile prawns. Analysis of variance and least significant difference test were applied, to find out whether the lipid sources in diets had any significant influence on the survival, growth, FCR, PER and chemical composition of the prawns.

Diets containing marine animal lipids and those containing mixture of marine animal lipid and plant oils (Diets 9 to 16) produced significantly ($P<0.05$) higher rate of survival than only plant oil based diets (Diet 1 to 8) with the exception of mustard oil diet, which produced a survival rate of 90%. The lowest (36.67%) survival was observed, when cotton seed oil was used as lipid source (Diet 2). However the differences in survival among diets 8, 1 & 9 to 16 were statistically insignificant. In general survival of juvenile prawns was less with plant oil diets than with other diets.

Growth

The data for growth of juvenile prawns expressed as percentage mean gains in length, wet weight, and dry weight are given in Fig. 22. The growth was significantly ($P<0.05$) influenced by the dietary lipid source.

The variations observed in growth in between diets containing plant oils were statistically insignificant. Among the diets with plant lipids, diet containing corn oil (Diet 7) and linseed oil (Diet 8) produced relatively better growth, but the diets (1 and 2) containing mustard oil (Diet 1) and cottonseed oil (Diet 2) produced relatively poor growth in juvenile prawns. Thus the results indicate that among the plant oils linseed and corn oils are relatively better than all other sources of plant lipids, and that mustard and cottonseed oils are poor sources of lipids for promoting growth in the juvenile prawns.

Among the diets with individual animal oils, diet 15 with prawn head oil and diet 10 with codliver oil produced relatively more growth; whereas diets with sardine oil (diet 9) and sharkliver oil (diet 11) produced relatively poor growth in juvenile prawns. The diets with a mixture of plant and animal oils produced significantly greater growth than any of the diets containing individual plant or animal oils.

Among the diets with mixture of plant and animal oils, diet 16 with a mixture of codliver oil, soyabean oil and lecithin promoted significantly the best growth and diet 12 with sardine oil and sunflower oil produced relatively poor growth.

Food conversion ratio (FCR) and Protein Efficiency Ratio(PER):-

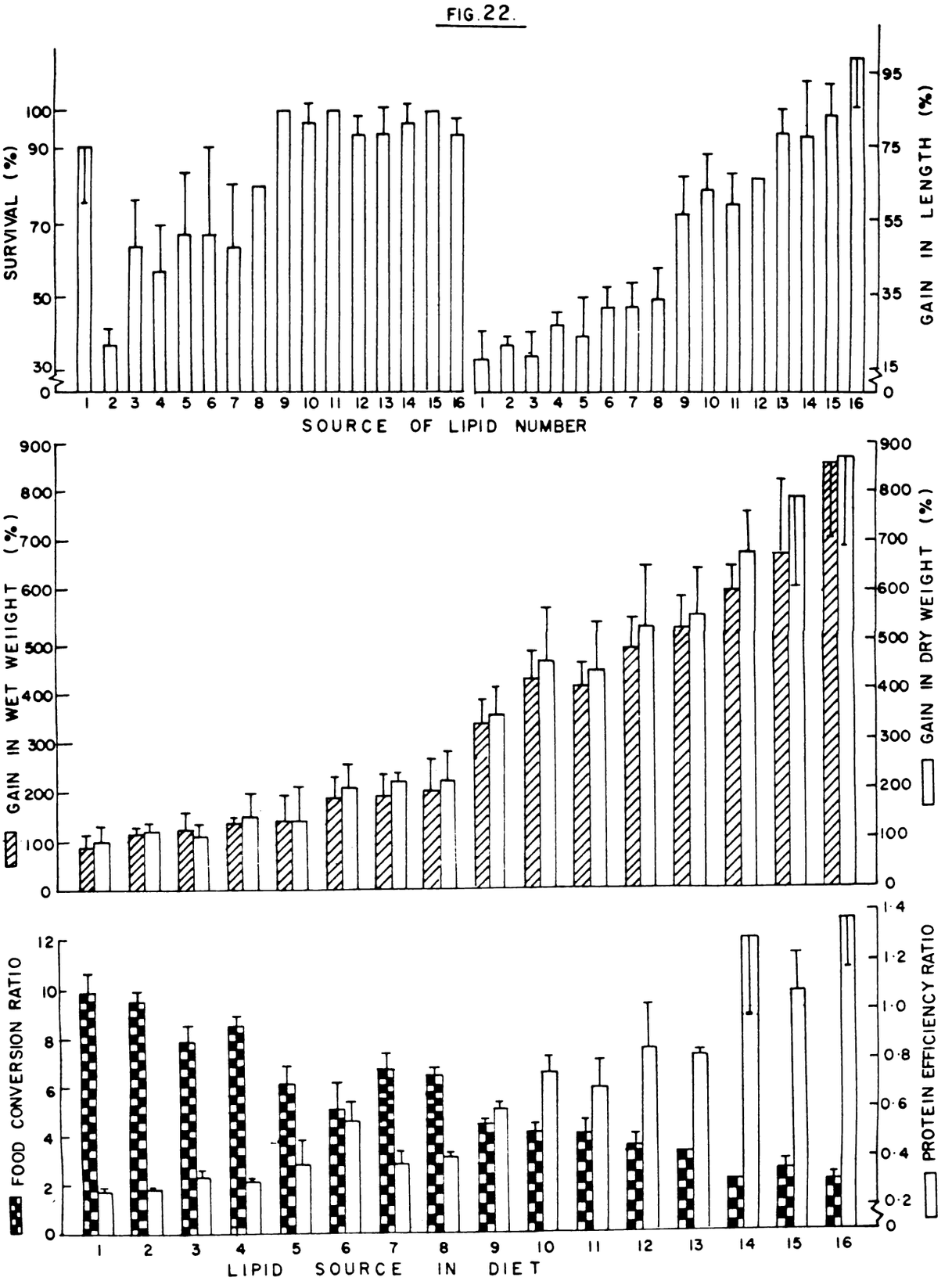
Food conversion ratios and protein efficiency ratios obtained for various diets are shown in Fig. 22. The diet 16, which produced the best growth and survival, also provided the best FCR (2, 0) and PER (1.377) among all the diets. The diets with mustard oil, cotton seed oil, soyabean oil and safflower oil gave significantly poor FCRs; whereas the diets containing mixture of plant and animal lipids produced significantly ($P < 0.05$) better FCR than remaining diets which had either plant or animal oils alone. Thus the food utilisation by prawn is greatly affected when plant oils are used as the sole lipid source (Diet 1 to 8). But the inclusion of marine animal lipids improved the food utilization by the prawns. It is also evident that the food conversion is considerably improved by the addition of prawn-head oil and mixtures of plant and animal lipids.

Diets with individual marine animal oils and mixture of animal and plant oils provided significantly ($P < 0.05$) better PER than those diets containing plant oils, with the exception of sunflower oil. Thus the protein utilization by

Fig. 22 Survival rate, growth, FCR and PER of juvenile prawns fed on the diets containing natural lipid source

Diet No.	Lipid source used in the diet
1	Mustard oil
2	Cotton seed oil
3	Soyabean oil
4	Safflower oil
5	Groundnut oil
6	Sunflower oil
7	Linseed oil
8	Corn oil
9	Sardine oil
10	Codliver oil
11	Sharkliver oil
12	Sardine oil + Sunflower oil
13	Sardine oil + Groundnut oil
14	Prawn head oil + Soyabean oil
15	Prawn head oil
16	Cod-liver oil + Soyabean oil + Lecithin

FIG. 22.


SURVIVAL (%)
GAIN IN LENGTH (%)
SOURCE OF LIPID NUMBER
GAIN IN WET WEIIGHT (%)
GAIN IN DRY WEIGHT (%)
FOOD CONVERSION RATIO
PROTEIN EFFICIENCY RATIO
LIPID SOURCE IN DIET

the prawn also appears to be significantly affected by most of the plant lipids. In contrast, the animal lipid sources and the mixtures of plant and animal lipid sources resulted in improved PERs. The results also suggest that among the lipid sources used in this experiment, the mixture of codliver oil, soyabean oil and lecithin (Diet 16), and prawn head oil (Diet 15) is significantly ($P<0.05$) superior as lipid source for utilization of dietary protein in the juvenile prawns.

Biochemical composition of prawn :-

The influence of various lipid sources upon the moisture, protein, lipid, cholesterol, carbohydrate and ash contents of prawn is shown in Fig. 23 and 24. The proximate composition of prawns was also influenced significantly ($P<0.05$) by the dietary lipid sources.

Diets containing various lipid sources had significant effect on moisture content of prawns, with the prawns fed on diets containing mustard oil (Diet 1), cotton seed oil(Diet 2), soyabean oil (Diet 3) and ground nut oil (Diet 5) having significantly higher moisture contents than prawns from other treatments. Besides, all the diets with plant lipids (Diet 1 to 8) produced prawns with relatively more moisture (72.89 to 76.58%) contents than the diets with animal lipids and those with a mixture of plant and animal lipids. In the latter groups moisture content was in the range of 72.3 to 73.9%.

Dietary lipid sources when included in the diet had also significant ($P<0.05$) effect on the protein deposition in the body of prawn. In general, protein content of prawns from various treatments with plant oils in diets was less than 67% and it varied from 60 to 67%, with the notable exception of prawns fed the sunflower oil diet, which had 68.10% protein. Protein content was in the range of 67 to 70% in prawns fed diets with either marine lipids (exception of sardine oil) or the mixture of marine lipids and plant lipids as sources of lipid.

Diets containing the mixture of plant and animal lipid sources promoted greater protein deposition in prawns than individual sources of lipids. In short the protein deposition in the body was less when the prawns were fed on diets with plant lipids than with diets containing animal or mixture of plant and animal lipids.

The prawns fed on diets containing the plant lipid sources with the exception of linseed oil diet had significantly ($P<0.05$) lower total lipid content than those fed diets with either marine lipid or a mixture of plant and marine lipids. There were no significant differences in the lipid contents in prawns among the dietary treatments 1 to 8. Similarly, the differences observed in the lipid content of prawns fed on diets 9 to 16 were not statistically

Fig. 23 Percent moisture, protein and lipid content of juvenile prawns fed on diets containing natural lipid sources.

Diet No.	Lipid sources used in the diet
1	Mustard oil
2	Cotton seed oil
3	Soyabean oil
4	Safflower oil
5	Groundnut oil
6	Sunflower oil
7	Linseed oil
8	Corn oil
9	Sardine oil
10	Codliver oil
11	Sharkliver oil
12	Sardine oil + Sunflower oil
13	Sardine oil + Groundnut oil
14	Prawn head oil + Soyabean oil
15	Prawn head oil
16	Codliver oil + Soyabean oil + Lecithin

significant. In general, the lipid content of prawns was in the range of 8 to 11.45% in the treatments 1 to 8 (diets incorporated with plant lipids), and in the range of 10.94 to 12.6% in the treatments 9 to 16 (diets containing either animal lipids or mixture of animal and plant lipids).

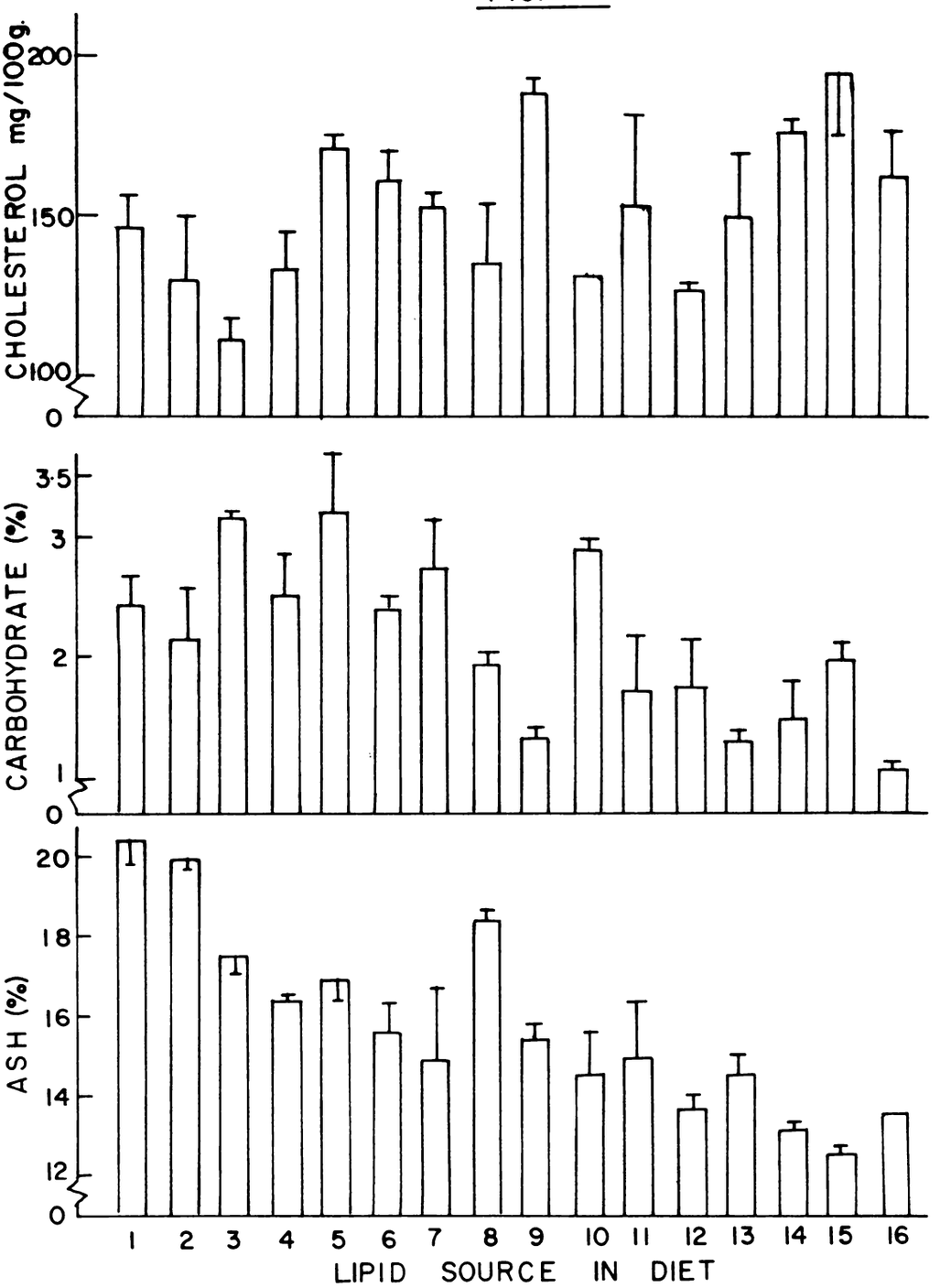
The cholesterol and carbohydrate contents in prawns (Fig.24) was not significantly influenced by the dietary lipid sources. Cholesterol content of prawn was in the range of 140 mg to 194 mg/100 mg of body weight (dry weight) and carbohydrate contents of the prawns was found in the range of 1.27 to 3.18%.

Dietary lipid sources had significant ($P \leq 0.05$)effect on the ash content of prawns. Diets containing prawn head oil (Diet 15), mixture of prawn head oil and soyabean oil (Diet 14) and codliver oil, soyabean oil and lecithin(Diet 16) produced prawns with significantly ($P < 0.05$) lower percentages of ash. Similarly, the diets containing mustard oil (Diet 1), cotton seed oil (Diet 2) soyabean oil (Diet 3) safflower oil (Diet 4) and ground nut oil (Diet 5) produced significantly higher level of ash in the body of prawn than the remaining diets. Ash contents of juvenile prawns was in the range of 12.5 to 14.5% in the treatments 9 to 16, (diets containing either marine lipid or mixture of plant and marine lipids) and in the range of 14.9 to 20.5% in

Fig. 24 Percent carbohydrate, ash and cholesterol content of juvenile prawns fed on diets containing natural lipid sources.

Diet No	Lipid sources used in the diet
1	Mustard oil
2	Cottonseed oil
3	Soyabean oil
4	Safflower oil
5	Groundnut oil
6	Sunflower oil
7	Linseed oil
8	Corn oil
9	Sardine oil
10	Codliver oil
11	Sharkliver oil
12	Sardine oil + Sunflower oil
13	Sardine oil + Groundnut oil
14	Prawn head oil + Soyabean oil
15	Prawn head oil
16	Cod liver oil + Soyabean oil + Lecithin

FIG. 24.


CHOLESTEROL mg/100g.
200
150
100
0
CARBOHYDRATE (%)
3.5
3
2
1
0
ASH (%)
20
18
16
14
12
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
LIPID SOURCE IN DIET

those prawns belonging to treatment 1 to 8 (diets containing plant lipids).

Thus the diets with animal lipid sources or mixture of animal and plant lipid sources produced prawns with relatively more protein and lipids, but with less moisture, ash and carbohydrate contents than the prawns fed on diets with plant lipid sources. Among all the sources of lipids used in this experiment a mixture of codliver oil, soyabean oil and lecithin (Diet 16) produced the best response in prawns by promoting growth and by providing improved FCR, PER and protein retention.

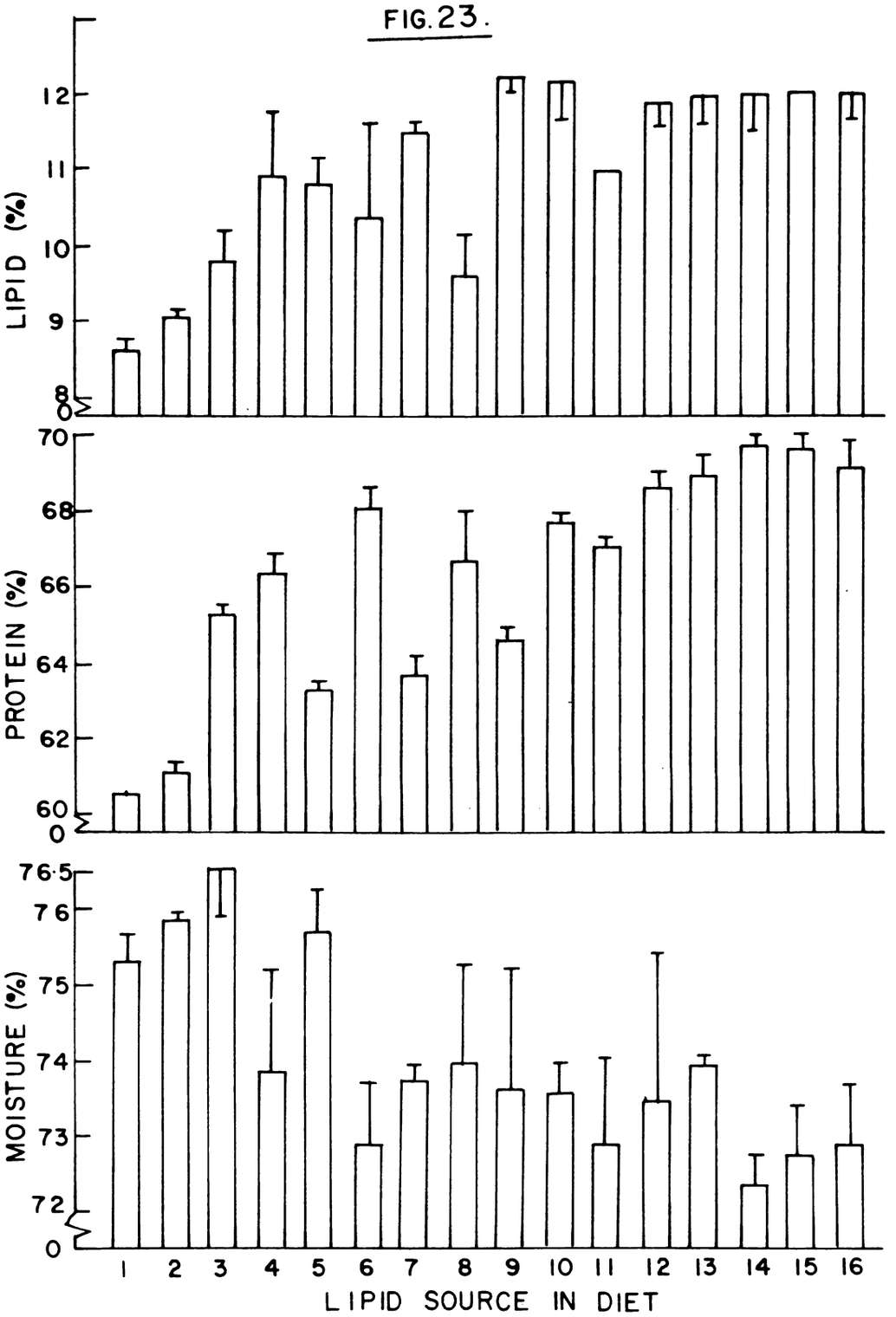
Apparent Digestibility coefficient of diets :-

The apparent digestibility coefficient data for various diets are given in the Table 31. Digestibility of the diets was also influenced by the sources of lipids incorporated in them. Digestibility observed in the present experiment shows some similarity with that of food conversion ratio and protein efficiency ratio. Digestibility of the food was relatively low in diets with plant oils, but it was improved by the addition of animal lipids in the diet. Digestibility of food was more than 70% when the mixture of plant and animal lipid was used in the diets of prawn. Diets with plant oils had lower digestibility (from 19 to 48%) as compared to

TABLE - 31 APPARENT DIGESTIBILITY COEFFICIENT OF FOOD(DRY MATTER) FOR THE JUVENILE PRAWNS FED ON DIETS CONTAINING NATURAL LIPIDS SOURCES

Diet No.	Name of Lipid Source	Apparent digestibility coefficient	Food conversion Ratio
1	Mustard oil	19.13 ± 1.114	9.833 ± 0.623
2	Cotton seed oil	23.46 ± 2.548	9.50 ± 0.408
3	Soyabean Oil	24.10 ± 3.04°	7.866 ± 0.634
4	Safflower oil	24.90 ± 3.18°	8.50 ± 0.408
5	Groundnut oil	41.67 ± 6.747	6.16 ± 0.776
6	Sunflower oil	47.633± 0.0°°	5.06 ± 1.020
7	Linseed oil	39.60 ± 5.533	6.48 ± 0.645
8	Corn oil	40.90 ± 3.210	6.455 ± 0.329
9	Sardine oil	55.34 ± 2.45°	4.416 ± 0.153
10	Codliver oil	61.23 ± 1.596	4.074 ± 0.319
11	Sharkliver oil	59.70 ± 3.47°	3.986 ± 0.598
12	Sardine oil + Sunflower oil	60.50 ± 5.374	8.506 ± 0.490
13	Sardine oil + Groundnut oil	62.4° ± 1.423	3.26 ± 0.094
14	Prawn head Oil + Soyabean oil	87.67 ± 3.721	2.066 ± 0.047
15	Prawn head Oil	76.60 ± 8.566	2.533 ± 0.368
16	Cod liver oil + Soyabean oil + Lecithin	90.76 ± 4.267	2.00 ± 0.311

FIG. 23.


LIPID (%)
12
11
10
9
8
0
PROTEIN (%)
70
68
66
64
62
60
0
MOISTURE (%)
76·5
76
75
74
73
72
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
LIPID SOURCE IN DIET

animal lipid (from 55 to 76.6%). Among diets with plant oils, diet with sunflower oil had better digestibility (47.63%) and diet with mustard oil had the lowest-digestibility (19.23%). The digestibility of food was better when animal oils were used in the diets. The diets with prawn-head oil had the highest digestibility of 76.60% and the diet with sardine oil resulted in relatively lower digestibility (55.34%). Among all the dietary lipid sources, the mixture of plant and animal oils and prawn head oil were relatively better sources of lipid for digestibility of food in the prawn. Digestibility was 60 to 90% for diets with mixtures of plant and animal oils. The diet with mixture of codliver oil, soyabean oil and lecithin was found to be best source of lipid for assimilation of ingested food (90.76%).

Fatty acid composition and nutritive value of dietary lipids

Results of the fatty acids analysis of the dietary lipid sources, post experimental prawns and reference prawn (wild prawn) are given in the Tables 32 and 33.

The fatty acid profiles of the selected plant lipids mainly composed of 14:0, 16:0, 18:0, 18:1 w9, 18:2w6 and 18:3w3; whereas fatty acid profile of selected marine lipids constituted of 14:0, 16:0, 16:1w7; 18:0, 18:1w9, 18:2w6, 18:3w3, 20:5w3, 22:5w3 and 22:6w3. The main difference

observed in between fatty acids contents of plant and marine animal lipids is in the composition of polyunsaturated fatty acids. With the exception of linseed oil and soyabean oil all the plant oils contained very high levels of linoleic acid (18:2w6) and very low levels or absence of linolenic, eicosapentaenoic acid and docosahexaenoic acids. In some plant oils like linseed oil and soyabean oil, linolenic acid (18:3w3) formed 41.05% and 7.38% respectively of the total lipids. Groundnut oil contained more percentage (50.94%) of oleic acid than linoleic acid (33.974%). Other plant lipids also had relatively higher levels of linoleic acid as in cottonseeed oil (52.2%), soyabean oil (57.8%), safflower oil (71.89%), sunflower oil (57.46%) and corn oil (50.02%). Gingely oil and sunflower oils had similar proportions of fatty acids with 52.3% of linoleic acid and 34.36% oleic acid. Mustard and rapeseed oils had low levels of linoleic acid but very high levels of erucic acid (22:0). The coconut oil had high level (90%) of saturated fatty acids and less than 1% of linoleic acid and other PUFA. Lecithin (phosphatidylcholine) had 59% saturated fatty acids 13.2% monounsaturated fatty acids and 25% polyunsaturated fatty acids (Table 32). In spite of this it also contain 3.6% choline, 2.2% inositol 1.2% sterol 3.1% phosphorus, 8% Ash and 1.1% Nitrogen (Conklin et al.,1980).

The prawns fed on diets containing plant oils had high percentages of linoleic acid (18:2w6) in their body tissues, when compared with that of the reference prawn. Among the various groups of prawns fed on diets with plant oils, linseed oil diet fed prawns had relatively higher levels of linolenic acid (18:3w3). Deposition of oleic acid (18:1w9) was observed in all the groups of prawns; but the prawn groups fed on the diet containing ground nut oil had higher level (26.77%) of oleic acid (18:1w9) than the prawns from other treatments. In general, the deposition of HUFA of w3 series fatty acids was less than linoleic acid(18:2w6) in all the groups of plant oil diet fed prawns. Neverthless, the diets containing plant oils as lipid sources induced relatively greater deposition of 18:2w6 and 18:3w3 than the marine oil diets. Besides, the deposition of 16:0 and 18:1w9 was relatively higher level in almost all the groups of prawn irrespective of type of lipid used in their diet. The concentration of saturated fatty acids in the body lipids of all the groups of prawns were similar, except for the very high levels (around 50%) in prawns fed the diets containing, sunflower oil or a mixture of sunflower oil and sardine oil. The concentration of saturated fatty acids in the body lipids of prawn was also relatively higher than the saturated fatty acid content of the dietary lipids, in almost all the groups of prawns.

The total monounsaturated fatty acid concentration in the body lipid was relatively greater in the prawns fed on diets containing marine animal lipids than plant lipids, except for corn oil, linseed oil and sunflower oil diets. Marine lipids used in these experimental diet contained 16:0, 18:0, 16:1w7, 18:1w9, 20:5w3 and 22:6w3 (Table 32). But the concentration of HUFA of w3 series (20:5w3 and 22:6w3) was higher and concentration of 18:2w6 and 18:3w3 was relative lower than that of plant oil diets. A similar pattern was observed in the fatty acids profile of prawns fed on diets containing fish and prawn lipids, with greater concentrations, of 16:1w7, 18:1w9, 20:5w3 and 22:6w3 and lower levels of 18:2w6 and 18:3w3. The fatty acids profile of the prawn groups fed on diets with prawn head oil (Diet 15) or diets with a mixture of codliver oil + soyabean oil + lecithin (Diet 16), resembled that of the dietary lipid source (Table 33). It is interesting to note that these two diets (Diet 15 and 16) produced significantly ($P < 0.05$) the highest growth, among all the diets used in this experiment.

The total contents of HUFA of w3 series (20:5w3 and 22:6w3) present in dietary lipids and percentage of HUFA of w3 series deposited in the body tissues of experimental prawns showed some similarity (Table 33), with some

exceptions. Although plant lipids did not contain 20:5w3 and 22:6w3, still some groups of prawns fed on the diet containing plant lipids (linseed oil, corn oil, soyabean oil) had small percentage of 20:5w3 and 22:6w3 fatty acids, particularly prawn fed on linseed oil diet contained about 6.183% of 20:5w3 and 3.09%% of 22:6w3. But this concentrati of 20:5w3 and 22:6w3 is less as compared to the concentratio present in the reference prawn (11.24% 20:5w3, 11.00%, 22:6w The contents of HUFA of w3 series present in the groups of prawn fed on diets with fish/prawn lipids showed some resemblance to that of the reference prawn. The diet containing fish lipids (codliver oil) provided, 23.42% total w3 fatty acids and only 9.74% of total w6 fatty acids (in the ratio of 2.4:1 w3:w6) which is similar to that of reference prawn.

However, since the growth of prawn was relatively more when fed a diet with mixture of marine animal lipids and plant lipids, it is suggested that the prawn(P. indicus) needs both type of fatty acids (w3 and w6) i.e. 18:2w6, 18:3w3, 20:5w3 and 22:6w3 for normal growth. The diet 16 containing 31.88% saturated fatty acids 28.128% monounsaturated fatty acids, 3.116% linolenic acid, 18.10% linoleic acid and 4.03% 20:5w3 and, 6.95% 22:6w3 provided the best growth. This diet contained about 21.00% total w6 and 14% total w3 fatty acids with w6:w3 ratio of 3:2 .

FATTY ACIDS COMPOSITION (%) OF NATURAL LIPID SOURCES USED IN THE DIETS OF PENAEUS INDICUS.

Mustard Oil	Cotton seed oil	Soya-bean oil	Safflo-wer oil	Ground-nut oil	Sunflo-wer oil	Lin seed oil	Corn-oil	Sardine Oil	Cod-liver oil	Shark liver oil	Sardine Oil+Sun-flower oil	Groundnut Oil +Sar-dine oil	Prawn head oil + soyabean oil	Prawn head oil	Codli-ver oil + Soya-bean oil +Lecithin	Coco-nut oil	Rap-seed Oil	Gingely Oil	Leci-thin
0.027	0.474	-	0.262	0.313	-	0.012	-	0.067	0.276	-	0.335	0.19	0.008	0.016	4.80	42.89	-	-	-
0.0618	1.278	0.373	0.929	0.216	0.044	0.075	0.054	4.94	5.43	0.813	2.47	2.57	2.113	3.833	5.78	19.90	-	0.032	-
-	0.17	-	0.04	-	-	-	-	0.158	-	-	0.079	0.079	0.165	0.33	-	-	-	-	-
-	0.145	-	-	-	-	-	-	0.398	0.322	-	0.199	0.19	0.68	1.36	0.10	-	-	-	-
2.633	20.61	11.77	7.213	13.44	6.35	9.56	15.116	13.693	10.03	14.98	10.084	11.85	17.313	22.85	10.52	9.0	2.0	10.14	20.20
9.478	1.581	-	0.161	-	-	-	-	7.136	11.35	1.51	3.068	3.76	5.269	10.539	6.883	-	-	0.05	-
-	0.182	-	0.282	-	-	0.037	-	0.745	0.80	0.278	0.372	0.37	0.78	1.56	0.45	-	-	0.027	--
-	0.573	-	-	-	1.241	-	-	0.933	-	-	0.466	0.46	0.64	1.294	0.04	-	-	-	-
-	3.07	3.664	2.18	1.958	-	3.744	2.004	12.797	1.796	4.38	7.716	8. 34	6.29	8.929	2.591	3.0	-	5.00	-
9.478	18.32	22.98	15.52	50.943	34.54	23.067	29.83	19.648	23.61	39.22	31.149	35.29	16.8	12.638	21.59	8.63	15.0	35.92	8.8
16.711	52.22	51.80	71.896	33.074	57.46	22.288	50.021	1.528	3.178	3.20	24.619	17.73	27.00	2.92	18.10	4.22	16.0	47.164	60.1
26.298	00.681	7.38	1.118	3.636	0.34	41.059	2.83	9.01	0.506	1.274	4.68	4.50	5.33	1.161	3.116	0.9	7.0	0.90	7.3
-	-	-	-	-	-	-	-	1.219	-	-	0.604	0.609	0.304	-	6.25	-	-	-	-
-	-	-	-	-	-	-	-	0.993	11.264	1.445	0.496	0.496	0.240	4.59	-	c8=4.0	-	-	-
-	-	-	-	-	-	-	-	0.08	-	-	0.3	0.041	0.655	3.286	2.89	c10=5.0	-	-	8.0
0.219	-	-	-	-	-	-	-	8.25	10.412	3.041	9.50	4.12	2.605	5.875	4.03	-	-	-	-
-	-	-	-	-	-	-	-	7.046	-	12.0	-	5.36	2.98	0.597	-	-	-	-	-
-	-	-	-	-	-	-	-	0.543	-	0.321	-	0.271	-	-	-	-	-	-	-
1.68	-	-	-	-	-	-	-	10.734	12.508	10.674	3.523	3.523	7.417	14.837	0.95	-	-	-	-
24.635	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Euric 15.13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.38	-	54.0	-	-
-	-	-	-	-	-	-	-	-	1.356	-	-	-	-	-	-	-	-	-	-
42.49	25.775	15.80	10.87	19.848	9.17	13.42	2.706	38.86	18.35	19.28	21.78	24.139	27.21	38.53	31.88	83.79	-	15.24	20.20
9.47	20.644	22.98	26.59	50.94	42.65	23.067	15.116	28.86	46.76	42.17	35.25	40.09	23.14	25.32	28.12	-	15.0	35.92	8.8
16.71	52.22	51.80	71.89	33.074	47.71	41.059	50.021	1.528	3.178	3.20	24.619	17.73	27.29	2.92	18.10	4.22	16.0	47.16	60.1
26.29	0.681	7.38	1.118	3.636	0.35	22.28	2.83	9.01	0.506	1.274	4.68	4.55	5.33	0.514	3.11	0.90	7.0	0.90	7.3
16.71	52.22	51.803	71.89	33.07	47.46	41.28	50.021	2.15	9.743	3.20	24.93	18.04	20.545	6.206	21.0	4.22	16.0	47.16	60.1
26.5	0.681	7.38	1.118	3.636	0.35	22.28	2.83	26.50	23.426	5.31	17.695	17.51	18.332	22.47	14.0	0.90	7.0	0.90	7.3
-	--	-	-	-	-	-	-	26.654	24.286	37.36	13.327	13.32	13.708	24.597	13.72	-	-	-	8.0

TABLE -33 FATTY ACID COMPOSITION (%) OF NATURAL LIPID USED IN DIET AND LIPID FROM THE WHOLE BODY OF POST EXPERIMENTAL JUVENILE PENAEUS INDICUS.

Diet No.			1		2		3		4		5		6		7		8	
Retention time	Fatty Acids	Wild Prawn Standard	Mustard Oil	Prawn Body Lipid	Cotton Seed	Prawns Lipid	Soyabean oil	Prawns Lipid	Safflower oil	Prawn Lipid	Groundnut oil	Prawns Lipid	Sunflower oil	Prawn Lipid	Linseed Oil	Prawn Lipid	Corn Oil	P L
2.42	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	-	
3.88	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	
4.34	14.1	-	-	-	0.170	-	-	-	0.040	0.322	-	-	-	-	-	0.233	-	
4.94	15.0	-	-	0.465	0.145	0.609	-	0.138	-	0.253	-	0.611	-	-	-	0.822	-	
6.44	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	1
7.61	16.1w7	7.53	9.478	1.118	1.581	0.609	-	0.519	0.161	0.461	-	-	-	1.626	-	1.776		
8.32	17.0	2.24	-	-	0.182	-	-	-	0.282	1.590	-	1.664	-	-	0.0376	1.908		
9.86	17.1	0.94	-	-	0.573	-	-	-	-	-	-	-	1.241	-	-	-		
11.04	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	
12.87	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	2
15.94	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	2
19.10	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	
21.55	20.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
25.38	20.1w9	1.39	-	0.698	-	1.244	-	1.138	-	0.829	-	0.946	-	0.812	-	-	-	
27.52	20.4w6	8.68	-	2.132	-	8.050	-	0.796	-	0.783	-	0.165	-	1.536	-	1.451	-	
33.37	20.5w3	11.24	0.2196	2.480	-	0.40	-	1.492	-	0.783	-	2.307	-	3.298	-	6.183	-	
38.23	22.5w3	1.88	-	-	-	-	-	1-591	-	0.783	-	-	-	-	-	-	-	
43.85	22.4w6	-	-	2.795	-	-	-	-	-	6.222	-	-	-	4.732	-	-	-	
49.81	22.6w3	11.00	1.688	0.093	-	0.316	1.601	0.412	-	1.451	-	0.303	-	2.65	-	3,096	-	
Total Saturated		27,04	42.493	37.228	25.775	38.848	15.809	49.65	10.874	43.877	19.848	34.824	9.170	44.60	13.421	35.414	2.076	
Total Monounsaturated		24.97	35.776	18.824	20.644	19.093	22.984	19.147	25.599	15.047	50.943	27.72	42.65	17.033	23.067	21.37	15.116	
	18:2w6	4.29	16.711	14.957	52.224	18.326	51.803	20.74	71.896	25.004	33.074	24.668	47.71	18.435	22.288	9.574	50.021	
	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	
Total w6		12.97	16.711	19.884	52.224	26.376	51.803	21.674	71.896	26.455	33.074	25.136	47.467	19.969	22.288	10.954	50.021	
Total w3		23.27	5.378	8.49	0.681	6.723	7.380	7.689	1.118	5.668	3.636	9.367	0.350	10.366	41.059	28.853	2.031	
Total PUFA > 20C		32.00	15.13	7.50	..(w6)	8.788w6	-	5.914 w6&w3	—(w6)	9.144	-	3.482	-	9.576	-	-	-	

Table 33 continued

8	9				10		11		12		13		14		15		16	
Corn Oil	Prawn Lipid	Sardine oil	Prawn Lipid	Fatty Acid	Codliver oil	Prawns Lipid	Shark Liver Oil	Prawn Lipid	Sardine + Sunflower oil	Prawn Lipid	Groundnut + Sardine oil	Prawn Lipid	Prawn Head oil +Soyabean oil	Prawn Lipid	Prawn-head Oil	Prawn Lipid	Codliver + Soyabean Oil + Lecithin	Prawn Lipid
-	5.203	0.0677	2.870	12:0	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
0.054	5.203	4.945	4.583	14:0	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	-	14:1	-	-	-	-	0.079	-	0.079	0.263	0.165	-	0.33	-	-	00085
-	0.485	0.398	2.99	15:0	0.322	-	-	-	0.199	-	0.199	-	0.68	2.064	1.36	-	0.10	0.459
15.166	14.332	13.693	5.99	16:0	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
	2.006	7.136	5.405	16:1	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
	1.212	0.745	7.507	17:0	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	-	17:1	-	-	-	-	0.466	-	00.460	-	0.642	3.456	1.294	-	0.04	1.254
2.004	4.52	12.797	2.535	18:0	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
29.831	21.30	19.648	17.625	18:1w9	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
50.021	23.086	1.528	9.494	18:2w6	3.178	4.449	3.2055	5.577	28.619	3.134	17.73	10.62	27.20	18.51	2.92	9.135	18.10	19.026
2.837	9.005	0.5	1.6	18:3w3	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	20:0	-	-	-	-	0.604	0.142	0.609	-	0.304	-	-	0.815	6.25	0.748
-	1.2 4	0.993	9.616	20:1w9	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	20:4w6	-	-	-	0.877	0.041	0.77	0.041	-	0.655	-	-	-	2.89	0.54
-	2.026	8.250	7.065	20:5w3	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
-	2.756	7.046	1.895	22:5w3	-	-	-	-	-	-	-	-	2.98	3.82	0.597	1.059	-	-
-		0.542	-	22:4w6	-	-	12.0	0.414	0.271	-	0.271	-	-	-	-	-	-	-
-	3.352	10.734	8.18	22:6w3	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
2.076	30.955	33.864	28.56	Total Saturated	18.35	34.90	19.28	34.859	21.78	50.625	24.139	42.55	27.214	32.856	38.525	34.82	31.885	32.977
15.116	24.43	28.868	32.646	Monounsaturated	46.76	36.071	42.175	29.216	35.259	23.99	40.099	30.003	23.144	24.838	25.322	32.273	28.128	23.373
50.021	23.086	1.528	9.494	18:2w6	3.178	4.449	3.2055	15.577	24.619	13.134	7.73	10.62	27.29	18.51	2.92	9.135	**18.10**	**19.026**
2.831	5.005	9.01	9.646	18:3w3	0.506	5.695	1.274	0.243	4.68	0.182	4.50	2.50	5.33	4.54	0.514	2.365	**3.116**	**3.982**
50.021	23.080	2.152	10.43	Total w6	9.743	4.449	3.2055	16.454	24.9318	13.904	18.042	10.62	27.345	18.00	6.206	11.254	**21.00**	**19.57**
2.031	17.138	26.5	19.68	Total w3	23.426	25.421	5.31	20.875	17.695	17.894	17.51	14.618	19.221	20.33	21.225	15.685	**14.00**	**14.793**
-	8.133	26.654	15.942	PUFA / 20	24.286	21.986	37.365	22.101	13.327	9.957	13.322	12.108	11.218	16.332	24.00	15.439	13.723	11.355

DISCUSSION

Studies on the fatty acids requirement of crustaceans have suggested that the nutritive value of lipids primarily depends upon the type and content of essential fatty acids (Kanazawa, 1985). The significantly higher survival and growth of larvae, post-larvae and juveniles on diets containing mixtures of animal and plant lipids clearly indicate the need for a blend of fatty acids of w6 and w3 series in the diets of P. indicus. The poor response in larval, post-larval and juvenile prawns to diets containing only plant lipids is mainly due to the deficiency of HUFA of w3 series such as eicosapentaenoic and docosahexaenoic acids. Examination of the fatty acids profile of the plant lipids used in the experiment (Table 32) indicate that linoleic acid (18:2w6) is most abundant in most cases. Besides, plant lipids containing linolenic acid (18:3w3) such as linseed oil had no significant influence on the animals when compared to lipids of marine origin or mixtures of plant and marine lipids. Data obtained from the experiment revealed that lipids of marine origin are superior to plant lipids. The major difference observed between these two groups of lipids is in their fatty acids profile (Table 32). The marine oils had high levels of HUFA of w3 series (eicosapentaenoic and

docosahexaenoic) and these seems to have induced superior response in the animals, as they presumably satisfied the essential fatty acid needs to a greater extent. However, the best response obtained with mixtures of lipids of plant and marine origin, particularly the diet containing a mixture of cod liver oil, soyabean oil and lecithin, demonstrates that the prawns have dietary requirement for a blend of lipids containing adequate levels of linoleic acid (18:2w6); linolenic acid (18:2w3), eicosapentaenoic acid (20:5w3), and docosahexaenoic acid (22:6w3) and arachidonic acid (20:4w6). This is clearly evident from the profile of fatty acids in the various lipids.

According to Deshimaru and Kuroki (1974a) and Deshimaru et al. (1979) prawns require lipid sources which can supply all the essential fatty acids in proper proportions and adequate levels. If the lipid is deficient in any of the essential fatty acids growth is affected. Plant lipids do not contain the w3 HUFA, essential for prawns, though they are rich in linoleic acid (18:2w6) and or linolenic acid (18:3w3) (Kanazawa et al., 1979f). Similarly, marine animal lipids alone could not produce the best growth in P. indicus as they do not contain the required level of 18:2w6 and 18:3w3, although marine lipids sources are rich in HUFA of w3 series. Conversely, growth is promoted by the inclusion of marine lipids like short necked clam or pollack liver oil

in P. japonicus (Kanazawa et al.,1977 b). Thus P. indicus being a omnivore (Hall, 1962) seems to require 18:2w6, which is rich in plant lipids, along with marine animal lipids (Read, 1981). Therefore, a mixture of plant and animal(marine) lipids in the diet promoted growth in prawns most efficiently, as this mixture provides the required blend of saturated, monounsaturated, and polyunsaturated fatty acids of both w3 and w6 series.

Although, all plant oils were unable to produce maximum growth, some oils like corn oil and linseed oil produced better growth than their counterparts (cotton seed oil, mustard oil, coconut oil and ground nut oil). Apparently, the former two oils contain linolenic acid (18:w3) in good amounts (Table 32) in addition to 18:2w6, usually present in all other plant oils. Studies with purified fatty acids (Chapter 3) clearly showed 18:3w3 to some extent promote growth and perhaps acts as a precursor for w3 HUFAS, which are essential fatty acids, for prawns. Fatty acid profile of prawns fed on diets containing these oils expressed small amounts of 20:5w3 and 22:6w3 i.e., 5.433% with corn oil diet and 9.279% with linseed oil diet. However, in view of the slow rates of conversion or lack of efficient system of conversion of 18:3w3 the animals could not produce superior growth. Earlier studies by Guary et al. (1976a), Aquacop (1978), Colvin (1976 b) and Read (1981) also showed that corn oil and linseed oil are better sources among the plant lipids.

It is generally assumed that the fatty acid needs of a species to a great extent reflects the fatty acid pattern of the animals. If this be so, the prawn head oil should satisfy mostly the essential fatty acids requirement of the prawn and perhaps the response accrued in prawns fed the prawn head oil could be due to this aspect. Besides the fatty acid patterns, the content of phospholipids, in the prawn head oil may be an additional factor contributing to the better performance of the animals as these phospholipid molecules are essential for prawns (vide Chapter 2). Similar conclusions have also been drawn by Kanazawa et al. (1979 a and 1985) for P. japonicus. The growth promoting effect of prawn head oil in prawns has also been reported by Sandifer and Joseph (1976).

Among marine lipids, sardine oil, prawn head oil and codliver oil produced better growth and survival in larvae, post-larvae and juveniles of P. indicus than the remaining individual animal lipids. The superior response may be because these oils are rich sources of 18:1w9 and essential fatty acid such as 20:5w3, 22:6w3; besides these oils also contain small percentage of other PUFA (Table 32). Codliver oil is reported as a standard lipid source for fish(Watanabe, 1982) and prawn (Aquacop, 1978) nutritional studies, as it was found to be a good lipid source. However, larval survival though improved by prawn head oil and codliver oil, sardine oil produced better survival and growth in larvae as well as

post-larvae of P. indicus may be because the larvae may have greater demand for w3 HUFA as they are oceanic (Menon, 1937; 1955). Jones et al. (1979b) and Teshima and Kanazawa (1984) also pointed out the necessity of w3 HUFA for growth and survival of larval stages of the prawn P. japonicus. Although, larval survival is comparatively less than post-larvae and juveniles, when same dietary lipid is provided; in general larval survival showed similar trend with better results when animal lipid or mixtures of animal and plant lipids were provided in their diet than that of plant lipid diets. From the results it is evident that P. indicus may require both plant (18:2w6, 18:3ω3) and marine animal lipids (20:5w3 and 22:6w3) in right proportion because significantly better performance in growth, FCR and PER in P. indicus was obtained with those diets containing mixture of plant and animal lipids. These observations agrees with the observations of Deshimaru et al. (1979). Data obtained from the fatty acid analysis of the dietary lipids and post experimental prawns (Table 33) further support the above observation.

The poor response produced by the diet containing mustard oil may be because of the large amounts of erucic acid (22:0) in the mustard oil, which seems to have growth inhibiting effect on prawns at the lipid level used in the experiment. Similarly, the presence of cyclopropenoic and malvalic fatty acids in cottonseed oil may have reduced the

biological value of the feed as it is known to produce undesirable biological activity (Lee and Sinnhuber, 1972). Sinnhuber *et al.*(1968) found cyclopropenoic fatty acid to reduce the growth rate of trout. Similarly coconut oil contain mostly saturated and monounsaturated fatty acids and levels of essential fatty acids are negligible. So this may be the reason coconut oil diet could not produce better growth in *P. indicus*.

Similarly, among the fish lipids shark liver oil produced poor response because it contains large quantity of squalene in spite of the high levels of essential fatty acids. This squalene has growth inhibiting activity as reported for the prawn *P. merguiensis* by Aquacop (1978) and in fish by Kayama (1964). On the contrary the diets containing other marine lipids, promoted significantly better growth FCR and PER in prawns than those of plant lipid diets. Marine lipids used in this experiment had greater percentage of HUFA of w3 series than that of plant oils, and the growth promoting effect of marine lipids could primarily be due to the presence of these essential fatty acids. Thus the present study shows that plant lipids (containing 18:2w6 and 18:3w3) are less effective in augmenting growth in *P. indicus* compared to marine lipids containing 20:5w3 and 22:6w3. In *P. japonicus* also similar results have been obtained by Kanazawa *et al.* (1978). Thus it is assumed that 18:2w6 and 18:3w3 are found to be less effective than 20:5w3 and 22:6w3 in *P. indicus* also.

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 33) 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). Thus dietary fatty acids are reflected in the tissue lipids of prawns.

The response of prawns to diets containing mixtures of plant and animal lipids clearly indicate that P. indicus require both plant and marine lipids sources, providing 18:2w6, 18:3w3, 20:5w3 and 22:6w3, for promoting growth, FCR, PER and for higher protein deposition in the body. Similarly, the dietary fatty acids are depositid in the tissue fatty acids with little modifications. Although the mixtures of plant and marine animal lipids had a good proportion of w6 fatty acids, this was not proportionately found in the tissue fatty acid profiles. Perhaps the great percentage of ingested w6 fatty acids are utilised for production of energy. But the presence of high percentage of w3 fatty acids in the prawn tissues indicate w3 fatty acids are preferentially retained for body building. Joseph and William (1975) and Sandifer and Joseph (1976) also made similar observations in Macrobrachium rosenbergii.

Prawns from various treatments had higher percentage of saturated fatty acids in the body when compared to their

dietary lipids but the percentages of PUFA in the body tissues was less than dietary PUFA content. These results indicates *de novo* synthesis of saturated fatty acids in the prawns and absence of *de novo* synthesis of 18:2w6, 18:3w3, 20:5w3 and 22:6w3 in adequate levels. Earlier studies have also shown the absence of *de novo* synthesis of HUFA in penaeid prawns (Kanazawa and Teshima, 1977; Kanazawa *et al*., 1979 b and c). However, the prawns fed on diets containing some plant oils expressed small percentage of w3 HUFA in their tissue lipids (5.963%w3 HUFA with sunflower oil 5.433% w3 HUFA with corn oil diet and 9.279 w3 HUFA with linseed oil diet). The possible reason can be (1) synthesis of w3 HUFA from 18:3w3 in small amounts and (2) the cannibolism prevailing during moulting resulting in cumulative accumulation of w3 HUFA in the body of prawn *P. indicus*. Among the mixtures of plant and animal lipids used in this experiment the mixture of codliver oil soyabean oil and lecithin in the ratio 6.67: 3.33: 2 provided the best growth, FCR, PER and protein retention in *P. indicus*. This ratio of lipids source provides 21% w6 fatty acids and 14% w3 fatty acids of the total lipids and thus seems to meet the fatty acid needs of prawns. Similarly, the diet with a mixture of prawn head oil and soyabean oil containing 27.9% w6 and 19.22% w3 fatty acids provided superior growth. In both these cases the fatty acid deposition in prawns showed some similarity with that of the reference prawn.

Thus it is clear from the study that if any one of the essential fatty acid is deficient or present in inadequate levels then their respective functions will be affected with the ultimate reduction in growth. According to New (1976) the ratio of w3:w6 fatty acids in the dietary lipids is important for penaeid prawns than the levels of each of these series fatty acids. Since the mixture of codliver oil, soyabean oil and lecithin provided the best response in all stages of P. indicus it can be assumed that the ratio of fatty acids of w6 and w3 series present in this mixed lipid source is adequate for P. indicus. Besides the fatty acids pattern, the above mixture provided phospholipids, essential for the prawn. Thus the mixture of codliver oil, soyabean oil and lecithin was most effective in promoting growth and survival in view of the balance of fatty acids and content of phospholipids.

Many studies carried out in the past on essential fatty acid requirements of crustaceans suggested that the nutritive value of lipid source for prawn is primarily related to the types and content of essential fatty acids (Kanazawa, 1985). Earlier studies (Kanazawa et al., 1970, 1977b; Guary et al., 1976a; Sick and Andrews, 1973; Joseph and William, 1975; Sandifer and Joseph, 1975; Castell and Covey, 1976; Aquacop, 1978; Tridell and Castell, 1980) have demonstrated that marine lipids containing w3 HUFA such as codliver oil, pollack liver oil, prawn head oil, short necked clam oil, and sardine oil have superior dietary value in nutrition of prawns and

lobsters, and plant lipids containing 18:2w6 having inferior dietary value. On the other hand Deshimaru and Kuroki(1974a), Deshimaru et al. (1979) and Read (1981) have shown that mixed lipid sources (marine animal lipid and plant lipid) are better in the diet of prawns. New (1976) in his review also suggested the importance of w6 fatty acids along with w3 fatty acid for prawn nutrition.

Thus all these studies clearly indicate that the mixture of plant and animal lipid sources are superior to individual lipid sources for prawns. The present study besides agreeing with the observations of above authors also indicate that phospholipids are essential constituentsfor P. indicus.

Food and protein utilization (FCR & PER), protein retention and digestibility of food in prawns are influenced by the dietary lipid source. Apparent digestibility of diets containing plant lipids appears to be poor as compared to that of marine lipids, may be because the marine lipids contain w3 HUFA having low melting point as compared to plant lipids. The digestibility of lipid is reported to decrease with increase in melting point (Takeuchi et al., 1979). The increase in digestibility of the diet resulted in better food conversion ratio and protein efficiency ratio on feeding diet with marine lipids. As a result of poor digestion of the ingested food containing plant lipids, the food conversion

ratio, protein efficiency ratio and protein retention in the body of animal appears to be poor.

The apparent digestibility, FCR, PER and protein retention in the body were better when prawns were fed on a diet containing mixture of codliver oil soyabean oil and lecithin than all other dietary lipid sources, used in this experiment. Feed efficiency and growth of the prawn P. japonicus was also shown to be better when Deshimaru et al. (1979) fed diet with mixture of pollack liver oil and soyabean oil than diets with any plant lipid (soyabean oil) or only fish lipid (pollack liver oil). These results agrees with the present findings with P. indicus. Thus the growth digestibility FCR, PER and protein retention were significantly influenced by the quality of lipid and not by the quantity of lipid in the diet. Thus it is clear that nutritive value of lipid depends primarily upon the essential fatty acid content of dietary lipid source (Watanabe,1982). Phospholipids in the diets also have growth promoting effect perhaps by promoting intestinal absorption and interorgan transport of dietary lipids and cholesterol (Kanazawa et al.,1985)

Thus present study with various lipid sources indicate that prawn P. indicus require lipid sources which provide w6 and w3 HUFA in adequate levels and in optimum proportions, and optimum contents of phospholipids in the diets.

CHAPTER - V

CHOLESTEROL REQUIREMENTS

INTRODUCTION

Sterols are solid alcohols containing hydroxyl groups. Steroids are the derivatives of saturated tetracyclic hydrocarbons, perhydrocyclopentanophenanthrene. The most abundant steroid in animal tissues is cholesterol (Lehninger, 1984). It occurs in the plasma membranes of many animal cells, in the lipoprotein of blood plasma and large quantities occur in the brain and the nerve tissues (Lehninger, 1984). Vertebrates are known to biosynthesize cholesterol from precursors such as acetate and mevalonate. But crustaceans do not have the ability to biosynthesize cholesterol (Zandee, 1964, 1966a; Van den Oord, 1964; Whitney, 1969; Kanazawa et al., 1971a and b; Teshima et al., 1983; D'Abramo et al., 1984). But cholesterol is found to be an essential nutrient for growth and survival of crustaceans (Kanazawa et al., 1970, 1971a, b; Deshimaru and Kuroki, 1974b; Castell et al., 1975; Teshima et al., 1983; D'Abramo et al., 1984).

Cholesterol, when mixed with fat or oil, has the peculiar property of enabling fat or oil to absorb water, thus helps in transportation of lipids in the body of animals. It is also a poor conductor of electricity and serves as an insulator against electrical discharge, especially in the brain, where it acts as insulator against nerve impulses which are electrical in character. In crustaceans, cholesterol is also the precursor

for various physiologically important compounds like steroid hormones, brain and moulting hormones and vitamin D(Kanazawa et al.,1971a; New, 1976). Erogosterol a precursor of cholesterol has been shown to get converted into vitamin D on irradiation by sunlight (Lehninger, 1984). Guary and Kanazawa (1973) investigated the role of cholesterol in the hypodermis formation during moulting in P. japonicus, as sterols are important components in the cellular and subcellular membranes, particularly in the hypodermis in Arthropoda (Gilbert, 1969; New, 1976).

Sterols are found to be the precursors of moulting hormones - ecdysone and ecodysterone in Arthropoda (Gilbert, 1969). Several workers have reported the induction of moulting in crustaceans by administration of ecdysones (Karlson and Skinner, 1960; Kurata, 1968; Krishnakumaran and Scheiderman, 1968; 1970; Jegala et al.,1972) as well as ecdysterone (Kanazawa et al., 1972). Detailed investigations on the uptake and turnover of cholesterol by the crab Hemigrapsus nudus during moulting has been made by Spaziani and Kater (1973) and they indicated the role of cholesterol as precursor for moulting hormones. Kanazawa and Teshima (1971) established that the lobster Panulirus japonica contain the enzyme system required for elaboration of several steroid hormones, although the initial substrate cholesterol can not be synthesized de novo by the lobster. Thus it is well established that crustaceans are

incapable of de novo sterol biosynthesis. They thus resemble the arachnids (Zandee, 1964), which rely upon dietary source for sterol. It has been demonstrated that (1-14C) acetate was not incorporated into squalene or sterol in the crayfish, Astacus astacus (Zandee, 1966a) the lobster, Homarus vulgaris (Zandee, 1964) or the crab, Cancer pagurus (Van den oord, 1964), and the lobster, Homarus gammarus (Zandee, 1967). Similarly, larvae of mud crab, Rhithropanopeus harrissii and the spider crab, Libinia emerginata also failed to utilize labelled acetate for sterol elaboration (Whitney, 1969). Finally Teshima and Kanazawa (1970, 1971a,b) have demonstrated the inability of a brine shrimp (Artemia salina), a prawn (Penaeus japonicus), a lobster (Panulirus japonica) and a crab (Portunus trituberculatus) to synthesise sterols. Incapability of sterol biosynthesis de novo has also been shown more recently by Teshima et al. (1983) in the case of larvae of P. japonicus and by D'Abramo et al. (1984) in juvenile lobster Homarus sp. and Duglass et al. (1981) in lobster. Thus all the studies so far conducted with crustaceans established the need for an exogenous source of sterol for survival. It is suspected, but yet to be proved that the crustacean tissues may lack specific enzyme systems required for biosynthesis of cholesterol from non-sterol substances.

Earlier investigations established cholesterol to be the most abundant sterol in crustaceans (Goad, 1976). More

recent detailed studies, particularly, those of Idler and Wiseman (1971), Teshima and Kanazawa (1971b), and Gagosian (1975) have confirmed that cholesterol is indeed the predominant sterol in crustaceans, although several crustaceans contain desmosterol in relatively greater quantities in some tissues. Thompson (1964) who studied the total cholesterol content of the body of few shellfishes reported cholesterol contents of 98 mg/100 g in Callinectes sapidus, 156 mg/100 g in the prawn Penaeus aztecus and 157 mg/100 g in P. setiferus. In P. japonicus sterol constituted about 0.17% of the biomass on wet weight basis (Kanazawa et al., 1971 a). New (1976) reported that the sterol content of the body was not affected by the type of dietary sterol, and cholesterol formed 96 to 99% of total sterol in shrimps.

The cholesterol content of prawn has also been found to vary with increase in body weight. Variations in concentration of cholesterol in the different tissues was also observed in the prawn P. aztecus (Krishnamoorthy et al., 1982) and the lobster Panulirus japonica (Teshima, 1972). The cholesterol content was highest in gonad, heart, intestine and hepatopancreas but was lowest in muscle and exoskeleton in the lobster (Teshima, 1972). The cholesterol content was highest in the eye-stalks of the prawn P. japonicus (Kanazawa et al., 1976a) and P. aztecus (Krishnamurthy et al., 1982).

The foregoing information suggests that cholesterol is a very important constituent in the body of crustaceans and that synthesis of cholesterol from non-sterol nutrients in the body is not evident. Thus studies have shown that crustaceans require a dietary source of sterol for normal growth and survival. New (1976), and more recently Kanazawa (1985) reviewed the information available on the sterol requirements of crustaceans. A dietary sterol requirement by the prawn P. japonicus was demonstrated by Kanazawa et al.(1970,1971a,b). These authors found that the growth rate of the prawn fed on the sterol free diet was poor, but it grew normally on a diet supplemented with 0.5% cholesterol. These authors also studied the efficacy of different sterols and found that ergosterol, sitosterol and stigmasterol promote good survival rate, but produce inferior growth to that of cholesterol. Besides, irrespective of the type of dietary sterol, the prawn had a similar pattern of tissue sterol composition with cholesterol consisting 96 to 99% of sterol indicating the synthesis of cholesterol from the other tested sterols. However a mixture of phytosterols composed of β-sitosterol and campesterol did not adequately substitute for cholesterol in the diet for juvenile lobster and it was suggested that dietary sterol requirement of Homarus sp. can be satisfied only by cholesterol (D'Abramo et al.,1984). Besides, the sterol composition of lobster was not affected by the quality or quantity of sterol

used in their diet (D'Abramo *et al.*,1984). *Artemia salina* could convert dietary ergosterol to cholesterol (Teshima and Kanazawa, 1971b). The prawn *P. japonicus* was able to absorb the dietary sterols even other than cholesterol but the percentage of absorption of dietary sterol was reduced when dietary level was more than 2% (Teshima *et al.*,1974). In contrast to the juveniles, the prawn larvae were able to utilize other sterols as substitutes for cholesterol suggesting that *P. japonicus* larvae probably possess the ability to convert certain C_{28} and C_{29} sterols to cholesterol (Teshima *et al.*,1983). Absorption rate of cholesterol was improved by the presence of other lipids such as phosphatidylcholine (lecithin). The high content of soya lecithin in the diet facilitated uptake of cholesterol in the lobster *Homarus* sp. (D'Abramo *et al.*,1982)

Studies have also shown variations in quantitative requirements of cholesterol by crustaceans. While Shudo *et al.*(1971) reported fastest growth in juvenile *P. japonicus*, when fed a diet containing 0.1% cholesterol, Kanazawa *et al.* (1971a) found 0.5% cholesterol to be best for the juveniles of the same species. In contrast, Deshimaru and Kuroki (1974b) found that the best relative growth was achieved with 2% cholesterol in the juvenile *P. japonicus*. Recently, Teshima *et al.* (1983) reported optimum growth and survival

of prawn larvae (P. japonicus), when fed a diet with 1% cholesterol. But a sterol free diet resulted in poor survival and growth. Survival and growth rate of the prawn, Artemisia longinaris was improved by feeding a diet containing 0.5% cholesterol (Petriella et al., 1984). Optimum level of dietary cholesterol required for juvenile lobster was also 0.5% (dry weight) and lower level (0.2%) of cholesterol produced inferior growth (Castell et al., 1975). D'Abramo et al. (1984) reported 0.12% level of cholesterol to be adequate for performing better growth and survival in the case of Homarus sp. Ponat and Adelung (1983) claimed that optimum dietary cholesterol level in the synthetic food fed to crab Carcinus maenas was 1.4 to 2.1%, however they have not tested lower levels of cholesterol. But the growth of the crab increased as the dietary cholesterol level increased.

From the foregoing review, it is apparent that crustaceans are incapable of biosynthesizing cholesterol from nonsterol nutrients but cholesterol is an important component of tissues, present in sufficiently high level in the body and serves as a precussor for steroid hormones and vitamin D. The above review also reveals that sterols other than cholesterol are less effective in promoting growth and improving survival. Thus cholesterol is an essential nutrient in the diet of crustaceans for proper growth and

survival. Besides, the quantitative requirement for cholesterol by the crustaceans depends upon the type of species and stage in life-cycle. In spite of the importance of cholesterol in prawns there is no information on the sterol requirement of Indian penaeid prawns. Therefore, the present study was undertaken to determine the dietary cholesterol requirement of larvae and juveniles of P. indicus. I have selected cholesterol, among the sterols, since earlier studies by Kanazawa et al. (1971a) and Teshima et al. (1983) clearly established its superiority in promoting growth in the prawn, P. japonicus compared to all other types of sterols.

MATERIALS AND METHODS

Four sets of experiments were conducted to determine the dietary cholesterol requirements of larvae, post-larvae 1-10, post-larvae 11-25, and juveniles of P. indicus by using approximately isocaloric and isonitrogenous diets with graded levels of cholesterol ranging from zero to four percent. Basal composition of the diets is same as that of reference diet in Table 2. Since lecithin enhances the cholesterol solubilization and transport in crustaceans (Lester et al., 1975) and it is found to be essential for growth in juveniles

(Kanazawa et al.,1979e and larval P. japonicus (Kanazawa et al.,1985), and for survival in the lobster Homarus americanus (Conklin et al.,1980), soya-lecithin (phosphatidylcholine) was incorporated at 2.0% level for larvae and post-larvae 1-10 and 4% for post-larvae 11-25 and juvenile prawns in addition to the basal lipid level of 8% which constituted to codliver oil and soyabean oil in the ratio of 2:1.

Seven isonitrogenous and isocaloric diets were prepared by using graded levels of cholesterol from zero to four percent viz. 0.0% (Diet 1), 0.5%(Diet 2), 1.0%(Diet 3) 1.5% (Diet 4), 2.0%(Diet 5) 3.0%(Diet 6) 4.0%(Diet 7). The energy content of the diet was maintained by adjusting carbohydrates levels particularly glucose, sucrose and cellulose powder with that of cholesterol.

While NPCL-17, a compounded diet from CMFRI, Cochin was used as a control (Diet 8) in post-larval and juveniles experiments, Phytoplankton was used as a control in larval experiments. These control diets are kept to have an idea about the environmental parameters.

Details about experimental animals stocking density, feeding and rearing techniques, duration of experiments, methods of preparation of diets, collection of data on

TABLE - 34 ENVIRONMENTAL FACTORS, STOCKING DENSITY PER TREATMENT, MEAN INITIAL LENGTH AND WEIGHTS OF ANIMALS, AND FEEDING LEVEL FOR THE EXPERIMENT ON CHOLESTEROL REQUIREMENTS

Parameters	Stage of the prawn			
	Larvae	Post-larvae 1-10	Post-larvae 11-25	Juveniles
Salinity (‰)	34.0 ± 2	32.0 ± 2	20.0 ± 2	20.0 ± 2
Temperature (°C)	29.0 to 31.0	28.5 to 30.0	26.2 to 28.2	28 to 31
pH	7.8 to 8.2	7.5 to 8.2	7.5 to 8.3	7.9 to 8.3
Dissolved oxygen in water (Mg/l)	5.1 to 8.2	4.7 to 6.7	4.7 to 6.2	4.8 to 6.2
Total ammonia -N in Seawater (ppm)	0.02 to 0.08	0.03 to 0.09	0.03 to 0.011	0.02 to 0.11
Initial number of prawn	150	60	45	30
Average initial length (mm)	-	6.00	12.40	28.35
Average initial wet weight (mg)	-	0.475	6.266	81.90 to 172
Average initial dry weight (mg)	-	0.110	1.50	27.16
Feeding level % of biomass	100	30 to 40	30 to 40	20 to 30

survival, growth and proximate composition, and analysis of data are described previously in the general materials and methods section (PP15-29).

Water quality parameters such as salinity, temperature, dissolved oxygen content, pH and ammonia concentration in the water were monitored regularly and found that these environmental variables were more or less similar among all the treatments of each of the experiments (Table 34). Initial mean length, wet weight and dry weight of experimental animals are given in Table 34. The differences found in means of initial length, wet weight and dry weight in between the treatments were statistically insignificant.

R E S U L T S

LARVAE

The results of the feeding experiment conducted in the larvae of *P. indicus* are shown in Table 35A. All the protozoea-I larvae in treatment 9, where food was not supplied, died within 3 days without metamorphosis. In treatment 8(control), where phytoplankton was fed, 34% of the larvae (protozoeaI) attained the post-larval stage I in 10 days. However the cholesterol deficient diet (Diet 1), when fed to the larvae, caused mortality at various larval stages with complete

mortality at mysis stage I on the 7th day of the experiment. Maximum mortality of larvae occurred at protozoea II stage and it was relatively less in protozoea III stage and increased again resulting in complete mortality at mysis stage I. The larvae grew to post-larvae 1 in 9 days, with 20.6% survival on the diet containing 0.5% cholesterol (Diet 2). Similarly, the diet with 1% cholesterol (Diet 3) produced 17.6% survival at the post-larval I stage. The survival of larvae was relatively low in treatments 4 to 7 as it ranged from 10.6% to 5.34%. Thus inclusion of cholesterol at levels above 1% in the diet did not promote larval growth and survival, but rather resulted in decreased rate of survival.

The data for final survival rates were subjected to analysis of variance and the significant difference between treatments if any, were determined by the least significant difference test. The results indicate that survival of larvae in treatment 2 and 3 was significantly ($P < 0.05$) higher than all other treatments, but significantly lower than treatment 8 (control). There was no significant difference in the survival rate between treatments 2 and 3, as well as treatments 3, 4, 5 and 6. These results indicate that P. indicus larvae require a dietary source of cholesterol

TABLE - 35A GROWTH AND SURVIVAL OF P. INDICUS LARVAE FED ON DIETS CONTAINING GRADED LEVELS OF CHOLESTEROL.

Diet No.	Cholesterol %	Survival rates (%) of various developmental stages of prawn larvae							
		P1	P2	P3	M1	M2	M3	PL1	Feeding Period days
1	0.0	100	27.34	14.67	2.00	-	-	-	6
2	0.5	100	52.00	41.34	38.67	36.67	33.34	20.60	9
3	1.0	100	52.67	40.67	36.00	30.00	24.00	17.34	9
4	1.5	100	33.33	23.33	20.66	18.00	16.00	10.67	10
5	2.0	100	38.67	28.67	23.33	18.00	14.67	9.34	10
6	3.0	100	40.67	21.33	12.67	10.67	6.67	5.34	11
7	4.0	100	29.34	25.34	21.34	10.00	9.34	8.67	12
8	Control	100	80.67	80.67	66.00	57.34	45.34	34.00	9
9	No Food	100	-	-	-	-	-	-	-

P1, P2, P3 = Protozoeal stages of larvae
M1, M2, M3 = Mysis stages of larvae
PL1 = Post-larva 1

as an essential nutrient and a minimum level of about 0.5% of cholesterol in the diet is sufficient enough to improve growth and survival of larvae.

In Table 35B the survival rates of larvae at various stages in life cycle are given. These results indicate the trend in occurrence of mortality during larval development and growth from protozoea I to post-larva I. Diets containing 0.5 and 1% cholesterol and the control diet were found to be better than all other treatments. The control diet of phytoplankton produced significantly the highest survival rates at protozoea II stage (80.67%). However heavy mortality occurred during the metamorphosis from protozoea III to Mysis I stage, thereby a survival rate of 66% only was attained. From Mysis 1 to post-larvae 1 stage there was considerable decline in the survival with the result only 34% of the protozoea reached the PL-1 stage. This shows that the mortality was more in zoeal stages than at mysis stages. Survival of larvae in treatment 2 with 0.5% cholesterol and treatment 3, 1% cholesterol in the diets showed some similarity. In these two treatments about 50% of the larvae died during metamorphosis from protozoea 1 to II stage and the survival was around 40% from protozoea-I to protozoea-III stage and finally 20.6% post-larvae survived in treatment 2 and 17.34% in treatment 3. It was also observed that the mortality of

TABLE - 35B SURVIVAL RATE (%) OF LARVAE AT VARIOUS DEVELOPMENTAL STAGES DURING METAMORPHOSIS.

Diet No.	Cholesterol Level %	Survival rate (%) of larvae at various developmental stages				
		P1	From P1 to P3	From P3 to M1	From M1 to M3	From M3 to PL1
1	0.0	100	14.67	13.63	-	-
2	0.5	100	41.33	93.54	86.20	62.00
3	1.0	100	40.67	88.52	66.67	72.22
4	1.5	100	23.33	88.57	77.41	66.67
5	2.0	100	28.66	23.33	62.85	63.63
6	3.0	100	21.33	59.37	52.63	80.00
7	4.0	100	25.33	21.33	83.75	93.00
8	Control	100	80.67	81.81	68.68	75.00
9	No Food	100	-	-	-	-

larvae was high during zoeal stages than during mysis stage in most of the treatments (Table 35B). The mortality of larvae remained constant from Mysis-I to Mysis-III and increased insignificantly from mysis-III to post-larvae I in almost all the treatments, except treatment-I with cholesterol free diet.

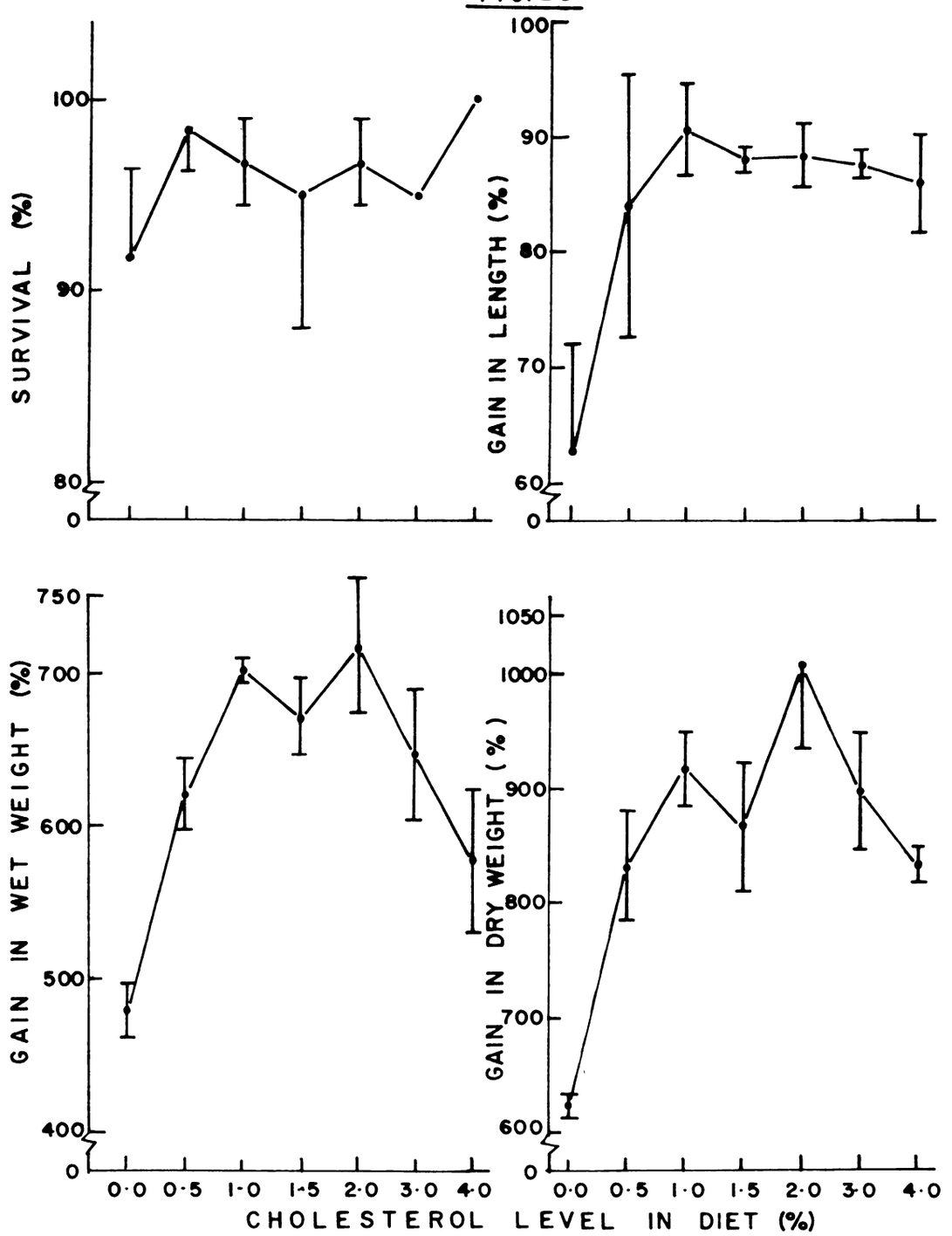
POST-LARVAE 1-10

The results of the feeding experiment conducted in post-larvae 1-10 of P. indicus with diets containing graded levels of cholesterol ranging from zero to four percent are shown in Fig. 25. Survival rate of post-larvae was uniformly high in all the treatments and it ranged from 91.65% to 100%. Statistical analysis of data did not reveal any significant differences in survival rates between most of the treatments.

The growth rates of post-larvae represented as the mean percent gains in length, wet weight and dry weight are shown in Fig. 25. Cholesterol concentration in the diet significantly ($P < 0.05$) influenced the growth of post-larvae. The cholesterol deficient diet (Treatment 1) produced significantly ($P < 0.05$) lower mean percent gains in length, wet weight and dry weight than all other diets. Supplementation of cholesterol in the diets resulted in significant increase in length, wet weight

Fig. 25 Survival rate, and growth of post-larvae 1-10 fed on diets containing graded levels of cholesterol

FIG. 25.


SURVIVAL (%)
100
90
80
0
GAIN IN LENGTH (%)
100
90
80
70
60
0
GAIN IN WET WEIGHT (%)
750
700
600
500
400
0
GAIN IN DRY WEIGHT (%)
1050
1000
900
800
700
600
0
0·0 0·5 1·0 1·5 2·0 3·0 4·0
CHOLESTEROL LEVEL IN DIET (%)

and dry weight of the post-larvae. The diet containing 0.5% cholesterol produced significantly greater ($P < 0.05$) growth than the cholesterol free diet. The wet weight gain of the post-larvae 1-10 was significantly ($P < 0.05$) higher with the diet containing 1% cholesterol, but gain in length and dry weights were not significantly higher than the post-larvae fed diet containing 0.5% cholesterol. Although the growth of post-larvae increased continuously corresponding to the increase in cholesterol level in diet, this trend in growth did not differ significantly between diets containing various levels of cholesterol. However, inclusion of cholesterol at a level of 2% in the diet gave significantly higher mean wet weight gain and dry weight gain than the diet with 0.5% cholesterol.

POST-LARVAE 11-25

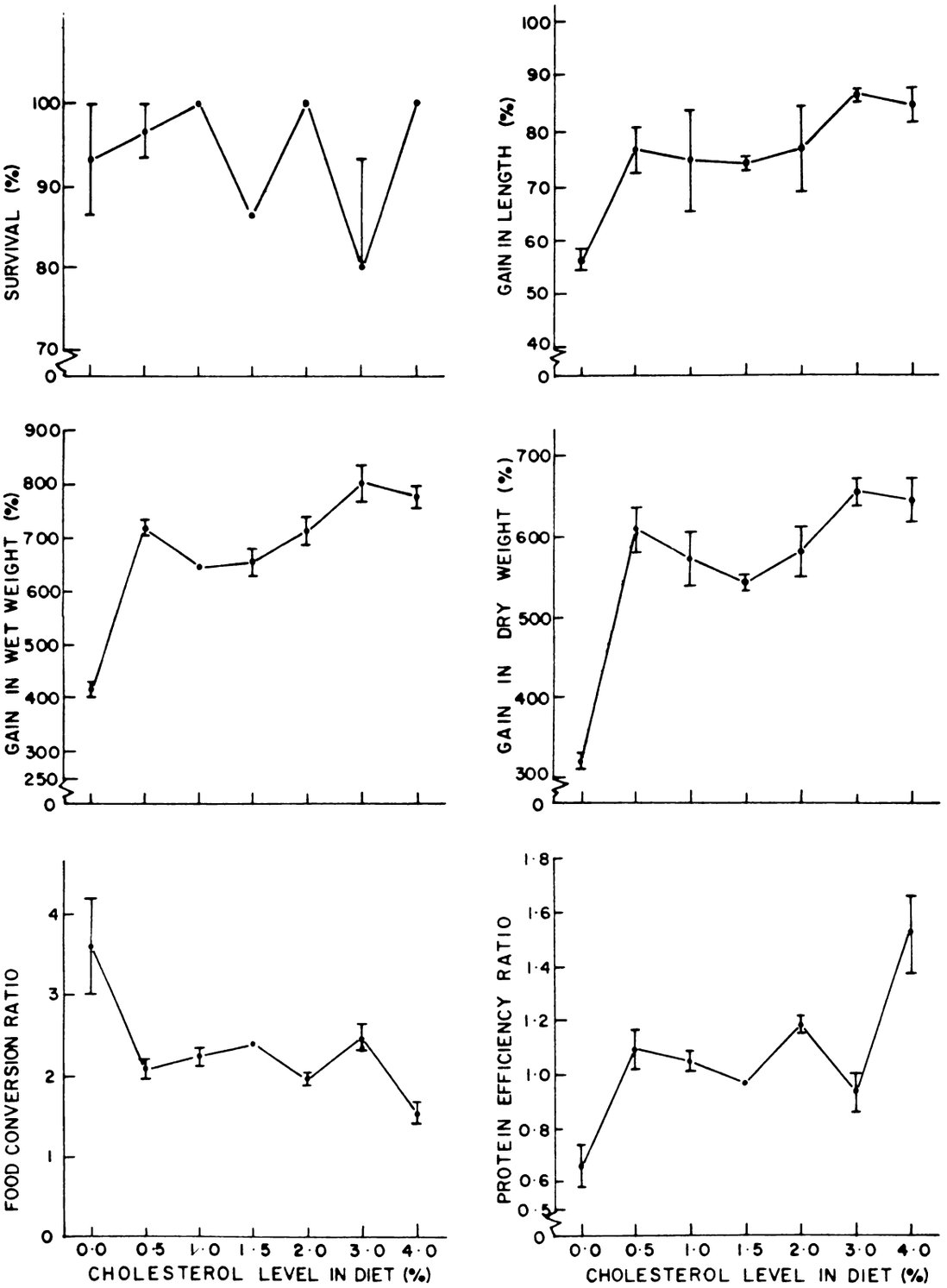
The results of the feeding experiment conducted in post-larvae 11-25 of *P. indicus* with diets containing various levels of cholesterol, viz.,0.0, 0.5, 1.0, 1.5, 2.0. 3.0, 4.0 g per 100 g of diet, are shown in Table 36 and Figure 26. Survival rates of post-larvae recorded from various treatments ranged from 80% to 100% and statistical analysis of the data showed that the cholesterol level in the diets did not significantly affect the survival rates of post-larvae.

Data for mean percent gains in length, wet weight and dry weight of post-larvae from various treatments are illustrated in Fig. 26. Analysis of variance of the data showed that the growth of prawns was significantly ($P < 0.05$) influenced by the dietary cholesterol level. The cholesterol free diet produced relatively poor growth. Post-larvae fed the diet containing 0.5% cholesterol had significantly ($P < 0.05$) higher mean percent gains in length, wet weight and dry weight than those fed the cholesterol free diet (Diet-1). Incorporation of cholesterol in diets at levels above 0.5% did not significantly augment growth. Although slight differences were observed in the growth of post-larvae fed on diets containing various other levels of cholesterol, the observed differences were not statistically significant.

The food conversion ratio (Fig.26) was significantly high but the PER (Fig.26) was significantly low in treatment 1, in which cholesterol-free diet was fed to post-larvae indicating that the utilization of ingested food and protein was greatly affected by the deficiency of cholesterol in the diet. The food conversion and protein efficiency ratios were significantly improved by the inclusion of cholesterol at a level of 0.5% in the diet. However, increasing the cholesterol level in the diet beyond 0.5% did not significantly influence the food conversion or protein efficiency ratios, except for the diet with 4% cholesterol which had significantly ($P < 0.05$)

Fig. 26 Survival rate, growth, FCR and PER of post-larvae 11-25 fed on diets containing graded levels of cholesterol

FIG. 26.


SURVIVAL (%)
100
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70
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GAIN IN LENGTH (%)
100
90
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GAIN IN WET WEIGHT (%)
900
800
700
600
500
400
300
250
0
GAIN IN DRY WEIGHT (%)
700
600
500
400
300
0
FOOD CONVERSION RATIO
4
3
2
1
0
PROTEIN EFFICIENCY RATIO
1·8
1·6
1·4
1·2
1·0
0·8
0·6
0·5
0
0·0 0·5 1·0 1·5 2·0 3·0 4·0
CHOLESTEROL LEVEL IN DIET (%)
0·0 0·5 1·0 1·5 2·0 3·0 4·0
CHOLESTEROL LEVEL IN DIET (%)

TABLE - 36 EFFECTS OF DIETARY CHOLESTEROL LEVELS ON THE BIOCHEMICAL COMPOSITION OF THE POST-LARVAE 11-25

Diet No.	Cholesterol Level in the diet (%)	Moisture (%)	Percentage on dry weight basis				
			Protein	Lipid	Carbohydrate	Ash	Cholesterol mg/100 g
1	0.00	76.77	60.15	7.65	3.08	20.40	60.00
		±0.14	±0.05	±0.55	±0.10	±0.50	±20.0
2	0.50	75.45	69.10	11.40	1.61	16.30	185.00
		±0.60	±0.10	±0.20	±0.39	±0.10	±5.0
3	1.00	75.67	68.30	11.85	2. 19	16.85	155.00
		±0.04	±0.50	±0.95	±0.06	±0.05	±35.00
4	1.50	75.71	67.80	11.10	3.05	17.00	133.00
		±0.61	±1.10	±0.10	±0.08	±0.30	±10.00
5	2.00	76.29	67.95	12.25	3.19	16.95	150.00
		±0.24	±0.05	±0.25	±0.01	±0.94	±10.00
6	3.00	76.63	68.05	12.00	3.15	15.95	135.00
		±0.13	±0.15	±0.10	±0.05	±0.25	±5.00
7	4.00	75.54	67.40	13.10	2.90	17.20	140.00
		±0.17	±0.50	±0.30	±0.10	±0.60	±0.00

higher PER and lower FCR than the diet with 0.5% cholesterol.

The moisture, protein, lipid carbohydrate ash and cholesterol contents of post-larvae obtained after the experiment are shown in Table 36. The proximate composition of post-larvae was also affected by the dietary levels of cholesterol. The post-larvae fed on the cholesterol deficient diet had significantly ($P < 0.05$) low protein, lipid and cholesterol but high moisture, ash and carbohydrate contents. Whereas the post-larvae fed on the diet containing 0.5% cholesterol had relatively high protein and cholesterol but low moisture and carbohydrate contents. Supplementation of cholesterol in the diet resulted in relatively greater deposition of protein, lipid and cholesterol in the body. Although slight differences were observed in protein deposition in post-larvae fed on diets containing various levels of cholesterol (0-5 to 3%), the observed differences were not statistically significant.

JUVENILES

The results of the feeding experiment conducted in juveniles are shown in Fig. 27 and 28. The survival rate of juvenile prawns was not significantly affected by dietary cholesterol level. The survival rates were very high in all the treatments as it ranged from 93.3% to 100%. The percent mean gains in length, wet weight and dry weight were considered

for assessing growth of juvenile prawns and the data are illustrated in Fig. 27. Analysis of variance of the data showed that the dietary level of cholesterol significantly ($P < 0.05$) affect the mean percent gain in length, wet weight and dry weight of juveniles. From Fig. 27 it is evident that the cholesterol deficient diet (Diet 1) produced relatively poor growth and that the inclusion of cholesterol at a level of 0.5% (Diet 2) significantly increased the growth of juvenile prawns. Least significant difference test showed that the mean percentage gains in length, wet weight and dry weight of prawns fed the cholesterol deficient diet were significantly lower than those fed diets containing various levels of cholesterol. Though the mean percent gain in length and wet weight were significantly ($P < 0.05$) higher at 3% cholesterol in the diet than that at 0.5% cholesterol, there was no significant difference in the dry weight gains of prawns between these two dietary treatments. However, inclusion of 4% cholesterol in the diet resulted in reduced growth (Fig. 27). The mean percent gain in length and wet weight of prawns increased with the dietary level of cholesterol upto 3% and further increase in cholesterol level in the diet resulted in reduced growth. The mean percent gain in dry weight of prawn was the lowest in cholesterol free dietary treatment (85%), and it sharply increased to 398% in treatment - 2(0.5% cholesterol diet) and reached the maximum (458.95%) in treatment-3 with 1% cholesterol and

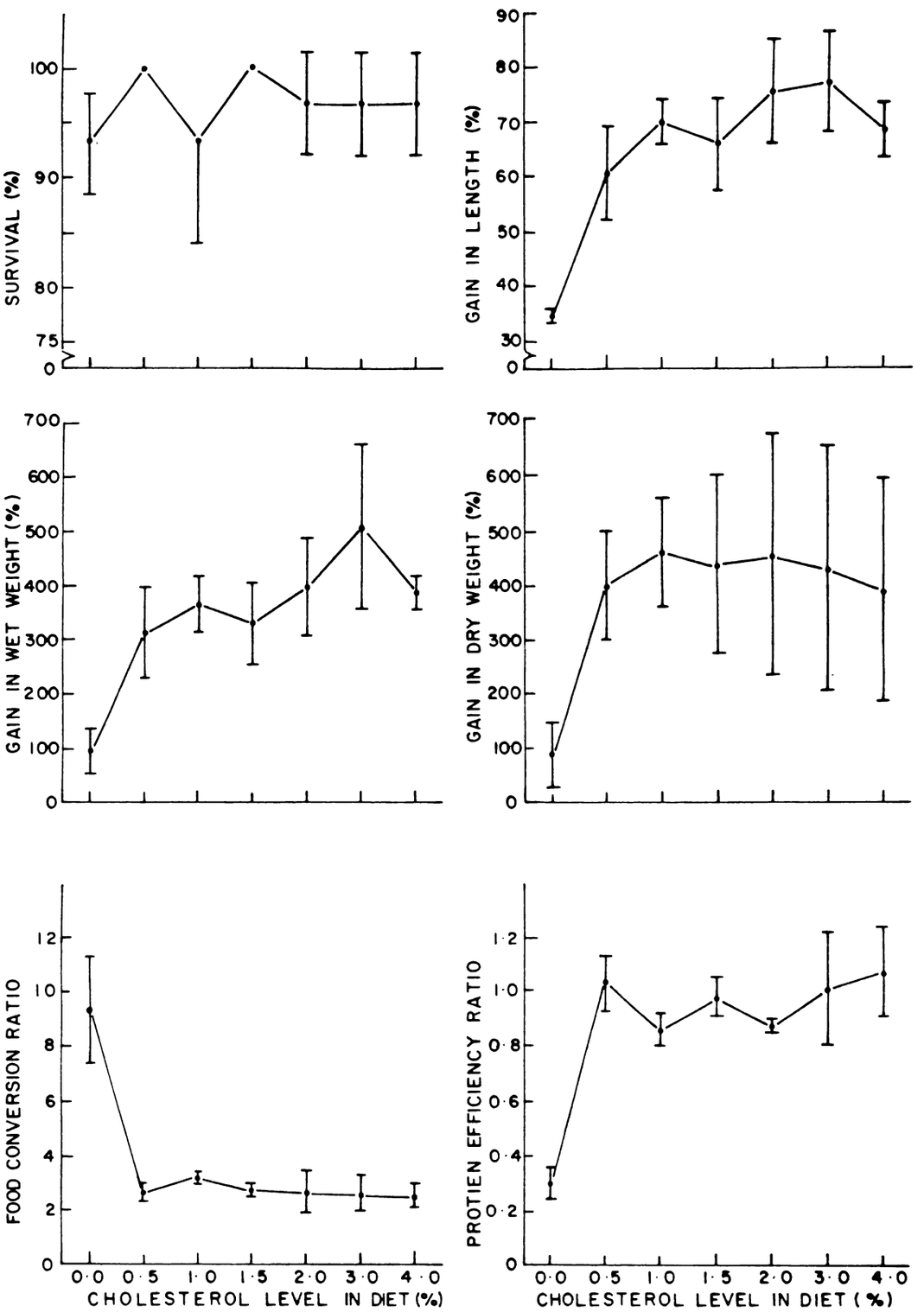
further increase in cholesterol level in the diet did not improve the mean percent dry weight gain and cholesterol level of 4.0% produced relatively less dry weight gain. However the observed differences in the mean percent dry weight gain among prawns receiving diets containing cholesterol level ranging from 0.5 to 4% were not statistically significant.

While the food conversion ratio (Fig. 27) was significantly higher ($P < 0.05$), the protein efficiency ratio (Fig.27) was significantly lower for diet 1 (cholesterol free diet). The food conversion and protein efficiency ratios were significantly ($P < 0.05$) improved by the inclusion of cholesterol at a level of 0.5% in diet. However, inclusion of increasing levels of cholesterol in the diets 2 to 7 did not significantly influence the food conversion or protein efficiency ratios. These results indicate that cholesterol is essential for proper utilization of the ingested food and protein, and that an optimum level of 0.5% cholesterol is sufficient enough to provide better food conversion and protein efficiency ratios.

The moisture, protein, lipid, carbohydrate, ash and cholesterol content of the juvenile prawns subjected to various experimental diets are shown in Fig. 28. Statistical analysis of the data showed that the proximate composition of prawns was also significantly ($P < 0.05$) affected by the dietary level of cholesterol. Compared to the prawns fed the diets

Fig. 27 Survival rate, growth, FCR and PER of juvenile prawns fed on diets containing graded levels of cholesterol

FIG. 27.


SURVIVAL (%)
100
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GAIN IN LENGTH (%)
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40
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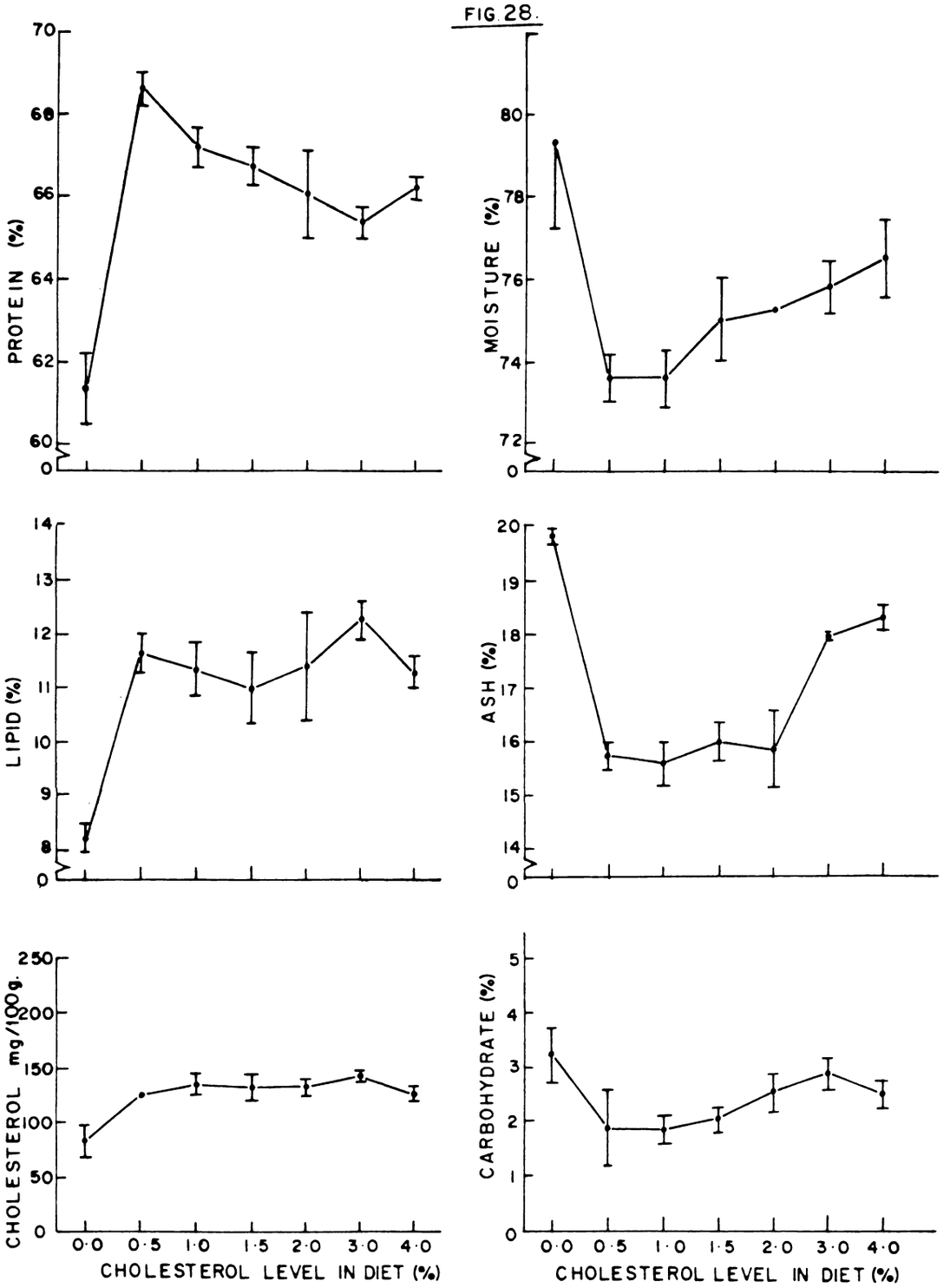
GAIN IN WET WEIGHT (%)
700
600
500
400
300
200
100
0
GAIN IN DRY WEIGHT (%)
700
600
500
400
300
200
100
0
FOOD CONVERSION RATIO
12
10
8
6
4
2
0
PROTIEN EFFICIENCY RATIO
1·2
1·0
0·8
0·6
0·4
0·2
0
0·0 0·5 1·0 1·5 2·0 3·0 4·0
CHOLESTEROL LEVEL IN DIET (%)
0·0 0·5 1·0 1·5 2·0 3·0 4·0
CHOLESTEROL LEVEL IN DIET (%)

containing various concentrations of cholesterol, the cholesterol deficient diet fed prawns had significantly ($P < 0.05$) lower protein, lipid and cholesterol contents but significantly ($P < 0.05$) higher moisture, ash and carbohydrate contents. Inclusion of cholesterol in the diet, even at the lowest level of 0.5% resulted in reduced moisture and ash contents, but caused increased accumulation of protein, lipid and cholesterol contents.

The moisture content of prawns fed the diet containing 0.5% cholesterol was similar to that fed diet with 1% cholesterol. Though there was slight increase in the moisture content of prawns fed the diet with 1.5% cholesterol it was not significantly different from that of prawns fed higher levels of cholesterol (2% and above) in the diets. Though the protein contents of prawns fed diets containing 0.5 and 1% cholesterol were relatively higher, there were no significant differences in the protein content of prawns between various dietary treatments. The highest lipid content was observed in prawns fed the diet containing 3% cholesterol but this was not significantly ($P > 0.05$) different from the lipid content of prawns fed with various other concentrations of cholesterol. The cholesterol content of prawns from treatments 2-7 were also not significantly different from each other. The ash content was significantly ($P < 0.05$) higher in prawns from treatment 1, 6 and 7 than that of prawns from other

Fig. 28 Biochemical composition of juvenile prawns fed on diets containing graded levels of cholesterol.

FIG. 28.


PROTEIN (%)
70
68
66
64
62
60
0
MOISTURE (%)
80
78
76
74
72
0
LIPID (%)
14
13
12
11
10
9
8
0
ASH (%)
20
19
18
17
16
15
14
0
CHOLESTEROL mg/100g.
250
200
150
100
50
0
0·0 0·5 1·0 1·5 2·0 3·0 4·0
CHOLESTEROL LEVEL IN DIET (%)
CARBOHYDRATE (%)
5
4
3
2
1
0
0·0 0·5 1·0 1·5 2·0 3·0 4·0
CHOLESTEROL LEVEL IN DIET (%)

treatments. Although significant differences in the proximate composition of prawns were not observed between diets containing more than 0.5% cholesterol, the protein content of prawns from treatment 2 (0.5% cholesterol) was significantly greater than that of treatments 4, 5, 6 and 7.

DISCUSSION

The results of the present experiments clearly demonstrate that cholesterol is an indispensable nutrient in the diet for the larvae, post-larvae and juveniles of the penaeid prawn, P. indicus. The growth, survival and metamorphosis of larvae seems to be greatly affected by cholesterol deficiency in the diet. The study further reveals that there is no beneficial effect when diets containing more than 0.5% are fed to the larvae. Besides, it is also evident that protozoeal stages are the worst affected by cholesterol deficiency, since the highest mortality rates occurred at this stage. The essentiality of cholesterol in the diet have also been reported for P. japonicus larvae (Teshima et al.,1983). However in P. japonicus best growth and survival were observed when 1% cholesterol was used in the diet (Jones et al.,1979a; Teshima et al.,1982b) and Teshima et al. (1983).But in the present study no advantage was observed by inclusion of cholesterol at levels greater than 0.5%, though 1% cholesterol

did not in any way affect the results. The differences in the quantitative difference could be mostly attributed to the species differences. Although none of the larvae reached the post-larval 1 stage, when cholesterol was excluded from the diet a small percentage of larvae could metamorphose and grew to mysis 1 stage. The survival and metamorphosis of larvae to mysis 1 may be due to the presence of trace amounts of sterol in the basal lipid used (codliver oil and soyabean oil) in the diet. However this amount seems to be indequate for the larvae as they could not grew up to post-larval 1 stage.

In general, mortality of larval prawns was relatively more during protozoeal stages when compared to mysis stages in almost all the treatments where cholesterol containing diets were fed. It is probable that the purified nature of the diet and its particle size had some adverse effect on the survival of protozoeae. Probable reasons for the high mortality rates during the protozoeal stages may include the non-availability of adequate quantity of the desired particle size of the food in the vicinity of the mouth of the larvae, thus subjecting them to obligatory fast; and leaching of essential nutrients from the micro-particulate diet. As compared to protozoeae, mysis larvae are bigger and have appendages to collect and hold the food and ingest it more efficiently (Muthu, 1983) which perhaps resulted in relatively less mortality during mysis stage. Earlier studies have

shown that the larvae of the prawn, Peneeus japonicus require an exogenous source of sterol in the diet for normal survival, growth and metamorphosis. Teshima et al. (1983) reported that the growth and survival of prawn larvae fed a cholesterol deficient diet were very poor, but the larvae grew and survived well on a diet supplemented with 1% cholesterol; but 5% cholesterol produced poor growth and survival. These results agrees with my observations on the larvae of P. indicus.

The survival of post-larvae 1-10 and 11-25 as well as juveniles was not significantly influenced by the cholesterol content of the diet; even the cholesterol deficient diet produced good survival. It is suepected that trace levels of cholesterol present in the basal lipid (codliver oil and soyabean oil) might have sustained such high survival rates even in the cholesterol deficient diet fed prawns. But growth of post-larvae as well as juveniles was significantly affected when they were fed on the cholesterol deficient diet. Growth increased significantly when 0.5% cholesterol was added in the diet. However high levels of cholesterol in the diet could not produce significantly higher growth in the post-larvae and juveniles. The cholesterol level in the diet also significantly influenced the FCR, PER and protein retention in the body in the post-larvae 11-25 and juvenile prawns. Exclusion of cholesterol from the diet resulted in poor FCR,

PER and protein retention. These results indicate that post-larvae and juvenile of P. indicus require cholesterol in the diet as an indispensable nutrient and 0.5% level of cholesterol in the diet appears to be most effective for promoting growth, food and protein utilization, and for protein deposition in the body.

Thus it is apparent that P. indicus do not have the capacity of synthesize cholesterol de novo and dietary cholesterol is essential. Thus the larvae and juveniles of the species conforms to the pattern observed for P. japonicus by Kanazawa et al. (1971a) Teshima and Kanazawa (1971a) and Teshima et al. (1983) as well as the observations of many other authors with crustaceans (Ponat and Adelung, 1983; D'Abramo et al., 1984) that crustaceans have a requirement for cholesterol in the diet.

Comparing the efficacy of a number of sterols for P. japonicus Kanazawa et al. (1971a) and Teshima et al. (1983) found that cholesterol is the best source of sterol for prawn. These authors found that growth rate of prawn P. japonicus fed on diet containing ergosterol, stigmosterol or sitosterol was inferior to diet with cholesterol (Kanazawa et al., 1971a). In Homarus sp. replacement of cholesterol with other sterols In the diet resulted in poor growth (D'Abramo et al., 1984). These studies confirmed the essentiality of cholesterol for

normal survival and growth (D'Abramo *et al.*, 1984) of crustaceans in general. Though the efficacy of other sterols were not elucidated during the present study, considering the above findings, it is obvious that cholesterol might be the ideal sterol for *P. indicus* also.

Feeding experiments using artificial diets have shown that *P. japonicus* juveniles require an optimum level of 0.5% cholesterol in the diet (Kanazawa *et al.*, 1971a) for normal growth and survival. Supplementation of 0.05 or 0.1% cholesterol resulted in poor growth, while 1% cholesterol produced no improvement over 0.5% level, 5% dietary cholesterol depressed growth. These findings agrees with the present results on post-larval and juvenile *P. indicus*. Castell *et al.* (1975) also reported 0.5% cholesterol in the diet could produce superior growth in the lobster when compared to 0.2% cholesterol in the diet and suggested to be the optimum level of cholesterol in the diet for better growth in the lobster, which was subsequently confirmed by D'Abramo *et al.* (1981b) in the diet of the lobster, *Homarus* sp. In contrast to the above observations Deshimaru and Kuroki (1974b) reported relatively higher level of (2.1%) dietary cholesterol for promoting best growth in juvenile *P. japonicus*. All these observations indicate the need for optimum cholesterol in the diet of crustaceans. Read (1981) also empirically used 2% cholesterol in his compounded diet prepared for *P. indicus* and

observed better growth with this diet, though no comparison was made to find out the influence of inclusion of cholesterol at lower levels. Whereas Shudo et al. (1971) reported relatively lower level (0.1%) as dietary cholesterol requirement for juvenile P. japonicus.

These differences in dietary cholesterol requirement can be attributed to the differences in the composition of the basal diet used as well as due to differences in quality and quantity of basal lipid used in the diet (D'Abramo et al.,1984). Kanazawa et al. (1971a) used 8% lipid in the diet as compared to 6% lipid used by Deshimaru and Kuroki (1974b). The increased requirement of cholesterol (2.1%) reported by Deshimaru and Kuroki (1974b) for juvenile P. japonicus may be due to the relatively low lipid content in their diets. Where as the relatively lower dietary cholesterol requirements reported by Kanazawa et al. (1971 a) may be due to relatively higher lipid in the diet. Dietary lipid is presumed to contain a certain level of cholesterol (Kanazawa, 1985). Thus optimum cholesterol requirement in the diet also depends upon other ingredients used in the diet. These observations indicate that in the presence of adequate lipid level in the diet, about 0.5% of cholesterol would be adequate to promote maximum growth and survival of penaeid prawns. In the present experiment with P. indicus I have used a basal lipid level

of 12% constituting 5.34% cod liver oil, 2.66% soyabean oil and 4% lecithin. It is assumed that 0.5% cholesterol along with the mixture of lipid used in the present study appears to be sufficient enough for producing maximum growth in the penaeid prawn P. indicus.

Teshima and Kanazawa (1983) have also demonstrated that the absorption rate of dietary cholesterol is improved by the presence of other lipids. The high content of dietary lecithin in purified lobster diet has been presumed to facilitate uptake of cholesterol (D'Abramo et al., 1982). Lester et al., (1975) observed that lecithin enhanced cholesterol solubilization when associated with the crustacean emulsifier N-N-dodecanosacrosyl taurine (DST). Absence of the phospholipid, phosphotidylcholine has been found to restrict the effective transport of cholesterol within the body of prawn. In the present study diets had 4% lecithin which certainly would have helped in the effective utilization of cholesterol by the prawns. Thus effective utilization of cholesterol depends upon the presence of phospholipids in the diet, as well as on the presence of polyunsaturated fatty acids (PUFA) D'Abramo et al., 1982). These observations (D'Abramo et al., 1982) further support the use of cod-liver oil (a source of PUFA) and lecithin (phospholipid) as a basal lipid source for the present study to determine the cholesterol requirement of P. indicus.

The proximate composition of *P. indicus* was also influenced by the dietary level of cholesterol. The rate of deposition of protein, lipid and cholesterol was relatively low in prawns fed on the cholesterol deficient diet, when compared to prawns fed on cholesterol diets. But there were no significant differences in the chemical composition of prawns between diets containing cholesterol levels from 0.5 to.4%. The FCR and PER significantly improved on inclusion of cholesterol in the diet of prawn which resulted in more deposition of protein in the body. It appears that at optimal concentrations cholesterol has protein sparing action, as the protein content of prawn increased when fed on the diet containing cholesterol (0.5%). The increased protein deposition may be due to the accelaration in the anabolic processes in the tissues as a result of stimulating effects of the steroid hromones synthesized from the dietary cholesterol. Thus the enhanced growth attained on addition of 0.5% cholesterol in diet might be because of the better utilization of food and protein.

Studies have shown cholesterol is used in hypodermis formation (Guary and Kanazawa, 1973; Goad, 1976). Besides the sterols are important as elements of cellular and subcellular structures in arthropods (Lasser *et al.*, 1966). Several workers have also reported sterol are found to be

precursor of moulting hormone in arthropoda (Gilbert,1969) as well as brain hormone in prawns (Kanazawa et al.,1971a; New,1976). Kanazawa et al. (1971a) reported that frequency of moulting increased in P. japonicus when fed on a diet containing cholesterol indicating the involvement of cholesterol in moulting. Further studies by Kanazawa et al. (1972) have demonstrated that ecdysterone induce moulting in P. japonicus and sterols are found to be precursor of ecdysterone, a moulting hormone (Gilbert, 1969). Deficiency of cholesterol in tissues has been shown to cause moult death syndrome in the lobster (D'Abramo et al.,1982). Since moulting is an essential physiological process in prawns, preceeding synthesis of new tissues in the body, the significant increase in growth as well as in protein content, as observed in the present study in prawns, can be expected by the addition of cholesterol which is the precursor for the steroid hormones.

Prawns fed on the cholesterol free diet retained relatively lower levels of tissue cholesterol than those fed on cholesterol supplemented diets in the case of post-larvae and juveniles of P. indicus. This observation is similar to that observed in the prawn P. japonicus (Kanazawa et al., 1971b; New 1976) and lobster, Homarus sp. (D'Abramo et al., 1988). Thompson (1964) reported that total cholesterol content of the body of various prawns was around 156 mg/100g

(*P. aztecus*) and 157 mg/100 g(*P. setiferus*). The quantity of cholesterol found in *P. indicus* during the present study also agrees with the cholesterol content of the above prawns.

The results of these experiments clearly indicate the essentiality of cholesterol for proper survival and growth of larvae, and growth of post-larvae 1-10, and 11-25, and juvenile of *P. indicus* and 0.5% of cholesterol in the diet was found to be most effective for promoting growth significantly in larvae, post-larvae and juvenile prawn was well as for better food conversion ratios, protein efficiency ratio and for more protein retention in post-larvae 11-25 and juvenile prawns.

SUMMARY

SUMMARY

Recent studies with prawns indicate that their growth, metamorphosis, maturation and moulting are affected by the type and level of lipids supplied in the diets. Despite the recognition of the importance of lipids in the diets of prawns there is no information on the essentiality and quantitative lipid requirements of Indian penaeid prawns. Therefore, during the present study about 24 laboratory experiments were conducted to determine the essentiality and dietary requirements of total lipids, phospholipids, fatty acids, cholesterol, and to ascertain the nutritional value of natural lipid sources for the larvae, post-larvae and juveniles of one of the most suitable cultivable species of penaeid prawns, P. indicus.

All the experiments were conducted in the laboratory following standard procedures, using isonitrogen and approximately isocaloric purified diets. Changes were made in the ingredients as required for specific experiments. For the larvae diets of particle size $< 37\mu$ were fed. For the post-larvae and juveniles pellet feed was given. While data on survival and growth of larvae and post-larvae 1-10 were recorded, data were collected on the survival, growth, food conversion ratio, protein efficiency ratio and biochemical composition

of the body for post-larvae 11-25 and juveniles. The influence of fatty acid pattern of dietary lipid sources on the fatty acids profile of prawns were also studied in the case of juvenile prawns. Analysis of variance and least significant difference test were employed to determine the significant differences between treatments in the observed parameters with the help of a Hewlett Packard Master Computer.

The salient findings from the studies are given below:

1. Experimental results clearly indicate the essentiality of lipid for proper survival, growth, conversion of food and protein, and for increased retention of protein in the body of P. indicus.

2. Deficiency of lipid in diets induced heavy mortalities in larvae and post-larvae, besides severely affecting the growth and metamorphosis. Sub-optimal lipid levels also affected the survival and growth of larvae and post-larvae. The highest growth as well as survival in groups of larvae and post-larvae 1-10 fed diets containing 10% lipid suggest that this may be the optimum level for these stages of P. indicus.

3. Although survival and growth of post-larvae 1-10, 11-25 and juveniles were very poor when fed on a lipid free diet, survival and growth were significantly improved by inclusion of 6% lipid in the diet, suggesting this may be the minimum

level required for these stages for maintenance and growth. However, for optimum growth performance, efficient food and protein conversion, and for protein synthesis, a dietary lipid level of 9 to 12% for post-larvae 11-25 and juveniles are required.

4. Supra-optimal levels of lipids had no beneficial effect though the post-larvae and juveniles could tolerate dietary lipid levels as high as 14% to 18% without any deleterious effect on growth.

5. The study also revealed the protein sparing action of dietary lipids. The poor response obtained in low lipid diets (>6%) is ascribed to the utilization of increased levels of protein for metabolic energy.

6. Lecithin (phosphatidylcholine) is found to be an indispensable dietary nutrient for larvae, post-larvae and juveniles of P. indicus. The growth, survival and metamorphosis of larvae and post-larvae 1-10, and growth FCR and PER of post-larvae 11-25 and juveniles seems to be greatly affected by lecithin deficiency in diet.

7. It is evident that for promoting high survival and growth larvae and post-larvae require a dietary level of 2% lecithin, while for juvenile prawns 1% lecithin in the diet is found to be optimum for normal growth.

Inclusion of more than 2% lecithin in the diet has no beneficial effect on survival and growth of larvae, post-larvae and juveniles of P. indicus. More than 4% lecithin in the diet produced reduced growth and poor survival in larvae and post-larvae.

8. The essentiality of phospholipid in the diets is ascribed to the limited ability of the prawn for phospholipid biosynthesis at an adequate level from other food ingredients, as well as the inability of endogenous synthesis of specific types of phospholipids that may be necessary as constituents of lipoproteins which play important role in transport of lipid.

9. Experimental studies clearly demonstrate the essentiality of a blend of polyunsaturated fatty acids of w3 and w6 series (18:2w6, 18:3w3, 20:5w3 and 22:6w3) for proper survival, growth, FCR, PER and retention of protein and lipid in various stages of P. indicus.

10. Diets containing purified fatty acids are poorly accepted by the prawn larvae as inclusion of these fatty acids in the diet caused complete mortality of larvae. But survival, growth and metamorphosis were improved by the inclusion of a mixture of codliver oil, soyabean oil and lecithin which are sources of essential fatty acids, such as 18:2w6, 18:3w3, 20:5w3 and 22:5w3 for the prawn.

11. The data on survival and growth of post-larvae 1-10, and survival, growth, FCR and PER of post-larvae 11-25 indicate that dietary linolenic acid requirement of post-larvae 1-10 and 11-25 may be about 1% and that excess dosage (above 1%) of linolenic acid in the diet significantly depress the growth. Similarly 1.0% linolenic or linoleic or mixture of linoleic and linolenic in the ratio of 0.5:0.5 appears to be optimum level in the diet for juvenile prawns for promoting growth, FCR and PER.

12. A total of 12% lipid in the diet providing 31.88% saturated fatty acids, 28.8% monounsaturated fatty acids, 18.1% linoleic acid, 3.12% linolenic acid, 11.9% of eicosapentaenoic acid and docosahexaenoic acid appears to be beneficial in the diet for post-larvae and juvenile prawns.

13. Among the natural lipid sources used in the present study a mixture of marine animal lipids and plant lipids proved to be superior lipid sources when compared to individual plant and marine animal lipid sources. Plant oils do not contain 20:5w3 and 22:6w3, which are found to be important essential fatty acids for P.indicus. Although animal (marine) lipids contain high levels of 20:5w3 and 22:6w3, they contain relatively low levels of 18:2w6 and 18:3w3 which are also required for various stages of the prawn.

14. Among the plant oils, sunflower oil, corn oil and linseed oil appears to be better sources as these plant oils contain relatively higher percentages of 18:3w3 in addition to 18:2w6, usually present in most of the plant oils. Linolenic acid (18:3w3) has been found to have superior essential fatty acid activity when compared to 18:2w6 for P. indicus.

15. The diets containing coconut oil, mustard oil, cotton seed oil and shark liver oil produced poor growth in larvae post-larvae and juvenile prawns. The reason being that coconut oil contain mostly saturated fatty acids, mustard oil contain high levels of erucic acid and cotton seed oil contain cyclopropenoic acid and malvalic acid. Among the marine animal lipids sharkliver oil contains high levels of squalene. Erucic acid, cyclopropenoic acid, malvalic acid and squalene have been found to produce growth inhibitory effect on animals and a similar response was observed in P. indicus.

16. In general, all the diets containing marine animal lipid sources produced better growth, FCR, PER and protein retention in prawns than the diets containing only plant oil as lipid source due to the presence of higher levels of 22:5w3 and 22:6w3 in marine animal lipids, which have growth promoting effect in prawns.

17. Among the individual marine animal lipids prawn-head oil appears to be a better lipid source for producing superior survival, growth, FCR, PER and protein retention, as it meets mostly the essential fatty acid requirements of the prawn. Besides the fatty acid pattern the content of phospholipid in prawn-head oil may be another factor contributing for the better performance in P. indicus.

18. The diets containing a mixture of plant and animal lipids produced superior growth than individual plant or animal lipids. Among the mixture of lipid sources a mixture of codliver oil, soyabean oil and lecithin produced significantly higher growth and survival in larvae, post-larvae 1-10 and also, significantly higher survival, growth, FCR, PER and protein retention in post-larvae 11-25 and juveniles of P. indicus. Similarly, a mixture of prawn-head oil and soyabean oil, also is a better lipid source for promoting growth, FCR, PER and retention of protein in the prawn.

19. The mixture of cod liver oil soyabean oil and lecithin in the ratio of 56:28:16 at a total lipid level of 10 or 12% can be successfully used as lipid source in compounding practical diets for larvae, post-larvae and juveniles of the prawn.

20. The fatty acid profiles of the selected plant oils composed of 14:0, 16:0, 18:0, 18:1w9, 18:2w6, 18:3w3, where

as marine animal lipids composed of 14:0, 16:0, 16:1w7, 18:0, 18:1w9, 18:2w6, 18:3w3, 20:5w3 and 22:6w3. The main difference observed between plant oils and marine animal lipid is the relatively high levels of linoleic acid and/or linolenic acid, and absence of eicosapentanoic acid and docosahexaenoic acid in the plant lipids.

21. The diets containing plant oils induced relatively greater deposition of 18:2w6 and 18:3w3 than the diets with marine oils. But diets with marine animal lipids induced greater deposition of 20:5w3 and 22:6w3 as compared to plant oil diets. Thus the fatty acid pattern of the prawns to a greater extent depended upon the fatty acids profile of dietary lipids.

22. The concentration of saturated fatty acids in the body lipids of all the groups of prawn, irrespective of their fatty acids, was relatively higher than the saturated fatty acid contents of dietary lipids suggesting synthesis of saturated fatty acids in the body of prawn from other dietary ingredients. The low concentration of 20:5w3 and 22:6w3 in the body lipids of prawn fed on diets with only plant oil indicates absence of slow rate of biosynthesis of these fatty acids from their precursor (18:3w3).

23. Diet with a mixture of codliver oil, soyabean oil and lecithi and that with a mixture of prawn-head oil and soyabean oil

provided prawns with fatty acid pattern almost similar to dietary lipids, suggesting that P. indicus needs natural lipid sources which can supply 18:2w6, 18:3w3, 20:5w3 and 22:6w3 in proper proportions.

24. Cholesterol is found to be an essential nutrient in the diet for larvae, post-larvae, and juveniles of P. indicus.

25. Survival, growth, and metamorphosis of larvae, post-larvae 1-10 and growth, survival, FCR, PER and protein retention of post-larvae 11-25 and juvenile prawns were greatly affected by cholesterol deficiency.

26. The growth, FCR, PER and protein retention were significantly improved on inclusion of 0.5% cholesterol in the diet of prawn, which resulted in more protein deposition in the body. It appears that optimal concentration of cholesterol has protein sparing action. The increased protein deposition may be due to the accelaration in the anabolic process(es) in tissues as a result of stimulating effect of steroid hormones synthesized from dietary cholesterol.

27. The optimal cholesterol requirement for larvae, post-larvae and juvenile prawns seems to be 0.5% of the diet, as high survival and growth in larvae, post-larvae and juvenile prawns and better FCR, PER and higher protein retention in post-larvae 11-25 and juvenile prawns were

recorded at this concentration.

28. Supra-optimal cholesterol levels in the diet has no beneficial effect on growth, FCR, PER and protein retention of various stages of *P. indicus*

Thus the present study has revealed the essentiality of various types of lipids for *P. indicus*. Besides, it is clear that optimum levels of lipids are essential for optimum performance of the animals. It is suggested that a mixture of plant and marine lipids, which provide a blend of polyunsaturated fatty acids such as linoleic (18:2w6) linolenic (18:3w3) eicosapentaenoic acid (20:5w3) and decosahexaenoic acid (22:6w3) should be included in the diets of various stages of *P. indicus* for achieving maximum production. It is also suggested that cholesterol and phospholipids should be included in the diets at optimal levels to achieve optimum performance.

REFERENCES

ABDEL-RAHMAN, S.H., S.KANAZAWA and S.TESHIMA, 1979. Effects of dietary carbohydrate on the growth and the levels of the hepatopancreatic glycogen and serum glucose of prawn. Bull. Jap. Soc. Sci. Fish., 45(12):1491-1494.

ACKMAN, R.G., 1967. Characteristics of the fatty acid composition and biochemistry of some freshwater fish oils and lipids in comparison with marine oils and lipids. Comp. Biochem. Physiol. 22: 907-922.

AHAMAD ALI, S., 1982 . Effect of carbohydrate (starch) level in purified diets on the growth of P. indicus. Indian J. Fish., 29(1 and 2): 201-208.

ALFIN-SLATER, R.B. and L.AFTERGOOD, 1968. Essential Fatty acids reinvestigated. Physiol. Rev., 48: 758-784.

ALLEN, W.V., 1972. Lipid transport in the dungeness crab, Cancer magister Dana. Comp. Biochem. Physiol., 43B:193-207.

ANDREWS, J.W., L.V.SICK and G.J.BAPTIST, 1972. The influence of dietary protein and energy levels on growth and survival of penaeid shrimp. Aquaculture, 1: 341-347.

ANON, 1986. Marine Fish production in India during 1983-84 and 1984-85. Mar. Fish. Infor. Serv. T and E Ser., No.67: 1986. 78P.

AOAC, 1965. Official Methods of Analysis of the Association of official Agricultural Chemists 1965, 10th Edition Published by the Association of official Agricultural chemists Washington, DC.

AOAC, 1975. Official Methods of Analysis of the Association of Official Agricultural Chemists. AOAC. Washington. D.C. 13th Edn., 1094P.

AQUACOP, 1978. Study of nutrition requirements and growth of Penaeus merguiensis in tanks by means of purified and artificial diets. Proc. World Maricult. Soc. 9:225-234.

BALAZS,G.H., E.ROSS, and G.C.BROOKS, 1973. Preliminary studies on the preparation and feeding of crustacean diets. Aquaculture, 2: 369-377.

BALAZS,G.H., and E.ROSS, 1976. Effect of protein source and level on growth and performance of the captive freshwater prawn Macrobrachium rosenbergii. Aquaculture, 7: 299-313.

BARLOW,J., and G.J.RIDGWAY, 1969. Changes in serum lipoprotein during molt and reproductive cycles of the American lobste (Homarus americanus) J. Fish. Res. Bd. Canada, 26:201-2109

BERHNARD,M., and A.ZATTERA, 1970. The importance of avoiding chemical contamination for a successful cultivation of marine organisms. Helgolander Wissenschaftliche Meeresunter sunhungen 20(1-4): 655-675.

BIDDLE,G.N., 1977. The nutrition of Macrobrachium species. In: Shrimp and Prawn Farming in the Western Hemisphere, J.A. Hanson and H.L. Goodwin (Eds.) Dowsden Hutchison and Ross Inc., Pennsylvania, pp. 272-291.

BLIGH E.G. and W.J. DYER, 1959. A rapid Method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37(8): 911-917.

BLIGH,E.G. and M.A.SCOTT, 1966. Blood lipids of lobster Homarus americanus J. Fish. Res. Bd. Can. 23: 1629-1637

BOGHEN,A. and J.D.CASTELL, 1980. Considerations of the lecithin and protein requirements of juvenile lobsters Homarus americanus. In Proceedings of the 1980 lobster nutrition workshop, University of Maine. Publ. Marine Sea Grant, Walpole ME (USA), 1980: 21-28 pp.

BORDNER C.E. and D.E.CONKLIN, 1981. Food Consumption and growth of juvenile lobster. Aquaculture, 24: 285-300.

BOTTINO,N.R., J.GENNITY, M.L.LILLY, E.SIMMONS and G.FINNE, 1980. Seasonal and nutritional effects on the fatty acids of three species of shrimp, Penaeus setiferus, P. aztecus and P. duorarum. Aquaculture, 19: 139-148.

BOWSER,P.R. and R.ROSEMARK, 1981. Mortalities of cultured lobster, Homarus, associated with a molt death syndrome Aquaculture, 23: 11-18.

BRAND,C.W. and L.B.COLVIN, 1977. Compounded diets for early post-larval Penaeus californiensis. Proc. World Mariculture Soc., 8: 811-820.

BROMLEY,P.J., 1980. Effect of dietary protein, lipid and energy content on the growth of turbot (Scophthalmus maximus). Aquaculture, 19: 359-369.

BURR,G.O. and M.M.BURR, 1930. On the nature and role of the essential fatty acids in nutrition. J. Biol. Chem., 86: 587-621.

CASTELL,J.D. and J.F.COVEY, 1976. Dietary lipid requirements of adult lobsters Homarus americanus. J. Nutr., 106: 1159-1165.

CASTELL,J.D., S.D.BUDSON,E.G.MASON and J.F.COVEY, 1975. Cholesterol requirements in the juvenile lobster H. americanus. J. Fish. Res. Bd. Can., 32: 1431-1435.

CASTELL,J.D., R.O.SINNHUBER, J.H.WALES and J.D. LEE, 1972. Essential Fatty acids in the diet of rainbow trout (Salmo gairdneri): Growth, feed conversion and some gross deficiency symptoms. J. Nutr., 102: 77-86.

CASTELL J.D., D.E.CONKLIN, J.CRAIG, K.MORMAN-BOURDEAU and S.P.LALL, 1981. Nutrition in Aquaculture. World conference on Aquaculture and International Aquaculture Trade Show, Venice, Italy 21-25 September, 1981 Reviews p. 19.

CECCALDI,H.J. and J.L.M.MARTIN, 1969. Evolution des proteins de l'hemolymphe chez Carcinus maenas durant l'ovogenese. C.R. Soc. Biol., 163: 2638-2641

CHARLES JOHN, BHASKER T.I. and S.AHAMAD ALI, 1984. Studies on the protein requirement of post-larvae of the penaeid prawn, Penaeus indicus H. Milne Edwards using purified diet. Indian J. Fish, 31(1): 74-81.

CLARKE,A. and J.F.WICKINS, 1980. Lipid content and composition of cultured Penaeus merguiensis fed with animal food. Aquaculture, 20: 17-27.

CLIFFORD,H.C. and R.W.BRICK, 1978. Protein utilization in the freshwater shrimp Macrobrachium rosenbergii. Proc. World Maricult. Soc., 9: 195-208.

CMFRI News Letter, 29-30, 1985. Role of Nutrition in Aquaculture CMFRI news letter No. 29 and 30 July-December 1985: 7-11

COLVIN,P.M., 1976a. Nutritional studies on penaeid prawns: Protein requirements in compounded diets for juvenile Penaeus indicus Milne Edwards. Aquaculture, 7:315-326.

_____, 1976b. The effect of selected seed oils on the fatty acid composition and growth of Penaeus indicus. Aquaculture, 8: 81-89

COLVIN,L.B. and C.W. BRAND, 1977. The protein requirement of penaeid shrimp at various life-cycle stages (with compounded diets) in controlled environment systems. Proc. World Maricult. Soc., 8: 821-840.

CONKLIN D.E., 1980a. Recent progress in lobster nutrition at Bodega marine laboratory. 1980. Lobster nutrition workshop proceedings. Publ. Marine Sea Grant, Technical Report, 58. 1980 p. 29-32.

_____, 1980b. Nutrition. In the Biology and Management of Lobster. Cobb, S.J. and Philips, B.F. (Eds) Vol. I. Academic Press. Inc New York. 366 pp.

_____, L.R.D'ABRAMO, C.E.BORDNER and N.A. BAUM, 1980. A successful purified diet for the culture of juvenile lobsters: the effect of lecithin. Aquaculture, 21: 243-249.

COWEY, C.B. and J.R.M.FORSTER, 1971. The essential amino acid requirements of the prawn Palaemon serratus. The growth of prawns on diet containing proteins of different amino-acid compositions. Mar. Biol., 10:77-81.

D'ABRAMO, L.R., C.E.BORDNER, G.R.DAGGETT, D.E.CONKLIN and N.A. BAUM, 1980. Relationship among dietary lipids, tissue lipid and growth in juvenile lobster. Proc. World Maricult. Soc., 11: 335-345.

_____ & _____, D.E.CONKLIN and N.A.BAUM, 1981a. Essentiality of dietary phosphatidylcholine for the survival of juvenile lobsters. J. Nutr., 111:68-69.

_____, D.E. CONKLIN, C.E. BORDNER, A.B.NANCY and K.A. NORMAN-BOUDREAU, 1981b. Successful artificial diets for the culture for juvenile lobster. J. World Maricult. Soc., 12(1): 325-332.

_____, C.E. BORDNER and D.E.CONKLIN, 1982. Relationship between dietary phosphatidylcholine and serum cholesterol in lobster Homarus sp. Mar. Biol., 67(2):231-235.

D'ABRAMO, L.R., C.E.BORDNER, D.E.CONKLIN and N.A. BAUM, 1984. Sterol requirement of juvenile lobster Homarus sp. Aquaculture, 42(1): 13-25

DALL, W. and D.J.W. MORIARTY, 1983. Functional aspects of nutrition and digestion. In: The Biology of Crustacea. Dorothy E. Bliss (Ed.) Academic Press, New York, 5:215-264.

DESHIMARU, O. and K. KUROKI, 1974a. Studies on a purified diet for prawn-I: Basal composition of diet. Bull. Jap. Soc. Sci. Fish., 40(4): 413-419.

_____, _____, 1974b. Studies on a purified diet for prawn-II: Optimum contents of cholesterol and glucosamine in the diet. Bull. Jap. Soc. Sci. Fish., 40(4): 421-424.

_____, _____, 1974c. Studies on a purified diet for prawn-III: A feeding experiment with amino acid test diets. Bull. Jap. Soc. Sci. Fish., 40(11):1127-1131.

_____, _____, 1975a. Studies on a purified diet for prawn-IV: Evaluation of protein, free amino acids and their mixture as nitrogen source. Bull. Jap. Soc. Sci. Fish., 41(1): 101-103.

_____, _____, 1975b. Studies on a purified diet for prawn-V. Evaluation of casein hydrolysates as a nitrogen source Bull. Jap. Soc. Sci. Fish., 41(3):301-304.

_____, _____, 1976. Studies on a purified diet for prawn-VIII. Adequate dietary levels of ascorbic acid and inositol. Bull. Jap. Soc. Sci. Fish., 42(5): 571-576.

_____, _____, 1979. Requirement of prawn for dietary thiamine, pyridoxine, and choline choloride. Bull. Jap. Soc. Sci. Fish., 45(3): 363-367.

DESHIMARU, O. and K.SHIGUENO, 1972. Introduction to the artificial diet for prawn, Penaeus japonicus. Aquaculture, 1: 115-133.

_____, and Y. YONE, 1978a. Requirements of prawn for dietary minerals. Bull. Jap. Soc. Sci. Fish., 44(8): 907-910.

_____, _____, 1978b. Effect of dietary carbohydrate source on the growth and feed efficiency of prawn. Bull Jap. Soc. Sci. Fish., 44(10): 1161-1163.

_____, _____, 1978c. Optimum level of dietary protein for prawn. Bull. Jap. Soc. Sci. Fish., 44(12): 1395-1397.

_____, K. KUROKI and Y. YONE, 1979. The composition and level of dietary lipid appropriate for growth of prawn. Bull. Jap. Soc. Sci. Fish., 45(5): 519-594.

_____, _____, S.SAKAMOTO and Y. YONE, 1978. Absorption of labelled calcium, ^{45}Ca by prawn from seawater. Bull. Jap. Soc. Sci. Fish., 44(9): 975-977.

DOUGLASS, T.S., W.E.CONNER and D.S. LIN, 1981. The biosynthesis, absorption, and origin of cholesterol and plant sterols in the Florida land crab. J. Lipid Res., 22: 961-970.

DUBOIS,M., K.A.GUILLES, J.K.HAMILTON, P.A.REGERS and I.SMITH, 1956. Colorimetric method for determination of sugars and related substances. Analyst. Chem., 28: 350-356.

FENUCCI,J.I., A.L.LAWRENCE and Z.P.ZEIN-ELDIN, 1981. The effect of fatty acid and shrimp meal composition of prepared diet on growth of juvenile shrimp Penaeus stylirostris J. World. Mariculr. Soc., 12(1): 315-324.

FISHER,L.R. 1960. Vitamins. In: The Physiology of crustacea' T.H. Waterman(Ed.) Volume I, Academic Press, New York, N.Y., pp. 259-289.

FOLCH,J., M. LEES and G.H.STOARE-STANLEY, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226:497-508.

FORSTER,J.R.M., 1972. Some methods of binding prawn diets and their effects on growth and assimilation. J. Cons. Inst. Explor. Mer., 34: 200-216.

_____, 1976. Studies on the development of compounded diets for prawns. Proc. First Intl. Conf. on Aquaculture Nutr., Delaware, NOAA (Sea Grant), 14-15 October 1975 pp. 229-248.

_____, and T.W. BEARD, 1973. Growth experiment with Palaemon serratus Pennant, fed with fresh and compounded foods. Fishery Invest. London, Ser. II, 27(7), 16 pp.

_____, and P.A. GABBOT, 1971. The assimilation of nutrients from compounded diets by prawns, Palaemon serratus and Pandalus platyceros. J. Mar. Biol. Assoc., U.K., 51: 943-961.

GAGOSIAN,R.B., 1975. Sterols of the lobster (Homarus americanus) and the shrimp (Pandalus borealis) Experientia, 31:878-880

GALLAGHER,M. and W.D.BROWN, 1975. Composition of San Francisco Bay brine shrimp (Artemia salina). Agric. Food Chem., 23: 630-633.

GIESE, A.C.,1967. Some methods for study of the biochemical constitution of marine invertebrates. Oceanogr. Mar. Biol. Ann. Rev., 1967, 5: 159-186.

GILBERT,L.I., 1969. Cited by Gilbert L.I.and J.D.O'Connor in "Chemical Zoology". Florkin, M. and B.T. Scheer, (Eds) Academic Press, New York, Vol. 5, pp. 229-254.

_____ and J.D. O'CONNOR, 1970. Lipid Metabolism and Transport in Arthropods. In: "Chemical Zoology", M. Florkin and B.T. Scheer (Eds.) Academic Press New York. 5 pp. 229-253.

GOAD J., 1976. Steroids of marine algae and invertebrate animals. In: Biochemical and Biophysical Perspectives in Marine Biology. Malins, D.C. and J.R. Sargent (Eds) Academic Press, London 3: 213-319.

GOPAKUMAR, K. and M.R.NAIR, 1975. Lipid composition of five species of Indian prawns. J. Sci. Food Agric. 26:319-325.

GUARNERI,M. and R.M.JOHNSON, 1970. The essential fatty acids In: Adv. Lipid. Res., 8, 115-174, Paoletti, R. and D. Kritchevsky (Eds.) Academic Press. New York. 8: 115-174.

GUARY, J.C. and A. KANAZAWA, 1973. Distribution and fate of exogenous cholesterol during the moulting cycle of the prawn, Penaeus japonicus. Comp. Biochem. Physiol., 46a: 5-10.

_____, M. KAYAMA, Y. MURAKAMI and H.J.CECCALDI, 1976a. The effect of a fat-free diet and compounded diets supplemented with various oils on moults, growth and fatty acid composition of prawn, Penaeus japonicus. Aquaculture, 7: 245-254.

GUARY, M., A.KANAZAWA, M.TANAKA and H.J.CECCALDI, 1976b. Nutritional requirements of prawn-VI Requirement for ascorbic acid. Mem. Fac. Fish., Kagoshima Univ., 25(1):53-57.

HALL,D.N.F., 1962. Observation on the taxonomy and biology of some Indo-West Pacific Pensaeidae (Crustacea Decapoda). Fishery Publ. Colon. Off. No.17, H.M.S.O., London 229 pp.

HALVER,J.E., 1972. The Vitamins. In: Fish Nutrition. J.E. Halver (Ed), Academic Press Inc., New York, pp. 29-104.

HANSON, J.A. and H.L.GOODWIN, 1977. Shrimp and prawn farming in the Western Hemisphere. Dowden, Hutchinson and Ross, Inc., Pennsylvania, 439 pp.

HATCH,F.T., and R.S.LEES, 1968. Practical Methods for plasma lipoprotein analysis. In: "Advances in Lipid Research". PAOLETTI R. and D. KRIJCHEVSKY (Eds) 6: 1-68. Academic Press, New York.

HESTRIN,S., 1949. Cholesterol estimation for tissue. J. Biol. Chem., 180:249.

HILDITCH,T.P. and P.N. WILLIAMS, 1964. The chemical constitution of Natural Fats. 4th Ed. John Wiley, and Sons, New York. pp. 30-56.

HUNER, J.V. and L.B. COLVIN, 1977. A short term study on effect of diets with varied calcium: Phosphorus ratios on the growth of juvenile shrimp Penaeus californiensis penaeidae, Crustacea Proc. World Mariicul. Soc.,8:775-778.

IDLER,D.R. and P.WISEMAN, 1971. Sterols of Crustacea. Int. J. Biochem., 2: 91-98

IMAI,T.,1977. Aquaculture in shallow seas. Progress in shallow sea culture. Amerind publishing Co.(Pvt). Ltd., New Delhi pp. 1-613.

JEGLA, T.C., J.D. COSTLOW and J.ALSPAUGH, 1972. Effect of ecdysones and some synthetic analogs on horseshoe crab larvae Gen. Comp. Endocrinol., 19: 159-166.

JONES,D.A., A.KANAZAWA and S.ABDEL-RAHMAN, 1979a. Studies on the presentation of artificial diets for rearing the larvae of Penaeus japonicus (Bate). Aquaculture, 17:33-4

_____, _____and K. ONO, 1979b. Studies on the nutritional requirements of the larval stages of Penaeus japonicus using microencapsulated diets. Mar. Biol. 54: 261-267.

JOSEPH J.D. and J.E.WILLIAMS, 1975. Shrimp head oil, A potential feed additive for mariculture. Proc. World Maricult. Soc., 6: 147-155.

KANAZAWA, A., 1982. Penaeid nutrition In: Proc. Second Intl. Conf. on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition, pp. 87-105. G.J.Pruder,C.J.Langdon and D.E.Conklin(Eds.) Louisiana State Univ., Baton Rouge, Louisiana.

_____, 1983. Effect of phospholipids on aquatic animals. Feed Oil Abst., B(18): 1-5

_____, 1985. Nutrition of penaeid prawns and shrimps. Proc. of the First Intl, Conf. on the Culture of Penaeid prawns/shrimps. Illoilo city, Philippines, 1984. pp.123-43

_____ and S.TESHIMA, 1971. In vivo conversion of cholesterol to steroid hormones in the spiny lobster, Panulirus japonica, Bull. Jap. Soc. Sci. Fish., 37:891-898.

_____, _____, 1977. Biosynthesis of fatty acids from acetate in the prawn, P. japonicus. Mem. Fac. Fish., Kagoshima Univ., 26: 49-53.

_____, _____, 1981. Essential amino acids of the prawn. Bull. Jap. Soc. Sci. Fish, 47(10):1375-1337.

KANAZAWA A. and S. TESHIMA, 1983. Development of microparticula diets for the larvae of fish, crustaceans, and shell fis Yoshoku, 20(11): 97-101.

_____, M. SHIMAYA, M. KAWASAKI and K. KASHIWADA, 1970. Nutritional requirements of prawn-I. Feeding on artificial diet. Bull. Jap. Soc. Sci. Fish., 36:949-954.

_____, N. TANAKA, S. TESHIMA and K. KASHIWADA, 1971a. Nutritional requirements of prawn-II. Requirement for sterols. Bull. Jap. Soc. Sci. Fish., 37(3): 211-215.

_____, _____, _____, _____, 1971b. Nutritional requirements of prawn-III. Utilization of the dietary sterols. Bull. Jap. Soc. Sci. Fish., 37(10):1015-1019.

_____, _____ and K.KASHIWADA, 1972. Nutritional requirements of prawn. IV: The dietary effect of ecdysones. Bull. Jap. Soc. Sci. Fish., 38:1067-1071.

_____, S. TESHIMA, Y. SAKAMATO and J.B. GUARY, 1976a. The variation of lipid and cholesterol contents in the tissue of prawn, P. japonicus during moulting cycle Bull. Jap. Soc. Sci. Fish., 42(9): 943-1064.

_____, S. TESHIMA and N. TANAKA, 1976b. Nutritional requirement of prawn - V. Requirements for choline and inositol. Mem. Fac. Fish. Kagoshima Univ., 25(1):47-51.

_____, _____ and S. TOKIWA, 1977a. Nutritional requirements of prawn - V. Requirements of choline and inositol. Mem. Fac. Fish., Kagoshima Univ., 25(1):47-51.

KANAZAWA, A., S. TESHIMA and S.TOKIWA, 1977b. Nutritional requirement of prawn - VIII. Effect of dietary lipids on growth. Bull. Jap. Soc. Sci. Fish., 43(7): 849-856.

_____, _____, M. ANDO and M. KAYAMA, 1978. Effects of eicosapentaenoic acid on growth and fatty acid composition of the prawn. P. japonicus. Mem. Fac. Fish., Kagoshima Univ., 27(1): 35-40.

_____, _____, _____, 1979a. Requirements of prawn, P. japonicus for essential fatty acids. Mem. Fac. Fish. Kagoshima Univ., 28:27-33.

_____, _____ and S. TOKIWA, 1979b. Biosynthesis of fatty acids from palmitic acid in the prawn, Penaeus japonicus. Mem. Fac. Fish. Kagoshima Univ., 28:17-20.

_____, _____, and K.ONO and K.CHALAYONDEJA, 1979c. Biosynthesis of fatty acids from acetate in the prawns, Penaeus monodon and Penaeus merguiensis. Mem. Fac. Fish. Kagoshima Univ., 28:21-26.

_____, _____, S.TOKIWA and H.J.CECCALDI, 1979d. Effects of dietary linoleic and linolenic acids on growth of prawn. Oceanol. Acta, 2(1): 41-48.

_____, _____, _____, M. ANDO and F.A.ABDUL RAZEK, 1979e. Effect of short-necked clam phospholipids on the growth of prawn. Bull. Jap. Soc. Sci. Fish.,45(8): 961-965.

_____, _____, _____, M. KAYAMA and M. HIRATA, 1979f. Essential fatty acids in the diets of prawn-II. Effect of docosahexaenoic acid on growth. Bull. Jap. Soc. Sci. Fish., 45(9):1151-1153.

KANAZAWA A., S.TESHIMA and K.ONO. 1979g. Relationship between essential fatty acid requirements of Aquatic animals and capacity for bioconversion of linolenic acid to highly unsaturated fatty acids. Comp. Biochem. Physiol. 63B: 295-298.

_____, _____, S.MATSUMOTO and T.NOMURA, 1981. Dietary protein requirement of the shrimp, Metapenaeus monoceros. Bull. Jap. Soc. Sci. Fish., 47(10):1371-1374.

_____, _____, H.SASADA and S. ABDEL-RAHMAN, 1982a. Culture of the prawn larvae with micro-particulate diets. Bull. Jap. Soc. Sci. Fish., 48(2): 195-199.

_____, R.PAULRAJ and S. AHAMED ALI, 1982b. Preparation of artificial diet for nutritional studies. CMFRI Spl.Publn. 8: 43-51.

_____, S.TESHIMA and M.SASAKI, 1984. Requirements of potassium, copper, manganese, and iron for Penaeus japonicus. Mem. Fac. Fish. Kagoshima Univ., 33(1):63-71.

_____, _____ and M. SAKAMOTO, 1985. Effects of dietary lipids, fatty acids, and phospholipids on growth and survival of prawn Penaeus japonicus larvae. Aquaculture, 50: 39-49.

KARLSON, P. and D.M.SKINNER, 1960. Attempted extraction of crustacean moulting hormone from isolated Y organs. Nature (London), 185, 543-544.

KAYAMA, M. 1964. Fatty acid metabolism in fish. Bull. Jap. Soc. Sci. Fish., 30: 647-659.

KINNE, O., 1977. Cultivation of Animals. In: Marine Ecology, Vol. III Cultivation, Part 2. O.Kinne (Ed) Wiley London pp. 599-1293.

KITABAYASHI, K., H. KURATA, K.SHUDO, K.NAKAMURA and S.ISHIKAWA, 1971a. Studies on formula feed for Kuruma prawn-I. On the relationship among glucosamine, phosphorus and calcium. Bull. Tokai Reg. Fish. Res. Lab., (65): 91-107.

_____, K. SHUDO, K.NAKAMURA and S.ISHIKAWA, 1971 b. Studies on formula feed for Kuruma prawn-III: On the growth promoting effects of both arginine and methionine. Bull. Tokai Reg. Fish. Res. Lab., 65: 119-127.

_____, _____, _____ _____, 1971c. Studies on formula feed for Kuruma prawn-II. On the utilization values of glucose. Bull. Tokai. Reg. Fish. Res. Lab., 65: 109-118.

KRISHNAKUMARAN A. and H.A.SCHNEIDERMAN, 1968. The chemical control of molting in arthropods. Nature, 220:601-602.

_____, _____, 1970. Control of molting in mandibulate and chelicerate arthropods by ecdysones. Biol. Bull., 139: 520-538.

KRISHNAMOORTHY, R.V., A. VENKATARAMIAH, G.J.LAXMI and P.BIESOIT, 1982. Effect of starvation and algal feeding on the tissue cholesterol levels in commercial shrimp, Penaeus aztecus. Proc. Symp. Coastal Aquaculture 1. 303-309.

KURATA, H., 1968. Induction of molting in prawn P. japonicus by inokosterone injection. Bull. Jpn. Soc. Sci. Fish. 34: 909-914.

LASSER, N.L., A.M.EDWARDS and R.B.CLAYTON, 1966. cited by Gilbert L.I. and D.O'Connor In: "Chemical Zoology" Florkin M. and B.T. Scheer (Eds) Academic Press, New York, Vol.5, pp 229-254.

LASSER,G.W. and W.V.ALLEN, 1976. The essential amino acid requirements of the dungeness crab, Cancer magister. Aquaculture, 7: 235-244.

LEE, D.L., 1970. Study on digestion and absorption of protein in artificial feeds by four species of shrimps. Collect. Rerr. Tungkang. Mar. Lab., 1: 77-84.

_____, 1971. Studies on the protein utilization related to growth of Penaeus monodon Fabricius. Aquaculture, 1: 1-18.

LEE,R.F. and D.L. PUPPIONE, 1978. Serum lipoprotein in the spiny lobster, Panulirus interruptus. Comp. Biochem. Physiol., B 59B: 239-243.

LEE,D.J. and G.B.PUTNAM, 1973. The response of rainbow trout to varying protein/energy ratios in test diet. J. Nutr., 103: 916-922.

LEE D.J. and R.O. SINNHUBER, 1972. Lipid requirements. In Fish Nutrition, J.E. Halver (Ed). Academic Press, New York, pp. 145-181.

LEHNINGER, A.L., 1984. Biochemistry. 4 Ed. Kalyani Publisher, Ludhiana. India. 1104 P.

LESTER, R., M.C.CARY, L.A. COOPERSTEIN and S.R.DOND, 1975. Crustacean intestinal detergent promotes sterol solubilisation. Science, New York, 189: 1018-1100.

LIGHTNER, D.V., B.HUNTER, P.C. MAGARELLI Jr. and L.B.COLVIN, 1979. Ascorbic acid: Nutritional requirement and role in wound repair in penaeid shrimp. Proc. World. Mariculr. Soc. 10: 513-518.

LOWRY, O.H., N.J.ROSEBROUGH, A.L.FARR and R.J.RANDALL, 1951. Protein measurement with the Folin-phenol reagent. J. Biol. Chem., 193: 275-275.

MAGARELLI, P.C., Jr., B.HUNTER, D.V.LIGHTNER and L.B.COLWIN, 1979. Black death: An ascorbic acid deficiency disease in penaeid shrimp. Comp. Biochem. Physiol., 63A: 103-108.

MARSHALL, S.M. and A.P.ORR, 1960. Feeding and nutrition In: The physiology of Crustacea, Vol. 1, T.H.Waterman (Ed.), Academic Press, New York, NY pp. 227-258.

MENON, M.K., 1937. The scyphomedusae of Madras and the neighbouring coast. Bull. Madras Govt. N.S., Nat. Hist.Sect., 3: 1-55.

_____, 1955. Identification of Marine and Inshore prawns of commercial value in India. Proc. Indo-Pacific Fish. Coun., Sec. III:345-346.

_____ and K. RAMAN, 1962. Observations on the prawn fishery of the Cochin backwaters with special reference to the stake-net catches. Indian J. Fish., 8(1):1-23.

MIYAJIMA, L.S., G.A.BRODERICK and R.D.REIMER, 1976. Identification of essential amino acids of the freshwater shrimp Macrobrachium ohione. Proc. World Mariculture Soc., 7: 699-704.

MOHAMED, K.H. and P.V.RAO, 1971. Estuarine phase in the life history of the commercial prawns of the west coast of India. J. Mar. Biol. Ass. India, 13(1&2):149-161.

_____, M.S.MUTHU, N.N.PILLAI, S.K.PANDIAN and S.AHMED ALI, 1983. Simplified hatchery technique for mass production of penaeid prawn seed using formula feed. Indian J. Fish., 30(2): 320-322.

MORRIS, R.J. and J.R.SARGENT, 1973. Studies on the lipid metabolism of some oceanic crustaceans. Mar. Biol., 22: 77-83.

MORRISON, W.R. and L.M.SMITH, 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipid with boron floride methanol. J. Lipid Res., 5: 600-608.

MUTHU, M.S., 1983. Development and culture of penaeid larvae. A Review. In: Progress in Invertebrate Reproduction and Aquaculture. T. Subramonian and Sudha Varadarajan (Eds) pp. 203-226.

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

_____, 1980. A bibliography of shrimp and prawn nutrition. Aquaculture, 21: 101-128.

O'CONNOR, J.D., and L.I.GILBERT, 1968. Aspects of lipid metabolism in crustaceans. Am. Zool., 8: 529-539.

_____, _____, 1969. Alteration in lipid metabolism associated with pre-molt events in a land crab and freshwater cray fish. Comp. Biochem. Physiol. 29: 889-904.

PAGE, J.W. and J.W.ANDREWS, 1973. Interactions of dietary levels of protein and energy on channel catfish (Ictalurus punctatus) J. Nutr., 103:1339-1346.

PASCUAL, F.P., R.M.COLOSO and C.T.TAMSE, 1983. Survival and some histological changes in Penaeus monodon(Fabricius) juvenile fed various carbohydrates. Aquaculture, 31: 169-180.

PAUL RAJ, R., 1976. Studies on the penaeid prawns of Pulicat Lake South India. Ph.D.Thesis. Univ. Madras 241 pp.

PETRIELLA,A.M., M.I.MULLER, J.L.PENUCCI and M.B.SAEZ, 1984. Influence of dietary fatty acid and cholesterol on the growth and survival of the Argentina prawn Artemesia longinaris. Aquaculture, 37(1): 11-20.

PETROLS,J., CECCALDI,H.J., T. ANDO, A.KANAZAWA, S.TESHIMA, 1978. Variation in lipid synthesis from acetate during the moulting cycle of prawn. Bull. Jap. Soc. Sci.Fish. 44(2): 139-141.

PONAT,A. and D.ADELUNG, 1980. Studies to establish an optimal diet for Carcinus maenas II. Protein and lipid requirement. Mar. Biol., 60: 115-122.

_____ _____, 1983. Studies to establish an optimal diet for Carcinus maenas. Mar. Biol., 74: 275-279.

PROVASOLI,L., 1975. Nutritional aspects of crustacean aquaculture. Proc. First Int. Conf. Aquacult. Nutr., pp. 13-21.

READ,G.H.L., 1977. Aspects of lipid metabolism in Penaeus indicus Milne Edwards. M.Sc. Thesis, University of Natal., 106 pp.

_____, 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 M.S.CAULTON, 1980. Changes 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.

RENAUD, L., 1949. Le cycle des reserves organiques chez. Les crustacea Decapodes. Ann. inst. Oceanog. (Paris)(N.S) 24: 259-357.

SANDIFER, P.A. and J.D. JOSEPH, 1976. Growth response and fatty acid composition of juvenile prawn (Macrobrachium rosenbergii) fed a prepared ration augmented with shrimp-head oil. Aquaculture, 8: 129-139.

SARGENT, J.R., 1976. The structure, metabolism and function of lipids in marine organisms. In: Biochemical and Biophysical perspectives in Marine Biology, D.C. Malins and J.R. Sargent (Eds.) Academic Press, London, 3: 150-212.

SHEWBART, K.L. and W.L.MIES, 1973. Studies on nutritional requirement of brown shrimp. The effects of linolenic acid on growth of P. aztecus. Proc. World Maricult. Soc., 4: 277-287.

_____, _____, and P.D.LUDWIG, 1972. Identification and quantitative analysis of the amino acids present in the protein of the brown shrimp, Penaeus aztecus. Mar. Biol., 16:64-67.

_____, _____, _____, 1973. Nutritional requirements of the brown shrimp. P. aztecus. US Dept. Commerce. Rep. No. COM-73-11794, NOAA, Office of Sea Grant. Rockville Md. 52 pp.

SHIMENO, S., H.HOSOKAWA, M.TAKEDA and H.KAJIYAMA, 1980. Effect of calorie to protein ratios in formulated diet on the growth, feed conversion and body composition of young yellow tail. Bull. Jap. Soc. Sci.Fish., 46:1083-1087.

SHUDO, K., K.NAKAMURA, S.ISHIKAWA and K.KITABAYASHI, 1971. Studies on formula feed for Kuruma prawn-IV: On the growth promoting effects of both squid-liver oil and cholesterol. Bull. Tokai Reg. Fish. Res. Lab., (65): 129-137.

SICK, L.V. and J.W.ANDREWS, 1973. Effects of selected dietary lipids, carbohydrates and proteins on the growth, survival and body composition of Penaeus duorarum. Proc. World. Mariculr. Soc., 4: 263-276.

_____, _____ and D.B.WHITE, 1972. Preliminary studies on selected environmental and nutritional requirements for the culture of penaeid shrimp. Fish. Bull., 70: 101-109.

_____, D. WHITE and G. BAPTIST, 1973. The effect of duration of feeding, amount of food, light intensity and animal size on rate of ingestion of pelleted food by juvenile penaeid shrimp. Proc. Fish. Cult., 35: 22-26.

SILAS, E.G., M.J.GEORGE and T.JACOB, 1984. A review of the fisheries of India: a scientific basis for the management of the resources. In: J.A. Gulland and B.J.Rothschild (eds), Penaeid shrimp their biology and Management. Fishing News Books Ltd., Farman, Survey, England pp.83-10

SINNHUBER, R.O., 1969. The role of fats In: Fish in Research. O.W. Neuhaus and J.E.Halver (Eds) Academic Press. New York pp. 245-261.

_____, J.H. WALES and D.J.LEE, 1968. Dietary factors and hepatoma in rainbow trout (Salmo gairdneri). II. Co-carcinogens by cyclopropenoid fatty acids and the effects of gossypol and altered lipids in aflatoxin induced liver cancer. J. Nat. Cancer Inst., 41(6): 1293-1301.

SPAZIANI,E., and S.B.KATER, 1973. Uptake and turnover of Cholesterol-^{14}C in Y-organs of crab Hemigrapsus rudus as a function of the molt cycle. Gen. Comp. Endocr., 20: 534-549

STICKNEY,R.R., 1979. Principles of warm water aquaculture. A wiley-Interscience publication, John Willey and sons Inc., pp. 1-373.

TAKEDA,M., S.SHIMENO, H.HOSOKAWA, H.KAJIYAMA and T.KAISYO, 1975. The effect of dietary calorie-to-protein ratio on the growth, feed conversion and body composition of yellow tail. Bull. Jap. Soc. Sci. Fish., 41: 443-447.

TAKEI,M. and AI,M., 1971. Studies on fishes favourite food-VI; Response of walking legs to substances in Kuruma prawn. Bull. Tokai Reg. Fish. Res. Lab., 68:61-69.

TAKEUCHI, T., T.WATANABE and C.OGINO, 1978a. Supplementary effect of lipid in a high protein diet of rainbow trout. Bull. Jap. Soc. Sci. Fish., 44: 677-681.

_____, _____, _____, 1978b. Optimum ratio of protein to lipids in diets of rainbow trout. Bull. Jap. Soc. Sci. Fish., 44: 683-688.

_____, M.YOKOYAMA, T.WATANABE and C.OGINO, 1978c. Optimum ratio of dietary energy to protein for rainbow trout. Bull. Jap. Soc. Sci. Fish., 44: 729-732-

_____, T. WATANABE and C.OGINO, 1979. Digestibility of hydrogenated fish oils in carp and rainbow trout. Bull. Jap. Soc. Sci. Fish. 45: 1521-1525.

TUSHIMA, S., 1972. Sterol metabolism in crustaceans. Mem. Fac. Fish. Kagoshima Univ , 21: 69-147.

TESHIMA, S., 1978. Requirement of essential fatty acids and sterols in crustaceans In: Fish culture and Dietary Lipids (Edited by Japanese Society of Scientific Fisheries), Suisangaku Ser. No. 22: 60-77 Koseisha Koseikaku, Tokyo, Japan.

_____ and A.KANAZAWA, 1970. Utilization and biosynthesis of sterols in Artemia salina. Bull. Jap. Soc. Sci. Fish. 37: 720-723.

_____, _____, 1971a. Biosynthesis of sterols in lobster, Panulirus japonica, the prawn Penaeus japonicus, and a crab Portunus trituberculatus. Comp. Biochem. Physiol., 38B: 597-602.

_____, _____, 1971b. Bioconversion of the dietary ergosterol to cholesterol in Artemia salina. Comp. Biochem Physiol., 38B, 603-607.

_____, _____, 1978a. Hemolymph lipids of the prawn Bull. Jap. Soc. Sci. Fish., 441: 925.

_____ _____, 1978b. Release and Transport of lipid in the prawn. Bull. Jap. Soc. Sci. Fish., 44(11) 1269-1274.

_____, _____, 1979. Lipid transport mechanism in the prawn. Bull. Jap. Soc. Sci. Fish., 45: 1341-1346.

_____, _____, 1980a. Transport of dietary lipids and role of serum lipoproteins in the prawn. Bull. Jap. Soc. Sci. Fish., 46: 57-55.

_____, _____, 1980b. Lipid constituents of serum lipoproteins in the prawn. Bull. Jap. Soc. Sci. Fish., 46: 57-62.

TESHIMA, S. and A.KANAZAWA, 1983. Digestibility of dietary lipids in the prawn. Bull. Jap. Soc. Sci. Fish.49:963-966

_____, _____, 1984. Effects of protein, lipid and carbohydrate levels in purified diets on growth and survival rates of prawn larvae. Bull. Jap. Soc. Sci. Fish., 50(10):1709-1715.

_____, _____and H.OKAMOTO, 1974. Absorption of sterol and cholesteryl esters in prawn Penaeus japonicus. Bull. Jap. Soc. Sci. Fish., 40:1015-1019.

_____, _____, _____, 1977. Variation in lipid classes during the moulting cycle of the prawn, Penaeus japonicus. Mar. Biol., 39: 129-136.

_____, _____,and M. SAKAMOTO, 1982a. Microparticulate diets for the larvae of aquatic animals. Min. Rev. Data File Fish. Res., 2: 67-86.

_____, _____, H.SASADA and M. KAWASAKI, 1982b. Requirements of the larval prawn, P. japonicus for cholesterol and soyabean phospholipids. Mem. Fac. Fish. Kagoshima Univ., 31: 193-199.

_____, _____, _____, 1983. Nutritional value of dietary cholesterol and other sterols to larvae of the prawns P. japonicus (Bate). Aquaculture, 31: 159-167.

THOMSON, M.H., 1964. Cholesterol content of various species of shellfish (1) Method of analysis and preliminary survey of variables. (Fishery Industrial Res.,2(3): 11-15

TRIDER,D.J. and J.D. CASTELL, 1980. Some current findings of the Halifax lobster nutrition group. In: 1980 Lobster nutrition workshop, proceedings Bayer R.C. and D' Agostino A (Eds) Technical Report 58 Maine Sea Grant Publication, pp. 36-40.

TYAGI, A.P. and A.PRAKASH, 1967. A study on the physiology of digestion in Freshwater prawn, Macrobrachium dayanum. J. Zool. Soc. India. 19: 77-83.

VAN DEN OORD, A.H.A., 1964. The absence of cholesterol synthesis in the crab, Cancer pagurus. Comp. Biochem. Physiol., 13: 461-467.

VENKATARAMIAH, A., G.J.LAKSHMI and G.GUNTER, 1975. Effect of protein level and vegetable matter on growth and food conversion efficiency of brown shrimp. Aquaculture, 6: 115-125.

VILLEGAS, C.T. and A.KANAZAWA, 1980. Rearing of the larval stages of prawn, Penaeus japonicus (Bate), using artificial diet. Mem. Kagoshima Univ. Res. Center S. Pac., 1(1): 43-49.

VONK, H.J., 1960. Digestion and metabolism. In: The Physiology of Crustacea. Volume I. T.H.Waterman (Ed) Academic Press, New York, N.Y., pp. 291-316.

WATANABE, T. 1982. Lipid nutrition in Fish. Comp. Biochem. Physiol., 73B(1): 3-15.

_____, _____, T.TEKEUCHI and C.H.OGINO, 1979. Studies on the sparing effect of lipids on dietary protein in Rainbow trout, Salmo gairdneri. Proc. world symp. on finfish nutrition and fish feed Technology, Hamburg 20-23 June Berlin 1979. pp. 118-125.

WEST, E.S., W.R.TODD, H.S.MASON and T.T.V.BRUGGEN, 1970. Text book of biochemistry Fourth Edition. The MacMillan Company, London 1595p.

WHITNEY, J.O., 1969. Absence of Sterol synthesis in the larvae of the mud crab Rhithropanopeus harrisii and spider crab Libinia emarginata. Mar. Biol. 3: 134-135.

ZANDEE, D.I., 1964. Absence of sterol synthesis in some arthropods. Nature (London), 20: 1315-1336.

_____, 1966a. Metabolism in cray fish Astacus astacus. III. Absence of cholesterol synthesis. Arch. Int. Physiol. Biochem., 74: 435-441.

_____, 1966b. Metabolism in the cray fish Astacus astacus IV. The fatty acid composition and the biosynthesis of the fatty acids. Arch. Inst. Physiol. Biochem. 74: 614-626

_____, 1967. Absence of cholesterol synthesis as contrasted with the presence of fatty acid synthesis in some arthropods Comp. Biochem. Physiol., 200: 811-822.

ZEIN-ELDIN, Z.P. and J.CORLIS, 1976. The effect of protein levels and sources of growth of Penaeus setaceus. Proc. FAO Tech. Conf. on aquaculture FIR:AQ/Conf/76/E33:10 pp.

_____, MEYERS, S.P., 1973. General considerations of problems in shrimp nutrition. Proc. World Mariculture Soc., 4: 299-317.