EFFECT OF CUTTLEFISH LIVER LIPID ON THE GROWTH OF *MACROBRACHIUM ROSENBERGII* (de Man) JUVENILES

BY

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THESIS

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Dedicated to my parents and brother

DECLARATION

I hereby declare that this thesis entitled " EFFECT OF CUTTLEFISH LIVER LIPID ON THE GROWTH OF *MACROBRACHIUM ROSENBERGII* (de Man) JUVENILES" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

Place : Panangad

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CERTIFICATE

Certified that this thesis, entitled " EFFECT OF CUTTLEFISH LIVER LIPID ON THE GROWTH OF *MACROBRACHIUM ROSENBERGII* (de Man) JUVENILES" is a record of research work done independently by Kum. Shyla. G. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associate-ship to her.

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1. INTRODUCTION

The ever increasing human population has made the food supply critical, forcing the society to explore various avenues of food production and to develop methods to augment the yield to meet the demand. The role of aquaculture in increasing food production is well known. It is specially significant at this time, since many of the natural resources are overexploited and the yield from capture fisheries is heading towards stagnation. Crustaceans play a very important role among cultured organisms. Culture of Giant freshwater prawn is a recent development in India, which has got a tremendous potential.

Giant freshwater prawn, *Macrobrachium rosenbergii* (de Man) is a suitable species to culture in inland water bodies as well as in low saline waters. Its wide acceptance is due to the desirable characters like fast growth rate, hardy nature, low protein requirement compared to penaeid species, resistance to diseases, compatibility with other species and high demand in export and local market. Although there are more than 34 species in India only 7 species are having commercial importance. But it is *M. rosenbergii* which monopolizes the freshwater prawn farming.

The breakthrough in seed production and larval rearing has helped to improve the status of freshwater prawn culture. Although it is commercially cultured in South East Asian countries, in India it has been a neglected resource till recently. But due to an increase in export market and setback faced in penaeid prawn farming, the value of this species is being realized now. There is a growing interest in the establishment of commercial hatcheries of this species. Aquaculture production of freshwater prawn in India is about 1507 MT, out of the global production of 1,30,313 MT (Anon, 1998). The present production in India is very low compared to the resource potential. Hence, India may be considered as a 'Sleeping Giant' as far as freshwater prawn farming is concerned.

Freshwater prawn is an omnivorous and coprophagous animal, which can also readily accept artificial feed. For successful rearing of *M. rosenbergii* nutritionally balanced formulated feed is a prime necessity. For the development of nutritionally balanced formulated diet one should have a clear understanding of the nutritional requirement of the species. Even though numerous studies have been reported about the protein requirement of freshwater prawn, there is still considerable uncertainty as to the prawn's quantitative dietary requirement of protein. There are numerous non-dietary factors which will interfere with the free responses of an organism to dietary protein (Anon, 1990). The optimum level of protein requirement reported for *M. rosenbergii* adults are in the range of 29-30%. In the case of juveniles the requirement is somewhat higher (New, 1976). Efficiency of utilization of carbohydrates as a source of energy depends on the source and level of carbohydrates. Utilisation of polysaccharides such as dextrins and starch are better than monosaccharides in prawns (Lovell, 1998). Incorporation of polysaccharides at the level of 30-40% of the diet is most common in crustacean feed. There are only very few published data on the requirement of lipid for freshwater prawns. A wide range of 2 – 15% of lipid levels have been reported both in research work and in commercially available freshwater prawn feed (New, 1994). Unlike fish, crustaceans cannot tolerate high levels of dietary lipid. n-3 and n-6 fatty acids having carbon more than or equal to 20 will improve the growth of juvenile prawns (D'Abramo and Sheen, 1993). Shrimps and prawns can absorb minerals directly from water through gills and body surface. Therefore, dietary mineral requirement depends on mineral content of water. There are only very few studies in cultured crustaceans regarding mineral nutrition. Vasaquez *et al.* (1989) found optimal growth of *M. rosenbergii* at water hardness less than 53 mg per litre of $CaCO_3$. Growth rate did not change significantly at lower hardness, but declined at higher levels. Under high stocking density and limited food supply vitamins are essential for normal growth.

For the formulation of a good prawn feed in addition to nutritional requirement, availability of ingredients, cost of ingredients, effect of ingredients and feed production technology should also be taken into

consideration. Cost of feed is one of the major input cost factor raising up the operation cost. Cost of supplementary feed accounts to nearly 40 – 60% total production cost of intensive farming operation (Anon, 1983). The high cost of feed is a problem in large scale farming. The feed cost can be reduced by using locally available cheap materials for feed formulation.

In this experiment an attempt has been made to develop a nutritionally balanced feed for juveniles of *M. rosenbergii* by using locally available cheap materials. For this cuttlefish liver was used as a lipid source, which is an unutilized processing waste in sea food industry. This is also a rich source of polyunsaturated fatty acids, which is an essential component for better growth and survival of *M. rosenbergii* juveniles. The main objective of the experiment was to find out the effect of feed containing different concentrations of cuttlefish liver lipid on the growth and survival of *M. rosenbergii* juveniles and to assess the possibility of utilization of cuttlefish liver as a source of lipid in the feed of prawns. The use of cuttlefish liver as a source of lipid is expected to increase the nutritional value of feed without increasing the cost of production.

2. REVIEW OF LITERATURE

2.1 Feed and feeding of crustaceans

From the field observation it is found that *Macrobrachium spp.* are omnivorous and they consume aquatic insects and larvae, nuts, grains, seeds, small mollusks, crustaceans, fish flesh and compounded pelleted feeds. According to Schroeder (1983) and Weidenbach (1982) prawns mainly depend on natural feed independently of the presence or absence of pelleted food. Feed is normally the largest single item in the running expenditure of a shrimp farm. Hence the suitability and cost effectiveness of feed is very important for the commercial success of a farm.

For developing a successful crustacean feed, a clear and thorough knowledge about the nutritional requirements of the organism is essential. Lack of standardization in experimental design, culture conditions and analytical techniques has limited the value of published information on shrimp and prawn nutrition and hence comparison of the results become difficult (New,1976). In recent years some studies have been carried out to understand the nutritional requirement of crustaceans with diets of different levels and composition of protein, lipid, carbohydrate, mineral and vitamin. Reference diet developed by Castell *et al.* (1989) have been successfully evaluated with *M. rosenbergii* by Reed and D' Abramo (1989).

2.2 Protein requirement

The source, dietary level and amino acid composition of proteins in relation to shrimp and prawn nutrition has received much attention, because it is the largest and most expensive component in the feed (New , 1976).

Shrimps do not have an absolute requirement of protein but require a balanced mixture of indispensable amino acids. The optimum dietary protein requirement of shrimp varies from 28-60% according to species, size, quality of protein and palatability of feed and availability of natural food organism (Lovell, 1998).

The protein content of commercial prawns feeds have been reported as 23.8-38.5% in Hawaii (Corbin *et al.*, 1983), 28.36% in Taiwan (Hsieh *et al.*, 1989) and 25-30% in French Guiana (IFREMER, 1989). Clifford and Brick (1979) reported that optimum growth of prawns were achieved with diet having 25% protein and 1:4 lipid : carbohydrate ratio. Under laboratory condition optimum protein level of 30-35% have been demonstrated by D' Abramo and Reed(1988) and Fruechtenicht *et al.* (1988). Even though numerous studies have been conducted on the growth rate and feed efficiencies at different levels of dietary protein. Still there exist a considerable uncertainty about the quantitative dietary protein requirement. Many studies suggested that the optimal dietary range of protein is 29% and no additional growth response at higher levels of protein greater than 40%. (Anon, 1990). According to New (1976) the optimal level of dietary protein for different species of prawns is between 27% and 35%. In the case of juvenile *M. rosenbergii* the requirement may be somewhat higher. According to Behanan and Mathew (1995) a diet containing 30 – 40% of protein produces better growth in *M. rosenbergii* . A diet containing 25% crude protein proved to be superior in respect of FCR, PER and SGR in freshwater prawn (Sharma and Reddy, 1996). Growth rates and feed utilization of freshwater prawn were significantly improved with increasing dietary protein levels upto 35%. Beyond this no significant improvement was evident. (El - Sayed, 1997).

Ravishankar and Keshavanath (1988) found that *M. rosenbergii* utilized feed pellets containing silk worm pupae and shrimp waste more efficiently and gave a higher specific growth rate than diets having fish meal, silk worm pupae alone or silkworm pupae plus clam meat. Unnikrishnan *et al.* (1992) were able to substitute extracted silk worm pupae for extracted clam meal as semi purified diet for *M. rosenbergii* post larvae with out any detrimental effect on the survival growth rate and protein efficiency ratio. Boonyaratpalm and New (1980) suggested that desirable protein level of feed from economical stand point was 15%, at least for the first four months of rearing. Supplementary prawn feeds were investigated by Perry and Tarver (1984) and found that the prawns fed with marine ration achieved the largest size and greatest biomass.

Koshio *et al.* (1992) compared the nutritive value of soybean protein and crab protein in *M. rosenbergii.* Protein content of test diet varied from 30-55% and weight gain for the group fed with crab protein concentrate seemed to be higher than that of soyabean protein but statistical significance was not detected. Behanan *et al.*

(1992) conducted another experiment on post larvae of *M. rosenbergii* by using pelleted feeds with 33 – 44% of protein and found that the diet containing 33% protein was ideal. James *et al.* (1992) found that *Spirulina fusiformis* can be used as a supplementary protein but cannot be secured as a sole protein source. Good growth and survival have been obtained by feeding shrimp with proteins having amino acid profile similar to the tissues of shrimp itself (Kanazawa, 1992).

Several materials have been defined as suitable source of protein for shrimp, which include squid, soybean meal, shrimp meal, and several type of fish meals. Reports on the optimum protein level for penaeid diets are conflicting, probably due to different basal diets used. The optimum protein requirement of penaeid prawns are 35 to 40%(Ali, 1995) and 32% (Kureshy *et al.*, 2000)

Protein level above 28% in the diet of *Penaeus setiferus* based on menhaden meal resulted a reduced growth rate unless carbohydrate or lipid level were also increased (Andrews *et al.*, 1972). According to Sick and Andrews (1973) *P. duorarum* showed a better growth when fed with a diet containing soybean meal at a protein level of 28-30%. Kanazawa *et al.* (1970) agreed that soybean was a superior protein source for penaeid shrimp nutrition while Deshimaru and Shigueno (1972) reported that any diet with an amino acid profile similar to shrimp tissue and rich in basic amino acids would produce relatively good growth.

Shigueno *et al.* (1972) reported that diets containing high level of protein from several sources including squid meal, white fish meal, dried euphausia were produced good results. Squid has also been featured as a protein source in shrimp studies (Subrahmanyam and Oppenheirmer, 1969, Kitabayashi *et al.*, 1971d, Deshimaru and Shigueno,1972, Kittaka, 1976,and Fenucci and Zein- Eldin, 1976). The inclusion of 56-74% squid meal by Kitabayashi *et al.* (1971 d) and 20-47% by Deshimaru and Shigueno (1972) gave good results in *P. Japonicus.* According to Fenucci *et al.* (1980) presence of 5-6% squid meal is advantageous in the feed of *P. stylirostis* and *P. setiferus* at a protein level of 30-35%. Growth promoting nature of squid protein is due to its nutritional value which depends on the digestibility co–efficient and amino acid profile (Fernandez-Palacois *et al.* 1997).

2.3 Amino acid requirement

The essential amino acids for shrimp are qualitatively similar to those for other animals. Cowey and Forster (1971) and Shewbart *et al.* (1972) found that arginine,

histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine and valine were essential for *Palaemon serratus* and *Penaeus aztecus*.. Similar results were obtained for *M. Ohione* by Miyajima *et al.*, (1975) except that no data was obtained for tryptophan. Watanabe (1975) showed that *M. rosenbergii* had qualitative requirement for the same amino acids except for lysine. Data was not obtained for threonine and tryptophan.

Quantitative requirement of essential amino acids for various shrimp species have not yet been determined. In the absence of this knowledge, it was found that better growth and survival was obtained by feeding shrimp with proteins having similar amino acid profile to that of shrimp tissue itself (Kanazawa, 1992). Attempt to supplement amino acid deficient diets with crystalline amino acid or basal diet consisting entirely of crystalline amino acid have not been successful with shrimp (Lovell.1998). Combination of various high quality proteins having an amino acid profile similar to clam meat produces a similar effect as that of a diet with clam protein (Deshimaru, 1982). Mai *et al.* (1988) found that juvenile *P. orientalis* did not absorb methionine and lysine simultaneously with the protein bound amino acids. Kanazawa (1992) found that methionine enriched soyabean protein was preferable to crystalline methionine when used to supplement a methionine deficient diet. The inability of utilization of free amino acid may be due to the difference in the rate of absorption of free amino acids and protein bound amino acids and also due to leaching of nutrients in to water before ingestion.

2.4 Carbohydrate

Shrimps and prawns utilize carbohydrate as an energy source but the efficiency of utilization varies depending on source and level of carbohydrate. They are able to utilize complex carbohydrates better than glucose (New, 1976). Wheat, starch, dextrin and oyster glycogen are completely assimilated by *Palaemon serratus* (Forster and Gabbott, 1971).

Carbohydrate digestion in crustacea has been demonstarated by Kooiman (1964) and the presence of many carbohydrases including α and β amylase, maltase, saccharase, chitinase and cellulase. Freshwater prawns have cellulase (Noborikawa, 1978) and chitinase used for the digestion of cellulose and chitin and carbohydrate can be used to spare protein. Barley seems to be superior than that of wheat as a

carbohydrate source due to its lower gelatinisation temperature. (Ashmore *et al.*, 1985).

Addition of 20% glucose to menhaden based diet for *Penaeus aztecus* reduced the growth rate, while inclusion of 30% of starch in diets with lower protein content increased the growth rate (Andrews *et al.*, 1972). In the diet of *P. monodon* level of protein can be reduced from 40 to 30% by increasing the starch content from 20 to 30% (Shiau and Peng, 1992).The reason for poor utilization of glucose by shrimp is due to rapid and ineffective absorption for energy metabolism whereas glucose from digested polysaccharides was absorbed more slowly and effectively. Andrews and Sick (1972) found that carbon from intact starch is incorporated in to the tissue of *P. setiferus* at a higher rate than from glucose. Inclusion of 40% cornstarch in a casein based diet for *Penaeus duorarum* produced faster growth than 10% or 0% starch (Sick and Andrews, 1973).

Replacement of glucosamine by glucose checked growth rate in *P. japonicus* (kitabayashi *et al.*, 1971b). The effect was partially overcome by the addition of further glucose. Dietary glucosamine increases growth rate at an optimum level of 0.53% in *P. japonicus* (Kitabayashi *et al.*, 1971a). But Deshimaru and Kuroki (1974a) could not observe growth improvement in juvenile *P. japonicus* fed with higher rate of glucosamine. A suitable source of dietary carbohydrate is necessary to spare or preclude the use of carbon chains from amino acids for chitin synthesis (Coway and Forster, 1971).Shrimps can utilize carbohydrate but the efficiency of utilization varies according to source. The ability to digest specific sources of carbohydrates varies between species.

2.5 Vitamins

Few qualitative and quantitative studies have been carried out on vitamin requirements of shrimp. The metabolic functions of vitamins in crustacea have been discussed by Fisher (1960). He reported that most of the B vitamins were required in crustacean diet, in addition to Vitamin C and E. Among 15 vitamins, 14 have been demonstrated to be dietary essentials for shrimp, which was determined according to the weight gain (Lovell, 1998). The optimum levels reported are 100 mg per kg of diet for vitamin E (Kanazawa, 1985) 4,000 IU per kg of vitamin D (Shiau and Hwang, 1994) and 30 mg per kg of vitamin K (Shiau and Liu, 1994).

Dietary requirement of water soluble vitamins are 15-100 mg per Kg for thiamine and 80mg per kg diet for pyridoxine (Deshimaru and Kuroki, 1979), 20 mg. per kg for riboflavin (Chen and Hwang, 1992), 400 mg per kg for niacin, less than 4 mg per kg for biotin (Kanazawa, 1985), 0.2 mg per kg for vitamin B12 (Shaiu and Lung, 1993) 2000-4000 mg/kg for inositol and 600 mg per kg for choline chloride (Kanazawa *et al.*, 1976). Folic acid and pantothenic acids are essential but the requirement value has not been determined.

Vitamin A is probably not essential in shrimp diets but its immediate precursor may be. Fisher *et al.* (1957) detected low level of vitamin A in several species of penaeid species especially in eyes, while higher levels were found in free swimming deep dwelling species indicates a visual function for vitamin A. Fisher (1960) reported that the source of Vitamin A in shrimp is often, β-carotene obtained from phytoplankton, other crustacea or from detritus. Shrimps possess the enzymes to convert vitamin A precursor to the vitamin itself.

Addition of vitamin C to squid based diet for *P. japonicus* accelerated growth rate (Kitabayashi *et al.*, 1971b). But excess of vitamin C inhibited growth. Shrimp grew best at inclusion levels of 0.22% Vitamin C deficiency in shrimp causes black death syndrome which is characterised by blackened lesions in subcuticular tissues of body surface, in the walls of oesophagus, stomach, hindgut, gills and in gill cavity (Lightner *et al.*, 1977). Vitamin C deficiency in *P. japonicus* causes discolouration on the margin of carapace, lower part of abdomen and on the tip of walking legs (Deshimaru and Kuroki, 1976).

Very little is known about the vitamin requirements of freshwater prawns. Vitamin C deficiency syndrome cause failure of moult. At least 50-100mg / kg of available ascorbic acid is essential for *M.rosenbergii* (New, 1994). Ittoop (1996) reported maximum weight gain in *M. rosenbergii* juveniles when fed on diet with 15mg of vitamin C in the form of CVC – F90 (hydrogenated vegetable oil form of Vitamin C) /kg dry diet. Survival rate of *M. rosenbergii* juveniles increase as the level of vitamin C increased from 0 – 100 mg/kg diet and optimum requirement is 104 mg/kg diet (D' Abramo *et al.*, 1994).

Dietary level of various vitamin requirements for shrimps is considerably higher than those of fishes. This may be due to high levels of requirement for metabolism or may be lost into water during ingestion (Lovell, 1998).

2.6 Minerals

References to minerals in literature on shrimp nutrition are scarce. Shrimps can absorb several minerals from surrounding water. *P. japonicus* grown in sea water do not have a dietary requirement for Calcium, Magnesium, Iron and Manganese. But requires Phosphorus, Potassium and several trace minerals in the diet. *P. vannamie* has a dietary requirement of Calcium and Iron in marine condition (Deshimaru and Yone, 1978 and Kanazawa *et al.* 1984).

Calcium is not essential for marine shrimp but it should be added in the diet to maintain Ca:P ratio 1:1 to 1:2. Phosphorus requirement varies according to Calcium content of the diet. Maximum growth was obtained with a dietary level of 0.34% phosphorous in the absence of Ca but in the presence of .5% Ca maximum growth occurred with 0.1% of phosphorous (Davis *et al.*, 1993).According to Kitabayashi *et al.* (1971a), the best growth rates were achieved with diets for *P. japonicus* when supplementary levels of 1.04% Phosphorus and 1.24% Calcium were added.

Regarding the mineral requirement of *M. rosenbergii*, not much details are available. According to Sze (1973) the ash content of *M. rosenbergii* is as high as the lipid level (15.9% dry weight of body). Forster and Gabbott (1971) found that *Palaemon serratus* apparently digested about 30% of the ash fraction of the diet having an ash content of 15% (w/w). A diet with low Calcium content is best in water having higher hardness. (Zimmermann *et al.*, 1994).

2.7 Lipid

2.7.1 Quantitative requirement of lipid

Lipids are essential nutrients in diets of shrimps and prawns for their energy value and as a source of essential fatty acids, fat soluble vitamins, sterols and phospholipids (Lovell, 1998). In addition to this, lipids play an important role in crustacean digestion, growth and reproduction. Lipids provide flavour and textural quality of the feed (Paulraj, 1987). Limited capabilities of crustaceans for *de novo* synthesis of lipid shows the importance of lipid in the diet of crustaceans. Crustacea lack the ability to synthesize sterols (Zandee, 1966a, Kanazawa *et al.*, 1971) and *de novo* synthesis of polyunsaturated fatty acid is virtually absent in shrimp *P. japonicus* (Kanazawa and Teshima, 1977) and cray fish *Astacus astacus* (Zandee, 1966b).

Dietary lipids have a sparing effect on the utilization of dietary protein. But shrimps cannot tolerate high levels of lipids (Lovell, 1998). Palmitic acid incorporated into the tissue of *Penaeus setiferus* at over twice the rate of tripalmitin following the ingestion of labeled diets, indicating the limited lipase activity in shrimp (Andrews and Sick, 1972).

A dietary lipid level in excess of 10% tends to depress growth of shrimps (Lovell, 1998). Addition of 10% supplemental lipid, consisting of equal parts of beef tallow, menhaden oil and corn oil, to diets of *P. setiferus* had an adverse effect on growth and survival (Andrews *et al.*, 1972). Inclusion of a lipid supplement at levels of 10% or more in the diet adversely affected growth and survival of *P. setiferus* (Anon, 1990). Supplementing a diet which had shrimp meal as protein source with either corn or cod liver oil conferred no advantage at 7.5% level but depressed the growth of *Palaemon serratus* at 15% level (Forster and Beard, 1973). Whether growth retardation was caused by suppressed metabolism, toxic effects, reduced consumption or poor digestability was not determined. Accumulation of lipid may affect the rate of metabolism. Additional food intake is generally thought to be influenced by energy content and excess dietary lipid may affect feed consumption (Church and Pond, 1982) and ultimately causes nutrient deficiencies.

Several studies using different lipid sources or combinations have suggested that a lipid level of 6-10% is optimum (Lovell, 1998). Better growth and survival rate was observed in *Penaeus monodon* juveniles fed with a diet containing 2% soylecithin and 3.8% crude degummed soybean oil (Piedad-Pascual, 1986). Kanazawa *et al.* (1977b) reported maximum growth with 12% lipid and Deshimaru *et al.* (1979) reported good growth with 6% lipid level in *Penaeus japonicus*. Lipid requirement of *Penaeus indicus* fed with a diet containing a mixture of cod liver oil, soybean oil and lecithin was found to be optimum at 10% (Chandge and Raj, 1990). According to Chandge and Raj (1997) there was an increase in growth, survival and protein efficiency ratio of *P. indicus* with increase in lipid level from 0 –12%. They also observed that no significant improvement in growth and a decline in protein content of the prawns by increasing the lipid level above 12%.

Guary *et al.* (1976) observed that the shrimps fed with a diet containing unsaturated fatty acids of the linolenic family, supplied by 4% linseed oil, 4% sardine oil or 4% mollusc oil grew faster in length and weight than feed supplemented with soybean oil and 8% tripalmitin. Deshimaru and Kuroki (1974a) found that better

growth of *P. japonicus*, fed with a diet containing Pollack liver oil and soybean oil having a lipid content of 6% than those with 12% lipid or without lipid supplementation. Deshimaru *et al.* (1979) found that a mixture of Pollack liver oil and soybean oil in a ratio ranging from 3:1 to 1:1 containing approximately 20 – 30% ω-6 and 10 – 20% ω-3 fatty acids is desirable as a dietary lipid source for *P. japonicus* at a level of 6%. Kanazawa *et al.* (1977a) reported highest growth in *P. japonicus* fed with a diet containing 8% short necked clam lipid. Kanazawa *et al.* (1977a) observed a reduction in body weight gain of *P. japonicus* when levels of Pollack residual oil powder was increased from 12 % to 16%.

Castell and Covey (1976) evaluated 1,5,10 and 15% levels of cod liver oil as a source of lipid in diets of adult *Homarus americanus*. Based upon evaluation of mean weight gain, feed conversion and number of moults, the 5% dietary lipid was found to be most beneficial. No significant improvement in the response occurred when the level of dietary oil was increased to either 10 or 15%. Briggs *et al.* (1994) showed that post larvae of *P. monodon* have a higher weight gain with diet containing 3% basal lipid and 3% lecithin at a total lipid level of 6.5%. Inclusion of oils (6% dry weight) in a purified diet fed to juvenile lobsters caused significant increment in growth (D' Abramo *et al.* 1980). Davis and Robinson (1986) added menhaden fish oil as a lipid source in diets of juvenile cray fish *Procambrus acutus acutus*. Lipid levels varied from 0 to 15% in 3% increments and reduction in growth was observed for those animals fed diet containing 9% or more lipid. The experiment conducted by Sheen and Wu (1999) for evaluating the lipid requirement in the diet of juvenile mud crab by using cod liver oil /corn oil. (2:1) showed that an optimum level of dietary lipid level for crab was ranging from 5.3 to 13.8%.

New (1994) reported a wide range of lipid levels of 2 to 15% both in research work and in commercially available prawn feeds. Lipid levels of commercial prawn feeds vary from 6 – 9% in Thailand (ASEAN/UNDP/FAO, 1988), 5 to 8% in French Guiana (IFREMER, 1989) and 2 to 4% in Taiwan (Hsieh *et al.*, 1989). Fatty acid composition is more important than total lipid content. Using 2:1 cod liver oil/corn oil mixture, Sheen and D' Abramo (1989) found that 6% inclusion rate was optimal and 0%, 10% and 12% levels were found to depress the growth of *M. rosenbergii*. Sandifer and Joseph (1976) had reported prawn feed impregnated with 3% shrimp head oil produced maximum growth in *M. rosenbergii* juveniles. A dietary lipid level ranging from 2% to 10% under a wide range of dietary lipid: carbohydrate ratio

appears to be satisfactory, indicating that juvenile freshwater prawn do not require a specific level of dietary lipid (Sheen and D' Abramo, 1991). Behanan and Mathew (1995) reported that *M. rosenbergii* requires 30 to 40% protein and 4 - 8% lipid in the diet. High rate of growth and survival was observed in post larvae of *M. rosenbergii* when fed with egg custard and cod liver oil during day time and *Moina micrura* at night (Alam *et al.*, 1995). Tiwari and Sahu (1999) conducted a study on post larvae of *M. rosenbergii* by using seven experimental diets with graded levels of soylecithin (0 to 6%) by replacing equal amounts of cod liver oil and ground nut oil mixture (1:1) for 60 days. Maximum weight gain was obtained with the feed containing 5% soylecithin along with 1% cod liver oil and ground nut oil. But survival was not affected by supplementation of soylecithin.

Sze (1973) reported 5.8% (dry weight) lipid level in body composition of juvenile *M. rosenbergii* referred to the contention of Scheer and Scheer (1951). Scheer *et al.* (1952) and Neiland and Scheer (1953) reported that protein rather than fat or carbohydrate was the primary energy source for crustaceans. However, Sick and Andrews (1973) reported that *Penaeus duorarum* fed a casein based diet with 1% cholesterol grew and survived significantly better when 10% fat was added. They found that lipid sources and levels have a marked effect on body lipid levels. Shrimps fed with no supplementary lipid had a lower body lipid level than fed with a diet containing 10% lipid.

Total tissue lipid content of *M. rosenbergii* fed on diets with different levels of lipids were very similar (Joseph and Williams, 1975). They also reported that the dietary ω-3 fatty acids were retained in the body , while ω-6 fatty acids were mobilized for energy production. Similar result was also observed by Sandifer and Joseph (1976) in long term feeding of *M. rosenbergii* fed with a diet impregnated with 3% shrimp head oil.

As per Table 1, the quantitative dietary lipid requirement of shrimps and prawns ranges from 3 to 12% of dry weight. According to New (1976), Biddle (1977) and D' Abramo (1989) this variation in optimum lipid level is due to the inter relationship between different classes of lipid which influences the requirement and the difference due to age and species.

Table 1 Quantitative dietary lipid requirements of prawns and shrimps

Species	Source of lipid	Optimum level	Reference
Homarus americanus	Cod liver oil	5%	Castell and Covey (1976)
Procambarus acutus	Menhaden fish oil	0 – 6%	Davis and Robinson (1986)
Penaeus japonicus	*PLO + soybean oil, 1:1 ratio	6% of PLO : soy oil	Deshimaru *et al.* (1979)
	Soybean oil, PRO** and SNCO***	8%	Kanazawa *et al.* (1977a)
	Cod liver oil and soybean oil 1:1	6%	Deshimaru and Kuroki (1974)
	Short necked clam	8%	Kanazawa *et al.* (1979a)
P. indicus	-	10 – 12%	Chandge and Raj (1997)
	Cod liver oil, soybean oil and lecithin	10%	Chandge and Raj (1990)
	Cod liver oil, prawn head oil, sardine oil and soybean lecithin (1:1:1:1)	6%	Ali (1990)
P. setiferus	Beef tallow, Menhaden oil and corn oil (1:1:1)	10%	Andrews *et al.* (1972)
P. dourarun	Beef tallow or linseed oil	10%	Sick and Andrews (1973)
Palaemon serratus	Corn oil and cod liver oil	> 7.5%	Forster and Beard (1973)
M. rosenbergii	Shrimp head oil	3%	Joseph and Willliam (1975) and Sandifer and Joseph (1976)
	-	>10%	Biddle (1977)
	Cod liver oil, corn oil and lecithin	> 10%	Hilton *et al.* (1984)
	Cod liver oil and corn oil	6%	Sheen and D' Abramo (1991)

* Pollack Liver Oil
** Pollack Residual Oil
*** Short Necked Clam Oil

2.7.2 Qualitative requirement of lipid

Colvin (1976) found that no significant difference in growth of *P. indicus* juvenile, when fed with a diet containing sunflower oil, linseed oil, soybean oil or ground nut oil at the rate of 5% level for a period of 35 days. Powdered Pollack residual oil, pollack residual oil and short necked clam lipid were better dietary lipid sources than soybean oil provided at a level of 8% in the diet of juvenile *P. japonicus* (Kanazawa *et al.*, 1977a). These studies showed the superiority of marine derived oils versus vegetable oil in growth of crustacea. Diets containing a mixture of marine and vegetable oil yielded best results. Deshimaru *et al.* (1979) found that 6% of a mixture of pollack liver oil and soybean oil provided in ratio between 3:1 and 1:1 was associated with comparatively higher growth rate and feed efficiency for *P. japonicus*. Similar results obtained for a feed containing a mixture of cuttle fish liver oil and soybean oil in the same ratio.

2.7.3 Fatty acids

According to Castell (1983) fatty acids can be divided into three different groups as follows :

1. Fatty acids that can be synthesized *de novo* from acetate. This group includes all even number carbon, straight chain, saturated fatty acids, including those composed of 20 or 22 carbons. The most abundant is 16:0 (palmitic acid). Crustaceans can convert these saturated fatty acids to mono unsaturated form, which contain one double bond.
2. Unusual fatty acids composed an odd number of straight chain carbon atoms and non-methylene interrupted fatty acids which have two or more double bonds that are separated by more than three carbons and cyclic forms.
3. Essential fatty acids (EFA) composed of the linoleic (n-6) and linolenic (n-3) families of poly unsaturated fatty acids (PUFAs). These fatty acids have two or more unsaturated bonds. The first double bonds of a fatty acid of the linoleic family occurs at the sixth carbon from the methyl end of the molecule while linolenic (n-3) family have their first double bond located between the third and fourth carbon from the methyl end. Marine animals derive the greatest EFA value from this family.

Fatty acids of these two families that consists of a chain of 20 or more carbon atoms and more than 3 double bonds are called highly unsaturated fatty acids (HUFAs).

Body tissue of marine crustaceans contain higher levels of HUFA and PUFA of the linolenic family than that of freshwater crustaceans. (Castell, 1983 and Chaumugam *et al.*, 1983). The linolenic family has been observed to have the greatest essential fatty acid value for marine animals. (Castell and Boghen, 1979).

2.7.3.1 Biosynthesis

Neither the linolenic nor linoleic families of fatty acids are synthesized *de novo* by crustaceans (Kayama *et al.*, 1980). Kanazawa and Teshima (1977) used acetate^{14}C and found its activity almost exclusively associated with the saturated (16:0, 18:0) and mono unsaturated (16:1, 18: n-9, 20: n-9) fatty acids and less than 2% of the total activity was found in each of the 18:2n-6, 18:3n-3, 20: 5n-3 and 22: 6n-3 fatty acids. Kanazawa *et al.* (1979c) found that *P. japonicus* can convert palmitic acid (16:0) to saturated and mono unsaturated fatty acids. Little or no activity originating from labeled palmitic acid was found in lonolenic, linoleic, eicosapentaenoic and docosa hexaenoic acid. These studies showed that dietary source of HUFAs and PUFAs are essential nutrients for penaeid shrimps.

Many studies suggested that crustaceans have little or no ability to produce n-3 and n-6 HUFAs from n-3 and n-6 PUFAs. Read (1981) stated that juvenile *P. indicus* had limited ability for chain elongation and desaturation of linoleic and linoleinic acid to 20C and 22C HUFAs. Colvin (1976) compared fatty acid profile of experimental shrimp and that of animals caught from the wild and found evidence of a limited capacity of shrimps for bioconversion of 18C : n-6 or n-3 fatty acids to carbon $\geq$ 20 n-6 or n-3 fatty acids. *Penaeus setiferus*, *P. aztecus* and *P. duorarum* can not convert 18C PUFAs to 20C and 22C HUFAs (Bottino *et al.* 1980). Kanazawa *et al.* (1979b) found that *P. japonicus* had some ability to convert linolenic acid to 20.5 n-3 and 22: 6n-3 fatty acids. Juvenile freshwater prawns have an extremely limited capacity to convert C18 to C $\geq$ 20 fatty acids of n-3 and n-6 families. However no evidence for the synthesis of 20:5n-3 from 22:6n-3 fatty acids.

2.7.3.2 Digestibility

Digestibility of saturated fatty acids decreased with increasing chain length while digestibility of monoenoic fatty acids increased with increasing chain length. Digestibility of fatty acid was influenced by the presence of other fatty acids and source of lipid. Dall (1981) found that digestion and absorption of C ^{14} tripalmitate

was completed in Norvegian lobsters *Nephrops norvegicus* within 8 – 12 hours. Teshima and Kanazawa (1983) found that apparent digestibilities of variety of lipids exceeded 80% when included at a level 8% in diets of *P. japonicus*. Inclusion of phospholipids accelarate the metabolism of dietary triglycerides and cholesterol in *P. japonicus* (Teshima *et al.*, 1986b, c, d). Digestibility of fatty acids of squid liver oil full fat soya, fish and squid are high, when these ingredients were included in the diet of *P. monodon* (Merican and Shim, 1995).

2.7.3.3 Nutritive value of essential fatty acids

Kanazawa *et al.* (1977b, 1978, 1979c, 1979d) showed that juveniles of *P. japonicus* had a higher weight gain with a diet containing 18:2n-6, 18: 3n-3, 20:5n-3 or 22: 6n-3 than with diet containing oleic acid indicating the necessity of n-3 fatty acids especially n-3 HUFAs. The optimum level of 20:5 n-3 and 22:6n-3 in the diets of *P. japonicus* juveniles was found to be about 1%. Shewbart and Mies (1973) found linolenic acid incorporated diet at levels ranging from 1-2% produced better growth in juveniles of *P. aztecus* and depressed growth at levels exceeding 2%. Read (1981) found that 1% addition of either 18:2n-6 or 18:3n-3 to diets fed to *P. indicus* improved growth and survival. Kanazawa *et al.* (1979d) conducted a study about the effects of dietary linoleic and linolenic acids in the growth of juvenile *P. japonicus* by using various test diets with different lipid sources. They found that both are effective but the effect of linoleic acid is inferior to that of linolenic acid. Growth of *P. japonicus* fed with a diet containing high levels of 18:3n-3 fatty acid was better than that achieved with equal levels of 18:2n6 (Guary *et al.*, 1976). A similar result was observed by Kanazawa *et al.* (1977b) and Xu *et al.* (1994a) for *P. chinensis*. Xu *et al.* (1994a) reported that a combination of 18:2n-6 and 18:2n-3 fatty acids achieved better survival in *P. chinensis* than either fatty acid provided alone.

A dietary requirement of 1-2% linolenic acid is needed for prawns (Anon, 1990). In prawns linolenate (n-3) as a sole lipid source depress the growth, while linoleite enhances the growth (New,1994). Martin (1980) conducted an experiment with *Palaemon serratus* fed with diets containing different proportions of 18: 2n-6 and 18: 3n-3 by varying the amount of soybean oil and linseed oil as a lipid source. He found that the best growth was achieved with a 18: 2n-6 / 18: 3n-3 ratio of 2:2.

D' Abramo and Sheen (1993) observed that the addition of a mixture of HUFAs or either of 20:4n-6,or 22:6n-3 fatty acid alone in the diets fed to juvenile *M. rosenbergii* showed a significant increase in weight compared to equivalent level of 18:3n-3 or 18:2n-6. Reigh and Stickney (1989) found that growth of post larvae of *M. rosenbergii* were reduced at 1% dietary level of 18: 3n-3 but this response was lacking at an equivalent level of 18:2 n-6. Teshima *et al.* (1992) found the highest weight gain of *M. rosenbergii* was achieved when fed with a diet containing a mixture of 18: 2n-6 and 18: 3n-3 in a ratio of 12:1. From these observations it is found that highest weight gain of *M. rosenbergii* would be achieved at a high ratio of 18C n-6 to 18C n-3 PUFAs. Shivanandamurthy (1998) reported that a combination of both n-3 and n-6 PUFAs with n-6 / n-3 ratio 2.6 was found to be optimum for the development and survival of *M. rosenbergii* larvae. Feeding experiment of Querijeo *et al.* (1997) showed that *M. rosenbergii* juveniles fed on diet with 2% stearic acid and 35% crude protein gave higher weight gain and FCR than those fed on diet with 7% stearic acid and 50% crude protein. According to him *M. rosenbergii* juveniles utilize stearic acid as an energy source but its contribution as an energy source is lower than that of carbohydrates.

In marine shrimps 20C and 22C HUFAs are having higher nutritive value than 18C PUFA. Kanazawa *et al.* (1977a) suggest that the lower nutritive value of soybean oil relative to Pollack residual oil is due to the small amount of n-3 HUFAs. But growth can be improved by adding 3% of oil rich in HUFA along with 18 : 2n-6. A diet containing 8% of short necked clam oil reduced optimum growth in prawns. Kanazawa *et al.* (1978, 1979c) observed that n-3 HUFAs possess higher activity as essential fatty acids than that of n-6 and n-3 PUFAs.

D' Abramo and Sheen (1993) found that dietary carbon more than or equal to 20 : n-3 or n-6 fatty acids were essential for juvenile *M. rosenbergii,* and showed significant increase in weight because prawns have an extremely limited capacity to convert C18 to C $\geq$ 20 fatty acids of n-3 and n-6 families. They also observed that the dietary levels of HUFAs as low as 0.75% were effective in producing a significant increase in weight of juvenile *M. rosenbergii* relative to a control diet containing a mixture of saturated and monounsaturated fatty acids. The best growth and survival of larval *P. japonicus* was achieved with 0.5 to 1.0 % n-3 PUFAs in the presence of a 3% level of lecithin (Kanazawa *et al.*, 1985).

Sandifer and Joseph (1976) reported that the n-3 fatty acids are used for the biosynthesis of long chain PUFAs which are deposited in tissue, whereas n-6 fatty acids were utilized as energy sources. HUFAs are preferentially incorporated and conserved in the polar lipid of crustacean tissue (Clarke, 1979, D' Abramo *et al.*, 1980, Kanazawa *et al.*, 1977a and D' Abramo and Sheen, 1993).

The role of temperature in influencing the essential fatty acid requirements of crustaceans is reported by the results of studies conducted by Cossins (1976), Farkas (1979) and Farkas and Nevenzel (1981). At lower temperature there is an increase of the proportion of monounsaturated and poly unsaturated fatty acids in tissue lipids. Hence changes in culture temperature of crustaceans changes the requirement of dietary levels of essential fatty acids.

Dietary polyunsaturated fatty acids such as 20: 4n-6, 20: 5 n-3 and 22: 6n-3 are necessary for successful ovarian maturation and spawning of *P. setiferus.* (Middleditch *et al.*, 1979, 1980). They also suggested the need for Arachidonic acid as a precursor to prostaglandins which appear to be important in crustacean reproduction and moulting. *M. rosenbergii* brood stock fed with high amount of HUFA showed high fecundity and increased hatchability(Cavalli *et al.* , 1999). The ovarian development of *P. japonicus* was retarded in the absence of n-3 HUFAs (Alva *et al.*, 1993). Xu *et al.* (1992, 1994b) suggested that dietary 20: 5n-3 and 22: 6n-3 positively influence fecundity and egg hatchability in *P. chinensis.*

2.7.4 Phospholipids

In addition to essential fatty acids marine shrimps seems to have a dietary requirement for phospholipids (Lovell, 1998). But precise requirements have yet to be established. Variable responses of feeding phospholipids have been noticed in crustaceans in relation to growth., stage of animal and composition and source of other dietary ingredients.

2.7.4.1 Phospholipid requirement

The prawn *P. japonicus* (Kanazawa *et al.*, 1979a, Kanazawa, 1983 and Teshima *et al.* 1986a) lobster *H. americanus* (Conklin *et al.*, 1980, Bowser and Rosemark, 1981and D' Abramo *et al.*, 1981) and crab *Portunus trituberculatus*

(Kanazawa *et al.*, 1983) have been shown to require a dietary source of some phospholipids for growth and survival. Feeding a casein based purified diet without phospholipid reduces the survival rate of juvenile lobster and also causes 100% mortality of larval prawns prior to attaining mysis stage (Kanazawa, 1993 and Kanazawa *et al.* , 1985).

In several studies phospholipids of unspecified composition such as lecithin are used, as a result of which it is difficult to draw conclusions with regard to the optimal level and types of phospholipid required for penaeid shrimps.(Tackaert *et al.*, 1991). The optimum level of dietary soybean lecithin for juvenile *P. japonicus* was 3% by Teshima *et al.* (1986a). The phospholipid deficiency in juvenile *P. japonicus* may not be related to a shortage of either choline or inositol (Kanazawa *et al.*, 1976 and Deshimaru and Kuroki, 1979). In lobsters the characteristic symptom of phospholipid deficiency is failure of the juveniles to extricate itself completely from its old exoskeleton (Conklin *et al.*, 1980) and this syndrome has been termed as moult death syndrome by Bowser and Rosemark (1981). Theshima *et al.* (1986b,c,d) observed that total lipid concentrations were higher in *P. japonicus* when fed with a diet containing 3% soybean lecithin. Kanazawa *et al.* (1985) reported poor growth and survival of *P. japonicus* larvae fed with a diet containing .5%, 1% and 2% of n-3 HUFA and either of 1% or no phospholipid. But showed better growth at 1% n-3 HUFA alongwith 3% phospholipid or 6% phospholipid alone.

Recently the necessity of dietary phospholipids such as soybean lecithin and phosphatidylcholine for better growth and food conversion ratio was found in juvenile *P. pencillatus* (Jenn, 1989 and Chen and Jenn, 1991), *P. monodon* (Piedad – Pascual, 1985 and 1986) and *P. chinensis* (Kanazawa, 1993). Piedad-Pascual (1986) has pointed out that growth of juvenile *P. monodon* increased with increase in soybean lecithin levels from 0 to 2% regardless of the lipid source. Jenn (1989) showed that the efficiency of dietary soybean phosphatydilcholine was influenced by protein quality. These studies showed that dietary phospholipids in crustaceans are nutritionally and biochemically unique.

In contrast to these studies some investigations suggest the non-essentiality of dietary phospholipids for growth of some crustacean species. Kean *et al.* (1985) found that the lobster *H. americanus* did not have a dietary requirement of lecithin and exhibited no moult death. Hilton *et al.* (1984) also reported that supplemental lecithin was not required by freshwater prawn *M. rosenbergii*. No significant

difference was detected in either weight gain or mortality of prawns provided with diets containing different levels of soybean lecithin ranging from 0-10%. No sign of deficiency and moult death syndrome was also observed. Briggs *et al.* (1988) found that supplementation of soybean lecithin at 5% conferred no advantage in freshwater prawn. Results of recent investigation conducted by Kanazawa (1993) also suggested that the supplementation of 1 and 2% soybean lecithin to either casein or crab protein based diet did not improve the growth rate and survival of freshwater prawn, *M. rosenbergii.* Cavalli *et al.* (2000) suggested that the basal level of 0.8% phospholipid was sufficient to meet dietary demand of prawn broodstock. Supplementation of lecithin at levels of 2.5% in the diet can accelerate the growth and food conversion ratio during early post larval phase of *M. rosenbergii.* After that phospholipids have no effect on growth of *M. rosenbergii* (Mahesh, 1996).

Dietary requirement of phospholipid in most of the crustaceans are in the range of 1-3%. Phosphatidylcholine and phosphatidylinositol are the most efficient in crustaceans. Phospholipids are superior to neutral lipid as a source of essential fatty acids and energy due to better digestibility. Table 2: shows the sources and optimal level of phospholipid requirement of crustacea.

Several studies have investigated the interaction between phospholipid requirement and other nutrients such as cholesterol and protein. Teshima *et al.* (1982) showed the effects of cholesterol in improving growth and survival of *P. japonicus* larvae which are unaffected by dietary soy lecithin levels (0-6%). Briggs *et al.* (1988) reported that the growth and survival of juvenile *M. rosenbergii* were not influenced by the effects of supplementary soybean lecithin and cholesterol.

In lobster, the growth promoting effect of dietary soybean lecithin was influenced by change in source and quality of protein (Conklin *et al.*, 1980 and Kean *et al.*, 1985) and the same effect was found in *P. penicillatus* by Jenn (1989).

Table 2 Quantitative dietary phospholipid requirements of prawns and shrimps

Crustacean species	Phospholipid source	Optimum level (%)	Reference
Juvenile lobster	Soybean lecithin; a mixture of soybean oil, PC, PE and others	7.5	Conklin *et al.*(1980)
Juvenile lobster	Refined soy lecithin; 95% PC	2	D' Abramo *et al.* (1981)
Juvenile lobster	Soy lecithin or crab phospholipids	No effect: 0 –6	Kean *et al.* (1985)
Kuruma prawn (larvae)	Soybean lecithin; a mixture of PC 24%, PE 30%, PI 18% and other PL such as SM, PS and LPC	3	Teshima *et al.* (1983)
P. monodon	Soybean lecithin	2	Piedad-Pascual (1986)
P. penicillatus	Soybean PC; a mixture of 80% PC and 20% LPC	1.2	Jenn, (1989), Chen and Jenn (1991)
P. stylirostris	Soybean lecithin	1.5	Bray *et al.* (1990)
P. chinensis	Soybean lecithin	2	Kanazawa (1993)
M. rosenbergii	Refined soybean lecithin	No effect: 0 – 10	Hilton *et al.* (1984)
M. rosenbergii	Soybean lecithin	No effect 0-5	Briggs *et al.* (1988)

PC – Phosphatidylcholine
PE – Phosphatidylethanolamine
PI – Phosphatidylinositol
LPC - Lysophosphatidylcholine

2.7.4..2 Effect on lipid concentration of midgut gland and serum

In juvenile lobsters absence of phospholipids in diet causes a significant decrease of total cholestrol and phospholipid in serum compared to control diet with 8% of refined soybean lecithin (D'Abramo *et al.*, 1982). *P. japonicus* fed with a diet deficient in phospholipid causes a decreased body retention rate of dietary cholesterol and lipids (Teshima *et al.* , 1986e). These results showed that the prawns are incapable of effectively utilizing dietary lipid especially cholesterol. Addition of phosphatidylcholine at the rate of 3% in the feed of juvenile prawns were found to increase the concentration of lipids such as triglycerides, cholesterol and phosphatidylcholine in hepatopancreas and haemolymph. (Teshima *et al.*, 1986b). Hence phosphatidylcholine helped the mobilization of lipids from midgut gland to haemolymph and other extra hepatic tissues.

2.7.4.3 Effect on transport of cholesterol.

Teshima *et al.* (1986d) reported that cholesterol was retained longer in gut and midgut gland of *P. japonicus* juveniles fed with phospholipid deficient diet and inclusion of 3% soybean lecithin increased the cholesterol level of haemolymph. D' Abramo *et al.* (1985a) also observed a reduction in transport rate of cholesterol of the midgut gland in juvenile lobster fed with a diet deficient in soy lecithin. Lipid transport in crustaceans seems to be basically conducted in the form of lipoproteins containing abundant phospholipids rich in phosphatidylcholine and phosphatidylinositol. (Lee and Puppione, 1978 and Teshima and Kanazawa, 1979, 1980a,b). Hence lack of phospholipid in diet reduces the level of phospholipid in the midgut gland, thereby restricting the formation of lipoproteins that serve to transport cholesterol in the haemolymph.

2.7.4.4 Role of Phospholipid in crustaceans

Insufficient transport of lipids especially cholesterol has been assumed to retard growth and high mortality in crustaceans which are receiving phospholipid deficient diet (D'Abramo *et al.*, 1985b, Teshima *et al.*, 1986d and Teshima and Kanazawa, 1988). In *P. japonicus* dietary phospholipids specifically

phosphatidylcholine containing unsaturated fatty acids as a constituent of lipoprotein is responsible for cholesterol transport (Teshima, 1985 and Teshima and Kanazawa, 1988). Baum *et al.* (1990) have shown that cholesterol levels in serum, lipoprotein and cholesterol excretion of lobster are influenced by dietary lecithin but not by change in the source of protein.

2.7.5 Sterols

In addition to essential fatty acids and phospholipids shrimps seems to have a dietary requirement for sterols. Because they cannot synthesise sterols from acetate or mevalonate as in the case of fin fishes (Douglass *et al.*, 1981 and Teshima, 1991).

2.7.5.1 Nutritive value of sterols

Crustaceans are unable to synthesize sterols *de novo* (Teshima, 1972). Hence sterols are considered as an essential nutrient for crustaceans because of the important role of sterols such as cholesterol as a cell constituent, as a metabolic precursor of steroid hormones and moulting hormones etc. (Teshima, 1972). Various studies conducted by Teshima (1978, 1983, 1985) revealed the importance of dietary sterols in growth of *P. japonicus*, planktonic crustaceans and in American lobster. Cholesterol is necessary for normal metamorphosis of nauplii to post larvae and survival of larval prawn. *P. japonicus* (Teshima *et al.*, 1982), juvenile lobsters (Conklin *et al.*, 1980 and Bowser and Rosemark, 1981) and cray fish *Pacifastcus leniusculus.* Crustaceans possess the ability to dealkylate some C28 and C29 sterols to cholesterol (Teshima, 1972). Hence cholesterol requirement of juvenile *P. japonicus* may be met by feeding sterols other than cholesterol (Teshima, 1978, Teshima *et al.*, 1983 and Teshima and Kanazawa, 1986). D' Abramo *et al.* (1984) showed that a mixture of dietary phytosterols comprised primarily of sitosterol could not replace cholesterol to satisfy the sterol requirement of lobsters *Homarus* spp. D' Abramo *et al.* (1985a) determined that a mixture of phytosterols was as effective as cholesterols in the partial satisfaction of the sterol requirement in crayfish.

Partial replacement of cholesterol with sitosterol in diet of *P. japonicus* slightly retard the larval development and in juvenile reduced the growth rate

(Teshima *et al.*, 1989). In crustaceans cholesterol is nutritionally superior than that of other sterols and sitosterol.

2.7.5.2 Quantitative requirement of cholesterol

The optimum level of dietary cholesterol reported for different crustacean species ranged from 0.12 to 2% of diets. Better growth and survival of Argentine prawn, *Artemesia longinaris* was obtained at a level of 0.5% - 2% of cholesterol in the diets (Romerio *et al.*, 1991). Cholesterol supplemented diet did not show significant growth improvement in Banana shrimp, *P. merguiensis*. Because the other sterols might have satisfied the cholesterol requirement or excess of cholesterol might have caused some adverse effect. (Thongrod and Boonyaratpalin, 1998).

In juvenile freshwater prawn *M. rosenbergii* a supplementation of 0.5 and 1 % cholesterol to a semipurified diet does not improve the growth (Briggs *et al.*, 1988). Inclusion of 5% cholesterol for the prawn *P. japonicus* (Kanazawa *et al.*, 1971), 2.0% cholesterol for the American lobster *H. americanus* (Castell *et al.*, 1975) and 1.45% sterols (a mixture of 0.47% cholesterol and 1.39% of sitosterol) for cray fish (D'Abramo *et al.*, 1985b) will inhibit the growth. Castell and Covey (1976) noted that a dietary supplementation of cholesterol did not improve growth of adult lobster, *H. americanus*, 300-600 mg in body weight, in contrast to the reported requirement of juveniles by Castell *et al.* (1975) and Bordner *et al.* (1986). From these studies it is clear that cholesterol requirement of lobsters vary with age. Deshimaru and Kuroki (1974b) reported that the growth promoting effect of cholesterol for the juvenile *P. japonicus* was inhibited by the presence of glucosamine in the diet.

In the case of *M. rosenbergii* there is no advantage conferred by supplementing cholesterol or lecithin to the basal diet containing 0.12% indigenous cholesterol and 0.048% total lipids. A trend towards enhancement of growth was noticed when supplemented lecithin was increased from 0-5% at 0.5 and 1% levels of supplementary cholesterol. This trend was reversed when cholesterol was absent (Anon, 1990).

Post larvae of *M. rosenbergii* fed with a diet containing cholesterol at 0 and .5% showed better growth than those at 1% and 5% levels, showing growth inhibition at higher levels but have no effect on survival rate. (Sherief *et al.*, 1992). Teshima *et al.* (1997) conducted an experiment on the daily requirement of cholesterol for the

freshwater prawn *M. rosenbergii.* The result indicated that juvenile *M. rosenbergii* was capable of *de novo* cholesterol synthesis. In contrast to other prawn species, *M. rosenbergii* required a dietary source of cholesterol for the maximum growth. Optimum dietary cholesterol levels for the juvenile *M. rosenbergii* was found to be 0.11 to 0.26% in the diet.

3. MATERIALS AND METHODS

The experiment was done to work out optimum level of lipid required in the feed of *M. rosenbergii* and to assess the commercial possibility of utilization of the cuttlefish liver as a source of lipid in the formulated feed for *M. rosenbergii.* The experiment was conducted at the freshwater prawn hatchery, College of Fisheries, Panangad, Cochin during the period from 5th February to 5th April, 2001.

3.1 Experimental animal

M. rosenbergii larvae derived from a single female hatch were reared in a fibre glass tank till it became post larvae. From this 500 post larvae were transferred to a flat bottomed fibre glass tank of 1.2 tons. The tank was filled with filtered freshwater and gentle aeration was provided using air diffusion stones. The post larvae were fed twice daily with granulated artificial feed having clam meat as the chief source of protein. Left over feed and waste were removed daily by siphoning out and 75% of water was renewed every day. Tiles and PVC pipes were provided at the bottom of the tank in order to reduce the cannibalism. 30 days old post larvae collected from this were used for the present experiment. 10 juveniles each were randomly distributed to all tanks after recording their initial weight.

3.2 Experimental set up

The diet evaluation experiment with *M. rosenbergii* juveniles was performed in the hatchery of College of Fisheries, Panangad. Flat bottomed circular fibre glass tanks having the following specifications were used for rearing the test animals.

Capacity of the tank	83 lit.
Diameter	55 cm
Height	35 cm
Thickness of wall	1 mm
Rim width	3 cm
Colour	Aquamarine

Clear well water filtered through a close meshed nylon blotting silk was used for filling the tanks up to a height of 25 cm. Mild uniform aeration was provided in the tanks with air diffusion stones and control valves. Flat square shaped tiles were kept at the bottom of the tanks in a slanting position for providing shelter to the animals.

3.3 Extraction of cuttle fish liver lipid

Cuttlefish liver lipid was extracted by solvent extraction method of Radin (1981) from the liver of *Sepia pharaonis* (Ehrenberg) collected from a seafood processing plant. 500 gm of liver was minced in a mortar. After mincing Hexane: Isopropanol (3:2) mixture was added to this at the rate of 18 ml for each gram of tissue. The mixture was homogenized thoroughly and filtered through a filter paper. The filtrate was evaporated to get the lipid. Yield of cuttlefish liver lipid was 20% on wet weight basis.

3.4 Experimental feed

3.4.1 Feed ingredients

Six experimental diets (T_1-T_6) were prepared for *M. rosenbergii* juveniles at graded level of lipid. The percentage composition of the diets are given in Table 3. Control diet (T_1) was prepared by using clam meat, ground nut oil cake, wheat bran, tapioca powder, vitamin-mineral mixture and cellulose powder without supplementing lipid. Different test diets (T_2-T_6) were prepared by adding different levels (1-5%) of cuttlefish liver lipid by replacing equal amount of cellulose powder.

Table 3. Percentage composition of experimental diets

Ingredients	Control diet	Percentage weight.				
	T1	T2	T3	T4	T5	T6
Clam meat powder	40	40	40	40	40	40
Ground nut oil cake	22	22	22	22	22	22
Wheat bran	22	22	22	22	22	22
Tapioca powder	10	10	10	10	10	10
Vitamin mineral mixture	1	1	1	1	1	1
Cellulose powder	5	4	3	2	1	0
Cuttlefish liver lipid	0	1	2	3	4	5

Plate I Liver of cuttlefish, *Sepia spp.*

3.4.2 Diet formulation, processing and storage

All the solid feed ingredients were dried, finely powdered and sieved through a 250μ sieve separately. All the ingredients were weighed accurately in an electronic balance according to percentage composition of the feed. Table 3 gives the percentage composition of experimental diets. Then all the ingredients other than vitamin mineral mixture (SuppleVit-M) and cuttlefish liver lipid were mixed well in a mortar and made a dough by adding distilled water at the rate of 125 ml for 100 gm of ingredients. The dough was transferred to a glass beaker and steamed for 30 minutes in an autoclave at atmospheric pressure. The steamed dough was cooled under fan. Vitamin-mineral mixture and cuttlefish liver lipid were added to this and mixed well. Then it was extruded through a noodling machine, spreading in an enamel tray and dried in an oven at 65°C for 7 hours for reducing the moisture to less than 12%. After drying, the pellets were broken into small pieces and kept at a temperature of less than 4°C and stored in air tight plastic containers until fed to the experimental animals. All the test diets were formulated to be isonitrogenous and isoenergetic.

3.4.3 Proximate Analysis of feed

Proximate analysis of the feed was done for evaluating the nutritional status of the feed. For each feed three replications of analysis were done and their mean was taken as the result. The methods used for the analysis are shown below:

Moisture content of the feed %	:	Drying the sample at 105 °C till a constant weight was arrived
Crude fat %	:	Solvent extraction using petroleum ether (B.P. 40-60°C) in a soxhelet extraction apparatus for 6 hrs.
Crude proteins %	:	Microkjeldhal's method (AOAC, 1984)
Ash content %	:	Burning the sample at 550°C ± 10°C for 6 hrs in a muffle furnace.

Carbohydrate %	:	100 - (% protein +% lipid + % Ash) by difference method (Hasting, 1976)
Water stability %	:	Percentage dry matter obtained after exposing pellets in water for 6 hrs. (Jayaram and Shetty, 1981)

3.5 Experimental design and procedure

Flat bottomed circular fibre glass tanks were used for the experiment. 180 members of healthy uniform sized juvenile prawns having average wt. .066 g were selected from a population of 500 number and 10 number each randomly distributed in eighteen experimental tanks. The experiment was conducted in a completely randomized design with six treatments and three replications each. Before the commencement of feeding with the experimental diet, the juveniles were conditioned with the control diet for 5 days. After conditioning, the experimental animals were starved for 24 hrs in order to empty the gut. Then 10 prawns from each tank were collected and weighed together in an electronic balance with a precision of 0.001 g. Before weighing water was blotted from the animals. Initial average weight of the juveniles was 0.066 g (66mg).

Each treatment group of animals was fed *ad libitum* with corresponding diets, twice a day at the rate of 15% of biomass for the first 15 days. After that the feeding ration was reduced to 10% for 15 days and then to 8% till the end of the experiment. Before giving the feed, leftover feed was collected daily and dried at 60°C for estimating the quantity of feed consumed.

Every morning before feeding the sides of the tanks were scrubbed to remove the algal growth, waste was siphoned out and 75% of water exchange was done by adding freshwater.

Sampling was done fortnightly. During each sampling the weight of all survivors in each tank were determined in order to adjust the quantity of feed according to the biomass. Growth assessment was done according to the procedures described above. Temperature of the water used for the experiment was checked daily while dissolved oxygen and pH were monitored at weekly intervals.

Duration of the feeding study was 60 days. At the end of the experiment prawns were starved for 24hrs. The number of prawns in each tank were counted, weighed collectively and the average weight was recorded . At the beginning and end of the experiment prawns were subjected to carcass proximate analysis.

3.6 Water quality measurement

During the experiment water quality parameters such as temperature, pH and dissolved oxygen were checked periodically by the following methods.

Temperature : By using Mercury thermometer of 0.10°C.

pH : By using universal indicator solution.

Dissolved Oxygen : Winkler's method (Strickland and Parsons,1972)

3.7 Carcass proximate analysis of test animals

Before and after the experiment the prawns were subjected to biochemical analysis for estimating proximate body composition by the methods already described.

3.8 Evaluation indices

The parameters evaluated are percentage weight gain, food conversion ratio, food conversion efficiency, net protein utilization, protein efficiency ratio, survival rate and carcass composition of test animals.

3.8.1 Percentage weight gain

Percentage weight gain of test animals were calculated by using the following formula.

$$\text{Percentage weight gain} = \frac{(\text{Average final weight} - \text{Average initial weight}) \times 100}{\text{Average initial weight}}$$

3.8.2 Food conversion ratio (FCR)

Food conversion ratio is the ratio between the weight of food consumed and the weight gain of the animal which often serves as a measure of efficiency of the diet.

$$FCR = \frac{\text{Average weight of food consumed in dry weight}}{\text{Average live weight gain}}$$

3.8.3 Food conversion efficiency (FCE)

FCE is the wet weight gain of animal from one unit of food consumed and this was calculated using the following formula.

$$FCE = \frac{\text{Wet weight gain of prawn (g) during the sampling period}}{\text{Dry weight of food consumed (g) during the sampling period}}$$

3.8.4 Survival rate (SR)

Mortality of the prawns during the experimental period was noted from each tank and percentage survival was calculated as follows .

$$\text{Percentage survival} = \frac{\text{Number of prawns retrieved}}{\text{Number of prawns stocked}} \times 100$$

3.8.5 Protein efficiency ratio (PER)

PER is the ratio between the weight gain of prawn and the amount of protein consumed. It was calculated by employing the formula of Hepher (1988).

$$PER = \frac{\text{Wet weight gain of prawn (g)}}{\text{Crude protein consumed (g)}}$$

3.8.6 Net protein utilization (NPU)

NPU is the ratio of protein retained to the protein consumed expressed as percentage of latter.

$$NPU = \frac{\text{Protein retained}}{\text{Protein consumed}} \times 100$$

3.9 Statistical analysis

The feed study was conducted by using completely randomized design (CRD). All the evaluation indices were analysed by using Analysis of variance (ANOVA) at 5% level significance. Pair-wise comparison of treatments were done using least significant difference. Arc-sine transformation was used for analyzing the survival rate of the experimental animals.

4. RESULTS

The effect of experimental diets having graded levels of lipid extracted from cuttlefish liver (0-5%) on growth, food conversion ratio, food conversion efficiency, protein efficiency ratio, net protein utilization, survival rate and body composition of *Macrobrachium rosenbergii* juveniles was evaluated. Total lipid content of the test diets denoted by T_1, T_2, T_3, T_4, T_5 and T_6 were 7.53%, 8.61%, 9.85%, 10.73%, 12.01% and 13.41% respectively. The results obtained are presented below:

4.1 Proximate composition of feed

In this experiment six practical diets having different levels of lipid were used. Proximate analysis of feeds were done and the results are given in Table 4. Lipid and ash were estimated by using moisture free samples. From the analysis it was found that the moisture content of the feed varied between10.65 % and 11.27%, while crude protein, lipid, ash and carbohydrates varied between 34.100% and 36.055%, 7.53% and 13.41%, 5.98% and 6.48% and 35.81% and 38.665%, respectively.

4.2 Water stability of feed

Test for water stability of different test diets were done for 1 hour, 2 hours, 3 hours and 6 hours. The results are given in Table 5. Maximum water stability was for the feed T_5 and minimum for the control diet T_1.

Table 4 Proximate composition of test diets

Percentage composition	Feeds					
	T_1	T_2	T_3	T_4	T_5	T_6
Moisture	11.27	11.23	11.05	11.01	10.65	10.68
Crude protein	36.055	35.330	35.104	34.467	34.100	34.12
Lipid	7.53	8.61	9.85	10.73	12.01	13.41
Ash	6.48	6.41	6.42	6.45	6.40	5.98
Carbohydrate	38.665	38.420	37.576	37.343	36.84	35.81

Table 5 Percentage water stability of pelleted feeds

Feed	1 hour	2 hour	3 hour	6 hour
T_1	91.64	89.625	89	88.796
T_2	95.249	93.455	92.059	88.826
T_3	96	94.563	93.824	89.611
T_4	96.071	93.159	93.695	91.466
T_5	97.628	97.228	96.443	94.455
T_6	97.576	94.048	93.004	92.218

4.3 Biological evaluation of feeds

4.3.1 Effect of dietary lipid levels on percentage weight gain

The data corresponding to the weight of the experimental animals at the beginning and the end of the experiment, growth in terms of percentage weight gain and mean are given in Table 6. Maximum weight gain was observed for the animals fed with the diet containing 2% of cuttlefish liver lipid (T_3) followed by 3% of lipid (T_4) and minimum for the control diet (T_1). Graphical representation of percentage weight gain of the juveniles are also shown in Figure 1. From the Analysis of variance (Table 7) it is found that the growth of the juveniles of *M. rosenbergii* were significantly different between the treatments. The highest weight gain was noticed for the treatment T_3 and it is entirely different from all other treatments. T_2 and T_6 showed the same effect on weight gain, while T_5 and T_4 showed almost the same.

4.3.2 Effect of dietary lipid levels on food conversion ratio

The data on average initial body weight, final body weight, average increment in weight, average weight of feed consumed, food conversion ratio and mean food conversion ratio of various treatments are given in Table 8. From this table it is clear that the least food conversion ratio is for the diet T_3, followed by T_4 and the highest value for the control diet. Graphical representation of food conversion ratio for various diets are shown in Figure 2. The Analysis of variance (Table 9) of data on the food conversion ratio of the prawns showed that the mean of the food conversion ratio was significantly different for feeds containing cuttlefish liver lipid when compared to controlled diet. The treatments having cuttlefish liver lipid formed a homogeneous group in the case food conversion ratio.

4.3.3 Effect of dietary lipid levels on food conversion efficiency

Food conversion efficiency of various test diets were evaluated and the data are given in Table 10. These data showed that maximum food conversion efficiency was for the diet supplemented with 2% of cuttlefish liver lipid (T_3) and least for the control diet. Graphical representation of food conversion efficiency of various diets is given in Figure 3. The Analysis of variance (Table 11) revealed that there was

significant effect of lipid on food conversion efficiency. Here all the treatments with cuttlefish liver lipid showed the same effect.

4.3.4 Effect of dietary lipid levels on survival rate

The data on stocking number, percentage survival and mean of survival rate of juvenile *M. rosenbergii* fed with diets with different concentrations of lipid are given in Table 12. Duration of the experiment was 60 days. Maximum survival rate was recorded for the diet T_3 and T_4 and minimum for the diet T_6. The Analysis of variance (Table 13) was carried out after Arc-sine transformation. And the result showed that there was no significant difference between treatments on survival.

4.3.5 *Effect of dietary lipid levels on protein efficiency ratio*

Protein efficiency ratio obtained for various treatments are shown in Table 14. From these data it is clear that protein efficiency ratio is maximum for the diet (T_4), followed by T_3 and minimum for the control diet. Graphical representation of protein efficiency ratio for various treatments are given in Figure 4. The Analysis of variance (Table 15) of data showed that there is significant variation between the treatments. Pair-wise comparison showed that all the treatments are at par except the control diet.

4.3.6 Effect of dietary lipid levels on net protein utilization

The data of protein retained in the body, protein consumed by the animals and net protein utilization are given in Table 16. Maximum net protein utilisation was for T_4 followed by T_3 and minimum for the control diet. Graphical representation of net protein utilization for various diets are shown in Figure 5. From the Analysis of variance (Table 17) of data about net protein utilization for various treatmental diets, it was clear that there is significant variation between the treatments. No statistical difference was observed between T_3 and T_4.

Table 6 Percentage weight gain of experimental animals

Treatment	Replication	Average initial wt. (g)	Average final wt. (g)	Average wt gain (g)	Percentage wt. gain	Mean ±S.D.
T_1	T_{11}	0.0664	0.3102	0.2438	367.1687	371.2169 ± 3.11
	T_{12}	0.0683	0.3222	0.2539	371.7423	
	T_{13}	0.0673	0.3195	0.2522	374.7399	
T_2	T_{21}	0.0633	0.3513	0.2880	454.9763	456.1478 ± 1.38
	T_{22}	0.0659	0.3660	0.3001	455.3870	
	T_{23}	0.0625	0.3488	0.2863	458.08	
T_3	T_{31}	0.0668	0.3941	0.3273	489.9701	489.0623 ± 2.52
	T_{32}	0.0633	0.3707	0.3074	485.6240	
	T_{33}	0.0678	0.4011	0.3333	491.5929	
T_4	T_{41}	0.0662	0.3830	0.3168	478.5499	476.3431 ± 1.92
	T_{42}	0.0633	0.3650	0.3017	476.6193	
	T_{43}	0.0658	0.3776	0.3118	473.3602	
T_5	T_{51}	0.0675	0.3809	0.3134	464.2963	467.6216 ± 2.99
	T_{52}	0.0640	0.3629	0.2989	467.0313	
	T_{53}	0.0657	0.3755	0.3098	471.5373	
T_6	T_{61}	0.0648	0.3598	0.2950	455.2469	459.927 ± 3.73
	T_{62}	0.0640	0.3612	0.2972	464.3750	
	T_{63}	0.0625	0.3501	0.2876	460.16	

Table 7 ANOVA of percentage weight gain of juvenile *M. rosenbergii*

Source of variance	Sum of squares	Degrees of freedom	Mean sum of squares	F. value
Treatment	26413.9	5	5282.78	475.2112*
Error	133.4	12	11.1167	
Total	26547.3	17		

*Significant at 5% level

Comparison of treatment means based on critical difference

Critical difference : 9.332

Treatment means $\overline{T_1}$ $\overline{T_2}$ $\overline{T_6}$ $\overline{T_5}$ $\overline{T_4}$ $\overline{T_3}$

Underscored means are not significantly different.

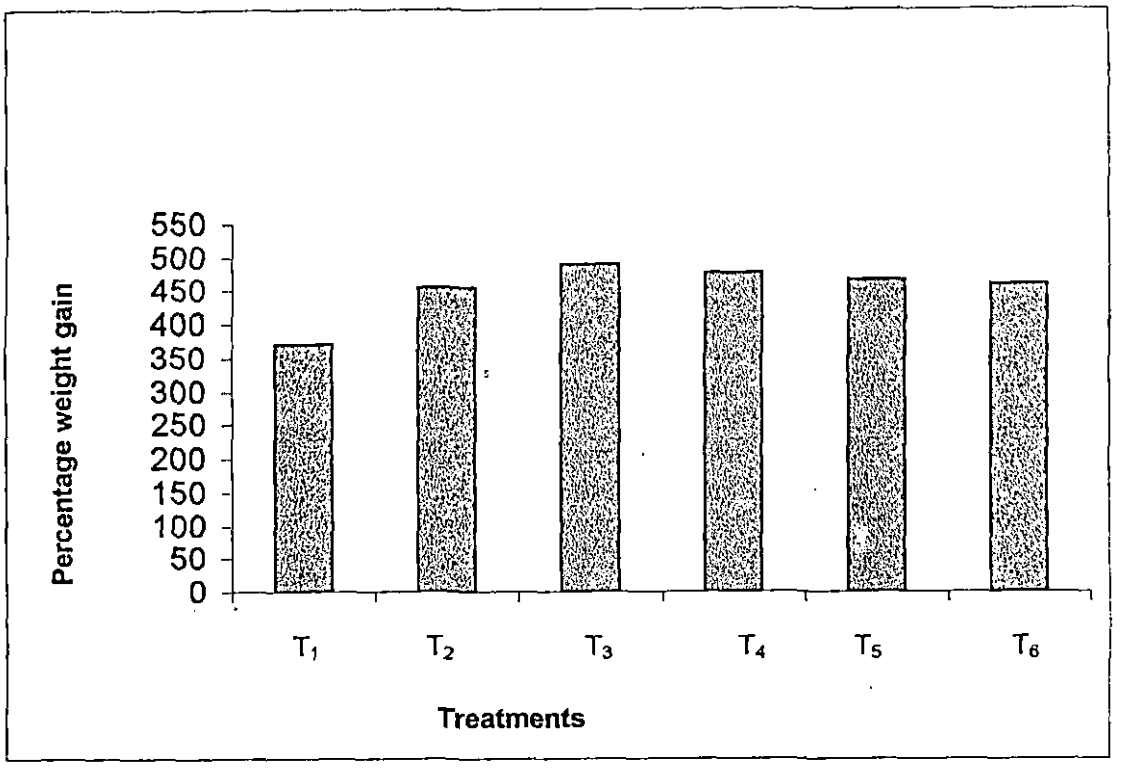

Fig. 1 Effect of dietary lipid levels on percentage weight gain of *M. rosenbergii* juveniles

Table 8 Food conversion ratio of different treatments

Treatment	Replication	Average initial biomass (g)	Average final biomass (g)	Average increment in biomass (g)	Average feed consumed	Food conversion ratio	Mean ± SD
T_1	T_{11}	0.0664	0.3102	0.2438	1.1681	4.791	4.417 ± 0.297
	T_{12}	0.0683	0.3222	0.2539	1.1160	4.395	
	T_{13}	0.0673	0.3195	0.2522	1.025	4.064	
T_2	T_{21}	0.0633	0.3513	0.2880	1.1247	3.905	3.887 ± 0.042
	T_{22}	0.0659	0.3660	0.3001	1.1491	3.829	
	T_{23}	0.0625	0.3488	0.2863	1.124	3.926	
T_3	T_{31}	0.0668	0.3941	0.3273	1.1200	3.422	3.553 ± 0.094
	T_{32}	0.0633	0.3707	0.3074	1.118	3.637	
	T_{33}	0.0678	0.4011	0.3333	1.2001	3.6007	
T_4	T_{41}	0.0662	0.3830	0.3168	1.1190	3.532	3.595 ± 0.0661
	T_{42}	0.0663	0.3650	0.2987	1.101	3.686	
	T_{43}	0.0658	0.3776	0.3118	1.112	3.566	
T_5	T_{51}	0.0675	0.3809	0.3134	1.204	3.842	3.719 ± 0.128
	T_{52}	0.0640	0.3629	0.2989	1.059	3.543	
	T_{53}	0.0657	0.3755	0.3098	1.169	3.773	
T_6	T_{61}	0.0648	0.3598	0.295	1.122	3.803	3.847 ± 0.068
	T_{62}	0.0640	0.3612	0.2972	1.128	3.795	
	T_{63}	0.0625	0.3501	0.2876	1.134	3.943	

Table 9 ANOVA of food conversion ratio

Source of variance	Sum of squares	Degrees of freedom	Mean sum of squares	F. value
Treatment	1.4750	5	0.295	9.5008*
Error	0.3726	12	0.03105	
Total	1.8476	17	0.1087	

*Significant at 5% level

Comparison of treatment means based on critical difference

Critical difference 0.4932

Treatment means $\underline{T_1}$ $\underline{T_2}$ $\underline{T_6}$ $\underline{T_5}$ $\underline{T_4}$ $\underline{T_3}$

Underscored means are not significantly different.

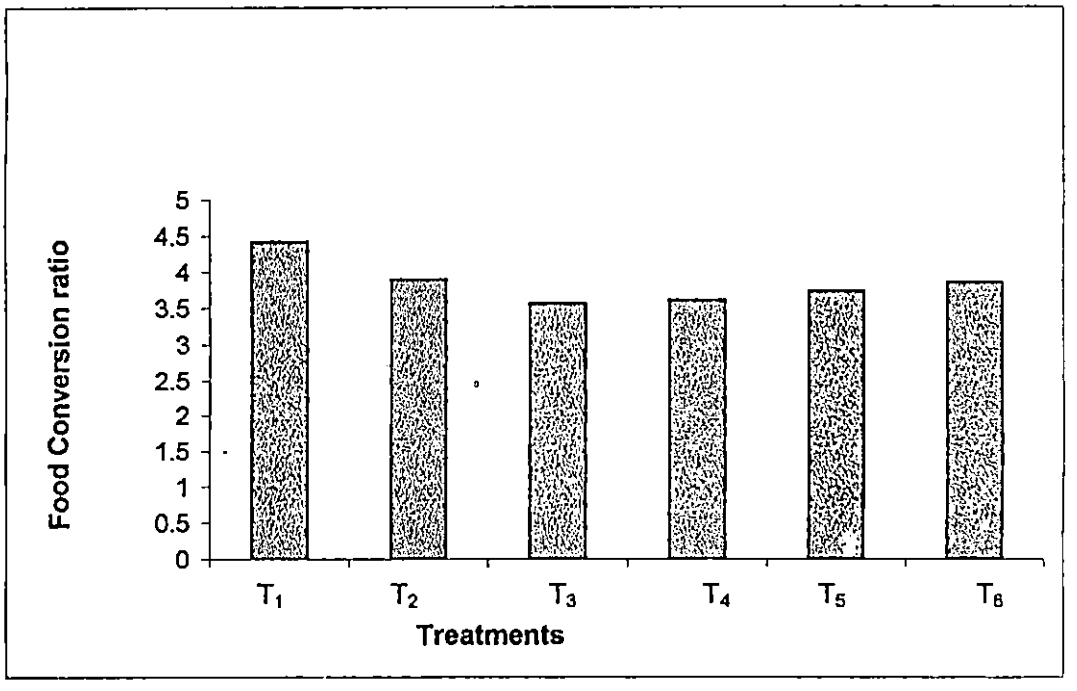

Fig. 2 Effect of dietary lipid levels on food conversion ratio of *M. rosenbergii* juveniles

Table 10 Food conversion efficiency of various treatments

Treatment	Replication	Food conversion efficiency	Mean ± SD
T_1	T_{11}	0.2087	0.2274 ± 0.0153
	T_{12}	0.2275	
	T_{13}	0.2461	
T_2	T_{21}	0.2561	0.2573 ± 0.0028
	T_{22}	0.2612	
	T_{23}	0.2547	
T_3	T_{31}	0.2922	0.2816 ± 0.0076
	T_{32}	0.2750	
	T_{33}	0.2777	
T_4	T_{41}	0.2831	0.2783 ± 0.0050
	T_{42}	0.2713	
	T_{43}	0.2804	
T_5	T_{51}	0.2603	0.2692 ± 0.0094
	T_{52}	0.2822	
	T_{53}	0.2650	
T_6	T_{61}	0.2630	0.2600 ± 0.0046
	T_{62}	0.2635	
	T_{63}	0.2536	

Table 11 ANOVA of food conversion efficiency of various feeds

Source of variance	Sum of squares	Degrees of freedom	Mean sum of squares	F. value
Treatment	0.005767	5	0.001153	10.4628*
Error	0.001323	12	0.0001102	
Total	0.007090	17		

*Significant at 5% level

Comparison of treatment means based on critical difference

Critical difference = 0.0294

Treatment means $\underline{T_1}$ $\underline{T_2\ T_6\ T_5\ T_4}$ $\underline{T_3}$

Underscored means are not significantly different.

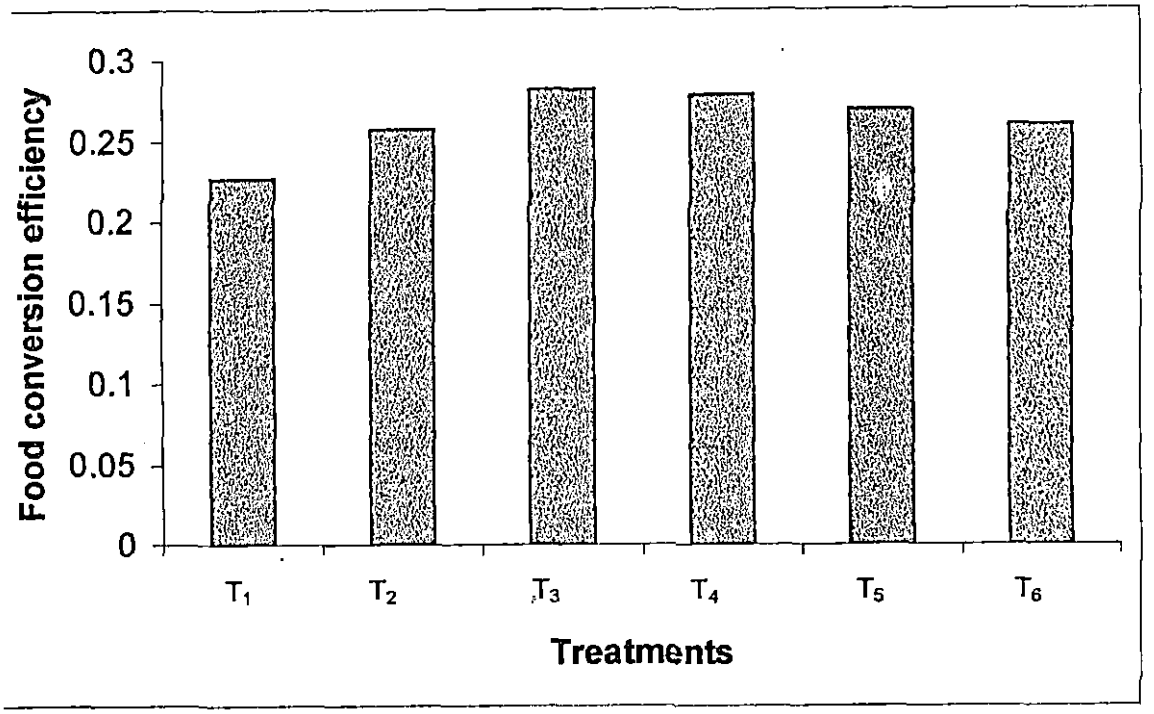

Fig. 3 Effect of dietary lipid levels on food conversion efficiency of *M. rosenbergii* juveniles

Table 12 Survival rate of different treatments

Treatment	Replication	Initial no.	Final no.	% survival	Mean ± SD
T_1	T_{11}	10	7	70	73.33 ± 4.71
	T_{12}	10	7	70	
	T_{13}	10	8	80	
T_2	T_{21}	10	7	70	80 ± 14.142
	T_{22}	10	7	70	
	T_{23}	10	10	100	
T_3	T_{31}	10	7	70	80 ± 14.142
	T_{32}	10	7	70	
	T_{33}	10	10	100	
T_4	T_{41}	10	5	50	76.667 ± 18.856
	T_{42}	10	9	90	
	T_{43}	10	9	90	
T_5	T_{51}	10	6	60	63.33 ± 4.714
	T_{52}	10	7	70	
	T_{53}	10	6	60	
T_6	T_{61}	10	6	60	53.33 ± 9.428
	T_{62}	10	6	60	
	T_{63}	10	4	40	

Table 13 ANOVA of percentage survival

Source of variance	Sum of squares	Degrees of freedom	Mean sum of squares	F. value
Treatment	1310.0468	5	262.0094	1.224
Error	2572.1356	12	214.3446	
Total	3882.1824	17		

No significant difference between the treatments.

Table 14 Protein efficiency ratio of different treatments

Treatment	Replication	Average weight gain (g)	Crude protein (g)	Protein efficiency ratio	Mean ± SD
T_1	T_{11}	0.2438	0.4212	0.5788	0.6307 ± 0.0423
	T_{12}	0.2539	0.4024	0.6310	
	T_{13}	0.2522	0.3696	0.6824	
T_2	T_{21}	0.2880	0.3974	0.7247	0.7283 ± 0.0079
	T_{22}	0.3001	0.4060	0.7392	
	T_{23}	0.2863	0.3971	0.7210	
T_3	T_{31}	0.3273	0.3932	0.8324	0.8022 ± 0.0216
	T_{32}	0.3074	0.3925	0.7832	
	T_{33}	0.3333	0.4213	0.7911	
T_4	T_{41}	0.3168	0.3857	0.8214	0.8073 ± 0.0147
	T_{42}	0.2987	0.3795	0.7871	
	T_{43}	0.3118	0.3833	0.8135	
T_5	T_{51}	0.3134	0.4106	0.7633	0.7894 ± 0.0277
	T_{52}	0.2989	0.3611	0.8277	
	T_{53}	0.3098	0.3986	0.7772	
T_6	T_{61}	0.295	0.3828	0.7706	0.762 ± 0.0132
	T_{62}	0.2972	0.3849	0.7721	
	T_{63}	0.2876	0.3869	0.7433	

Table 15 ANOVA of protein efficiency ratio

Source of variance	Sum of squares	Degrees of freedom	Mean sum of squares	F. value
Treatment	1.518	5	0.3036	346.9714*
Error	0.0105	12	0.000875	
Total	1.5281	17		

*Significant at 5% level

Comparison of treatment means based on critical difference

Critical difference = 0.083

Treatment means	$\overline{T_1}$	$\overline{T_2}$	$\overline{T_6}$	$\overline{T_5}$	$\overline{T_3}$	$\overline{T_4}$

Underscored means are not significantly different

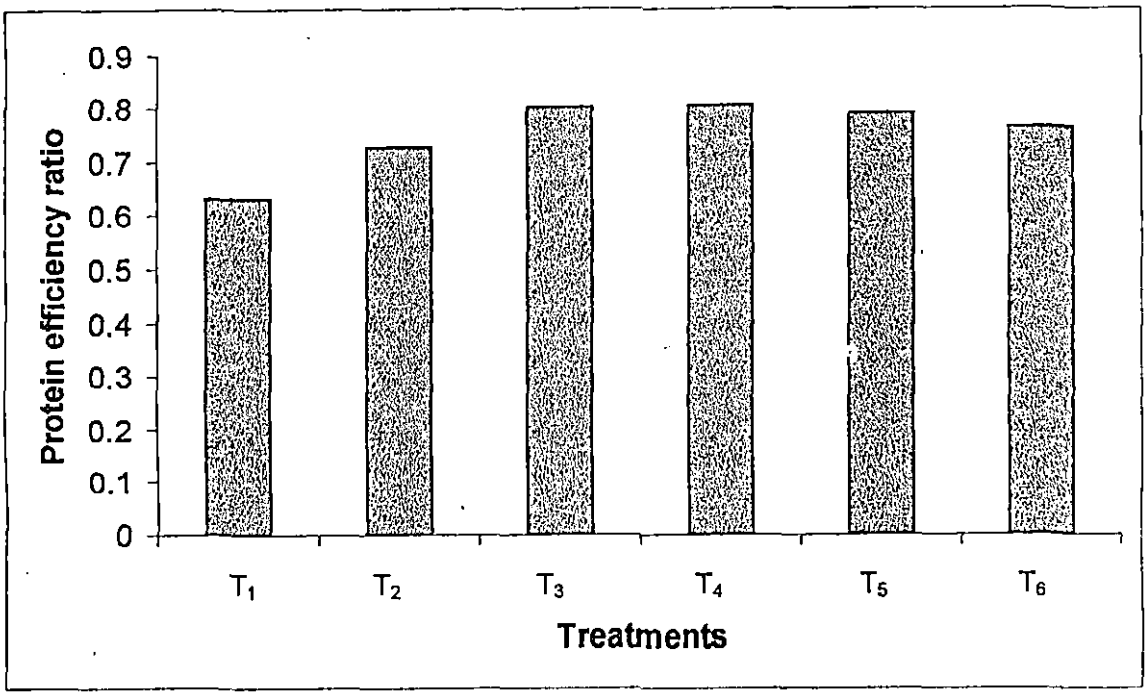

Fig. 4 Effect of dietary lipid levels on protein efficiency ratio of *M. rosenbergii* juveniles

Table 16 Net Protein Utilization of different treatments

Treatment	Replication	Protein retained (g)	Protein consumed (g)	NPU	Mean ± SD
T_1	T_{11}	0.543	0.4212	128.9174	133.4218
	T_{12}	0.541	0.4024	134.4433	±
	T_{13}	0.506	0.3696	136.9048	3.34
T_2	T_{21}	0.598	0.3974	150.4781	149.2127
	T_{22}	0.592	0.4060	145.8128	±
	T_{23}	0.601	0.3971	151.3473	2.43
T_3	T_{31}	1.9253	0.3932	489.6490	478.2058
	T_{32}	1.9523	0.3925	497.4013	±
	T_{33}	1.8856	0.4213	447.5671	21.89
T_4	T_{41}	1.8875	0.3857	489.370	491.9345
	T_{42}	1.8975	0.3795	500.00	±
	T_{43}	1.8645	0.3833	486.4336	5.83
T_5	T_{51}	1.795	0.4106	437.165	441.8661
	T_{52}	1.611	0.3611	446.1368	±
	T_{53}	1.763	0.3986	442.2980	3.68
T_6	T_{61}	1.552	0.3828	405.4337	404.931
	T_{62}	1.561	0.3849	405.5599	±
	T_{63}	1.5623	0.3869	403.7994	0.8

Table 17 ANOVA of Net Protein Utilization

Source of variance	Sum of squares	Degrees of freedom	Mean sum of squares	F. value
Treatment	405783.83	5	81156.766	596.8313*
Error	1631.7522	12	135.9794	
Total	407415.58	17		

*Significant at 5% level

Comparison of treatment means based on critical difference

Critical difference = 32.6386

Treatment means $\underline{T_1 \quad T_2}$ $\quad T_6 \quad T_5 \quad \underline{T_3 \quad T_4}$

Underscored means are not significantly different

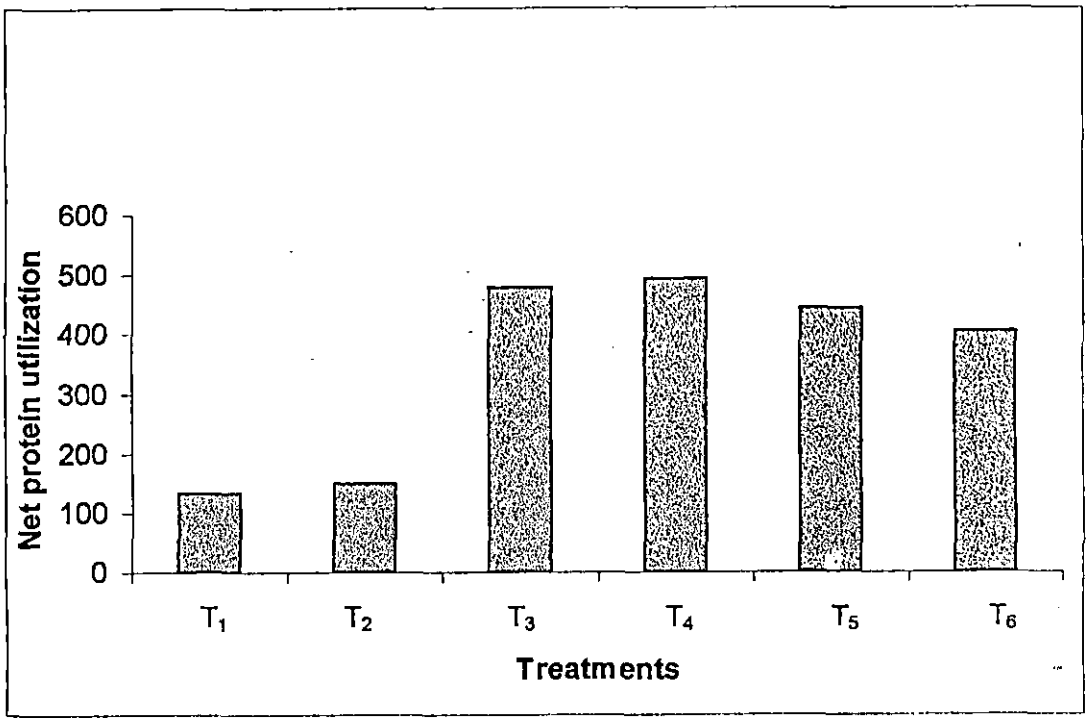

Fig. 5 Effect of dietary lipid levels on net protein utilization of *M. rosenbergii* juveniles

4.4 Carcass proximate composition

At the beginning and end of the experiment the prawns were subjected to biochemical analysis. Crude lipid, protein, ash and carbohydrate are expressed as percentage of dry body weight.

4.4.1 *Initial carcass proximate composition of test animals*

The carcass composition of the test animals were estimated and the corresponding data are given in Table 18. Moisture, protein, lipid, ash and carbohydrate levels were 78.7311%, 64.1233%, 1.7742%, 14.826% and 19.2762%, respectively.

4.4.2 Final carcass proximate composition of test animals

Final carcass proximate composition of juvenile *M. rosenbergii* were evaluated after the experimental period of sixty days and the data are given in Table:19.

From the data it is clear that moisture and carbohydrate of body of test animals were maximum for the control diet (T_1) and minimum for T_6 having 5% cuttlefish liver lipid. But crude fat content of body increased with increase of dietary lipid and was maximum for the treatment T6. While crude protein content of the body of test animal showed an increment with increase of dietary lipid level up to the test diets T_3 and T_4. Ash content did not show such steady variation with different levels of lipid in feed.

The result of analysis of variance (ANOVA) of moisture, crude protein, crude fat, ash and carbohydrates are given in Tables 20, 21, 22, 23 and 24 respectively. All these parameters showed significant variation among treatments. Graphical representation of percentage moisture, fat and protein content of body of test animals are given in Figure 6.

Table: 18 Initial carcass composition of juvenile *M. rosenbergii*

	Moisture %	crude protein %	crude fat %	ash (%)	carbohydrate %
Mean ±S.D	78.7311± 0.4617	64.1233± 0.0021	1.7742± 0.0038	14.826± 0.0256	19.2762± 0.0235

Dry weight basis

Table:19 Final carcass composition (Mean ± S.D)* of juvenile *M. rosenbergii*

Parameters (%)	T_1	T_2	T_3	T_4	T_5	T_6
Moisture ±S.D	82.144 ±0.0475	80.111 ±0.2157	77.652 ±0.2555	76.192 ±0.0646	74.148 ±0.1076	71.76 ±0.1062
Crude protein ±S.D	64.653 ±0.017	64.717 ±0.003	66.044 ±0.043	66.044 ±0.0274	65.813 ±0.0774	65.681 ±0.0045
Crude fat ±S.D	1.311 ±0.0078	1.512 ±0.013	1.854 ±0.0312	2.672 ±0.0849	2.842 ±0.0142	3.265 ±0.0123
Ash ±S.D	13.31 ±0.0037	13.058 ±0.0553	12.404 ±0.0234	13.182 ±0.0703	13.187 ±0.0463	13.027 ±0.0698
Carbohydrate ±S.D	20.726 ±0.0729	20.713 ±0.02639	19.698 ±0.2158	18.168 ±0.0249	18.158 ±0.0297	18.027 ±0.0293

*Average of these replications expressed as dry weight basis.

Result of analysis of variance (ANOVA) of the data on final carcass composition of juvenile *M. rosenbergii* fed with various diets having different levels of lipid.

Table : 20 ANOVA of moisture content of body

Source of variance	Sum of squares	Degrees of freedom	Mean sum of squares	F. value
Treatment	218.3542	5	43.67083	1237.8939*
Error	0.42334	12	0.035278	
Total	218.7775	17		

*Significant at 5% level.

Comparison of treatment means for moisture based on critical difference

Critical Difference = *0.525711*

Treatment means $\overline{T_6}$ $\overline{T_5}$ $\overline{T_4}$ $\overline{T_3}$ $\overline{T_2}$ $\overline{T_1}$

Table 21 ANOVA of crude protein content of body

Source of variance	Sum of squares	Degrees of freedom	Mean sum of squares	F. value
Treatment	6.16076	5	1.232152	561.5762*
Error	0.026806	12	0.002234	
Total	6.187567	17		

*Significant at 5% level

Comparison of treatment means for crude protein based on critical difference
critical difference = 0.13293

Treatment means $\underline{\bar{T}_1 \quad \bar{T}_2} \quad \underline{\bar{T}_6 \quad \bar{T}_5} \quad \underline{\bar{T}_4 \quad \bar{T}_3}$

Underscored means are not significantly different

Table 22 ANOVA of crude fat content of body

Source of variance	Sum of squares	Degrees of freedom	Mean sum of squares	F. value
Treatment	9.366116	5	1.873223	854.3345*
Error	0.0026311	12	0.002193	
Total	9.392428	17		

*Significant at 5% level

Comparison of treatment means for crude fat based on critical difference .

Critical difference = 0.13107

Treatment means $\bar{T}_1 \quad \bar{T}_2 \quad \bar{T}_3 \quad \bar{T}_4 \quad \bar{T}_5 \quad \bar{T}_6$

Treatments are significantly different.

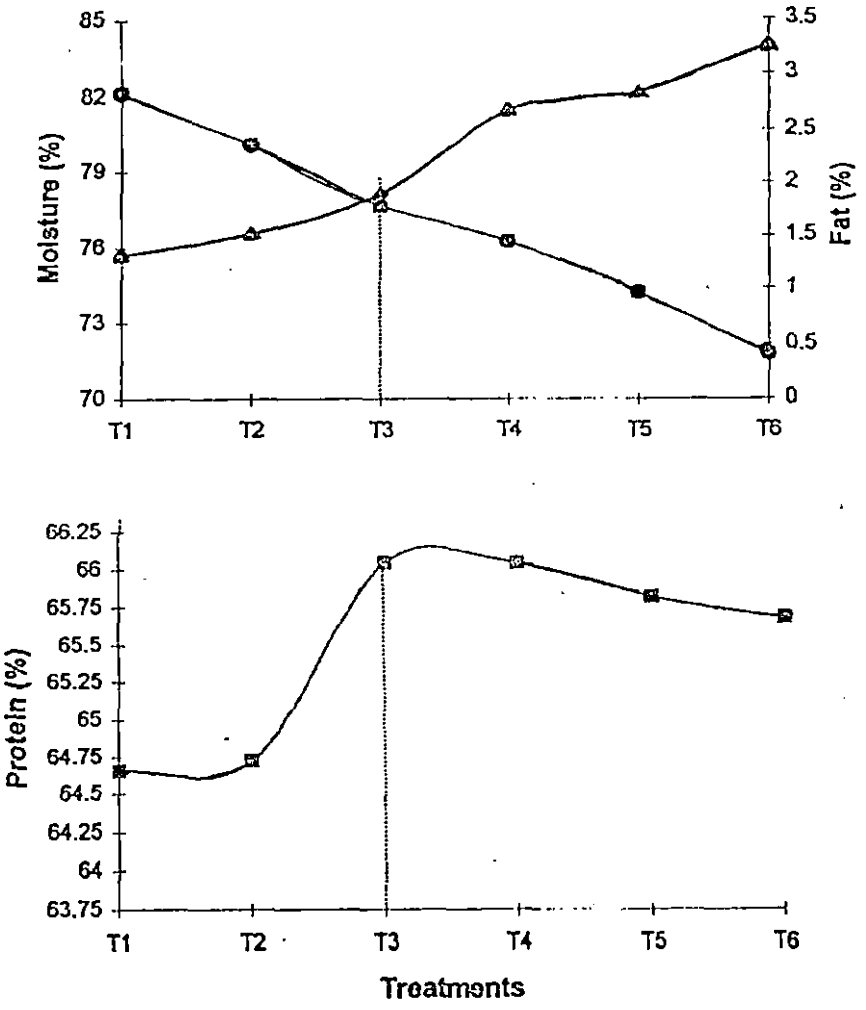

Figure 6 Effect of dietary lipid level on body composition of juvenile *M. rosenbergii*

Fat Moisture

Table 23 ANOVA of Ash content of body

Source of variance	Sum of squares	Degrees of freedom	Mean sum of squares	F. value
Treatment	1.603618	5	0.320724	82.34591*
Error	0.046738	12	0.003895	
Total	1.650356	17		

*Significant at 5% level

Comparison of treatments for ash based on critical difference

Critical difference = 0.17468

Treatment means $\underline{T_3 \quad T_6 \quad T_2 \quad T_4 \quad T_5} \quad \underline{T_1}$

Underscored means are not significantly different.

Table 24 ANOVA of carbohydrate content of body

Source of variance	Sum of squares	Degrees of freedom	Mean sum of squares	F. value
Treatment	25.1361	5	5.02722	162.3337*
Error	0.371621	12	0.030968	
Total	25.50772	17		

*Significant at 5% level

Comparison of treatment means based on critical difference

Critical difference = 0.49255

Treatment means $\underline{T_6 \quad T_5 \quad T_4} \quad \underline{T_3 \quad T_2} \quad \underline{T_1}$

Underscored means are not significantly different.

4.5 Water quality parameters

The mean and range of various water quality parameters are given in Table 25.

4.5.1 Temperature

Maximum and minimum temperature recorded during the experimental period were 26.1 and 30.3°C, respectively.

4.5.2 pH

pH of water was almost uniform in all tanks and varied from 7.0 – 8.2 during the experimental period.

4.5.3 Dissolved oxygen:

The maximum value of dissolved oxygen recorded was 8.5 mg/l and minimum 7.0 mg./l.

Table 25 Water quality parameters in the experimental tanks during the study period

Parameters		Weeks							
		1	2	3	4	5	6	7	8
	Mean	26.8	28	27.9	26.1	28.6	29	30.3	29.5
Temp	Range	26.7 - 26.9	27.6 - 28.4	27.7 - 28.1	26 - 26.2	28.3 - 28.9	28.8 - 29.2	30 - 30.6	29.3 - 29.7
pH	Mean	7.2	7.8	8.1	7	8.2	8	7.9	8.2
	Range	7 – 7.4	7.6 – 8.0	8 – 8.3	6.9 – 7.1	8 – 8.3	7.9 – 8.1	7.8 – 8	8 – 8.4
D.O.	Mean	8	7.6	7.8	8.5	7.4	7.5	7	7.2
	Range	7.9 – 8.1	7.3 – 7.9	7.6 - 8	8.4 – 8.6	7.2 – 7.6	7.3 – 7.7	6.9 – 7.1	7 – 7.4

5. DISCUSSION

In crustacean nutrition lipids are the second largest component after protein and they have a variety of roles serving as a source of energy, as a source of essential fatty acids, sterols, phospholipids, as a component of biomembrane and as carrier of fat soluble vitamins (Teshima and Kanazawa, 1980a,b and Nichols *et al.*, 1998). The present study also revealed the essentiality of lipid in the diet of prawns and its effect on various factors such as growth, food conversion ratio, food conversion efficiency, survival rate, protein efficiency ratio, net protein utilization and body composition of the test animals. Addition of cuttlefish liver lipid to clam meat-based diet has got influence on growth, food utilization efficiency and body composition of *M. rosenbergii* juveniles.

5.1 Percentage weight gain

In the present experiment highest weight gain was recorded for the prawns fed on a diet containing 2% cuttlefish liver lipid and having a total lipid content of 9.85%. The total lipid levels in the diets varied from 7.53% to 13.41%. As the percentage lipid level increased, the percentage weight gain also increased and the maximum was observed at 9.85% total lipid with 2% as cuttlefish lipid. Thus, the present study reveals that the optimum level of lipid in the diet of *M. rosenbergii* is 9.85%. The growth rate of prawns fed with test diets varied significantly from control diet, while very low and high concentration of lipid in feed reduced the growth rate of prawns. Percentage weight gain obtained with the feed T_3 was 117.845% higher than that of control diet T_1.

While studying the effect of lipid on growth of shrimps and prawns many workers suggested a dietary lipid requirement varying from 3 – 12% of dry weight. Lipid requirement may vary according to the interrelationship between different classes of lipids, age and species of animals (New, 1976, Biddle, 1977 and D' Abramo, 1989). Joseph and William (1975) and Sandifer and Joseph (1976) reported maximum growth of *M. rosenbergii* when fed with a diet containing 3% shrimp head oil which is rich in ω-3 fatty acids. According to Biddle (1977) and Hilton *et al.*(1984) the optimum dietary requirement of lipid for freshwater prawn is > 10%.

Sheen and D' Abramo (1991) used cod liver oil and corn oil as a source of lipid in nutrition of *M. rosenbergii* and found that 6% lipid produced optimum growth for *M. rosenbergii*. They also reported that a dietary lipid level ranging from 2 – 10% under a wide range of dietary lipid : carbohydrate ratio appears to be satisfactory. Clifford and Brick (1979) found that protein utilization was better when enough amount of fat and carbohydrates were provided in the diet. Similar result was obtained in *P. indicus* larvae and post larvae when fed with 10% of lipid (Chandge and Raj, 1990).

In the present study, gain in weight was reduced when supplementation of cuttlefish liver lipid exceeded 2% (total lipid more than 9.85%) in diet of *M. rosenbergii*. Other studies have also indicated that high dietary lipid levels depressed crustacean weight gain (Castell and Covey, 1976, Davis and Robinson, 1986 and Sheen, 1997). This reduced weight gain might be due to insufficient lipid utilization particularly when other energy sources are available. Kanazawa *et al.* (1977a) reported poor growth with lipid free diet and maximum with 10% lipid (mixture of Pollack residual oil and soy oil), which agrees with the present observation of maximum growth for 9.85% and minimum for control diet without cuttlefish liver lipid. From the study it is clear that diet with graded level of lipid has significant effect on growth. Optimum level of lipid in diet is 2% of cuttlefish liver lipid with total lipid content of 9.85%.

The present study also reveals addition of 2% cuttlefish lipid to the control diet enhances growth, food conversion efficiency and net protein utilization in *M. rosenbergii* juveniles. This may indicate, as described earlier, the addition of 2% cuttlefish liver lipid to the control diet having a lipid 7.53% satisfies the optimum requirement of *M.rosenbergii* (9.85%) under the present set of conditions or this may be due to the presence of typical types of poly unsaturated fatty acids, sterols and phospholipids in cuttlefish liver lipid. The composition of cuttlefish and squid lipid are different from that of marine fish oil. Cuttlefish lipid is a rich source of 18:1, 20:1, 20:5, 22:6 fatty acids (Nair and Gopakumar, 1977). Effective utilization of lipids depends mainly on its composition. Besides essential fatty acids, adequate level of phospholipids and sterols present in the lipid may also influence the utilization of diet (Chandge and Raj, 1990). According to Kanazawa (1985) the type and content of essential fatty acids dominate the nutritive value of lipid. Kanazawa *et al.* (1985) reported that lipid components such as phospholipids and sterols are also equally important.

5.2 Food conversion ratio and food conversion efficiency

All the test diets (T_2 to T_6) with graded level of cuttlefish liver lipid showed the same effect but are significantly different from the control diet. Highest food conversion efficiency and least food conversion ratio were for the diet T_3 having 2% of cuttlefish liver lipid. This might be due to the optimum level of lipid in the diet which contributed to optimum food utilization. The present study, thus showed that the growth and utilization of feed were influenced by the lipid content of the diet.

The effect of dietary lipids and its level on growth and feed efficiency was studied in different species of prawns by different workers. Deshimaru *et al.* (1979) reported that a diet containing a mixture of Pollack liver oil and soy bean oil in a ratio of 3:1or 1:1 (both having a total lipid level of 6%) gives the highest growth and feed efficiency in *P. japonicus*. Chandge and Raj (1997)showed that food conversion ratio changes with lipid level in the diets of *P. indicus*. Tiwari and Sahu (1999) reported the effect of different levels of lecithin on food conversion ratio and food conversion efficiency of *M. rosenbergii* juveniles. Their results showed 5% soy lecithin supports maximum food conversion efficiency and minimum food conversion ratio. In the present study, further addition of cuttlefish liver lipid above 2% does not significantly affect food conversion ratio and food conversion efficiency. That means the addition of 2% cuttlefish liver lipid to a control diet having 7.53% lipid can meet the requirements of *M. rosenbergii* for essential fatty acids, sterols and phospholipids.

5.3 Survival rate

In the experiment, the percentage survival of different treatments varied between 53.33% and 80% which was not statistically different. This shows that a dietary lipid level more than 7.53% does not influence survival rate. This in turn indicates that there is no added benefit on survival rate by supplementing the control diet with cuttlefish liver lipid. Sandifer and Joseph (1976) and Sheen and D' Abramo (1991) reported similar results. They found that survival rate of *M. rosenbergii* was not influenced by level of lipid in diet. Tiwari and Sahu (1999) also found that survival rate of post larvae of *M. rosenbergii* was not affected by soy lecithin supplementation. Dietary lipid level ranging from 5.3 to 13.18% does not affect the survival rate of juvenile crab *Scylla serrata* (Sheen and Wu, 1999).

5.4 Protein efficiency ratio

The effect of adding different levels of cuttlefish liver lipid in the control diet of *M. rosenbergii* juveniles on protein efficiency ratio was also investigated. The investigation showed that addition of cuttlefish liver lipid enhances protein efficiency ratio. This enhancement in protein efficiency ratio was statistically significant. However protein efficiency values did not vary among the different test diets, though the mean values were higher for T_3 and T_4. This again indicates that the addition of 2% cuttlefish liver lipid to the control diet benefits the prawn with respect to protein efficiency ratio also.

Several investigators suggest that optimum protein levels depend upon a proper balance of lipid and carbohydrates sources. Tiwari and Sahu (1999) reported similar observations in *M. rosenbergii* fed with a diet containing graded levels of soy lecithin. They found increase of protein efficiency ratio with increase in levels of soy lecithin .It is thus clear from the present study that lipid at adequate levels will increase the protein efficiency ratio. The lipid content in T_3 and T_4 might have provided energy, essential fatty acids and phospholipids for the growth of prawns and increased protein sparing action. It was also found that adequate level of lipid will significantly spare the protein in fish (Watanabe, 1982).

5.5 Net protein utilization

The effect of cuttlefish liver lipid on net protein utilization of juvenile *M. rosenbergii* was studied. Result of the present experiment showed that the supplementation of cuttlefish liver lipid promoted net protein utilization. Net protein utilization values of control diet T_1 and diet with 1% cuttlefish liver lipid were statistically not different. Net protein utilization ratio was highest for 2%and 3% cuttlefish liver lipids and the values at these levels were not significantly different. This indicates that at 2% added cuttle fish liver lipid, the net protein utilization is optimum.

This can be explained on the basis of protein sparing action of lipid. Increase in level of lipid will act as an energy source during moulting. Hence more protein can be spared for growth. Protein utilization was found to be better when enough fat and carbohydrate were provided in the feed (Millikan *et al.*, 1980). Similar observation

was also found in the case of *M. rosenbergii* by Clifford and Brick (1979). They found that protein utilization was better when enough amount of fat and carbohydrates are provided in the diet. *P. indicus* larvae and post larvae require 10% lipids for proper utilization of protein and growth was found to be better at this level (Chandge and Raj, 1990). Chandge and Raj (1997) observed that *P. indicus* juvenile require 12% lipid for proper utilization of protein. Similar effect of dietary lipid in sparing protein has also been reported for fish diet by Watanabe (1982). According to him addition of lipid containing essential fatty acid help in effective utilization of dietary protein in fish.

5.6 Carcass composition of juvenile *M. rosenbergii*

Carcass proximate analyses of experimental animals were done. From the result it was clear that prawns fed with a diet containing optimum level of lipid showed proper utilization of feed and protein. The chemical composition of body of prawns is significantly influenced by the dietary lipid level.

5.6.1.Protein

The data of present study clearly indicate that for efficient protein synthesis lipid should be present in adequate level. The results showed that protein content of the test animals increased with increase of lipid level in diet and this reached a maximum at 2% cuttle fish liver lipid with a total lipid level of 9.85%. This supports the results obtained for protein efficiency ratio and net protein utilization.

Chandge and Raj (1997) reported steady increase in growth and protein content of prawns with increase in dietary lipid level. This may be due to the protein sparing action of dietary lipid which will help to increase the protein content of body. It is thus clear from the present study that optimum dietary lipid can spare protein in the body.

5.6.2 Lipid

Lipid content in the whole body of the prawns at the end of the feeding experiment were estimated and found that all the treatments were significantly different with the values ranging from 1.211% to 3.684% (dry weight). From the data

it is clear that the lipid content of body increased with increase of dietary lipid. Sheen and D' Abramo (1991) reported similar results of body fat ranging from 1.42 – 3.7% with increase of dietary lipid level. Poly unsaturated fatty acids are functionally incorporated and conserved in polar lipid of crustacean tissue (Sandifer and Joseph, 1976, Kanazawa *et al.,* 1977b, Clarke, 1979, D' Abramo *et al.,* 1980 and D' Abramo and Sheen, 1993). These results support present observation on body lipid content of *M. rosenbergii.* D' Abramo *et al.* (1980) found a higher lipid content in hepatopancrease and remaining body of juvenile lobster fed with a diet containing higher concentration of lipid. According to them the metabolism, deposition and transport of lipid in lobster are diet dependant. Sandifer and Joseph (1976) and Hilton *et al.* (1984) indicated that lipid content of tissue of *M. rosenbergii* increased with increasing dietary lipid. The present study also demonstrate a similar relationship between lipid content of diet and body fat. This lipid accumulation may be due to high calorie intake or due to imbalance in dietary fat.

5.6.3 Moisture

The effect of dietary lipid on moisture content of body showed significant variation between treatments. The moisture content of body is found to be decreased with increase of lipid in diet. Chandge and Raj (1997) reported high moisture content of tissue of prawn fed with a lipid free diet than those fed on a diet containing lipid. This might be due to the inverse relationship between fat and moisture content. Minimum moisture content of body of animal fed with a diet containing maximum lipid.

5.6.4 Carbohydrate

From the present experiment it was found that carbohydrate content of body of experimental animal was maximum for T_1 where the lipid content was less and minimum for the diet T_6. Similar observation was also reported by Chandge and Raj (1997).

From the combined graph (Fig.6) it is easy to assess the best diet having optimum dietary lipid for growth, food conversion efficiency, protein utilization

and ideal body composition of *M. rosenbergii*. Maximum body protein was observed for the animals, which were fed with the diet T_3. Hence it is clear that the test diet T_3 having 2% of cuttlefish liver lipid, which maximizes the protein deposition of body of test animals is the most suitable one. The moisture and crude fat contents of body of test animals were also at desirable levels for this treatment.

5.7 Water quality parameters

5.7.1 Temperature

Water temperature is an important parameter, which affects the growth and survival of aquatic organisms. According to New and Singholka (1985) *M. rosenbergii* can tolerate a wide range of temperature 18 – 34°C and optimal level is 29-31°C. Temperature below 18°C and above 33°C are lethal for freshwater prawns (Farmanfarmain and Moore, 1978). According to Barnabe (1990) optimum level of temperature of freshwater prawn is 25-31°C and can be adapted to temperature up to 20°C. In the present study the mean temperature of tanks observed was within the optimal range suggested for freshwater prawn.

5.7.2 pH

Malecha *et al.*(1980) and Sandifer and Smith (1985) found that high pH value adversely affected the growth of *M. rosenbergii*. Hsieh *et al.* (1989) and New and Singholka (1985) reported that optimum pH for the growth of *M. rosenbergii* is within the range of 7.0 –8.5. In the present study, water pH recorded was almost uniform in all the tanks and mean pH varied from 7.2 – 8.5, which were within the optimal level.

5.7.3 Dissolved oxygen

According to Barnabe (1990) for freshwater prawn the concentration of dissolved oxygen should be 5mg/l but it can temporarily withstand 3 mg /l. New and Singholka (1985) reported that optimal dissolved oxygen level in culture pond of *M. rosenbergii* was above 75% saturation. In the present study, weekly range of dissolved oxygen was always above 5 mg/l. Hence it was within the tolerable limit.

6. SUMMARY

Cuttlefish liver is a cheap source of lipid rich in polyunsaturated fatty acids, which is essential for the growth of freshwater prawn, *Macrobrachium rosenbergii.*

In the present study juveniles of *M. rosenbergii* having an average weight of 0.066g were used as experimental animals. The experiment was conducted in completely randomized design with six treatments having three replications each for a period of 60 days.

The control diet.(T_1) was not supplemented with cuttlefish liver lipid, while test diets(T_2 to T_6) were impregnated with graded levels of lipid derived from cuttlefish liver with an increment of 1%.

Water quality parameters were checked during the experiment and were found to be with in the tolerance limit for optimum growth of *M. rosenbergii.*

Various evaluation indices viz. percentage weight gain, food conversion ratio, food conversion efficiency, protein efficiency ration, net protein utilization, survival rate and carcass proximate composition of the test animals were determined.

Maximum growth, food conversion efficiency and protein utilization were obtained for the diet T_3 having 2% cuttlefish liver lipid (total lipid 9.85%).

From the study it is clear that formulated prawn feed with 2% cuttlefish liver lipid is a cheap and potentially balanced feed which promote good growth and protein efficiency ratio in *M. rosenbergii.*

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EFFECT OF CUTTLEFISH LIVER LIPID ON THE GROWTH OF *MACROBRACHIUM ROSENBERGII* (de Man) JUVENILES

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ABSTRACT OF THESIS

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ABSTRACT

Giant freshwater prawn is an important species suitable to culture in both fresh and low saline waters. For successful farming of freshwater prawns, nutritionally balanced cheap diet is a prime necessity. The present study was designed to develop a nutritionally balanced feed for freshwater prawn by using cuttlefish liver as a cheap source of lipid.

In this study juveniles of *Macrobrachium rosenbergii* were used for evaluating the effect of various levels of lipid on growth, food conversion ratio, survival rate, protein utilization and body composition. For this six experimental diets designated as T_1 to T_6 were prepared by using clam meat, groundnut oil cake, wheat bran, tapioca powder, vitamin mineral mixture and cellulose powder. Test diets (T_2 to T_6) were prepared by adding different levels (1 to 5%) of cuttlefish liver lipid with an increment of 1% by replacing equal amount of cellulose powder. Total lipid content of the diets T_1, T_2, T_3, T_4, T_5 and T_6 were 7.53%, 8.61%, 9.85%, 10.73%, 12.01% and 13.41%, respectively. Completely randomized design with six treatments each having three replications was used for analyzing the results. Ten numbers of juveniles were randomly distributed in each tank Feeding was done for a period of 60 days.

Test animals fed with a diet containing 2% of cuttlefish liver lipid with a total lipid content of 9.85% showed better growth, food conversion efficiency and protein utilization. But survival rate was not affected by the addition of cuttlefish liver lipid. The protein content of body of test animals was maximum for the treatment, T3 having 2% cuttlefish liver lipid. Lipid and moisture contents of the body were at desirable levels for this treatment. Hence, it is found that, the diet containing 2% of cuttlefish liver lipid with a total lipid of 9.85% is good for *M. rosenbergii.*