EVALUATION OF DIFFERENT PROTEIN SOURCES IN THE FORMULATED FEED FOR *MACROBRACHIUM ROSENBERGII* (De Man) JUVENILES

By
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THESIS

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CERTIFICATE

Certified that this thesis entitled **EFFECT OF DIFFERENT PROTEIN SOURCES IN THE FORMULATED FEED FOR *MACROBRACHIUM ROSENBERGII* (DE MAN) JUVENILES** is a record of research work done independently by Sri **ANILKUMAR P** under my guidance and supervision and that it has not previously formed ~~the~~ the basis for the award of any degree fellowship or associateship to him

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DECLARATION

I hereby declare that this thesis entitled **EFFECT OF DIFFERENT PROTEIN SOURCES IN THE FORMULATED FEED FOR *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 formed the basis for the award to me of any degree, diploma, associateship, or other similar title, of any other University or Society

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I INTRODUCTION

Freshwater prawns are well distributed throughout the tropical and subtropical zones There are over 100 species belonging to the genus *Macrobrachium* (New 1990) of which the giant freshwater prawn *Macrobrachium rosenbergii* is considered to be the most important from aquaculture point of view due to its fast growth rate, omnivorous feeding habit, successful reproduction in captivity, low protein requirement compared to penaeid shrimps and high consumer preference and market price Thus in recent years the culture of this species has received wide acceptance and popularity in many countries around the world making freshwater prawn farming as synonymous to the culture of *M rosenbergii*

The cost of feed is one of the items pushing up the operational cost of prawn farming, accounting for 40-60% of variable cost in intensive aquaculture enterprises This makes it highly desirable to have a close look into the nutrient requirements of the cultivable species so as to formulate feeds that can sustain good growth with maximum conversion efficiency Developing such feeds using locally available cheap ingredients and industrial wastes and by products, will reduce the cost of production in prawn farming Among the various ingredients, protein is the most expensive component in the formulated feeds and therefore, it is not surprising that more time effort and resources have been devoted to the investigations on protein nutrition of prawn than any other dietary components Formulation of a balanced feed containing low cost protein ingredients can bring down the cost of the supplementary feed to a great extent

In the present study an attempt has been made to evaluate the nutritional quality of certain locally available, comparatively less expensive sources of protein in the formulated feed of *M rosenbergii* such as clam meat, squid and shrimp head wastes of processing industry squilla and silkworm pupae The black clam, *Villorita cyprinoides* which is the main stay of clam fisheries in India accounting 64% of the total annual clam production (Narasimham 1991), is the most common feed traditionally used in shrimp farming and it is one of the cheap source of protein in our locality Squilla is another source of protein which is locally available abundantly and cheaply Large quantities of Squilla obtained as by catch in trawl fishing at present is not properly utilized Similarly, shrimp head which is produced as a waste by the processing industry in large quantities is available in plenty Squid and other cephalopod wastes are also available for use as ingredients for prawn feed The sericulture industry in India which is growing at a faster rate during the past few years has a lot of waste after silk extraction in the form of silkworm pupae which is not found to be used productively

Utilization of these waste products for feed formulation has dual advantages 1) forming valuable feed ingredients for the rapidly growing shrimp and prawn rearing enterprises of our country thereby helping to conserve our valuable foreign exchange spent at present for importing formulated feed and 2) helping to save the amount spent on waste disposal and/or solving environmental problems

Carbohydrates are the the most economical sources of dietary energy They appear to be well utilized by shrimps and feed containing relatively high levels of carbohydrate appear to have acceptable palatability and support good growth (Pascual *et al* , 1983, Catacutan,1991, Shiau *et al* 1991, Shiau and Peng, 1991) The giant freshwater prawn, *M rosenbergii* being omnivorous may be able to utilize carbohydrate for meeting its energy requirements so that costly protein can be spared for growth Thus by including sufficient carbohydrate in its diet, the level of protein can be minimized substantially However, the information on carbohydrate utilization and metabolism of prawn is scanty and require further studies

Limitations in our knowledge on nutrition of shrimp and prawn underscore the need to continuously develop, test and apply new nutritional concepts This is particularly true for the rapidly growing shrimp feed industry where feed formulations are presently based on assumptions and ' unknown growth factors ' rather than the science of nutrition

The present study in the giant freshwater prawn has been undertaken to evaluate the nutritional quality of certain locally available cheap protein sources, and to find the requirement of carbohydrate to know the level at which it can be utilized for energy metabolism, so as to spare the protein for achieving maximum growth

II REVIEW OF LITERATURE

2 1 Nutritional requirements of shrimps and prawns

The literature regarding nutritional requirements of shrimps and prawns has been reviewed by New (1976, 1980) Biddle (1977) Forster (1976), Sick and Millikin (1983) and Corbin *et al* (1983) Recently, practical manuals on the preparation of diets and feeding of prawn have been brought out by New and Singholka (1985) and New (1987 1988 and 1990), in which there are few examples of formulations of compounded diets

2 1 1 Protein

Protein is one of the most expensive components in animal feeds and therefore, it is not surprising that more time resources and effort have been devoted to investigations on protein requirement of prawn, than any other dietary component (Hanson and Goodwin, 1977) The source, dietary level and amino acid composition of proteins in relation to shrimp nutrition have received most attention from nutritionists so far

Since the early works of Subrahmanyam and Oppenheimer (1970) Kanazawa *et al* (1970) and Deshimaru and Shigueno (1972), numerous studies have been undertaken on growth rates and feed efficiencies of various crustacean species, fed with different levels of dietary protein Table 1 gives an account of the wide range of optimum dietary protein levels observed with special reference to the source of protein used

Table 1 Protein requirement of commercially important prawns and shrimps of aquaculture importance with special reference to the source of protein

Species	Protein source(s)	Suggested optimum protein level (%)	Reference
M rosenbergii	Fish meal Soybean meal Shrimp meal	35	Balazs *et al* (1973)
,	Soybean meal Tuna meal	35	Balazs & Ross (1976)
,,	Menhaden meal Soybean meal	35	Clifford & Brick (1979)
,	Menhaden meal Soy protein	40	Millikin *et al* (1980)
,,	Shrimp meal Fish meal Peanut meal Soy meal	15	Boonyaratpalin & New (1982)
,,	Fish meal Fish solubles Blood meal Cotton seed meal	25	Perry & Tarver (1987)
,,	____	14	Antiporda (1986)
,,	Casein	13 25	Gomez *et al* (1988)
,,	Crab protein	33 35	D Abramo & Reed (1988)
,,	Crab protein	30	Freuchtenicht *et al* (1988)

Table I contd

Species	Protein source(s) employed	Suggested optimum protein level (%)	Reference
P japonicus	Squid meal Mysid shrimp meal Brine shrimp meal Fish meal Gluten Whale meal, Casein Soy protein Activated Sludge Petroleum yeast	>60	Deshimaru & Shigueno (1972)
,	Fish meal Soybean meal Shrimp meal Brewer s yeast	40	Balazs *et al* (1973)
,	Casein, Egg albumen + Pure amino acids	50	Deshimaru & Kuroki (1974)
,,	Casein, Egg albumen	52 57	Deshimaru & Yone (1978)
,,	Casein	45 55	Teshima & Kanazawa (1984)
,,	Crab protein	42	Koshio *et al* (1993)
P monodon	Casein	35	Bages & Sloane (1981)
,	Fish meal Egg albumen	46	Lee (1971)
,,	Casein Squid meal Fish meal Shrimp meal Soybean meal	40	Alava & Lim (1983)
,,	Casein Gelatin	40 50	Bautista (1986)
,	Casein	40 44	Shiau *et al* (1991)
,	Casein	36 40	Shiau & Chou (1991)

Table 1 Contd

Species	Protein source(s) employed	Suggested optimum protein level (%)	Reference
P indicus	Prawn meal, Yeast	43	Colvin (1976)
,,	Mantis shrimp Ground nut oil cake	42 9	Ali (1982a)
,,	Egg albumen	25	Ali (1988)
,,	Casein	29	Ali (1988)
P merguiensis	Casein	50 55	AQUACOP (1978)
,,	Mussel meal	34 42	Sedgwick (1979)
P vannamei	Shrimp meal, Fish meal, Soybean meal	30 35	Colvin & Brand (1977)
P stylirostris	Shrimp meal Fish meal, Yeast	30 35	Colvin & Brand (1977)
P setiferus	Menhaden meal	28 32	Andrews *et al* (1972)
P aztecus	________	23 31	Shewbart *et al* (1973)
,,	Shrimp meal, Fish meal, Soy meal	25	Balazs *et al* (1973)
,,	Fish protein	40	Venkataramiah *et al* (1975)
,,	Squid mantle meal	36 5	Fenucci & Zein Eldin(1976)
P duorarum	Soybean meal	28 30	Sick & Andrews (1973)
P californiensis	Shrimp meal Menhaden meal Soybean meal	30 35	Brand & Colvin (1977)

Based on the information presented in the table 1 it would appear that, there is still uncertainty as to the quantitative dietary protein requirements of shrimps and prawns. At one extreme there are those studies that suggest increased growth rates and feed efficiencies with increased protein levels even at levels in excess of 60% of the diet. At the other extreme, there are studies suggesting that optimum dietary protein levels are more in the range of 20 to 30%. In this latter category, several of the reports show no additional growth response at higher levels and indicate decreased growth rates or feed efficiencies when protein exceeds 40% of the diet. The wide range in optimum protein requirement noted in these studies, could mainly be attributed to the widely varying protein sources employed (New, 1976; Biddle, 1977., Corbin *et al.*, 1983; Chen, 1993), the stage of experimental animals used (New, 1976) and the variations in the experimental procedures followed, including unstandardized way of expressing the proximate composition of test diets.

Non-protein energy is yet another factor that influence the optimum protein requirement. The combination of high dietary proteins in the presence of low levels of non-protein energy can force the crustacean to deaminate significant portion of the protein, thus yielding the carbon fragments required for cellular energy metabolism (Hanson and Goodwin, 1977).

In his review on research in shrimp nutrition, New (1976) has pointed out the lack of standardization in experimental design, culture conditions and analytical techniques, which had limited the value of published information and made comparison of research results from different laboratories difficult or impossible.

It can be seen that even for a single species of shrimp there is a wide variation in the reported protein requirement. For instance, the reported level for *P. japonicus* ranges from 42 to over 60%. Deshimaru and Shigueno (1972) reported the optimum protein requirement of this species as over 60%, which seems to be too high when compared to that of other penaeid shrimps. The reason for this higher requirement has been attributed to be their carnivorous feeding habit (Deshimaru and Kuroki, 1974; Deshimaru and Yone, 1978). All the investigations, after the pioneering work on the protein requirement of *P. japonicus* by Deshimaru and Shigueno (1972), have reported the quantitative requirement of protein as below 60% (vide table 1). Balazs *et al.* (1973) reported protein requirement of this species as more than or equal to 40%, which was the highest level tested in their experiment; while Deshimaru and Kuroki (1974) found the optimum as 50% in the same species. On the other hand, Deshimaru and Yone (1978) recommended the optimum level of protein as 52 - 57% for *P. japonicus*. Teshima and Kanazawa (1984) who had conducted trials using purified diets with carragenan as binder on *P. japonicus* post-larvae found levels of protein around 45, 45-55 and 55% or more as optimum when diets contained carbohydrate at 25, 15 and 5% levels respectively. Very recently, Koshio *et al.* (1993) reported maximum growth of *P. japonicus* at a level of 42% dietary protein in the diet.

The variation in optimum protein requirement of *P. japonicus* in many cases can be traced down to the source of protein employed by various workers. The higher values were obtained when poor quality protein sources were used; for instance casein. According to New (1976) diets based on casein have generally produced poor result with shrimp. Penaflorida (1989) opined that arginine, an essential amino acid is deficient in casein. Arginine is required by all crustaceans primarily due to the lack of urea cycle which is necessary for arginine biosynthesis from ornithine (Miyajima *et al.*, 1977). When casein was used as protein source for juvenile lobsters, poor growth and survival associated with 'moult death' syndrome were experienced (Bowser and Rossenmark, 1981).

In a recent report by Koshio *et al.* (1993), wherein crab protein was used for *P. japonicus* an optimum protein requirement of 42% was obtained. Crab protein appears to be better utilized by crustaceans than many commercially available protein and protein extracted from many marine organisms tested (Bogen *et al.*, 1982). Crab protein has been later selected for the preparation of standard reference diet (SRD) for crustacean nutrition studies - HFX CRD 84 (Halifax crab protein reference diet). The use of crab protein as protein source could be the possible reason for the lower optimum protein requirement (42%) reported by Koshio *et al.* (1993) compared to many works suggesting it as over 50% and in some cases as over 60%.

In general freshwater prawns (*Macrobrachium* spp.) require less protein in their diet, nearly a third of that required for penaeids (Pandian, 1989). In the case of giant freshwater prawn, *M. rosenbergii*, the optimum protein requirement suggested by various workers ranges from 13 - 40% which is a very wide range.

A critical analysis of available information of protein requirement on *M. rosenbergii* presented in table 1 reveals that, most of the workers put it in the range of 13-25%, while Millikin *et al.* (1980) reported the optimum as high as 40%. On the other hand, others such as Balazs *et al.* 1973; Balazs and Ross (1976); D'Abromo and Reed (1988) and Freuchtenicht *et al.* (1988) have observed the optimum protein requirement as between 30 and 35%.

The reasons for this unusually wide variation in the reported values of optimum protein requirement of *M. rosenbergii* can be attributed to the variation in protein source used. Among the various reports the lowest level of protein requirement for *M. rosenbergii* is the one suggested by Gomez *et al.*

(1988) which is 13-25% using a casein based diet. Gomez *et al.* (1988) appear to have used a moist diet with moisture content in the range of 45.4 - 49.4% and reported the optimum protein requirement on as fed basis, rather than following the standard way of expressing proximate composition in dry matter basis. By applying the formula suggested by New (1987), the expression on as fed basis can be converted to dry matter basis. By doing so, it could be found that the optimum protein requirement observed by Gomez *et al.* (1988) is in the range of 23.85 - 47.76% on a dry matter basis. The best growth response was observed in the upper limit of the range (ie, 47.76%) suggested by Gomez *et al.*(1988). This high requirement of protein could possibly be due to the use of casein as the protein source.

However, Boonyaratpalin and New (1980) evaluated the effect of three protein levels (15, 25 and 35%) on *M. rosenbergii* reared in outdoor concrete ponds and suggested 15% as the desirable level of protein from economic point of view, as there was not much variation in the performance of the diets differing in protein levels tested. Similarly, Antiporda (1988) reported that 14% protein level could sustain good growth in *M. rosenbergii* and Barlett and Enkerlin (1983) have produced satisfactory growth of this species, in asbestos, asphalt or polythene covered earthern ponds at a protein level as low as 14%. All these studies emphasize the importance of natural food in prawn rearing as suggested by New (1990).

Among the various works on protein requirement of *M. rosenbergii*, the highest level is being reported by Millikin *et al.* (1980) using fish meal and soybean meal protein sources. But according to Deshimaru and Shigueno (1972) predominance of fish meal in prawn feed never brought good results because it is low in basic amino acids (arginine, histidine and lysine) and phenyl alanine. Colvin (1976) who got a similar observation explained that fish meal is deficient in amino acids like tyrosine and phenyl alanine. Ali (1982a), while experimenting with *P. indicus* observed lowest growth response with fish meal in the diet compared to mantis shrimp, clam meal and prawn meal. Later, Sherif (1989) also reported that fish meal based diets are inferior to clam based diet for *M. rosenbergii*. These reports suggest that fish meal is deficient in certain amino acids and that could be the possible reason for this elevated protein requirement observed by Millikin *et al.* (1980)for *M. rosenbergii* (40%). Millikin *et al.* (1980) based on their experiment pointed out that this protein requirement of 40% could be lowered, if the amino acid balance of protein sources is adjusted. Balazs *et al.* (1973) suggested that a dietary protein concentration in excess of 35% might be required for maximum growth rate among juvenile *M. rosenbergii*. The protein source used seems to have a profound influence on the protein requirement, as in that study it was found that an all vegetable diet gave better result compared to a fish meal- soybean meal diet, but inferior to a fish meal-soybean meal- shrimp meal diet, at 35% protein level. This experiment also suggests that fish meal is an inferior source of protein but the use of shrimp meal along with fish meal could ameliorate the deficiency in certain amino acids particularly the basic amino acids.

Using crab protein which was found to be superior for crustaceans by Bogen *et al.* (1982) and later selected for the preparation of standard reference diets for crustacean nutrition studies, D'Abramo and Reed (1988) and Freuchtenicht *et al.*(1988) reported the optimum protein requirement of *M. rosenbergii* juveniles as 33-35% and 30% respectively.

A critical evaluation of the results of various experiments reveals the importance of evaluation of the quality of protein sources in the formulated feed of prawn, as the protein requirement is dependent on the source of protein. In this regard, the essential amino acids of prawn and the similarity of amino acid profile of prawn with protein source also gain importance.

2.1.2 Essential amino acid

Protein requirement is significantly affected by the quality of dietary protein which in turn is dependent on the level, balance and bio-availability of essential amino acids. The essential dietary amino acids for shrimps and prawns are quantitatively similar to those for other animals (New, 1976).

~~quantitatively similar to those for other animals (New, 1976).~~ Cowey and Forster (1971), Shewbart *et al.*(1972) and Kanazawa and Teshima (1981) have showed that arginine, histidine, isoleucine, leucine, lysine, methionine, phenyl alanine, threonine, tryptophan and valine are essential for *Palaemon serratus*, *Penaeus aztecus* and *P. japonicus* respectively. Similar results were obtained for *Macrobrachium ohione* by Miyajima *et al.*(1977), except that no data was obtained for tryptophan. According to the authors, acid hydrolysis used in the experiment presumably destroyed all tryptophan and opined that tryptophan is probably essential for *M. ohione*. Torres (1973), using fluctuations observed between values in the free amino acid pool of *P. kerathurus* found only eight amino acids as essential viz, histidine, isoleucine, lysine, methionine, phenyl alanine, threonine and valine; interestingly, arginine was not found to be essential in this study. According to Miyajima *et al.*(1977), all crustaceans require arginine primarily due to the lack of urea cycle in crustaceans which is necessary for arginine biosynthesis from ornithine.

In *M. rosenbergii*, Watanabe (1975) reported an essentiality for tyrosine in addition to arginine, histidine, isoleucine, methionine, phenyl alanine, tryphtophan and valine. A conspicuous variation in *M. rosenbergii* is the apparent nonessentiality for lysine, which has been reported to be essential for all other animals studied so far (Watanabe, 1975). In his review on prawn and shrimp nutrition, New (1976) has pointed out that this apparent non-essentiality for lysine obsered by Watanabe (1975) was probably due to analytical difficulties. This observation was further evaluated by Stahl and Aheran (1978) using purified amino acid test diets and reported that lysine is not essential for the growth and survival of *M. rosenbergii*. The difference in growth in terms of percentage increment in length of prawns receiving various levels of amino acid(0, 0.7, 1.4 and 2.8 %) for a period of 30 days was found to be statistically not significant. The authors also investigated the essentiality of arginine, histidine and tryphtophan which were already reported to be essential for *M. rosenbergii* and found all these three amino acids as being nonessential. But the authors while giving these results exercised some caution by stating gut and / or tank bacteria may have supported the prawn with amino acids in amount sufficient for growth. Biddle (1977) also cautioned drawing conclusion on essentiality of amino acids on the basis of short term growth studies before considering pre-nutritional status of the prawn, likely existence of nutrient reserves in the body of the prawn, the ability of the prawn to preferentially conserve a specific nutrient that is deficient in diet and the possibility of adequate quantity of the amino acids made available for absorption from microbial synthesis in the gut of the prawn.

Certain amino acids could be spared by other amino acids which are summarized by Paulraj (1993). For instance, methionine could be spared by cystine, thereby the level of cystine in the feed should be considered while adjusting the methionine level in the feed (Paulraj, 1993). Phenyl alanine is spared by tyrosine in fish, but in prawn, phenyl alanine is converted into tyrosine by an irreversible pathway (Paulraj, 1993). So adequate level of phenyl alanine must be ensured in the feed of prawn.

Bio-availability of amino acid is yet another factor important while considering the amino acid requirement in the feed. Methionine may undergo oxidation during processing of ingredients and feed, to methionine sulphoxide or methionine sulphone and the bio-availability of these compounds to fish and prawn is largely unknown (Paulraj, 1993). According to Halver (1980), methionine sulphoxide may have some biological value for fishes which have limited capacity to reconvert it to methionine and thus particularly make up for some of the methionine oxidized during processing. It is therefore, essential to determine the level of methionine after preparing the feed to have an idea about the available form of this amino acid in the feed after processing. Lysine is a basic amino acid having two amino groups. The second (epsilon) amino group must be free and reactive for effective utilization of lysine by prawn (Paulraj, 1993). During the manufacture of feed, the second amino group may react with non-protein molecules present in the feed ingredients to form compounds that render the lysine biologically unavailable So, the biologically available lysine in finished feed is more important than ch micaliy measurable lysine in feed ingredients

Reports on the quantitative amino acid requirement of shrimps and prawns are scanty (New, 1976, Biddle, 1977, Hanson and Goodwin, 1977 and Corbin *et al* ,1983) This is mainly due to the problems associated with the use of pure amino acids in the test diets of shrimps and prawns Deshimaru and Kuroki (1975) and Deshimaru (1982) have showed that diets containing only amino acids instead of protein brought about a poor growth and high mortality in feeding trials of *P japonicus*

Based on their study of amino acid supplementation to the diets of *M rosenbergii*, Stahl and Aheran (1978) suggested that searching for optimum amino acid levels for incorporation into commercial feeds may be less important than it was earlier thought Thus evaluation of quality of various protein sources which are found to be suitable for incorporation into practical diets, based on growth studies gain importance, rather than trying to find quantitative amino acid requirement and balanc ing their level in the commercial feed of shrimps and prawns

2 1 3 Protein sources

Fish meal is one of the most common ingredients used in the commercial shrimp feed possibly due to its large scale availability The proportion of fish meal in commercial feed ranges between 10 and 40% (Akiyama *et al* 1992) In view of the hıgh cost of good qualıty fısh meal of relatıvely constant chemical composıtıon, ıt is not surprısıng that feed cost account for 40 60% of total operatıng cost ın intensıve aqua culture enterprıses (ADCP 1983) Clearly, alternatıve and ıdeally less expensıve proteın sources of good qualıty must be found (Tacon and Jackson, 1985)

Approaches employed for partıal or total replacement of fish meal ın commercıal aquafeeds fall ınto two broad catego rıes, namely the use of conventıonal feed ıngredıents havıng hıgher proteın levels and the use of a new generation of uncon ventıonal high proteın feed ıngredıents

Based on orıgın, proteın sources used for compounding feeds can be dıvıded ınto plant protein sources and anımal protein sources

2 1 3 1 Plant Proteın sources

Accordıng to Tacon and Jackson (1985) apart from amıno acıd profıles whıch are often ımbalanced, endogenous antı nutrıtıonal factors also create problems lımıtıng the use of plant feed stuffs ın aquafeeds at hıgh levels Feed stuffs of plant orıgın are, as a whole, lower ın proteın content compared to those of anımal orıgın In addıtion the presence of hıgh amount of carbohydrate, fıbre and other molecules such as glucosıdes, phytates and cyclopropanes ın these sources present the nutrıtıonıst wıth problems that are generally not encoun- tered wıth sources of anımal orıgın (Spınellı *et al* , 1979)

According to Hanson and Goodwin (1977) feeds of plant origin alone seem not to result, acceptable growth and survival in prawns Most of the plant proteins have shown to yield poor growth rates in prawns when used individually excepting a few like soybean meal (Kanazawa *et al* 1970 Sick and Andrews 1973 Millikin *et al* , 1980, Fernandez and Lawrence 1988, Akiyama *et al* 1988 and Akiyama 1989) wheat gluten (Forster and Gabbot 1971 and Deshimaru and Shigueno, 1972) and peanut meal (Lee 1971 Sick *et al* 1972 Forster and Beard, 1973 Balazs *et al* , 1973 and Gopal, 1986)

The improved growth rates produced by some of the plant sources have been attributed to their high polysaccharide content compared to monosaccharide (Forster and Gabbot, 1971, Kitabayashi *et al* , 1971 Andrews *et al* , 1972 and Sick and Andrews, 1973) Deshimaru and Shigueno (1972) reported that, soybean meal, though a protein of plant origin, has an amino acid profile quite similar to that of prawns Venkataramiah *et al* (1975) showed that plant material is an essential part of prawn diet and found that it specifically improves feed conversion efficiency and survival in brown shrimp, *P aztecus* Since the animal protein sources are relatively expensive, protein sources of plant origin have been used by many workers in the shrimp feeds in an attempt to minimize the cost of production

2 1 3 2 Animal protein sources

A variety of animal protein sources and the substitution effect of one source with another in the formulated feeds of many species of shrimps and prawns, have been evaluated by various investigators with varying degrees of success

2 1 3 2 1 Fish meal

Fish meal made from good quality whole fish that is properly processed is the best quality protein source commonly available to aquafeed manufacturers Fish meal made from whole fish contains 60 80% protein It is also a rich source of energy and minerals and is highly digestible and palatable for most of the species It is high in available lysine and methio nine the two amino acids most deficient in plant feed stuffs (Lovel, 1989) Marine fish meal contains 1 1 25% n-3 fatty acids, which are essential for most of the species Fish meal made from wastes of fish processing and canning plants is lower in quality and quantity in respect of protein

Because of its high cost fish meal is generally used only in limited quantities in commercial feeds The dependence of fish meal may cause economic constraints in some areas, especially in developing countries, where it is less abundant, is of lower quality and expensive too Also in the more devel oped countries, difficulties are sometimes experienced due to fluctuations in fish meal supply and price, when supply is short (Lovel, 1989)

Generally, the essential amino acid profile of the protein of the animal s body closely approximates its dietary requirement The fish meal normally does not provide all essential amino acids to the required level and is found to be generally poor in threonine, phenyl alanine arginine and histidine (Lovel, 1989)

Fish meal a high quality protein source for finfishes seems to have lower nutritional value for shrimps and prawns, especially when used as a sole protein source This has been reported for *P duorarum* (Sick and Andrews, 1973), *P japonicus* (Deshimaru and Shigueno,1972) *P indicus* (Colvin, 1976) *P monodon* (Pascual and Destajo, 1979), *Palaemon serratus* (Forster and Beard, 1973) and *M rosenbergii* (Sherif, 1989) Deshimaru and Shigueno (1972) attributed this to the shortage of phenyl alanine and the basic amino acids (arginine histidine and lysine) in fish meal

Deshimaru and Shigueno (1972) found that diets prepared with fish meal were inferior to short necked clam for *P japonicus* and attributed it to the different amino acid profile of fish meal when compared to the species tested Colvin (1976) while working on *P indicus* got similar observation and explained that fish meal is deficient in amino acids like tyrosine and phenyl alanine Ali (1982a) while experimenting on *P indicus* observed lowest growth response with fish meal in the diet compared to mantis shrimp clam meal prawn meal and the control fresh clam meat Sick and Beaty (1975), while comparing different protein sources for *M rosenbergii* obtained poor weight gain with fish meal, lower even compared to a plant protein source, soybean meal The apparent protein digestibili ty of fish meal was found to be less than that of soybean meal in the case of *P vannamei* (Akiyama *et al* 1988) Sherif (1989) also reported poor performance of fish meal compared to clam meat in the diet of *M rosenbergii*

The observed poor performance of fish meal in shrimp and prawn feeds may be related to its amino acid profile Fish meal is lower in basic amino acids which are apparently essential for shrimps (Deshimaru and Shigueno ,1972 New 1976, Brand and Colvin 1977 and Colvin and Brand 1977) and /or due to an imbal ance in mineral content (Colvin and Brand 1977)

On the other hand Sick and Andrews (1973) working on *P duorarum* compared the dietary value of menhaden meal with other protein sources and found menhaden meal as superior to shrimp meal casein and maize gluten meal, but inferior to soybean meal

The freshness of raw material and the method of drying affect the quality of fish meal Wood *et al* (1991) have pointed out the quality problems of Indian fish meal Most of Indian fish meal is made by sundrying and grinding Due to the poor handling and processing the Indian indigenous fish meal is of low quality with less protein content of about 45% compared to 65% or more of the first grade fish meal in the international market Most of Indian fish meal is also reported to have high microbial count and contain considerable impurities including sand and salt These are major problems for commercial feed manufacturers, affecting the quality of feed formulation as well as the life of their processing equipments Hence, commercial shrimp feed manufacturers mostly rely on imported fish meal of high quality the price of which is high Vacuum dried and steam dried fish meals are recommended for shrimp feeds (Akiyama *et al* , 1992) Flame dried fish meal is exposed to high temperature which makes protein less available, oxidizes lipids and produce antinutritional factors like histamine (Akiyama *et al* , 1992) Although fish meal is reported to be a poor source of protein, it is one of the most common ingredients in commercial shrimp feed at 10 40% inclusion level (Akiyama *et al* , 1992), possibly due to its bulk availability

2 1 3 2 2 Molluscs

Short necked clam, *Tapes philippinarum* has traditionally been considered as an excellent feed for *P japonicus* Kanazawa *et al* (1970) had shown that *P japonicus* grows well on diets with short necked clam Deshimaru and Shigueno (1972) and Deshimaru (1981 and 1982) attributed the reason for the superiority of short necked clam as its similarity in amino acid profile to that of *P japonicus* Sherif (1989) reported that, dried clam meat as principal protein source gave better results in terms of growth and survival compared to a diet based on fish meal for *M rosenbergii*

High nutritional value of squid meal as a protein source was demonstrated by several authors for several penaeids viz, *P japonicus* (Kitabayashi *et al* 1971 Deshimaru and Shigueno 1972) *P setiferus* and *P stylirostris* (Fenucci *et al* ,1980), *P aztecus* (Fenucci and Zein Eldin 1976) *P monodon* (Cruz-Ricque and AQUACOP 1987), *P vannamei* (Dokken and Lawrence, 1985) and recently for *P indicus* (Cruz Ricque *et al* 1987)

Deshimaru and Shigueno(1972) found that squid meal has an excellent nutritive value for *P japonicus* and stated that it is due to the the essential amino acid composition of squid protein which is similar to that of *P japonicus* Cruz Ricque *et al* (1987) suggested that this growth promoting effect is due to a protein fraction of squid tissue and not related to amino acid balance They suggested the existence of an unknown growth factor in squid Cruz Suarez *et al* (1987) demonstrated that the growth promoting effect of squid protein is not because of amino acid supply but due to an increase in metabolic activity and protein synthesis per cell, since it tends to induce a hypertrophy of cells and not hyperplasy

Growth promoting effect of squid protein had been evaluated at different levels by many authors Kitabayashi *et al* (1971) used 56 74% level of squid meal in their experiment with *P japonicus*, while Deshimaru and Shigueno (1972) used at lower rates (20-47%) for the same species However, using a series of diets for *P aztecus* containing 31 5% shrimp meal, Fenucci and Zein Eldin (1976) obtained best growth with diet containing 5 or 15% squid meal, while larger proportions of squid meal of 30 and 49% resulted in high mortality and de creased growth rate

Cruz Ricque *et al* (1987) evaluated the growth response to supplementation of mixed diets, with protein extracted from frozen squid at different levels (1 5 3, 6, 16%) in the diet of four species of shrimps In *P stylirostris* and *P vannamei*, growth rates were significantly improved even at lowest level of supplementation Improvement was obtained only with 6 and 16% supplementation in *P monodon* while, no significant im provement was observed in *P indicus* This clearly suggests the existence of species specificity in the growth promoting effect of squid protein The growth promoting effect of squid protein at 16% of the diet could be explained by its nutritional value, particularly amino acid content However result obtained at lower levels of squid seems to be due to an unknown growth factor According to Akiyama *et al* (1992), this growth factor, which is believed to be small peptides, increases the digestive efficiency of shrimps and enhances growth rate On the other hand, Fenucci and Zein Eldin (1976) attributed higher amount of arginine in squid as the possible reason for the enhanced growth rate in *P aztecus* Squid meal is also an excellent attractant and squid meal levels in commercial shrimp feeds usually ranges from 2 to 10% There is no nutritional limita tion in the use of squid meal in shrimp feed however, its use is limited by price and availability (Akiyama *et al* ,1992)

2 1 3 2 3 Crustaceans

Crustacean meal has been tested extensively by many workers in shrimp feeds expecting it to produce better result owing to its similarity in amino acid profile with the body protein of shrimp although contradictory results have also been reported

Sick and Andrews (1973) observed higher growth rate for *P duorarum* with shrimp meal diet, than casein and maize gluten meal but poor growth response than with soybean meal and fish meal Forster and Gabbot (1971) reported that in *Palaemon serratus* the assimilation efficiency for shrimp meal was poorer than that of casein gelatin, egg albumen freeze dried egg, mussel mantle, fish meal, maize gluten meal, groundnut meal and bacterial protein In *Pandalus platyceros* also a lower assimilation efficiency of shrimp meal has been reported compared to casein soybean meal and bacterial protein, but higher to that of white fish meal (Forster and Gabbot, 1971) But Forster and Beard (1973) reported a better performance for shrimp meal over fish meal in the diet of *Palaemon serratus* On the other hand Balazs *et al* (1973) observed no benefit when shrimp meal was used along with tuna and soybean meal at 25 and 35% protein levels According to Akiyama *et al* (1988), apparent protein digestibility of shrimp meal was reported to be poor than that of soybean meal Similarly AQUACOP *et al* (1989) reported lower digestibility of shrimp meal compared to fish meal, squid meal and soybean meal for *Penaeus monodon*, *P vannamei* and *P stylirostris* On the other hand, Cruz Suarez *et al* (1993) observed a positive dose response relationship in the growth rate of *P vannamei* when shrimp meal was incorporated in the diet along with other sources The best growth response in the study was obtained when shrimp meal was used at 18% of the diet, which was also the highest level tested Earlier, Nair and Thampy (1987) reported superiority of diet based on shrimp meat (*Metapenaeus dobsoni*) over other sources of protein tested for the larvae of *Macrobrachium rosenbergii*

Shrimp meal is high in crude protein content and several essential amino acids (Forster 1976) besides, it is a good source of fatty acids (Sandifer and Joseph 1976) and appear promising for compounding shrimp feed (Venkataramiah *et al* , 1978 and Ali, 1988) However shrimp meal as a single source of protein has been reported to result poor rate of growth in *P indicus* (Raman *et al* , 1982) and *P monodon* (Pascal and Destajo, 1979) Commercial shrimp feed usually contains 5 15% shrimp meal (Akiyama *et al* 1992)

Another potential crustacean source of protein which is locally available and inexpensive is the stomatopods *Oratosquilla nepa* the mantis shrimp is a stomatopod caught in abundance in early fishing season for shrimps and large quantities of this occur in the trawl catches along with shrimp during November to January (Ali and Mohamed, 1985) At present, this species is not properly utilized Ali (1982a) used squilla protein in the formulated feed of *P indicus* after coagulating the protein and drying and compared it with other compounded feed based on clam meal, prawn waste, fish meal and a control (fresh clam meat) The best growth response was observed for the mantis shrimp based diet, closely followed by clam meal diet But the feed efficiency was better for clam meal based diet

Later Ali and Mohamed (1985) tried four combinations of prawn waste and mantis shrimp in the formulated feed for *P indicus* Unlike as in the previous case, in this experiment, the mantis shrimp they have used was in the form of a meal prepared by sundrying and pulverizing Prawn waste meal was also prepared by the same method Best growth response and feed efficiency for *P indicus* was noted when 25% prawn waste and 35% mantis shrimp were used together in the feed

2 1 3 2 4 Other sources

Silkworm pupae is yet another source of protein which seem to be potential for shrimp and prawn feed But the literature on the use of silkworm pupae in the formulated feed of shrimps and prawns is scanty Ali (1988) explored the possibility of incorporating silkworm pupae as a protein source in the formulated feeds of *P indicus* juveniles But the result showed that feeding with silkworm pupae diet results in low digestibility, protein efficiency ratio and biological value for *P indicus* He attributed it to the high chitin content of silkworm pupae According to Hanson and Goodwin (1977) penaeids appear to have two chitinase systems one in hepatopancreas and the other centred around the chitinoclastic bacteria in the digestive gland Several studies have reported measurable levels of chitin degrading enzymes in the alimentary tract of various crustaceans (Chandramohan and Thomas, 1984, Lynn, 1990; Spindler Bath *et al* , 1990) In addition it is commonly observed that shrimps and prawns consume their exuviae following ecdysis These observations suggest that crustaceans may be able to utilize the dietary chitin Vaitheswaran and Ali (1986) reported that glucosamine and chitin have a growth promoting effect in *P indicus* It may also be seen that the purified diets proposed for the use in nutritional studies with shrimps and prawns contain 0 5% glucosamine (Kanazawa *et al* , 1970 and Kanazawa, 1982) Recently Unnikrishnan *et al* (1992) reported high nutritional value of silkworm pupae in the formulated feed of *M rosenbergii* and pointed out that it is comparable to clam meal based diet

To find an alternative to fish meal, many workers have tried various unconventional sources of protein in the formulated feeds with variable success Sultan *et al* (1982) obtained encouraging results with frog flesh waste in the formu lated feed for *P indicus* and *P monodon* Forster and Gabbot (1971) evaluated the digestibility of whale meal and slaughter house wastes in the feed of *Palaemon serratus* and found them to be least assimilated among the various sources tested According to Deshimaru and Shigueno (1972), although the quantity of total amino acids in whale meal is high, its amino acid pattern differ from that of prawns Goswami and Goswami (1979 and 1982) pointed out the possibility of using slaughter house waste in shrimp feed

Nutritional response of post larvae of *P vannamei* to various levels of meat and bone meal indicated that it could be cost effectively used as partial substitute to marine protein in shrimp feeds (Lawrence and Castille, 1988) Blood meal has been found to be an inferior source of protein in the diet of *P paulensis* when it replaced fish meal shrimp meal clam meal, soymeal or rice bran (Marchiori *et al* , 1982) Brand and Colvin (1977) observed growth depression in *P californiensis* when blood meal was included at 5 15% of the diet Dominy and Ako (1988) opined that blood meal which contains co-valently attached methionine, might be a potentially useful way to supplement limiting amino acids in feeds According to Akiyama *et al* (1992) blood meal is not commonly used in commercial feed and when used the level should not exceed 7% of the diet

Yet another group of unconventional ~~group of~~ protein is single cell protein such as algal powders, yeast and bacterial protein Bacterial protein was found to be among the best assimilated protein in the diet of *Palaemon serratus* and *Pandalus platyceros* (Forster and Gabbot, 1971) Superior performance of petroleum yeast over white fish meal in the diet of *P japonicus* was reported by Deshimaru and Shigueno (1972) Brand and Colvin (1977) observed better growth and survival while using yeast in the diet of shrimp Akiyama *et al* (1992) is of the view that utilization of yeast in the aquafeeds is limited by palatability (bitter flavour) of the feed In commercial feeds the level of yeast ranges from 2 5% and it is pointed out that it should not exceed 5% owing to palatability considerations

Among algal powders, *Spirulina* was found to promote growth in *P japonicus* (Cuzon *et al* , 1981) and *P indicus* (Ali, 1988) James *et al* (1992) while investigating the possibility of using *Spirulina fusiformis* as protein source in the feed of *M rosenbergii* suggested that, it cannot serve as a sole protein source, but can be used effectively as a supplementary protein source

2 1 2 Carbohydrate

Although the protein requirement of shrimps and prawns has been studied extensively the information on the carbohydrate nutrition is scarce The carbohydrate requirement of various species has not been well defined so far But there are few studies which evaluated the quality of various carbohydrate sources

Knowledge of optimal level of protein and protein sparing effects of non protein nutrients such as carbohydrate and lipid, is effective in reducing cost of feed Protein, the expensive fraction could be optimally utilized for growth rather than for maintenance of the prawn, if there is better understanding of protein sparing capacity of other components like carbohydrate and lipid

The feed with high dietary protein, in the presence of low levels of non protein energy may force the crustaceans to deaminate significant proportions of protein thus yielding the carbon fragments required for cellular metabolism Hysmith *et al* (1973), while experimenting with *P aztecus* observed that low protein/ high energy and high protein/ low energy diets gave better growth rate than low protein/ low energy or high protein/high energy diets The higher amount of energy needed by prawn at higher levels of protein to attain approximately the same growth rate obtained at a lower protein level, implies that when non protein energy sources are limiting protein is catabolised (Bautista, 1986) According to Shiau and Chou (1991), when energy level of the diet is kept at a level of 330 kcal/100g, the dietary protein level of *P monodon* can be lowered from 40 to 36 % Therefore, it is critical to obtain the proper protein energy (P/E) ratio in a diet, for the most economical production of shrimp Reduction of any excess protein in the diet will help in reducing the quantity of ammonia, being excreted by shrimp (Shiau and Chou 1991)

Crustaceans can utilize dietary carbohydrates as energy source, polysaccharides such as starch and dextrin, being better than monosaccharides Glucose in the diet at the level of 10% or above is known to suppress growth (Lovel 1989) Addition of 20% glucose to a menhaden meal based diet for *P aztecus* produced reduced growth rate, while inclusion of 30% starch in the diet with lower protein diet increased growth rate (Andrews *et al* 1972)

Abdel Rahman *et al* (1979) have shown that *P japonicus* juveniles had a better weight gain on diets containing disaccharides such as sucrose, maltose and trehalose and polysaccharides such as dextrin and starch than on diets containing monosaccharides such as glucose galactose and fructose They attributed the reason for the higher nutritive value of di and polysaccharides than monosaccharides for *P japonicus* as the gradual absorption of these compared to the quick absorption of glucose in the stomach and release all at once into haemolymph When a large quantity of glucose is added to diets, blood glucose levels get elevated to abnormally high levels, whereas di and polysaccharides are not absorbed from the stomach They are digested to glucose and trehalose in the midgut and hepatopancreas and then released gradually into haemolymph

AQUACOP (1978) also suggested that a carbohydrate such as starch appears more suitable than glucose in the diet for shrimps

The dietary characteristics of seven carbohydrate sourc es viz, glucose fructose galactose maltose, sucrose glyco gen and starch were evaluated for *P indicus* by Ali (1993) and observed superior growth for shrimp fed with diets having starch and maltose Among the different carbohydrates tested, diets having glucose, fructose, galactose (monosaccharides), sucrose and glycogen show poor growth Monosaccharide also showed poor feed conversion ratio than di and polysaccharides Among the various combinations of carbohydrates, sucrose maltose starch mixture at equal proportion was found to be the best compared to others (Ali 1993)

Ali (1982 b) while experimenting with *P indicus* at various levels of starch observed best growth response while using 40% starch in a casein based diet

2 1 3 Lipid

Lipids are required in the diet of shrimps and prawns, not only for their energy value but also as sources of essential fatty acids fat soluble vitamins sterols and phospholipids However, high levels of lipids are usually associated with significant retardation of growth (Forster and Beard 1973 Deshimaru and Kuroki 1974 Kanazawa *et al* 1977a)

Kanazawa *et al* (1977a) obtained best growth for *P japonicus* when 8% short necked clam oil was included in the diet while 16% inclusion produced growth inhibition among the three levels (8, 12, 16%) tested Deshimaru *et al* (1979) obtained best growth for the same species when 6% lipids was used in the diet among four levels (3, 6 9 12%) tested Mendoza (1982) found that a diet containing 11 7% lipid gave maximum growth and feed efficiency for *P monodon* juveniles Catacutan and Kanazawa (1985) showed that the best growth of *P monodon* juvenile was obtained with lipid source containing high amounts of highly unsaturated fatty acids of n 3 series in semipurified diet at 10 11% level in their diet Sheen and D'Abramo (1991) while experimenting *M rosenbergii* juveniles tested 6 lipid levels (0 12%) with cod liver oil and corn oil in 2 1 ratio and observed significant reduction in weight when 10 or 12% lipid level was tried

Polyunsaturated fatty acids (PUFA) of linolenic (n 3) and linoleic (n 6) families (18 2n 6, 18 3n 3, 20 5n 3, 22 6n 3) have been recognized as very important nutrients for the growth of crustaceans (D'Abramo and Sheen, 1993) Growth enhancing response to dietary oils containing comparatively high levels of $C \geq$ 20n 3 PUFA (shrimp head anchovy sardine, clam or cod liver oils) was observed by Sandifer and Joseph (1976) for *M rosenbergii* Kanazawa *et al* (1977b) Guary *et al* (1976) for *P japonicus* Read (1981) for *P indicus* and by

Martin (1980) for *Palaemon serratus*

Analysis of the diet influenced on fatty acid profiles of crustacean tissue has led to a better understanding of fatty acid metabolism This approach led to the conclusion that *P japonicus* (Kanazawa *et al* 1979b) and *M rosenbergii* (Reigh, 1985) have the ability to synthesize saturated and monounsaturated fatty acids from 16 0 (Palmitic acid) but these fatty acids are not in turn transformed into 18 2n 6 18 3n 3 20 5n 3 or 22 6n 3 Analysis of the fatty acid profile of tissue of white shrimp *P setiferus* brown shrimp, *P aztecus* and pink shrimp *P duorarum* by Bottino *et al* (1980) and *P indicus* by Read (1981) revealed that these species possess a limited capacity to convert C18 PUFA into C$\geq$20 PUFA Using labeled 18 3n 3, Kanazawa *et al* (1979a) identified a similar metabolic limitation in *P japonicus*

Growth of crustaceans has been improved through dietary additions of 18 3n 3 and/or 18 2n 6 and the level of the measured response of dietary 18 3n 3 generally exceeds that to 18 2n 6 (Shewbart and Mies 1973 Read 1981 Guary *et al* , 1976, Kanazawa *et al* 1977a) In contrast, *P indicus* and *M rosenbergii* appear to prefer dietary 18 2n 6 over 18 3n 3 in satisfying a C18 PUFA requirement (Read 1981 and Reigh and Stickney 1989)

Kanazawa *et al* (1978 1979a) concluded that either 20 5n 3 (eicosapentaenoic acid) or 22 6n 3 (docosahexaenioc acid) also termed highly unsaturated fatty acids (HUFA) have higher nutritive value than 18 2n 6 or 18 3n 3 PUFA for *P japonicus* The unique nutritional value of 20 5n 3 and 22 6n 3 relative to 18 2n 6 and 18 2n 3 was also reported for *Palaemon serratus* by Martin (1980) Fenucci *et al* (1981) recommended a ratio of n 3 fatty acids to 18 2n 6 ranging from 1 00 to 1 18 to achieve the best growth response of *P stylirostris*

2 1 4 Vitamins

The present understanding of the qualitative vitamin requirements of penaeid shrimps has been based almost entirely on the studies on *P japonicus* which is considered as the best documented species (Kanazawa *et al* , 1976 and Deshimaru and Kuroki, 1976) These studies seemed to indicate that shrimps have much higher vitamin requirements than other aquatic animals such as fish (Chen,1993) This variation is suggested to be caused by differences in feeding behaviour , size, growth rate, environmental factors and nutrient interrelationships Since shrimp is a slow feeder prolonged suspension of feed in water increases the leaching of nutrients Hence a higher vitamin requirement for crustaceans than for fish

Vitamins are essential for normal growth maintenance and reproduction of animals The metabolic functions of vitamins in shrimps and prawns has been discussed by Paulraj (1993) New (1976) drew attention to the paucity of knowledge on the qualitative and quantitative vitamin requirements of shrimp

Specific quantitative vitamin requirements are yet to be determined for most of cultivable species In the case of shrimps and prawn, data available are only for a few vitamins, that too, for postlarval and juvenile stages of few species At present, vitamin premixes are used while formulating the feeds Since vitamins or their precursors are often present in the raw materials, the 'blanket' application of vitamin premixes to multi ingredient shrimp diet may result in some excesses (New, 1976) Conversely, while the vitamin requirements of shrimp remain unknown diets may still be deficient in other vitamins even after supplementation As in the case of mineral metabolism, the study of the vitamin requirements of shrimp remains a neglected field (New 1976)

At low stocking densities, natural food may provide a good proportion or all of the vitamins required by the shrimps and prawns However at high stocking densities as in semi intensive and intensive systems when natural food is limited to sustain the entire population vitamin supplementation in the diet is important

In crustaceans, vitamin C deficiency leads to the inhibition of alkaline phosphatase activity, resulting in poor chitin synthesis (Paulraj 1993) Vitamin C is required for hydroxylation of proline and lysine in the formation of collagen Deficiency of this vitamin has been characterized by poor growth, reduced moulting frequency or incomplete moulting, high incidence of post moult death poor feed conversion decreased resistance to stress, impaired collagen synthesis and wound healing melanized lesions underneath the exoskeleton blackening of gills and in extreme cases black death disease which causes high mortality rate (Paulraj 1992 and He and Lawrence 1993)

Kitabayashi *et al* (1971) observed accelerated growth in *P japonicus* fed with diets containing vitamin C the optimum level was found to be 0 22% Deshimaru and Kuroki (1976) have shown that juveniles of *P japonicus* requires 3000 10 000 mg of vitamin C, 600 mg of choline 2000 4000 mg of inocitol 60 120 mg of thiamine and 120 mg of pyridoxine per kg of feed Recently, Shiau and Jan (1992) found that *P monodon* requires 2000 mg of vitamin per kg of feed which is closer but slightly lower to the optimum suggested by Kitabayashi *et al* (1971) but much lower than the optimum requirement suggested by Deshimaru and Kuroki(1976) for *P japonicus* One factor that may be involved in the apparent difference in dietary vitamin C requirement may be the more omnivorous nature of *P monodon* compared to *P japonicus* (Chen 1993) An important factor to be considered in the vitamin C requirement is the source and its stability during processing and subsequent storage Ascorbic acid is the most sensitive vitamin to degradation during feed processing and storage As much as 80 100% of the initial amount of supplemental L ascorbic acid can be lost during processing and subsequent storage (Grant *et al* ,1989) L ascorbyl 2 polyphosphate (APP) is relatively resistant to oxidation When this form of vitamin C was used the requirement of vitamin C for *P japonicus* was found to be 100 mg per kg of feed (Shigueno and Itoh 1988) much lower than the requirement suggested by Deshimaru and Kuroki (1976) for the same species using L ascorbic acid (3000 10 000 mg/kg) Recently vitamin C requirement of *P vannamei* was determined as 120 mg /kg of feed (He and Lawrence, 1993)

In a study depicting the effects of individual deletions of water soluble vitamins from semi purified diets on juvenile *P monodon*, Catacutan and Cruz (1989) found that, growth was poorest for treatments without vitamin supplement and inocitol or choline deficient diets Enhanced growth was observed in riboflavin deficient diet Severe cases were found in treatments without vitamin supplement, followed by deletion of inocitol, choline or vitamin C

2 1 5 Minerals

As with most aquatic animals, shrimps and prawns can absorb or excrete minerals directly from or to aquatic environment through the gills and body surface Therefore, the dietary requirements of minerals is largely dependent on the mineral concentration of water in which they are reared

Investigations on the mineral requirement of shrimps and prawns are scarce, although many workers have used mineral supplements (New 1976) These supplements have mainly been used for other farm or experimental animals

The best growth rates were achieved with diets for *P japonicus* when supplementary levels of 1 04% phosphorus and 1 24% calcium were added (Kitabayashi *et al* ,1971) When the calcium-phosphorus ratio was increased to 2 1, growth was inhibited and pigmentation decreased Five percent inclusion of a mineral premix in a casein based diet for *P aztecus* gave 18% increase in biomass (Sick *et al* 1972) and no increase when the premix was omitted The supplement had a 1 3 1 calcium phosphorus ratio and provided 0 66% calcium and 0 51 % phosphorus to the final diet Deshimaru and Kuroki (1974) using a casein egg albumen diet for *P japonicus* reported that highest growth increment was noted when the calcium phosphorus ratio was maintained at 0 76 1 Shewbart *et al* (1973) postulated that calcium potassium, sodium and chloride requirements for *P aztecus* might be satisfied through osmotic regulation Phosphorus, however, may be essential in the diet because it is present in large quantities in shrimp, but not in seawater

Nutritional studies of shrimp were initiated in the early 1970 s However, comparisons of various studies were found to be difficult or impossible due to differences in methodologies and absence of standard reference diet However, these studies have been used in commercial feed formulations

Limitations in our knowledge of nutrition of prawn underscore the need to continuously develop, test and apply new nutritional concepts This is particularly true for the rapidly expanding shrimp feed industry where feed formulations are presently based largely on assumptions and unknown growth factors' rather than nutritional science

III MATERIAL AND METHODS

Macrobrachium rosenbergii juveniles of 2 5 3 0 cm size were used for conducting two sets of experiments The first experiment was to evaluate the quality of certain inexpensive and locally available sources of protein, such as Clam meal Squid meal Squilla meal, Shrimp head meal and Silkworm pupae in their formulated feeds The second experiment aimed at evaluating the effect of partial substitution of protein with carbohydrate was conducted using the best protein source determined from the first experiment

3 1 Experimental animals

The juveniles of M rosenbergii used for the experiment were the ones reared in FRP tank for 30 40 days in the Macrobrachium hatchery of College of Fisheries, from the post larvae obtained from a private hatchery near Cochin The post larvae, transported in polythene bags partly filled with freshwater and inflated with oxygen were introduced into an oval, flat bottom fibre glass tank of 1 2 t capacity filled with freshwater from the supply system of the hatchery up to half the capacity The water was kept aerated and the post larvae fed with dried and pulverized clam meat once daily The feed remnants were ~~to be~~ siphoned out daily prior to feeding and water replaced partially with filtered freshwater

3 2 Experimental rearing facilities

The experiments were conducted in the *Macrobrachium* hatchery of the College of Fisheries, using cylindrical fibre glass tanks having a diameter of 55 cm and a height of 35 cm

The water used was drawn from the freshwater distribution system of the hatchery taken from a well It was filtered using bolting silk before use During the course of experiments, water used to be completely replaced with filtered water once every week A depth of about 20 cm was maintained throughout the experiment (about 47 5 l water in a tank)

Aeration was provided to individual tanks through PVC tube and diffusion stones from the air distribution system of the hatchery Gentle air supply was maintained, uniformly throughout the experimental period using air regulators except during feeding cleaning of tanks water changing and removal of left over feed and feacal matter, during which periods aeration was stopped

Each tank was provided with a V' shaped tile, placed in an inverted position to provide hiding place especially for newly moulted prawns since provision of artificial substrata has been reported to reduce cannibalism and improve survival rate of *M rosenbergii* post larvae and juveniles (Smith and Sandifer 1975 Ra anan *et al* 1984 Gomez *et al* 1988)

During experimental period the tanks were covered with an opaque polythene sheet, to prevent prawns from jumping out and deposition of dust in tanks This was also found helpful in reducing sun light which causes algal growth in rearing water The growth of algae by serving as food for prawn, is likely to affect the result of feeding study, apart from causing wide fluctuations in physico chemical parameters of the water used in the experiment

3 3 Experimental diets

3 3 1 Major protein sources

Clam meal, Squid meal Squilla meal Shrimp head meal and Silkworm pupae served as major protein sources respectively in the five compounded test diets formulated for evaluating their quality

3 3 1 1 Processing of major protein sources

The protein sources used for the feed formulation, were processed following a uniform method, so as to avoid variations in quality due to processing

CLAM MEAL Live clam *Villorita cyprinoides* collected by local fishermen from Cochin backwater were steamed in an autoclave for 15 minutes at ambient pressure and the meat separated was dried in an electric dryer for 12 hours at $60^{o}C$

SQUID MEAL Processing waste of Squid, *Loligo* sp was collected from a private seafood exporting company in Cochin and transported in a styrofoam box with crushed ice In the laboratory, it was steamed in an autoclave for 15 minutes at ambient pressure and dried in an electric dryer for 12 hours at 60°C

SQUILLA MEAL Whole squilla *Oratosquilla nepa* was collected from Cochin fisheries harbour and transported in a styrofoam box with crushed ice It was washed thoroughly steamed in an autoclave for 15 minutes and dried in electric dryer for 24 hours at 60°C The duration of drying was higher, than that used for other protein sources, since bigger sized whole squilla was used

SHRIMP HEAD MEAL Head waste of shrimp *Parapenaeopsis stylifera* was collected from a local shrimp peeling shed It was steamed in an autoclave for 15 minutes at ambient pressure and dried in an electric dryer for 12 hours at 60°C

SILKWORM PUPAE Live pupae of silk worm, *Bombyx mori* used for quality assessment of silk at the Cocoon Reeling Centre, Pattanakad (Alapuzha district) were brought in a carton to the laboratory They were steamed for 15 minutes in an autoclave at ambient pressure, dried for 12 hours in an electric dryer at 60°C and then partially deoiled by solvent extraction method using petroleum ether since they contain high level of lipid

3 3 2 Other ingredients

Besides the major protein sources ingredients such as groundnut oil cake, wheat bran, tapioca flour sardine oil maize oil mixture and vitamin mineral mixture were also used for compounding the test diets Tapioca flour served as major carbohydrate source as well as binder while groundnut oil cake and wheat bran were included to provide plant protein as well as carbohydrate Sardine oil and maize oil mixture at 2 1 ratio was used as supplementary lipid source Sardine oil was obtained from Central Institute of Fisheries Technology (CIFT) Cochin and 'Maize Magic brand maize oil marketed by Karnataka State Agro corn Products Limited Bangalore purchased locally Supplevite M a vitamin mineral mixture (Sarabhai Chemicals, Bombay) was included in the test diets to provide the necessary vitamins and minerals Cellulose (Loba Chemie Indoaustranal Co, Bombay) was used as filler while compounding the diets

3 3 3 Storage of ingredients

The dry ingredients used for the formulation of feeds were packed in polythene bags after powdering in a grinder and passing through a sieve of 250 microns while wheat bran was directly sieved The packing was done using a sealing machine to make it hermetic after excluding the air pockets to the possible extent Polythene bags containing individual ingredients kept in plastic jars were stored in a refrigerator till they were used for feed formulation The oils and Supplevite M were also stored in a refrigerator and used as such

3 3 4 Proximate composition of feed ingredients

Analyses of proximate composition of ingredients were done prior to feed formulation and is presented at table 2

The analyses were done following standard A O A C (1980) methods and results were expressed on dry matter basis except for moisture which was on as fed basis

1 The moisture content by drying the sample at 105°C until a constant weight was reached

2 Crude protein using Micro Kjeldahl s method

3 Crude fat by solvent extraction method using petroleum ether(B P 40 60°C) in a soxhlet extraction apparatus for 6 hours

4 Crude fibre content by the method of Pearson (1976)

5 The ash content by incinerating the sample at 550°C for 6 hours in a muffle furnace

The carbohydrate content (Nitrogen free extract NFE) was calculated using difference method (Hasting 1976)

Table 2 Proximate composition of the ingredients used in the feed formulation

INGREDIENT	Moisture	Crude Protein	Crude Fat	Crude Fibre	Ash	NFE
Clam meal	7 65	58 70	11 55	0 88	7 93	20 94
Squid meal	6 60	83 60	6 92	0 69	4 65	4 14
Squilla meal	6 95	52 75	8 87	6 35	25 70	6 33
Shrimp head meal	8 20	51 20	8 34	7 26	24 36	8 84
Silkworm pupae	8 45	64 50	3 86	6 42	21 94	3 28
Groundnut oil cake	8 65	43 65	9 50	9 65	13 34	23 86
Wheat bran	7 95	19 20	5 89	24 70	27 57	22 64
Tapioca flour	8 20	3 86	2 16	2 98	2 58	88 42

3 3 5 Formulation and processing of test diets

Two types of test diets were formulated for

1 evaluating the quality of protein sources and

2 studying the effect of partial substitution of protein with carbohydrate in the formulated feeds of *M rosenbergii* juveniles

3 3 5 1 Test diets for evaluation of protein sources

For evaluating the quality of different protein sources, 5 isonitrogenous (35% crude protein) isolipidic(9% crude fat) and isocalorific(375 kcal/100g) feeds were formulated (experiment I)

The five feeds were given abbreviated names shown as below and used subsequently for easy documentation

1 CLM (Clam meal as major protein source)

2 SQM (Squid meal as major protein source)

3 SLM (Squilla meal as major protein source)

4 SHM (Shrimp head meal as major protein source)

5 SWP (Silkworm pupae as major protein source)

Table 3 gives the proportion of the ingredients used for the preparation of test diets

The respective ingredients were weighed accurately in an electronic balance and the dry ingredients except the Supplevite M were mixed well in a clean dry ceramic mortar The dry mixture was made into a soft dough consistency by adding sufficient distilled water (1 1 3 W/V) and mixed well in

Table 3 Composition of test diets (%) formulated for the evaluation of quality of protein sources and their proximate composition

INGREDIENTS	TEST DIETS				
	CLM	SQM	SLM	SHM	SWP
Clam meal	48 92				
Squid meal		34 35			
Squilla meal			54 45		
Shrimp head meal				56 10	
Silkworm pupae					44 50
Groundnut oilcake & Wheat bran (1 1)	20	20	20	20	20
Tapioca flour	7 90	18 00	15 75	14 00	18 00
Cellulose	19 78	21 55	6 16	6 12	10 70
Supplevite M	1	1	1	1	1
Lipid mix *	2 40	5 10	2 64	2 78	5 80
* Sardine oil and Maize oil @ 1 1					
PARAMETER	PROXIMATE COMPOSITION OF TEST DIETS				
Moisture	8 26	8 74	8 38	8 46	8 63
Crude Protein	34 72	34 91	35 10	34 93	35 05
Crude Fat	8 81	9 10	8 93	8 86	9 20
Crude Fibre	21 34	23 60	13 21	13 73	16 98
Ash	11 98	9 97	10 71	19 55	15 52
Carbohydrate (NFE)	23 15	22 42	23 05	22 93	23 25
Gross Energy *	374	375	377	375	380

* Gross energy (kcal/100g) is calculated based on following calorific values Protein 5 65 kcal/g Lipid 9 45 kcal/g Carbohydrate 4 10 kcal/g (Kosutarak *et al* 1993)

the mortar The dough was transferred into a glass beaker and steam cooked for 30 minutes in an autoclave at ambient pressure The cooked dough was rapidly cooled under an electric fan and again mixed well in a dry mortar along with Supplevite-M, and sardine oil maize oil mixture

The well homogenized mixture was pelletized using a hand operated house hold extruder with a die of 2 mm diameter onto a clean tray as a single layer and dried at 60 °C for 12 hours in an electric dryer with forced air convection The dried pellets had a diameter of 1 mm They were manually broken into small pieces of about 5mm length and was packed in small polythene bags and hermetically packed using a sealing machine after excluding air pockets to the possible extent These bags were kept in plastic jars and stored in a refrigerator

3.3 5 2 Test diets for evaluating the effect of partial substitution of protein with carbohydrate

Clam meal which was found to be the best protein source in the first experiment, was used as major protein source for the second experiment

9 test diets were formulated, with 3 protein levels viz, 25, 30 and 35% each at 3 levels of carbohydrate(20, 25 and 30%), thus forming 9 combinations of protein and carbohydrate

For easy documentation and better understanding these diets were given 9 abbreviated names based on conventional method of statistics with two factors Protein is designated by the letter P and carbohydrate with C Levels of protein and carbohydrates are indicated by numbers 1, 2 and 3 For instance P1 represents the first level of protein in this trial which is 25% protein For 30% protein level P2 was used, which is the second level of protein in this experiment and P3 the third level of protein Similarly carbohydrate levels were designated as C1 C2 and C3 to indicate the levels of carbohydrate which are 20, 25 and 30% respectively in this experiment So each test diet is denoted by a combinaton of 2 letters and 2 numbers such as P1C1, P1C2 P1C3, P2C1, P2C, P2C3, P3C1, P3C2 and P3C3 as given below

DIET	PROTEIN LEVEL (%)	CARBOHYDRATE LEVEL(%)
P1C1	25	20
P1C2	25	25
P1C3	25	30
P2C1	30	20
P2C2	30	25
P2C3	30	30
P3C1	35	20
P3C2	35	25
P3C3	35	30

The lipid level was kept at 9% in all the test diets and the composition of test diets is presented in the table 4

Table 4 Composıtıon of test dıets (%) formulated to study the effect of partıal substıtutıon of proteın wıth carbohydrate and theır proxımate composıtıon

INGREDIENT	TEST DIETS							
	P C1	P1C	P1C3	P2C1	P2C2	P2C3	P3C1	P3C2	P3C3
Clam meal	38 25	38 25	38 25	46 75	46 75	46 75	55 80	55 80	55 80
GOC &Wheat bran (1 1)	10	10	10	10	10	10	10	10	10
Tapıoca	8 80	14 45	20 10	7 80	13 40	19 0	5 65	11 30	16 95
Cellulose	37 85	32 2	26 55	31 30	25 70	20 10	25 40	19 75	14 10
Supplevıte	1	1	1	1	1	1	1	1	1
Oıl mıx *	4 1	4 1	4 1	3 15	3 15	3 15	2 15	2 15	2 15
Sardıne oıl and Maı e oıl mıxture @ 1 1									
PARAMETER	PROXIMATE COMPOSITION OF TEST DIETS								
Moısture	8 94	8 71	8 82	8 65	8 58	8 73	8 60	8 56	8 42
Proteın	25 24	24 88	25 10	29 75	30 20	29 80	34 64	34 90	35 15
Fat	8 96	8 80	8 87	8 78	8 88	8 92	8 95	8 73	8 91
Fıbre	31 64	28 80	24 72	8 31	23 40	20 11	23 13	18 75	14 93
Ash	12 66	11 17	9 51	11 36	10 96	9 92	11 53	11 31	9 36
NFE	21 5	26 35	31 80	21 80	26 56	31 25	21 75	26 31	31 65
Energy	315	332	356	340	363	381	370	388	412
P/E ratıo	80 0	75 0	70 5	87 4	83 1	78 3	93 8	90 0	85 2
C P/CHO	1 17	0 94	0 79	1 36	1 14	0 95	1 59	1 33	1 11

Gross Energy (kcal/100g) ıs calculated based on following calorıfıc values
Proteın 5 65 kcal/g Lıpıd 9 45 kcal/g Carbohydrate 4 10 kcal/g
(Koshıo *et al* 1993)

The method of formulation processing and storage of test diets were same as in the case of first experiment

3 3 6 Proximate composition of test diets

The analyses of the proximate composition of test diets were conducted to ascertain the nutrient status The methodology employed was the same as that for the proximate composition analysis of ingredients The proximate composition of test diets for evaluating the quality of protein sources is presented in table 3, and the proximate composition of test diets used for evaluating the effect of partial substitution of carbohydrate for protein in table 4

3 4 Experimental procedure

3 4 1 Evaluation of quality of protein sources

240 numbers of *M rosenbergii* juveniles were selected from the middle of the size range of the stock population and 12 prawns each were stocked in the 20 cylindrical FRP tanks The respective feeds were randomly allowed to each tank and the experimental animals were weaned on to respective diets for 7 days from dried clam meat which was used for stock rearing On 8^{th} day they were starved for 24 hours, prior to the start of actual feeding experiment Out of the 12 prawns in each tank, 10 uniform sized apparently healthy ones were selected and weighed collectively after carefully blotting dry, between the

folds of a filter paper using an electronic balance (Shimadzu Labror AEU 130) with an accuracy of 0 00001 g The average size of juvenile prawns at the beginning of the experiment was 26 ±1mm length and 0 09183 ± 0 00517g weight

For each treatment, 4 replicates were maintained in separate tanks Hence 40 prawns were used for each treatment The duration of the feeding experiment was 45 days

Intermediate growth assessments were made on 15th and 30th day and final assessment on the 45th day The prawns were fed with respective diets slightly in excess , twice daily in a Petri dish kept at the centre of each tank Feeding was done in the morning between 8 and 9 a m and in the evening between 5 and 6 p m Before feeding, left over feed was removed and dried in an oven at 60°C to constant weight

3 4 2 Evaluation of effect of partial substitution of protein with carbohydrate

As in the case of the first experiment the prawns were weaned on to the respective diets using 12 prawns in each tank Out of this, 10 prawns were selected and weighed after starving them for 24 hours, and the actual feeing experiment was started with respective diets The average size of juvenile prawns at the beginning of the experiment was 28±2mm length and 0 11108± 0 005566g weight

For each treatment 3 replicates were maintained in separate tanks Hence a total of 30 prawrs were used for each treatment The duration of the experiment was 45 days as in the case of first one Growth assessments and schedule of feeding were the same as that of first experiment

3 5 Physico chemical parameters

Physico-chemical parameters of water used for this experiment were monitored using following methods

1	Temperature	Mercury bulb thermometer with accuracy of 0 1^{o}C
2	pH	Universal indicator solution
3	Dissolved oxygen	Standard Winkler method

3 6 Evaluation criteria

3 6 1 Survival rate

During the course of each experiment mortality of prawns was noted down and percentage survival rates were calculated at the end of each experiment for each replication as follows, from initial and final numbers of experimental animals

$$\text{Survival rate (\%)} = \frac{[\text{ Initial no } - \text{ No of dead prawn(s)}] \times 100}{\text{Initial no of prawns}}$$

3 6 2 Growth rate

Growth in feeding experiments is expressed as absolute weight gain percentage growth rate or more recently as Specific growth rate (SGR) The expression of growth in terms of SGR is an improvement over the former ones (Hepher, 1988) So for comparison of different treatments growth in terms of SGR was preferred in the present study while respective weight gain nd percentage growth rates were also given along with SGR values

$$SGR(\%) - \frac{[\ln W1 \quad \ln W0]}{t} X 100$$

where SGR(% day ^{1}) is the percentage growth rate, W0 the initial weight of experimental animals, W1 the final weight of experimental animal and t the duration of the experiment in days

The calculated value gives the average percentage increase in body eight over the experimental period

3 6 3 Feed efficiency

Feed efficiency has traditionally been given special attention but unfortunately often presented in an undefined form (Boonyaratpalin, 1989) Two terms which dominate the literature are Feed conversion ratio (FCR) and Feed conversion efficiency (FCE)

$$FCR = \frac{\text{Feed intake on a dry matter basis}}{\text{Weight gain on wet matter basis}}$$

$$FCE = \frac{\text{Weight gain on wet matter basis}}{\text{Feed intake on a dry matter basis}}$$

As they contain the same factors in inverse relation, they are frequently mixed up FCE has been recently preferred since it is positively correlated with growth and protein efficiency ratio In addition if weight gain is zero weight gain / feed intake is zero but feed intake / weight gain is infinite, which is difficult to interpret biologically (Boonyaratpalin, 1989) So in the present study FCE is preferred over FCR, and to avoid any confusion, the respective FCR values are also given along with FCE values

3 6 4 Protein efficiency ratio (PER)

Efficiency of protein utilization is expressed as protein efficiency ratio (PER)

$$PER = \frac{\text{Wet weight gain of experimental animal}}{\text{Crude protein intake on a dry matter basis}}$$

3 7 Statıstıcal methods

The experiments were planned ın the form of Randomızed block desıgn (RBD) Analysıs of variance (Snedecor and Cochran 1968) was carrıed out for the results of each experıment and treatment dıfferences studıed at 1% level of sıgnıfıcance Paırwıse comparısons of treatment mean values were done usıng crıtıcal difference based on Student s t at 1% level of sıgnıfıcance

The data on peıcentage survıval rate were analyzed after effectıng Arc sıne transformatıon of values as gıven below

$$Y - \sin^{1} (X / 100)^{0\ 5}$$

where Y ıs the transformed value and X the percentage survıval rate

For statıstıcal analysıs of data,computer progıamme was wrıtten ın BASIC language and result of analysis obtained ın the form of prınt out

IV RESULTS

4 1 Experiment to evaluate the quality of protein sources in the formulated feeds

The quality of protein sources in the formulated test diets for the juveniles of *M rosenbergii* was evaluated based on the following criteria

1 Survival rate

2 Growth measured as SGR (% day ^{1})

3 Feed conversion efficiency (FCE)

4 Protein efficiency ratio (PER)

4 1 1 Effect of protein sources on survival rate

The percentage survival rates of prawns, fed with diets differing in protein sources are given in table 5 and graphically presented in fig 1

Analysis of variance of data (table 6) indicates that no significant variation exists among the various sources of protein used in the experiment as far as their influence on survival rate is concerned

Among the feeds tested the highest survival rate of 95% was recorded for shrimp head meal based diet (SHM) and silk worm pupae diet (SWP), while test diets based on clam meal (CLM), squid meal (SQM) and squilla meal (SLM) produced survival rates to the extent of 92 5%

Table 5 Details of initial number and percentage survival rate *M rosenbergii* juveniles fed with diets differing in protein sources

FEED	R*	No of Prawns		Survival rate (%)	Mean survival rate
		Initial	Final		
CLM	1	10	9	90	92 50
	2	10	10	100	
	3	10	9	90	
	4	10	9	90	
SQM	1	10	8	80	92 50
	2	10	10	100	
	3	10	10	100	
	4	10	9	90	
SLM	1	10	9	90	92 50
	2	10	10	100	
	3	10	10	100	
	4	10	8	80	
SHM	1	10	9	90	95 00
	2	10	10	100	
	3	10	10	100	
	4	10	9	90	
SWP	1	10	10	100	95 00
	2	10	9	90	
	3	10	9	90	
	4	10	10	100	

R* Replication

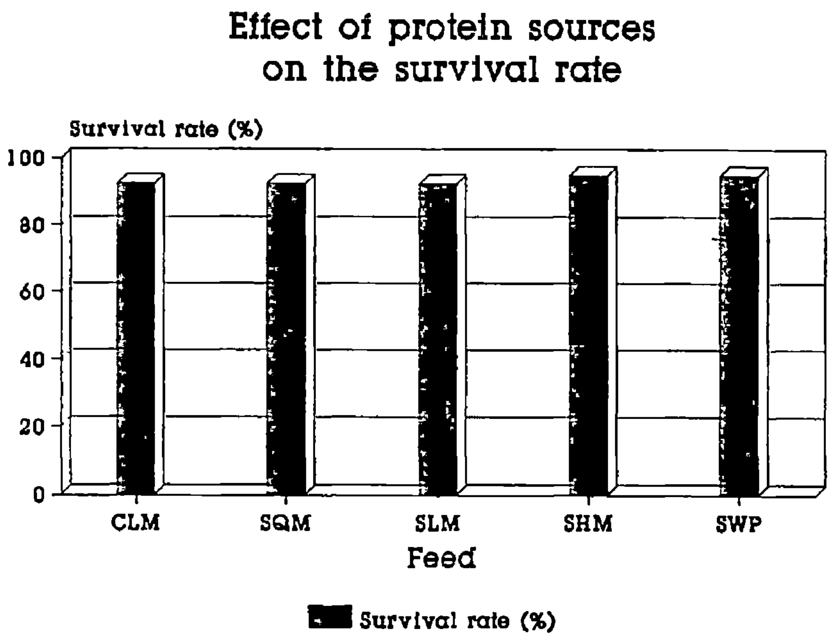

Fig 1 Survival rate (%) of *M rosenbergii* juveniles fed with diets differing in protein sources

Table 6 Result of analysis of varıance of data to compare the effect dıfferent Proteın sources on the survıval rate of *M rosenbergıı* juvenıles

(Data subjected to Arc sıne tıansformatıon)

Source of variatıon	Degrees of freedom	Sum of squares	Mean sum of squares	F ratio
Replıcation	3	621 81250	207 27080	1 78807
Between feeds	4	57 79688	14 44922	0 12465
Error	12	1391 02300	115 91860	
Total	19	2070 6330		

(Calculated F value < table value at 1% level of sıgnificance)

4 1 2 Effect of protein sources on growth rate

The data on initial weight of *M rosenbergii* juveniles and of their growth in terms of specific growth rate (SGR) are presented in table 7 and fig 2 Growth increment during the 45 day experimental period is graphically represented in fig 3

A significant difference in the influence (P <0 01) of dietary protein sources is observed in SGR of prawns (table 8) The prawn fed with clam meal based diet (CLM) produced the highest SGR of 2 97 followed by the squid meal diet (SQM) with 2 83, though not significantly different from the former Squilla meal diet (SLM) is not different from that of squid meal diet (SQM) and shrimp head meal diet (SHM), with respect to SGR values (2 83 and 2 38 respectively) Silkworm pupae diet (SWP) produced the lowest SGR (2 23) but not significantly lower when compared with shrimp meal diet

4 1 3 Effect of protein sources on feed conversion efficiency (FCE)

The data on feed conversion efficiency of *M rosenbergii* juveniles over the experimental period in response to the different protein sources are given in the table 9 and graphically represented in fig 4

The analysis of variance of data on FCE showed that there is a significant variation (P<0 01)between diets of various protein sources (table 10) Diets based on clam meal and squid meal gave relatively higher FCE values of 0 372 and 0 369 respectively and in pairwise comparison these did not differ significantly In the case squilla and shrimp head meal

Table 7 Specific growth rate (SGR) of *M rosenbergii* juveniles fed with diets differing in protein sources

FEED	R*	WEIGHT OF PRAWN (g)			FINAL WEIGHT 45th day	WEIGHT GAIN (g) 0 45 days	SGR (% day^{-1})
		Inital	15th day	30th day			
CLM	1	0 09332	0 16505	0 23854	0 32941	0 23609	2 80282
	2	0 08358	0 15064	0 23028	0 32452	0 24094	3 01454
	3	0 09710	0 17143	0 24502	0 34269	0 24559	2 80241
	4	0 08264	0 15908	0 22945	0 35852	0 27588	3 26109
MEAN		0 08916	0 16155	0 23582	0 33879	0 24963	2 97022
SQM	1	0 09058	0 15813	0 22408	0 31424	0 22366	2 76427
	2	0 08407	0 14529	0 23946	0 32543	0 24136	3 00777
	3	0 09555	0 16405	0 22614	0 31801	0 22246	2 67207
	4	0 093304	0 15504	0 23550	0 33806	0 24502	2 86710
MEAN		0 09081	0 15563	0 23103	0 33069	0 23313	2 82780
SLM	1	0 09461	0 15048	0 23319	0 27816	0 18355	2 39652
	2	0 08803	0 14058	0 22872	0 30152	0 21349	2 73591
	3	0 09469	0 15628	0 22665	0 29032	0 19563	2 48972
	4	0 09276	0 14607	0 21243	0 29946	0 20670	2 60437
MEAN		0 09252	0 14835	0 22275	0 29237	0 19984	2 55663
SHM	1	0 08170	0 14021	0 19878	0 27839	0 19669	2 72438
	2	0 09407	0 15433	0 20534	0 28350	0 18943	2 45150
	3	0 09493	0 14568	0 22650	0 26145	0 16652	2 25134
	4	0 09834	0 15433	0 21139	0 25137	0 15303	2 08554
MEAN		0 09226	0 14864	0 21050	0 26868	0 17642	2 37819
SWP	1	0 09308	0 13169	0 17436	0 23921	0 14613	2 09752
	2	0 09340	0 13108	0 19950	0 27995	0 18655	2 43938
	3	0 09640	0 12803	0 20634	0 26552	0 16912	2 25152
	4	0 09479	0 13964	0 17932	0 24537	0 15058	2 11356
MEAN		0 09442	0 13261	0 18988	0 25751	0 16310	2 22550

Table 8 Result of analysis of variance of data to compare the effect of different protein sources on the specific growth rate (SGR) of *M rosenbergii* juveniles

Source of variation	Degrees of freedom	Sum of squares	Mean sum of square	F ratio
Replication	3	0 14984	0 04995	1 42046
Between feeds	4	1 51979	0 37995	10 80545 *
Error	12	0 42195	0 03516	
Total	19	2 09158		

Critical difference for comparison of treatment mean values-0 4050 (at 1% level)

Pairwise comparison of mean values of SGR using critical difference

Feed	CLM	SQM	SLM	SHM	SWP
Mean values of SGR	2 97022	2 82780	2 55663	2 37819	2 22549
	———	———			
		———	———		
			———	———	
				———	———

Underscored mean SGR values are not significantly different at 1% level

* Significantly different at 1% level

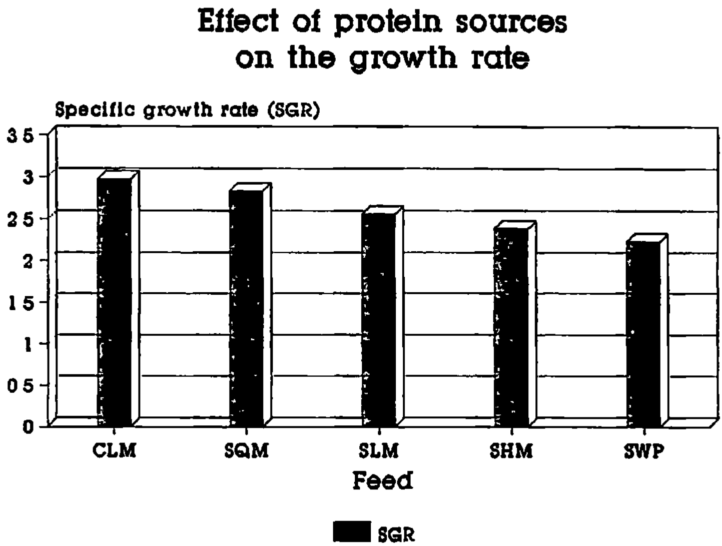

Fig 2 Specific growth rate (SGR) of *M rosenbergii* juveniles fed with diets differing in protein sources

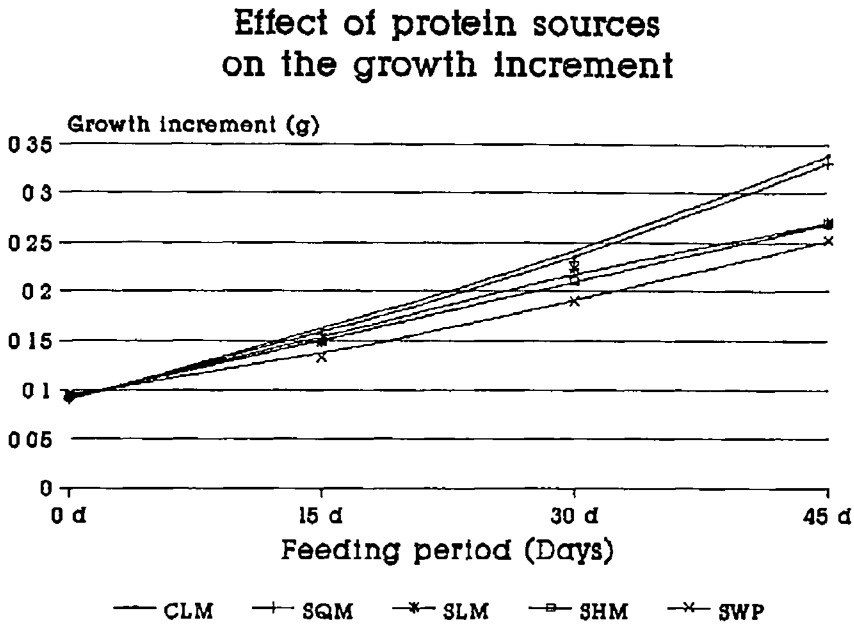

Fig 3 The pattern of weight increase with time when *M rosenbergii* juveniles were fed with diets differing in protein sources

Table 9 Feed conversion efficiency of *M* *rosenbergii* juveniles fed with diets differing in protein sources

FEED	R *	WEIGHT GAIN (g) (A)	FEED INTAKE (g) (B)	FCE (A/B)	MEAN FCE	FCR (B/A)	MEAN FCR
	1	2 03149	5 68048	0 35763		2 79621	
	2	2 40943	6 29762	0 38259		2 61374	
CLM	3	2 11321	5 79420	0 36471	0 37164	2 74190	2 69308
	4	2 40024	6 28971	0 38161		2 62045	
	1	1 60813	4 56259	0 35246		2 83720	
	2	2 41358	6 38959	0 37774		2 64735	
SQM	3	2 22455	5 819884	0 38223	0 36081	2 61621	2 71220
	4	2 11214	5 80869	0 36362		2 75014	
	1	1 55736	5 35133	0 29102		3 43615	
	2	2 13487	6 48660	0 31181		3 20703	
SLM	3	1 95625	6 56537	0 29796	0 29527	3 35610	3 39178
	4	1 46809	5 23793	0 28028		3 56785	
	1	1 68851	5 99759	0 28153		3 55200	
	2	1 89436	6 45847	0 29331		3 40932	
SHM	3	1 66514	5 74912	0 28963	0 28540	3 45263	3 50560
	4	1 27889	4 61842	0 27713		3 60846	
	1	1 46122	5 48434	0 26643		3 75326	
	2	1 58555	6 06466	0 26144		3 82496	
SWP	3	1 42568	5 67909	0 25104	0 26167	3 98340	3 82406
	4	1 50579	5 62355	0 26777		3 73462	

R* Replication

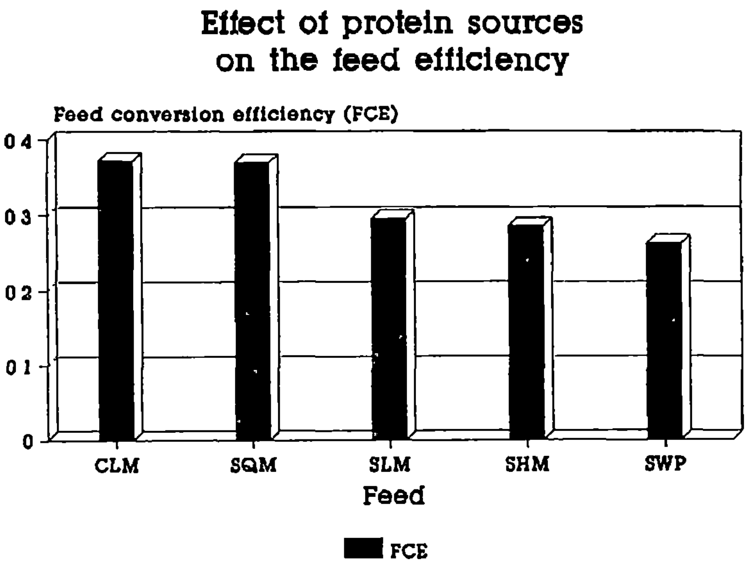

Fig 4 Feed conversion efficiency (FCE) of *M rosenbergii* juveniles fed with diets differing in protein sources

Table 10 Result of analysis of variance of data to compare the effect of different protein sources on the feed conversion efficiency (FCE) of *M rosenbergii* juveniles

Source of variation	Degrees of freedom	Sum of squares	Mean sum f squares	F- ratio
Replication	3	0 00065	0 00022	2 10975
Between feeds	4	0 04089	0 01022	99 76207 *
Error	12	0 00123	0 00010	
Total	19	0 04276		

Critical difference for comparison of treatment mean values=0 02187 (at 1%level)

Pairwise comparison of mean values of FCE with critical difference

Feed	CLM	SQM	SLM	SHM	SWP
Mean values of FCE	0 37164	0 36901	0 29527	0 28540	0 26167

(CLM and SQM underscored together; SLM and SHM underscored together)

Underscored mean FCE values are not significantly different at 1% level

* Significantly different at 1% level

diets, FCE were 0 295 and 0 285 respectively which also not vary significantly from each other Silkworm pupae diet recorded lowest FCE (0 262) among the various feeds differing in protein sources tried and differed significantly from all other treatments

4 1 4 Effect of protein sources on protein efficiency ratio (PER)

The data on protein efficiency ratio of *M rosenbergii* juveniles recorded for feeds based on different protein sources (table 11) showed a significant variation as given in table 12 The variation in PER with respect to protein sources are depicted in fig 5

Diets formulated from clam meal produced highest PER (1 070), closely followed by squid meal diet (1 057), though not significantly different from the former Squilla meal and shrimp head meal diets produced PER values at 0 841 and 0 817 respectively, which are not significantly different Silkworm pupae based diet registered the lowest PER (0 747), which is significantly lower than all other treatments

4 2 Experiment for evaluation of partial substitution of protein with carbohydrate in the formulated feeds

The effect of partial substitution of protein with carbohydrate in the formulated feed of *M rosenbergii* juveniles was evaluated following the same criteria used in the first experiment such as survival rate growth rate, feed conversion efficiency ratio and protein efficiency ratio

Table 11 Protein effıcıency ratıo (PER) of *M rosenbergii* juvenıles fed wıth dıets dıfferıng in proteın sources

FEED	R*	WEIGHT GAIN (g)	FEED INTAKE (g)	PROTEIN INTAKE (g)	PER	MEAN PER
CLM	1	2 03149	5 68048	1 97226	1 03003	1 07038
	2	2 40943	6 29762	2 18653	1 10194	
	3	2 11321	5 79420	2 01175	1 05044	
	4	2 40024	6 28971	2 18379	1 09912	
SQM	1	1 60813	4 56259	1 59280	1 00962	1 05703
	2	2 41358	6 38959	2 23061	1 08203	
	3	2 22455	5 81988	2 03172	1 09491	
	4	2 11214	5 80869	2 02781	1 04158	
SLM	1	1 55736	5 35133	1 87832	0 82913	0 84123
	2	2 13487	6 84660	2 40316	0 88836	
	3	1 95625	6 56537	2 30444	0 84890	
	4	1 46809	5 23793	1 83851	0 79852	
SHM	1	1 68851	5 99759	2 09496	0 80599	0 81707
	2	1 89436	6 45847	2 25594	0 83972	
	3	1 66514	5 74912	2 00817	0 82919	
	4	1 27889	4 61842	1 61196	0 79338	
SWP	1	1 46122	5 48434	1 92226	0 76016	0 74656
	2	1 58555	6 06466	2 12566	0 74591	
	3	1 42568	5 67906	1 99052	0 71623	
	4	1 50579	5 62355	1 97105	0 76395	

R* Replıcatıon

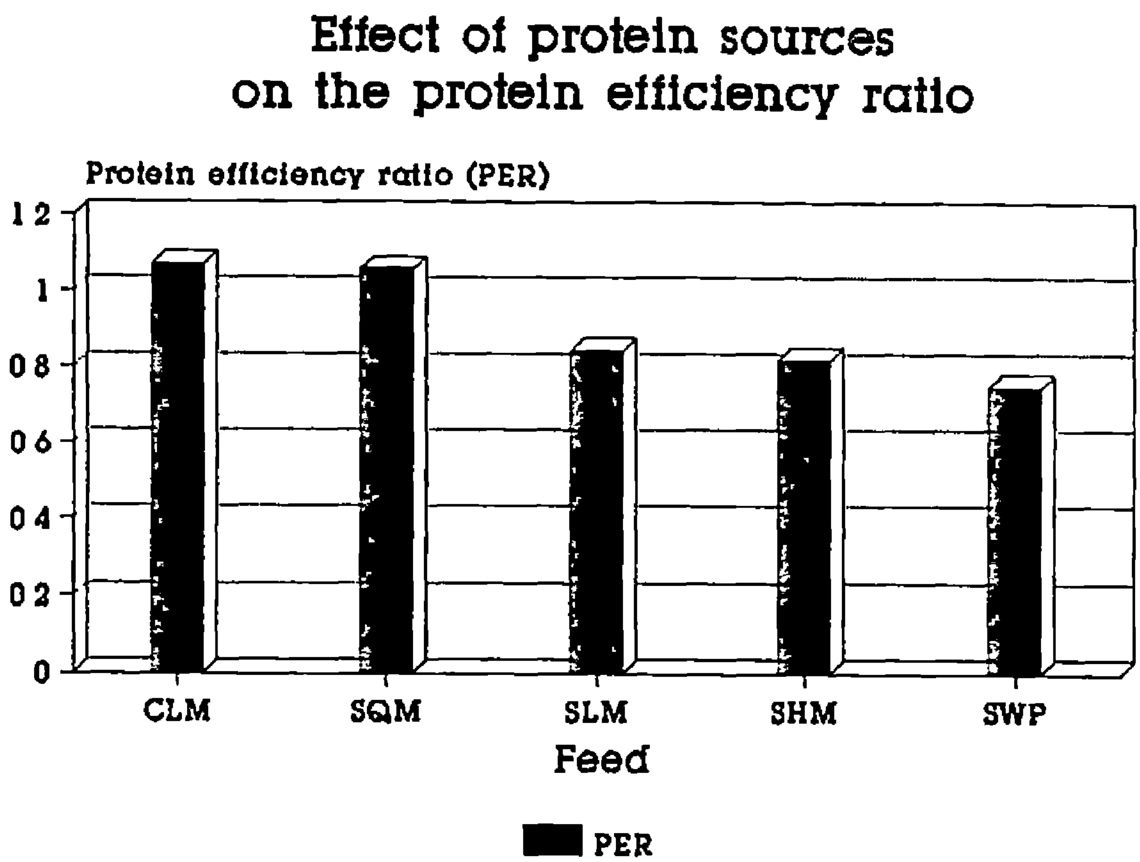

Fig 5 Protein efficiency ratio (PER) of *M rosenbergii* juveniles fed with diets differing in protein sources

Table 12 Result of analysis of variance data to compare the effect of different protein sources on the protein efficiency ratio (PER) of *M rosenbergii* juveniles

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F ratio
Replication	3	0 00532	0 00177	2 11052
Between feeds	4	034942	0 08736	104 00 *
Error	12	0 01008	0 00084	
Total	19	0 36482		

Critical difference for comparison of treatment mean values-0 0626 (at1% level)

Pairwise comparison of mean values of PER using critical difference

Feed	CLM	SQM	SLM	SHM	SWP
Mean values of PER	1 07038	1 05703	0 84123	0 81707	0 74656

Underscored mean PER values are not significantly different at 1% level

* Significantly different at 1% level

4 2 1 Effect of partial substitution of protein with carbohydrate on survival rate

The percentage survival rates of *M rosenbergii* fed on diets differing in carbohydrate levels are given the table 13 and graphically represented in fig 6

The analysis of variance of data showed that the influence of partial substitution of carbohydrate for protein on survival rates of prawns is not varying significantly (table 14)

4 2 2 Effect of partial substitution of protein with carbohydrate on growth rate

The specific growth rate (SGR) recorded for *M rosenbergii* juveniles fed on respective diets are given in table 15 and graphically presented in fig 7

The analysis variance data suggests that dietary carbohydrate substitution for protein in the test diets of prawns has a significant influence on SGR (table 16)

The pattern of SGR values shows that, at each level of protein, additional dietary carbohydrate produced increased growth rate, besides higher growth rate at successively higher protein levels used in the trials

At 25% protein, increasing the level of dietary carbohydrate produced only a non significant increase in SGR

But at 30% protein with 20% carbohydrate (P2C1), SGR recorded did not differ significantly from that of 25% protein and 30% carbohydrate (P1C3) But P2C2 having 30% protein and 25% carbohydrate and P2C3 with 30% protein and 30% carbohydrate recorded significantly higher SGR than that of P2C1

Table 13 Details of initial number and percentage survival rate of *M rosenbergii* juveniles fed with test diets to study the effect of partial substitution of protein with carbohydrate

Feed	R*	No of prawn		Survial rate (%)	Mean survial rate
		Inital	Final		
P1C1	1	10	10	100	93 33
	2	10	9	90	
	3	10	9	90	
P1C2	1	10	9	90	96 67
	2	10	10	100	
	3	10	10	100	
P1C3	1	10	9	90	96 67
	2	10	10	100	
	3	10	10	100	
P2C1	1	10	9	90	93 33
	2	10	10	100	
	3	10	9	90	
P2C2	1	10	10	100	96 67
	2	10	9	90	
	3	10	10	100	
P2C3	1	10	10	100	90 00
	2	10	8	80	
	3	10	9	90	
P3C1	1	10	10	100	96 67
	2	10	10	100	
	3	10	9	90	
P3C2	1	10	9	90	93 33
	2	10	9	90	
	3	10	10	100	
P3C3	1	10	9	90	96 67
	2	10	10	100	
	3	10	10	100	

R* Replication

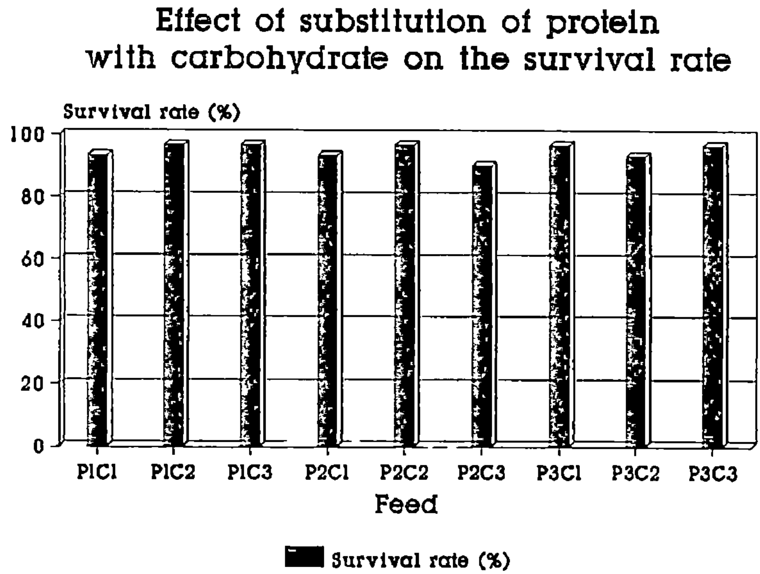

Fig 6 Survival rate (% of M rosenbergii juveniles fed with test diets to study the effect of partial substitution of protein with carbohydrate

Table 14 Result of analysis of variance of data to compare the effect of partial substitution of protein with carbohydrate on survival rate of *M rosenbergii* juveniles

(Data subjected to Arc sine transformation)

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F ratio
Replication	2	7 85938	3 92969	0 03228
Between feeds	8	542 01560	67 75195	0 55658
Error	16	1947 65600	121 72850	
Total	26	2497 53100		

(Calculated F value < table value at 1% level of significance)

Table 15 Specific growth rate (SGR) of *M rosenbergii* juveniles fed with test diets to study the effect of partial substitution of protein with carbohydrate

FEED	REP	WEIGHT OF PRAWN (g) Initial	15th day	30th day	FINAL WEIGHT 45th day	WEIGHT GAIN 45 day	SGR (% day⁻¹)
P1 C1	1	0 11023	0 15194	0 19534	0 24732	0 13709	1 79581
	2	0 11601	0 14202	0 18198	0 23109	0 11508	1 53140
	3	0 10478	0 15340	0 19636	0 25[illegible]47	0 15169	1 98922
MEAN		0 11034	0 14912	0 19123	0 24496	0 13462	1 77214
P1 C2	1	0 11601	0 16146	0 20172	0 26250	0 14619	1 81461
	2	0 10792	0 15402	0 20530	0 26031	0 15239	1 95663
	3	0 10483	0 14536	0 19548	0 25439	0 14056	1 89002
MEAN		0 10959	0 15361	0 20083	0 25607	0 14648	1 88709
P1 C3	1	0 10893	0 15637	0 20887	0 26358	0 15465	1 96367
	2	0 11200	0 15133	0 20231	0 26136	0 14936	1 88311
	3	0 12141	0 17006	0 22428	0 27704	0 15563	1 83331
MEAN		0 11411	0 15925	0 21182	0 26733	0 15321	1 89336
P2 C1	1	0 11203	0 16016	0 23204	0 29216	0 18013	2 13008
	[illegible]	0 11939	0 16867	0 24356	0 30581	0 18642	2 09015
	3	0 11[illegible]	0 17132	[illegible]	0 2[illegible]	0 17[illegible]	2 09313
MEAN		[illegible]	0 16[illegible]72	0 23599	[illegible] 29513	0 18064	2 10445
P2 C2	1	[illegible] 10830	[illegible] 15945	0 23801	0 31292	0 201[illegible]	2 35787
	2	0 10962	0 [illegible]	[illegible]	0 [illegible]	0 198[illegible]	2 29840
	3	0 102[illegible]	0 15436	0 2[illegible]	0 29815	0 19570	2 37383
MEAN		0 10[illegible]79	0 15958	0 22504	0 30[illegible]48	0 19969	2 34337
P2 C3	1	0 11048	0 17017	0 27128	0 35616	0 21568	2 60121
	2	0 10692	0 16601	0 26216	0 34274	0 23582	2 58865
	3	0 12307	0 17824	0 28403	0 36936	0 24629	2 44226
MEAN		0 11349	0 17147	0 27219	0 35609	0 24260	2 54404
P3 C1	1	0 10322	0 17499	0 2[illegible]500	0 35847	0 2[illegible]25	2 76663
	2	0 11809	0 18930	0 2714[illegible]	0 37[illegible]31	0 2612[illegible]	2 59313
	3	0 11038	0 18296	0 27924	0 36806	0 25768	2 67626
MEAN		0 11056	0 18242	0 27190	0 36861	0 25865	2 67867
P3 [illegible]2	1	0 10341	0 18934	0 2[illegible]	0 37817	0 27476	2 88143
	2	0 11707	0 19758	0 [illegible]	[illegible]	[illegible]	2 71101
	3	0 11238	[illegible] 18960	0 [illegible]352	0 381[illegible]	0 [illegible]	2 71582
MEAN		0 110[illegible]5	0 19217	0 2[illegible]873	0 38538	0 27413	2 79642
P3 C3	1	0 11721	0 21358	0 31917	0 42501	0 31280	2 95942
	2	0 10601	0 20740	0 30890	0 [illegible]0413	0 29535	2 95850
	3	0 10984	0 21765	0 32272	0 41337	0 30413	2 94837
MEAN		0 10935	0 21288	0 31693	0 41345	0 30409	2 95543

[illegible]

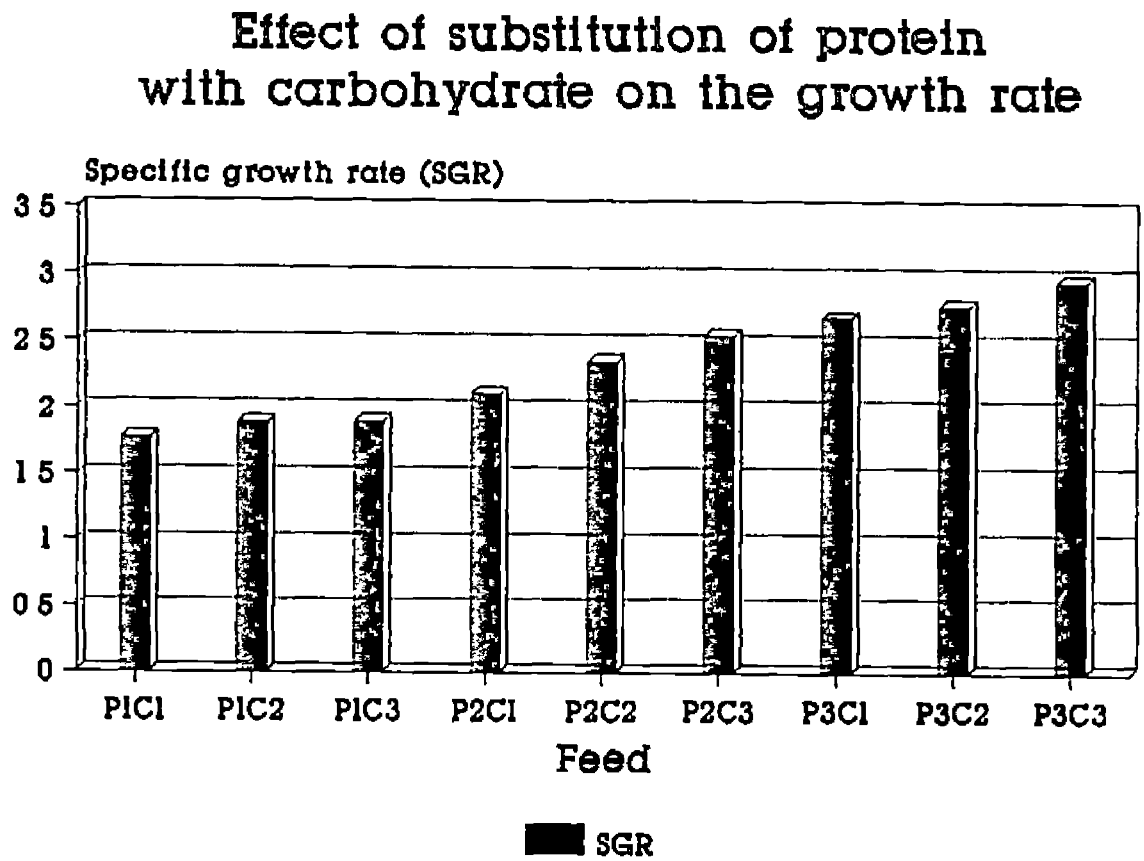

Fig 7 Specific growth rate (SGR) of *M rosenbergii* juveniles fed with test diets to study the effect of partial substitution of protein with carbohydrate

Table 16 Result of analysis of variance of data to compare the effect of partial substitution of protein with carbohydrate on specific growth rate (SGR) of *M rosenbergii* juveniles

Source of variation	Degrees of freedom	Sum of of squares	Mean sum of squares	F ratio
Replication	2	0 02423	0 01212	1 25982
Between feeds	8	4 50201	0 56275	58 51725 *
Error	16	0 15387	0 00962	
Total	26	4 68011		

Critical difference for comparison of treatment mean values-0 23389 (at 1% level)

Comparison of mean values of SGR with critical difference value

Feed	Mean SGR values
P1C1	1 77214
P1C2	1 88709
P1C3	1 89336
P2C1	2 10445
P2C2	2 34337
P2C3	2 54404
P3C1	2 67867
P3C2	2 76942
P3C3	2 95543

Sidescored mean SGR values are not significantly different at 1% level

* Significantly different at 1% level

At 35% protein level, 20 and 25% carbohydrate levels (diets P3C1 and P3C2 respectively) registered SGR values which are not significantly different from each other as well as that of diet with 30% protein and 30% carbohydrate (P2C3) Highest value of SGR was recorded by *M rosenbergii* juveniles fed on diet with 35% protein and 30% carbohydrate (P3C3)

4 2 3 Effect of partial substitution of protein with carbohydrate on feed conversion efficiency (FCE)

The details of feed consumption, weight gain and FCE of *M rosenbergii* juveniles fed with the test diets for evaluating the effect of partial substitution of protein with dietary carbohydrate presented in the table 17 and the pattern of FCE values plotted in fig 8, which suggest a progressive rise in FCE at successively higher levels of protein and of carbohydrate levels at each level of protein

Analysis of variance suggests that the treatments had a profound influence on FCE of *M rosenbergii*(table 18)

At 25% protein the lowest level of carbohydrate (20%) produced a significantly lower FCE of 0 299 compared to 0 314 and 0 321 respectively obtained for higher levels of carbohydrate (25 and 30%), the latter two being not significantly different from each other

At 30% protein the lowest carbohydrate level of 20% produced a FCE of 0 334 which is not significantly higher than that observed for P1C3 (25% protein and 30% carbohydrate) At this level of protein (30%) higher carbohydrate levels of 25 and 30% gave FCE values of 0 35 and 0 357 respectively which do not differ significantly

Table 17 Feed conversion efficiency (FCE) of *M rosenbergii* juveniles fed with test diets to study the effect of partial substitution of protein with carbohydrate

FEED	R*	WEIGHT GAIN (g) (A)	FEED INTAKE (g) (B)	FCE (A/B)	MEAN FCE	FCR (B/A)	MEAN FCR
P1C1	1	1 37090	4 48813	0 30545	0 29922	3 27386	3 34271
	2	0 91966	3 11270	0 29545		3 38462	
	3	1 26049	4 24740	0 29677		3 36964	
P1C2	1	1 20242	3 78923	0 31733	031448	3 15134	3 18022
	2	1 52384	4 82492	0 31583		3 16629	
	3	1 40568	4 53056	0 31027		3 22304	
P1C3	1	1 28300	3 94352	0 32534	0 32110	3 07368	3 11461
	2	1 49358	4 69950	0 31782		3 14647	
	3	1 55622	4 86113	0 32014		3 12368	
P2C1	1	1 50919	4 40843	0 34234	0 33435	2 92106	2 99190
	2	1 86425	5 70168	0 32697		3 05843	
	3	1 46610	4 39276	0 33375		2 99622	
P2C2	1	2 04623	5 85103	0 34972	0 35127	2 85942	2 84730
	2	1 67912	4 69522	0 35762		2 79624	
		1 95707	5 64859	0 34647		2 88625	
P2C3	1	2 45680	6 92719	0 35466	0 35658	2 81960	2 80549
	2	1 67277	4 57119	0 36594		2 73271	
	3	2 09349	5 99607	0 34914		2 86415	
P3C1	1	2 55250	7 28264	0 35049	0 36071	2 85314	2 77350
	2	2 61222	7 11725	0 36703		2 72460	
	3	2 20876	6 05807	0 36460		2 74275	
P3C2	1	2 36938	6 54946	0 36177	0 36624	2 76421	2 73078
	2	2 39795	6 55523	0 36581		2 73368	
	3	2 69075	7 25006	0 37113		2 69444	
P3C3	1	2 70304	6 62328	0 40811	0 39446	2 45031	2 537 o
	2	2 95342	7 75677	0 38075		2 62637	
	3	3 04130	7 70909	0 39451		2 53480	

R* Replication

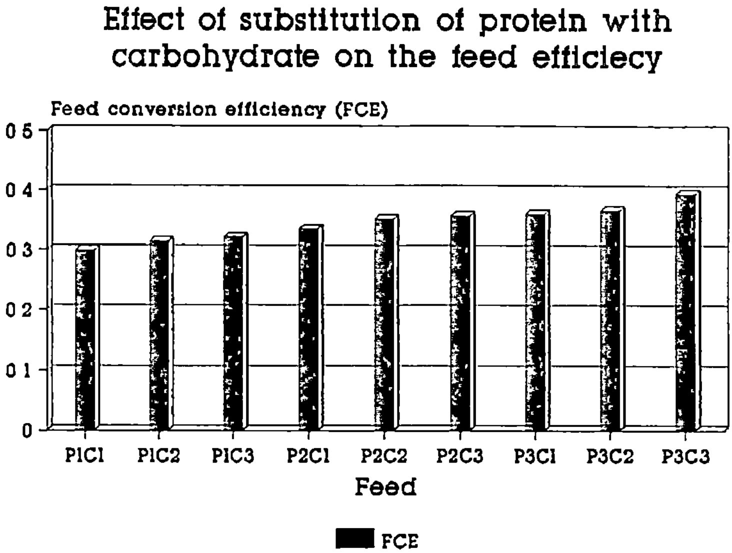

Fig 8 Feed conversion efficiency of *M rosenbergii* juveniles fed with test diets to study the effect of partial substitution of protein with carbohydrate

Table 18 Result of analysis of variance of data to compare the effect of partial substitution of protein with carbohydrate on feed conversion efficiency (FCE) of *M. rosenbergii* juveniles

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-ratio
Replication	2	0.00005	0.00002	0.40655
Between feeds	8	0.02107	0.00263	43.18788 *
Error	16	0.00098	0.00006	
Total	26	0.02210		

Critical difference for comparison of treatment mean values=0.01461 (at 1%level)

Comparison of mean FCE values with critical difference value:

Feed	Mean values of FCE
P1C1	0.29922
P1C2	0.31448
P1C3	0.32110
P2C1	0.33435
P2C2	0.35127
P2C3	0.35658
P3C1	0.36071
P3C2	0.36624
P3C3	0.39446

Sidescored mean FCE values are not significantly different at 1% level

* Significantly different at 1% level

At the highest level of protein (35%), the observed value of FCE with 20% carbohydrate (P3C1) was not significantly higher than that of the diet with 30% protein and 30% carbohydrate (P2C3). At this level of protein (35%), it was found that , 20 and 25% carbohydrate levels produced FCE values which do not differ much.

The feed having 35% protein and 30% carbohydrate (P3C3), has given the highest FCE value (0.394), which is significantly higher than all other combinations of protein and carbohydrate levels.

4.2.4 Effect of partial substitution of protein with carbohydrate on protein efficiency ratio (PER)

The data on observed PER values for the various diets are presented in table 19 and graphically represented in fig. 9.

Analysis of variance of data suggests that the various treatments had a significant influence on PER of *M. rosenbergii* (table 20).

The trend of PER shows that, successively higher protein levels produced lower efficiency of protein utilization for growth, but at each level of protein, increasing the carbohydrate levels effected better utilization of protein for growth.

At 25% protein, the lowest PER was noted for diet with 20% carbohydrate (P1C1), which is significantly lower than that of higher carbohydrate levels tried. These two latter PER values were not significantly different.

Table 19. Protein efficiency ratio (PER) of *M. rosenbergii* juveniles fed with test diets to study effect of *partial substitution of protein with carbohydrate*

FEED	R *	WEIGHT GAIN (g) (A)	FEED INTAKE (g)	PROTEIN INAKE (g) (B)	PER (A/B)	MEAN PER
P1C1	1	1.37090	4.48813	1.13280	1.21018	1.18551
	2	0.91966	3.11270	0.78565	1.17058	
	3	1.26049	4.24740	1.07204	1.17578	
P1C2	1	1.20242	3.78923	0.94276	1.27542	1.26396
	2	1.52384	4.82492	1.20044	1.26940	
	3	1.40568	4.53056	1.12720	1.24705	
P1C3	1	1.28300	3.94352	0.98982	1.29619	1.27928
	2	1.49358	4.69950	1.17957	1.26620	
	3	1.55622	4.86113	1.22014	1.27544	
P2C1	1	1.50919	4.40843	1.31151	1.15073	1.12388
	2	1.86425	5.70168	1.69625	1.09904	
	3	1.46610	4.39276	1.30685	1.12186	
P2C2	1	2.04623	5.85103	1.76701	1.15802	1.16315
	2	1.67912	4.69522	1.41796	1.18418	
	3	1.95707	5.64859	1.70587	1.14725	
P2C3	1	2.45680	6.92719	2.06430	1.19014	1.19658
	2	1.67277	4.57119	1.36221	1.22798	
	3	2.09349	5.99607	1.78683	1.17162	
P3C1	1	2.55250	7.28264	2.52271	1.01181	1.04130
	2	2.61222	7.11725	2.46542	1.05955	
	3	2.20876	6.05807	2.09852	1.05253	
P3C2	1	2.63938	6.54946	2.28576	1.03658	1.04939
	2	2.39795	6.55523	2.28778	1.04816	
	3	2.69075	7.25006	2.53027	1.06342	
P3C3	1	2.70304	6.62328	2.32808	1.16106	1.12221
	2	2.95342	7.75677	2.72650	1.08323	
	3	3.04130	7.70909	2.70975	1.12236	

R* Replication

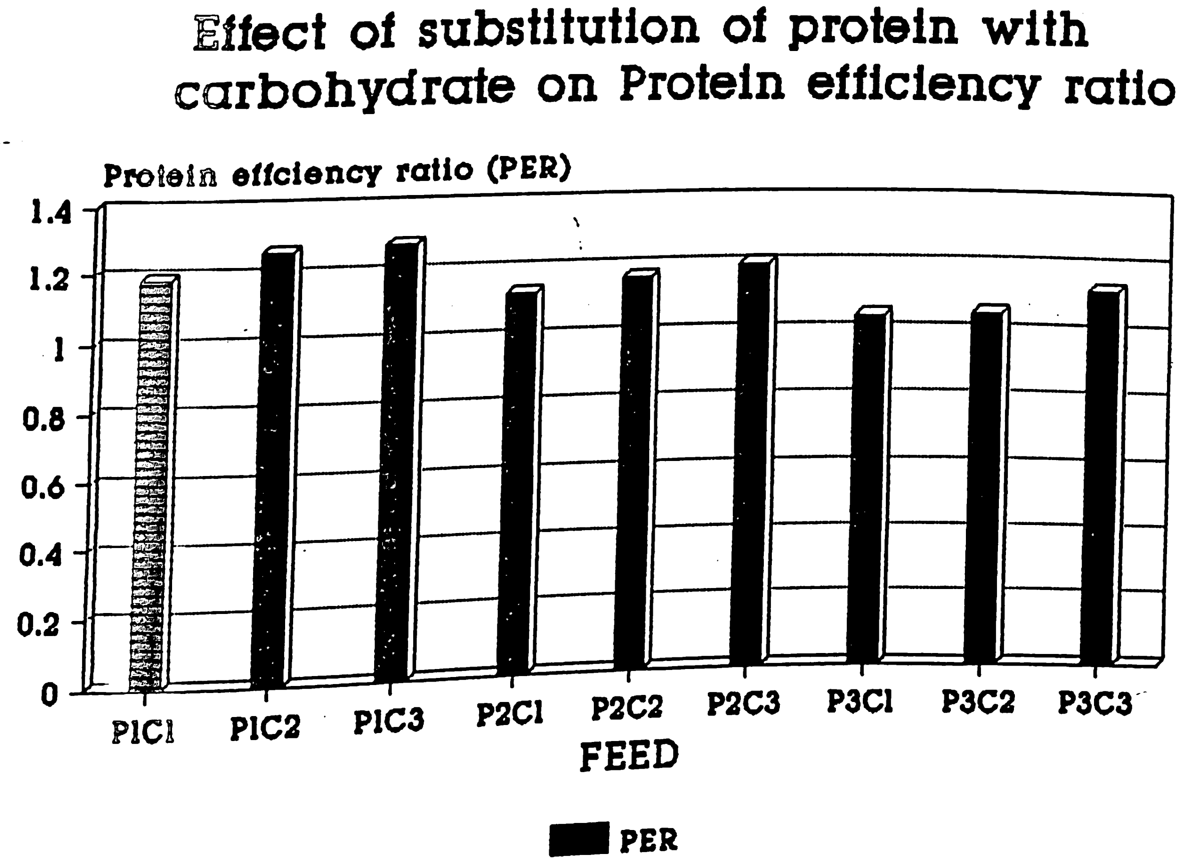

Fig.9 Protein efficiency ratio of *M. rosenbergii* juveniles fed with test diets to study the effect of partial substitution of protein with carbohydrate

Table 20 Result of analysis of variance of data to compare the effect of partial substitution of protein with carbohydrate on protein efficiency ratio (PER) of *M. rosenbergii* juveniles

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-ratio
Replication	2	0.00075	0.00038	0.63090
Between feeds	8	0.16810	0.02101	35.28183 *
Error	16	0.00953	0.00060	
Total	26	0.17838		

Critical difference for comparison of treatment mean values=0.04565 (at 1% level)

Comparison of mean values of PER with critical difference value:

Feed	Mean values of PER
P1C1	1.18551
P1C2	1.26396
P1C3	1.27928
P2C1	1.12388
P2C2	1.16315
P2C3	1.19658
P3C1	1.04130
P3C2	1.04954
P3C3	1.12222

Sidescored mean PER values are not significantly different at 1% level

* Significantly different at 1% level

At 30% protein, two adjacent levels of carbohydrate (20 & 25% and 25 & 30 %) did not differ each other in terms of PER, ie.,P2C1 & P2C2 are equivalent; so also, P2C2 & P2C3, with respect to PER.

At 35% protein, the PER values observed for diet with first two levels of carbohydrate (20 & 25%), are not different pairwise. But diet with 30% carbohydrate is significantly higher than the other two diets (P3C1 & P3C2), in terms of PER.

The highest PER, was recorded by the diet, P3C3 (25% protein and 30% carbohydrate) which was 1.279; while the lowest was by P3C1 (35% protein and 20% carbohydrate).

4.3 Physico-chemical parameters

The physico-chemical parameters were monitored during the feeding experiment. It was observed that the temperature of rearing water varied between 27 and 31°C while pH ranged between 7 and 8. The dissolved oxygen values were found to be in the range of 6-7 ppm.

V DISCUSSION

5.1 Evaluation of quality of protein sources

The present study conducted to evaluate the nutritional quality of different locally available sources of protein, viz, clam meal, squid meal, squilla meal,shrimp head meal and silkworm pupae in the formulated feed of the juveniles of *M. rosenbergii*, based on survival rate, growth, feed conversion efficiency and protein efficiency ratio had shown clam meal followed by squid meal as better sources of protein.

5.1.1 Survival rate

High survival rate (>90%) was observed for *M. rosenbergii* juveniles fed with diets differing in protein sources, suggesting that the different protein sources tested do not show any variation with regard to their influence on survival rate and can be incorporated in the diet without any deleterious effect on the rate of survival. New (1976), while reviewing the dietary studies of shrimps and prawns, opined that mortalities in nutritional studies are rare, unless the diet is grossly deficient in nutrients; being particularly true when animals are individually housed. Although, variations in growth rate and feed efficiency were observed when diets differing in protein sources were tested, the high survival rate observed in the present study, irrespective of the source of protein, suggests that the sources evaluated in general are not grossly deficient in their nutritional quality.

Biddle (1977) has cautioned drawing conclusions regarding the quality of diets used, on the basis of short term growth studies before considering the likely existence of nutrient reserves in the body of prawns, their ability to preferentially conserve specific nutrient that is deficient in the diet and the possibility of adequate quantity of amino acids being made available for absorption from microbial synthesis in the intestine of prawn. The short duration of the experiment in the present case may be a factor which has contributed to the absence of any significant variation in the rate of survival of *M. rosenbergii* juveniles fed on diet differing in the source of protein.

5.1.2 Growth rate

Among the different feeds tested in the present study, the diet based on clam meal gave the highest the growth rate, closely followed by squid meal diet; the difference in response being insignificant. Thus, these two can be considered more or less equivalent as far as the quality of protein is concerned. The lowest growth rate was recorded for silkworm pupae diet; while intermediate growth was obtained for the two crustacean meal diets tested namely, squilla meal and shrimp head meal diets.

Fresh clam meat is conventionally used for feeding shrimps. Kanazawa *et al*(1970) reported that fresh short necked clam, *Tapes philippinarum* gave superior growth compared to other compounded diets for *P. japonicus*. Deshimaru and Shigueno (1972) attributed the reason for superiority of short necked clam as the similarity in amino acid profile of this with that of *P. japonicus*. Forster and Beard (1973) also reported higher growth rate and survival for *Palaemon serratus* when fresh clam was used. Ali (1982a) while evaluating the nutritional quality of certain protein sources for *P. indicus* juveniles observed good growth with clam meat based diet which was on par with that of squilla based diet. But superior feed efficiency was noted for clam meal diet. Josekutty (1991) found clam meal as the best source of protein for *P. monodon* among the various sources tested. Sherief (1989) observed clam meat as principal protein source giving better result in terms of growth and survival compared to a diet based on fish meal in the case of *M. rosenbergii*.

Recently, Rao (1994) stated that fresh clam meat along with mantle fluid could be incorporated along with other low quality locally available ingredients, the fresh clam serving as an attractive flavouring agent. In the present study clam meal would have served also as an attractant. But the quality of clam meal as a better source was established beyond doubt as the highest protein efficiency and feed efficiency were recorded for clam meal based diet. Narayana and Alexander (1972), while investigating biochemical composition of different molluscs found the black clam, *Villorita* as a good source of protein and rich in free amino acid. Deshimaru *et al.*(1985) demonstrated that fresh clam could produce superior growth, survival and feed efficiency compared to a series of compounded diets in *P. monodon* juveniles and stated that fresh short necked clam is rich in essential amino acids with high ratios of methionine and arginine to total amino acid content, besides being rich in polar lipids and sterols. Prawns and shrimps are known to rely mainly on trypsin and to a lesser extent, chymotrypsin for protein digestion (Lee *et al.*,1980; Lan and Pan, 1993). Trypsin cleaves protein at the peptide bonds adjacent to the basic amino acids while, chymotrypsin cleaves at aromatic amino acids (Lan and Pan, 1993). Since basic amino acids are the specific sites for action of trypsin, the main proteolytic enzyme of prawns and shrimps, higher content of basic amino acid in the feed provides more sites available for trypsin action. Available literature suggests that clam is a rich source of basic amino acids especially arginine and this could be one of the possible reasons for better growth, protein utilization and feed efficiency observed for clam in the present study. Earlier, Deshimaru and Shigueno (1972); New (1976); and Colvin and Brand (1977) found the nutritive value of fish meal, deficient in arginine - a basic amino acid, as inferior to that of squid meal which is rich in basic amino acids, as a dietary protein source for *P. japonicus*. It was further demonstrated that diets enriched with arginine by compounding feed with ingredients such as clam, prawn meal and squid meal rich in arginine improved growth and feed efficiency of shrimp (Deshimaru *et al.*,1985). Penaflorida (1989) opined that arginine is the most limiting amino acid of prawn diet and squid and shrimp meal are close to arginine level of prawn muscle. Arginine is reported to be required by all crustaceans primarily due the lack of urea cycle which is necessary for arginine biosynthesis from ornithine (Miyajima *et al.*,1977).

Apart from the quality of the protein, clam meal is also rich in polyunsaturated fatty acids (PUFA), especially rich in n-3 PUFA which is reported to have a growth enhancing effect on *M. rosenbergii* as reported by Sandifer and Joseph (1976).

Superiority of squid meal as a protein source has been demonstrated in many species of shrimps. Kitabayashi *et al.*(1971) and Deshimaru and Shigueno (1972) in *P. japonicus*; Fenucci and Zein-Eldin (1976) in *P. aztecus*; Fenucci *et al.*(1980) in *P. setiferus* and *P. stylirostris*; Cruz-Ricque and AQUACOP (1987) in *P. monodon*; Dokken and Lawrence (1985) in *P. vannamei* and Cruz-Ricque *et al* (1987) in *P. indicus* had found squid meal as a superior protein source.

In the present study, the nutritional quality of squid meal has been found to be equivalent to that of clam meal for *M rosenbergii* Deshimaru and Shigueno (1972) who found squid meal as an excellent protein source for *P japonicus* stated that the superiority of squid meal is due to the essential amino acid composition of squid protein which is similar to that of *P japonicus* Fenucci and Zein Eldin (1976) attributed higher amount of arginine in squid protein as a possible reason for enhanced growth rate in *P aztecus* Deshimaru *et al* (1985) also opined that squid is rich in arginine, an essential amino acid Cruz Ricque *et al* (1987) evaluated the growth response of supplementation of a mixed diet with protein extracted from frozen squid at different levels (1 5 3, 6 and 16%) in the diet of four species of shrimps In *P stylirostris* and *P vannamei* growth rates were significantly improved even at the lowest level of supplementation Improvement was obtained only with 6 and 16% in *P monodon* while no improvement was observed in *P indicus* suggesting a species specific growth enhancement effect of squid protein The growth promoting effect for squid protein at 16% supplementation could be explained by its nutritional value, particularly amino acid content However, at lower levels the result obtained seemed to be due to an unknown growth factor Based on their study Cruz Ricque *et al* (1987) suggested this growth promoting effect to be due to a protein fraction of squid tissue and not related to amino acid balance

According to Akiyama *et al* (1992) this growth factor, which is believed to be small peptides increases the digestive efficiency of shrimp Cruz Suarez *et al* (1987) observed that the growth promoting effect of squid is not due to an amino acid supply, but due to increase in metabolic activity and protein synthesis per cell, since it tends to induce hypertrophy of cell and not hyperplasy Later, Cruz Rique *et al* (1989) found levels of glucose and amino acids in the haemolymph of *P japonicus* receiving diet containing squid decreasing faster, indicating an enhancing effect of squid protein on the metabolism of shrimp Squid meal is also an attractant and its level in commercial feeds ranges from 2 to 10% There is no nutritional limitation in squid meal, however, its use is normally limited by price and availability as pointed out by Akiyama *et al* (1992)

Crustacean meals have been tested extensively by many in shrimp feeds expecting them to produce better result owing to their similarity in amino acid profile with the body protein of shrimp In the present study the two crustacean meals namely, squilla and shrimp head meals produced only an intermediary growth rate among the five protein sources tested

Ali (1982a) while comparing different sources of protein, found squilla as the best protein source for *P indicus* being slightly better than that of clam meal But feed efficiency was found to be better for clam based diet From the results of the present study it can be seen that clam meal is superior in terms of growth and feed efficiency in the formulated feed of *M rosenbergii* Better growth response of squilla diet obtained by Ali (1982a) might be due to the use of purified protein which was obtained by boiling squilla followed by drying of coagulated protein In the present study whole squilla was used and the result obtained is in agreement with that of Ali (1988) who observed superiority of clam meal over squilla in terms of growth and feed efficiency when whole squilla was incorporated in the diet of *P indicus* Squilla is a locally available source of protein which is also inexpensive and at present not properly utilized Although squilla as a single protein source resulted in lower growth rate in the present study, partial substitution of this for fish meal could be done for compounding commercial shrimp feeds as in the case of shrimp meal Squilla was categorized by Wood *et al* (1991) as a potential, at the same time cheap and locally available protein source for compounding cost effective practical shrimp feed Ali and Mohamed (1985) suggested the possibility of using a combination of shrimp and squilla in the practical feed of shrimp The over dependence on fish meal may cause economic constraints in some geographic areas especially in developing countries, where fish meal is less abundant Also in the more developed countries difficulties are some times encountered due to fluctuations in fish meal supply and its price when supply is short (Lovel, 1989) So, partial substitution of fish meal with squilla meal appears to be promising when used in combination

Shrimp meal has been regarded as a suitable source of protein in shrimp feed Deshimaru and Shigueno (1972) attributed similarity in amino acid profile as the possible reason for better performance of shrimp fed with shrimp meal diet Sick and Andrews (1973) obtained higher growth and survival in *P duorarum* fed with shrimp meal diet Ali (1988) while evaluating different protein sources for *P indicus* observed best growth and survival for shrimp meal diet although feed efficiency was better for clam based diet In *Palaemon serratus*, Forster and Beard (1973) found better performance for shrimp meal than with fish meal in the diet Contrary to this Pascual and Destajo (1979) observed poor survival and growth of *P monodon* postlarvae fed on diet prepared exclusively using shrimp meal since according to them shrimp meal alone cannot fully satisfy the nutritional demands of shrimp Josekutty (1991) also observed lower growth in *P monodon* when shrimp meal was used as protein source compared to clam meal and slaughter house waste

Shrimp meal is high in crude protein content and several amino acids (Forster 1976) It is a good source of fatty acids (Sandifer and Joseph 1976) and appears promising for compounding shrimp feed (Venkataramaigh *et al* 1978 Ali 1988

Table 20 Result of analysis of variance of data to compare the effect of partial substitution of protein with carbohydrate on protein efficiency ratio (PER) of *M rosenbergii* juveniles

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F ratio
Replication	2	0 00075	0 00038	0 63090
Between feeds	8	0 16810	0 02101	35 28183 *
Error	16	0 00953	0 00060	
Total	26	0 17838		

Critical difference for comparison of treatment mean values-0 04565 (at 1% level)

Comparison of mean values of PER with critical difference value

Feed	Mean values of PER
P1C1	1 18551
P1C2	1 26396
P1C3	1 27928
P2C1	1 12388
P2C2	1 16315
P2C3	1 19658
P3C1	1 04130
P3C2	1 04954
P3C3	1 12222

Sidescored mean PER values are not significantly different at 1% level

* Significantly different at 1% level

At 30% protein, two adjacent levels of carbohydrate (20 & 25% and 25 & 30 %) did not differ each other in terms of PER, ie ,P2C1 & P2C2 are equivalent so also, P2C2 & P2C3, with respect to PER

At 35% protein, the PER values observed for diet with first two levels of carbohydrate (20 & 25%), are not different pairwise But diet with 30% carbohydrate is significantly higher than the other two diets (P3C1 & P3C2) in terms of PER

The highest PER, was recorded by the diet, P3C3 (25% protein and 30% carbohydrate) which was 1 279 while the lowest was by P3C1 (35% protein and 20% carbohydrate)

4 3 Physico chemical parameters

The physico-chemical parameters were monitored during the feeding experiment It was observed that the temperature of rearing water varied between 27 and 31°C while pH ranged between 7 and 8 The dissolved oxygen values were found to be in the range of 6 7 ppm according to Richards (1990) is a natural source of choline which is an essential component of phospholipid and acetyl choline, choline playing a vital role in the maintenance of cell structure, transmission of nerve impulses synthesis of methionine and transport of lipid within the body of shrimp

A major disadvantage of shrimp meal is stated to be its high fibre and chitin content The true protein value of shrimp waste is reported to be only about 50 70% of its apparent or crude protein content, depending on the proportion of meat portion to shells in the original material, as much of the crude protein is derived from chitin (Richards 1990) Processing methods followed are also reported to affect the nutritional quality of shrimp meal (Cruz Suarez *et al* 1993) According to Hanson and Goodwin (1977), penaeids appear to have two chitinase systems, one largely in the hepatopancreas and the other centered around chitinoclastic bacteria in the digestive gland Many reports showing measurable levels of chitin degrading enzymes in the alimentary tract of various crustaceans (Chandramohan and Thomas,1984 Lynn,1990 and Spindler Bath *et al* 1990) are available In addition, it is commonly observed that shrimps and prawns consume exuviae following ecdysis These clearly suggest that crustaceans may be able to utilize dietary chitin Vaitheswaran and Ali(1988) reported that glucosamine and chitin have a growth promoting effect in *P indicus*

Among the various protein sources tried in the present study diet based on silkworm pupae produced lowest growth rate Ali(1988) while experimenting with *P indicus* juveniles observed poor growth rates when silkworm pupae were used as protein source But later Unnikrishnan *et al* (1992) explored the possibility of using silkworm pupae in the diet of *M rosenbergii* postlarvae and reported that the growth and survival rates equivalent to clam meal diet were obtained

The results obtained in the present study indicate that although the rate of survival was not affected, the growth rate of those fed with silkworm pupae feed was markedly affected and it was the lowest compared to the other sources of protein It may be due to the fact that among the various sources of protein tested in the present study, silkworm pupae was the only one that is of terrestrial origin Deshimaru and Shigueno (1972) opined that proteins of marine origin are rich in essential amino acids compared to terrestrial origin which determine their quality as feed ingredient for shrimp The proteins of marine origin have a relatively high proline and arginine content, which may account for the good growth of prawn fed with protein from these sources (Dall *et al* , 1990) Ali (1988) has attributed the high lipid content in silkworm pupae, which produce an off flavour due to rancidity of lipid making the feed not attractive or acceptable to the shrimp, as the reason for poor growth The silkworm pupae used in the present study was solvent extracted to remove the excess lipid So the possibility of producing off flavour is not there The highest feed conversion ratio observed for silkworm pupae based diet in the present study also suggests that the palatability of the diet was not affected due to any off flavour The result of the present study thus indicates that silkworm pupae as a single protein is not ideal for compounding feed However, it may be possible to use it in combination with other protein sources

5 1 3 **Feed conversion efficiency**

The feed conversion efficiency values obtained in the present study using different protein sources fall into three categories clam meal and squid meal diets registering best feed efficiency silkworm pupae based diet producing lowest feed efficiency and squilla meal and shrimp head meal diets showing intermediate feed efficiency The better feed efficiency obtained for prawns fed with clam meal and squid meal diets might be due to better quality of protein from these sources Feeds that are nutritionally poor are likely to be consumed more by prawns, provided they are palatable High diet intake does not necessarily bring about rapid growth (Deshimaru and Shigueno 1972) According to Colvin (1976) protein sources that are deficient in essential nutrient produce less efficient feed conversion ratio Ali (1982a) while evaluating the nutritional quality of various protein sources obtained best feed efficiency for clam based diet for *P indicus* juveniles Later, the same author observed clam meal based diet producing the best feed conversion in *P indicus* (Ali,1988) Similarly Josekutty (1991) found that clam meal based diet had given the best feed conversion ratio among the various sources tested in *P monodon* The most efficient conversion of feed observed for clam meal diet in this study is in agreement with these observations

Although the feed efficiency value recorded for squilla meal diet is significantly lower than that of squid meal diet the observed growth rates are comparable This pattern suggests that by consuming more squilla meal diet a growth rate comparable with that of squid meal diet could be obtained A similar pattern can be seen between shrimp head meal and silkworm pupae diets

5 1 4 Protein efficiency ratio

The results of the presents study have indicated that prawns fed on diets based on clam meal produced highest protein efficiency ratio, closely followed by squid meal diet, though not significantly different from the former This suggests that both these sources are efficient in converting dietary protein for growth Squilla meal diet and diet based on shrimp head meal produced lower protein efficiency ratio, while silkworm pupae diet had given the lowest protein efficiency ratio among the various test diets tried in this experiment Ali(1988) obtained highest protein efficiency ratio for *P indicus* fed on clam based diet, while lowest protein efficiency ratio was noted for silkworm pupae diet Josekutty(1991) found clam meal diet giving highest efficiency of protein utilization for growth in *P monodon*

Steffens(1981) reported that the protein efficiency ratio can be used to evaluate the quality of protein in the feed those with higher protein efficiency ratio being of good quality and those with low values being of poor quality According to Boonyaratpalin (1989) feed conversion efficiency and protein efficiency ratios are positively correlated In the present study also this was evident

5 2 Evaluation of substitution of protein with carbohydrate in the formulated feed of *M rosenbergii* juveniles

The evaluation of the effect of substitution of protein with carbohydrate using clam meal which was established as the best protein source by the first experiment indicated that it is economically advantageous thing, as reduction in protein requirement of *M rosenbergii* is possible

5 2 1 Survival rate

In all the protein levels viz 25 30 and 35% the combination of various carbohydrate levels such as 20,25 and 30% produced high survival rate (>90%) of *M rosenbergii* juveniles In statistical terms, these survival rates were found to be not significantly different among themselves This result indicates that lowering of protein level from 35 to 25% and substituting with carbohydrate could provide enough energy required for maintenance and some growth Ali (1982b) also observed no variation in survival rate when the protein level in the diet of *P indicus* varied from 20 6 to 46 5% Koshio *et al* (1993), while experimenting with *P japonicus* found no significant variation in survival rate when the protein level of diet ranged from 21 to 60 7% Recently, Nair and Sherief (1993) showed that survival rate of *M rosenbergii* was not affected significantly when fed with a series of diets of varying protein levels from 24 75 to 50 5% In the present study also, no significant variation in survival rate was observed among the three protein levels tested viz 25, 30 and 35%

5 2 2 Growth rate

The observed growth rate in the present study in terms of specific growth rate suggests that there is a corresponding increase in growth rate of *M rosenbergii* juveniles when they are fed on diets with increasing level of protein, as well as carbohydrate levels at each level of protein The highest growth rate was observed when 35% protein and 30% carbohydrate were included in the test diet The growth studies of *M rosenbergii* by Balazs *et al* (1973), Balazs and Ross (1976) Clifford and Brick (1979) D Abramo and Reed (1988) and Freuchtenicht *et al* (1988) conducted in tanks where natural food is lacking had shown that the optimum protein level of the species is in the range of 25-35% As stated by Gomez *et al* (1988) information on carbohydrate nutrition of prawn is scanty Akiyama *et al* (1992) opined that carbohydrate utilization and metabolism by shrimp feeds to be further studied

Bautista (1986) reported that carbohydrate above 20% depressed the growth rate of *P monodon* and suggested that energy requirement for efficient utilization of protein would have already been achieved at 20% level of sucrose Alava and Pascual (1987) also observed highest growth rate in *P monodon* when fed with 20% level of carbohydrate in the form of trehalose than with 10 or 30% Contrary to this Shiau and Peng (1991) observed that growth of *P monodon* was not significantly affected when the dietary protein level was reduced from 40 to 30% and increasing the corn starch level from 20 to 30% Similarly Andrews *et al* (1972) obtained increased growth rate ın *P aztecus* when 30% starch was ıncluded ın the dıet Ali (1982b) observed best growth response whıle usıng 40% starch ın a caseın based diet ın *P ındıcus*

These varıatıons ın carbohydrate requırement of dıfferent specıes of shrımp may be due to the source of carbohydrate and / or feedıng habıt of these species tested Abdel-Rahman *et al* (1979) have shown that *P japonıcus* juveniles had a better weight gain on diets containıng dısaccharıdes such as sucrose, maltose and trehalose and polysaccharides such as dextrın and starch than on dıet contaınıng monosaccharıdes such as glucose, galactose and fructose Andrews *et al* (1972) observed reduced growth when 20% glucose was ıncluded ın the dıet of *P aztecus* while, ınclusıon of 30% starch ıncreased the growth rate Accordıng to Lovel(1989) glucose in the dıet at levels of 10% or more suppresses the growth of shrımp Bautısta(1986) also opıned that more complex carbohydrates may provıde better proteın sparıng than sucrose whıch was used as the carbohydrate ın that study AQUACOP (1978) suggested that carbohydrate such as starch appears ore suitable than glucose ın the diet of shrımp

The gıant freshwater prawn, *M rosenbergıı* ıs an omnıvorous specıes (New, 1990) Thıs fıeld observatıon has been confırmed by enzyme studies of Lee *et al* (1980) Gomez *et al* (1988) has suggested the avaılabılıty of varıous carbohydrases among the digestive enzymes of *M rosenbergii* These observations suggests that *M rosenbergii* may be able to utilize carbohydrate for energy metabolism so that costly protein can be spared for growth Thus by including sufficient carbohydrate in the diet, the level of protein can be minimized substantially In the present experiment, the pattern of specific growth rate observed shows that, at each level of protein, addition of carbohydrate produced increased growth within the levels tested At 25% protein increasing the level of dietary carbohydrate produced only a non significant increase in specific growth rate But at this level of protein, with 30% carbohydrate (P1C3) the growth rate recorded did not differ significantly from that of 30% protein and 20% carbohydrate (P2C1) which suggests that by using 30% carbohydrate along with 25% protein a growth equivalent to feeding a diet with 30% protein and 20% carbohydrate could be achieved In practical terms this mears that 5% protein could be substituted with 10% carbohydrate A similar observation was made by Shiau and Pong (1991) on *P monodon* wherein they found that the dietary protein level could be lowered from 40 to 30% by increasing the corn starch level from 20 to 30% It was reported that this substitution has not found to affect the growth significantly At the second level of protein (30%) tested in the present study 25 and 30% carbohydrate levels recorded significantly higher growth than at 20% level

At 35% protein level 20 and 25% carbohydrate levels (P3C1 and P3C2 respectively) gave growth rates which are not significantly different from each other as well as with that of the diet with 30% protein and carbohydrate each (P2C3) showing that 30% protein will be sufficient if 30% carbohydrate is included in the diet At the same time if a higher level of 30% carbohydrate could be provided as increase in protein level to 35% would be more beneficial as a significantly better growth was obtained with a feed of this combination (P3C3)

A critical analysis of the pattern of growth rates shows that the requirement of carbohydrate is dependent on the level of protein In practical terms this means that, the optimum level of carbohydrate varies at different levels of protein For instance at 25% protein 20% carbohydrate is sufficient and increasing the level of carbohydrate beyond this level at this level of protein does not produce any significant increase in growth rate In statistical terms, all carbohydrate levels tried (20, 25 and 30%) at 25% protein are equivalent so far as growth is concerned But at 30% protein, the growth rate is found to be higher with diets having 25 or 30% carbohydrate, the latter two are found to be significantly different from each other suggesting that at 30% protein a higher level of 25% carbohydrate is required But at 35%

protein only at a still higher level of 30% carbohydrate there is a positive influence on growth in *M rosenbergii* Thus it could be seen that at higher protein levels the carbohydrate requirement tends to be higher Increased carbohydrate requirement at higher protein level points to the higher energy requirement at higher level of protein In practical terms this means to utilize higher protein level effectively for growth rather than for energy purpose higher non protein energy in the form of carbohydrate is to be included in the feed Higher energy requirement by shrimp at higher levels of protein was observed by Bautista (1986) who found that at 50% protein level (370kcal/100g of feed) the growth was only more or less equivalent to 40% protein (330kcal/100g of feed) as the excess protein was catabolized for energy purpose His explanation that when non protein energy sources are limiting protein is catabolized for energy purpose seems to be true in the case of *M rosenbergii* also as in the present study by increasing the carbohydrate level to 30% the higher level of protein was effectively utilized for achieving better growth

5 2 3 Feed conversion efficiency

The present study to evaluate the effect of partial substitution of protein with carbohydrate on feed conversion efficiency had revealed that at a level of 25% protein, an equal amount of carbohydrate is required for efficient feed conversion At this level of protein an increase of carbohydrate from 20 to 25% is found to produce more efficient feed conversion, although for better growth rate 20% carbohydrate is found to be sufficient since the increase in growth rate produced at 25% level carbohydrate is found to be statistically not significantly higher than that of 20% carbohydrate level This suggests that more feed is consumed at 25% protein level with 20% carbohydrate to achieve an equivalent growth of 25% carbohydrate level at this protein level Since economy of farming is more dependent on feed conversion, it may be desirable to provide 25% carbohydrate at 25% protein level from an economic point of view since carbohydrate is a cheap ingredient among the various components of feed But increasing the level of carbohydrate to 30% at 25% protein was not found to yield significantly higher growth or feed efficiency

It could be seen from the results of the present study that by increasing the carbohydrate level from 20 to 30%, the protein requirement of *M rosenbergii* could be reduced from 30 to 25% without affecting the growth or feed efficiency significantly In practical terms, this means that a saving of 5% protein could be achieved with addition of 10% carbohydrate which is more economical A similar observation was made in *P monodon* by Shiau and Peng (1991) who found that growth and feed conversion efficiency of *P monodon* were not significantly affected when the dietary protein was reduced from 40 to 30% by increasing the corn starch level from 20 to 30% Similarly, the protein level of *M rosenbergii* could be reduced from 35 to 30% by increasing the level of carbohydrate from 20 to 30% Dietary protein is generally one of the most expensive components of compounded diets So if increasing increments of dietary protein level do not result in concomitant increase in production the economic success of aquafarming will be affected (Zeitoum *et al* 1976) The feed with high dietary protein in the presence of low levels non protein energy may force the crustaceans to deaminate sufficient proportions of protein Supplementation of dietary carbohydrate has been reported to improve feed conversion efficiency of *P indicus* (Ali,1982b) This was also evident in the present study as at each level of protein, increasing the carbohydrate level from 20 to 30% produced an apparent increase in feed efficiency The low feed efficiency obtained with prawn fed on diet with lower carbohydrate implies that *M rosenbergii* ate more food to overcome energy inadequacies as noted in the case of *P monodon* by Bautista(1986) Hanson and Goodwin (1977) pointed out that the efficiency with which protein is assimilated by shrimps is most likely to be affected by the relative proportion of carbohydrate and lipid in the formulation as well as the amino acid composition of the protein source Sedgwick (1979) found that optimal utilization of protein by *P merguiensis* is closely related to energetic value of diet and that carbohydrate and lipid could also increase growth efficiency at sub optimal levels of protein Bautista (1986) noticed higher protein sparing action of carbohydrate over lipid in the case of *P monodon* Carbohydrates are the cheap and most economical source of dietary energy (Ali,1982b and Davis and Arnold,1993) They appear to be well utilized by shrimp and feeds containing relatively high levels of carbohydrates appear to have acceptable palatability and support good growth (Pascual *et al* 1983 Shiau *et al* 1991 Catacutan 1991 and Shiau and Peng 1991)

5 2 4 Protein efficiency ratio

Protein efficiency ratio was found to decrease with increase in dietary protein concentration Millikin *et at* (198) and Boonyaratpalin and New (1982) observed an inverse relationship between protein level and protein efficiency ratio Similarly Colvin (1976) found a decline in protein efficiency ratio with successive increase in protein level while experimenting with *P indicus* Ali (1988) also observed higher efficiency of protein utilization at lower dietary protein in *P indicus* In another study Ali (1982b) obtained the highest protein efficiency ratio at intermediate level of protein tested (20 5%) in the same species But Alava and Lim (1983) observed an increase in protein efficiency ratio for *P monodon* with increase in dietary protein level up to 40%

In the present study decreasing the protein level from 35 to 25% produced an increase in protein utilization Highest protein efficiency ratio was noted when lowest level of protein (25%) was used along with 30% carbohydrate which was the highest level of carbohydrate tried in this experiment Clifford and Brick(1979) studied the metabolic response of *M rosenbergii* to various levels of dietary protein and concluded that higher carbohydrate levels resulted in better utilization of protein for growth Similarly, Gomez *et al* (1988) suggested that dietary protein is spared by supplementation of starch in the diet of *M rosenbergii* In *P monodon* protein utilization was found to be increased as the available energy level in the diet increased (Bautista, 1986) Hajra *et al* (1988) reported an increase in protein efficiency ratio and growth in *P monodon* concomitant with an increase in carbohydrate and attributed it to the protein sparing action of metabolizable energy from carbohydrate Sedgwick (1979) found that optimal utilization of protein by *P merguiensis* was closely related to energy level of diet and carbohydrate and lipid can increase growth efficiency at sub optimal levels of protein The efficiency by which protein is assimilated by prawn is most likely affected by the relative proportion of lipid and carbohydrate in the diet as well as the composition of the source of protein used (Hanson and Goodwin 1977) Insufficient non protein energy in the diet can lead to catabolism of dietary protein for energy in crustaceans (Capuzzo and Lancaster, 1979) and lower protein efficiency ratios

5 3 Physico-Chemical parameters

The physico chemical conditions under which the experiments were conducted are in general conducive for growth and survival of *M rosenbergii* The water used for the experiment was drawn from the freshwater distribution system of the *Macrobrachium* hatchery, College of Fisheries The temperature of the rearing water varied between 27 and 31^{o}C which was within the optimum range reported for the growth of *M rosenbergii* (New and Singholka 1985 Sandifer and Smith 1985) In the present experiment the pH of the water used for the rearing of prawn was found to be slightly alkaline (7 5 + 0 5) throughout the experimental period New and Singholka (1985) and Sandifer and Smith (1985) have suggested a pH range of 7 8 5 as optimum for rearing of *M rosenbergii* New (1976) also recommended a pH range of 7 8 5 for nutritional studies of prawn Dissolved oxygen values (6 7 ppm) observed during the present study were also within the range required for optimum growth of *M rosenbergii* (New,1976, 1990)

VI SUMMARY

1 The present study was aimed to evaluate the nutritional quality of certain inexpensive and locally available sources of protein viz, clam meat squid waste squilla shrimp head waste and silkworm pupae in the formulated feed of *M rosenbergii*

2 Five test diets differing in source of protein were formulated with clam meal squid meal, squilla meal, shrimp head meal and silkworm pupae as main protein sources along with groundnut oil cake wheat bran tapioca flour sardine and maize oils and Supplevite M (a commercial vitamin mineral mixture) 3 The protein sparing action of carbohydrate was then evaluated by partial substitution of protein with carbohydrate using 9 test diets with different combination of protein (25 30 and 35%) and carbohydrate (20 25 and 30%)

4 The survival rate of *M rosenbergii* was not found influenced substantially by the different protein sources tried, showing that they are not grossly deficient in nutrients and suggesting that these sources could be used for compounding feeds without any deleterious effect on survival rate of *M rosenbergii*

5 The protein sources tested had a profound influence on growth rate of *M rosenbergii* Clam meal and squid meal had given best growth squilla and shrimp head meals intermediate growth and silkworm pupae the lowest growth among the various

sources tested

6 Diets based on clam meal and squid meal were found to produce best feed conversion efficiency suggesting that these proteins are of better quality Squilla and shrimp head meal diets recorded lower feed efficiency while, lowest feed efficiency among various sources of protein was shown by the silkworm pupae diet

7 Although the feed efficiency recorded by squilla meal diet is significantly lower than that of squid meal diet the observed growth rates were found to be comparable suggesting that, by consuming more squilla meal diet which is lower in nutritional value, a growth comparable with that of squid meal diet could be achieved A similar pattern can be seen between shrimp head meal diet and silkworm pupae diet

8 Protein efficiency ratio was found to be positively correlated with feed conversion efficiency, the pattern of values observed among the various protein sources being similar to that of feed conversion efficiency Higher values of protein efficiency ratio recorded for clam and squid meal diets, points to the better quality of protein from these sources

9 Squilla meal, shrimp head meal and silkworm pupae as single sources of protein were found to yield lower growth and feed efficiency but it may be possible to use them in combination with other sources in the formulated feed of prawn

10 Shrimp head meal is found helping to improve pigmentation of prawn as it is a good source of carotenoid pigments besides acting as an attractant

11 Lowering of protein level from 35 to 25% with various combination of carbohydrate was found to be not affecting the survival rate of *M rosenbergii*

12 Growth response as well as feed efficiency studies have shown that the protein level could be lowered from 35 to 30% by increasing the carbohydrate level from 20 to 30% Similarly protein level could be reduced to 25 from 30% by increasing the level of carbohydrate from 20 to 30% without affecting growth, survival and feed efficiency of *M rosenbergii*

13 Carbohydrate requirement of *M rosenbergii* was found to be dependent on the level of protein in the diet Growth response and feed efficiency suggest that at 25% protein 20 25% carbohydrate is required while at 30% protein 25% carbohydrate is the optimum At 35% level of protein 30% carbohydrate is found to be required

14 Increasing the carbohydrate level at sub optimum level of protein was found to help in improving the feed conversion efficiency and protein efficiency ratio, suggesting that protein could be spared for growth rather than for using in the maintenance, provided an adequate level of carbohydrate is included in the feed

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VIII ABSTRACT

The nutritional quality of five locally available sources of protein such as clam meat squid waste, squilla, shrimp head waste and silkworm pupae was evaluated in the feed for the giant freshwater prawn *Macrobrachium rosenbergii* with a view to develop a feed which is cost effective The feeds were prepared using these locally available ingredients along with groundnut oil cake wheat bran tapioca flour Supplevite (a commercially available vitamin mineral mixture) and sardine oil and maize oil mixture

The protein sources tested were not found to be grossly deficient in their nutritional quality as good survival rates of over 90% were obtained when they were used as the main protein sources in the feed of juvenile prawn Highest growth rate was obtained for clam and squid based diets intermediate growth response with squilla and shrimp head based diets and the lowest growth among the various sources was given by silkworm pupae diet

Feed conversion efficiency was higher for clam and squid based diets while intermediate feed efficiency was noted for squilla and shrimp head meal diets Lowest feed efficiency was observed for silkworm pupae diet Protein efficiency ratio showed a similar pattern

In the second experiment, substitution with carbohydrate for protein was found to be promising from an economic point of view It was found that lowering of protein level from 35 to

30% could be achieved by increasing the carbohydrate level from 20 to 30% without affecting the growth, survival and feed efficiency Similarly protein level could be lowered from 30 to 25% by increasing carbohydrate by 10% from 20% level This suggest that a 5% reduction of costly protein could be achieved by including 10% more of carbohydrate which will be able to reduce the cost of the feed with out sacrificing the nutritional quality of the formulated feed