MACROBRACHIUM ROSENBERGII (DE MAN)

BY

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THESIS

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DECLARATION

I hereby declare that this thesis entitled **Protein Requirement of the Postlarvae and Juveniles of *Macrobrachium rosenbergii* (De Man)** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship, or other similar title,of any other university or society.

Panangad
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CERTIFICATE

Certified that this thesis, entitled **Protein Requirement of the Postlarvae and Juveniles of *Macrobrachium rosenbergii* (De Man)** is a record of research work done independently by Mr. Sahadevan, P. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to him.

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1 INTRODUCTION

Aquaculture of shrimps and prawns has made significant advances during the last decade. With a production of 27,065 metric tonnes/year(New, 1990a; Sebastian, 1990),the farming of freshwater prawns (*Macrobrachium* spp.) contributes only to about 6% of the global production of shrimps and prawns through aquaculture. However, freshwater prawn farming is of considerable local importance in isolated parts of the world (New, 1990a).

Of the well over 125 species of *Macrobrachium*, the giant freshwater prawn, *Macrobrachium rosenbergii* is considered to be the most important one from a culture point of view, owing to its fast growth rate, hardy nature, omnivorous feeding habit, economic and efficient food conversion and relatively good productivity per unit area of water in mono as well as polyculture systems. Other attributes which make this species suitable for warmwater aquafarming include its relatively tame and less cannibalistic nature, comparatively short larval period, tolerance to a wide temperature and salinity regimes, successful reproduction in captivity, availability of commercial techniques for seed production, absence of major disease problems, wide consumer acceptability and high market value with export potential. *M. rosenbergii* is also of economic importance in sport fisheries (Liao, 1990) and as a bio-agent for the control of schistosome water snails in fish ponds (Lee *et al.*, 1982).

Significant advances have been made in recent years in understanding the biology, seed production and farming of *M.rosenbergii*. Notwithstanding the success in acquiring basic information about this species there still remains a paucity of information especially regarding the nutritional requirement of the species.

The cost of feeds and feeding represents one of the largest recurring expenditures of a prawn farm (New, 1976a; Biddle, 1977; Shang and Fujimura, 1977; Wyban *et al.*, 1988; Abesamis, 1989; Pascual, 1989; Akiyama *et al.*, 1991). Hence the suitability and cost effectiveness of the ration are of paramount importance to commercial success. The yield of prawn and, in turn, the profitability of the farming operation depend to a large extent on the quality and quantity of the feeds used. This applies alike to the conventional pond rearing and to the more evolved, restrictive and rigidly controlled intensive prawn farming. However, the more intensive the aquaculture system, the greater is the importance of quality feeds and greater the proportion of feed cost to the total cost.

A comprehensive knowledge of the nutritional requirement and related aspects of the species selected for culture and the composition of the raw materials used in the formulation of the compounded diet, is an essential pre-requisite for evolving low-cost balanced feeds. Protein is the most dominent and the most expensive component of any crustacean diet and hence studies directed to investigate the protein requirement of the species both in qualitative and quantitative terms require special emphasis. The results of such studies would help develop low-cost special feeds for the different life stages which would help reduce the total period of time for obtaining marketable size prawns and in turn bring down the cost of production to considerable extent.

Except for a few penaeid species, precise nutritional studies on prawns and shrimps are few or fragmentary (Forster, 1976; New, 1976a, 1980a; Wickins, 1976; Hanson *et al.*, 1977a,b; Ceccaldi, 1978; Kanazawa, 1980, 1982, 1984; Bages and Sloane, 1981; Castell, 1982; Teshima, 1984). Indeed a few studies to ascertain the protein need of the giant freshwater prawn, *M. rosenbergii* using compounded diets have been reported (Balazs and Ross, 1976; Millikin *et al.*, 1980; Boonyaratpalin and New, 1982). However, these investigations are confined to the juvenile stages of the prawn whose requirement is expected to differ from that of the faster growing early postlarval stages. Moreover, the use of compounded diets with widely varying protein sources limits the usefulness of these results (New, 1976a; Biddle, 1977; Corbin *et al.*, 1983).

Undoubtedly these indicate the need for the use of a universally acceptable, completely defined, chemically purified diet in nutritional studies with prawns and shrimps. Since casein is the only protein source available in highly purified form, its use as a protein source reduces considerably the extraneous nutritional factors (Halver, 1957; Kanazawa *et al.*, 1971, 1976; Gopal, 1986; Kiron, 1988) which markedly alter the protein requirements of the prawn. Thus casein based diets allow precise nutritional studies, whereby relationship between particular dietary ingredients and physiological indices can be observed (D'Abramo *et al.*, 1982). The present study is undertaken to elucidate the optimum level of dietary protein required by the early postlarvae and juveniles of the prawn *M. rosenbergii* using purified diets based on casein and amino acid mixture. The effect of different protein to energy ratios on the performance of the two life stages of the prawn is also considered. Further, in an effort to bring down the cost of feeds in commercial farming of *M. rosenbergii*, the effect of substitution of protein from animal sources with that from the relatively cheap and locally available plant sources was also assessed. Probably for the first time in palaemonid prawns an attempt has also been made to quantify the maintenance protein requirement in *M. rosenbergii*.

2 REVIEW OF LITERATURE

Although there are several facets of the nutrition of prawns which contrast with those of the higher vertebrates, that which has the greatest significance, atleast from the view point of aquafarming, is their high requirement of dietary protein. The point is also of academic interest in that the amount of essential amino acids required by crustaceans seems vastly to exceed their requirements for protein synthesis, thereby indicating the apparent preferential use of protein for energy purposes. It is therefore necessary to ascertain with confidence the protein and amino acid requirement of farmed crustaceans.

Obviously the experimental method and the make up of the test diet used to determine the amino acid and protein requirement of crustaceans influence the protein requirement value significantly. As such, the values reported for many species of crustaceans by various authors differ considerably and in a few cases recent studies have confirmed only a lower requirement value than was earliar reported (see Colvin, 1976 and Ahamed Ali, 1988 in *Penaeus indicus*; Balazs and Ross, 1976; Millikin *et al.*, 1980 and Gomez, *et al.*, 1988 in *M. rosenbergii*). This review is limited to an evaluation of the general methods employed to find out the amino acid and protein requirement of aquatic organisms with special reference to shrimps and prawns and the results obtained in the area of amino acid and protein nutrition. An attempt has also been made to briefly review the relevant literature on the use of various sources of protein in crustacean feeds.

2.1 Amino acid Requirement

2.1.1 *Qualitative Amino acid Requirement.*

The overall role of dietary protein is to provide sufficient quantities of those amino acids essential to the normal health and survival of an animal. Traditionally, we think of an essential amino acid as one that cannot either be synthesised at any level within the body, or is synthesised in only limited quantities which are inadequate to meet the physiological need of the organism. Thus there is a necessity for the particular amino acid to be supplied in the diet of the consuming organism. Therefore in order to establish the quantitative amino acid requirement of the prawn, one must first demonstrate the qualitative requirement of the specific amino acid in question.

The essentiality of various amino acids for prawns may be studied either by growth studies or by ^{14}C labelling studies (Wilson, 1989). The former involves feeding purified diets containing crystalline amino acids and determining the essentiality of each amino acid based on the growth response associated with the deletion of that amino acid from the diet.

Nutritionists were unable to utilize crystalline amino acid test diets to study amino acid requirement of crustaceans (Wilson, 1989). Deshimaru and Kuroki (1974c) prepared a test diet to mimic the amino acid composition of a formulated dry feed to study the amino acid requirement of *P. japonicus* and it was found to give very poor growth rates, high mortalities and reduced food intakes. Further studies by Deshimaru and Kuroki (1975 a, b) and Deshimaru (1981, 1982) confirmed that pure amino acids and peptides are inferior to intact proteins. Diet based on a mixture of crystalline amino acids approximating the amino acid content of casein has also been reported to result in poor performance in the larvae of *P. japonicus* (Teshima *et al.*, 1986). Similarly Andrews and Sick (1972) found that *P. setiferus* could incorporate protein more fully from intact yeast protein than from yeast hydrolysates. Deshimaru (1981) has also shown that the growth of the prawn juvenile was not improved by supplementation with L-arginine in a casein-albumen diet as compared to protein of the short neck clam. Further more, he has demonstrated that the assimilation rate of dietary L-arginine to muscle protein by prawn juveniles was extremely low compared to that of proteinous arginine. Cowey and Forster (1971) failed to augment the nutritive value of a diet composed of gelatin and zein by

supplementing with crystalline amino acids suggesting that the ability of the palaemonid prawn, *Palaemon serratus* to utilize crystalline amino acids is rather limited. In *M. rosenbergii*, Stahl and Ahearn (1978) reported poor growth rates when fed with crystalline amino acid diet compared to diets containing intact protein.

The observed poor performance of amino acid based purified diets compared to intact protein diets in prawns and shrimps may be related to the immediate availability of the amino acids for absorption and consequent saturation of the absorption site, shorter retention time, amino acid imbalance, alteration of the intestinal pH condition and the related electrolyte movement, lower palatability of the diets, leaching of individual amino acid or to artefact.

An alternative way to determine the essential amino acid requirements in shrimps and prawns is to administer appropriate ^{14}C labelled substrate such as glucose or acetate into the given organism and then trace its incorporation into amino acids within the body. Those which accept the labelled carbon can be synthesized by the organism concerned and hence are non-essential. On the other hand, those amino acids which do not accept the labelled carbon cannot be synthesized within the body and is considered as essential.

Several workers have employed [U-^{14}C] glucose, [3-^{14}C] pyruvate and either [^{14}C] or [^{3}H] acetate successfully for studying the qualitative amino acid requirements of crustacea (Wilson,1989). Thus radio-isotopic method was used successfully by Zandee (1966) in *Astacus astacus*, Cowey and Forster (1971) in *Palaemon serratus*, Shewbart *et al.* (1972) in *Penaeus aztecus*, Gallagher and Brown (1975) in *Homarus americanus*, Van marrewijk and Zandee (1975) in *Astacus leptodactylus*, Claybrook (1976) in *Uca pugilator*, Lasser and Allen (1976) in *Cancer magister*, Miyajima *et al.* (1976) in *Macrobrachium ohioni*, Coloso and Cruz (1980) in *Penaeus monodon*, Kanazawa and Teshima (1981)in *P.japonicus* and He (1988) in *P.orientalis*

On the basis of the various studies 10 amino acids viz., arginine, histidine, isoleucine, leucine, lysine,methionine, phenyl alanine, threonine, tryptophan, and valine are found to be essential for prawns and shrimps (New, 1976a; Biddle, 1977; Wilson, 1989).

The qualitative amino acid requirement has so far been investigated in only 9 species of prawns and shrimps. These include *P.serratus* (Cowey and Forster, 1971),*P.aztecus*(Shewbart *et al.*, 1972), *P.*kerathurus (Torres,1973), *M.ohione* (Miyajima *et al.*, 1976), *M. rosenbergii* (Watanabe, 1975; Stahl and Ahearn, 1978), *P.monodon* (Coloso and Cruz, 1980), *P.japonicus* (Kanazawa and Teshima, 1981), *Acetes japonicus* (Yongquan and Jinglin, 1987) and *P.orientalis* (He, 1988). Similar studies on qualitative amino acid requirement have also been conducted in other related crustaceans like *Astacus astacus* (Zandee, 1966), *A.leptodactylus* (Van Marrewijk and Zandee, 1975), *Cancer magister* (Lasser and Allen, 1976), *Uca pugilator* (Claybrook, 1976), *Homarus americanus* (Gallagher and Brown, 1975) and *Pseudocuphasia sinica* (Yongquan and Jinglin, 1987).

Cowey and Forster (1971) gave intrahaemocoelic injections of [U-^{14}C] acetate to *P.serratus*. The prawns were fed with an artificial diet consisting of freeze dried cod meal as the principal protein source. The prawns were then sacrificed, a sample of the protein was hydrolysed and the constituent amino acids were seperated and analysed for radio activity. Little or no ^{14}C had been incorporated into arginine, histidine, methionine, valine, threonine, isoleucine, leucine, lysine, phenyl alanine and tryptophan implying that the prawns have an absolute dietary requirement for them. Torres (1973) using fluctuations observed between extreme values in the free amino acid pool of *P.kerathurus* indicated

only eight amino acids viz., histidine, isoleucine, leucine, lysine, methionine, phenyl alanine, threonine, and valine as essential.

Miyajima *et al.* (1976) used labelled glucose ($U^{-14}C$ glucose) to study the indispensable amino acids in the freshwater shrimp, *M.ohione*. Presumably due to the destruction by the acid hydrolysis (Miyajima, *et al.*,1976; Farmanfarmaian and Lauterio, 1980) tryptophan analysis could not be conducted in this experiment. The result was similar to that of *P.serratus* except for a requirement for tyrosine and it has been suggested that tyrosine is synthesized from dietary concentration of phenyl alanine by non hydroxylation reaction whose biosynthetic pathway involves the enzyme phenyl alanine monoxygenase (Lehninger, 1970) as has been demonstrated by Zandee (1966) in the crayfish, *A.astacus*. However, the feeding of tyrosine may have a sparing action on phenyl alanine by reducing the quantity of phenyl alanine converted to tyrosine, as has been indicated by West *et al.* (1974) in higher vertebrates.

Recently by employing labelled glucose He (1988) found tyrosine and glycine to be essential in addition to the ten amino acids already mentioned above. Though tyrosine has been suggested to be synthesized from phenyl alanine, non incorporation of radio activity in glycine seems to be some what unexpected. This observation was, however, found to be in concord with that by Claybrooks (1976) in *U.pugilator* which also showed a dietary essentiality for glycine. It is of some interest to note that this amino acid is also essential for the chick.

In *M.rosenbergii* Watanabe (1975) and Watanabe *et al.* (1976) reported an essentiality for tyrosine in addition to arginine, histidine, methionine, leucine, isoleucine, valine, tryptophan, and phenyl alanine. A striking deviation in the prawn, *M.rosenbergii* is the apparent non-essentiality for lysine, which has been reported to be essential for all animals so far studied. This unique observation was further evaluated by Stahl and Ahearn (1978) using purified amino acid test diets and could surprisingly find that dietary lysine was not indispensible for the growth and survival of *M.rosenbergii*. The difference in the percentage increment in length which reflects the growth was found to be statistically not significant in prawns receiving the various levels of amino acid viz., 0, 0.7, 1.4 and 2.8% for a period of 30 days in a thrice replicated experiment. These authors also investigated the essentiality of arginine, histidine and tryptophan which have already been reported to be essential for *M.rosenbergii*, in studies similar to that employed for lysine but of varying durations from 6 to 12 weeks and found all the three to be non-essential.

Biddle (1977) cautioned drawing conclusion on the essentiality of amino acids on the basis of short term growth studies before considering the pre nutritional status of the prawn, likely existence of nutrient reserves in the prawn body, the ability of the prawn to preferentially conserve a specific nutrient if that is deficient in diet and the possibility of adequate quantity of the amino acid made available for absorption from microbial synthesis in the prawns' gut. However, except for the possibility of supply of the amino acid synthesized by the symbiotic intestinal microflora, in the experiment by Stahl and Ahearn (1978), other possibilities seem to be improbable. Stahl and Ahearn (1978) were of the view that prior nutritional reserves of the prawn are unlikely to provide sufficient quantities of the missing amino acid to support significant growth in the prawn, during the study period. The ability of an organism to store amino acids as such, unabsorbed and unutilized by the body is limited (West *et al.*, 1974). Further, if the amino acid was derived from the body protein significant growth of the organism could not have been registered in the above mentioned experiment. However, the importance of bacteria either present in the intestinal tract or those in the rearing water, on the sides of the rearing enclosures etc. which the prawns might have consumed cannot be overlooked. Bacteria which could survive the digestive process have been isolated from the gastro intestinal tract of the marine shrimp, *P.setiferus* (Hood *et al.*, 1971, Hood and Meyers, 1973) and are shown to elaborate an array of extracellular material

that assists the crustacean in obtaining nutrients which might not otherwise be available to it. Based on the conclusion made by Mittler (1971) on aphides, Myzus persicae which are found to grow for two generations on diets from which arginine, leucine, lysine, phenyl alanine, threonine, tryptophan and valine were omitted individually, Stahl and Ahearn (1978) suggested that the gut bacteria, in *M.rosenbergii* might be capable of synthesizing extracellular amounts of the deleted amino acids and leaking them to the host prawn. The possible contribution of amino acids by the intestinal gut flora, particularly bacteria, has also been indicated by Farmanfarmaian and Lauterio (1980) and Fair and Sick (1982) in *M.rosenbergii*.

In an attempt to estimate the metabolic requirements for selected amino acids in *M.rosenbergii*, changes in concentrations of free amino acids in the haemolymph after a relatively short period of starvation (5 days) which reflects the natural amino acid fluxes between haemolymph and tissues during a period of stress, has been measured by Fair and Sick (1982). These authors suggested that *amino acids* rapidly removed from serum during short term starvation were probably most critical for tissue metabolism and indicated relative metabolic requirements for proline, glutamic acid, methionine, phenyl alanine and leucine. However, this does not give an idea about the dietary essentiality of these amino acids for the prawn, *M.rosenbergii* as some of these can be readily synthesized from other tissue components (Fair and Sick, 1982).

Although some innate metabolic differences among the crustacean species may also have contributed to variations in essential amino acid requirements reported by various investigators, differences in experimental design and methodology probably contributed to most of the discrepancies (Fair and Sick, 1982). Further, monitoring the assimilation of radio activity labelled amino acid may result in anomalous interpretations of data due to critical differences in equilibria between stable and isotopically labelled metabolic pools of organic compounds (Willis and Jones, 1977). The physiological adaptations of euryhaline crustaceans like *M.rosenbergii* may also alter classic concepts of "essential" and "non-essential" amino acids (Fair and Sick, 1982; Sick and Millikin, 1983). These authors are of the view that although metabolic requirement of amino acids at the cellular level would be some what similar in all organisms, adaptations to a particular aquatic environment and the unique features of periodic moulting may necessitate specific amino acid requirement that are unique for crustaceans. Though the free amino acids associated with these physiological functions in crustacea are considered "non-essential" the rate of rapid turnover for the purpose may more than offset their rate of synthesis from the precursors which would necessitate a dietary essentiality for many of them necessitating their grouping under "truely semi essential amino acids" as suggested by Maynard and *Loosli (1969)* and West *et al.* (1974) and as proposed for *Macrobrachium* sp. by Fair and Sick (1982).

The foregoing review undoubtedly indicates that the issue of amino acid indispensability is still confusing in crustacea. However, it may be noted here that the issue is unsettled in higher vertebrates, too. According to Jackson (1983) and Laidlaw and Kopple (1987) the classical perception of dispensability is too narrow. The division between indispensable amino acids and dispensable amino acids presumes that dispensability is conferred by the carbon skeleton (Rose, 1957). With ^{15}N it has been shown that there is specific channelling of amino groups between amino acids (Jackson and Golden, *1980; Jahoor et al.* (1988). Accroding to Jackson (1983) as the *movement of amino* groups between amino acids is not free, both the carbon skeleton and the amino group can confer indispensability, giving rise to a classification with four categories of amino acids (Table *1*). On this basis, only lysine and threonine can be considered to be absolutely indispensable, and only alanine, glutamate and aspartate, which are formed by transamination from readily available intermediates, dispensable. *All other* dispensable amino acids are conditionally indispensable, being derived from indispensable amino acids or exhibiting occasions in which the demand for them exceeds the capacity for their synthesis (Laidlaw and Kopple, 1987; Millward *et al.*, 1989).

Table 1. Classification[1] of amino acids according to dispensability of carbon skeleton and amino group

Amino group	Carbon skeleton	
	Indispensable	Dispensable
Indispensable	lysine threonine	serine glycine cysteine
Dispensable	BCAA tryptophan phenyl alanine methionine	gluatamate alanine aspartate

BCAA, branched chain amino acids
[1]Adopted from Millward *et al.* (1989)

2.1.2 *Quantitative Amino acid Requirement.*

Quantitative requirement of amino acids may be studied by gradual increase in the amount of each of these amino acids to a test diet deficient in the respective amino acids and following the effect of the added amount on the growth and performance of the species under study. This procedure has been used among finfishes in chinook, coho and sockeye salmon, Japanese eel, catfish etc. (Halver *et al.*, 1959; Halver, 1965; Chance *et al.*, 1964; Wilson *et al.*, 1977; Nose, 1979) and among crustaceans in *M.rosenbergii* (Stahl and Ahearn, 1978).

An alternative method tried by some investigators in prawns (Kitabayashi *et al.*, 1971b; Colvin and Brand, 1977; Farmanfarmaian and Lauterio, 1979; Farmanfarmaian, 1980) to estimate the quantitative amino acid requirement is the use of semipurified and practical type test diets. The semipurified type diet involves using imbalanced protein supplemented with crystalline amino acids in the test diets. The practical type diet involves using normal feed stuffs to furnish the bulk of the amino acids in the test diets which may be formulated either to make up a fixed amount of the desired protein level with the remaining amount of the protein equivalent being made up of crystalline amino acids or to be deficient in only the amino acids being studied. This method has also been used to investigate the amino acid requirement by finfish nutritionists (Kaushik, 1979; Dabrowski, 1981; Jackson and Capper, 1982; Hughes *et al.*, 1983; Ketola, 1983; Walton *et al.*, 1984; Thebault *et al.*, 1985). However, Wilson (1985) suggested the results of these as only estimated values because of many inherent problems in the procedure like, 1. protein digestibility coefficient may vary 2. amino acid availability within a protein may vary 3. the rate of passage and digestion of various proteins vary and may be slower than the rate of supplemented amino acids and 4. the imbalanced protein used may contain high levels of certain amino acids which may depress the rate of absorption of other amino acids. Cowey and Tacon (1983) and Cowey and Luquet (1983) have reviewed various problems involved in the accurate determination of the amino acid requirement of fish based on growth studies. Some of these include lack of precision in the interpretation of the growth response curves, the growth rates commonly observed with amino acid test diets are generally lower than those observed with intact protein diets, and there is always the possibility that some of the crystalline amino acids in the test diets may be leached during the feeding studies.

While not used to date with crustaceans the serum or tissue free amino acid studies and the amino acid oxidation studies may also be employed to quantify the amino acid requirement. Such studies have been successfully used in various finfishes (Harding *et al.*, 1977; Wilson *et al.*, 1977, 1978, 1980; Kaushik, 1979; Thebault, 1985; Thebault *et al.*, 1985).

Reports on the quantitative amino acid requirement of prawns and shrimps are scarce (New, 1976a; Biddle, 1977; Hanson and Goodwin, 1977; Corbin *et al.*, 1983; Sick and Millikin, 1983). Colvin and Brand (1977) employed a practical shrimp diets supplemented with the respective amino acid to investigate the amino acid requirements in the penaeid prawn, *P.californiensis* and found a requirement value of 1.5-2% of protein as methionine and less than 5% of the protein as lysine. SEAFDEC (1986) reported an arginine requirement value of approximately 5.76% for *P.monodon*. Studies on the required amount of arginine and histidine in the diet for *P.monodon* showed that a level nearest to the amount in the amino acid pattern of the prawn postlarvae gave the best results (Pascual,1989). Kitabayashi (1971b) showed that supplements of arginine and methionine (0.83 and 0.52% respectively) improved the performance of a diet containing 50% squid meal. The recommended essential amino acid requirement value (as percent of protein) for commercial shrimp feeds include arginine (5.8%), histidine (2.1%), isoleucine (3.5%), leucine (5.4%) lysine (5.3%), methionine (2.4%),methionine-cystine (3.6%), phenyl alanine (4.0%), phenyl alanine-tyrosine (7.1%), threonine (3.6%), tryptophan (0.8%) and valine (4.0%), on as fed basis (Akiyama *et al.*, 1991).

Watanabe *et al.* (1976) and Stahl and Ahearn (1978) reported a tentative requirement of 1.4% lysine for *M.rosenbergii*. Farmanfarmaian and Lauterio (1979) tested the amino acid balance of a commercially available pellet and found that several amino acids were in insufficient supply in the ration. Ration performance was significantly improved by fortification of the diet with 1% arginine, phenyl alanine, leucine and isoleucine.

New (1976a) commented that while it is unlikely that any multi ingredient shrimp ration will be qualitatively deficient in any of the essential amino acids, it is still impossible to formulate rations with a balanced amino acid composition without providing excess of one or the other. Some nutritionists suggest that amino acid composition of formulated diet should closely mimic the amino acid pattern of the cultured species (Phillips and Brockway, 1956; Ogino, 1963; Kitabayashi *et al.*, 1971b; Deshimaru and Shigueno, 1972; Deshimaru, 1982; Hew and Cuzon, 1982; Sherief, 1987; Chiu, 1988; Kompiang, 1990).

2.2 Protein Requirement

2.2.1 *Quantitative Protein Requirement.*

The protein requirement of any organism need not only to satisfy the organism's need for substrates for maintenance and growth, but also a need for the provision of nutrients to exert a regulatory influence on the organism which activates the various processes associated with growth (Millward, 1989). Like other animals, crustaceans do not have a true requirement for proteins but have a requirement for a well balanced mixture of essential and non-essential amino acids.

Based on feeding techniques pioneered and developed for terrestrial animals and for fishes, the dietary protein requirements were investigated for various species of prawns and shrimps by various authors. The prawns were fed a balanced diet containing graded levels of a high quality protein over a definite period of time and observed protein levels giving maximum growth and, more recently tissue retention or nitrogen balance was taken as the requirement. Dietary protein requirements are normally expressed in terms of a fixed dietary percentage or as a ratio of protein to dietary energy.

Numerous investigators used various semipurified and, less commonly, purified diets to estimate the protein requirement of the various species of prawns and shrimps. One of the earliest published compounded rations for shrimp was that of Kanazawa *et al.* (1970) which was designed for *P.japonicus* and formulated with reference to the composition of short neck clam (*Tapes philippinarum*), a widely used but relatively expensive commericial diet for shrimp in Japan. The feed consisted of purified soybean protein, together with a few minor sources of nitrogen, and a few amino acids and must have contained around 55% protein (New, 1976a). On the basis of a feeding study for 30 days in which the best diet produced growth rates of 72% of the control diet (fresh short neck clam) Kanazawa *et al.* (1970) suggested that an artificial diet consisting of purified soybean protein (50.0%), glucose (5.6%), sucrose (10.0%), starch (4.0%), chitin (4.0%), glucosamine (1.5%), cellulose powder (4.0%), methionine (1.0%) , tryptophan (0.2%), glutamic acid (0.2%), glycine (0.1%), citric acid (0.3%), succinic acid (0.3%), refined soybean oil (8.0%), cholesterol (0.5%), salt mixture (7.7%), vitamin mixture (2.6%) and binder agar (4.0%) is sufficient for the feed trials as to the nutritional requirement of the prawn, *P.japonicus*. Later Kanazawa *et al.* (1976) modified the original composition by substituting soybean protein with casein. Castell and Budsen (1974) and Shlesser and Gallagher (1974) tried casein based semi purified diet for nutritional studies on lobster (*Homarus* sp.)

Later many investigators have used test diets varying greatly in composition, amino acid make up, texture and other physical properties to study the protein requirement of the various species of shrimps and prawns. A few workers have also employed practical commercial diets with graded levels of protein for the purpose. The sources of protein employed by various authors in nutritional studies with prawns and shrimps include casein, fish meal, soybean meal, shrimp meal, crab protein, squilla meat, clam meat, mussel meat, squid meat, ground nut oil cake, pea nut cake, single cell proteins, leaf proteins etc. singly or as combinations of two or more of the above, supplemented with or without the amino acids deficient in the respective proteins.

New (1976a) criticized the lack of standardization in experimental design, test feeds employed, culture conditions and analytical techniques which limited the value of published information on shrimp and prawn nutrition and made direct comparisons of the results from different laboratories difficult. He also gave a brief account of the data required from dietary trials for comparative purposes. Recognizing the shortcomings in past research, the World Mariculture Society (now the World Aquaculture Society) established a Nutrition Task Force to develop guidelines for standardizing aquaculture nutrition research methodology (Conklin and Beck, 1979). Castell and Tiews (1980) presented report of the the European Inland Fisheries Council (EIFAC), International Union of Nutrition Scientists (IUNS) and International Commission for Exploration of the Sea (ICES) Working group on Standardization of Methodology in Fish Nutrition Research. These working groups advocated the establishment of a standard reference diet as an important first step in facilitating direct comparison of results among laboratories, experiments and species. Boghen *et al.* (1982) discussed the need for a standard reference diet for nutritional studies with crustaceans. They tested purified protein concentrates from a number of marine organisms and reported that diets containing rock crab (*Cancer irroratus*) gave superior results. Kanazawa *et al.* (1982) gave the composition and methods of preparation of casein based purified diet for nutritional studies with prawn. Castell *et al.* (1989a)) evaluated two dietary formulations, one based principally on casein (BML 81 S) and the other on purified crab protein (HFX CRD 84) which on the basis of data on growth and survival of a variety of crustacean species viz., *Pandalus danae, Penaeus monodon, P.stylirostris, P.vannamei, P.brasiliensis, P.setiferus, P.aztecus and H.americanus*, were suggested as acceptable standard reference diets. Castell *et al.* (1989b) also gave details of selection of a purification procedure for production of the rock crab (*Cancer irroratus*) protein ingredient to be used in standard reference diets for crustaceans. Reed and D'Abramo (1989), Morrissy (1989) and Bordner (1989) evaluated the performance of these diets viz., BML 81 S and HFXCRD 84

in nutritional studies with the freshwater prawn (*M.rosenbergii*), the freshwater cray fish (*Cherax tenuimanus*) and the Dungeness crab (*Cancer magister*) respectively.

Recently purified diets based on casein were also successfully tried by many investigators in nutritional studies on various penaeid prawns (Aquacop, 1978; Gopal, 1986; Ahamed Ali, 1988; Clark and Lawrence, 1988a,b; Civera and Guillaume, 1989; Diminy and Lim, 1989), on *M.rosenbergii* (Hilton *et al.*, 1984; Heinen, 1988; Briggs *et al.*, 1988; Gomez *et al.*, 1988; Reigh and Stickney, 1989) and on other crustaceans (Conklin *et al.*, 1980; Baum *et al.*, 1989).

The maintenance protein requirement of an animal is the protein intake required to maintain nitrogen equilibrium. It reflects catabolism and turnover of body protein, irrespective of nutritional status of the prawn. The amount of protein required for maintenance in prawns and shrimps can be measured by feeding a diet containing just enough protein to balance the loss of protein due to recycling of tissues, enzymes etc., so that the protein content of the body remains unchanged. Since loss of endogenous nitrogen in faeces and metabolic excretion continues irrespective of protein intake, very often protein requirement for maintenance is determined by measuring the nitrogen loss on prawns fed a protein-free diet (Hepher, 1988). The rate of protein loss can be measured either by determining the content of body nitrogen on successive samples or by determining the content of nitrogen in the excretions. These data can also be combined with nitrogen retention data obtained by feeding increasing protein level and obtaining nitrogen or protein intake which results in nitrogen equilibrium. The methodology used to evaluate the excretion of endogenous nitrogen has been reviewed by Luquet and Kaushik (1981), Hepher (1988) and Wilson (1989). Kaushik (1980) and Ogino *et al.* (1980) compared the methods based on nitrogen excretion and change in body carcass when fed with zero protein diet and found a small difference in the results.

Studies on maintenance nitrogen requirment of prawns and shrimps are very few. Perhaps the report of maintenance nitrogen (protein) requirement in *P.indicus* by Ahamed Ali (1988) stands as a single report in the literature. Forster and Gabbott (1971) and Ahamed Ali (1988) reported the metabolic faecal nitrogen in *P.serratus* and *P.indicus* respectively.

Since the early work of Subrahmanyan and Oppenheimer (1969), Kanazawa *et al.* (1970) and Deshimaru and Shigueno (1972), numerous studies have been reported on the growth rates, feed efficiencies etc. of various crustaceans fed different levels of dietary protein. Most of these studies on the protein requirement of the prawns and shrimps have been reviewed in detail by New (1976a,b, 1979, 1980a), Forster, (1976); Wickins (1976),Rao (1983), Kanazawa (1984), Gopal (1986) and Ahamed Ali (1988). Many of these authors also discussed the problems associated with conducting basic nutrient requirement studies in crustacea. New (1980b) also published a bibliography of shrimp and prawn nutrition providing an exhaustive coverage of literature on the subject of protein nutrition published till the date. Biddle (1977), Corbin *et al.* (1983), and Sick and Millikin (1983) have given a comprehensive review of the protein requirement and feeding practices exclusively for the gaint freshwater prawn, *M.rosenbergii*. Recently a practical manual on the preparation and feeding of diets for prawns (and fish) in which some examples of formulations for compounded shrimp diets were provided has also been published (New, 1987).New (1988) and Sebastian (1990) also briefly discussed the crude protein requirement and feeding practices for the giant freshwater prawn, *M.rosenbergii*.

Rather than paraphrase such findings on the crude protein requirement, for brevity, an abbreviated table has been presented to illustrate the wide range of dietary protein requirement values reported by the investigators for the various species of shrimps and prawns (Table 2). Also presented in the table are the principal sources of protein used by these investigators in their respective diets, which have been reported to affect the protein requirement values considerably (Zein-Eldin and Meyers, 1973; New, 1976a; Kanazawa, 1984; Tacon and Cowey, 1985; Wilson, 1989).

Table 2. Summary of dietary protein levels evaluated in feeding studies with various species of shrimps and prawns

Species	Life stage	Protein source	Optimum[1] level(%)	Reference
P.monodon	juvenile	casein & fish meal	45.0	Lee (1971)
"	postlarva	fish meal	30.0	Khannapa (1977)
"	juvenile	casein	30.0-40.0	Khannapa (1979)
"	postlarva	"	55.0	Bages & Sloane (1981)
"	juvenile	---	35.0	Lin *et al.* (1981)
"	"	casein,squid meal, fish meal, shrimp meal, soybean, & bread flour	40.0	Alava & Lim (1983)
"	"	casein & gelatin	40.0-50.0	Bautista (1986)
"	"	prawn muscle, casein, gelatin & egg protein	55.0	Nezaki (1986)
"	brood stock	fish meal, squid meal & shrimp head meal	50.0-55.0	Millamena *et al.*(1986)
P.indicus	juvenile	prawn meal	43.0	Colvin (1976)
"	"	leaf protein, copra meal powder, prawn head powder, soybean, squid & clam meat	50.0-60.0	Sambasivam *et al.* (1982)
"	"	mantis shrimp & ground nut oil cake	42.9	Ahamed Ali (1982)
"	early postlarva	casein	40.0	Bhasker (1982)
"	late postlarva/ juvenile	"	30.0	"
"	juvenile	"	35.0-40.0	Gopal (1986)
"	"	albumen	25.0	Ahamed Ali (1988)
"	"	casein	29.0	"
P.merguiensis	"	"	50.0-55.0	Aquacop (1978)
"	"	mussel meal	34.0-42.0	Sedgwick (1979)

Table 2. Summary of dietary protein levels evaluated in feeding studies with various species of shrimps and prawns (*Contd.*)

Species	Life stage	Protein source	Optimum level(%)	Reference
P.japonicus	juvenile	silk worm, brine shrimp & fish meal	55.0	Kanazawa *et al.* (1970)
,,	—	—	53.5	Kitabayashi *et al.* (1971c)
,,	juvenile	squid meal (principally)	60.0	Deshimaru & Shigueno (1972)
,,	—	squid meal, fish meal, mysid meal, sludge & yeast	60.0	Shigueno *et al.* (1972)
,,	juvenile	soybean, fish & shrimp meal	40.0	Balazs *et al.* (1973)
,,	,,	casein & egg albumin	50.0	Deshimaru & Kuroki (1974a)
,,	,,	,,	52.0-57.0	Deshimaru & Yone (1978b)
,,	larva	casein	45.0	Teshima & Kanazawa (1984)
P.aztecus	—	—	23.0-31.0	Shewbart *et al.* (1973)
,,	—	—	40.0	Balazs *et al.* (1973)
,,	postlarva	fish protein	40.0	Venkataramiah *et al.* (1975b)
,,	juvenile	soyflour	51.5	Zein-Eldin & Corliss (1976)
,,	,,	squid mantle meal	36.5	Fenucci & Zein-Eldin (1976)
P.setiferus	,,	menhaden meal	28.0-32.0	Andrews *et al.* (1972)
,,	—	—	30.0	Fenucci *et al.* (1980)
P.duorarum	juvenile	soybean meal	28.0-30.0	Sick & Andrews (1973)
P.stylirorostris	postlarva	shrimp meal, fish meal & yeast	30.0-35.0	Colvin & Brand (1977)
,,	—	—	30.0	Fenucci *et al.* (1980)
P.californiensis	early postlarva	shrimp meal, fish meal, soybean meal & yeast	44.0	Colvin & Brand (1977)
,,	late postlarva	shrimp meal, fish meal,	30.0-35.0	Brand & Colvin (1977)

Table 2. Summary of dietary protein levels evaluated in feeding studies with various species of shrimps and prawns (*Contd.*)

Species	Life stage	Protein source	Optimum[1] level(%)	Reference
		soybean meal, wheat, whole egg protein	(closer to 35)	
P.vannamei	postlarva	shrimp meal, fish meal & soybean meal	30.0-35.0	Colvin & Brand (1977)
"	larva	—	37.6	Smith & Lawrence (1988)
M.monoceros	juvenile	casein	55.0	Kanazawa *et al.* (1981)
M.macleayi "		prawn meal & wheat	27.0	Maguire & Hume (1982)
P.serratus	—	fish meal & shrimp meal	30.0-40.0	Forster & Beard (1973)
M.nobili	juvenile	casein	35.0	Murugadas & Pandian (1987)
"	—	—	30.0	Pandian & Murugadas (in prep., Pandian, 1989)
M.rosenbergii	juvenile	soybean, tuna & shrimp meal	35.0	Balazs & Ross (1976)
"	—	—	25.0	Manik (1976)
"	larva	—	15.0-20.0	Sick (1976)
"	juvenile	—	25.0	Clifford & Brick (1979)
"	"	fish meal & Soybean	40.0	Millikin *et al.* (1980)
"	juvenile/ sub adult	shrimp meal pea nut meal & soybean meal	15.0	Boonyaratpalin & New (1982)
"	—	—	25.0	Perry *et al.* (1984)
"	—	—	14.0	Antiporda (1986)
"	juvenile	crab protein	33.0-35.0	D'Abramo & Reed (1988)
"	"	casein	13.0-25.0	Gomez *et al.* (1988)
"	"	crab protein	30.0	Freuchtenicht *et al.* (1988)

[1] Wherever range is given the middle value is used for calculations

It may be noted from Table 2 that though 32 species of shrimps and prawns have been investigated and cultured throughout the world (Liao, 1987) dietary protein requirement has been estimated only for about 15 species. In general, the protein requirement values reported vary from 14 to 60% of the dry diet. The average protein requirement value for the 57 cases cited in Table 2 is 37.9% .This value is around 200% of the protein requirement values reported for terrestrial animals but slightly less than the average value (41.9%) reported by Hepher (1988) for finfishes.

Many authors attributed the higher protein requirement of most of the finfishes and shellfishes to their carnivorous/omnivorous feeding habits (Cowey, 1975; Watanabe *et al.*, 1979; Tacon and Cowey, 1985) and to the apparent preferential use of protein to carbohydrate and lipids as a source of energy (Gerking, 1955; Kutty and Mohamed, 1975; Ahamed Ali, 1988).

Mertz (1968) related the high protein requirement to the high amino acid level in the plasma of the fish, which is three to six times higher than that in mammals. He assumed it to be due to a deficiency in deamination enzymes and high recycling of amino acids. However, there is no experimental evidence to support this assumption either in finfishes (Hepher, 1988) or in crustaceans. In crustaceans the relatively high protein need may also be related to the nitrogen cost of moulting.

The observed higher percentage protein requirement of shrimps and prawns compared to homeothermic terrestrial animals may atleast in part be linked to the method of expressing the protein requirement in relative terms. The energy need of crustaceans is relatively less compared to the homeothermic terrestial animals which inturn apparently increases the protein need when expressed in terms of percentage of dry weight of the food. It may be noted that the dietary protein requirements of farmed crustaceans are not far dissimilar from those of terrestrial animals when expressed in absolute amount of protein per unit body weight of the animal or to bring about unit increase in body weight. Thus to allow valid comparisons between results from different laboratories protein requirement can no longer be simply expressed solely in percentage terms or as a protein energy ratio (Tacon and Cowey, 1985).

Further as has been opined by Wilson (1989) many of the observed protein requirement values for crustaceans are perhaps over estimated because of inadequate considerations of factors like energy concentrations in the diet, and amino acid composition and digestibility of the dietary protein. Pandian (1987) and Tacon and Cowey (1985) also observed that the high protein requirement values suggested by various authors might in part be a function of the methodology employed for its determination.

It may be seen that in comparison to the penaeid shrimps the protein requirement of the *Macrobrachium* spp. is relatively low. New (1988) and Pandian (1989) also made a similar conclusion by analysing the available protein requirement values for maximum growth in *Macrobrachium* spp. and penaeied spp. and the latter author attributed this observation to the faster growth potential and lower food conversion efficiency of the penaeied spp. compared to *Macrobrachium* spp. This may also be associated to the difference in the feeding habits of the two groups of prawns. Thus in comparison to the more carnivorous shrimp, *P.japonicus* (Reymond and Legardere, 1990) the protein need of the truely omnivorous *M.rosenbergii* (John, 1957; Ling, 1969; Lee *et al.*, 1980) is strikingly less. Omnivorous/herbivorous fish are reported to economize their protein requirement by reabsorbing digestive enzymes and by reducing protein metabolites (Pandian, 1989). Herbivorous fish, especially those with a long intestine, reabsorb over 90% of the digestive enzymes, as against 40% by the carnivores (Hofer and Schiemer, 1981; Pandian, 1989). The fraction of food nitrogen lost through ammonia is in the range of 3.5% for herbivores (Caulton, 1978, Hofer *et al.*, 1985), which is half of that (7%) excreted by carnivores (Brett and Groves, 1979). The lower protein requirement of the truely omnivorous *M.rosenbergii*

bergii may also be associated to the less preferential use of protein for energy production in the species. Clifford and Brick (1983) on the basis of an oxygen-nitrogen ratio of 22.1:1 that they obtained, observed in *M.rosenbergii* that the energy metabolism in this species is dominated by the oxidation of carbohydrate with the lipids and proteins forming secondary or tertiary substrates. Similarly high oxygen-nitrogen ratio was also reported in *M.rosenbergii* by Stern *et al.* (1984) and Venugopalan (1988).

There exists considerable disagreement among investigators regarding the gross protein requirement of various species of shrimps and prawns. At one extreme there are those reports that suggest increased growth rates and efficiencies even at levels in excess of 50% of the dry diet (Kanazawa *et al.*, 1970, 1981; Kitabayashi *et al.*, 1971c; Deshimaru and Shigueno, 1972; Shigueno *et al.*, 1972; Zein-Eldin and Corliss, 1976; Aquacop, 1978; Deshimaru and Yone, 1978b; Bages and Sloane, 1981; Sambasivam *et al.*, 1982; Nezaki, 1986; Millamena *et al.*, 1986) the results of which show that with the increase in protein content in the diet, there is an increase in growth rate of the prawn. At the other extreme are the studies suggesting that optimum dietary protein levels are more in the range of 20-30% (Andrews *et al.*, 1972; Shewbart *et al.*, 1973; Sick and Andrews, 1973; Manik, 1976; Sick, 1976; Clifford and Brick, 1979; Ahamed Ali, 1988). The results of these studies show that with increase in protein content in the diet, there is an increase in growth and efficiencies only upto a certain level of protein, beyond which the growth rates show a diminishing trend. While reviewing the published literature on the subject, New (1976a) observed that a consensus of openion on the optimum protein requirement for shrimps and prawns suggest a value near to 27-36% of the dry diet.

Descrepancies also exist in the reported protein requirement values for the same species by different investigators and in a few instances by the same worker(s) when different sources of protein were used (Table 2.). In *P.indicus* Ahamed Ali (1982) reported a protein requirement value of 42.8% using a test diet based on mantis shrimp and ground nut oil cake. However, in the same species Ahamed Ali (1988) found values of 25% and 29% using purified diets based on egg albumen and casein respectively. Employing a practical diet based on 70% animal and 30% plant proteins, however, Ahamed Ali (1988) found a protein requirement of 30% for *P.indicus*.

For the freshwater prawn *M.rosenbergii* using a diet based on soybean meal, tuna meal and shrimp meal Balazs and Ross (1976) reported a protein requirement value of more than 35% of the dry diet. Millikin *et al.* (1980), by using menhaden meal and soybean protein found a protein requirement value of 40% to bring about maximum growth. However studies on prawn growth in concrete ponds by employing a diet based on shrimp meal, fish meal, peanut meal, and soybean meal (5:2:1:1) indicated a low level of protein (15%) to bring about optimum growth in the prawn *M.rosenbergii* (Boonyaratpalin and New 1982). Further studies in asbestos asphalt or earthern bottom ponds (Bartlett and Enkerlin, 1983) and aquaria (Antiporda, 1986) indicated favorable results at dietary crude protein levels as low as 14%. Low protein requirement (13-25%) has also been reported by Gomez *et al.* (1988) by using a test diet based on casein. However studies using purified crab protein by D'Abramo and Reed (1988) and Fruechtenicht *et al.*, 1988, found optimum dietary protein levels of 33-35% and 30% respectively in *M.rosenbergii*.

The variety of ingredients used, the number of experimental rations and also the non uniform experimental procedures and conditions have made the direct comparisions of the results from different laboratories difficult (Zein-Eldin and Meyers, 1973; New, 1976a; Biddle, 1977; Kanazawa, 1984; Wilson, 1985, 1989). Undoubtedly, the use of improperly balanced protein sources, widely varying in metabolizable energy content of the diets, absence or presence of some non-protein nutrients and the use of different life stages of the prawn and their physiological states have also resulted in the observed variation in the protein requirement values. Various authors (New, 1976a; Biddle, 1977) criticised and cautioned drawing con-

clusions on the basis of such studies. Thus it may be noted that without understanding more about the relationship between protein requirement and factors affecting it, which may not be strictly dietary in nature, the result of these experiments cannot generally be applied.

Most protein requirement values for crustaceans are based on levels of protein that result in maximum growth with little or no index of protein utilization (Wilson, 1989). Growth rate in terms of weight is one of the most common criteria by which the diets and its protein levels are evaluated in prawns and shrimps. However weight increment need not always be due to protein anabolism alone (Hepher, 1988) and, hence, complete dependence on weight increment provide a some what inaccurate picture of protein requirement values.

Protein digestibility and their absorption by the crustacean species under consideration may vary considerably depending on the source of protein. Thus, the source of protein and its amino acid make up has got profound influence on the optimum dietary protein required by the prawn. Dietary availability of essential as well as non-essential amino acids (Hepher, 1988) and their presentation for absorption was found to affect the metabolic performance of animals (Murai *et al.*, 1981, 1982; Hepher, 1988). The dietary concentration of the various amino acids may also play a significant role in the palatability of the test diets (Takei and Ai, 1971). More over the rate of passage of various proteins through the gastro intestinal tract of the organism under study varies. If a source of protein that is evacuated at a much higher rate is used to evaluate the protein requirement in prawns, a higher level of protein may be needed to meet the body requirement for maximum growth.

Hanson and Goodwin (1977) quoted Zein-Eldin to opine that the difference in protein requirement levels are a function of the feed formula used. According to her *P.aztecus* showed depressed growth rates when protein in one experimental formula dropped below 53% while 32% protein was adequate in another formula. The minimum protein requirements of shrimps were also reported to be dependent upon the presence of other dietary components from which carbon chains can be catabolized for basal energy requirements (Andrews *et al.*, 1972). The ratio of protein to total energy of the diet has also been shown to affect the protein requirement in prawns (Bages and sloane, 1981; Hajra *et al.*, 1986; Gomez *et al.*, 1988). Excess energy in the food may limit consumption, for prawns like other animals feed to satiate energy requirements. On the other hand, sub optimum level of non-protein energy may necessitate additional amount of protein to satisfy the energy needs.

Many investigators state that they have used isoenergetic diets to determine the protein requirements. However, since, the metabolizable energy of the various ingredients has not yet been determined for most crustaceans, these workers have used various estimated physiological fuel values in expressing the protein requirement in relation to the dietary energy level. The use of same energy values for the dietary nutrients of different sources for different species of crustaceans may lead to inconsistent results because these values are mostly species and protein source specific. In many cases the energy sources are reported to have a sparing action on protein (Hysmith *et al.*, 1972; Capuzzo and Lancaster, 1979; Fair *et al.*, 1980; Bhasker and Ahamed Ali, 1984; Gomez *et al.*, 1988).

The physical characteristics of diets have a direct bearing on consumption rates and digestibility of ingested food (Biddle, 1977) and thus inturn on protein requirement. The use, by different authors, of widely varying binding materials and their levels in their test diets might also have contributed to the observed variation in protein requirement values. Sick and Beaty (1975) observed in the juveniles of *M.rosenbergii* that a diet based on soybean meal with 5% collagen did not result in gain in weight while that with long chain amylose starch did. Farmanfarmaian and Lauterio (1979) reported that more efficient feed conversions and higher growth rates were obtained using algin rather than carboxy methyl cellulose as a binder.

The use of various levels of dietary inert materials such as cellulose to adjust the nutrient balance may lead to inconsistent results on protein requirement of prawns. Excessive and varying levels of dietary cellulose, for example, in test diets for shrimp is objectionable in the light of the recent report by Herald (1989) in higher vertebrates that dietary fiber can have an adverse effect on mineral bio availability. Biddle *et al.* (1977) reported negative effect of fibre on growth and survival of blue crab (*Callinectes sapidus*). Fair *et al.* (1980) observed a decrease in nitrogen assimilation with increasing dietary fibre. Gomez *et al.* (1988) noticed physiological abnormalities in *M.rosenbergii* fed with diets having high cellulose content. Borrer and Lawrence (1989) also found higher level of cellulose to affect the dry matter digestibility in *M.rosenbergii*. In addition, cellulase enzyme has been reported to be present in shrimp (Naborikawa, 1978; Fair *et al.*, 1980). *M.rosenbergii* is reported to be capable of utilizing cellulose atleast to a limited extent (Fair *et al.*, 1980; Lee *et al.*, 1980), which thus can lead to an "unaccounted" dietary energy contribution to the animal thereby leading to a change in the protein-metabolizable energy ratio of the test diets.

Apart from these factors the formulation and preparation of the diets, feeding regime, the quantity of feed offered etc. can also affect the protein requirement of prawns. According to Tacon and Cowey (1985), Pandian (1987, 1989) most workers have used fixed and low ration in nutritional studies. Tacon and Jackson (1985) pointed out the importance of the initial grinding and the consequent particle size of the feed material which affect the efficiency of heat processing, bio-availability of nutrients of individual feed ingredients, pellatability, feed acceptability by the animal and the digestibility.

In addition, age and size of the animal, environmental parameters such as temperature, salinity, dissolved oxygen and pH of water are known to influence the growth, food conversion efficiency and protein requirement of animals. In general the protein requirement of the fast growing young stage will be higher than that of the later relatively slow growing stage indicating the importance of conducting protein requirement studies specific to each life stage and physiological state of the organism concerned. Bhasker and Ahamed Ali (1984) demonstrated that the dietary protein requirement of the postlarvae of *P.indicus* decreased with an increase in their age. These authors found the protein requirement of the early postlarvae of the prawn to be 40% compared to 30% in the case of the late postlarval stage. Goodwin and Hanson (1975), Colvin and Brand (1977), Deshimaru and Yone (1978b), Khannapa (1979) and Sedgwick (1979) also reported a reduction in the protein requirement value with age in penaeid prawns. Balazs *et al.* (1974), Farmanfarmaian and Lauterio (1979) Millikin *et al.* (1980) and Sick and Millikin (1983) reported similar trend in protein requirement of *M.rosenbergii*.

Sick *et al.* (1972) studied selected environmental and nutritional requirement of penaeid shrimp and Venkataramiah *et al.* (1975a) reviewed the influence of environmental and nutritional factors on *P.aztecus*. Changes in water temperature, have been shown to alter the metabolic activity markedly and influence the dietary protein requirements (De Long *et al.*, 1958; Millikin, 1982, 1983). According to Seagrave (1988) in all fishes the feeding stimulus is primarily determined by water temperature. Kalyanaraman (1983) while studying the effect of salinity on food intake, growth, conversion efficiency and proximate composition reported that the salinity had profound influence on them. He also observed increased ammonia excretion in juvenile *P.indicus* at sub and supra optimum salinity levels and related it to the increased catabolism of amino acids at these salinity levels. Stern *et al.* (1984) and Venugopalan (1988) observed, on the basis of oxygen-nitrogen ratios under different salinity conditions, salinity to have influence on the protein catabolism in *M.rosenbergii*, . Light intensity may also significantly affect assimilation values (Sick *et al.*, 1973). Light intensity is known to be directly related to ingestion rate among juveniles and postlarval *P.duorarum* (Sick and Baptist, 1973) and *M.rosenbergii* (Sick and Beaty, 1975).

2.2.2 *Qualitative Protein Requirement.*

The performance of a shrimp diet is influenced not only by the dietary protein concentration, but the amino acid composition and the presence in the diet of other nutritional factors as well. Many authors emphasized the importance of a protein source having an amino acid profile similar to that of the shrimp under consideration to bring about maximum growth (Kitabayashi *et al.*, 1971b; Deshimaru and Shigueno, 1972; Aquacop 1976; New, 1976a; Wickins, 1976; Deshimaru, 1981, 1982; Hew and Cuzon, 1982; Ahamed Ali, 1988). However the digestibility and the amino acid availability for absorption by the organism must also be considered while assessing a source of protein to be successfully incorporated in shrimp diets. Some proteins are biologically unavailable for the animals due to alteration in the amino acid composition during processing by combining with other compounds, thereby becoming resistant to proteolytic enzymes (Cowey and Sargent, 1972; Maynard *et al.*, 1979) or due to the destruction of heat sensitive amino acids (Smith and Circle, 1972). Protein from different sources may thus be utilized by shrimps to different extents and affect growth rates differently.

A variety of sources of protein and the substitution effect of one source with another in various species of shrimps and prawns have been evaluated by various investigators with varying degrees of success. There exists variation among species in the ability to utilize protein from different sources and hence the extrapolation of results across species lines is not desirable.

In general casein as a single source of protein was reported to be poorly utilized by shrimps (Kanazawa *et al.*, 1970; Sick *et al.*, 1972; Sick and Andrews, 1973; New, 1976a). *P.aztecus* grew little on diets with casein as the major source of protein and the growth was poorer than that for *P.japonicus* fed with such diets (Kanazawa, *et al.*, 1970). The poor performance of casein as the sole source of protein may principally be attributed to the suboptimum level of sulpher containing amino acids or other essential nutrients that the diet contain for crustaceans (Baum *et al.*, 1989). Casein has been reported to be well assimilated by *P.serratus* and gave the best result compared to 14 other diets belonging to plant as well as animal origin. Teshima *et al.* (1986) reported better performance of casein as a single source of protein in the diets for the larvae of *P.japonicus* compared to gelatin and crystalline amino acids and produced comparable results with white fish meal. In the juveniles *of P.indicus* Ahamed Ali (1988) compared the performance of casein based diet to that with egg albumen, fibrin and gelatin and found casein to be utilized well second only to egg albumen. For diets containing purified ingredients, Akiyama *et al.* (1988) observed no differences in protein digestibility between casein, wheat gluten, gelatin and soy protein. Casein based diets were also reported to be utilized well by the postlarvae (Reed and D'Abramo, 1989) and juveniles (James *et al.*, 1990) of *M.rosenbergii*. Attempts to improve the dietary value of casein by supplementing with other protein sources or crystalline amino acids which are otherwise deficient in casein have also been reported by many (Deshimaru and Kuroki, 1974a, 1975a; Teshima *et al.*, 1986). Alternatively report of successful supplementation with casein to improve the dietary value of other protein sources have also been published (Koshio *et al.*, 1989). Reports of the use of casein incorporated with or without crystalline amino acids or other protein sources as test diets to study the nutritional requirement of many species of crustaceans have also been published (Bhasker and Ahamed Ali, 1984; Hilton *et al.*, 1984; Gopal, 1986; Ahamed Ali, 1988; Clark and Lawrence, 1988a,b; Gomez *et al.*, 1988; Bordner, 1989; Castell *et al.*, 1989a,b; Civera and Guillaume, 1989; Morrissy, 1989; Reed and D'Abramo, 1989; Reigh and Stickney, 1989).

An analysis of the results on the use of casein based diets in crustacean nutrition studies indicates its high dietary value when supplemented with the limiting nutrient and protected against leaching of nutrients in water, though the effects may vary in different species.

Egg albumen was also used by various authors singly or in combination with other protein sources. In comparison with casein, egg albumen as the sole source of protein resulted in better performance in the juveniles of *P.indicus* (Ahamed Ali, 1988), but apparently poorer in the larvae of *P.japonicus* (Teshima, *et al.*, 1986). Egg albumen has also been featured as a feed attractant in shrimp diets (Sick and Beaty, 1975). In the larval rearing of *M.rosenbergii* successful use of egg albumen as a supplemental source of protein is common (New and Singholka, 1982).

Presumably because of the dissimilar amino acid profiles with that of the prawn body protein gelatin has resulted in poorer results in trials with shrimps and prawns (Cowey and Forster, 1971; Ahamed Ali, 1982, 1988; Teshima *et al.*, 1986). Forster and Gabbott (1971) reported gelatin to be the best digested protein of a series of 16 diets tested in *P.serratus*. Akiyama (1988) observed good apparent digestibility of gelatin in *P.vannamei*.

Various marine proteins have been widely evaluated in shrimp diets, with varying degrees of success. In *P.serratus* Cowey and Forster (1971) observed freeze dried cod muscle to result in highest weight gain compared to casein, gelatin, gelatin supplementated with tryptophan, zein, zein supplemented with lysine and tryptophan. Sick and Andrews (1973) working with *P.duorarum* compared the dietary value of menhaden meal with a number of alternative protein sources and found the fish meal to be superior to shrimp meal, casein and maize gluten meal but inferior to one from the plant origin viz., soybean meal. In *P.indicus* a diet based on fish meal was proved to be the best one among eleven feeds experimented with (Raman *et al.*, 1982). Ahamed Ali (1988) also observed fish meal to be the best, when compared to clam meat powder, prawn waste meal and mantis shrimp in *P.indicus*. *P.japonicus* also exhibited higher assimilation efficiency when fed with fish meal (Nose, 1964).

In nutritional studies with prawns and shrimps results in contrast to the above mentioned observations were also reported. Thus a diet based principally on fish meal has been reported to be an inferior diet for prawns by Deshimaru and Shigueno (1972) and Colvin (1976). The apparent protein digestibility of fish meal was found to be less than that of soybean meal in *P.vannamei* for diets containing practical ingredients (Akiyama *et al.*, 1988). Teshima *et al.*(1986) also reported the poorer performance of a larval diet based on white fish meal. Mason and Castell (1980) indicated the toxic effect of fish protein concentrate in the juveniles of *H.americanus*. In *M.rosenbergii* an amaizo diet having fish meal as the principal source of protein resulted in poorer weight gains compared to many other sources of protein tested including one with soybean protein (Sick and Beaty, 1975). Sherief (1987) also reported the poorer performance of fish meal compared to clam meat in the postlarvae of *M.rosenbergii*.

The observed poorer performance of fish meal in shrimps and prawns may be related to its amino acid profile. Fish meal is low in basic amino acids which are apparently necessary for shrimps (Deshimaru and Shiguino, 1972; New 1976a; Brand and Colvin 1977; Colvin and Brand 1977) and/or due to an imbalance in the mineral content (Colvin and Brand, 1977). It may also be related to the components in it other than protein or to the difference in physical characteristics between diets (Sick and Andrews, 1973). The method of drying and the freshness of the raw material affect the quality of the fish meal. Vacuum and steam dried fish meals are recommended for use in shrimp diets (Akiyama *et al.*, 1991). Flame dried fish meal is exposed to higher temperature which renders protein less available, oxidizes lipids and produces anti nutritional factors ie., histamine (Akiyama *et al.*, 1991). Nevertheless, fish meal is one of the most common ingredients in commercial shrimp feeds and its level of inclusion usually ranges from 10-40% (Akiyama *et al.*, 1991).

Crustacean meals have also been tested extensively by many investigators in shrimp diets. In general, due to the more similar amino acid profile to that of prawn body protein shrimp meal is expected to produce the best results in prawns. However, contrasting results have been reported from different

laboratories. Thus in *P.duorarum* it resulted in a poorer growth performance than soybean and fish meal but better than casein and maize gluten meal (Sick and Andrews, 1973). Forster and Gabbott (1971) found the assimilation efficiency of shrimp meal in *P.serratus* to be poorer than that of casein, gelatin, egg albumen, freeze dried egg, mussel mantle, fish meal, soybean meal, maize gluten meal, ground nut meal and bacterial protein but better than that of two mammalian sources of protein and cotton seed meal tested in the trial. In *Pandalus platyceros* the same authors found the assimilation efficiency of shrimp meal to be inferior to that of casein, soybean meal and bacterial protein but superior to that of white fish meal. In the juveniles of *M.rosenbergii* Balazs *et al.* (1974) observed no beneficial effects when shrimp meal was combined with tuna and soybean meals at the 25 and 35% protein levels. Akiyama (1988) reported the apparent protein digestibility of shrimp meal to be poorer than that of soybean meal in *P.vannamei*. Aquacop *et al.* (1989) reported the lower digestibility coefficient of shrimp meal compared to fish meal, squid meal and soybean meal in *P.stylirostris, P.monodon* and *P.vannamei*. An examination of dietary essential amino acid composition indicates that, with the exception of tyrosine and possibly also phenyl alanine, prawn meal is a poorer source of proven essential amino acids compared to fish meal (Colvin, 1976). In *P.californiensis* Colvin and Brand (1977) obtained poorer growth performance when the marine proteins (fish meal and shrimp meal) exceeded a level of 40% in the diet. Dried *Acetes* was reported to produce comparable results with live feeds in *P.monodon* by Kungvankij *et al.* (1986). In *P.serratus* Forster and Beard (1973) reported the slightly better performance of shrimp meal over fish meal. Nair and Thampy (1987) also reported the superiority of a diet based principally on prawn meat (*Metapenaeus dobsoni*) over other sources of proteins tested, in the larvae of *M.rosenbergii*. Shrimp meal levels in commercial shrimp feeds usually range from 5-15% (Akiyama *et al.*, 1991).

In addition, the use of shrimp head waste in compounded ration appears promising (Venkataramaih *et al.* 1978 and Ahamed Ali,1988). Shrimp head waste is high in crude protein, several essential amino acids (Forster, 1975) and contains a fair amount of fatty acids (Sandifer and Joseph, 1976) and hence may be a relatively cheap nutritional source in shrimp diets. However shrimp head waste meal as a single source of protein has been reported to result in poor growth rate in *P.indicus* (Raman *et al.*, 1982) and in *P.monodon* (Pascual and Destajo, 1979).

A significant portion of nitrogen present in the shrimp head waste and to some extent the whole body protein is in the form of non-protein nitrogen which may not have much protein function. The ability of the organism to assimilate large quantity of chitin which, in general, is considered to be resistant to digestion must also be taken into account before any recommendation for the large scale use of this source of protein is made. Mention may also be made here of the observation by Venkataramiah *et al.* (1975b) that *P.aztecus* fed on an exclusive diet of shrimp meat to become more susceptible to chitino-clastic bacterial infection.

Crab protein has also been evaluated to be incorporated in shrimp diets by a few. Protein from the rock crab (*Cancer irroratus*) has been found to be of insufficient value except when supplemented with 20-40% casein for the larvae of *P.japonicus* (Koshio *et al.*, 1989). Mention may also be made on the use of purified crab protein in nutritional studies with a variety of crustaceans including *M.rosenbergii* (Boghen *et al.*, 1982; Freuchtenicht *et al.*, 1988; D'Abramo and Reed, 1988; Bordner, 1989; Castell *et al.*, 1989a,b; Morrissy, 1989; Reed and D'Abramo, 1989). Successful large scale use of other crustacean protein sources such as squilla (*Oratosquilla* spp.) in larval diets for the penaeid shrimp larvae was also documented (Alikunhi *et al.*, 1980). Ahamed Ali (1982, 1988) also found mantis shrimp based diet to perform well in grow out feeds for *P.indicus*.

Another important source of protein that has attracted the attention of shrimp nutritionists is the silk worm pupal proteins. Doubts may be raised on the ability of prawns to assimilate large quantity of

chitin present in the silk worm pupae. Penaeids as a matter of interest, appear to have two chitinase systems one largely in the hepatopancreas and the other centred around the chitino-clastic bacteria in the digestive glands (Hanson and Goodwin, 1977). However, the results on the effect of supplemental glucosamine, the monomer of chitin, on growth and survival of prawns is conflicting. Kitabayashi *et al.* (1971a) demonstrated that the addition of 0.52% glucosamine to diets improved growth but that of chitin inhibited growth. Deshimaru and Kuroki (1974b) has demonstrated an inhibitary action of glucosamine on the growth promoting effect of cholesterol. However Vaithaiswaran and Ahamed Ali (1984) reported glucosamine and chitin to have a growth promoting effect in the juveniles of *P.indicus*. It may also be seen that the purified diets proposed for use in nutritional studies with prawns and shrimps by Kanazawa *et al.* (1970) and Kanazawa *et al.* (1982) include 0.5% glucosamine . In growth studies with the juveniles of *P.indicus* silk worm pupa was found to be a poor protein source having low digestibility, protein efficiency ratio, net protein efficiency ratio and biological value (Ahamed Ali, 1988). Recently in *M.rosenbergii*, on the basis of a growth study for 21 days, Unnikrishnan *et al.* (1990) reported the high nutritional value of silk worm pupal protein.

Proteins from earth worm, marine annelids and mollusks like squid, clam, oyster, mussel etc. have also been featured as promising sources of protein in shrimp diets (Subrahmanyan and Oppenheimer, 1969; Cowey and Forster, 1971; Kitabayashi *et al.*, 1971c; Deshimaru and Shigueno, 1972; Forster and Beard, 1973, 1974; Kittaka 1976; Fenucci and Zein-Eldin, 1976; Murai and Andrews, 1978; Lim *et al.*, 1979; Fenucci *et al.*, 1980; Dokken and Lawrence, 1985; Pascual, 1985). Mollusks are reported to be excellent feed for penaeids (Forster, 1976). The inclusion of 56-74% squid meal by Kitabayashi *et al.* (1971c) and 20-47% by Deshimaru and Shigueno (1972) gave good results with *P.japonicus*. The latter authors have shown squid meal to have an amino acid profile closely similar to that of the prawn *P.japonicus*. However, using a series of diets for *P.aztecus* containing 31.5% shrimp meal a level of 5 and 15% squid meal gave better growth and survival than levels of 30% and 49% (Fenucci and Zein-Eldin, 1976). Many authors (Cruz-Saurez and Guillaume, 1983; Cruz-Ricque *et al.*, 1987, 1989; Cuzon and Aquacop, 1987) reported a growth promoting effect of squid protein extract even at a very low level of inclusion in the diet. This was attributed to an unknown growth factor contained in squid. This unknown growth factor, which is believed to be a small peptide increases the digestive efficiency of shrimp as well as enhances growth rate, and is more highly concentrated in the mantle as compared to the viscera (Akiyama *et al.*, 1991). Cruz-Suarez *et al.* (1987) found that the growth promotion by squid protein fraction was through cell hypertrophy rather than hyperplasy. A preliminary experiment by Cruz-Suarez *et al.* (1989) suggested an effect of protein and their extracts on the regulation of shrimp digestive enzyme activities. Cruz-Ricque *et al.* (1989) also suggested an effect of squid extract on the absorption of nutrients (internal transport or endocytosis). The growth promoting effect of squid may vary according to the species of prawn. Cuzon and Aquacop (1987) evaluated the growth promoting effect of squid protein extract in diets for *P.stylirostris, P.vannamei, P.monodon* and *P.indicus*. In *P.stylirostris* and *P.vannamei* growth rates were significantly improved by squid protein fraction at the lowest level of supplementation (1.5%). However in *P.monodon* growth improvement was obtained only with 6 and 16% levels while no significant response was observed in *P.indicus*. Squid meal levels in commercial feeds usually range from 2-10% (Akiyama *et al.*, 1991).

In addition, the use of bivalve mollusks either as a supplementary feed or as a principal source of protein in complete commercial feeds for prawns and shrimps is also common. Clam meat powder as a single source of protein has been found to result in superior growth in *P.indicus* (Ahamed Ali, 1982, 1988). Short neck clam (*T.philippinarum)* has been traditionally considered as an excellent feed for *P.japonicus*. Deshimaru and Shigueno (1972) compared the distribution and amount of amino acids in the flesh of *P.japonicus* with that of short neck clam and found a good similarity between them. *P.japonicus* has also been shown to grow well on diets with short neck clam (Kanazawa *et al.*, 1970) and amino acid pattern similar to that of the proteinaceous fraction of the short neck clam (Deshimaru,

1981). Nair *et al.* (1990) reported the superiority of dried clam meat as a single nutritional source compared to a commercial prawn feed with a protein concentration of 31.2% in *M.rosenbergii*. In the postlarvae of the same prawn Sherief (1987) also found a formulated feed with dried clam meat as the principal source of protein to give better results compared to the one based on fish meal. Minamizawa and Morizane (1970) obtained better results when *Artemia* nauplii were supplemented with chopped short neck clam in the larvae of *M.rosenbergii*.

Sultan *et al.* (1982) obtained encouraging results using frog flesh waste as a protein source in the feed for *P.indicus* and *P.monodon*. The use of mammalian meals and slaughter house waste has also been reported. Although the amount of total amino acids in whale meal is high, its amino acid distribution is far dissimilar from that of the prawns (Deshimaru and Shigueno, 1972). Forster and Gabbott (1971) has also evaluated the digestibility of whale meal and slaughter house offal in *P.serratus* and found them to be among the least assimilated ones. The authors attributed no reason for the observation. But the presence in the slaughter house waste of collagen a fibrous protein resistant to digestion, may atleast partly be responsible for it.Goswami and Goswami (1979, 1982) indicated the possibility of the use of slaughter house waste in shrimp diets. Graces and Heinen (1989) reported beef liver as an excellent supplemental feed for the postlarvae of *M.rosenbergii*. Increasing amount of beef liver in the diet generally gave increased mean weight and yield but most benefit was achieved at 15% level of inclusion of beef liver on a dry weight basis. However, the saturated lipid contents of the terrestrial animal by-products are believed to reduce the production performance of shrimp (Akiyama *et al.*, 1991).

Recently, Lawrence and Castille (1988) studied the nutritional response of postlarval *P.vannamei* to various levels of meat and bone meal and indicated that meat and bone meal could be cost effectively used as a partial substitute to the marine protein in shrimp diets. Blood meal, as a partial protein replacement for fish meal, has also been tried by a few. Brand and Colvin (1977) showed growth depression in *P.californiensis* at all levels of inclusion (5-10%). Blood meal has also been found to be inferior in the diet of *P.paulensis* when it replaced fish meal, shrimp meal, clam, soybean meal or rice bran (Marchiori *et al.*, 1982). Nevertheless, a grain based protein complemented with a blood meal supplement may prove to be an attractive cost effective diet with an amino acid profile effective for shrimp growth (Dominy and Ako, 1988). These authors observed that blood meal products could replace marine protein in grow out rations for *P.vannamei* and indicated that the blood meal product containing covalently attached methionine might be a potentially useful way to supplement limiting amino acids in shrimp diets. According to Akiyama *et al.* (1991) blood meal should not be used in commercial shrimp feeds at levels exceeding 7%.

Those of the animal origin being relatively expensive, proteins of plant origin have also been tried in shrimp diets by many workers in attempts to minimise the cost of production while maintaining adequate growth of shrimps. According to Hanson and Goodwin (1977) herbaceous feeds alone seem not to result in acceptable growth and survival in prawns. Studies by Venkataramiah *et al.* (1975b) showed that plant material is an essential part of prawn diet and specifically improves food conversion efficiency and survival rates in brown shrimp *P.aztecus*.

A somewhat unexpected observation in prawns and shrimps is the superior performance of soybean meal compared even to many animal sources of protein observed in almost all the prawn species tested so far. Sick and Andrews (1973) using *P.duorarum*, obtained relatively high growth rates employing soybean meal as a single protein source when compared to shrimp meal or fish meal. Progressive increment of soybean protein from 12-41% replacing shrimp meal and fish meal (1:1 ratio) has been reported to result in better growth and feed efficiency in the prawn *P.californiensis* by Colvin and Brand (1977) who attributed the result to a better amino acid profile and/or to mineral balance. In

P.japonicus an all-vegetable (soybean) based diet could produce comparable results to that with soybean, fish meal and shrimp meal. Fenucci *et al.* (1980) replaced 50% squid meal with a purified soy protein and obtained better growth, survival and feed conversion ratios in *P.setiferus* and *P.stylirostris*. Recently Fernandez and Lawrence (1988a) also reported the better growth supporting effect of soybean meal in a diet for *P.vannamei* and suggested an inclusion level of 45-60% and 25-53% soybean meal in diets having 35% and 25% protein levels respectively. In *P.vannamei* for the diets containing practical ingredients, soybean meal had a higher apparent protein digestibility than fish meal, squid meal, rice bran and shrimp meal (Akiyama *et al.*, 1988). For diets containing purified ingredients, these authors observed no differences between casein, wheat gluten, gelatin and soy protein. Akiyama (1988) reviewed several studies in which fish meal was replaced by soybean meal. In *P.monodon* Akiyama (1989) demonstrated the possibility of replacing considerable amount of white fish meal by soybean meal. Aquacop *et al.* (1989) also reported the higher digestion coefficient (99%) of soybean meal in *P.stylirostris*, *P.monodon* and *P.vannamei* compared to six other protein sources tested, including three of animal origin. Similarly Sick and Beaty (1975) reported the superior growth supporting effect of soybean in the juveniles of *M.rosenbergii*. Soy meal has been successfully used to replace fish meal and shrimp meal in *M.rosenbergii* by Balazs *et al.* (1973) and Balazs and Ross (1976). Deshimaru and Shigueno (1972) showed that soybean protein has a pattern of amino acid distribution more similar to the prawn than the brine shrimp (*Artemia* spp.) nauplii, another crustacean animal. Successful attempts to fortify the soybean meal with crystalline methionine or methionine analogue has also been reported in the juveniles of *P.vannamei* (Ako, 1988). There are species difference and size difference in the ability of marine shrimp to nutritionally utilize soybean meal (Akiyama, 1988). According to Kanazawa *et al.* (1970) soy protein lacks tryptophan and methionine for shrimp. Soybean meal levels in commercial shrimp feeds usually range from 10-25% (Akiyama *et al.*, 1991). According to Akiyama (1988) the maximum level of soybean meal in feeds should not exceed 40%.

In a search for alternative protein sources and to achieve a more balanced amino acid profile, coconut oil cake, copra meal, ground nut oil cake, gingely oil cake, pea nut cake, black gram husk etc. were also employed by a few investigators in shrimp diets (Lee, 1970; Forster and Gabbott, 1971; Balazs and Ross, 1976; Aquacop, 1976; Goswami and Goswami, 1979; Raman *et al.*, 1982; Sherief, 1987; Ahamed Ali, 1988).Report of successful use of dried distiller's grains, a byproduct of corn ethanol production in the diet of *M.rosenbergii* is also published (Kohler and Killian, 1988).

Various workers also indicated the potential possibility of use of mangrove foliage in the diet of the penaeid prawns like *P.indicus*, *M.monoceros* and *M.affinis* (Vijayaraghavan and Ramadhas, 1980; Goswami and Goswami, 1982; Sambasivam *et al.*, 1982; Thomas, 1985). Decayed mangrove leaves form the bulk of the natural food for prawns in the estuaries (Rajyalakshmi, 1982; Naskar, 1983). Quasim and Sankaranarayanan (1972) also observed that young specimens of the shrimp *M.dobsoni* could survive indefinitely on detritus pellets. Primavera and Gacutan (1985) reported the use of live and decaying *Ruppia* and *Najas* as diets for *P.monodon*. However, Venkataramiah *et al.* (1978) was unsuccessful in utilizing the decomposed high grass (*Spartina patens*) as a source of food in the culture of brown shrimp (*P.aztecus*). In *M.rosenbergii* leaf proteins like that from *Leucaena luecocephala*, acacia meal , troca meal etc. were also employed satisfactorily (Glude, 1975; Aquacop, 1976). Harpaz and Schmalback (1986) found the use of fresh leaves of *Ailanthus* and *Malva* in the feed to eliminate mouth-death syndrome and to reduce black spot disease in the prawn

A proteinaceous feed stuff that attracted considerable interest recently is single cell proteins such as algal powders and meals, yeasts, bacterial proteins etc. which have been reported to be easily and most efficiently assimilated by prawns and shrimps. The advantages of employing single cell proteins over the conventional plant and animal proteins in aquafeeds have been discussed by Tacon and Jackson (1985). Bacterial protein was found to be among the best assimilated proteins in *P.platyceros* and

P.serratus (Forster and Gabbott, 1971). Deshimaru and Kuroki (1974b) and Deshimaru and Shigueno (1972) indicated the superior performance of petroleum yeast over white fish meal in *P.japonicus*. The use of marine yeast for feeding the larvae of *P.japonicus* has been demonstrated by Furukawa (1972), Furukawa and Hidaka (1973) and Furukawa *et al.* (1973). Brand and Colvin (1977) and Cuzon *et al.* (1981) found inclusion of yeast in shrimp diets to promote better growth and survival. Yeast levels in commercial feeds usually range from 2 to 5%. The levels of yeast should not exceed 5% in feeds unless the yeast product used is palatable to shrimp (Akiyama *et al.*, 1991). Bender *et al.* (1988) have developed a simple silaging process for the large scale production of low cost bacterial protein by anaerobically digesting grass clippings or waste biomass, that may have large scale adoption by aquafeed manufacturers. Live algae like *Skeletonema, Tetraselmis, Chaetoceros* etc. were extensively being used as penaeid larval feeds in many parts of the world. Successful use of dried algae as a larval feed as complete or partial replacement of live algae was also reported (Thomsen *et al.*, 1988). Forster (1976) observed algae as an excellent food item for penaid prawns. Hajra *et al.* (1988) also employed algal powder in a diet for *P.monodon*. *Spirulina* was found to promote appreciable growth in *P.japonicus* (Cuzon *et al.*, 1981) and in *P.indicus* (Ahamed Ali, 1988). Recently James *et al.* (1990) investigated the possibility of using solar dried *Spirulina fusiformis* as a protein source in the feed for the postlarvae of *M.rosenbergii* and suggested that the alga cannot serve as a sole protein source for the prawn but can be effectively used as a supplementary protein. Cuzon *et al.* (1981) found the active fraction of *Spirulina* powder to be present in the lipid free fraction rather than the lipid fraction.

It may be noted that in comparison with conventional feed proteins , a significant proportion of the nitrogen present in single cell protein is in some form other than amino acids, the prominent form being nucleic acids and nucleotides (Tacon and Jackson, 1985). It has been suggested that the high nucleic acid content of single cell proteins may be deleterious for growth at high dietary inclusion levels (Sanchez-Muniz *et al.*, 1978; Tacon and Cooke, 1980).

The large scale use of raw herbaceous protein sources in shrimp and prawn diet is objectionable, for in addition to impairing the digestibility by the animals, they may also contain anti nutritional factors which can affect the growth of the organism concerned. This topic has been reviewed by Tacon and Jackson (1985) and Matty (1989a).

A careful evaluation of the forgoing review shows that prawns and shrimps in general and *M.rosenbergii* in particular can assimilate proteins from a wide variety of plant and animal sources efficiently.This may be further supported by the presence of a broad range of proteolytic enzymes, carboxy peptidases and specific amino acid peptidases (Murthy, 1977; Lee *et al.*, 1980). However, the ability of most of these sources of protein singularly to support acceptable growth in these animals is to be doubted in view of the deficiency of one or more amino acids in them compared to the amino acid requirement of the prawns. Hence, the most rationale approach is, undoubtedly, to combine many sources of protein so as to bring about one that more closely meets the amino acid requirement of the prawn species under consideration. Many authors reported the better growth supporting effect of mixed protein sources in penaeid shrimps (Deshimaru and Shigueno, 1972; Shigueno *et al.*, 1972; Conklin *et al.*, 1977; Alava and Lim, 1983; Gopal, 1986; Chiu, 1988; Goxe *et al.*, 1988; Pascual, 1988). In initial trials with *M.rosenbergii* a diet based on soybean, shrimp and fish gave superior growth compared to those based on either soybean or a combination of fish and soybean (Balazs *et al.*, 1973). Similarly Balazs *et al.* (1974) reported better growth in *M.rosenbergii* with a mixture of plant as well as animal sources of protein compared to either plant or animal source of protein. The Hawaiian pelleted feed for *M.rosenbergii* is also reported to be based on a large number of proteins mainly from vegetable components (Weidenbach, 1982). It may also be recalled here that the efficiency with which protein is assimilated by prawns and shrimps is very likely affected by the relative proportion of lipids, carbohydrates and the trace elements (Hanson and Goodwin, 1977). A synergistic effect between dietary components was also postulated by Fenucci and Zein-Eldin (1976).

3 MATERIAL AND METHODS

3.1 Experimental Prawns, their Nutritional History and Acclimation Procedure

The postlarvae and juveniles of the prawn *Macrobrachium rosenbergii* employed in the present investigations were obtained from the *Macrobrachium* Hatchery of the College of Fisheries, Panangad, Cochin, Kerala and were of known nutritional history. For the production of seeds in the hatchery, berried females collected from the Vembanad lake at Poothotta, about 25km from the Cochin bar-mouth were employed. The seed production technique followed in the hatchery has been discussed by Nair and Thampy (1987), Nair *et al.* (1989) and Sebastian (1990). The larvae were fed with a processed microparticulate suspension feed prepared from *Metapenaeus dobsoni* meat and hen's egg during the day time, at three hourly intervals followed by a night time feeding with freshly hatched nauplii of brine shrimp *(Artemia spp.)* supplied by Biomarine Inc., U.S.A. The larvae took 25 days to start metamorphosing to postlarval stage.

Since the life stages of the prawn is not clearly defined as postlarval, juvenile, adult stages etc., in the present study, the stage from the day of metamorphosis to the first 30-35 days was taken as postlarval and that beyond the 30 - 35 days till they were approximately 2 month - old was considered as juvenile stage. In fact, these two periods cover the nursery phase of the prawn *M.rosenbergii.*

For each experiment, full sibling prawn postlarvae and juveniles metamorphosed in the first two days (of metamorphosis) were selected. The animals intended for the study were gradually acclimated to freshwater from a salinity of 12-14 ppt used for larval rearing soon after the metamorphosis to synchronise with a salinity condition which is ideal for maximum growth (Wickins, 1972; Goodwin and Hanson, 1975; Perdue and Nakamura, 1976; Sandifer *et al.,* Ms.; Venugopalan, 1988).

The acclimation period in the case of the postlarvae lasted for five days. During the initial three days the postlarvae (mean weight, 0.00701 ± 0.00025g) intended to determine the protein requirement were fed *ad libitum* twice daily, with a commercial pelleted diet (Table 3) alternated with one of the purified diets employed in the present experiment to determine the protein requirement of the postlarvae and juveniles of the prawn. The commercial formulation was found to support acceptable growth in the juveniles of *M.rosenbergii* (Sherief, 1987). The diet had a crude protein content of 35.32%. The purified diet was based on casein and amino acid mixture (Table 4) and had 40.20% protein. During the last two days, the postlarvae were fed with the purified diet alone. The feeding procedure helped to get the postlarvae used to the purified diet. The postlarvae (mean weight, 0.00707 ± 0.00037g) meant for the study to evaluate the effect of substitution of protein of animal origin with that of plant origin, were fed *ad libitum* with the commercial pelleted diet mentioned above, twice daily during the entire acclimation period.

The juveniles of *M.rosenbergii*, which were reared in freshwater for approximately a month on the commercial pelleted diet were also subjected to the respective acclimation procedure described above for five days. The mean weight of the juveniles employed to determine the quantitative protein requirement and to find the effect of substitution of protein source were 0.15037 ± 0.00195g and 0.15848 ± 0.00821g respectively.

The mean weights of the postlarvae and juveniles employed in the short term digestibility study were 0.00712 and 0.15815g respectively.

For all studies, healthy animals were selected and assigned to each tank on a random basis. Before the start of the experiments, the prawns were starved for a day and 50% of the animals in each tank were weighed in an electric monopan balance nearest to 10 µg. The weighing of the animals was completed within seconds causing least stress to the animals.

Table 3. Composition (% dry weight) of the commercial diet employed during the acclimation period

Ingredient	Percentage
Clam meat powder	40.00
Ground nut oil cake	25.00
Rice bran	25.00
Tapioca	10.00
Proximate composition	
Moisture[1]	9.00
Crude protein	35.32
Crude fat	7.26
Ash	9.50

[1]% wet weight

3.2 Experimental Rearing Facilities

All the experiments were conducted in cylindrical fibre glass tanks with a diameter of 55cm and a height of 35cm and having a capacity of 83.0 litres. The interior of the tanks was aquamarine in colour.

The prawns were reared in tap water of zero salinity which was vigorously aerated for 24 hours in a 1.2 ton capacity fibre glass tank to remove any trace of chlorine present. Freshwater or low saline water has been reported to be ideal for the growth of the postlarvae and juveniles of *M.rosenbergii* (Wickins, 1972; Goodwin and Hanson, 1975; Perdue and Nakamura, 1976; Sandifer *et al.*, Ms.;Venugopalan, 1988). Before the use, the water was filtered through a bolting silk cloth and filled in each tank to the required capacity (75 litre).

Since the provision of artificial substrata has been reported to reduce cannibalism and to improve the yield of *M.rosenbergii* (Smith and Sandifer, 1975; Cohen *et al.*, 1983; Ra'anan *et al.*, 1984; Gomez *et al.*, 1988) each tank was provided with a meshed (0.5cm) artificial substratum made of polyvinyl chloride. The substratum is cylindrical in shape, having a height of 40cm and a diameter of 30cm and is black in colour. These were also provided with two septa, one at 10cm from the bottom edge and the other at 10cm from the top edge. Each septum was having a hole with a diameter of 10cm at the centre. In order not to hinder the free movement of the prawns at the bottom of the tanks, three equidistant 5x5cm slots were provided at the bottom edge of the substrata.

All the experiments were conducted inside a shed roofed with red mud plastic sheet intermittently provided with translusccnt fibre glass sheets to allow moderate light inside. No special effort was taken to control the light regime. However, to facilitate work during night hours a 100W electric bulb was lighted above the tanks, upto 22.00 hours. Immediately on completion of the work, all the lights were switched off. Aeration was provided from a 5 HP autostop pressure adjusted oil-free air compressor channelled throug PVC pipes and diffusion stones. The air supply was maintained uniformly throughout the experimental period, except during cleaning of the tanks and removal of leftover feed and faecal matter.

3.3 Device for Excreta Collection

For the collection of excreta of the prawns a special type of device described by Lazarus and Reddy (1988) and further modified slightly to suit the present requirement was used. The device is made of glass and consists of a cylindrical bulb of inner diameter 3.5cm and length 10cm which is

extended on the upper end in the form of a long tube with an opening. The tube is having an inner diameter of 1cm and a length of 25cm. The lower end of the bulb opens in the form of a conical flare of height 1.5cm and base 1cm. This flared inner opening of the bulb is continued as an inner narrow tube of inner diameter 0.5cm and a length of 8cm, which runs along the axis of the bulb. The base of the bulb, just above the conical flare, is fitted with a small side tube of 1cm diameter inclined at approximatly 45^{0} and is provided with a stopper which can be fitted air tight. They are also provided with two pairs of hooks, one pair each on the small tube and on the stopper. The hooks are provided on opposite sides, on the small tube and the stopper which help to make their fitting perfectly air tight by a ruhber band fastening the hooks on the same side, one on the small tube and the other on the stopper. The operation of the device is described by Lazarus and Reddy (1988).

3.4 Respirometer

Oxygen consumption and ammonia excretion of individual prawns were measured with respirometers described by Venugopalan (1988). The respirometer chamber consists of a flat bottom flask having 320ml capacity provided with an inlet, an outlet and an opening at the top for letting in air while drawing the samples, all of which are connected to plalstic tubings.

3.5 Experimental diets and their Preparation

In order to study the protein requirement of the postlarvae and juveniles of *M.rosenbergii* six isocaloric (metabolizable energy) purified diets for prawns with graded levels of protein (0, 10, 20,30, 40 and 50%) were formulated. The diets were formulated to contain all predicted essential nutrients in equal quantity except the level of protein and carbohydrate (Table 4 and 5). The purified diets were originally based on the formula recommended by Kanazawa *et al.* (1982) for nutrition studies with *Penaeus japonicus*. However, in the light of the recently published information on the specific nutrient requirement of the species, modifications were effected on the original composition.

Casein was selected as the principal protein source because it is available in highly purified form (Halver, 1957; Kanazawa *et al.*, 1971, 1976) and has been found to be assimilated satisfactorily by the prawn, *M.rosenbergii*, in a number of studies (Hilton *et al.*, 1984; Briggs *et al.*, 1988; Gomez *et al.*, 1988; Reigh and Stickney, 1989). Casein is low in arginine, tryptophan and methionine compared to the amino acid composition of the whole prawn muscle (Table 6). Since these amino acids were reported to be essential for shrimps and prawns (Watanabe, 1975; Miyajima *et al.*, 1976; New, 1976a; Biddle, 1977; Sick and Millikin, 1983; Wilson, 1989), they were incorporated in the experimental diets.

In comparison with monosaccharides prawns were reported to assimilate polysaccharides, as a carbohydrate source, better (Andrews and Sick, 1972; Forster and Gabbott, 1971; Sick and Andrews, 1973; New, 1976a; Aquacop, 1978; Deshimaru and Yone, 1978c; Abdel-Rahman, *et al.*, 1979; Pascual *et al.*, 1983; Alava and Pascual, 1987). Further, corn starch is available in relatively pure form and hence its inclusion in the diet is justified (Sick and Andrews, 1973; Hajra *et al.*, 1986).

The lipid source employed was a mixture of corn oil and cod liver oil which provides both ω_6 (linoleic) and, ω_3 (linolenic) polyunsaturated fatty acids which were reported to be essential for prawns (Colvin, 1976; Guary *et al.*, 1976; Kanazawa *et al.*, 1979a,b; Chiu, 1988; Reigh and Stickney, 1989). Further, a dietary total lipid level of 7-10% has been found to result in good growth in *M.rosenbergii* (Joseph and Williams, 1975; Sandifer and Joseph, 1976; Briggs *et al.*, 1988). Sheen and D'Abramo (1989) reported a lipid requirement value of less than 10% for *M.rosenbergii*.

Hilton *et al.* (1984) reported the dietary non essentiality of lecithin for the prawn *M.rosenbergii*. Hence no effort was taken to incorporate lecithin in the purified diets employed in the present study. The finding of Hilton *et al.* (1984) was further confirmed by Briggs *et al.* (1988).

Table 4.Composition (% dry weight) of the purified diets

Ingredient	Source[1]	Diet number					
		1	2	3	4	5	6
Fat free casein	A	0	10.5	21.0	31.5	42.0	52.5
Arginine	A	0	0.2	0.4	0.6	0.8	1.0
Methionine	A	0	0.2	0.4	0.6	0.8	1.0
Tryptophan	A	0	0.1	0.2	0.3	0.4	0.5
Starch	B	75.0	64.0	53.0	42.0	31.0	20.0
Glucosamine-HCl	C	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin mixture	D	4.2	4.2	4.2	4.2	4.2	4.2
Mineral mixture	D	4.4	4.4	4.4	4.4	4.4	4.4
Cod liver oil	E	6.0	6.0	6.0	6.0	6.0	6.0
Corn oil	F	3.0	3.0	3.0	3.0	3.0	3.0
Cholesterol	A	0.5	0.5	0.5	0.5	0.5	0.5
Cellulose	G	4.4	4.4	4.4	4.4	4.4	4.4
Carragenan (binder)	H	2.0	2.0	2.0	2.0	2.0	2.0
Total	-	100.00	100.00	100.00	100.00	100.00	100.00
Proximate composition							
Moisture[2]		9.20	8.30	8.80	8.00	8.40	8.90
Crude protein		-	10.10	19.20	30.50	40.20	50.60
Crude fat		10.80	11.00	11.50	11.20	11.70	11.50
Carbohydrate		78.70	67.90	57.50	47.20	36.30	25.60
Ash		10.50	11.00	11.80	11.10	11.80	12.30
Gross energy (k cal/100g)[3]		420.95	434.04	446.00	463.19	476.40	487.91
Metabolizable energy (k cal/100g)[3]		401.20	400.00	398.80	400.40	399.60	396.80
Protein-gross energy ratio (mg/k cal)[3]		-	23.27	43.04	65.84	84.38	103.71
Protein-carbo-hydrate ratio[3]		-	1:6.70	1:3.00	1:1.60	1:0.90	1:0.51

[1] A, Sisco Reserch Laboratories (P) Ltd., Bombay; B, E.Merck(India) Ltd., Bombay; C, Central Institute of Fisheries Technology (CIFT), Cochin; D, provided in Table 5; E, Universal Generics Pvt Ltd. (Seven Seas); F, local market; G, Romali, Bombay; H, Chemical Drug House, New Delhi; [2] % wet weight; [3] calculated value

Though very recent studies (Briggs *et al.*, 1988; Sherief *et al.*, 1990) have indicated the nonessentiality of dietary cholesterol for *M.rosenbergii*, at the start of the present experiments little was known on the subject. On the otherhand, there was a unifying evidence that many crustaceans utilize cholesterol for their normal growth and survival (Castell *et al.*, 1975) but are incapable of *de novo* synthesis from simple sugars like acetate or mevolonate (Van den oord, 1964; Zandee, 1964, 1966, 1967; Whitney, 1969; Teshima and Kanazawa, 1971. Kanazawa *et al.*, 1971). The reported cholesterol requirement levels of various species of crustaceans range from 0.1--1.4% (see, Kanazawa *et al.*, 1970, 1971; Shudo *et al.*, 1971; Deshimaru and Kuroki, 1974b; Teshima, 1982; Teshima *et al.*, 1982b, 1983; D'Abramo *et al.*, 1984, 1985; Kean *et al.*, 1985; Bordner *et al.*, 1986; Clark and Lawrence,1988a), though most values are nearer to 0.5%. On the basis of these studies a decision was taken to include cholesterol at 0.5% level in the purified diets employed in the present study.

Table 5. Composition of the vitamin and mineral mixture used in the purified diets

Ingredient	Source[1]	Quantity (mg/Kg finished diet)
Vitamin mixture		
Thiamine HCl	A	4.00
Riboflavin	A	8.60
P-aminobenzoic acid	B	10.00
Biotin	I	0.40
Inositol	A	400.00
Niacin	A	40.00
Calcium pantothenate	A	60.00
Menadione	I	4.00
β– Carotene	G	9.60
α – Tocopherol	I	30.00
Calciferol	G	1.20
Cyanocobalamin	I	0.08
Sodium ascorbate	A	3000.00
Folic acid	A	0.80
Choline chloride	I	600.00
Pyridoxine	A	12.00
Mineral mixture		
K_2HPO_4 -	I	1500.00
$Ca_3(PO_4)_2$	J	1500.00
$MgSO_4$	K	520.00
$NaH_2PO_4.2H_2O$	I	400.00
NaCl	B	240.00
$MnSO_4$	B	3.2
$FeSO_4$	B	100.00
KCl	I	200.00
$ZnSO_4$	B	20.0
KI	B	6.0
Na Citrate	K	300.0

[1] A,B and G, same as for Table 4; I, Loba Chemie (Pvt) Ltd., Bombay; J, Riedel (India) Chemicals, New Delhi.; K, Qualigens Fine Chemicals (Glaxo India Ltd.), Bombay

No studies exist on the individual mineral requirement of the prawn *M.rosenbergii*, though Heinen (1988) found growth reduction when trace mineral mix was omitted from the diet. So the mineral mixture used in the present study was based on some already published successful semipurified diets used in nutritional studies with *M.rosenbergii* (Stahl and Ahearn, 1978; Hilton *et al.*, 1984).

Heinen (1988) indicated that fat soluble vitamins were of not much importance in the diet of *M.rosenbergii*. He, however, reported the essentiality of water soluble vitamins in the diet of the prawn. Neverthless, in the purified diets all fat soluble as well as water soluble vitamins reported to be essential for other crustaceans were incorporated to avoid any possible growth retardation due to their deficiency. The levels of various vitamins incorporated were comparable to the purified diets prepared by Kanazawa *et al.* (1982) except sodium ascorbate and α- tocopherol which were provided at slightly higher levels. Glucosamine hydrochloride was incorporated in all purified diets at 0.5% level since it was reported to enhance growth in shrimps (Kitabayashi *et al.*, 1971a; Vaitheswaran and Ahamed Ali, 1984). Glucosamine hydrochloride is also assumed to improve the assimilation of dietary casein by the

prawn by promoting the growth of the intestinal *Lactobacillus* spp. of bacteria (Mukundan, M.K.,Pers. Comm.). Carragenan at 2% was included as a dietary binder since it has been reported to have good binding property and assumed not to be harmful to the prawn, *M.rosenbergii*. Carragenan was also featured as an acceptable diet coating material in shrimp diets (Teshima *et al.*, 1982a; Kanazawa, 1983; Koshio *et al.*, 1988, 1989).

Table 6. The percentage composition of essential amino acids in the tail muscle of *M.rosenbergii* and in the casein-amino acid based protein source employed in the formulation of purified diets

Essential amino acid	Tail muscle of *M.rosenbergii*[1]	Recommended level of essential amino acids in penaeid shrimp feed[2]	Casein[3]-amino acid mixture
Arginine	8.10	5.80	4.67
Histidine	1.78	2.10	3.73
Lysine	6.79	5.30	7.06
Tryptophan	-	0.80	1.46
Phenyl alanine-tyrosine	5.54	7.10	9.26
Methionine-cystine	2.544	3.60	4.62
Threonine	2.96	3.60	3.77
Leucine	5.79	5.40	8.37
Isoleucine	2.83	3.50	4.74
Valine	2.87	4.00	6.02

[1] Dry weight basis, Farmanfarmaian and Lauterio (1980); [2] As fed basis, Akiyama *et al.* (1991); [3] Dry weight basis, Penaflorida (1989); [4] Only methionine; cystine not determined

The purified diets were produced in the form of dry pellets (moisture, 8.60 ± 0.44%) due to its obvious advantages over moist diets. It is easier to keep, better suited for dietary experiments and when rehydrated acquires the same soft constituency. Chemical analysis of the two forms also showed better retention of nutrients by the dry ones (Bages and Sloane, 1981). It also helps to maintain better water quality than moist feeds (Matty, 1990).

Each ingredient used in formulating the purified diets was powdered separately, sieved through 250 micron sieve and weighed. The water soluble vitamins were dissoved in water and fat soluble vitamins in the lipid mixture. All the solid ingredients, including the mineral mixture were first mixed together and blended thoroughly in a kitchen mixie. The mixture was then steam cooked for 10 minutes. On reducing the heat, the oil mixture was added and thoroughly mixed. After cooling the mixture to room temperature, water soluble vitamins were added and again blended thoroughly. The pH of the diet was maintained near neutral. The binder was dissolved in approximately 400ml of water (per kilogram diet) heated to approximately 50-60°C. The diet mixture was then added to the binder solution and the diet was prepared into a dough. The dough was then extruded through a hand pelletiser, collected in suitable trays and dried in an electric oven at 50°C for 12 hours to obtain dry pellets (2mm diameter) with a moisture content of 8.6 ± 0.44%. The diets were then broken into small pieces and packed airtight in plastic containers and stored at 4°C in a refrigerator. All pellets remained stable in water for atleast 7-8 hours as a result of the carragenan binder and the finely ground nature of the dietary ingredients. The proximate composition, calculated gross energy values, metabolizable energy values, protein energy ratio and protein-carbohydrate ratio of the various diets are presented in Table 4. Studies on prawn feed development have indicated that successful prawn diets generally do not contain energy levels below 350 k cal/100g of diet (Colvin, 1976; Sedgwick, 1979; Alava and Lim, 1983; Hajra *et al.*,

1986, 1988; Gomez *et al.*, 1988). The selected energy range in the various purified diets employed in the present experiments was therefore kept above this level.

There is no published information available on the metabolizable energy value of dietary nutrients for *M.rosenbergii* or other species of shrimps and prawns. So it was decided to use the standard metabolizable energy values (4 k cal/g for protein and carbohydrate and 8 k cal/g for lipid) which had been used by many investigators in finfishes and shellfishes (De Long *et al.*, 1958; Ogino and Saito, 1970; Cowey *et al.*, 1972; Halver, 1972; Garling and Wilson, 1976; Clifford and Brick, 1978; Moore *et al.*, 1988). Using these values would allow the exchange of corn starch and the casein-amino acid protein on an equal weight basis and thus simplify the formulation of purified diets employed to investigate the quantitative protein requirement of the postlarvae and juveniles of *M.rosenbergii*. The gross energy of the various diets were calculated based on the following energy values; 4.10 k cal/g for carbohydrate, 9.10 cal/g for lipid and 5.5 k cal/g for protein(ADCP, 1983).

Inorder to evaluate the performance of the purified diets, a control ration (Table 3) based on clam meat and ground nut oil cake (Sherief, 1987) was also employed. The control ration is expected to cover all known nutritional requirements of *M.rosenbergii* with a certain degree of safety.

A short term digestibility study (for 10 days) was conducted to determine the digestibility of a few locally available protein sources singly or in combination by the postlarvae and juveniles of *M.rosenbergii*, the results of which along with the results of the experiments to determine the quantitative protein requirement would help fix the dietary protein level in the subsequent studies to evaluate the effect of substitution of animal source of protein with plant source. For the purpose, eight pelletted diets were formulated. The ingredients and proximate composition of the diets employed are presented in Table 7. Effort has been made to maintain almost the same level of the major nutrients except carbohydrate in all the diets. Since one of the ingredients (black gram) contain relatively lower level of protein (20%)it was decided to keep the protein level in all the diets employed in the digestibility study at 15% to make them isonitrogenous. Based on the results of the earlier experiments to determine the quantitative protein need of the postlarvae and juveniles of the prawn, and also the observations by Ahamed Ali(1988), Nose (1963) and Hepher (1988), the dietary density of protein was assumed not to have a significant influence on the digestibility of protein. Being a short term study no special attempt to balance the micronutrients in the diets was taken.

For formulation of feeds, the ingredients were individually powdered, seived (250 micron) and weighed according to the respective percentage composition. Ingredients were mixed together thoroughly and steam cooked for 30 minutes. The binder was separately melted in a small quantity of hot water (50-60^{0}C). To this, the dry ingredients were added and homogenised thoroughly. The resulted dough was extruded throgh a hand pelletiser using a 2mm diameter die. The pellets were dried in an electric oven at 50-60^{0}C for 12 hours to get dry pellets having a moisture content of approximately 8.64 $\pm$ 0.36%. The pellets were then broken and stored in airtight plastic containers in a refrigerator at 4^{0}C, till used. All pellets remained stable in water for atleast 8 hours.

For the experiment to evaluate the effect of substitution of protein of animal origin with that of plant origin six isonitrogenous practical diets varying in composition (Table 8) were formulated. The protein level in the diets was maintained based on the results of the experiment to evaluate the quantitative protein requirement of the prawn (3.6.1 and 3.6.2) and the short term digestibility study (3.6.3) conducted prior to this investigation. In order to simplify interpretations, however, as few ingredients as possible were used to formulate the diets. Further, since cost consideration restricted the choice of dietary protein sources, only prawn meat, clam meat, soybean, black gram and ground nut oil cake were selected as protein sources. The selection was also based on their wider availability in many areas of the world where there is a potential for prawn farming. Prawn and clam meat were documented as excellent sources of protein for many species of prawns (Kanazawa *et al.*, 1970; Forster

Table 7 Composition (% dry weight) of the diets employed in the digestibility study

Ingredient	Origin of protein	Diet number							
		1	2	3	4	5	6	7	8
Fat free casein	Animal	16.00	-	-	-	-	-	-	-
Prawn meat	Animal	-	23.00	-	-	-	-	13.50	-
Clam meat	Animal	-	-	33.00	-	-	-	13.50	-
Soybean	Plant	-	-	-	7.30	-	-	-	15.50
Ground nut oil cake	Plant	-	-	-	-	41.50	-	-	15.50
Black gram	Plant	-	-	-	-	-	74.90	-	15.50
Corn starch	-	75.00	70.00	60.00	59.70	52.50	17.00	66.00	48.50
Cod liver oil	-	7.00	5.00	5.00	1.00	4.00	6.00	5.00	3.00
Carragenan (binder)	-	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Proximate composition									
Moisture[1]	-	9.00	8.60	8.50	9.20	8.50	8.00	8.70	8.60
Crude protein	-	14.40	14.95	14.85	14.93	14.98	15.04	14.85	14.88
Crude fat	-	6.80	7.07	7.31	7.35	6.75	6.91	7.17	6.87
Carbohydrate	-	76.00	72.00	61.40	67.66	64.94	68.27	68.20	66.03

[1] % wet weight

and Gabbott, 1971; Forster and Beard, 1973; Sick and Andrews, 1973; Forster, 1976; Colvin and Brand, 1977; Lim *et al.*, 1978; Pascual, 1978; Villegas, 1978; Pascual and Destajo, 1979; Deshimaru, 1981; Ahamed Ali, 1982, 1988; Goswami and Goswami, 1982; Kungvankij *et al.*, 1986; Sherief, 1987; Hajra *et al.*, 1988; Aquacop *et al.*, 1989), but are relatively expensive. However, a decision to include these two sources of protein was taken since these have a closely mimicking amino acid pattern to that of the test animal and could serve as an ideal reference protein to study the substitution effect of other protein sources. Soybean was featured probably as the best source of protein of plant origin for shrimp (Kanazawa *et al.*, 1970; Balazs *et al.*, 1973; Forster and Beard , 1973; Sick and Andrews, 1973; Balazs and Ross, 1976; Colvin and Brand, 1977; Lawrence *et al.*, 1986; Pascual *et al.*, 1986; Akiyama *et al.*, 1988; Ako, 1988; Fernandez and Lawrence, 1988a; Penaflorida, 1989). Ground nut oil cake was also successfully employed by a few in shrimp diets (Ahamed Ali, 1982; Sherief, 1987). Black gram though not widely employed in shrimp diets, because it is relatively inexpensive and because it is available in many parts of the world, its use if found successful was expected to bring down the cost of shrimp diets considerably. New (1987) also documented black gram as a leguminous seed with potential in aquaculture feeds.

In order to ensure optimum nutrient balance and to simulate the amino acid pattern of the shrimp body protein to the maximum extent possible, mixed rather than single source of protein was employed. Thus a mixture of prawn and clam meat served as the animal source of protein and a mixture of soybean, black gram and ground nut oil cake served as the plant source. Tapioca was employed as the carbohydrate source. The lipid source employed was sardine oil. Vitamin mixture was also added at 2% level and carragenan (2%) formed the binding agent in all the diets.

Table 8. Composition (% dry weight) of the practical diets

Ingredient[1]	Diet number					
	1	2	3	4	5	6
Prawn meat	29.47	22.08	14.78	7.40	-	-
Clam meat	21.31	15.94	10.66	5.33	-	100
Soybean	-	7.49	14.98	22.50	30.00	-
Ground nut oil cake	-	7.49	14.98	22.50	30.00	-
Black gram	-	7.49	14.98	22.50	30.00	-
Sardine oil	4.16	3.33	2.50	1.65	0.80	-
Tapioca	41.09	32.18	23.12	14.12	5.20	-
Vitamin mixture[2]	2	2	2	2	2	-
Carragenan[3]	2	2	2	2	2	-
Proximate composition						
Moisture[4]	8.90	9.00	9.20	8.80	8.60	-
Crude protein	31.26	31.15	31.16	31.11	31.04	46.88
Crude fat	8.65	8.65	8.80	8.50	8.55	7.00
Carbohydrate	40.42	41.45	42.29	43.22	44.23	20.12
Ash	19.67	18.75	17.75	17.17	16.18	26.00
Gross energy (k cal/100g)[5]	416.37	419.99	424.85	425.66	429.87	404.03
Metabolizable energy (k cal/100g)[5]	355.92	359.60	364.20	365.32	369.48	324.00
Protein-gross energy ratio (mg/k cal)	75.08	74.17	73.34	73.09	72.71	115.80

[1]Local market; [2]Sisco Research Laboratories (P) Ltd., Bombay;[3]Chemical Drug House, New Delhi; [4]% wet weight; [5]calculated values

In order to inactivate the antinutritional factors that might be present in the plant sources of protein they were steam cooked for approximately one hour before use (Wee and Shu, 1989). The procedure adopted in the preparation of the pelleted diet was as described earlier. The proximate composition, calculated gross energy, metabolizable energy and the protein-energy ratio of the various diets are presented in Table 8. The selected energy range in the various diets was kept above the critical level suggested for shrimp and prawn diet. The Metabolizable and gross energy of the various diets were calculated based on the standard values discussed earlier. To study the performance of dried clam meat as a sole nutritional source it was offered to a seperate group of postlarvae and juveniles.

3.6 Experimental Procedure

3.6.1 *Study to Evaluate the Protein Requirement of the Postlarvae.*

The experiment was conducted in fibre glass tanks having an effective water capacity of 75 litre by maintaining 50 numbers of uniform sized postlarvae of the prawn in each tank.

Six isocaloric (metabolizable energy) purified diets for prawn with graded levels of protein (0, 10, 20,30, 40 and 50%) were formulated (Table 4). The experimental diets were assigned to each tank

randomly. According to Corbin *et al.* (1983) multiple feeding has specific advantages over one time feeding, in *M.rosenbergii*. Further, food consumption by prawns was found to be greater when feed was presented twice rather than once or thrice daily in the prawn (Taechenuruk and Stickney, 1982). Similarly in contrast to the use of fixed feeding regime in which the feeding level has been set arbitrarily which would influence the outcome of the observed dietary requirements, feeding to satiation is preferred in nutritional studies(Tacon and Cowey, 1985). In the light of these observations, in the present experiments prawns in each tank were provided with one of the six purified diets *ad libitum*, twice daily at 08.00 hours and 18.00 hours. In each tank, feed was provided in two petridishes, kept at opposite sides. Gentle aeration was provided in all the tanks.

Immediately before each feeding the uneaten food in each tank was collected. The faecal matter was collected 2-3 hours after each feeding,using a special type of device already described. The faecal matter sucked up was delivered on to a bolting silk strainer, wherein it was washed with a gentle stream of distilled water. It was then transfered to small airtight container and stored at $0^{o}C$ in a refrigerator.They were pooled for each sampling period and later subjected to appropriate gravimetric and/or biochemical analysis, on drying in an electric oven at $105^{o}C$.

Bacterial and algal growth is known to occur in rearing tanks during the feeding experiment which would serve as an unaccounted nutrient supply to the prawns. Effort has been made to avoid growth of bacteria and algae, as far as possible,by removing the sediments, if any, and leftover feed regularly. Further, more than 90% of the rearing water was exchanged every day with fresh filtered water. The sides of the tanks were cleaned every day to avoid the growth of periphyton. However, for fear of its possible effect on normal metabolism of prawns, no antibiotics were employed in the present experiment.

Sampling of the prawn was performed at the end of each 10 day period. Immediately prior to each sampling, the prawns were starved for 12 hours to avoid the possible influence of the food retained in the digestive system of the prawn. During sampling all the prawns were together weighed after blotting with a dry filter paper, to the nearest 10 µg, causing least stress to the animals. The results of weighing and biochemical analysis were expressed as the mean of the values for three similar treatments. At the termination of the experiments the prawns were weighed, an equal number of them were euthanized and subjected to biochemical analysis.

Oxygen-nitrogen ratio of the prawn was estimated at the end of the experiment. For the purpose,the oxygen concentration and ammonia excretion values for the postlarvae reared on various diets were determined. Before the beginning of the respirometry, the animals were starved for 24 hours. They were acclimated in the respirometer chamber for 1 hour before readings were taken and the chamber was flushed with freshwater throughout the period of equilibration. At the beginning of each run, water sample was drawn for estimating the dissolved oxygen and then the respirometer was closed. All the respirometers were placed in a water bath and maintained at a temperature of $30 \pm 1^{o}C$. After one hour a water sample was drawn for oxygen estimation. The difference between the two readings was considered to be the oxygen consumed by the animal during the period of experiment. Triplicate readings were taken in all cases, and were averaged. Between two runs, the water flow was allowed for 30 minutes for re-aeration.

Ammonia excretion was determined using water samples drawn from the respirometer chamber prior to and after each respirometry.

All the readings are taken during the dark phase of photoperiod, inorder to ensure uniformity of experimental conditions between each run.

3.6.2 *Study to Evaluate the Protein Requirement of the Juveniles.*

The procedure adopted was the same as in 3.6.1 except that the stocking density maintained was 25 juveniles per tank.

3.6.3 *Short term Study to Determine the Apparent digestibility of a few Protein Sources.*

This experiment was conducted to determine the efficiency of the postlarvae and juveniles of the prawn to assimilate proteins from various animal and plant sources. This was conducted also with a view to help fix the level of dietary protein in the diets for the subsequent studies to evaluate the effect of substitution of animal source of protein with plant sources.

The experimental facilities, feeding procedure etc. employed in this experiment was the same as those employed in 3.6.1. and 3.6.2. The stocking density of the postlarvae and juveniles maintained was 50 and 25 numbers in each tank respectively. Each treatment was replicated thrice. The prawns in each tank were offered one of the diets mentioned in Table 7. As under 3.6.1, the faeces and uneaten food were collected and pooled for the experimental period. The duration of each experiment was 10 days.

3.6.4 *Study to Evaluate the Effect of Protein Source on the Postlarvae.*

The experimental procedure adopted was the same as in 3.6.1 except that instead of feeding with the purified diets, the prawns in each tank was fed with one of the isonitrogenous practical diets mentioned in Table 8.

3.6.5 *Study to Evaluate the Effect of Protein Source on the Juveniles.*

The experimental procedure adopted was the same as in 3.6.2, except that instead of feeding with the purified diets, the prawns in each tank was fed with one of the isonitrogenous practical diets mentioned in Table 8.

3.7 Determination of Water Quality Parameters

Water quality was closely monitored throughout the experiment. Water temperature and pH were measured twice daily between 06.00 and 07.00 hours and 21.00 and 22.00 hours. Dissolved oxygen was measured once everyday in the morning (06.00 - 07.00 hours). Ammonia was measured once every week and total alkalinity, twice during the entire experimental period.

Temperature was measured by using mercury bulb thermometer with an accuracy of 0.1°C and pH by electrometric method using Elico digital pH meter (Model LI-122). Dissolved oxygen was measured by standard Winkler's method (Strickland and Parsons, 1972) and total ammonia by photometric measurement using indophenol method (Grasshoff *et al.*, 1983). Total alkalinity was determined by acidimetric titration method (APHA *et al.*, 1981).

3.8 Biochemical Analysis

Analysis of proximate composition of feed, excreta and body flesh was performed by employing standard AOAC (1980) method. All analyses were done in duplicate. The moisture content was determined by drying the sample at 105°C until a constant weight was reached. Total nitrogen was determined by microkjeldahl method in which samples were digested in concentrated sulphuric acid

and the resulting ammonium nitrogen was determined by titrating against standard sulphuric acid. The total nitrogen content multiplied by 6.25 was taken as the crude protein content. The crude fat was determined by extraction for 6 hours with petroleum ether (boiling point, 40-60°C) in a soxhlet apparatus. The ash content was determined by incinerating the sample at 600°C for 6 hours in a furnace and the carbohydrate (nitrogen free extract) was calculated by substracting the percentage of all other components put together from 100%. All ingredients and proximate composition (except moisture content) were expressed on dry weight basis.

3.9 Evaluation Criteria

In aquatic nutrition studies, a hierarchy of evaluation criteria and terms are being used. Jobling (1983) reviewed the terms used to indicate the rate or efficiency of a process for energy transformation and recommended specific terms as well as methodology to be used in fish growth and nutrition studies. Since a corresponding review for decapod is not available (Pandian, 1989), in the present study evaluation criteria and expression subjectively selected from published literature are employed.

3.9.1 *Percentage Survival.*

During the course of each experiment death of prawns, if any, were immediately noted in the survival charts maintained for the purpose. The percentage of survival was calculated at the end of each sampling period and at the termination of each experiment for each tank and the mean was computed as follows.

$$\text{Percentage survival} = \frac{\text{Initial number of prawns - Number of dead prawns x 100}}{\text{Initial number of prawns}}$$

3.9.2 *Growth rate.*

Growth in prawns is expressed as absolute gain in weight, percentage growth rate, or more recently as specific growth rate. Though expression of growth in terms of specific growth rate (*GW*) is an improvement over the former ones, a decrease in *GW* with increasing body weight was apparent in many growth studies (Cooper, 1961; Laarman, 1969; Winberg, 1971; Elliott, 1975) indicating that *GW* is far from expressing growth rate in meaningful terms (Hepher, 1988), especially when there is a difference in body weight of the prawn at each sampling time. In these circumstances, the use of specific growth coefficient (*SG*), as suggested by Jobling (1983) and Hepher (1988) was assumed to be more satisfactory. *SG* was calculated using the formula developed by Schroeder (quoted in Hepher, 1988).

$$SG \text{ (g/day)} = 3\,\frac{(W1^{0.33} - W0^{0.33})}{t}$$

Where W_1 is the mean weight (g) of the prawn at the end of each sampling period, W_0 is the mean weight (g) of the prawn at the start of each sampling period and t is the duration of each sampling period in days.

By multiplying *SG* by 100, the instantaneous percentage growth of prawn of unit weight (*IPGU*), usually 1g weight was obtained (Hepher, 1988).

However to avoid any confusion on the use of a relatively new index for expressing growth, specific growth rate of the prawn over the whole experimental period was also provided. Specific growth rate was calculated by using the formula,

$$GW\% = \frac{ln\ W_1 - ln\ W_0}{t}$$

Where *GW%* is the percentage specific growth rate, *ln* W_1 is the natural logarithm of the weight of the prawn at the termination of the experiment, *ln* W_0 is the natural logarithm of the weight of the prawn at the start of the experiment and t is the duration of the experiment in days.

3.9.3 *Food Intake.*

In order to avoid the influence of body weight the food intake of the prawn during each sampling was expressed as food intake of the prawn of unit weight (g) per day and is calculated using the following formula.

Food intake of the prawn of unit weight per day =

$$\frac{F_o - F_u}{t \times 1/2 (W_0 + W_t)}$$

Where F_o is the weight (g) of the food offered during the sampling period, F_u is the weight (g)of uneaten food collected during the sampling period, t is the duration of the sampling period, W_0 is the total weight (g) of the prawn at the beginning and W_t is the total weight (g) of prawn at the end of the sampling period.

3.9.4 *Food Conversion Efficiency.*

The food conversion efficiency (FCE) which is the wet weight gain of the prawn from one unit of food consumed was calculated using the following formula.

$$\text{Food conversion efficiency (\%)} = \frac{\text{Wet weight gain of prawn(g) during the sampling period} \times 100}{\text{Dry weight of food consumed (g) during the sampling period}}$$

3.9.5 *Protein Digestibility.*

Protein digestibility was expressed both in terms of apparent and true digestibility. Apparent digestibility of protein was calculated employing the following formula.

$$\text{Apparent digestibility (\%)} = \frac{(\text{Quantity of protein consumed} - \text{Quantity of protein in the faeces}) \times 100}{\text{Quantity of protein consumed}}$$

True digestibility was calculated employing the following formula.

$$\text{True digestibility (\%)} = \frac{(\text{Quantity of protein consumed} - \text{Quantity of protein in the faeces} + \text{Quantity of metabolically derived protein in the faeces}^{1}) \times 100}{\text{Quantity of protein consumed}}$$

[1]Quantity of protein excreted by the prawn when fed with zero protein diet.

3.9.6 *Faecal Nitrogen Excretion.*

Faecal nitrogen excretion was calculated hy the following formula.

$$\text{Faecal nitrogen excretion (mg N/100g diet)} = \frac{\text{Quantity of nitrogen in the faeces (mg)} \times 100}{\text{Quantity of food consumed (g)}}$$

3.9.7 *Prawn Carcass Composition.*

At the beginning and termination of each experiment equal number of prawn was subjected to biochemical analysis. The moisture content was expressed as percentage of wet body weight of prawns. Crude protein, crude fat and ash were expressed as percentage of dry body weight.

3.9.8 *Nitrogen Retention.*

Nitrogen retention (Nr) expressed as mg N/100g body weight of the prawn per day was calculated by employing the following formula.

$$Nr = \frac{(N_t - N_0) \times 100}{1/2 \times (W_0 + W_t)t}$$

Where N_t is the final body nitrogen of the prawn (mg), N_0 is the initial body nitrogen of the prawn (mg) and W_0 is the weight (g) of the prawn at the beginning of the experiment, W_t is the weight (g) of the prawn at the end of the experiment and t is the duaration of the experiment (days).

3.9.9 *Efficiency of Protein Utilization.*

Efficiency of protein utilization was expressed as protein efficiency ratio (PER) and was calculated by employing the following formula (Hepher, 1988).

$$\text{Protein efficiency ratio} = \frac{\text{Wet weight gain of prawn (g)}}{\text{Crude protein fed (g)}}$$

3.9.10 *Oxygen-Nitrogen ratio.*

From the oxygen consumption and ammonia excretion rates obtained following the procedure described in 3.6, the oxygen-nitrogen (O:N) ratio of individual prawn fed with the different diets were estimated using the method given by Bayne *et al.*(1985).

3.10 Statistical Analysis

The result of each experiment was subjected to either analysis of variance or regression analysis as the case may be, and where found necessary, differences studied at 5% level of probability (Snedecor and Cochran, 1967) except where indicated otherwise. Analysis of variance of the data was done in the form of a two way classification, the protein level / source and the time (sampling period) being the two factors under consideration. However, while discussing the results, the protein level / source factor alone was considered since the interpretation with reference to the time factor is not covered under the study. Wherever required, the data was subjected to angular transformation before performing the analysis of variance test. Pair-wise comparison of the data was performed by the multple t test technique (Snedecor and Cochran, 1967).

Inorder to determine the dietary protein concentration that result in maximum growth second order polynomial regression analysis method was employed. Second order polynomial regression method describes the relationship between protein level and growth rate / food conversion efficiency in a curvilinear fashion ($Y = a + bx + cx^2$) and is the most statistically valid method for the purpose (De Silva *et al.*(1989). This method has been reported to have specific advantages over other methods (Cowey *et al.*, 1972) and has been used by many investigators (Moore *et al.*, 1988; Kiron, 1988; De Silva *et al.*, 1989).

From the growth curve , the dietary protein level that result in maximum growth was calculated by employing the method of differential calculus.

As suggested by Zeitoun *et al.*(1976), the most economical dietary protein level was calculated from the growth curve, utilizing the 95% confidence limits. The 95% confidence limits were calculated for the curve using the method given by Snedecor and Cochran (1967).

4 RESULTS

Three sets of experiments were conducted with the postlarvae and juveniles of *Macrobrachium rosenbergii*. The first set of experiments was conducted to evaluate the quantitative protein requirement of the two life stages of the prawn. In the second set, a short term study was undertaken to determine the efficiency of assimilation of certain inexpensive and locally available sources of protein by the postlarvae and juveniles of *M.rosenbergii*. This experiment was conducted with a view to help fix the dietary protein level required to be maintained in the third set of experiments, which was intended to study the effect of substitution of protein of animal origin with that of plant origin, in the practical commercial diet for the prawn.

4.1 Study to Evaluate the Effect of Protein Concentration

The physico-chemical conditions under which the experiment to determine the quantitative dietary protein requirement of the postlarvae was conducted were salinity, 0 ppt; temperature, 27.90 ± 2.20°C; pH, 7.80 ± 0.48; dissolved oxygen, 6.60 ± 0.33 ppm; ammonia, 0.010-0.030 ppm and total alkalinity, 56.90 ± 6.06 ppm. The experiment to determine the quantitative dietary protein requirement of the juveniles of the prawn was conducted under the following physico-chemical conditions: salinity, 0 ppt; temperature, 28.40 ± 2.00°C; pH, 7.50 ± 0.30; dissolved oxygen, 6.20 ± 0.14 ppm; ammonia, 0.010-0.028 ppm and total alkalinity, 58.80 ± 2.05 ppm. No special effort was taken to control the light regime. The light regime was approximately 8 hours dark and 16 hours light.

4.1.1 *Effect of Protein Concentration on Survival.*

The data on the stocking number and the percentage survival of the postlarvae and juveniles of *M.rosenbergii* fed with different protein concentrations for a period of 40 days are presented in Table 9. The respective final percentage survival of the postlarvae and juveniles are presented in fig.1.

During the initial 10 days, the survival rate of the postlarvae and juveniles of the prawn was little influenced by the level of protein tested. However, thereafter, the postlarvae and juveniles which received a diet with zero percentage protein showed a sudden decline in the rate of survival reaching the respective final percentage survival values of 48 and 60%. These animals were observed to gradually become less and less active and finallly to succumb to death.

The analysis of variance of the data on the percentage survival of the postlarvae and juveniles of the prawn showed that the protein concentrations tested had a statistically significant ($P<0.01$) effect on percentage survival. Higher levels of protein (≥20%) resulted in better survival rates and final values ranged from 84 to 92% in the case of the postlarvae and 92 to 96% in the case of the juveniles. The best rate of survival in the case of the postlarvae was obtained when they were fed with diets having 30 or 40% level of protein. Similarly, in the case of the juveniles, diets containing 20, 30 or 40% level of protein resulted in the highest percentage survival. In both the cases increase in the dietary protein concentration to 50% resulted in an apparent reduction in the rate of survival. A pair-wise comparison of the data using multiple t-test technique revealed that the survival rate of the postlarvae and juveniles of the prawn fed with the zero protein diet was significantly ($P<0.01$) lower compared to that obtained when they were provided with the other diets. The survival rate of the postlarvae provided with 10% protein diet was not significantly different from that obtained when they were provided with 20 or 50% protein diet but significantly different from the survival rate obtained when they were provided with 30 or 40% protein diets. In the case of the juveniles, however, the difference in survival rate obtained when provided with 10 and 20% or 10 and 30% protein diets were significant, though that obtained with 10 and 40% or 10 and 50% were not. The apparent difference in the rate of survival of the postlarvae

and juveniles of the prawn which were fed with diets with 20, 30, 40 and 50% protein concentrations was statistically not significant among one another.

Table 9. Details of stocking number and percentage survival of the postlarvae (ordinary type) and juveniles (bold face type) of *M.rosenbergii* fed with different protein concentrations

Protein concentration (%)	Initial number stocked	Percentage survival of the prawn on day			
		10	20	30	40
0	50	96	76	62	48
	25	**96**	**76**	**64**	**60**
10	50	98	94	88	66
	25	**100**	**96**	**88**	**72**
20	50	100	98	92	84
	25	**100**	**100**	**96**	**96**
30	50	100	100	98	92
	25	**100**	**100**	**96**	**96**
40	50	100	100	96	92
	25	**100**	**96**	**96**	**96**
50	50	98	98	90	86
	25	**96**	**96**	**92**	**92**
Control	50	98	98	92	90
	25	**96**	**96**	**96**	**96**

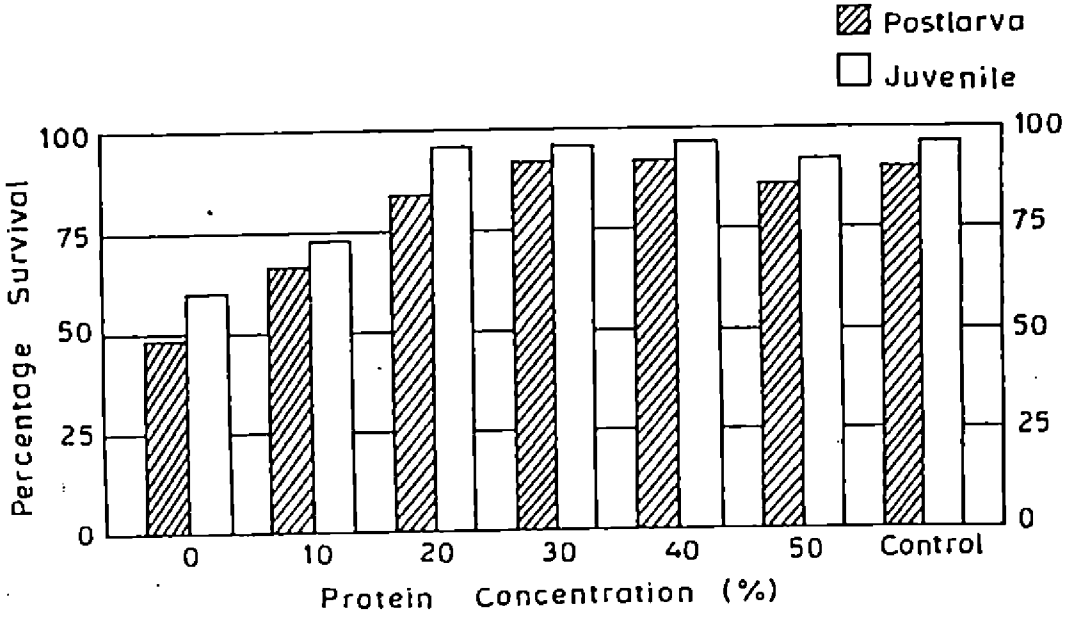

Fig.1. The final percentage survival of the postlarvae and juveniles of *M.rosenbergii* fed with different protein concentrations.

It could also be seen that the percentage survival of the postlarvae and juveniles of *M.rosenbergii* fed with the control diet was not significantly different from those obtained when the animals were fed with 20, 30, 40 or 50% dietary protein concentrations.

A comparative analysis of the mean final survival rates of the postlarvae and juveniles of the prawn using student t-technique also revealed that the effect of the low levels of protein tested (viz., 0 and 10%) on the final survival rate of the postlarvae is significantly different from that of the juveniles.. The low level of dietary protein is found to bring about a more pronounced decline in the survival rate of the postlarvae when compared to that of the juveniles.

4.1.2 *Effect of protein Concentration on Growth.*

The data on the initial weight of the postlarvae and juveniles of *M.rosenbergii* and their growth in terms of the instantaneous percentage growth of the prawn of unit weight (*IPGU*) for each sampling period are presented in Table 10. To avoid any confusion on the use of a relatively new index for expressing growth, the specific growth rate of the prawn over the whole experimental period is also provided (Table 10). The mean *IPGU* (specific growth coefficient x $10^{2)}$ of the postlarvae and juveniles of the prawn is presented in fig.2.

Table 10. Details of initial weight and growth of the postlarvae (ordinary type) and juveniles (bold face type) of *M.rosengergii* fed with different protein concentrations

Protein concen-tration (%)	Initial weight (g)	*IPGU* of the prawn				Percentage specific growth
		0-10	10-20	20-30	30-40	0-40
0	0.00680	(-0.02309)	(-0.04072)	(-0.06061)	(-0.06978)	(-0.25896)
	0.15242	**(-0.03184)**	**(-0.04782)**	**(-0.07334)**	**(-0.09094)**	**(-0.11547)**
10	0.00720	0.56189	0.83092	0.55871	0.32572	2.47669
	0.14962	**1.06389**	**1.04456**	**0.87210**	**0.68266**	**1.55939**
20	0.00690	0.62151	0.89702	1.05602	0.84735	3.50973
	0.15201	**1.38751**	**1.37832**	**1.29916**	**1.20394**	**2.14349**
30	0.00680	0.87829	1.09474	1.39144	1.46292	4.60021
	0.15063	**1.59418**	**1.97879**	**1.80837**	**1.74545**	**2.78174**
40	0.00740	0.95222	1.11853	1.39099	1.23630	4.41321
	0.14927	**1.60056**	**1.31807**	**1.25629**	**1.10958**	**2.16026**
50	0.00680	0.85503	0.69660	0.91683	1.15683	3.68893
	0.14687	**1.02123**	**0.85810**	**0.81047**	**0.60777**	**1.42533**
Cont-rol	0.00720	0.89617	1.20490	1.48357	1.53622	4.74019
	0.15174	**1.56044**	**1.92617**	**2.00948**	**2.03334**	**2.90629**

Protein inclusion was found to have a profound influence on the growth of the postlarvae and juveniles of the prawn. Protein exclusion from the diet (zero percentage protein) resulted in a well pronounced reduction in body weight of the postlarvae and juveniles, the respective percentage reduction in the body weight being 9.84 and 4.51% over the experimental period. A comparative analysis of the percentage decline in the body weight of the postlarvae and juveniles using student

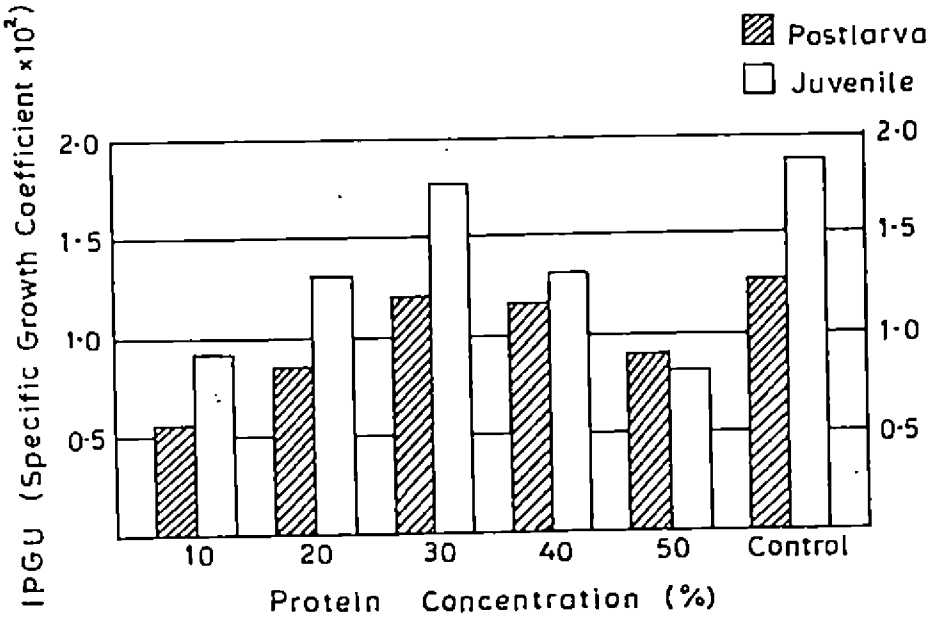

Fig.2. The mean *IPGU* (specific growth coefficient $\times 10^2$) of the postlarvae and juveniles of *M.rosenbergii* fed with different protein concentrations.

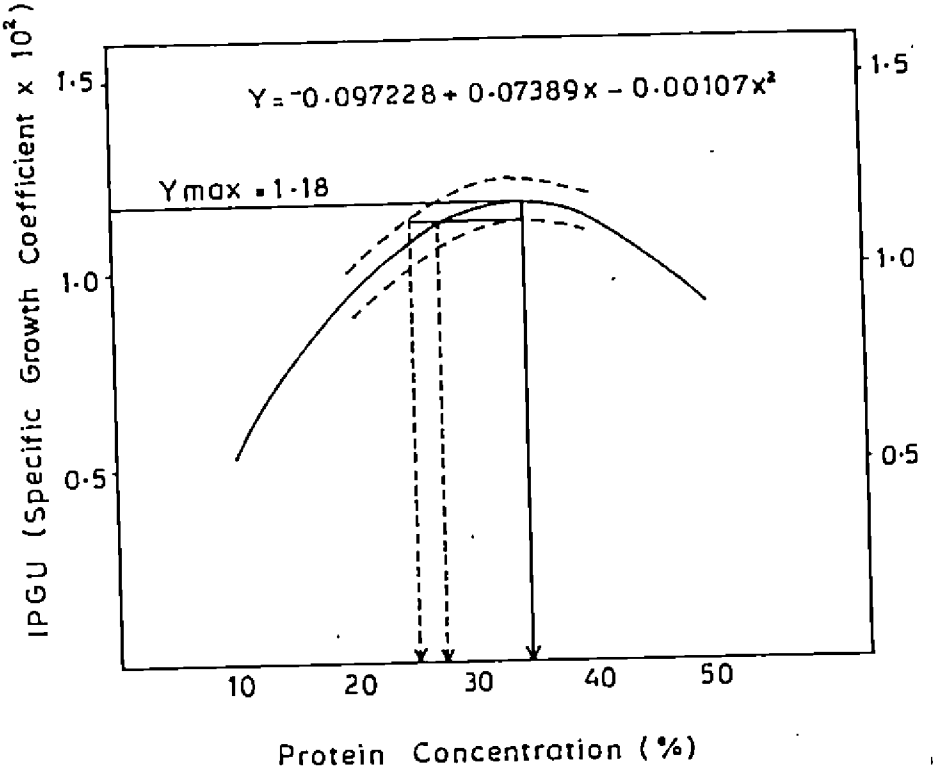

Fig.3. Second order polynomial relation (solid curve) and 95% confidence limits (dashed curved lines) of the instantaneous percentage growth of the postlarvae of *M.rosenbergii* of unit weight and dietary protein concentration.

t-technique revealed that the decline in body weight is more pronounced in the case of the former compared to the latter.

The analysis of variance of the data on *IPGU* of the postlarvae and juveniles of the prawn showed that the protein concentrations examined had a statistically significant ($P < 0.01$) influence on the growth of the prawn. The growth of the postlarvae was found to increase as the dietary protein concentration increased up to 30%. A further increase in the level of protein in the diet resulted in an apparent but statistically not significant reduction in the growth of the postlarvae. A pair-wise comparison of the data revealed that the difference in growth rate in response to the different dietary protein concentration is significant between the lower (≤ 0%) and the intermediate (20 and 30%) and the lower and the higher (≥40%) protein levels examined but not significant between the two higher levels (40 and 50%) of protein tested. The difference in growth was also significant between the two intermediate levels (20and 30%) but not significant between the intermediate and higher levels.

The juveniles of the prawn also showed a similar trend in growth. The best growth was obtained when the prawns were fed with 30% dietary protein level followed by 40 and 20% levels of protein. Here too, an increase in the dietary protein concentration beyond the 30% level resulted in a decrease in the rate of growth of the prawn. A pair-wise analysis revealed that the variation in growth in response to the difference in the dietary protein concentration is statistically significant ($P < 0.01$) among all the levels of protein tested, except between 10 and 50% or 20 and 40%.

The growth of the prawns provided with the control diet was found to be significantly different from that obtained when they were provided with the other diets except the ones with

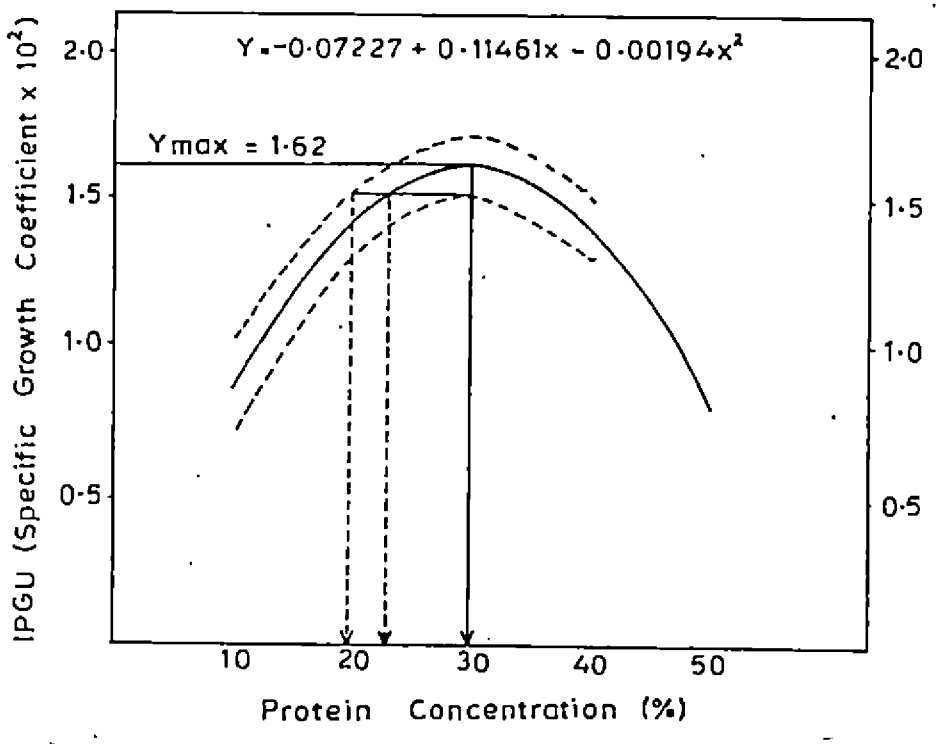

Fig.4. Second order polynomial relation (solid curve) and 95% confidence limits (dashed curved lines) of the instantaneous percentage growth of the juveniles of *M.rosenbergii* of unit weight and dietary protein concentration.

30 and 40% protein in the case of the postlarvae and 30% protein in the case of the juveniles.

Second order polynomial regression equations were established between the dietary protein concentrations examined and the corresponding growth (*IPGU*) of the postlarvae ($Y= -0.097228 + 0.07389x - 0.00107x^2$)and juveniles ($Y= -0.07227 + 0.11461x - 0.00194x^2$) of the prawn (fig.3 and 4 respectively). By employing the technique of differential calculus, the levels of protein that result in maximum growth in the postlarvae and juveniles of *M.rosenbergii* were theoretically determined to be 34.5 and 29.5% respectively.

The economic protein requirements viz., the dietary protein concentrations which minimize the cost while maintaining adequate growth were found to be 24.5-27.5% and 19.5-23.0% protein respectively in the postlarvae and juveniles, usually the higher levels being more acceptable ie., postlarvae, 27.5% and juveniles, 23.0%.

4.1.3 *Effect of Protein Concentration on Food Intake.*

The data on the food intake per day by the postlarvae and juveniles of the prawn of unit weight over the experimental period are presented in Table 11.

The mean food intake per day by unit weight of the postlarvae (r= -0.38) and juveniles (r= 0.41) of *M.rosenbergii* was found not to be correlated well with the dietary protein levels. In both the cases, analysis of variance of the data on the food intake have shown that the observed variation in the food intake according to the variation in the dietary protein concentration was statistically not significant.

Table 11. Details of food intake per day by the postlarvae (ordinary type) and juveniles (bold face type) of *M.rosenbergii* of unit weight fed with different protein concentrations

Protein concentration (%)	Food intake (g) per unit weight (g) of the prawn per day				
	0-10	10-20	20-30	30-40	Mean (0-40)
0	0.19746	0.18320	0.19207	0.17231	0.18626
	0.11542	**0.06490**	**0.07470**	**0.07780**	**0.08321**
10	0.23236	0.29424	0.17307	0.10266	0.20058
	0.08426	**0.08385**	**0.07730**	**0.06769**	**0.07828**
20	0.15629	0.18977	0.19597	0.15007	0.17303
	0.07934	**0.07367**	**0.06524**	**0.05880**	**0.06926**
30	0.15959	0.16648	0.18501	0.17238	0.17087
	0.08167	**0.09671**	**0.08364**	**0.07828**	**0.08508**
40	0.16860	0.16339	0.17370	0.13862	0.16108
	0.08653	**0.06806**	**0.06252**	**0.05478**	**0.06797**
50	0.22016	0.15471	0.18366	0.20082	0.18984
	0.10992	**0.11525**	**0.12153**	**0.08972**	**0.10911**
Control	0.16425	0.18729	0.20133	0.16767	0.18014
	0.07480	**0.09490**	**0.09548**	**0.10058**	**0.09144**

The intake of the control diet by the postlarvae and juveniles was also found not to be different from that of other diets. It could also be seen that the food intake per day by unit weight of the prawn was relatively more in the case of the postlarvae compared to the juveniles.

4.1.4 *Effect of Protein Concentration on Food Conversion Efficiency.*

The data on the food conversion efficiency of the postlarvae and juveniles of *M.rosenbergii* over the experimental period, in response to the different dietary protein concentrations are presented in Table 12. The mean food conversion values of the two life stages examined were presented in fig.5.

Table 12. The food conversion efficiency of the postlarvae (ordinary type) and juveniles (bold face type) of *M.rosenbergii* fed with different protein concentrations

Protein concentration (%)	Food conversion efficiency (%)			
	0-10	10-20	20-30	30-40
10	11.81	12.34	12.89	12.00
	23.04	**21.38**	**18.38**	**15.78**
20	19.56	20.59	20.54	19.31
	31.39	**31.05**	**30.81**	**29.79**
30	26.46	27.23	26.49	25.66
	34.87	**33.00**	**31.73**	**30.15**
40	26.27	27.41	29.39	26.69
	33.13	**32.00**	**31.04**	**19.55**
50	18.72	19.41	19.24	19.55
	17.08	**12.96**	**11.08**	**10.84**
Control	25.74	25.92	25.08	26.70
	37.23	**32.78**	**30.77**	**26.94**

The analysis of variance of the data on food conversion efficiency revealed that the protein density had a statistically significant ($P< 0.01$) influence on the food conversion efficiency in both the postlarvae and juveniles of the prawn. In the postlarvae, the food conversion efficiency was found to improve as the dietary protein concentration increased up to 40%. The food conversion value obtained when the postlarvae were fed with a diet with a protein concentration of 30% was found to be similar to that obtained when they were fed with a diet with 40% protein. However, a further increase in the dietary protein concentration to 50% resulted in a sharp decline in the food conversion efficiency value. A pair-wise comparison of the data revealed that the difference in the food conversion efficiency in response to the difference in the protein density is statistically significant among all levels of proteins tested except between 30 and 40%.

In the juveniles of the prawn, the best food conversion eficiency value was obtained when the prawns were fed with 30% protein diet, closely followed by the 40 and 20% protein diets. A pair-wise comparison, showed that the food conversion efficiency values obtained in response to 20, 30 and 40% protein diets were statistically not significant among one another. However, the diets with 10 and 50%. protein showed significantly ($P< 0.01$) lower food conversion efficiency.

In general, the food conversion efficiency of the postlarvae was relatively less when compared to that of the juveniles.

The food conversion efficiency value obtained in the case of the postlarvae when fed with the control diet was similar to that obtained when they were provided with the 30% protein diet, but significantly less than the value obtained when they were provided with the 40% protein diet. In the

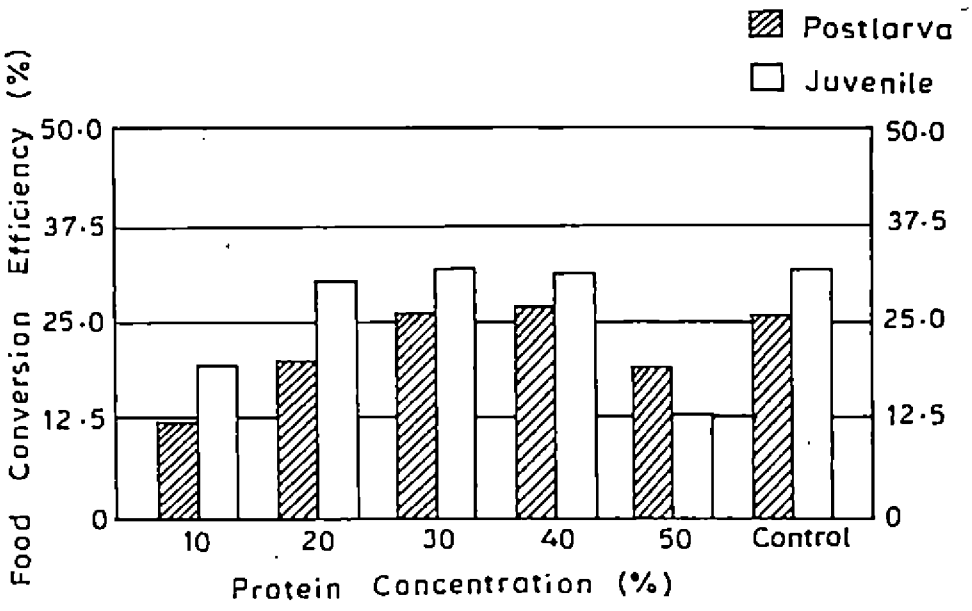

Fig.5. The mean food conversion efficiency of the postlarvae and juveniles of *M.rosenbergii* fed with different protein concentrations.

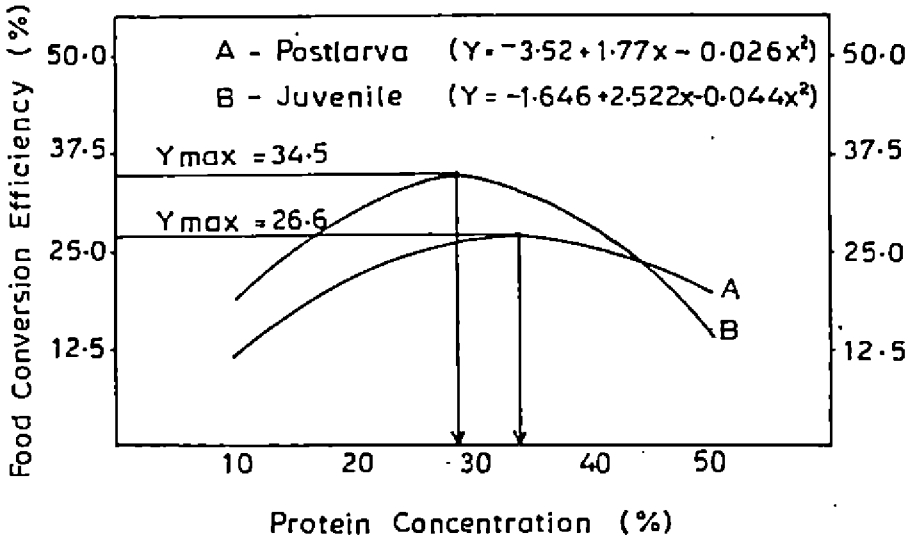

Fig 6. Second order polynomial relation of the food conversion efficiency of the postlarvae and juveniles of *M.rosenbergii* and dietary protein concentration.

case of the juveniles, however, the food conversion efficiency value obtained with the control diet was comparable to the values obtained when they were provided with the 20, 30 or 40% protein diets.

Second order polynomial regression equations were establisshed between the dietary protein concentration and the corresponding food conversion efficiency of the postlarvae ($Y= -3.52 + 1.77x -0.026x^2$) and the juveniles ($Y= -1.646 + 2.522x - 0.044x^2$) of the prawn. The relationship between the two parameters in the case of the postlarvae and juveniles are presented in fig.6. By employing the technique of differential calculus the levels of protein that result in maximum food conversion efficiency in the postlarvae and juveniles were determined to be 34.02 and 28.59% respectively.

4.1.5 *Effect of Protein Concentration on Digestibility of Protein.*

The data on apparent digestibility of protein by the postlarvae and juveniles of *M.rosenbergii* are presented in Table 13. In the case of both the life stages, the apparent digestibility of protein increased with increase in the dietary protein concentration, the mean lowest and highest values for the experimental period being 77.50% (at 10% level of protein) and 88.83% (at 50% level of protein) for the postlarvae (fig.7) and 85.27% (at 10% level of protein) and 95.51% (at 50% level of protein) for the juveniles (fig.8) of the prawn. It could be seen from the figures that the apparent digestibility values for the control diet were 88.83 and 93.67% respectively for the postlarvae and juveniles.

Table 13. The apparent digestibility of protein by the postlarvae (ordinary type) and juveniles (bold face type) of *M.rosenbergii* fed with different protein concentrations

Protein concentration	Apparent digestibility of protein (%)			
(%)	0-10	10-20	20-30	30-40
10	76.81	77.42	77.85	77.93
	84.39	**84.83**	**85.78**	**86.07**
20	83.77	84.87	83.40	83.50
	90.24	**90.62**	**92.10**	**92.69**
30	85.78	85.93	86.17	87.09
	91.93	**92.76**	**94.19**	**94.81**
40	87.38	87.08	86.94	88.29
	93.25	**93.66**	**96.64**	**96.04**
50	88.91	88.37	87.61	87.64
	94.40	**95.82**	**95.54**	**96.28**
Control	88.86	89.02	88.78	88.66
	93.08	**93.43**	**93.65**	**94.56**

The analysis of variance of the data on the apparent digestibility of the postlarvae and juveniles of the prawn showed that the efffect of the protein concentration on the apparent digestibility is statistically significant among all the levels of proteins tested except between 40 and 50%.

In the postlarvae the apparent digestibility of protein of the control diet was also found to be significantly different from that of all other diets except the ones with 30 and 40% protein. In the juveniles, the apparent digestibility of protein of the control diet was significantly different from that of all other diets.

The true digestibility of protein for each sampling period calculated on the basis of the respective metabolic faecal nitrogen value (see 4.1.6) for the postlarvae and juveniles are presented in Table 14. The mean true digestibility of protein by the postlarvae and juveniles are presented in figures 7 and 8 respectively. In the postlarvae and juveniles of the prawn, the variation in protein concentration had

Table 14. The true digestibility of protein by the postlarvae (ordinary type) and juveniles (bold face type) of *M.rosenbergii* fed with different protein concentrations

Protein concentration	True digestibility of protein (%)			
(%)	0-10	10-20	20-30	30-40
10	89.20	89.81	90.24	90.32
	93.83	**94.27**	**95.22**	**95.51**
20	89.96	91.07	89.60	87.70
	94.96	**95.34**	**96.82**	**97.41**
30	89.91	90.06	90.30	91.22
	95.08	**95.91**	**97.34**	**97.96**
40	90.48	90.18	90.04	91.39
	95.61	**96.02**	**96.00**	**98.40**
50	91.39	90.85	90.09	90.12
	96.29	**97.71**	**97.43**	**98.17**
Control	90.98	91.17	90.89	90.75
	94.98	**95.39**	**95.59**	**96.72**

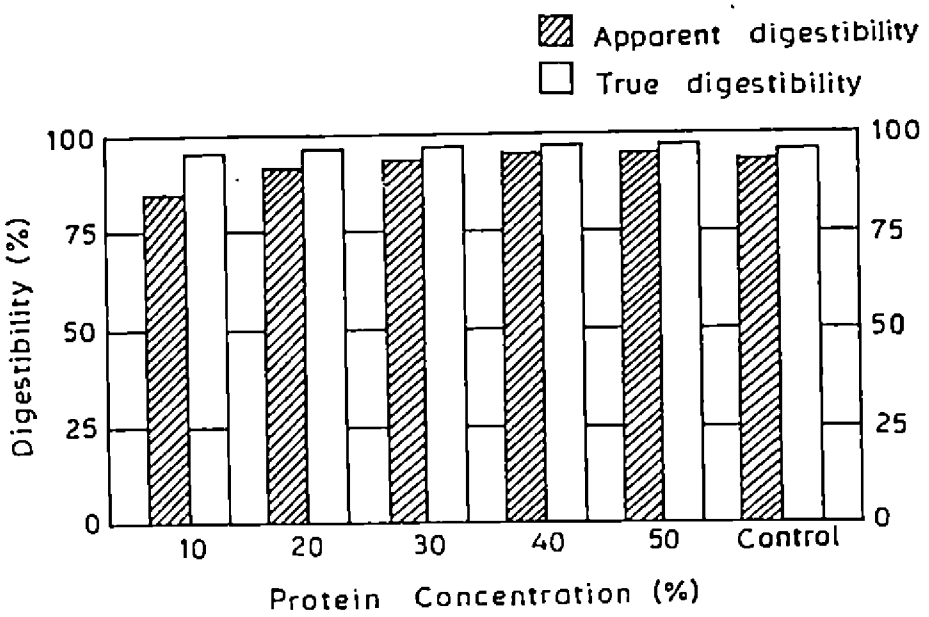

Fig 7. The mean apparent and true digestibility of protein by the postlarvae of *M.rosenbergii* fed with different protein concentrations.

little influence on the true digestibility of protein. The true digestibility of protein of the control diet was comparable to that of other diets.

In general, it could be seen that the digestibility of protein by the postlarvae is relatively lower compared to that by the juveniles.

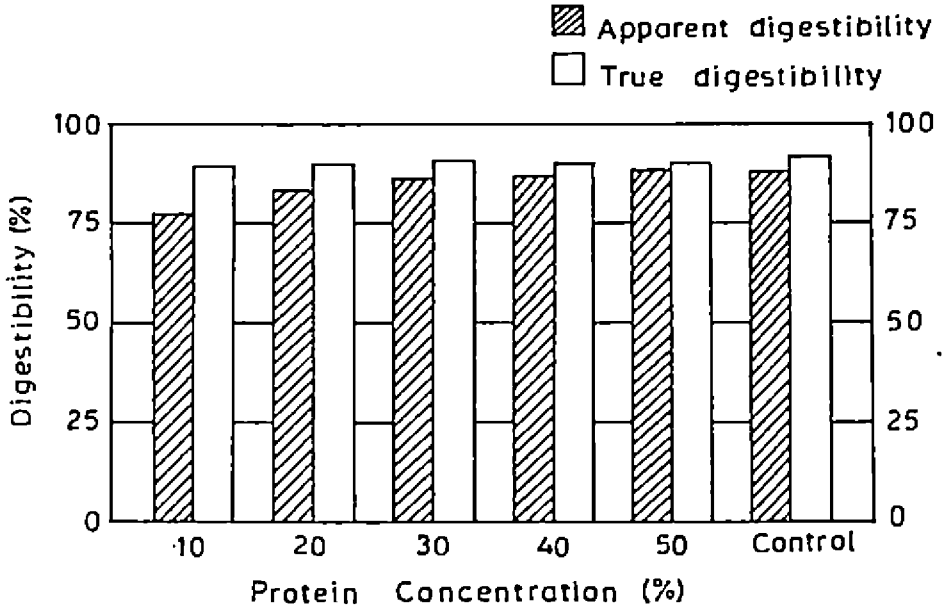

Fig 8. The mean apparent and true digestibility of protein by the juveniles of *M.rosenbergii* fed with different protein concentrations.

4.1.6 *Effect of Protein Concentration on Faecal Nitrogen Excretion.*

The data on faecal nitrogen excretion by the postlarvae and juveniles of *M.rosenbergii* are presented in Table 15.

The analysis of variance of the data on faecal nitrogen excretion (mg N/100g diet) by the postlarvae and juveniles of *M.rosenbergii* showed that the dietary protein concnetration had a statistically significant ($P< 0.01$) influence on the faecal nitrogen excretion. The relationship between the dietary protein concentration and the nitrogen excretion(mg N/100g diet) was found to be positively, linearly correlated with correlation coefficient values of 0.999 and 0.985 for the postlarvae and juveniles of the prawn, respectively. Further, it could be seen from Table 15 that the amount of faecal nitrogen excreted per 100g diet consumed by the postlarvae is relatively higher compared with that by the juveniles.

It could also be observed that even when the prawns were fed with a protein-free diet, some amount of nitrogen viz., 198mg N/100g diet in the postlarvae and 151mg N/100g diet in the juveniles was present in the faeces, which represent the metabolic faecal nitrogen.

The metabolic faecal nitrogen was also calculated by employing the indirect method of regressing nitrogen excretion against the dietary protein concentration for the postlarvae (fig.9) and the juveniles (fig.10). By this technique the values of metabolic faecal nitrogen of the postlarvae and juveniles of *M.rosenbergii* were determined to be 217.66 and 212.81mg N/100g diet respectively. These values are

9.93 and 40.93% higher than the respective values of metabolic faecal nitrogen obtained by employing the direct faeces collection method, in the case of the postlarvae and juveniles of the prawn respectively.

Table 15. Nitrogen excretion (faecal) by the postlarvae (ordinary type) and juveniles (bold face type) of *M.rosenbergii* fed with different protein concentrations

Protein concentration (%)	Faecal nitrogen excretion (mg N/100g diet)				
	0-10	10-20	20-30	30-40	Mean
0	188.00	191.10	200.20	212.70	198.00
	140.60	**148.00**	**160.00**	**155.40**	**151.00**
10	371.04	361.28	354.40	353.12	359.96
	249.76	**242.72**	**277.52**	**222.88**	**235.72**
20	519.68	484.16	531.20	528.00	515.76
	312.32	**300.16**	**252.80**	**233.92**	**274.85**
30	682.56	675.36	663.84	619.68	660.36
	387.36	**347.52**	**278.88**	**249.12**	**315.72**
40	807.68	826.88	835.84	749.44	804.96
	432.00	**405.76**	**215.04**	**253.44**	**326.56**
50	887.20	930.40	991.20	988.80	949.40
	448.00	**334.40**	**356.80**	**297.60**	**359.20**
Control	629.50	620.48	638.88	640.58	631.13
	390.85	**371.24**	**361.67**	**307.61**	**357.84**

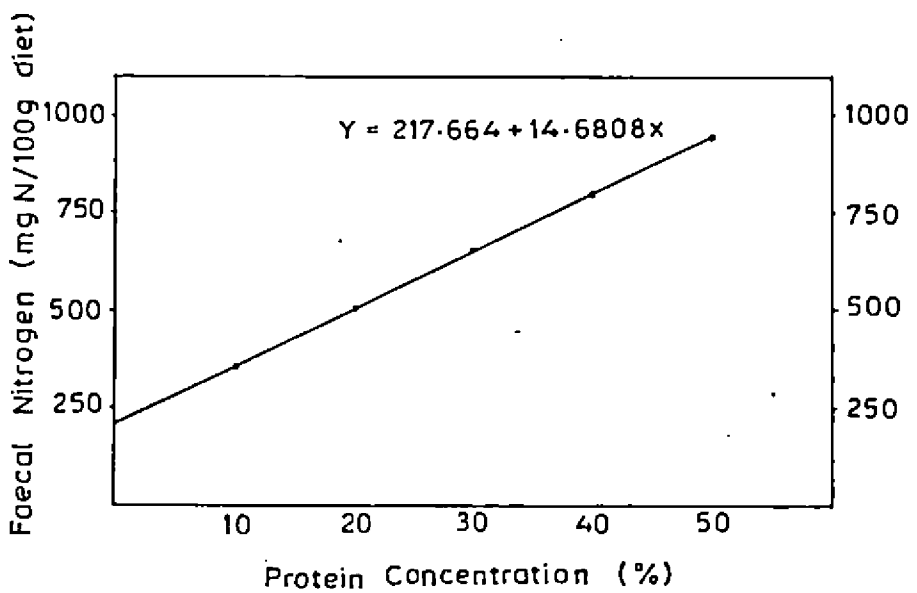

Fig 9. Linear relation between the faecal nitrogen excretion by the postlarvae of *M.rosenbergii* and the dietary protein concentration.

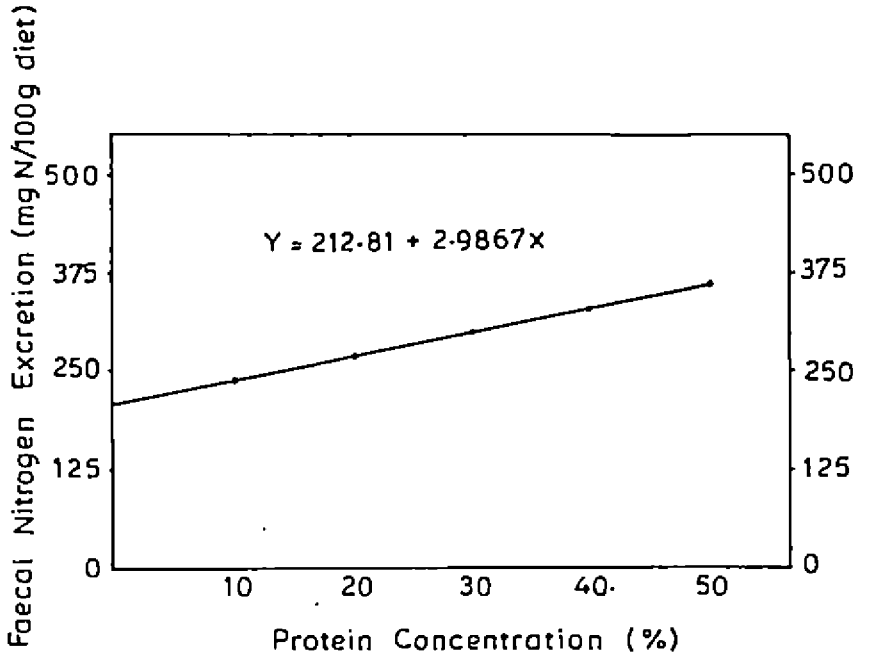

Fig 10. Linear relationship between the faecal nitrogen excretion by the juveniles of *M.rosenbergii* and the dietary protein concentration.

4.1.7 *Effect of Protein Concentration on Prawn Carcass Composition.*

The carcass composition of the postlarvae and juveniles of the prawn at the beginning and end of the study is presented in Table 16.

The moisture content of the prawn was found to vary between 80.80 and 82.00% in the case of the postlarvae and 76.22 and 81.00% in the case of the juveniles of the prawn. The variation in moisture content according to the dietary protein concentration was found to be statistically not significant. However, dietary protein concentration was found to result in an apparently more pronounced but statistically not significant variation in the body protein concentration in both the life stages of the prawn examined. Protein exclusion from the diet resulted in a decrease in the percentage of prawn carcass protein, the respective values being 52.83% for the postlarvae and 53.39% for the juveniles of the prawn. The highest percentage protein in the prawn body was obained when they were provided with 40% dietary protein concentration in the case of the postlarvae (63.31%) and 50% dietary protein concentration in the case of the juveniles (64.73%). The variation in the crude fat content of the body carcass was also found to be statistically not significant. The ash content, too, was found not to be significantly influenced by the variation in the dietary protein concentration. The carcass composition of the postlarvae and juveniles of the prawn fed with the control diet was also not significantly different from that obtained when they were provided with the other diets.

4.1.8 *Effect of Protein Concentration on Nitrogen Retention.*

The data on the nitrogen retention (= nitrogen balance) by the postlarvae and juveniles of *M.rosenbergii* against the respective digestible nitrogen intake are presented in Table 17.

It could be seen that in the case of the postlarvae, over the digestible nitrogen intake range of 267.55-759.51mg N/100g body weight/day, the relationship between digestible nitrogen intake and the

nitrogen retention was linear (r=0.95). However, beyond this range of nitrogen consumption the nitrogen retention value decreased sharply. Over the entire range of digestible nitrogen intake tested in the present experiment, the relationship between the nitrogen intake (mg N/100g body weight/day) and the nitrogen retention (mg N/100g body weight/day) could be represented by a second order polynomial regression equation; $Y = -24.31011 + 0.23732x - 0.0001506x^2$ (fig.11). From the equation, by applying the technique of differential calculus, digestible nitrogen intake of 787.90mg N/100g body weight/day can be found to result in maximum nitrogen retention in the postlarvae. From Table 17 it could also be observed that when the postlarvae were fed with no protein, it resulted in a loss of body nitrogen equal to 17.95mg N/100g body weight/day which represents the endogenous nitrogen excretion or the digestible nitrogen level that results in zero nitrogen balance in the postlarvae of the prawn, assuming an efficiency of utilization of protein very close to 100%.

Table 16. Carcass composition (% dry weight) of the postlarvae (ordinary type) and juveniles (bold face type) of *M.rosenbergii* fed with different protein concentrations

Protein concentration	Carcass composition (% dry weight) at the end of the study			
(%)	Moisture[1]	Crude protein	Crude fat	Ash
0	81.00	52.83	10.50	24.96
	80.17	**53.39**	**10.76**	**25.38**
10	81.90	53.58	10.20	24.50
	81.00	**54.98**	**9.90**	**23.80**
20	81.10	59.63	8.90	21.88
	80.00	**58.14**	**8.44**	**20.64**
30	81.12	61.10	8.50	20.80
	78.10	**61.28**	**8.30**	**20.35**
40	80.80	63.31	8.48	20.60
	76.22	**64.64**	**8.00**	**20.02**
50	82.00	56.62	8.90	22.56
	77.20	**64.73**	**7.80**	**20.00**
Control	80.99	60.64	8.30	21.00
	78.64	**64.70**	**8.24**	**20.94**
Initial carcass composition	78.31	61.40	9.00	21.00
	76.80	**63.26**	**9.20**	**20.00**

[1] % wet weight

Similarly, in the juveniles of *M.rosenbergii* the same relationship could be observed between the digestible nitrogen intake and the nitrogen retention. Over the range of 117.95-404.34mg N/100g body weight/day of nitrogen intake, the nitrogen retention by the juveniles was found to be linear (r=0.99). Here too, a higher digestible nitrogen intake value(858.04mg N/100g body weight/day) resulted in drastic decline in the nitrogen retention. The relationship between the two, could be explained by the equation, $Y = -15.20349 + 0.27566x - 0.00026248x^2$ (fig.12). By applying the technique of differential

calculus, the value of nitrogen intake to bring about maximum nitrogen retention was found to be 525.101mg N/100g body weight/day.

Table 17. Nitrogen retention (= nitrogen balance) in the postlarvae and juveniles of *M.rosenbergii* against the respective digestible nitrogen intake

Protein concent-ration (%)	Digestible nitrogen intake(mg N/100g body weight/day)		Nitrogen retained (mg N/100g body weight/day)	
	Postlarva	Juvenile	Postlarva	Juvenile
0	-	-	-17.95	-18.69
10	267.55	117.95	27.73	13.44
20	436.29	202.57	51.37	30.34
30	599.96	366.23	65.04	51.76
40	759.51	404.34	67.47	51.70
50	1178.08	858.04	46.53	28.25
Control	742.39	467.94	66.27	56.25

Feeding the juveniles with the zero protein diet resulted in a loss of body protein equivalent to 18.69 mg N/100g body weight which represents the endogenous nitrogen excretion.

In the case of both the postlarvae and juveniles of *M.rosenbergii* from figures 11 and 12 it could be seen that the more nitrogen (protein) is consumed, the more is retained over a wide range of protein consumption. In both the cases, the value of a, the Y-intercept in the regression equations viz., $Y= -24.31011 + 0.23732x - 0.0001506x^2$ and $Y= -15.20349 + 0.27566x - 0.00026248x^2$ for the postlarvae and juveniles respectively, are negative which represent the value of nitrogen lost (endogenous nitrogen excreted) when the prawns do not consume protein at all.

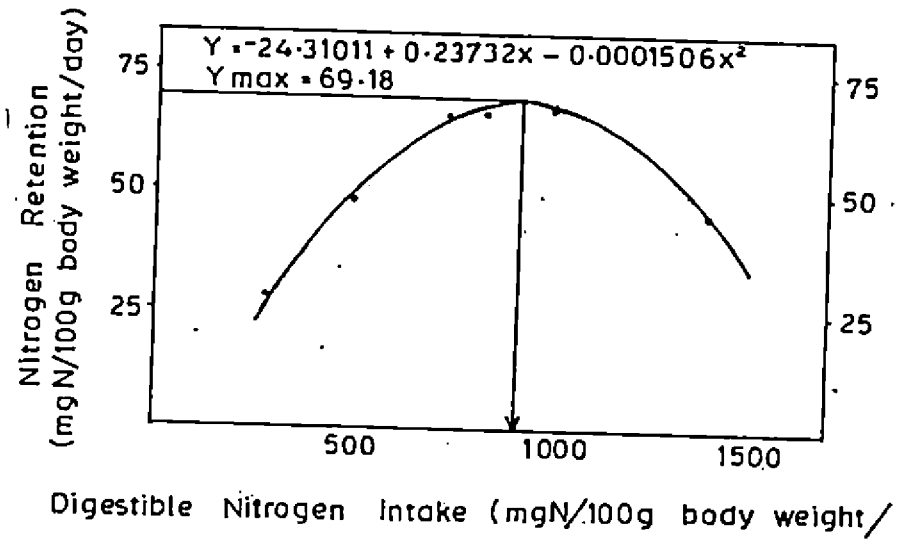

Fig 11. Second order polynomial relation of the diegestible nitrogen intake and the nitrogen retention in the postlarvae of *M.rosenbergii*.

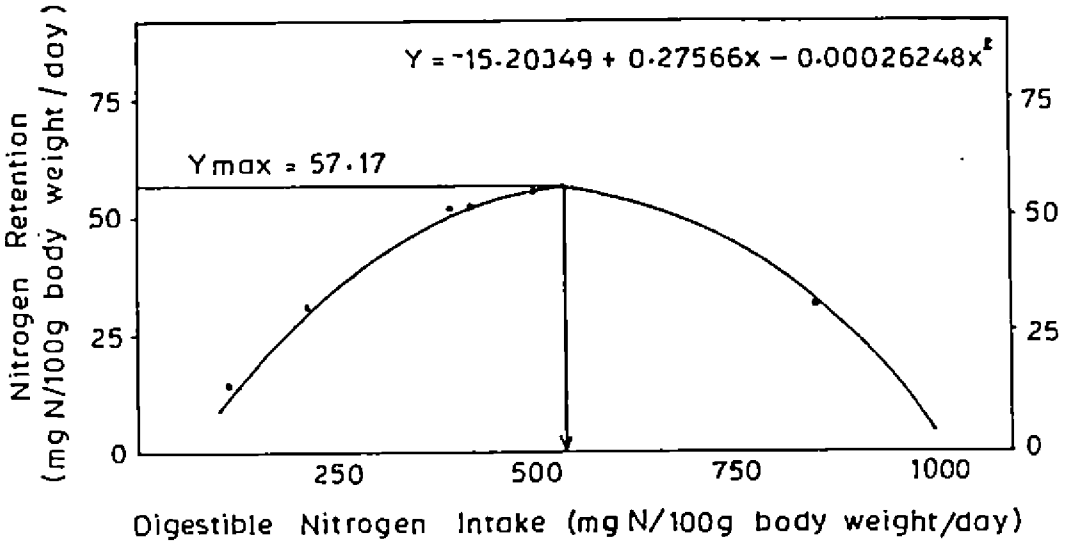

Fig 12. Second order polynomial relation of the diegestible nitrogen intake and the nitrogen retention in the juveniles of *M.rosenbergii*.

4.1.9 *Effect of Protein Concentration on Efficiency of Protein Utilization.*

The data on the protein efficiency ratio (PER) of the postlarvae and juveniles of the prawn over the experimental period are presented in Table 18. The mean PER of the animals corresponding to each protein concentration is presented in fig 13.

Table 18. Protein efficiency ratio (PER) of the postlarvae (ordinary type) and juveniles (bold face type) of *M.rosenbergii* fed with different protein concentrations

Protein concentration (%)	Protein efficiency ratio			
	0-10	10-20	20-30	30-40
10	1.181	1.234	1.289	1.200
	2.304	**2.189**	**1.838**	**1.578**
20	0.978	1.030	1.027	0.966
	1.567	**1.553**	**1.541**	**1.490**
30	0.882	0.908	0.883	0.855
	1.162	**1.100**	**1.058**	**1.005**
40	0.657	0.685	0.685	0.667
	0.828	**0.800**	**0.776**	**0.738**
50	0.374	0.388	0.385	0.391
	0.342	**0.259**	**0.222**	**0.217**
Control	0.728	0.731	0.709	0.763
	1.054	**0.928**	**0.871**	**0.763**

The PER of the postlarvae and juveniles of *M.rosenbergii* was significantly influenced by the dietary protein concentration. The best PER values were obtained when the prawns were provided with

a protein concentration of 10% in the diet. In both the life stages of the prawn, the mean PER values decreased as the dietary protein concentration increased form 10-50%. The relationship between the protein concentration and the PER values of the postlarvae and juveniles was found to be linear, with the corresponding correlation coefficient (r) of -0.991 and -0.997 respectively. The mean PER values for the control diet were 0.733 and 0.904 for the postlarvae and juveniles of the prawn respectively.

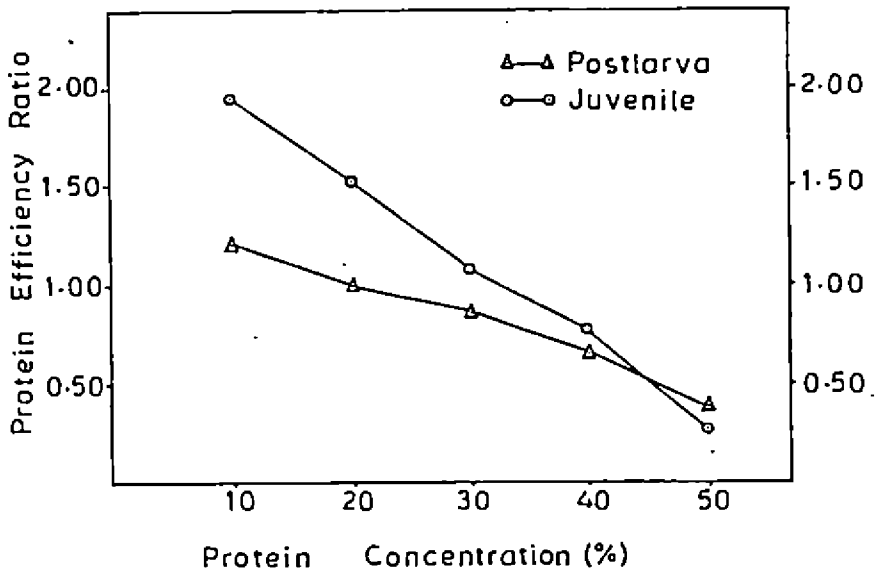

Fig 13. The mean protein efficiency ratio (PER) of the postlarvae and juveniles of *M.rosenbergii* fed with different protein concentrations.

4.1.10 *Effect of Protein Concentration on Oxygen-Nitrogen Ratio.*

The data on the oxygen-nitrogen ratio of the postlarvae and juveniles of *M.rosenbergii* in response to the different dietary protein concentrations are presented in Table 19.

Table 19. Oxygen-nitrogen ratio of the postlarvae and juveniles of *M.rosenbergii* fed with different protein concentrations

Protein concentration (%)	Oxygen-nitrogen ratio	
	Postlarva	Juvenile
0	36.20	33.43
10	32.61	29.46
20	29.80	27.24
30	28.43	27.12
40	28.25	26.06
50	25.17	23.38
Control	28.06	27.41

The oxygen-nitrogen ratio of the postlarvae and juveniles of *M.rosenbergii* was significantly influenced by the difference in the dietary protein concentration. The highest value for the oxygen-nitrogen ratio was obtained when the prawns were provided with a dietary protein level of 0%, the values being 36.20 for the postlarvae and 33.43 for the juveniles. The values were found to decrease

as the dietary protein concentration increased, in both the postlarvae and juveniles of the prawn. The lowest values were obtained when the prawns were fed with 50% protein diet. Further, a pair-wise comparison of the data revealed that the oxygen-nitrogen values obtained in response to the 20, 30 and 40% dietary protein concentrations were not significantly different from one another.

4.2 Short term Study to Determine the Apparent Digestibility of a few Protein Sources

The data on the apparent digestibility of protein by the postlarvae and juveniles of *M.rosenbergii* fed with protein from different sources for 10 days are presented in Table 20.

Table 20. The mean apparent digestibility of protein by the postlarvae and juveniles of *M.rosenbergii* fed with protein from different sources (short term study)

Protein source	Origin of protein source	Mean apparent digestibility (%)	
		Postlarva	Juvenile
fat free casein	animal	93.80	96.05
prawn meat	„	89.26	93.50
clam meat	„	91.40	94.60
soybean	plant	89.86	91.60
ground oil cake	„	90.27	92.20
black gram	„	89.50	90.80
prawn meat + clam meat	animal	91.40	94.20
soybean + ground nut oil cake + black gram	plant	90.50	90.90

The analysis of variance of the data on the short term study on the apparent digestibility of protein by the postlarvae and juveniles of *M.rosenbergii* fed with protein from different sources showed that the apparent protein digestibility was not significantly influenced by animal or plant feed stuff origin.

4.3 Study to Evaluate the Effect of Protein source

The physico-chemical conditions under which the experiment to determine the effect of progressive substitution of protein of animal origin with that of plant origin on the postlarvae and juveniles of *M.rosenbergii* was conducted were salinity, 0 ppt; temperature, 28.20±1.80°C; pH, 7.70±0.82;dissolved oxygen, 6.61±0.42 ppm; ammonia 0.015-0.030 ppm and total alkalinity, 58.50±6.00 ppm. The experiment on the juveniles was conducted under the following physico-chemical conditions: salinity, 0 ppt; temperature, 27.80±2.20°C; pH, 8.00±0.48; dissolved oxygen, 6.60±0.40 ppm; ammonia, 0.018-0.036 ppm and total alkalinity, 60.28±2.06 ppm. The light regime was as given in section 4.1.

4.3.1 *Effect of Protein Source on Survival.*

The data on the stocking number and the percentage survival of the postlarvae and juveniles of *M.rosenbergii* fed with protein from different sources are presented in Table 21. The respective final percentage survival of the postlarvae and juveniles are presented in fig 14.

The analysis of variance of the data on the percentage survival of the postlarvae and juveniles showed that the substitution of the protein of animal origin with that of plant origin had no statistically significant effect on the percentage survival. The final survival rate of the postlarvae varied from 90%

to 94% when provided with diets having protein from animal and plant source in different ratios. In the case of the juveniles the final survival rate varied between 92 and 96%.

The survival rate of the postlarvae and juveniles of *M.rosenbergii* when provided with the control diet (clam meat) was significantly lower compared to the respective survival rate of the animals fed

Table 21. Details of stocking number and percentage survival of the postlarvae (ordinary type) and juveniles (bold face type) of *M.rosenbergii* fed with protein from different sources

Diet number	Initial number stocked	Percentage survival of the prawn on day			
		10	20	30	40
1	50	98	98	96	92
	25	**100**	**96**	**96**	**96**
2	50	98	98	96	94
	25	**96**	**96**	**96**	**96**
3	50	100	98	96	94
	25	**96**	**96**	**96**	**96**
4	50	100	98	92	92
	25	**100**	**96**	**96**	**96**
5	50	100	98	94	90
	25	**96**	**96**	**96**	**92**
6	50	96	94	90	88
(Control)	**25**	**94**	**92**	**92**	**90**

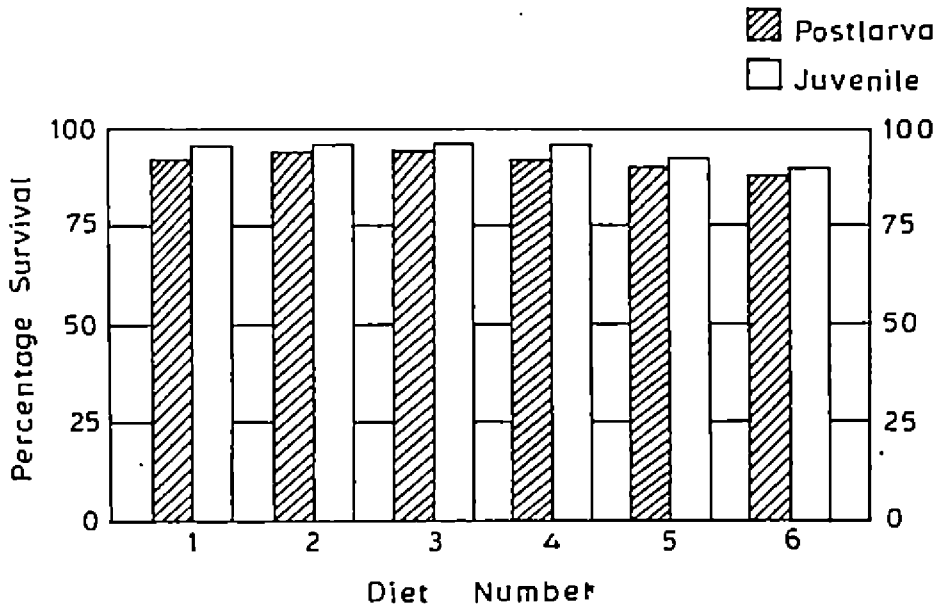

Fig.14. The final percentage survival of the postlarvae and juveniles of *M.rosenbergii* fed with protein from different sources.

with the compounded diets. The final survival rates of the postlarvae and juveniles provided with the control diet were 88% and 90% respectively.

4.3.2 *Effect of Protein Source on Growth.*

The data on the initial weight of the postlarvae and juveniles of *M.rosenbergii* and their growth in terms of the instantaneous percentage growth of the prawn of unit weight (*IPGU*) for each sampling period are presented in Table 22. Growth is also expressed as percentage specific growth (Table 22). The mean *IPGU* of the postlarvae and juveniles of the prawn is presented in fig.15.

Table 22. Details of initial weight and growth of the postlarvae (ordinary type) and juveniles (bold face type) of *M.rosenbergii* fed with protein from different sources

Diet number	Initial weight (g)	*IPGU* of the prawn				Percentage specific growth
		0-10	10-20	20-30	30-40	0-40
1	0.00720	0.849	1.196	1.448	1.541	4.680
	0.14826	**1.474**	**1.964**	**1.981**	**1.519**	**2.731**
2	0.00730	0.947	1.173	1.466	1.570	4.750
	0.15373	**1.606**	**1.985**	**2.028**	**1.693**	**2.826**
3	0.00690	0.950	1.126	1.520	1.404	4.720
	0.16207	**1.567**	**1.999**	**1.951**	**1.792**	**2.784**
4	0.00760	0.915	1.210	1.554	1.390	4.640
	0.15543	**1.564**	**2.018**	**1.965**	**1.704**	**2.798**
5	0.00680	0.849	1.164	1.412	1.408	4.600
	0.17216	**1.510**	**2.091**	**1.710**	**1.857**	**2.694**
6	0.00660	0.795	1.104	1.345	1.301	4.430
(Control)	**0.15924**	**1.299**	**1.528**	**1.912**	**1.497**	**2.500**

The analysis of variance of the data on *IPGU* of the postlarvae of the prawn showed that the substitution of protein of animal origin with that of plant origin had a statistically significant ($P<0.01$) effect on the growth. The best growth of the postlarvae was obtained when fed with a diet based on protein of animal and plant origin in 3:1 ratio viz., 75% protein of animal origin and 25% of plant origin. However, pair wise analysis of the data using multiple t-test technique showed that the growth of the postlarvae provided with the above mentioned diet was not significantly different from the growth rate of the postlarvae provided with the other diets except the one based entirely on plant protein. The growth rate of the postlarvae obtained in response to the other diets with various combinations of protein from animal and plant sources were also found to be not significantly different from one another.

In the juveniles of the prawn the substitution of dietary protein of animal origin with that of plant origin had no statistically significant effect on the growth rate. Nevertheless, a slight increment in growth rate was noticeable when the plant protein component was incorporated in the diet upto 50%.

The growth rate of the postlarvae and juveniles of the prawn provided with the control diet was found to be significantly less compared to those provided with the other diets.

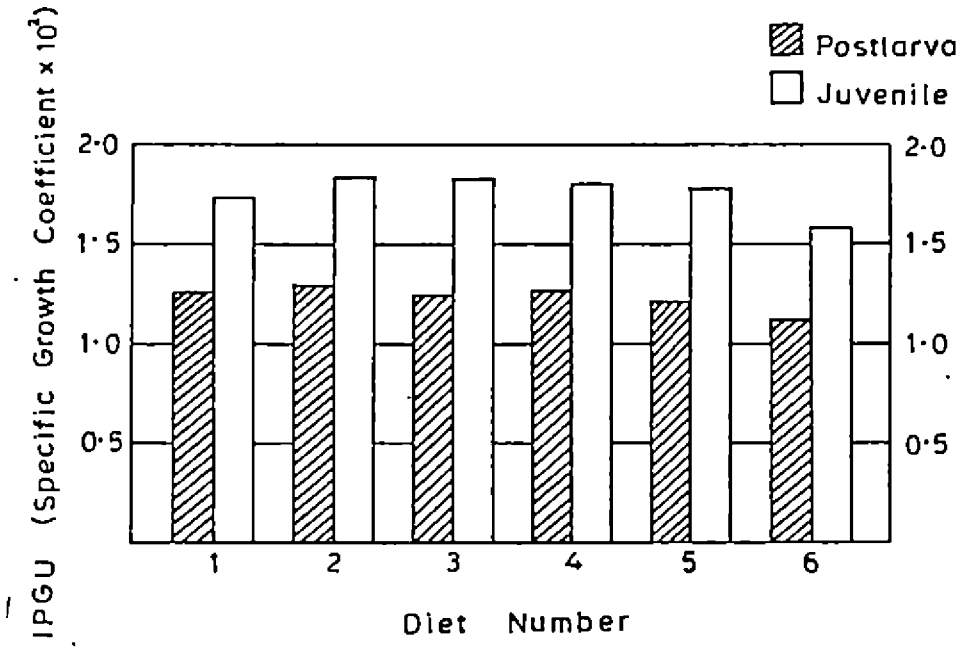

Fig.15. The mean *IPGU* (specific growth coefficient x 10^2) of the postlarvae and juveniles of *M.rosenbergii* fed with protein from different sources.

4.3.3 *Effect of Protein Source on Food Intake.*

The data on the food intake per day of the postlarvae and juveniles of the prawn of unit weight over the experimental period are presented in Table 23.

Table 23. Details of food intake per day by the postlarvae (ordinary type) and juveniles (bold face type) of *M.rosenbergii* of unit weight fed with protein from different soruces

Diet number	Food intake (g) per unit weight (g) of the prawn per day				Mean food intake (g) per unit weight (g) of the prawn per day
	0-10	10-20	20-30	30-40	0-40
1	0.14376	0.17839	0.17886	0.17556	0.16914
	0.08859	**0.08728**	**0.08280**	**0.06054**	**0.07980**
2	0.15317	0.16250	0.18279	0.17240	0.16772
	0.08164	**0.08935**	**0.08592**	**0.06842**	**0.08133**
3	0.15668	0.16620	0.19472	0.16609	0.17092
	0.07652	**0.09082**	**0.08282**	**0.07389**	**0.08101**
4	0.16164	0.17242	0.20494	0.16127	0.17507
	0.07871	**0.09271**	**0.08227**	**0.07060**	**0.08107**
5	0.15146	0.17810	0.22605	0.17391	0.18238
	0.07936	**0.10691**	**0.08040**	**0.08507**	**0.08794**
6	0.14741	0.17218	0.19310	0.16462	0.16933
(Control)	**0.07068**	**0.08229**	**0.09791**	**0.08244**	**0.08333**

The analysis of variance of the data on the food intake per day per unit weight of the postlarvae and juveniles of the prawn showed that the substitution of protein of animal origin with that of plant-origin had no statistically significant effect on the food intake. The food intake values of the postlarvae and juveniles provided with dried clam meat (control) were also found not to be significantly different from those obtained with the other diets.

4.3.4 *Effect of Protein Source on Food Conversion Efficiency.*

The data on the food conversion efficiency of the postlarvae and juveniles of *M.rosenbergii* in response to the substitution of animal source of protein with plant source are presented in Table 24. The mean food conversion efficiency values of the two life stages examined are presented in fig.16.

Table 24. The food conversion efficiency of the postlarvae (ordinay type) and juveniles (bold face type) of *M.rosenbergii* fed with protein from different sources

Diet number	Food conversion efficiency (%)			
	0-10	10-20	20-30	30-40
1	28.00	27.20	27.80	25.80
	30.00	**36.70**	**35.30**	**34.20**
2	28.90	28.88	27.20	26.42
	34.90	**35.60**	**34.20**	**33.00**
3	28.80	27.60	26.80	25.02
	35.80	**35.20**	**33.80**	**32.00**
4	26.23	27.80	25.30	24.89
	35.20	**34.80**	**34.60**	**32.20**
5	27.02	27.00	25.60	24.43
	32.70	**30.40**	**30.20**	**28.60**
6 (Control)	26.40	27.04	25.00	24.60
	32.60	**30.40**	**29.20**	**25.10**

The analysis of variance of the data on food conversion efficiency of the postlarvae and juveniles of *M.rosenbergii* showed that the substitution of protein of animal origin with that of plant origin had a statistically significant influence on the food conversion efficiency. The postlarvae fed with a diet based on 75% animal protein and 25% plant protein (diet 2) showed the highest mean food conversion efficiency. However,pair wise analysis of the data showed that the food conversion efficiency of the postlarvae fed with diets with protein derived 100% from animal source (diet 1), 75% from animal and 25% from plant source (diet 2) and 50% each from animal and plant source (diet 3) was not significantly different from one another. An increase in the plant protein component in the diet beyond 50% resulted in a statistically significant decline in food conversion efficiency. The food conversion efficiency of the postlarvae fed with diet 4 (25% animal protein and 75% plant protein) was not significantly different from that obtained when the postlarvae were provided with diet 5 (100% plant protein).

The postlarvae fed with the dried clam meat showed a significantly lower food conversion efficiency compared to those fed with diet 1, 2 or 3. However, the food conversion efficiency of the postlarvae fed with the dried clam meat was not significantly different from those fed with diet 4 or 5.

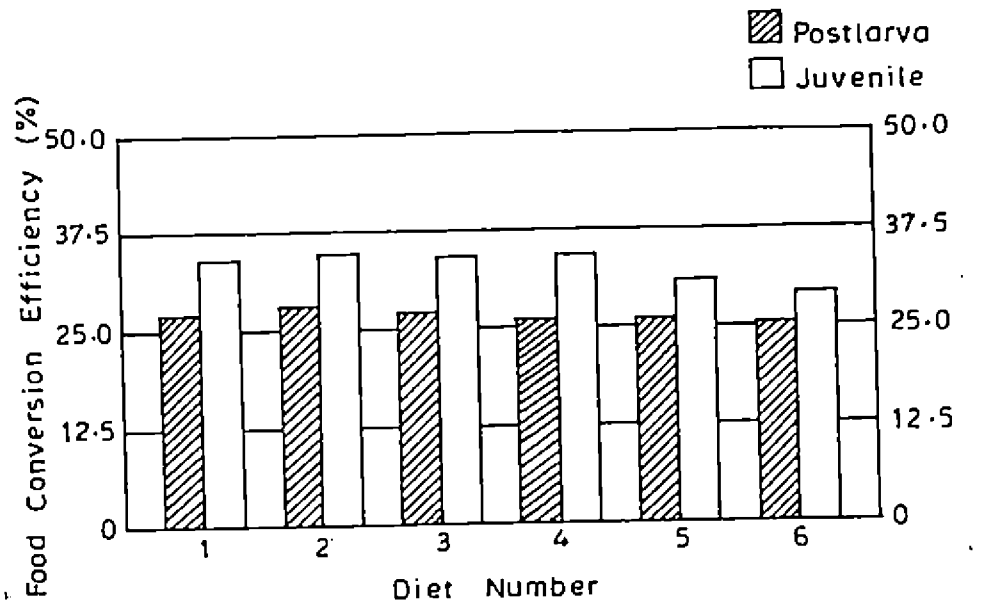

Fig. 16. The mean food conversion efficiency of the postlarvae and juveniles of *M.rosenbergii* fed with protein from different sources.

In the juveniles too, the substitution of protein of animal origin with that of plant origin had a statistically significant effect on the food conversion efficiency. Pair-wise analysis of the data showed, however, that the difference in food conversion efficiency is not statistically significant when animal protein was substituted with plant protein upto 75%. The decline in the food conversion efficiency when the juveniles were fed with a 100% plant protein diet was statistically significant. Feeding with the dried clam meat resulted in a significantly less food conversion efficiency compared to other diets except the one based entirely on plant protein (diet 5).

4.3.5 *Effect of Protein Source on Digestibility of Protein.*

The data on the apparent digestibility of protein by the postlarvae and juveniles of *M.rosenbergii* fed with protein from different sources are presented in Table 25.

The progressive incorporation of protein of plant origin by replacing that of animal origin resulted in a gradual decrease in the apparent digestibility of protein by the postlarvae and juveniles of the prawn.

The analysis of variance of the data showed that the substitution of protein from animal source with that from plant source had a statistically significant ($P< 0.01$) effect on the apparent digestibility of protein by the postlarvae. The diet having protein entirely of animal origin (diet 1) had the highest apparent digestibility. However, the apparent protein digestibility of this diet was not significantly different from the apparent digestiblity of protein of the diet having 75% protein of animal origin and 25% of plant origin (diet 2). Further, no significant difference could be observed in the apparent protein digestibility of diets 2 and 3, diets 3 and 4, diets 3 and 5 and diets 4 and 5. The apparent protein digestibility of all other diets were significantly different from one another.

In the juveniles too, the diet with 100% protein of animal origin (diet 1) had the highest apparent digestibility. This was followed closely by one with 75% protein of animal origin and 25% of plant origin (diet 2) and one with 50% protein each of plant and animal origin (diet 3). However, pair-wise comparison of the results revealed that the difference among the apparent digestibility of protein of the three diets were not statistically significant. The difference in the apparent digestibility of all other diets were significant.

The apparent digestibility of protein of the control diet was found to be significantly different from that of other diets in the postlarvae and juveniles of the prawn.

The true digestibility of protein by the postlarvae and juveniles of *M.rosenbergii* when provided with the various diets is presented in Table 26. The true digestibility was calculated based on the matabolic faecal nitrogen value of *M.rosenbergii* as 198 and 151mg N/100g diet (see 4.1.6).

Table 25. The apparent digestibility of protein by the postlarvae (ordinary type) and juveniles (bold face type) of *M.rosenbergii* fed with protein from different sources

Diet number	Apparent digestibility of protein (%)			
	0-10	10-20	20-30	30-40
1	87.84 **93.24**	89.11 **92.72**	89.47 **91.87**	88.55 **91.69**
2	89.11 **91.80**	87.69 **91.72**	87.48 **91.20**	89.85 **91.10**
3	88.12 **91.73**	86.60 **91.88**	86.44 **90.54**	87.46 **91.12**
4	86.88 **88.98**	86.01 **88.90**	88.39 **90.70**	86.88 **89.81**
5	87.15 **88.34**	86.92 **88.38**	86.13 **89.27**	84.57 **86.96**
6 (Control)	91.15 **93.80**	90.56 **92.86**	91.18 **94.48**	91.79 **95.58**

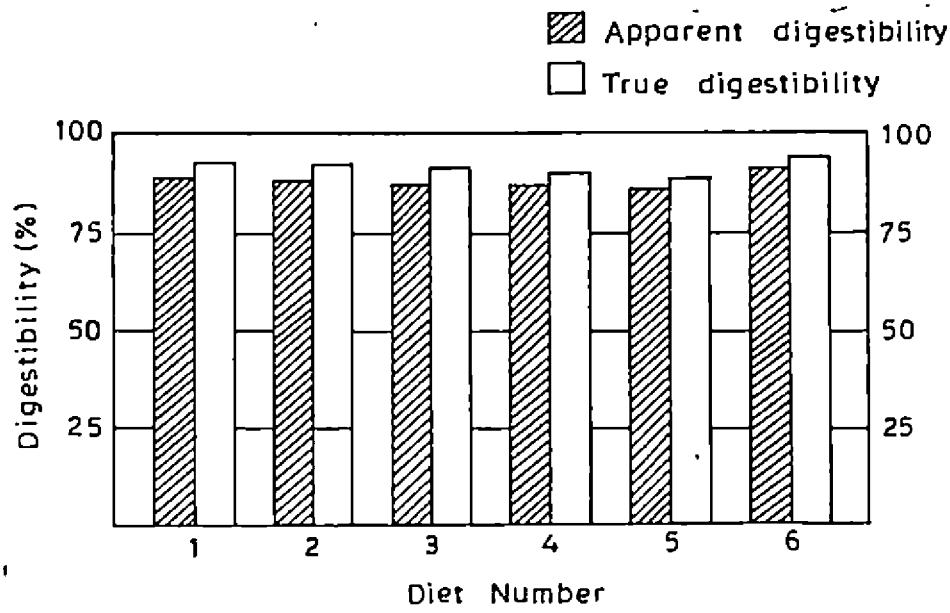

Fig.17. The apparent and true digestibility of protein by the postlarvae of *M.rosenbergii* fed with protein from different sources.

Figures 17 and 18 show the mean apparent and true digestibility of protein by the postlarvae and juveniles of *M.rosenbergii* respectively.

Table 26. The true digestibility of protein by the postlarvae (ordinary type) and juveniles (bold face type) of *M.rosenbergii* fed with protein from different sources

Diet number	True digestibility of protein (%)			
	0-10	10-20	20-30	30-40
1	91.37 **96.41**	92.64 **95.89**	93.00 **95.04**	92.08 **94.86**
2	92.61 **94.90**	91.19 **94.82**	90.98 **94.30**	93.35 **94.20**
3	91.68 **94.87**	90.16 **95.02**	90.00 **93.68**	91.02 **94.26**
4	90.49 **92.16**	89.62 **92.08**	92.00 **93.88**	90.49 **92.99**
5	90.64 **91.43**	90.41 **91.47**	89.62 **92.36**	88.06 **90.05**
6 (Control)	93.97 **95.94**	93.38 **95.00**	94.00 **96.62**	94.61 **97.12**

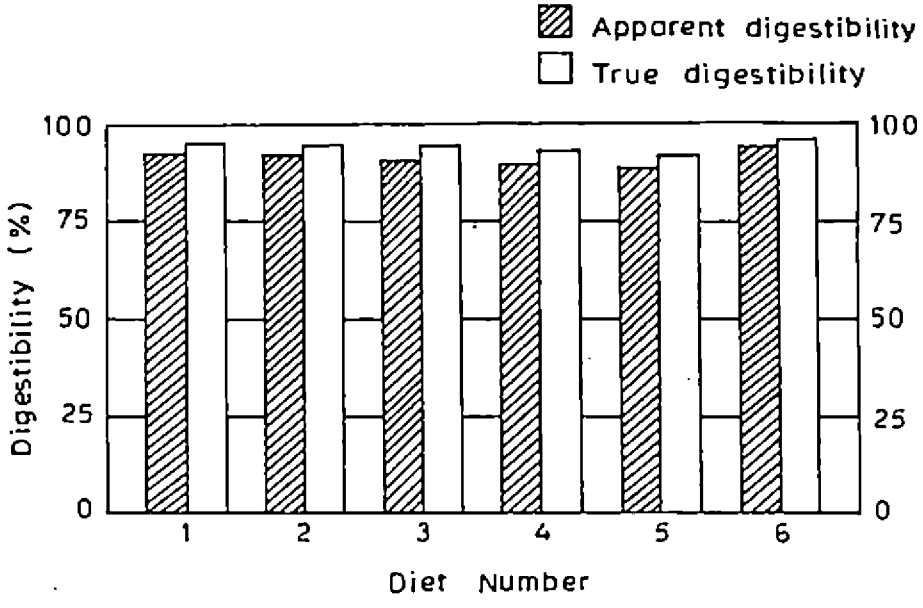

Fig.18. The apparent and true digestibility of protein by the juveniles of *M.rosenbergii* fed with protien from different sources.

The substitution of animal source of protein with plant source had statistically significant influence on the true digestibility of protein by both the postlarvae and juveniles of the prawn. Pair-wise comparison of the data on true digestibility of protein by the postlarvae revealed that the true digestibility of protein is significantly different among all the diets except between diets 1 and 2, diets 2 and 3, diets 2 and 4,diets 3 and 4, diets 3 and 5 and diets 4 and 5. In the juveniles, pair wise comparison of the results showed that the true digestibility of protein by the juveniles was significantly different among all diets except between diets 1 and 2 and diets 2and 3. The true digestibility of the dried clam meat was significantly higher than that of all other diets in the postlarvae and all other diets except diet 1 in the juveniles of the prawn.

4.3.6 *Effect of Protein Source on Prawn Carcass Composition.*

The biochemical composition of the postlarvae and juveniles of *M.rosenbergii* before and after the study is presented in Table 27.

The moisture content of the prawn was found to vary from 80.20 to 81.80% in the case of the postlarvae and 77.60 to 79.24% in the case of the juveniles when protein of animal origin was substituted with protein of plant origin in different ratios. The crude protein content of the postlarvae and juveniles of the prawn varied from 62.80 to 64.40% and 63.30 to 65.10% respectively.The crude fat content varied from 7.80 to 8.60% and 7.30 to 8.80% in the postlarvae and juveniles respectively. The ash content was found to vary from 20.50 to 21.80% in the postlarvae, and 20.60 to 21.90% in the juveniles.

Table 27. Carcass composition of the postlarvae (ordinary type) and juveniles (bold face type) of *M.rosenbergii* fed with protein from different sources

Diet number	Carcass composition (% dry weight) at the end of numberthe study			
	moisture[1]	crude protein	crude fat	ash
1	80.80	62.80	8.60	21.80
	78.20	**64.20**	**8.80**	**21.00**
2	80.20	63.00	8.40	21.60
	77.60	**64.00**	**7.90**	**21.90**
3	81.10	64.40	7.90	20.80
	77.70	**63.30**	**7.60**	**21.20**
4	81.00	62.40	8.00	20.50
	79.24	**65.10**	**7.30**	**20.80**
5	81.80	61.30	7.80	21.70
	78.16	**64.00**	**8.10**	**20.60**
6 (Control)	80.20	63.90	8.90	21.20
	79.98	**64.80**	**8.30**	**20.90**
Initial carcass composition(%)	79.00	62.00	8.80	22.00
	77.00	**63.00**	**9.20**	**20.80**

[1]%wet weight

juveniles. The analysis of variance of the data on the carcass composition of the postlarvae and juveniles provided with the various diets having different ratio of animal and plant sources of protein showed that the observed difference in the carcass compositions is statistically not significant.

The carcass composition of the postlarvae and juveniles of the prawn fed with dried clam meat was also found to be not significantly different from that obtained when they were provided with the other diets.

4.3.7 *Effect of Protein Source on Efficiency of Protein Utilization.*

The data on the protein efficiency ratio (PER) of the postlarvae and juveniles of the prawn fed with diets having different ratio of protein of animal and plant origin are presented in Table 28. The mean PER of the postlarvae and juveniles are presented in fig.19.

Table 28. Protein efficiency ratio (PER) of the postlarvae (ordinary type) and juveniles (bold face type) of *M.rosenbergii* fed with protein from different sources

Diet number	Protein efficiency ratio(PER)			
	0-10	10-20	20-30	30-40
1	0.90 **0.96**	0.87 **1.17**	0.89 **1.13**	0.83 **1.09**
2	0.93 **1.12**	0.93 **1.14**	0.87 **1.10**	0.85 **1.06**
3	0.92 **1.15**	0.89 **1.12**	0.86 **1.08**	0.80 **1.03**
4	0.84 **1.13**	0.89 **1.12**	0.81 **1.11**	0.80 **1.04**
5	0.87 **1.05**	0.87 **0.98**	0.82 **0.97**	0.79 **0.92**
6 (Control)	0.56 **0.70**	0.58 **0.65**	0.53 **0.62**	0.52 **0.54**

The dietary proteins tested had a statistically significant ($P< 0.01$) influence on the PER of the postlarvae and juveniles of *M.rosenbergii*. In the case of the postlarvae, the best PER was obtained when they were provided with a diet having 75% protein of animal origin and 25% of plant origin (diet 3). The lowest PER value in the case of the postlarvae was obtained with the diet having 100% protein from plant source (diet 5). Comparison of the data employing multiple t-test technique revealed that the effect of substitution of protein of animal origin with that of plant origin on PER was significant among all diets except between diets 1 and 2, diets 1 and 3, diets 1 and 4, diets 2 and 3, diets 3 and 4, diets 3 and 5, and between diets 4 and 5.

In the juveniles the PER values obtained when diets 1, 2, 3 and 4 were offered to them were similar. However, substitution of animal protein beyond 75% resulted in statistically significant

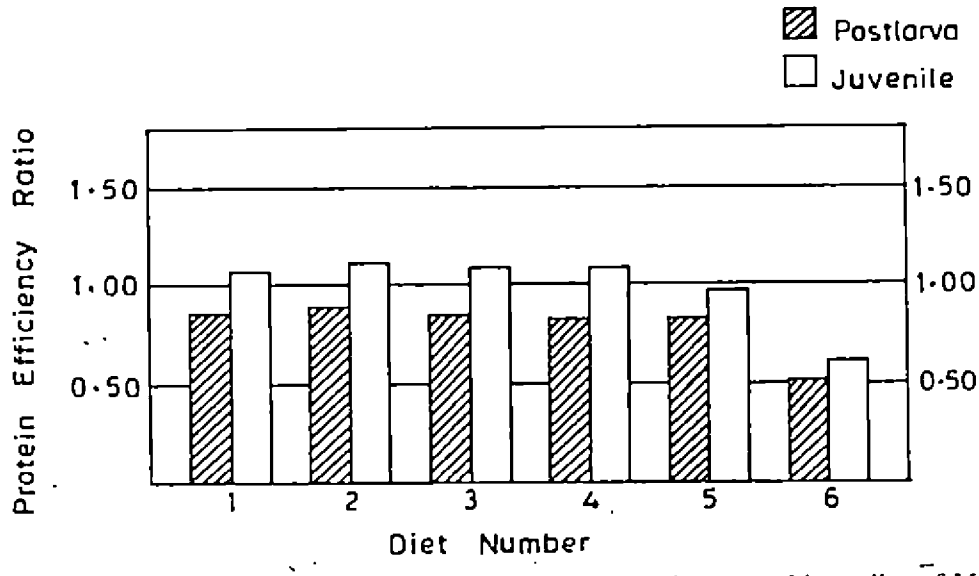

Fig.19. The mean protein efficiency ratio (PER) of the postlarvae and juveniles of *M.rosenbergii* fed with protein from different sources.

reduction in the PER values. In the case of both the postlarvae and juveniles, the PER obtained when provided with the control diet was the lowest and was significantly different from those obtained with other diets.

4.3.8 *Effect of Protein Source on Oxygen-Nitrogen Ratio.*

The data on the oxygen-nitrogen ratio of the postlarvae and juveniles of *M.rosenbergii* in response to the different dietary protein sources is presented in Table 29.

Table 29. Oxygen-nitrogen ratio of the postlarvae and juveniles of *M.rosenbergii* fed with protein from different sources

Diet number	Oxygen-nitrogen ratio	
	Postlarva	Juvenile
1	31.00	32.80
2	32.60	32.00
3	30.00	33.00
4	29.00	28.62
5	27.20	26.80
6 (Control)	22.46	20.50

The oxygen-nitrogen ratio of the postlarvae and juveniles of *M.rosenbergii* was significantly ($P<0.01$) influenced by the substitution of dietary protein of animal origin with that of plant origin. The highest value for the oxygen-nitrogen ratio was obtained when the postlarvae were provided with diet 2 (O:N value, 32.60) and the juveniles with diet 3 (O:N value, 33.00). The value of O:N ratio declined when protein from animal source was substituted with that from plant source beyond 25% in the case of the postlarvae and 50% in the case of the juveniles. The diet based on protein entirely of plant origin

(diet 5) resulted in a relatively very low O:N ratio in both the postlarvae and juveniles. A pair-wise comparison of the data showed that the differences in the O:N value of the postlarvae fed with the various diets were significant among one another except between diets 1 and 2, diets 1 and 3 and diets 3 and 4. Similarly the differences in the O:N ratio of the juveniles fed with the various diets were significant among one another except between diets 1 and 2, diets 1 and 3, diets 2 and 3 and diets 4 and 5. The O:N value of the postlarvae and juveniles were minimum when provided with the dried clam meat as the sole nutritional supply (control). These values were significantly less compared to the O:N values obtained for other diets.

5 DISCUSSION

The physico-chemical conditions under which the experiments were conducted are in general most conducive to obtain maximum growth in the postlarvae and juveniles of *Macrobrachium rosenbergii*. The experiment was conducted in freshwater. Various authors observed a salinity range of 0-2ppt as ideal for the growth of *M.rosenbergii* (Wickins, 1972; Goodwin and Hanson, 1975; Perdue and Nakamura, 1976; Sandifer, *et al.*, MS; Venugopalan, 1988). New (1976a) recommended the use of slightly saline (2ppt) water in nutrition studies on *M.rosenbergii*. The temperature of rearing water varied between 27 and 31°C which is within the optimum range reported for the growth of *M.rosenbergii* (Raman, 1967; Uno *et al.*, 1975; Armstrong, 1978; Farmanfarmaian and Moore, 1978; New and Singholka, 1982; Sandifer and Smith, 1985). The pH of the water in which the prawns were reared varied between 7.2 and 8.5. Though no specific studies relating water pH to growth and survival of *M.rosenbergii* have been conducted, New and Singholka (1982) and Sandifer and Smith (1985) suggested a pH range of 7.0-8.5 as the optimum for *Macrobrachium* ponds. Cripps and Nakamura (1979), Sarver *et al.* (1979, 1982), Malecha *et al.* (1980) and Sandifer and Smith (1985) observed that high pH values are not favourable for the growth of *M.rosenbergii*. Natividad (1982) reported that in Philippine rivers, *M.rosenbergii* prefers a wide range of pH (4.0-8.5). New (1976a) also recommended a pH range of 7.0-8.5. for nutrition studies on *M.rosenbergii*.

The dissolved oxygen values (6.1-7.0 mg/litre) were also within the range required for optimum growth in the prawn (Smith *et al.*, 1976,1978,1981 and Subrahmanyam, 1987). New (1976a) suggested 75% saturation of dissolved oxygen as ideal for nutrition studies on the freshwater prawn. New and Singholka (1982) also reported an oxygen concentration of 75% saturation as a provisional value for *Macrobrachium* ponds. The total alkalinity levels were not beyond the safe level (180ppm) as reported by Sandifer and Smith (1985). New and Singholka (1982) recommended levels between 40 and 100mg/litre calcium carbonate for successful farming operation. Brown *et al.* (1991) supported the above mentioned recommendation. Frequent water exchange also helped to maintain the ammonia concentrations below the harmful level. The acute ammonia toxicity value to the postlarvae of *M.rosenbergii* is 1.75 mg NH_3-N/litre (unionized) at a water pH of 7.6 (Armstrong *et al.*, 1978). The light regime was approximately 8 hours dark and 16 hours light as suggested by New (1976a) for nutritional studies on *M.rosenbergii*.

5.1 Protein Concentration

The importance of dietary protein inclusion was revealed by the fact that the survival of the postlarvae and juveniles of the prawn *M.rosenbergii* was the lowest when protein component was excluded from the diet. The utilization of protein from the body tissues of the prawn fed with zero protein diet which resulted in well pronounced negative growth might have rendered the animals weak and vulnerable to infection leading to higher mortality rate. A similar observation was also made by Gopal (1986) and Ahamed Ali (1988) in *P.indicus*. Kiron (1988) also observed higher mortality rate when the brackishwater finfish *Liza parsia* was fed with a zero protein diet. Possibly due principally to the difficulty in feeding some fishes and prawns a diet without protein, studies involving feeding the prawns and fishes with such a diet are very few. In the present study the postlarvae showed a more pronounced decline in survival rate compared to the juveniles, the former being the more critical protein requiring life stage (Venkataramaih *et al.*, 1975a).

An increase in the protein concentration in the diet improved the final percentage survival of the postlarvae and juveniles of the prawn. Thus in the postlarvae the best survival rate of 92% was obtained at a dietary protein level of 30 and 40%. In the juveniles, however, a dietary protein density of 20, 30 and 40% produced the highest percentage survival (96%). In both the cases, a further increase in the

dietary protein concentration resulted in a statistically not significant reduction in the percentage survival.

The final percentage survival values of the postlarvae and juveniles of *M.rosenbergii* obtained in the present study was relatively high which may be attributed to the better balancing of nutrients in the experimental diets, maintenance of water quality within the desirable range throughout the experimental period by daily exchange of almost the entire quantity of the rearing water and also to the provision of artificial substrata in each rearing tank. Provision of artificial substrata has been reported to increase the survival rate, and inturn the yield of *M.rosenbergii* (Smith and Sandifer, 1975; Cohen *et al.*, 1983; Ra'anan *et al.*, 1984; Sandifer and Smith, 1985; Gomez *et al.*, 1988). Forster (1970) and Rickards (1971) reported increased survival rate of *P.serratus* and *P.duorarum* respectively when provided with artificial substrata. Segal and Roe (1975) also reported that artificial shelters can improve survival rate in prawns.

The trend in the surivival rate of the postlarvae and juveniles of *M.rosenbergii* observed in the present experiment is an increase in the survival rate with an increase in the dietary protein density upto a certain optimum level and a decrease, when the dietary protein density increased further. This is in perfect concord with the observations made by various investigators in various species of prawns and shrimps (Andrews *et al.*, 1972; Venkataramiah *et al.*, 1975b; Colvin and Brand, 1977; Khannapa, 1977; Ahamed Ali, 1982; Bages and Sloane, 1981; D'Abramo and Reed, 1988; Gomez *et al.*, 1988).

Dietary protein concentration had a significant effect on the growth of the postlarvae and juveniles of *M.rosenbergii*. The growth of the postlarvae and juveniles of the prawn expressed in terms of the mean *IPGU* over the experimental period showed a parabolic relationship with the dietary protein concentration. The growth rate improved as the level of dietary protein increased upto a certain optimum level. A further increase in the protein level resulted in a decline in the growth rate of the animal. In the case of the postlarvae the best growth rate was obtained when they were fed with a diet having 30% protein, closely followed by the one with 40% protein. It was observed that the difference in the growth of the prawn when fed with diets having a protein concentration of 20, 40 or 50% is statistically not significant. Similarly the best growth of the juveniles of *M.rosenbergii* was obtained when fed with a diet with 30% protein followed closely by those with 40 and 20% protein. Here too, the difference between the latter two values are statistically not significant. These findings quite interestingly indicate that there is no significantly added advantage in feeding the postlarvae and juveniles of *M.rosenbergii* with diets having either 40 or 50% protein concentration over the one with 20% protein concentration. This observation is important from the view point of commercial aquaculture.

In the postlarvae and juveniles of the prawn protein levels below and above 30% resulted in reduction in growth. The former may be attributed to the insufficient protein for optimum amino acid synthesis to bring about maximum growth. It may also be related to the supra-optimum levels of carbohydrate present in these diets. Feeding with diet containing 40% carbohydrate has been reported to result in growth retardation in finfish by Shimeno *et al.* (1979). While the optimum dietary carbohydrate for the prawns remains near 20%, (Alava and Pascual, 1987) the carbohydrate levels in certain diets employed in the present experiment reached upto 70% which, especially in the presence of low protein concentration might have a deleterious effect on the growth of the prawn. Alava and Pascual (1987) have found a reduction in the growth of *P.monodon* when the dietary carbohydrate level exceeded 20%.

The observation that a dietary protein level of 40 or 50% resulted in decreased growth rate may be attributed to the presence of supra-optimum level of dietary protein and the availability of low non-protein dietary energy. Lovell (1973) indicated that diets with high levels of protein and low amount of non-protein energy might be toxic to channel catfish (*Ictalurus punctatus*). According to Wilson

(1989) if too much protein is supplied in the diet, only part of it will be used to make new proteins and the remainder will be converted to energy. Possibly the combination of high dietary protein in the presence of low non-protein energy may force the crustaceans to deaminate significant portion of the protein thus yielding the carbon fragments required for cellular energy metabolism. As a result of this altered metabolism, the animal could be expected to show reduced growth rates and efficiencies. Sedgwick (1979) and Bautista (1986) observed a higher non-protein energy need in the prawn at higher levels of dietary protein to attain approximately the same growth rate obtained at lower protein levels implying that when non-protein energy sources are limiting, protein is catabolized for energy production. Bages and Sloane (1981) also found the need for incorporation of enough carbohydrate to compensate for the high quantity of protein in the diet for *P.monodon*. This is further evident from the observation of increased rate of production of ammonia and consequent decline in the oxygen-nitrogen ratio observed in the postlarvae and juveniles of the prawn, when fed with 40 or 50% dietary protein concentration, in the present experiment (see 4.1.10). The decline in growth rate associated with an excess of dietary protein concentration may also be attributed to the increased cost of catabolism of protein for energy production. Hajra *et al.* (1986) observed steady increase in growth of *P.monodon* concomitant with an increase in total carbohydrate. An excess of dietary protein may increase the energy cost of assimilation by hiking the specific dynamic action (Le Grow and Beamish, 1986). Fenucii and Zein-Eldin (1976) also postulated the synergistic effect between dietary components affecting growth. Andrews *et al.* (1972) reported inclusion of 30% starch in diets with lower protein content increased growth rate in prawn. Clifford and Brick (1978) observed in *M.rosenbergii* that a fat-carbohydrate ratio of 1:3-1:4 resulted in a more efficient utilization of dietary protein than ratios of 1:1 or 1:2.

A similar trend in growth according to change in dietary protein density has been reported by many other investigators. Many of the experiments, the results of which are quoted in Table 2 showed that, with an increase in protein content in the diet, there was an increase in growth rate of the prawns, only for a part of the protein level tested beyond which the prawns exhibited only reduced growth rate. (Lee, 1971; Andrews *et al.*, 1972; Venkataramiah *et al.*, 1975b; Zein-Eldin and Corliss, 1976; Deshimaru and Yone, 1978b; Millikin *et al.*, 1980; Bages and Sloane, 1981; Alava and Lim, 1983; Antiporda, 1986; Bautista, 1986; Gopal, 1986; Ahamed Ali, 1988; D'Abramo and Reed, 1988; Freuchtenicht *et al.*, 1988).

The size of the juveniles of *M.rosenbergii* employed in the present study is similar to that used by Balazs and Ross (1976) and Millikin *et al.*(1980). Nevertheless, the protein concentration required for maximum growth is substantially less than the value reported by the above mentioned authors. The difference may be due to the difference in the experimental conditions and/or due to the difference in the dietary composition especially that of the amino acid pattern. Millikin *et al.* (1980) noted that optimum protein requirement will vary considerably with alterations of the dietary amino acid profile, variations of dietary supply of macro and micro nutrients other than protein and changes in selected environmental factors.

Growth rates of postlarvae and juveniles of *M.rosenbergii* obtained in the present study is difficult to compare with those of others because of the varieties of conditions, specified and unspecified, under which they were grown and the varieties of measurements used to describe growth. Growth rate is also related to the food consumption (Segal and Roe, 1975), apart from nutritional quality of food.

Prawns given a protein-free diet showed negative growth during the experimental period. The observation was true for both the postlarval and juvenile stages and the decline in body weights were 9.84 and 4.51% respectively. The loss of body weight of the prawn when fed with zero protein diet was obviously due to the use of body tissues for the essential maintenance nitrogen needs of the organism. Inadequate protein in the diet results in a reduction or cessation of growth and a loss of weight due to

withdrawal of protein from less vital tissues to maintain the functions of more vital tissues (Wilson 1989). This observation is in absolute agreement with that by Lee (1971) and Ahamed Ali (1988). Ahamed Ali (1988) observed a decline in body weight of 5.4% in live weight when *P.indicus* was fed with zero protein diet for 30 days. Cuzon *et al.* (1980) also found a statistically not significant decrease in body weight in *P.japonicus* starved for 28 days. Loss of body weight of protein-starved prawns were also reported by other investigators (Lee, 1971; Krishnamoorthy *et al.* 1982). The difference in the percentage reduction in the body weight in the present experiment compared to those obtained by the above mentioned authors may be related to the difference in the species and also to the difference in the initial body weight of the animals used in the studies. However, Gopal (1986) observed growth in shrimps provided with a protein-free diet which he attributed to the cannibalism by the co-habiting shrimps.

In agreement with the present observation, among finfishes Pfeffer *et al.* (1977) reported loss of body weight when subjected to protein starvation. However, the present observation is not in agreement with those by Ogino *et al.* (1976), Sen *et al.* (1978) and Kiron (1988) in finfishes. These authors found a slight increment in weight of the fish when fed with zero protein diet and attributed the weight gain to the deposition of lipids in the body which is mainly derived from carbohydrate metabolism. But it must also be pointed out that in the studies by the above mentioned nutritionists since frequency of water exchange was limited, the animals could have had access to extraneous nutritional supply, may be in the form of unicellular organisms such as algae and bacteria which might have, though on a limited scale, satisfied the protein need of the organism. However, in the present experiment, since rearing water was exchanged almost completely everyday the possibility of such an extraneous protein supply was insignificant, if not absent.

The observation in the present experiment that prawn loses weight when subjected to protein starvation should be of interest to the prawn farmer since it allows him to calculate the "price" in terms of body weight to be paid for a period of protein starvation.

As must be expected, the absolute quantity of feed consumed by the postlarvae and juveniles of the prawn increased with an increase in the size of the prawn. However it may be observed that the quantity of food consumed by unit weight of the prawn per day remained not significantly influenced by the dietary protein concentration. This observation is true for both the postlarvae and juveniles of the prawn, under the present experimental conditions. Further, the mean food intake (g) per unit weight of the prawn (g) per day was found to be relatively high for the postlarvae when compared to the juveniles. Deshimaru and Shigueno (1972) reported similar observation in *P.japonicus*. Sinha (1979) also reported a decrease in the rate of food consumption with size in the carp. It is also known that optimum feeding rates decrease as the fish grow (Moore *et al.*, 1988). The relatively higher food intake per unit weight of the postlarvae than that of the juveniles may be attributed to the higher metabolic activity of the former compared to the latter. Since the energy requirement of an animal is directly proportional to its metabolic activity the feed intake will be at gradually declining rates with increasing size (Tacon, 1987a).

The observation that food consumption remains not significantly influenced by the dietary protein concentration in the postlarvae and juveniles of the prawn corroborates the fact that prawns, like other animals feed to satiate the energy needs. In that case, it is the total available energy content of the diet that determines the amount of feed consumed by an organism and not the protein content. It may be noted that the diets employed in the present experiment contain similar metabolic energy concentrations, though the ratio of protein to energy in them varies. Calorie concentration of diet plays a major role in affecting the amount of food eaten by a fish whereas dietary protein level is not important unless excessively high (Sinha, 1979). De la Higuera et al. (1989) reported that dietary protein had no significant influence on the dietary food intake.

observations quite in contrast to the above were reported by a few investigators.In *P.japonicus* Deshimaru and Yone (1978b) noted a decrease in daily food intake with an increase in dietary protein level.Gopal (1986) found the highest specific food consumption in *P.indicus* when provided with zero protein diet followed by 10% protein diet. He obtained the lowest specific food consumption when provided with 40% protein diet. A further increment in dietary protein level resulted in a slight increment in specific food consumption. On the otherhand a tendency of increasing percentage daily food intake with increasing protein level in the diet was observed by Balazs and Ross (1976). This observation was attributed to a better palatability of diets with higher protein concentration or to the decreasing content of carbohydrate. It has been postulated that the higher percentage of carbohydrate in lower protein diets affect the appetite control centres as happens in mammals and a few other fish species. According to Martin and Gallego (1987) amino acids are the most important chemical signals for appetite centres.

The food conversion efficiency increased with increase in the dietary protein concentration upto 40% in the postlarvae and upto 30% in the juveniles of the prawn. In both the cases, the food conversion efficiency value obtained when the prawns were fed with 30% protein diet was similar to that obtained when they were provided with 40% protein diet. However, in the postlarvae and juveniles of the prawn a further increase in the dietary protein concentration to 50% resulted in a significant decline in food conversion efficiency. Similar observation of increase in food conversion efficiency with increase in protein level only upto the optimum level of dietary protein was made by Colvin and Brand (1977) in *P.californiensis*, *P.stylirostris* and *P.vannamei*. In the latter two species, the decrease in food conversion efficiency when the protein level increased beyond the optimum level was not statistically significant. Deshimaru and Shigueno (1972) also observed an increase in food conversion efficiency with an increase in dietary protein concentration. Similar trends in improvement of food conversion efficiency with an increase in dietary protein concentration upto a certain level and a decline in food conversion efficiency when the dietary protein concentration increased further were also reported in *P.aztecus* by Venkataramiah *et al.* (1975b), in *P.japonicus* by Deshimaru and Yone (1978b), in *P.monodon* by Alava and Lim (1983) and in *P.indicus* by Gopal (1986) and Ahamed Ali (1988).

The observation that sub-optimum level of protein resulted in a decline in food conversion efficiency may be attributed to the insufficient supply of protein to obtain optimum growth in the animal. The amount of palatable formulations eaten by a prawn is likely to increase if the diet is nutritionally poor (Grajeer and Neal, 1972) and high feed intake does not necessarily bring about rapid growth (Deshimaru and Shigueno, 1972). Deshimaru and Shigueno (1972) found that the feed conversion efficiency in general increased as the proportion of crude protein in the diet increased. The observed lower food conversion efficiency of the postlarvae and juveniles of *M.rosenbergii* when provided with sub-optimum dietary protein concentration may also be attributed to the higher carbohydrate content of these diets. Shimeno *et al.* (1979) observed that high levels of carbohydrate in the diet had a negative effect on food conversion efficiency. The poorer food conversion efficiency of *M.rosenbergii* at lower dietary protein level may also be partly related to the apparently lower protein digestibility at lower dietary protein levels, as observed in the present experiment.

The decline in the food conversion efficiency corresponding to supra-optimum dietary protein concentration may be attributed to the increase in the metabolic cost associated with the catabolism of the excess protein and to the altered cellular metabolism itself. The presence of sub-optimum level of dietary carbohydrate might also have contributed to the decline in food conversion efficiency. Supplementation of dietary carbohydrate has been reported to improve the food conversion efficiency in *P.indicus* (Ahamed Ali, 1982).

Food utilization, expressed as food conversion efficiency is known to be affected by a number of factors which include body weight (Pandian, 1967), ration size (Condrey, 1982), temperature

(Menzel, 1958) and salinity (Kinne, 1960; De Silva and Perera, 1976). Generally food utilization becomes less efficient with increasing weight (De Silva, *et al.*, 1989); when the prawn is small in size higher efficiency of feeding is obtained with a given amount of food. Accordingly when the culture period is prolonged, the feed efficiency throughout the period tends to be lowered. In the present experiment, however, the food conversion efficiency of the postlarvae remained more or less unchanged during the course of the experiment. On the other hand, the food conversion efficiency of the juveniles decreased as they grew. The general trend for food utilization to decrease with increasing body weight is to be expected because metabolic processes are known to diminish and energy expenditure per unit body weight declines with increasing body weight and/or age and as such will be reflected in the food conversion efficiency. The best food conversion efficiency exhibited by the postlarvae and juveniles in the present experiment is comparable to the values reported by Shang and Fujimura (1977), Boonyaratpalin and New (1982) and Gomez *et al.* (1988) in the juveniles of the prawn provided with optimum dietary protein concentration. However, these values are slightly lower than the food conversion values reported by Balazs and Ross (1976), Roberts and Bauer (1978), Millikin *et al.* (1980), Smith and Sandifer (1980) and Perry and Tarver (1987) which may be attributed to the difference in the nutritional composition of the respective diets and the rearing conditions. The food conversion values reported by Roberts and Bauer (1978), Smith and Sandifer (1980) and Perry and Tarver (1987) are for the pond rearing trials employing compounded rations where the prawns had unlimited supply of natural shrimp food organisms.

Data on food conversion ratios of various species of penaeid prawns in response to various dietary formulations have been published by Andrews *et al.* (1972), Venkataramiah *et al.* (1975b), Colvin (1976), Royan *et al.* (1977), Aquacop (1978), Fenucci *et al.* (1980), Goswami and Goswami (1982), Alava and Lim (1983), Gopal (1986), Ahamed Ali (1988) and Jaenike (1989). However, the results of these experiments cannot directly be compared to the present results due to the difference in the composition of the diets, species, size of animals and other environmental parameters involved, each of which has a varying effect on the food conversion efficiency (Menzel, 1958; Kinne, 1960; Pandian, 1967; Hysmith *et al.*, 1972; De Silva and Perera, 1976; New, 1976a; Condrey, 1982; Goswami and Goswami, 1982; De Silva *et al.*, 1989).

The apparent digestibility of protein in the postlarvae of the prawn increased from 77.50 to 88.83% when the dietary protein concentration increased from 10 to 50%. An increase in the apparent protein digestibility was observed in the juveniles also, the values being 85.27 and 95.51% for the 10 and 50% dietary protein concentrations respectively. A similar trend of increasing apparent digestibility coefficients with increasing dietary protein concentration was observed by Nose (1963, 1964); Lee (1970); Page and Andrews (1973); Wee and Tacon (1982) and Kiron (1988). According to Kiron (1988) the low apparent digestibility of protein at lower protein levels might be due to the higher carbohydrate levels in these diets. The studies by Shimeno *et al.* (1979) have shown that high levels of carbohydrate had a negative effect on protein digestibility. Akiyama *et al.* (1991) opined that diets with lower protein and higher carbohydrate result in lowering of apparent digestibility of protein. However, the variation in apparent digestibility in the present experiment may, atleast partly, be related to the lower level of inclusion of metabolic faecal nitrogen whose amount is influenced by the amount of food ingested rather than the composition of the food (Hepher, 1988). Nose (1967) discussed this topic in detail.

The true digestibility of protein by the postlarvae and juveniles of the prawn was found to be little influenced by the dietary level of protein. This observation was in confirmation with that of Ahamed Ali (1988) who observed in the juveniles of *P.indicus* that the influence of protein levels on the true digestibility of protein was statistically not significant. Similar observation was also made by Nose (1963) and Ogino and Chen (1973).

However, in the present experiments it may be noted that the true digestibility, though not significantly affected by the level of protein, shows a general trend to increase with an increase in the level of protein. This may be attributed to the increased secretion of protease enzymes at increased

protein concentration of the diets. Yonge (1937) suggested that dietary composition has a direct effect on digestive enzyme activities in invertebrates. Grossman *et al.* (1943), Kawai and Ikeda (1972), Mukhopadhyaya *et al.* (1978) and Steffens (1981) also reported a positive relationship between dietary protein concentration and protease activities. Prosser and Van wheel (1958) and Van wheel (1959) reported that a continued high protein diet caused a suppression of proteolytic activity in the giant African snail (*Achitina fulica*). Lee *et al.* (1980) indicated a surplus production of protease relative to low dietary concentration of protein in *M.rosenbergii.*

Between postlarvae and juveniles of the prawn, it was observed, in general, that the protein digestion efficiency is less in the former. Hysmith *et al.* (1972) reported differences in the ability of *P.aztecus* to utilize a 45% protein diet, dependent on shrimp size. The dependence of protein utilization on fish size and other factors was shown by De Silva *et al.* (1989) and was attributed to lower enzyme action in smaller fish. This topic was reviewed in detail by Steffens (1981).

Studies on the digestibility of protein of various sources by various species of crustaceans are many (Forster and Gabbott, 1971; Forster, 1972,1976; Mason and Castell, 1980; Ashmore *et al.*, 1985; Gopal, 1986; Ahamed Ali, 1988; Akiyama *et al.*, 1988). Principally due to the difference in the sources of protein and other dietary nutrients, comparison of the results of these studies with those of the present study is not possible.

Further, the relatively high efficiency of digestion of *M.rosenbergii* compared to many penaeid species may be related to its true omnivorous feeding habit. Sather (1969) while investigating the amylases and proteinases of decapods discovered a higher protease activity in omnivores than in carnivores which might be explained on the basis of enzyme kinetics (Lee, *et al.*, 1980).

The amount of nitrogen excreted by unit weight of the postlarvae and juveniles is found to be influenced by the dietary level of protein. A high positive correlation between the dietary protein concentration and the nitrogen excretion (mg N/100g diet) was observed in the case of the postlarvae and juveniles of the prawn, in the present experiment. It indicates an increase in the level of catabolism of protein with an increase in dietary protein concentration. Millikin and Sick (1980) and Millikin *et al.* (1980) observed higher faecal nitrogen in prawns fed a 49% protein diet compared to those with 23, 32 and 40%. Further, the amount of faecal nitrogen excreted per 100g of diet consumed by the postlarvae was found to be higher compared to that by the juveniles. Atleast partly this observation can be attributed to the high inclusion level of metabolic faecal nitrogen and the less efficiency of protein digestion in the postlarvae compared to the juveniles.

The metabolic faecal nitrogen of the postlarvae and juveniles of the prawn found out by the direct faecal collection methods were 198mg N/100g diet and 151mg N/100g diet respectively. However the corresponding values obtained by the extrapolation of the graph between dietary protein concentration and the corresponding nitrogen excretion were found to be 217.66mg N/100g diet and 212.31mg N/100g diet respectively. These values are 9.93 and 40.93% higher than the respective values of metabolic faecal nitrogen obtained by employing the direct faeces collection method, in the case of the postlarvae and juveniles of the prawn respectively. The variation in the values of metabolic faecal nitrogen between the two techniques may be related to the difference in the methodology employed. In shrimps, dietary protein is not fully utilized for the synthesis of the tissues. The unutilized fraction of dietary nitrogen is excreted alongwith that originating from the recycled tissues. Metabolic faecal nitrogen excreted by shrimp fed a protein-free diet is therefore lower than that excreted by shrimp fed protein diets. Moreover, since with the consumption of protein there is an increased secretion of digestive enzymes and loss of intestinal epithelial cells excreted metabolically derived nitrogen measured on protein starved shrimps will be lower than that measured on protein fed shrimp. Yonge (1937), Grossman *et al.* (1943), Kawai and Ikeda (1972), Mukhopadhyaya *et al.* (1978) and Steffens (1981) observed a positive relationship

between dietary protein concentration and protease activities. Such variation in the matobolic faecal nitrogen values was obtained by Forster and Gabbott (1971) in *P.serratus* when two different methods were employed viz., direct faeces collection method and the regression method. The value obtained when the former method was used was approximately 472 % higher than that obtained using the latter method. Nose (1967) also observed difference in the value of metabolic faecal nitrogen when the two methods were used.

Studies on the faecal nitrogen excretion and the metabolic faecal nitrogen are few in shrimps and prawns. The only earlier attempt to determine the metabolic faecal nitrogen of a palaemonid prawn was that by Forster and Gabbott (1971) who reported a value of 185.2mg N/100g diet in *P.serratus*. Among penaeid prawns Ahamed Ali (1988) reported a metabolic faecal nitrogen value of 326.4mg N/100g diet in *P.indicus*. The difference in the metabolic faecal niotrogen between *M.rosenbergii* and *P.indicus* may be related to the difference in the species and to the feeding habits. *M.rosenbergii* is a true omnivore (John, 1957; Ling, 1969; Lee *et al.*, 1980). Omnivores are reported to economize their protein need by reabsorbing digestive enzymes and by reducing protein metabolites (Pandian, 1989). Studies on the metabolic faecal nitrogen of finfishes are many (Nose, 1967; Ogino *et al.*, 1973; Kiron, 1988). It may be observed that the metabolically derived nitrogen in the faeces is relatively more in prawns compared to finfishes. This may be due to the fact that, in addition to the residues of the digestive juices, epithelial cells abraded from the walls of the alimentary tract and bacterial residues (Maynard and Loosli, 1969) the metabolic faecal nitrogen in prawns would be contributed by the secretion of a chitinous peritrophic membrane around the faecal pellets too (Forster, 1953) and it is possible that this might result in quite large losses of nitrogen (Forster and Gabbott, 1971).

While the assessment of metabolic faecal nitrogen can throw some light on the potential contamination of faeces, and hence on the estimation of digestibility, it is quantitatively negligible and will bear little influence in nitrogen balance studies under normal feeding conditions (Forster and Gabbott, 1971 and Kaushik, 1989). It may be pointed out that the difference between true digestibility and apparent digestibility of protein will be more marked only when protein intake is low.

Carcass composition of the postlarvae and juveniles of *M.rosenbergii* was found to be little influenced by the dietary protein concentration. However among the different biochemical components, body protein showed a more apparent difference in response to the changes in dietary protein concentration. Protein exclusion from the diet resulted in a decline in the percentage of prawn carcass protein, the respective values being 52.83 and 53.39% for the postlarvae and juveniles of *M.rosenbergii*. The highest percentage protein in the body was obtained when provided with 40 and 50% dietary protein concentration in the case of the postlarvae and juveniles respectively. However, the variation in the body carcass protein in response to the variation in the dietary protein concentration was found to be statistically not significant.

Alava and Lim (1983) found an increase in carcass protein corresponding to an increase in dietary protein level upto 50% in *P.monodon*. A further increase in dietary protein was found to result in a statistically not significant decrease in the body protein. However, these authors could observe no significant differences in the body fat of shrimps fed graded levels of proteins. Gopal (1986) found the biochemical composition of *P.indicus* to be significantly influenced by the dietary protein concentration. Mason and Castell (1980) observed no significant effect of dietary protein concentration on the carcass composition of *H.americanus*.

A parabolic relationship exists betwen the nitrogen retention (mg N/100g body weight/day) and the digestible nitrogen intake (mg N/100g body weight/day) in the postlarvae and juveniles of *M.rosenbergii*. The relationship between the digestible nitrogen intake and the nitrogen retention can be explained by the equations $Y = -24.31 + 0.2373x - 0.0001506x^2$ and $Y = -15.203 + 0.2757x$

$-0.00026248x^2$ in the postlarvae and juveniles of the prawn respectively. It indicates that the more nitrogen is consumed the more is retained only upto a certain optimum level, beyond which the nitrogen retention diminishes. These values were found to be 787.8 and 525.1mg N/100g body weight/day in the case of the postlarvae and juveniles respectively.

The above mentioned findings are of immense commercial application in that, it gives an idea about the absolute quantitative protein requirement to result in maximum body nitrogen retention in the early life stages of *M.rosenbergii*. It may serve as a guideline for the determination of the quantity of proteins from different sources to be incorporated in the commercial diets for the postlarvae and juveniles of the freshwater prawn provided, their digestibility is known. For instance assuming a true digestibility of 90.30 and 96.41% for the casein-amino acid based diet for the postlarvae and juveniles (as obtained in the present experiment), it can be safely deduced that 872.43 and 544.65mg N/100g body weight should be incorporated in the diet for the postlarvae and juveniles respectively. Studies on the protein requirement of prawns in absolute quantitative terms are scarce. The common expresssion by nutritionists of nutrient requirement solely in terms of a dietary percentage has itself limited value unless it is related to the food intake and subsequent growth of the animal (Tacon and Cowey, 1985). It is necessary to express dietary nutrient requirements in terms of weight of nutrient required per 100g body weight per day. Tacon and Cowey (1985) presented the protein requirement of the various fish species examined as grams of protein required per Kilogram body weight per day which varies from 7.5 to 52.5g/kg body weight (viz., 120-840mg N/100g diet). A direct comparison of the results of the present experiment with the values presented by Tacon and Cowey (1985) is not possible, unless the true digestibility factor is also taken into account. However, it may broadly be taken that the absolute quantity of protein required by the early life stages of *M.rosenbergii* is not far dissimilar to that of the finfishes.

The maintenance nitrogen requirement (= endogenous nitrogen excretion) of the postlarvae and juveniles of *M.rosenbergii* based on the difference in the carcass nitrogen before and after feeding with a zero protein diet was found to be 17.95 and 18.69mg N/100g body weight/day. It assumes a 100% assimilation of protein by the animals. The values obtained from the regression equations for the postlarvae ($Y = -24.31 + 0.2373x - 0.0001506x^2$ and juveniles ($Y = -15.203 + 0.2757x - 0.00026248x^2$) were 24.31 and 15.203mg N/100g body weight. Owing obviously to the lack of published literature on the maintenance nitrogen requirement of prawns, a comparison of the result of the present experiment is not possible. The only investigation on the maintenance requirement of prawns seems to be that by Ahamed Ali (1988) who reported the maintenance requirement of juveniles of *P.indicus* as 22.5%. He obtained the value by the statistical method of regressing the dietary protein level (percentage) to the nitrogen balance. The nitrogen balance was calculated as the difference in the nitrogen of the diet and the nitrogen in the faeces. However, nitrogen intake value to maintain nitrogen equilibrium obtained by Ahamed Ali (1988) seems to be over estimated especially in view of the fact that the same author found a protein level of 25% to bring about maximum growth in the prawn, in the same study. Moreover, regressing the percentage level of dietary protein against the nitrogen balance (difference in the nitrogen of the diet and the nitrogen in the faeces) to arrive at the maintenance nitrogen requirement is rather erroneous. A better method would have been regressing the digestible nitrogen intake (mg N/kg body weight/day) against nitrogen balance (mg N/kg body weight/day).

Even though many investigators have studied endogenous excretion losses and nitrogen retention in a variety of finfishes, very few investigators have actually determined the maintenance requirement of protein using either purified or semi purified diets (Ogino and Chen, 1973; Kaushik *et al.*, 1981; Gatlin *et al.*, 1986; Kiron, 1988).

In the present study the endogenous nitrogen excretion of the postlarvae was found to be slightly higher than that of the juveniles. It may be explanined as due to the small average weight of the prawns in the former case, or due to the difference in the conditions under which the studies were conducted. Ehrlich (1974) and Dabrowski (1977) have reported higher values of endogenous nitrogen excretion in fishes having smaller sizes compared to larger ones and Dabrowski (1977) related it to the difference in body weight. Further, the endogenous nitrogen excretion was found to be sensitive to many factors including water temparature (Savitz, 1969; Ogino *et al.*, 1973).

The values of the endogenous nitrogen excretion obtained in the present study is slightly higher than the values reported in finfishes by many investigators (Nose, 1961; Ogino, *et al.*, 1973; Jauncey, 1982; Kiron, 1988). The raltively higher endogenous nitrogen excretion value for prawns may be due to the relatively higher level of metabolically derived faecal nitrogen in prawns.

Primarily due to the fact that the present study is a mass rearing experiment where the chances of feeding of the dead prawns by the survived ones cannot totally be ruled out, the values of endogenous nitrogen excretion (= maintenance nitrogen) obtained must be taken more as a practical figure rather than a precise theoretical one, for the determination of which data on individually housed animals under closely monitored experimental condition is needed. Obviously due to many practical problems, many aqua-nutritionists have employed the above mentioned procedure in studies with their animals (eg., Ahamed Ali, 1988; Kiron, 1988). Studies on maintenance nitrogen requirement viz., nitrogen intake needed to maintain nitrogen equilibrium, of the prawns have many practical applications in commercial aquafarming especially when stunting of growth of prawns is desired possibly to exploit the relatively faster growth phase of the post-stunting period or in hatcheries where large number of seeds have to be kept under 'no growth condition' due to practical space limitations. Periodic fasting practices of prawns have been reported to improve the growth of the shrimp (Lee *et al.*, 1974). Under such circumstances the prawns have to be fed with a low protein ration, which result in no appreciable gain in body weight but, ideally, in higher survival rate. The results of the present investigation indicate that for *M.rosenbergii* seeds, under the above mentioned conditions, a ration to supply 15-25mg N/100g body weight/day would suffice the requirement. However, in practical rations the digestibility of the source of protein employed, the bio-availability of nutrients, the influence of the water quality parameters etc. must also be taken into account. The loss of unutilized portion of the dietary protein is unavoidable and the utilization efficiency of the protein must be considered while calculating the maintenance nitrogen requirement (Hepher, 1988).

Protein efficiency ratio was found to decrease with increase in dietary protein concentration. The change in PER holds a linear relationship with that of dietary protein concentration. Dietary protein has been reported to affect the protein utilization efficiency differently in different species of crustaceans. Millikin *et al.* (1980) and Boonyaratpalin and New (1982) reported an inverse relationship between the dietary protein level and the PER in *M.rosenbergii*. In *P.indicus* Colvin (1976) found a decline in the efficiency of protein utilization (PER) with successive increase in the dietary protein level. Hajra *et al.* (1986) observed a steady increase in PER value concomittant with increase in total carbobydrate in the diet. Ahamed Ali (1988) observed higher efficiency of protein utilization in *P.indicus* at lower dietary protein levels.The efficiency decreased as the protein level increased. However, Ahamed Ali (1982) noticed the highest PER values at intermediate level of protein tested (20.5%) in the same prawn. In *P.monodon* Alava and Lim (1983) observed an increase in PER with an increase in dietary protein level upto 40%. When the protein level increased further the PER value decreased and reached the minimum corresponding to a dietary protein level of 60%. Gopal (1986) also observed an increase in PER when the dietary protein concentration increased upto 30% in *P.indicus*. A further increase in dietary protein resulted in a decrease in PER and the lowest value was obtained when the ptotein level reached 60%.

In the present experiment, the decrease in PER with an increase in the dietary protein may be related to the lower starch content of the high protein diets. Clifford (1979) studied the metabolic responses of juveniles of *M.rosenbergii* to various levels of dietary protein and concluded that higher carbohydrate levels resulted in a greater efficiency of protein utilization. Similarly Gomez *et al.* (1988) observed in the juveniles of *M.rosenbergii* that the PER increased with the higher dietary starch content suggesting that dietary protein is spared by supplementation of starch. In *P.monodon* juveniles, protein utilization improved as the available energy level in the diet increased (Bautista, 1986). Sedgwick (1979) found that optimal utilization of protein by *P.merguiensis* was closely related to the energetic value of the diet and that carbohydrate and lipid can also increase growth efficiency at sub-optimum level of protein. The efficiency by which protein is assimilated by prawn is most likely affected by the relative proportion of lipids and carbohydrates in the diet as well as the amino acid composition of the protein source employed (Hanson and Goodwin, 1977). Insufficient non-protein energy in the diet can lead to catabolism of dietary protein for energy in crustacea (Cupuzzo and Lancaster, 1979; Cupazzo, 1982) and hence lower protein efficiency ratio. In the juveniles of *P.monodon* Bautista (1986) found an improvement in protein utilization with an increment in the available dietary energy level.

The oxygen-nitrogen ratio of the postlarvae and juveniles of *M.rosenbergii* was found to be significantly influenced by the dietary protein concentration. The highest value for oxygen-nitrogen ratio was obtained when the postlarvae and juveniles were provided with a diet having 0% protein. The values were found to decrease as the dietary protein level increased progressively, indicating higher rate of catabolism of protein for energy purpose. Excretion of higher levels of nitrogen corresponding to higher levels of dietary nitrogen intake has already been discussed else where.

The oxygen-nitrogen ratio has been widely employed as a means of defining the nutritional status and metabolic equilibrium of various marine and terrestrial organisms (Corner and Newell, 1967; Conover and Corner, 1968; Snow and Williams, 1971; Wieser, 1972; Mayzaud and Dallot, 1973). From measurement of ammonia excretion and oxygen uptake it is possible to estimate the contribution of amino acid catabolism to energy production under aerobic conditions (Walton, 1985). Laxminarayana and Kutty (1982) reported a reduced oxygen consumption and increased ammonia excretion rate in prawns under stress condition. Venugopalan (1988) determined the oxygen-nitrogen ratio of the juveniles of *M.rosenbergii* reared at different salinity conditions. Gopal (1986) is of the openion that ammonia excretion rate is an indication of efficiency of protein metabolism in prawns. Measurement of the oxygen-nitrogen ratio of fed animals enables deductions to be made concerning the class of nutrients utilized as substrates in oxidative metabolism. Conover and Corner (1968) state that the catabolism of pure protein would result in an oxygen-nitrogen ratio of approximately 8, whereas the oxygen-nitrogen ratio from lipid or carbohydrate catabolism would approach infinity after corrections for endogenous nitrogen excretion. Complete starvation of a nutritionally stable animal will produce a downward trend in oxygen-nitrogen ratio as fat reserves are exhausted and utilization of stored protein increased. However, in the present experiment, since carbohydrate and lipid are available sufficiently in the protein-free diet the oxygen nitrogen ratio remained high. Clifford and Brick (1978) found oxygen-nitrogen ratio of *M.rosenbergii* to be inversely correlated to dietary protein levels. Millikin *et al.* (1980) found oxygen-nitrogen ratio not to be significantly affected when *M.rosenbergii* was fed with diets with protein levels varying from 23-49%. However, these authors found an apparently high oxygen-nitrogen value when the prawn were provided with low protein diet compared to high protein diet. Sick (1976) found the larvae of *M.rosenbergii* to show an oxygen-nitrogen ratio of 8 or lower for diets with 20% or less in total protein content indicating the inadequacy of dietary protein or poor amino acid balance. The oxygen-nitrogen ratio obtained in the present experiment is comparable to the values reported by other investigators (Clifford and Brick, 1978; Millikin *et al.*, 1980; Venugopalan, 1988) in this species but less than the values reported by Clifford and Brick (1983) in starved *M.rosenbergii*. Although precise theoretical calculation of oxygen-nitrogen ratios are possible the practical ap-

plicability by this index is at best qualitative in that it is a useful tool for qualitative description especially when used in conjunction with other indices of nutrient utilization. The relative contribution of the various nutrients to energy production are dependent on a number of factors including species, nutritional state, temperature etc. (Walton, 1985). Amino acid in excess of the requirements for the formation of structrural tissue proteins, enzymes, hormones etc. are deaminated to form ammonia and keto acids, thereby reducing the oxygen-nitrogen value.

On the basis of the growth and survival data and the data on the various nutritional indices it could be assumed that the protein requirement of the postlarvae and juveniles of *M.rosenbergii* lies close to 30%. In order to precisely ascertain the requirement values, second order polynomial regression equations were established between the dietary protein levels tested and the mean *IPGU*, on the basis of which the dietary protein concentrations which result in maximum growth have been found to be 34.5 and 29.5% respectively for the postlarvae and juveniles. Such a relationship between the dietary protein concentrations and the food conversion efficiency values was also established and the respective protein concentration to result in the best food conversion efficiency was found to be 34.02 and 28.59% .From the observations it could be safely deduced that the protein requirement for maximum performance of the early postlarvae lie between 34 and 35%, under the present experimental conditions. For the juveniles the requirement value is between 28 and 30%. It is emphasised here that the protein requirement values indicated above are on a dry weight basis. On an as-fed basis the protein requirement of the postlarvae and juveniles of *M.rosenbergii* lie between 30.6 and 31.5% and 25.2 and 27.0% respectively, considering a moisture content of 10% in the diet. These values lie within the protein requirement values reported for the species by earlier workers. Studies of Balazs and Ross (1976) indicated that a protein concentration of more than 35% is required to maintain adequate growth in the juveniles of *M.rosenbergii*. These authors employed a combination of soybean, tuna and shrimp meal as the protein source. Manik (1976) and Perry *et al.*(1984) reported a protein requirement value of 25% for *M.rosenbergii*. Clifford and Brick (1979) concluded that optimum conditions for the growth of *M.rosenbergii* were achieved with a 25% dietary protein level. The crude protein level of commercial diets being used in prawn farms in Hawai varied from 23.8 to 38.5% (Corbin *et al.,* 1983); though lower level may be satisfactory (New, 1988). Millikin *et al.* (1980) employing menhaden meal and soybean protein found a dietary protein requirement of 40% for the juveniles of *M.rosenbergii.* According to Sick and Millikin (1983) the protein requirement of early juvenile *M.rosenbergii* lies around 40% whereas that of larger prawn around 25-30%. In a pond rearing experiment where the animals could have unlimited access to natural food organisms, Boonyaratpalin and New (1982) found a dietary protein level of 15% to be satisfactory for the juvenile *M.rosenbergii.* Antiporda (1986) indicated a protein level of 14% to result in favourable growth in the prawn. Stahl (1979) had postulated that the earthern substrate in ponds appeared to supply a major growth factor lacking in the feeds applied in a simulated pond experiment. Employing casein based semipurified diet Gomez *et al.* (1988) found a protein level of 13.0-25.0% to bring about maximum growth in the juveniles of *M.rosenbergii.* However, studies using purified crab protein (D'Abramo and Reed, 1988) and, in aquaria (Freuchtenicht *et al.*, 1988) indicated optimum dietary protein levels of 33-35% and 30% respectively.

No studies on the dietary protein requirement of the early postlarvae of *M.rosenbergii* have been published. Sick (1976) reported a protein requirement of 15% for the larvae of *M.rosenbergii.*

Evidence to the effect that shrimp of different ages require different dietary protein levels is also gained in the present experiment. The observation may be attributed to the relatively faster growth rate of the early postlarvae compared to the juveniles. Similar observations on the higher protein requirement of the faster growing early life stages compared to the later stages have been reported by many investigators in various species of shrimps and prawns (Balazs *et al.*,1974; Colvin and Brand, 1977; Deshimaru and Yone, 1978b; Farmanfarmaian and Lauterio, 1979; Khannapa, 1979; Sedgwick, 1979;

Millikin *et al.*, 1980; Sick and Millikin, 1983 and Bhasker and Ahamed Ali, 1984). The practice of using relatively high protein diets for the rearing of early postlarval stages is very common in commercial shrimp culture (see Chiu, 1988; Hirano, 1989; Jaenike, 1989; Chen, 1990 and Akiyama *et al.*, 1991). Generally as the animals increase in size, protein requirement decreases relative to energy needs (Maynard and Loosli, 1969).

It could be seen that the protein requirement of the various species of penaeid prawns varies from 25-60% (Table 2). However, due to variations in the species, their feeding habits, the dietary protein sources employed and the conditions under which the experiments were conducted, a direct comparison of the results of those studies with the present one is difficult. Nevertheless, it may be mentioned that a protein requirement value close to 40% has been generally considered to be satisfactory for most of the penaeid shrimps except for *P.japonicus*. Most of the commercial diets available in the market meant for intensive cultivation of penaeid prawns have a protein concentration of 35-45% depending on the age of the shrimp.

These indicate that the dietary protein requirement of *M.rosenbergii* is relatively less compared to penaeid prawns. New (1988) and Pandian (1989) also made a similar conclusion by analysing the published protein requirement values for maximum growth in *Macrobrachium* spp. and penaeid species. The latter author attributed this to the faster growth potential and the lower food conversion efficiency of penaeid prawns.

Various techniques are employed to ascertain the protein need of animals. These include the analysis of variance, the broken line model, and the second order polynomial regression analysis (De Long *et al.*, 1958; Zeitoun *et al.*, 1973, 1976; Cowey, 1979; Anderson *et al.*, 1981). However, the polynomial regression method has some advantages over the other two methods. This method describes the relationship between protein concentration and dietary efficiency in a curvilinear fashion, which is more appropriate than the other methods (Cowey *et al.*, 1972). Polynomial regression analysis method has been employed by many nutritionists to determine the nutrient requirement for maximum growth in finfishes (Zeitoun *et al.*, 1976; Kiron, 1988; Moore *et al.*, 1988 and De Silva *et al.*, 1989. But its use in shrimp nutrition studies is not known. The dose response curve method described by Cowey *et al.* (1972) has also been used to determine the protein requirement of fishes.

The protein requirement value obtained in the present experiments and also those reported by many other authors must be taken as the dietary level to elicit maximum growth and the best performance. One of the fundamental aims of aquaculture is the evolution of commercial diets leading to the cost effective production of aquatic animals. The fact that the optimum dietary protein for a species may not be the most economic to use (New, 1987) necessitates the introduction of economic parameters in the protein requirement studies to evolve an 'economic protein requirement' viz., the dietary protein concentration which minimize the costs while maintaining adequate growth as suggested by Zeitoun *et al.* (1976). As such, the economic protein requirement of the postlarvae and juveniles of *M.rosenbergii* was found to be 27.5 and 23.0% (dry weight basis) respectively. It indicates that a dietary protein concentration of 27.5 and 23.0% can bring about economically acceptable growth in the postlarvae and juveniles, respectively of *M.rosenbergii*.

No reports on the economic protein requirement of *M.rosenbergii* or penaeid prawns are known. However, the studies by Boonyaratpalin and New (1982) takes into account the economic criteria in recommending the protein requirement value for *M.rosenbergii*.

Future attempts to establish protein requirement of prawns and shrimps must preferably be directed from three different angles viz., dietary protein requirement for maintenance, dietary protein requirement for growth maximization and the economic dietary protein requirements. These, if

expressed in relative and absolute quantitative terms, will provide data from a purely theoretical and practical aquafarming point of view.

Since, in addition to the variation in the dietary protein density, the diets used in the present investigation also vary in protein to energy ratio the results may be viewed from a different angle also. It may be noted that corresponding to the gradual change in protein (mg)-energy (k cal) ratio, from 23.27 to 107.71 the survival, growth rate and food conversion efficiency indices also varied considerably. An increase in the protein-energy ratio upto 65.84 improved the survival, growth and food conversion efficiency in the postlarvae and juveniles of the prawn. However a protein-energy ratio beyond 84.38 in the case of the postlarvae and 65.84 in the case of the juveniles resulted in a decline in the growth rate.

In common with other animals, shrimps eat to meet their energy requirements. The balance of energy components in diets is important since deficiency or excess of energy can result in reduced growth. When excess energy is supplied appetite or demand may be satisfied before a sufficient quantity of protein required for growth is ingested. Influence of changes of dietary protein-energy ratios on growth and protein utilization has been demonstrated in several species of finfishes (Lee and Putnam, 1973; Takeuchi *et al.*, 1978). The digestible energy requirement per unit of protein for warmwater fish ranges from 8.0-9.5 k cal per gram of protein (NRC, 1983) which is equivalent to a protein-energy ratio of 105-125mg/k cal. Alava and Lim (1983) reported that 30-40% protein wih energy levels ranging from 357-375 k cal/100g diet can maintain satisfactory resistance against moulting in prawns. However, protein-energy ratio has not been determined for any shrimp species (Akiyama *et al.*, 1991). This value would be dependent on "determined" digestible energy values for feed ingredients. Given that animals do not necessarily require protein, digestible energy requirement should be based on essential amino acids (Akiyama *et al.*, 1991). It is believed that higher energy feeds would require higher levels of the limiting essential amino acids. Sedgwick (1979) found that optimal utilization of protein by *P.merguiensis* was closely related to the energetic value of diet and that carbohydrate and lipid could also increase growth efficiency at sub-optimal quantities of protein.

It may be noted that most organisms which form food for the prawns are rich in protein. In them the fat content (the major energy yielding nutrient component) is however low. The protein-energy (metabolizable) ratio for natural food is about 130mg/k cal (Hepher, 1988; Matty, 1989b) whereas the present experiment indicates that for the optimal growth the postlarvae and juveniles require a protein-gross energy ratio of approximately 65mg/k cal viz., approximately 75mg/k cal of metabolizable energy (Table 4). This indicates an excess of protein in the natural food of the freshwater prawn. Hence in nature and in extensive farming when the maximum standing crop of the prawn is reached and natural food cannot support more growth, energy limits growth rather than protein. This deficit in energy can often be satisfied by carbohydrate rich food, for prawns do not tolerate high dietary lipid content.

Since the diets employed in the experiment is isolipidic, it may safely be deduced that a protein-carbohydrate ratio near to 1:1.6 would result in the best performance in the postlarvae and juveniles of *M.rosenbergii*. According to Bages and Sloane (1981) growth in prawn is protein dependent and not related with protein-starch ratio. Survival rates were affected by the amount of starch and was deduced that when the protein starch ratio was kept within a certain range (1.2-3.5) the survival of the postlarvae increased significantly (Bages and Sloane, 1981). Gomez *et al.*(1988) observed a protein-starch ratio of 1:1.1 to result in maximum growth in the juveniles of *M.rosenbergii*. In the present experiments the best results were obtained when the protein content was close to 30% and a carbohydrate to lipid ratio of 3-4:1. Clifford and Brick (1978, 1979) observed prawns (*M.rosenbergii*) fed diets having a protein concentration of 25% and 1:3 or 1:4 ratio of dietary lipid to carbohydrates

catabolised less protein than prawns fed 25% protein diets having higher ratios of lipid to carbohydrates. However, in a long term feeding study Millikin *et al.* (1980) reported better growth and feed conversion efficiency in *M.rosenbergii* fed with a diet having 32% protein plus a 1:3 lipid to carbohydrate ratio.

The results of the present experiments also show that the test diet employed would appear to be an adequate one in that neither the weight gain nor the survival rate of prawn reared on this diet was significantly different from that of the prawn reared on the controlled diet. Various authors indicated the need for developing a test diet which is satisfactory for use in nutritional studies on *M.rosenbergii*. (New, 1976a; Biddle, 1977; Conklin and Beck, 1979; Boghen *et al.*, 1982). Hilton *et al.* (1984) presented a casein and gelatin based semi-purified diet that may with some modifications be used in nutrition studies on *M.rosenbergii*. However, they indicated the possibility of some marginal deficiencies in the diet. Purified diets based on casein were also successfully used by many investigators in nutrition studies on *M.rosenbergii* (Heinen, 1988; Briggs *et al.*, 1988; Gomez *et al.*, 1988; Reigh and Stickney, 1989).

5.2 Protein Source

The effect of substitution of dietary protein of animal origin with that of plant origin was studied by gradually replacing the former by the latter in practical diets for the postlarvae and juveniles of *M.rosenbergii* and analysing the responses of the animals against each treatment.

In the case of both the postlarvae and juveniles the survival rate was not influenced by the substitution of protein from animal source with that from plant source. In the postlarvae the highest final survival rate was obtained when fed with a diet based on 75% protein of animal origin and 25% of plant origin and the one with 50% protein each of animal and plant origin. No difference in the final survival rate of the juveniles was observed when the protein from animal source was substituted with that from plant source upto 75%. A substitution level beyond this produced a slight but not significant decline in the survival rate.

The survival rates obtained in the present experiments were relatively high which may be attributed to the maintenance of good water quality and provision of artificial substrata which would have acted as hiding place for the newly moulted and weak animals which inturn could result in higher survival. Report of high survival rate in nutritional studies with shrimps and prawns employing compounded practical diets are many (Fujimura and Okamota, 1970; Deshimaru and Shigueno, 1972; Balazs and Ross, 1976; Colvin , 1976; Colvin and Brand, 1977; Millikin *et al.*, 1980; Hajra *et al.* 1988; Briggs *et al.*, 1988; Akiyama, 1989; D'Abramo and Sheen, 1989).

The lowest survival rate in the present experiment was obtained when dried clam meat (control diet) was offered as the sole nutritional source to the postlarvae and juveniles.

In the postlarvae the best growth rate was obtained when the diet contained a combination of protein of animal and plant origin in 3:1 ratio. However, the growth rate of the postlarvae provided with the above mentioned diet was found not significantly different from the growth rate of the postlarvae provided with the other diets except the one based entirely on plant protein. Exclusion of protein of animal origin in the diet resulted in statistically significant reduction in the growth of the postlarvae. However, it could be seen that the substitution of animal protein with plant protein had no statistically significant effect on the growth rate of the juveniles of the prawn. The control diet resulted in significantly reduced growth in the postlarvae as well as in the juveniles.

In the present study fairly good growth rate of the postlarvae and juveniles of *M.rosenbergii* was obtained. It may be due to the well balanced nature of amino acids in these diets obtained by the proper mixing of protein from various sources. Most of the plant proteins have been shown to yield poor growth

rates in prawns when used individually, excepting a few (Kanazawa *et al.*, 1970; Deshimaru and Shigueno, 1972; Balazs *et al.*, 1973; Sick and Andrews, 1973).Improved growth rates produced by some of the plant proteins have been attributed to their higher polysaccharides than to monosaccharides (Sick and Andrews, 1973). These plant proteins have been observed to promote superior growth rates when mixed with animal proteins.

No statistically significant variation in the food intake was observed in the postlarvae and the juveniles of the prawn when the animal proteins in the diet was progressively replaced by the plant proteins. This observation is quite interesting in that, in general many proteins from animal sources were documented to act as feed attractant in shrimps(Sick and Andrews, 1973; Shewbart *et al.*, 1973). However, the present experiments were conducted in small tanks where the chances of the prawns to encounter the feed is relatively more compared to the field condition, where the feed attractants have major roles to play.

The food conversion efficiency of the postlarvae was little influenced by the substitution of animal protein with plant protein upto 50%. However, a substitution level beyond 50% resulted in a significant decline in food conversion efficiency. In the juveniles, however, the incorporation of plant protein as a substitute to animal protein upto 75% resulted in no significant variation in the food conversion efficiency. These findings are of great economic significance in that plant protein component in general is less expensive compared to animal protein. Balazs *et al.*(1974) and Balazs and Ross (1976) observed that early life stages of *M.rosenbergii* could utilize different plant and animal proteins efficiently. The food conversion values obtained in the present experiments are similar to the values reported by various authors employing different formulations of compounded diets in *M.rosenbergii* (Farmanfarmaian and Lautero, 1979; Boonyaratpalin and New, 1982; New, 1988). These values are however lower than those reported by Balazs and Ross (1976), Millikin *et al.* (1980), Smith and Sandifer (1980) and Perry and Tarver (1987). In penacid shrimps employing various compounded feeds based on varying protein sources and under different experimental conditions various food conversion values were reported (Venkataramiah *et al.*, 1975b; Royan *et al.*, 1977; Rajyalakshmi *et al.*, 1979; SEAFDEC, 1981, 1983; Ahamed Ali, 1982; 1988; Goswami and Goswami, 1982; Raman *et al.*, 1982; Sultan *et al.*, 1982; Liu and Mancebo, 1983; Ahamed Ali and Mohammed, 1985; Gopal, 1986; Hajra *et al.*, 1986; Durairaj *et al.*, 1987 and Dominy and Ako, 1988). Obviosly because of the difference in the dietary formulation, comparing the food conversion efficiency values obtained in the present experiment with those obtained in penacid shrimps is not possible. Venkataramiah *et al.* (1975b) observed an increase in food conversion efficiency when vegetable matter was increased in the diet. However, diets with plant proteins were found to give relatively higher FCR (= lower food conversion efficiency) than those with animal proteins (Gopal, 1986). The amount of palatable formulations eaten by a prawn is likely to increase if the diet is nutritionaly poor, and high diet intake does not necessarily bring about rapid growth (Deshimaru and Shigueno, 1972). It may be noted that shrimp feeds containing same level of dietary nutrients and which result in similar survival and food conversion efficiency may cause significant difference in growth (Fujimura, 1989). Feeding with the dried clam meat resulted in relatively low food conversion efficiency. This may be attributed to the fact that nutrient content of the dried clam meat is dissimilar to the nutritional needs of the prawn. Clam meat contains relatively high protein level but sub-optimum level of many non-protein nutrients. The decline in food conversion efficiency corresponding to a supra-optimum level of dietary protein and a sub-optimum level of dietary carbohydrate has already been discussed.

The results of the short term digestibility study indicated that the apparent digestibility of protein was not significantly affected by the animal or plant origin of the feed stuff. The mean apparent protein digestibility varied between 89.50 and 93.80% in the postlarvae and 90.80 and 96.05% in the juveniles of the prawn. The results support the contention that ingredient origin has no effect on protein

digestibility. Nevertheless, in the subsequent growth study the apparent and true digestibility of protein by the postlarvae and juveniles of *M.rosenbergii* decreased progressively in response to a progressive substitution of protein of animal origin with that of plant origin. In general, protein of animal origin was found to be more digestible compared to that of plant origin. However, in the postlarvae substitution of animal protein with plant protein upto 25% resulted in no significant decline in the digestibility of protein. In the juveniles, a substitution level of 50% did not cause any significant difference in the apparent protein digestibility though the true digestibility showed a decline when the level of substitution reached 50%.

The apparent difference in the results of the short term digestibility study and the subsequent growth study though may be related to the short term nature of the former or to the compositional difference of the diets, is not exactly known. However, in general it may be concluded that *M.rosenbergii* can digest proteins from a wide variety of sources with relatively high efficiency. This may further be evident from the digestive enzyme studies in the prawn. Lee *et al.* (1980) found that *M.rosenbergii* possesses an intricate protease system which can hydrolyse complex proteins. These protease should enable the prawn to secure all dietary essential amino acids and from any diets composed of various proteins (Lee *et al.*, 1980). Similarly high efficiency of digestion of protein from various sources has been reported in shrimps and prawns (Nose, 1964; Lee, 1970; Ting, 1970; Forster and Gabbott, 1971; Condrey *et al.*, 1972; Fenucci *et al.*, 1982; Ahamed Ali, 1988; Ashmore *et al.*, 1985; Akiyama, 1988; Akiyama *et al.*, 1988). Results of the study by Akiyama *et.al.* (1988) indicated that the apparent digestibility of protein was not influenced by animal or plant ingredient origin. Such digestibility similarity has been reported by Lee (1970), Ting (1970) and Condrey *et al.* (1972); whereas Nose (1964), Forster and Gabbott (1971) and Fenucci *et al.* (1982) working with *P.japonicus, P.serratus* and *P. platyceros* and *P.stylirostris* respectively, reported that proteins of animal origin are better digested than proteins of plant origin. However, according to Ahamed Ali (1988) animal protein in general show lower digestibility. These conflicting observations concerning protein digestibility are possibly related to species examined, ingredient quality and composition of diets, other than proteins. The differences in digestion coefficient have also been attributed to microflora of intestine and digestive enzymes (Nail, 1962; Shell, 1967; Dabrowski, 1977). Digestibility may be limited due to incomplete digestive action or because of lack of complete absorption (Maynard and Loosli, 1969). Nose (1963) reported that an unfavourable amino acid composition of the diet may also affect the protein digestibility. According to Yonge (1937) diet has a direct effect on digestive enzyme activities in invertebrates. Although the protein level is known to influence the protease activity (Grossman *et al.*, 1943; Kawai and Ikeda, 1972; Mukhopadhyaya *et al.*, 1978; Steffens, 1981) there is no report to show any relationship between the protein source and protease activity in prawns and shrimps. Patra and Ray (1988) observed higher protease activity in the hepatopancreas, stomach and intestine of fish fed with animal source of protein. Variation in the enzyme activity related to the structure of protein and duration of retention of feed in the digestive tract which inturn depends on the fibre contents and physical consistency of the diet has been reported (Venkatesh *et al.*, 1986). It must be emphasised here that the digestibility of individual feed ingredients will vary depending on physical and nutritional characteristics of the material under test, the manufacturing process employed in the preparation of the feed ingredient, the dietary inclusion level of the feed ingredients, the development status and digestible capacity of the fish or shrimp species in question, the feeding method employed and the experimental technique employed for estimating nutrient digestibility (for reviews see Austreng, 1978; Cho *et al.*, 1982, 1985; Choubert *et al.*, 1982; Jobling, 1983; NRC, 1983; Tacon and Rodrigues, 1984; Talbot, 1985; Wilson and Poe, 1985; Smith *et al.*, 1980; Kirchgessner *et al.*, 1986; De la Noue and Choubert, 1986; Tacon, 1988). The knowledge of digestibility of dietary nutrients is essential for the study of prawn energetics and for the evaluation of different food stuff. In view of the difficulties encoutered with the quantitative collection of faeces within an aquatic environment and the different methods

employed by individual laboratories for estimating nutrient digestibility, further research is required in this subject area before confidence can be given to the digestibility coefficients obtained. The fact that the postlarvae and juveniles exhibited maximum digestibility when they were provided with dried clam meat as the sole food may be attributed to the presence of little carbohydrate in it. Presence of carbohydrate has been reported to lower the protein digestibility (Shimeno *et al.*, 1979; Kiron, 1988; Akiyama *et al.*, 1991).

The carcass composition of the postlarvae and juveniles of *M.rosenbergii* was found not to be influenced by the dietary source of protein, in the present experiment. Similar observation was made by Mason and Castell (1980). However, chemical content of the diet was found to influence the body composition of fish by Philips *et al.* (1966) and Jayaram and Shetty (1980). Gopal (1986) reported that prawns show variations in body composition depending on the quality of protein in the diets. He found higher body moisture content when the prawn was fed with plant protein. He observed higher protein deposition in prawns fed on protein from animal source and attributed it to the higher biological value of the animal protein. According to Cowey and Forster (1971) growth and deposition of protein in prawns are based on quality of protein intake. Cowey and Corner (1963) reported that variation in essential as well as non-essential amino acids present in the diet may affect the metabolic process which inturn influence the synthesis of body proteins.

In the postlarvae and juveniles of the prawn incorporation of plant protein component in the diet upto 75% resulted in no significant difference in the protein efficiency ratio. Steffens (1981) reported that the PER values can be used to evaluate the quality of protein in the diet, those with high PER values are of good quality protein and those with low PER values are of poor quality. The highest PER obtained when the prawns were provided with a diet with a mixed source of protein may be attributed to the better balancing of amino acids in the diet. The fact that the least PER values obtained in the postlarvae and juveniles when provided with the dried clam meat as the sole dietary source may rightly be attributed to the channelling of more protein for non-protein function. The efficiency with which protein is assimilated by prawn is most likely affected by the relative proportion of lipids and carbohydrates in the formula as well as the amino acid composition of whatever protein is employed (Hanson and Goodwin, 1977). Farmanfarmaian (1980) reported improvement of PER when supplemented with the deficient amino acid in a commercial diet. The PER values obtained in the present experiments are similar to those reported by different workers for the species when provided with different diets (Farmanfarmaian and Lautero, 1980; Millikin *et al.*, 1980; Boonyaratpalin and New, 1982; Gomez *et al.*, 1988).

Gopal (1986) is of the opinion that ammonia excretion rate is an indication of efficiency of protein metabolism in prawns. If prawn is fed on a good quality diet, the diet is efficiently utilised for better growth with minimum catabolism of protein, which inturn indicates the lower ammonia excretion rate and hence a higher oxygen-nitrogen ratio. In the present study upto an incorporation level of 50% plant protein , the oxygen-nitrogen ratio of the postlarvae and juveniles did not show any significant difference. Beyond this range the oxygen-nitrogen ratio declined significantly indicating higher level of catabolism of protein. The use of oxygen-nitrogen ratio as a means of defining the nutritional status and well being of invertebrateshas already been discussed.

The foregoing discussion reveals that progressive substitution of protein from animal source with that from plant source upto 50% in the case of the postlarvae and 75% in the case of the juveniles does not bring about any substantial decline in the performance of the prawn but may marginally improve their performance and inturn significantly cut down the cost of the feed component in the overall production economics of the *Macrobrachium* farms. However, substitution of animal protein with plant protein beyond the above mentioned levels resulted in a decline in growth and performance of the

postlarvae and juveniles of *M.rosenbergii* indicating the importance of animal protein in the commercial diets. Attempts to rear *M.rosenbergii* exclusively on plant derived material resulted in increased mortality and decreased weight (Stern *et al.*, 1976). Balazs *et al.* (1973) reported superior performance of a diet based on fish, soybean and shrimp to an all vegetable diet (soybean) or fish-soybean diet. Balazs *et al.* (1974) also reported the superior performance of a diet based on animal and plant origin to others based completely on vegetable origin. The better performance of the diets based on a mixture of animal as well as plant proteins may be related to the better balancing of amino acids in them. Ideally the amino acid profile of feeds should be balanced so that maximum growth can be attained at the lowest possible cost. Excessive or limiting levels of some amino acids may increase the apparent requirement for protein. Alava and Lim (1983) opined that diets containing protein from two or more sources are better utilized by shrimp than those containing protein from a single source. Ahamed Ali (1988) found mixed sources of protein with 70% animal and 30% plant origin to result in maximum growth in prawn, compared to either plant or animal protein. Addition of vegetable matter partly corrected the decrease in growth rate associated with increase in protein level beyond the optimum level (Venkataramiah, *et al.*, 1975b). Shigueno *et al.* (1972) reported that diets containing high levels of protein from several animal and plant sources were very efficient in shrimps. Deshimaru and Shigueno (1972), Conklin *et al.* (1977), Alava and Lim (1983), Gopal (1986), Goxe *et al.* (1988) and Pascual (1988) also reported the superiority of mixed sources of protein in shrimp diets. The Hawaian pelleted feed for *M.rosenbergii* is also reported to be based on a large number of proteins mainly from vegetable components (Weidenbach, 1982). Many authors postulated that the diet with an amino acid pattern closely resembling the animal body protein bring about maximum growth (Phillips and Brockway, 1956; Ogino, 1963, Kitabayashi *et al.*, 1971a; Deshimaru and Shigueno, 1972; Deshimaru, 1982; Hew and Cuzon, 1982; Sherief, 1987; Chiu, 1988; Kompiang, 1990). These authors also postulated that an admixture of certain protein sources with additives will have a special pattern of amino acid distribution similar to that of the prawn. The present observation is very relevant in commercial aquafarming, where the feed cost represents one of the largest recurring expenses. The cost of commercial shrimp feed is extremely time and place specific. It depends on a range of factors including ingredient and production costs, farm location in relation to manufacturing site and what the manufacturer perceives the farmer is able to pay (New,1990b). Progressive incorporation of relatively inexpensive, locally available plant proteins by replacing animal proteins results in a progressive reduction in the feed cost. Thus a decision based on biological evaluation data coupled with economic criteria will favour incorporation of protein from plant sources. However, a number of anti-nutritional factors exist in plant feed stuffs (Tacon and Jackson, 1985; Tacon, 1987b; Matty, 1989a) which limit their use in shrimp feeds. Anti-nutritional factors are also, to a lesser extent, found in feed stuffs of animal origin. This highlights one of the benefits of a multi-ingredient compound feed: risks are minimized by having small quantities of a variety of raw materials (New,1990b). Achieving nutritionally balanced diet is also easier when many ingredients are employed. Further, ideally, formulated diets in which optimum nutrient levels would be supplied by an appropriate but varying mixture of feed stuffs would be useful in reducing feed costs relative to the normal fluctuations within the commodities market (Conklin *et al.*, 1983).

The examination of natural food of *M.rosenbergii* supports the findings of the present experiment. Examination of stomach content in the field indicates that the species ingests a wide variety of food items of both plant and animal origin, as well as detritus thus functioning as a primary consumer, a secondary consumer and a detritivore in its natural environment (John, 1957; Rao, 1967). Common items of food of the prawn include aquatic worms, aquatic insects and insect larvae, small mollusks and crustaceans, flesh and offal of fish and other animals, grains, seeds, nuts, fruits, algae and tender leaves and stems of aquatic plants (Ling, 1969). John (1957) observed that the prawn's diet depends on its environment. She found the stomach contents of prawns caught in flooded paddy fields consisted of 60% partially digested paddy while the stomach contents of prawns collected from canal, rivers and

lakes consisted largely of organic debris, crustacean appendages, vegetable matter and molluskan fragments. In polyculture systems, Lilyestom and Romaire (1987) found *M.rosenbergii* to depend to a greater extent on the autotrophic food web for their diet. Seston and macrophytes contributed 18-75% of the prawn growth. The prawn were found to feed on seston at small sizes and consumed more aquatic macrophytes at larger sizes. Costa and Wanninayake (1986) found the food of *M.rosenbergii* to contain 44-55% detritus, 21-36% animal matter and 11-24% plant matter.

Evidence for the conjuncture that there exists difference in the utilization of protein from different sources depending on prawn size is also gained through the present experiments. Thus though substitution of animal protein with plant protein upto 75% did not result in significant reduction in growth in the juveniles, a substitution level beyond 50% led to relatively poorer growth in the postlarvae. The food habits of shrimp vary not only between species but also according to age (New, 1987). Lilystrom and Romaire (1987) showed by stomach content analysis that prawns increased their ingestion of macrophytes as they grew. Research on compounded diets for the early postlarvae of *M.rosenbergii* is completely lacking. There exist a few studies on the nutritional requirement of the early postlarvae of penacid prawns (Forster, 1972; Kittaka, 1976; Khannapa, 1977; Brand and Colvin, 1977; Colvin and Brand, 1977 and Bages and Sloane, 1981). Such studies on compounded diets on the early postlarvae would help prepare formulated diets which simplify the operation of hatcheries and nurseries and guarantee the production from ponds where natural food is lacking or inadequate.

The results of the present experiments also indicate the possibility of successful use of clam meat, prawn meat, soybean, ground nut oil cake and black gram in the commercial diets for the freshwater prawn. Prawn and clam meat have been reported as good sources of protein for shrimps and prawns (Kanazawa *et al.*, 1970; Forster and Gabbott, 1971; Deshimaru and Shigueno, 1972; Suryanarayana and Alexander, 1972; Forster and Beard, 1973; Sick and Andrews, 1973; Deshimaru, 1981; Ahamed Ali, 1982, 1988; Goswami and Goswami, 1982; Kungvankij *et al.*, 1986; Sherief, 1987; Nair and Thampy, 1987; Akiyama, 1988; Aquacop *et al.*, 1989). Nevertheless, their large scale use will become cost prohibitive because their supply based on known and accessible resources is limited and will not sustain a major increase in offtake (Wood and Coulter, 1988). Soybean has been featured probably as the best protein of plant origin for shrimp feeds (Kanazawa *et al.*, 1970; Deshimaru and Shigueno 1972; Balazs *et al.*, 1973; Forster and Beard, 1973; Sick and Andrews, 1973; Balazs and Ross, 1976; Sick and Beaty, 1975; Colvin and Brand, 1977; Fenucci *et al.*, 1980; Lawrence *et al.*, 1986; Pascual *et al.*, 1986; Akiyama *et al.*, 1988; Ako, 1988; Boonyaratpalin *et al.*, 1988; Fernandez and Lawrence, 1988a; Aquacop *et al.*, 1989; Penaflorida, 1989). However, amino acid profile of soybean shows a slight deficiency of tryptophan and methionine for prawns (Kanazawa *et al.*, 1970). Raw soybeans contain anti-nutritional substances which decrease animal growth and performance. The predominant anti-nutritional substance is trypsin inhibitor (Kunitz, 1945; Birk *et al.*, 1963) which may also cause pancreatic hypertrophy with excessive endogenous protein losses (Booth *et al.*, 1960; Rackis, 1972). Several less studied anti-nutritional substances have also been identified (Rackis, 1972; Liener, 1975). It has been well documented that heat treatment of soybean improves its utilization. Heat treatment primarily inactivates the heat labile trypsin inhibitor and denatures the soy protein making them more digestible (Kakade *et al.*, 1973). However, excessive overheating) may be undesirable because of the Millard reaction; the formation of unavailable sugar-amine complexes (Maynard *et al.*, 1979) and the destruction of heat sensitive amino acids (Smith and Circle, 1972). Wee and Shu (1989) reported that boiling full fat soybean for 1 hour at 100^{0}C inactivates 80% of the trypsin inhibitor activity. The use of soymeal in shrimp feeds has been limited partly due to the limited knowledge available on shrimp nutrition and the infancy of shrimp culture industry. Soymeal has been successfully used to replace marine proteins in *M.rosenbergii* (Balazs *et al.*,1973; Sick and Beaty, 1975; Balazs and Ross, 1976). The replacement of 50% of the fish meal and shrimp meal by soybean meal produced higher growth rates and better feed conversion ratios in *P.californiensis* (Colvin and Brand, 1977). Fenucci *et al.*

(1980) replaced 50% of squid meal with a purified soy protein and obtained better growth, survival and feed conversion ratios in *P.setiferus* and *P.stylirostris*. Sick and Andrews (1973) reported soy meal as a superior protein source to fish meal and shrimp meal in *P.duorarum* whereas Forster and Beard (1973) reported a reduction in growth when fish meal was completely replaced by soybean meal in *P.serratus*. High levels of purified soy protein (58%) has been attributed to a reduction of food intake in *P.aztecus*. (Fenucci and Zein-Eldin, 1976). Because of the limited knowledge in shrimp nutrition these observed differences may be due to dietary factors other than soybean meal protein (Akiyama, 1988). Akiyama *et al.* (1988) found soybean meal to have a higher apparent protein digestiblity than fish meal, squid meal and shrimp meal in *P.vannamei*. Akiyama (1988) observed that *P.monodon*, *P.vannamei* and *P.japonicus* digest soybean meal very efficiently and there is very slight species difference in the apparent digestibility of soybean meal by these prawns. Successful use of ground nut oil cake in shrimp diets was reported by many (Forster and Gabbott, 1971; Sherief, 1987; Ahamed Ali, 1988). However, ground nut oil cake is low in cystine, tryptophan, threonine, methionine and lysine (Easterson, 1987) and isoleucine and valine (New 1987). It may also contain anti-nutritional factors like protease inhibitors, phytohaemaglutinins, phytic acid and saponins (Tacon and Jackson, 1985). The successful use of black gram as a protein component in the present experiment has commercial importance in that black gram is one of the most inexpensive protein sources, incorporation of which bring down the cost of production of shrimp feed considerably. New (1987) documented black gram as a leguminous feed with potential in aquaculture feeds.

In the present experiment clam meat as a sole source of protein was found to result in poor performance in the postlarvae and juveniles of *M.rosenbergii*. This observation is in agreement with those of Kitabayashi *et al.* (1971c), Colvin (1976), Villegas (1978) Ahamed Ali (1982) and Gomez *et al.* (1988) but in disagreement with those of Kanazawa *et al.* (1970) and Forster and Beard (1973) who reported superior performance of fresh clam meat.

The observation that clam meat failed to bring about acceptable performance in *M.rosenbergii* may be related to the presence of anti-nutritional factors (Tacon and Jackson, 1985; Matty, 1989a) in it or to the lack of sufficient supply of non-protein nutrients including some vitamins. Supra-optimum level of protein in the presence of low non-protein energy may result in lower growth and performance in shrimps. This point has been discussed elsewhere. The finding suggests that clam meat or for that matter other fresh feeds of animal origin like trash fish, mussels, oysters etc. as sole nutritional source cannot sustain acceptable growth in the prawn in intensive culture systems. Though feeding of single ingredient to shrimp is often practised it can be a very inefficient use of feed because a single ingredient is most unlikely to supply all the nutrients required by the animal in the proportion in which it needs them (New, 1987). In extensive culture systems, where natural feeds contribute substantially to the nutritional needs of the prawn, fresh feeds may produce quite satisfactory results. The importance of fresh feeds cannot, however, totally be neglected in intensive systems as shrimp grown on pelleted feed are often reported to take longer time to reach market size (Chen, 1990) and may lack proper pigmentation as has been observed by Celada *et al.* (1989) in cray fishes. And hence, even in places, where shrimp feed production underwent considerable refinement, farmers using pelletized feed often present their shrimp with a fresh feed diet every four or five feedings or atleast once in a week to guarantee a nutritional balance (Chen, 1990).

6 SUMMARY

The present study is undertaken to elucidate the protein requirement of the early postlarvae and juveniles of the freshwater prawn, *Macrobrachium rosenbergii* (De Man). Three sets of experiments were conducted. The first set of experiments was conducted to determine the quantitative protein requirement of the two life stages of the prawn using casein and amino acid based isocaloric (metabolizable energy) purified diets having graded levels (0, 10, 20, 30, 40 and 50%) of protein. The effect of the various levels of protein on the survival, growth rate, food intake, food conversion efficiency, protein digestibility, faecal nitrogen excretion, carcass composition, body nitrogen retention, efficiency of protein utilization and the oxygen-nitrogen ratio of the postlarvae and juveniles were considered for a period of 40 days.The second set of experiments was conducted to determine the digestibility of certain locally available sources of protein which have potential for use in commercial shrimp diets viz., prawn meat, clam meat, soybean, groundnut oil cake and blackgram. Since this was undertaken also to fix the level of protein to be incorporated in the practical diets used in the third set of experiments, the digestibility of casein was also tested. Each experiment was conducted for 10 days. The third set of experiments (each for 40 days) was to evaluate the effect of progressive substitution of protein of animal origin with that of plant origin in the postlarvae and juveniles of *M.rosenbergii*.

The salient findings of the various experiments are summarised below:

1. During the initial 10 days the survival rate of the postlarvae and juveniles of *M.rosenbergii* was little influenced by the level of protein tested. However, thereafter, the postlarvae and juveniles which received a diet with zero percentage protein showed a sudden decline in the rate of survival reaching the respective final percentage survival value of 48 and 60%. These animals were observed to gradually become less and less active and finally to succumb to death.

2. The dietary protein concentrations tested had a statistically significant effect on the percentage survival of the postlarvae and juveniles of *M.rosenbergii*. The best survival rate in the postlarvae was obtained when they were provided with 30 or 40% level of dietary protein. In the juveniles, however, diets with 20, 30 or 40% protein resulted in the highest final survival.

3. Protein inclusion had a profound influnce on the growth of the postlarvae and juveniles of the prawn. Exclusion of protein from the diet resulted in a well pronounced reduction in the body weight of the postlarvae and juveniles of *M.rosenbergii*, the respective percentage loss of body weight over a period of 40 days being 9.84 and 4.51%.

4. The dietary protein concentration tested had a significant influence on the growth of the postlarvae and juveniles of the prawn. The growth of the postlarvae and juveniles showed a curvilinear relationship with the dietary protein concentraion. The growth of the postlarvae increased as the dietary protein concentration increased upto 30%. A further increase in the level of protein in the diet resulted in an apparent but statisitically not significant reduction in the growth of the postlarvae. The growth of the juveniles also increased corresponding to an increase in the dietary protein concentration upto 30%. A further increase in the level of dietary protein resulted in significant decline in the growth rate of the juveniles.

5. By establishing second order polynomial relationship between the growth rate of the postlarvae and juveniles of the prawn and the dietary protein concentration and by employing the differential calculus method the protein concentrations which result in maximum growth were determined to be 34.5 and 29.5% in the postlarvae and juveniles respectively. The economic protein requirement of the postlarvae and juveniles ie., the dietary protein concentration that minimize the cost while maintaining adequate growth were found to be 27.5 and 23.0% respectively.

6. Dietary protein levels had little influence on the food intake per day per unit weight of the postlarvae and juveniles of *M.rosenbergii*. In general the food intake per day by unit weight of the prawn was relatively more in the case of the postlarvae compared to the juveniles.

7. The food conversion efficiency of the postlarvae and juveniles of *M.rosenbergii* was influenced by the dietary protein concentration. There exists a curvilinear relationship between the food conversion efficiency and the dietary protein concentration. The food conversion efficiency increased with an increase in the dietary protein concentration upto 40% in the case of the postlarvae and upto 30% in the case of the juveniles. Further increase in the dietary protein concentration resulted in a decline in food conversion efficiency in both the life stages. The postlarvae showed the best food conversion efficiency (26.94%) when provided with a diet with 40% protein closely followed by the one with 30% protein concentration (26.46%). In the juveniles the best food conversion efficiency (32.44%) was obtained when fed with a diet having 30% protein followed by the one with 40% (31.41%) and the one with 20% protein concentration (30.76%).

8. By establishing second order polynomial relationship between the food conversion efficiency and the dietary protein concentration and by employing the differential calculus method, protein levels of 34.02 and 28.59% were found to result in maximum food conversion efficiency in the postlarvae and juveniles of *M.rosenbergii* respectively.

9. The protein concentration had a significant effect on the apparent digestibility of protein in the postlarvae and juveniles of *M.rosenbergii*. The apparent digestibility of protein improved as the dietary protein concentration increased, the lowest and highest values being 77.50 and 88.13% in the postlarvae and 85.27 and 95.51% for the juveniles of the prawn. However, the true digestibility was observed to be little influenced by the level of protein tested indicating that the effect of protein concentration on the apparent digestibility might have resulted from the difference in the inclusion level of metabolic faecal protein which is proportional to the amount of food ingested and not affected by the composition of the food.

10. The dietary protein concentration had a significant effect on the faecal nitrogen excretion, the relationship between the two being linear with correlation coefficient values of 0.999 and 0.985 respectively for the postlarvae and juveniles of the prawn.

11. Evenwhen the prawns were fed with a protein-free diet some amount of nitrogen (viz. 198mg N/100g diet in the postlarvae and 151mg N/100g diet in the juveniles) was present in the faeces. These represent the amount of metabolic faecal nitrogen.

12. The metabolic faecal nitrogen values calculated by employing the indirect method of regressing nitrogen excretion against the dietary protein concentration was 9.93 and 40.93% higher than the respective values obtained by the direct faeces collection method. The values of metabolic nitrogen excretion obtained by the former method are 217.66 and 212.81mg N/100g diet in the postlarvae and juveniles of the prawn.

13. The carcass composition of the postlarvae and juveniles of *M.rosenbergii* was not influenced by the variation in dietary protein concentration.

14. The more nitrogen is consumed the more is retained over a wide range of protein consumption in both the postlarvae (787.9mg N/100g body weight/day) and juveniles (525.0mg N/100g body weight/day) of *M.rosenbergii*. Beyond these values, the protein retention decreased sharply.

15. Feeding with zero protein diet resulted in a loss of body nitrogen equal to 17.95 and 18.69mg N/100g body weight/day in the postlarvae and juveniles respectively. These values represent the endogenous nitrogen excretion or the digestible nitrogen levels that results in zero nitrogen balance (maintenence nitrogen requirement) assuming an efficency of utilization very close to

100%. By employing the statistical method of regressing the digestible nitrogen intake against the nitrogen retention the endogenous nitrogen excretion was calculated as 24.31 and 15.20mg N/100g body weight/day.

16. The protein efficiency ratio of the postlarvae and juveniles of *M.rosenbergii* was significantly influenced by the changes in dietary protein concentration. The dietary protein concentration and the PER were negatively linearly correlated. The best PER values were obtained when the postlarvae and juveniles were fed with a diet having 10% protein concentration.

17. Dietary protein concentration had a significant influence on the oxygen-nitrogen ratio of the postlarvae and juveniles of *M.rosenbergii*. An increase in the dietary protein concentration resulted in a decrease in the oxygen-nitrogen ratio in both the postlarvae and juveniles. Thus the highest value for the oxygen-nitrogen ratio was obtained when the prawns were fed with zero protein diet and the lowest, when fed with 50% protein diet.

18. On the basis of the above mentioned observation on the growth and performance of the postlarvae and juveniles of *M.rosenbergii* in response to the various dietary protein concentrations, it may be concluded that the protein requirement of the postlarvae and juveniles lie close to 30%. However, for maximum growth and performance protein concentrations of 34-35% and 28-30% may be required for the postlarvae and the juveniles respectively of *M.rosenebergii*.

19. The results of the present experiment also show that the test diet employed in the present study is adequate for nutritional studies on the postlarvae and juveniles of *M.rosenbergii*. Neither the weight gain nor the survival rate of the prawns reared on the purified diet (with 30 or 40% protein) is significantly different from those obtained for the control diet. The food utilization efficiency indices obtained for the purified diet were also comparable to those obtained for the control diet.

20. The results of the short term digestibility study indicated that the apparent digestibility of protein was not significantly affected by the animal or plant origin of the feed stuff in the postlarvae and juveniles of the prawn.

21. The substitution of protein of animal origin with that of plant origin had no statistically significant effect on the percentage survival of the postlarvae and juveniles of *M.rosenbergii*.

22. Substitution of dietary protein from animal source with that from plant source upto 75% had no significant effect on the growth rate of the postlarvae of *M.rosenbergii*. However, the complete substitution of protein from animal source with that from plant source significantly reduced the growth rate. The best growth of the postlarvae was obtained when fed with a diet based on protein from animal and plant sources in 3:1 ratio. In the juveniles of the prawn the substitution of dietary protein of animal origin with that of plant origin had no statistically significant effect on the growth rate. Neverthless, a slight increment in growth rate was obtained when the plant protein component was incorporated in the diet upto 50%.

23. The food intake per day per unit weight of the prawn was not influenced by the plant or animal origin of protein.

24. Substitution of protein of animal origin with that of plant origin upto 50% resulted in no significant decline in the food conversion efficiency of the postlarvae of *M.rosenbergii*. In the case of the juveniles, however, a substitution level upto 75% resulted in no significant reduction in the food conversion efficiency. Substitution of animal protein with plant protein beyond these levels caused significant decline in the food conversion efficiency in the postlarvae and juveniles.

25. The progressive substitution of protein from animal source with that from plant source resulted in a gradual decline in the apparent and true protein digestibility in the postlarvae and juveniles. However, the decline in apparent digestibility corresponding to incorporation of plant protein

upto 25% in the postlarvae and upto 50% in the juveniles was not significant. In the postlarvae and juveniles of the prawn the true digestibility of protein remained little influenced by the substitution of protein from animal source with that from plant source upto 25%.

26. Carcass composition of the postlarvae and juveniles of *M.rosenbergii* was not influenced by protein of plant or animal origin.

27. Substitution of protein from animal source with that from plant source upto 75% resulted in no significant difference in the protein efficiency ratio (PER) of the postlarvae and juveniles of *M.rosenbergii*. In both the life stages, a 100% plant protein based diet produced the lowest PER value.

28. Oxygen-nitrogen ratio of the postlarvae and juveniles was not influenced by the substitution of protein of animal origin with that of plant origin upto 50%. In the postlarvae and juveniles, the highest oxygen-nitrogen ratios was obtained when provided with diets with protein of animal and plant origin in 3:1 and 1:1 ratio respectively.

29. On the basis of observation on growth and performance, a diet based on animal and plant protein in 1:1 and 1:3 are recommeded for the manufacture of compounded diets for the postlarvae and juveniles respectively of *M.rosenbergii*. In addition to its beneficial effects on the growth and performance of the postlarvae and juveniles these diets bring down the cost of feeds substantially.

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PROTEIN REQUIREMENT OF THE POSTLARVAE AND JUVENILES OF *MACROBRACHIUM ROSENBERGII* (DE MAN)

BY

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ABSTRACT

Three sets of experiments were conducted with the postlarvae and juveniles of the giant freshwater prawn, *Macrobrachium rosenbergii* (De Man). The first set of experiments was conducted to determine the quantitative protein requirement of the two life stages of the prawn using casein and amino acid based purified diets having graded levels (0, 10, 20, 30, 40 and 50%) of protein. Each experiment was conducted for 40 days and the effect of protein concentration on the survival, growth, food intake, food conversion efficiency, protein digestibility, nitrogen excretion, nitrogen retention, body carcass composition, efficiency of protein utilization and oxygen-nitrogen ratio was studied. Among the different diets the one with 30% protein produced the best results in the postlarvae and juveniles of the prawn. Using second order polynomial regression analysis and differential calculus methods the protein requirement for maximum growth in the postlarvae and juveniles of the prawn was calculated as 34.5 and 28.5% respectively. In absolute terms these represent 787.9 and 529.0mg protein per 100g body weight of the prawn per day. The economic protein requirement of the postlarvae and juveniles was found to be 27.5 and 23.0% respectively. The maintenance protein requirement of the postlarvae and juveniles was determined to be 17.95 and 18.69mg protein per 100g body weight per day assuming an efficiency of utilization of protein very close to 100%. The metabolic faecal nitrogen excretion was found to be 198 and 151mg N per 100g body weight per day for the postlarvae and juveniles respectively.

In the second set of experiments, short term (10 days each) studies were conducted to determine the efficiency of assimilation of certain locally available sources of protein by the postlarvae and juveniles of *M.rosenbergii*. These experiments were conducted also to help fix the levels of protein to be maintained in the third set of experiments. The protein sources evaluated were casein, prawn meat, clam meat, soybean, ground nut oil cake and black gram. The results of the study indicated that the apparent protein digestibility is not influenced by animal or plant origin of the ingredient.

The third set of experiments was conducted to evaluate the effect of substitution of protein of animal origin with that of plant origin in the postlarvae and juveniles of *M.rosenbergii*. Each experiment was conducted for 40 days and on the basis of data on survival, growth, food intake, food conversion efficiency, protein digestibility, carcass composition, efficiency of protein utilization and oxygen-nitrogen ratio, substitution of 50 and 75% protein of animal origin with that of plant origin was found to result in no significant decline in the overall performance of the postlarvae and juveniles respectively of *M.rosenbergii*.