EFFECT OF DIETARY PROTEINS ON GROWTH, FOOD CONVERSION AND DIGESTIVE ENZYME ACTIVITY OF JUVENILE MACROBRACHIUM ROSENBERGII

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BY

LIZY PAULOSE. C.

SCHOOL OF INDUSTRIAL FISHERIES
COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY
COCHIN - 682 016

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TO

My Beloved Father

CERTIFICATE

This is to certify that this thesis is an authentic record of research work carried out by Smt. Lizy Paulose. C. M.Sc. under my supervision and guidance in the School of Industrial Fisheries, Cochin University of Science and Technology in partial fulfilment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** and that no part thereof has been submitted for any other degree.

Kochi - 682016
November, 1996

SALEENA MATHEW
(Supervising teacher)

Dr. Saleena Mathew,
Reader,
School of Industrial Fisheries,
Cochin University of Science and Technology,
Finearts Avenue, Kochi - 682016.

DECLARATION

I, Lizy Paulose. C., do hereby declare that the work presented in this thesis is the result of my own investigation and neither the thesis nor any part thereof has been accepted, nor is beeing submitted for any other degree. All the sources of information have been duly acknowledged.

Lizy Paulose. C.

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CONTENTS

Chapter 1

GENERAL INTRODUCTION

India occupies a place of pride in the world map, as the largest producer and exporter of shrimps and, the major portion of India's production and export of shrimps is from Kerala. The giant fresh water prawn *Macrobrachium rosenbergii,* is widely distributed all over the country and is the most suitable for culture in fresh water ponds and reservoirs. A lot of extensive research has been carried out in the country on almost all commercially important species of marine and fresh water prawns in our waters, regarding their biology, breeding and development and fishery (Alikunhi, and Hameed (1984)

It has been observed that *Macrobrachium rosenbergii* is an ideal species which has been recently incorporated in the commercial aquaculture ventures (Rao, 1993). The culture of fish in fresh water and the traditional prawn farming carried out in inland water, are the well studied areas of aquaculture. For the last fifteen years, almost everywhere in the world, immense progress has been made in the development of new techniques, making it possible to use new areas of water, for cultivation (Barnabe, 1990).

An appropriate feeding is a major factor in the successful larval rearing. The time taken for the metamorphosis of a larval batch, varies according to the temperature and the feeding conditions. Under favourable conditions, the post larval stage is reached in 16 to 28

days. The post larvae can then be transferred to the fresh water (Pillay, 1990). Fresh water prawn production from India, had risen from a mere 200 ton in 1991 to 8000 ton in 1994. It is predicted that by the year 2000, the global production would reach one lakh ton and Asian production to about 68000 ton.

From the culture point of view, of the many species of Macrobrachium, *Macrobrachium rosenbergii* is considered to be the most ideal one owing to its fast growth rate, due to its hardy nature and omnivorous feeding habit, economic and efficient food conversion and relatively good productivity per unit area of water in mono as well as polyculture systems (New, 1990,1995). Due to its hardy nature, it withstands fluctuations in environmental conditions particularly salinity and temperature. The health of the farmed aquatic organisms in aquaculture systems, largely depends on the natural productivity of the systems, the quality and quantity of food available, nature and intensity of farming, variation in water and soil parameters, source of stocking material, site selection and the preparations leading to the stocking and management measures adopted. Food, both natural and supplemented, should provide all essential nutrients in the right quantities to the farmed organisms. Lack of essential nutrients leads to nutritional deficiency disorders (Murthy, 1996).

The dietary protein is necessary for the maintenance, tissue repair and growth. The optimum dietary protein level for the

growth of penaeid shrimps, has been reported to range from, 28% to 60% depending on the size, protein quality, level of non protein energy, feeding rate and availability of natural food organisms.

The optimum water quality management is a prerequisite for the success of any prawn hatchery. Water is the main source that carries diseases to the larvae and to the juvenile prawns. Therefore it is very important to maintain water quality, scrupulously.

The principal objectives of this study are:

☞ To obtain information concerning the changes in the digestive enzyme activities that occur in the digestive tract of *Macrobrachium rosenbergii* after giving a feed.

☞ To study the effect of dietary proteins on the activity of digestive enzymes and the relationship with other nutritional parameters such as growth and feed digestibility.

☞ To provide a method in selecting efficient protein diets for prawn culture, based on their capacity to promote production of digestive enzymes.

☞ To investigate the effect of diets, containing different dietary protein concentration, on the level of protein synthesis, RNA content and the ratio of RNA/DNA in the muscle tissue.

Fish growth, as defined by the increase in the fish flesh, is mainly accomplished through the synthesis of protein. A major advantage of RNA measurement is that, it could possibly be used to assess the growth rate occurring at a given time. Changes in the RNA-DNA ratio are considered to be a more accurate index of metabolic activity, than RNA concentration alone, since the ratio is not affected by the differences in cell numbers. The importance of digestive enzyme analysis as a tool in the study of nutrition, lies in the description of patterns in an animal dietary regimen, such as, ability to specifically hydrolyse individual materials in the diet, response to different nutrient sources and levels, bacterial contribution to digestion, cyclic secretion and changes in these, as the animal grows and matures (Lee et al, 1984). Since crustaceans are now being evaluated for commercial culture, the changes in the digestive enzyme activities during the life cycle and adaptation to new diets, need to be examined quantitatively.

Digestive enzyme activity may change with age, physiological state and season. Gastric digestion and evacuation are affected by a number of factors such as quantity and quality of food, the rate of secretion of gastric juice, gastric mobility and the capacity

of the intestine to accept chime from the stomach (Hepher, 1988). The hepatopancreas of *Macrobrachium rosenbergii*, as in several other crustaceans, contain enzymes such as carbohydrases, lipases and proteases.

Therefore, information on the digestive enzymes and their preferential conditions of activity, would greatly help in rationally adjusting the quality and quantity of the feed supplied to prawns, during the different stages in their life cycle. The study gives valid information on the relationships between dietary protein level, digestive enzyme status, protein efficiency ratio and other growth parameters. The results will also provide an easy method, to select efficient protein diets for prawn culture, based on their capacity to promote production of digestive enzymes.

Chapter 2

REVIEW OF LITERATURE

In this chapter, an attempt is made to make a thorough review of the literature on the various aspects relating to the culture, nutritional requirements, food and feeding, digestive enzyme activity, nucleic acid composition, water quality parameters and nutritional evaluation methods of *Macrobrachium rosenbergii*.

2.1 CULTURE OF MACROBRACHIUM ROSENBERGII

Fresh water prawn farming is one of the topics reviewed in a number of books on crustacean farming (Mcvey, 1993; Lee and Wickens, 1992) and generally on aquaculture (Pillay, 1990; Brown, 1991).

A review on prawn culture with particular reference to India is also available (Sebastian, 1990). In addition, several proceedings of the meetings devoted entirely to the topic on fresh water prawn culture have been published (New, 1982; Silas, 1992; and Thakur et al., 1994).

Research concerned with the culture of the fresh water prawn *Macrobrachium rosenbergii* and the status of its development globally up to 1989, has previously been reviewed by New (1990) and later by New (1995). A number of practical manuals in the culture of fresh water prawns are available. These include the general manual (New and Singholka, 1985)

and a manual on fresh water prawn farming for India (Sebastian, et al 1993). Useful information on recirculating prawn hatcheries, is also contained in Daniels, et al. (1992)

Alam, et al (1993) worked in Malaysia on improving larval nutrition by using different live food organisms for the culture of *Macrobrachium rosenbergii* larvae. In 1992, they worked on various aspects of its culture, physiology and dietary requirements. Angell, (1994) work in Bangladesh in popularising hatchery technology and promotion of farming *Macrobrachium rosenbergii* is noteworthy. Brown and his co-workers (1991) worked on the effect of various physio chemical parameters on growth and survival of the giant prawn in U.K.

Sterol and polyunsaturated fatty acid requirements of *Macrobrachium rosenbergii* and its polyculture with cray fish and channel cat fish were reported by D'Abramo, et al. (1994) in U.S.A. Gomez and his co-researchers (1990), have worked on the effect of parental history on larval development and on the effect of dietary carbohydrates and proteins on the growth of *Macrobrachium rosenbergii* in Japan.

Karplus et al., (1992) studied the effect of size and age at stocking on population structure and social control of

growth. Liao and Hsieh, (1990) in Taiwan worked on the tolerance of post larvae of *Macrobrachium rosenbergii*, to drugs.

New, (1995) has reviewed the status of fresh water prawn farming in the world. A stable water temperature for *Macrobrachium rosenbergii* during transport is essential for success. Schmitt and Uglow, (1993) have shown that sudden changes in temperature appear to cause stress, as exhibited by increased nitrogen effluence rates. Similarly stress was also caused by handling. Avault, (1986) reviewing seven years of research on *Macrobachium rosenbergii* production in Lousiana noted that, regardless of experimental design and salinity, prawns died when temperatures fell to about 13°C and required a minimum of 18°C for growth and dissolved Oxygen from 6-8 ppm levels below 25 to 30% saturation also caused visible distress and the optimum pH was 7.0 to 8.5.

Although the growth rate of individual *Macrobrachium rosenbergii* is rapid (up to 100 to 120g in 7 to 9 months) in a population, there are wide divergences in growth rate. Sorgeloos and Leger, (1992) have made concerted efforts in Belgium in improving the larval nutrition of *Macrobrachium rosenbergii* by using live food organism, artemia and rotifers with the help of enrichment techniques. Evaluation of distillery dried grains and solubles, fish meal as feed ingredients to the giant fresh water prawn in U.S.A was done

by Tidwell et al., (1993).

In India, research work was initiated much earlier by Subramaniam (1984). , on the use of different feeds for *Macrobrachium rosenbergii* at Kakinada and by Rao in 1991 on the use of Taifex worms as larval feed for *Macrobrachium rosenbergii* and *Macrobrachium malcolmsonii*. Trials on hatchery production and larval rearing nursery and grow out production of *Macrobrachium rosenbergii* were conducted at Kochi by Nair and Hameed in 1992 and by Thampy and Nair in 1992.

Similar work has been done by Reddy et al; (1991,1994), at the Central Institute of Fisheries Education at Mumbai, Sebastian et al, (1994) at Rosenfisheries, Trichur; Ravishankar and Keshavanath, (1988), Ahmed and Varghese, (1992), Shamila and Joseph, (1995) and Murthy, (1995) at College Of Fisheries, Mangalore; Rao, (1994) at CIBA, Vasudevappa et al; (1994) at Fisheries Research Station Bangalore.

In India freshwater prawns are traditionally cultured on a small scale. In the "Pokkali" fields of Kerala and the "Bheries" in West Bengal, along with several brackish water shrimps and

fish, a few species of fresh water prawns namely, *Macrobrachium rosenbergii* and *Macrobrachium rude* etc, also form part of the produce. Thakur, in 1993, found that in these systems, the production or the percentage contribution of fresh water prawn was limited due to the constrains in the farming system.

Subramaniam in 1984 reported a production of 280-700kg/ha of *Macrobrachium rosenbergii* in six months, depending on the conditions of the pond at a stocking rate of 30,000/ha following monoculture practice. Experiments conducted in Tamil Nadu have shown a production range of 350-725 kg/ha of *Macrobrachium rosenbergii* in 6-7 months under monoculture (Anon, 1990).

Durairaj et al., (1992) attempted fresh water prawn culture in Tamil Nadu and achieved production up to 630 kg/ha of *Macrobrachium rosenbergii* in 6 months under monoculture, while the production under polyculture was 411 kg of prawn and 840 kg of fish/ha/6 months.

According to Raje and Joshi (1992), the yield in prawn farming ranged between 535 kg/ha in 9 to 10 months of culture period under mono culture systems. Ahmed and Varghese (1992) based on their studies in Mangalore, reported that, survival and growth of prawns were better in polyculture with Indian and Chinese carps. Susheela et al., (1992) obtained production of *Macrobrachium rosenbergii* ranging

between 105.8 and 254 kg/ha/160 days in monoculture and 297-408kg/ha of prawns and fish together in 160 days.

Although research work was initiated as early as in 1970's, the culture of the giant fresh water prawn *Macrobrachium rosenbergii* in an organised and commercial scale, has not taken place in the country. Only small scale culture by farmers had been done here and there. The probable reasons for the slow pace of development appears to be the inadequate supply of seed and the lack of extension work. Although private hatcheries exist in the country, the production does not cater to the needs and demands of the prawn farmers. Considering the present scenario of Aquaculture in India, farming of *Macrobrachium rosenbergii* has a greater scope and potentiality.

2.2 NUTRITIONAL REQUIREMENTS OF MACROBRACHIUM ROSENBERGII

The nutritional requirements of crustacea have been reviewed by New (1976, 1980) and Conklin (1980) and they suggested that crustacea appear to have all the dietary requirements usually associated with complex metazoa (Dall and Moriarty, 1983). The nutrients required by fish and crustaceans for growth, reproduction and other normal physiological functions are similar to those of land animals. They need protein (amino acids), minerals, vitamins, lipids, growth factors and energy sources.

2:2:1 PROTEINS AND AMINO ACIDS.

According to Das (1993), dietary proteins are required for maintenance, tissue repair and growth. The utilisation of dietary protein is mainly affected by its amino acid composition, level of protein intake, calorie content of diet and physiological state of the animal.

Azad (1996) reported that protein requirements of fish and shellfish are high, both as a source of amino acids for protein synthesis and also for gluconeogenesis. The protein requirement of any organism need, not only to satisfy the organism's need for substrates, for the maintenance and growth, but also, to exert a regulatory influence on the organism which activates the various processes associated with the growth (Millward, 1989).

Since the early work of Subrahmanyam 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 on different levels of dietary proteins. Most of these studies on the protein requirement of the prawns and shrimps have been reviewed in detail by New (1976, 1979, 1980), Forster (1976), Wickens (1976), Rao (1983), Kanazawa (1984), Gopal (1986) and Ali (1988). Many of these authors also had discussed the problems associated with conducting the basic nutrient requirement studies in crustacea. 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 giant fresh water prawn *Macrobrachium rosenbergii*.

Gomez et al., (1988) found that the protein requirement of *Macrobachium rosenbergii* juveniles was 13-25%. Law et al, (1992) observed that a 40% protein diet supported optimal growth compared to 25, 30 and 50%. The results of the various experiments reveal the importance of evaluation of the quality of protein sources in the formulated feed of prawn, as the protein requirement is dependent on the source of protein. The essential aminoacids of prawn and the similarity of these amino acids of prawn with protein source also gain importance.

Bhasker and Ali (1984) demonstrated that the dietary protein requirement of the post larvae of *Penaeus indicus* decreased with an increase in their age. These authors found the protein requirement of the early post larvae of the prawn to be 40% compared to 30% in the case of the late post larval stage.

Goodwin and Hanson (1975), Colvin and Brand (1977), Deshimaru and Yone (1978), Khannapa (1979) and Segwick (1979) also reported a reduction in the protein requirement value with age in penaeid prawns. Balazs et al, (1974), reported a similar trend in protein requirement of *Macrobrachium rosenbergii*. It can be seen that even for a single species of shrimp, there is

wide variation in the reported protein requirement. The reported level of protein for *Penaeus japonicus*, ranges from 42 to over 60%.

Pandian (1989) reported that Macrobrachium species require less protein in their diet, nearly one third of that required for penaeids. According to Das (1993), the gross protein requirements of *Macrobrachium rosenbergii* for the first 119 days, post metamorphosis was 35% and ranged between 28 to 30% for the next 120 to 175 days.

A critical analysis of available information of protein requirement on *Macrobrachium rosenbergii* reveals that most of the researchers put it in the range of 13 to 25% while Millikin et al., (1980) reported the optimum as high as 40%. On the other hand others such as Balazs et al., (1973) and Balazs and Ross (1976), D' Abramo and Read (1988) have observed the optimum protein requirement as between 30 and 35%.

Commercial feeds, used for Macrobrachium species culture, varied in protein levels in different regions. The protein levels include 23.8 to 38.5% in Hawaii (Corbin et al., 1983), 22 to 30% in Thailand (Asian/UNDP/FAO 1988), 28 to 36 % in Taiwan (Hsieh et al., 1989) and 25 to 30% in French Guiana (Ifremer, 1989) for the culture of Macrobrachium species.

Interestingly, at 14% protein level also a satisfactory growth had been reported by Barlett and Enkerlin (1983).

Teshima and Kanazawa (1984) who had conducted trials using purified diet with carragenan as binder on *Penaeus japonicus* post larvae, found levels of protein around 45, 45 to 55 and 55% or more as optimum, when diets contained carbohydrates at 25, 15, and 5% respectively. Very recently Koshio et al; (1993) reported maximum growth of *Penaeus japonicus* at a level of 42% dietary protein in the diet.

According to New (1976) diets based on casein had generally produced poor result with shrimp, due to the low arginine content of casein. Fish meal, as a high quality protein source, seems to have lower nutritional value for shrimp, as reviewed by Sick and Andrews (1973), Colvin (1976) and Shigueno (1975).

All shrimp species that have been studied, require the same 10 essential amino acids as fin-fishes and terrestrial animals. (Lovell, 1989) Arginine, histidine, tryptophan, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine have been found to be essential for *Penaeus japonicus*. (Kanazawa and Teshima, 1981) *Penaeus aztcus* (Cowey and Forster, 1971) and *Penaeus monodon* (Coloso and Cruz, 1980).

Works on amino acid requirement of fresh water prawns are scarce. Watanabe (1975) and Stahl and Aheran (1978) reported that lysine is probably non essential and tyrosine is essential in addition to arginine, histidine, isoleucine, methionine, phenylalanine and valine for the giant prawn *Macrobrachium rosenbergii*.

The essentiality of various amino acids for prawns may be studied either by growth studies or by C-14 labelling studies (Wilson,1989). According to Miyajuna et al., (1977) and Penaflorida (1989) all crustaceans require arginine, primarily due to the lack of urea cycle in crustaceans which is necessary for arginine biosynthesis for ornithine. Although alanine is thought to have an osmoregulatory function, taurine is involved in cardiac and neural processes in prawns and, improvements due to supplementation of amino acid have been noted, and the quantitative dietary requirements of *Macrobrachium rosenbergii* remain to be defined. Prawns may have a dietary requirement for taurine and may be fulfilled by its biosynthesis (Smith et al., 1987).

Certain amino acids could be spared by other amino acid and that was summarised by Paul Raj (1993). Methionine could be spared by cystine, thereby the level of cystine in the feed should be considered while adjusting the methionine level in

the feed. Phenyl alanine is spared by tyrosine in fish, but in prawn, phenyl alanine is converted into tyrosine by an irreversible pathway. So adequate level of phenylalanine must be ensured in the feed of prawn.

Bioavailability of amino acid is yet another important factor while considering the amino acid requirement in the feed Deshimaru and Kuroki (1975). Deshimaru (1982) have shown that diets containing only amino acids instead of protein, brought about poor growth and high mortality in the feeding trials of *Penaeus japonicus*.

Based on their study of amino acid supplementation to the diets of *Macrobrachium rosenbergii*, Stahl and Aheran (1978) suggested that searching for optimum amino acid levels for incorporation into commercial feeds, may be less important than it was earlier thought.

The evaluation of the quality of various protein sources, which are found to be suitable for incorporation into practical diets, based on growth studies, gains importance rather than trying to find quantitative amino acid requirement and balancing their level in the commercial feed of shrimps and prawns.

2:2:2 CARBOHYDRATES

The energy value of a diet affects partitioning and utilization of protein, lipid and carbohydrate content of a diet. Das (1993) and Lovell (1989) reported that excessive levels of protein/energy ratios in the diet usually resulted in the reduction of growth rate.

According to Chuang et al., (1985), shrimps and prawns are known to be able to utilise complex polysaccharides better than simple ones, such as glucose. Carbohydrate sparing of protein for energy is possible, because prawn possesses both cellulases and chitinases. Furthermore, dietary fibre levels up to 30% have shown not to suppress growth experimentally. *Macrobrachium rosenbergii* has a much higher alpha-amylase and cellulase activities in either hepatopancreas, stomach or intestine than those of the marine shrimp *Penaeus monodon*, *P.japonicus*, *P.penicillatus* or *Metapenaeus monoceros*.

Briggs (1991) found that the best growth rate was achieved with wheat flour, followed by the pure polysaccharides dextrin and alpha-starch at 94% and the disaccharide sucrose at 90%. Alpha-cellulose, glucose and chitin were less effective. The results confirm that complex carbohydrates are utilised more effectively than simpler di or monosaccharides.

An important finding was that cellulose, often used as a "non nutrient" bulking ingredient in diets used for nutritional studies, may contribute significantly to the nutrition of prawns. The main substrate used by *Macrobrachium rosenbergii* post larvae and juveniles to meet metabolic requirements, was found to be carbohydrates by Diaz-Herrera et al., (1992) and Gomez et al., (1990), who examined the effect of various carbohydrate sources in a purified diet and found that glucose gave the lowest weight gain in juvenile *Macrobrachium rosenbergii*. After 60 days, the best weight gains were achieved by diets containing alpha-potato starch or soluble starch with dextrin while sucrose and glycogen were found to be less effective.

Hysmith et al., (1973) while experimenting with *Penaeus aztcus* observed that low protein/high energy and high protein/low energy diets gave better growth rate than low protein/low energy or high protein/high energy diets.

According to Shiau and Chou (1991), when energy level of the diet is kept at a level of 330 kcal/g, the dietary protein level of *Penaeus monodon* can be lowered from 40 to 36%. Therefore it is critical to obtain the proper protein energy P/E ratio in a diet, for the most economical production of shrimp.

The feed, with high dietary protein in the presence of low levels of non protein energy, may force the crustaceans to deaminate significant proportions of protein, and thus yielding the carbon fragments required for cellular metabolism (Lovell, 1989).

Sedgwick (1979) showed that, excessive energy in the diet, decreases feed intake and consequently limits the consumption of protein and other essential nutrients. Lim and Pascual (1979) observed that *Panaeus monodon* juveniles (about 1g) grew best when fed 38% protein diets having energy values of 3.2 to 3.6 kcal/g of diet.

The optimum energy to protein ratios for various species of shrimp at different sizes have not yet been defined. Addition of 1 to 2 parts of carbohydrate per one part of protein for *Penaeus monodon* diets (Bages and sloanes, 1981) or 25% carbohydrate in 45% protein diets for *Penaeus japonicus* (Teshima and Kanazawa, 1984), allowed satisfactory growth.

Andrews et al., (1972) showed that addition of 20% glucose to a menhaden meal based diet for *Penaeus aztcus*, produced a reduced growth rate, while an inclusion of 30% starch in the diet with lower protein diet, increased growth rate.

Rahman et al., (1979), Aquacop (1978), and Ali (1993) also reviewed that, *Penaeus japonicus* juveniles had a better weight gain on diets containing disaccharide and polysaccharides, than on diets containing monosaccharide. Among the various combinations of carbohydrates, sucrose, maltose, starch mixture at equal proportion was found to be the best, compared to others. Ali (1982) also showed that in *Penaeus indicus* the best growth response was observed while using 40% starch in a casein based diet.

Apart from the energy yielding function, carbohydrate, if properly gelatinised by heat treatment, may improve the water stability of shrimp diets. Inclusion of 40% corn starch in casein based diet for Penaeid duorarum produced a faster growth than 10 or 0% starch (Sick and Andrews, 1973). Rahman et al., (1979) had studied the effect of dietary carbohydrate on the body weight gain, survival, PER and the hepatopancreatic glycogen and serum glucose levels of the prawn *Penaeus japonicus*.

2:2:3 Lipids

Lipids are required in the diets of shrimps, not only for their energy value, but also as a source of essential fatty acids, fat soluble vitamins, sterols and phospholipids.

Das (1993) showed that, shrimps appear to have dietary requirements for fatty acids of the linoleic and linolenic series. It is generally accepted that, crustacea, cannot tolerate high levels of dietary fat and more than 10% is not recommended. High levels of lipids are usually associated with significant retardation of growth (Forster and Beard, 1973; Deshimaru and Kuroki, 1974; Kanazawa et al; 1977).

Mendoza (1982) found that, a diet containing 11.7% lipid gave maximum growth and feed efficiency for *Penaeus monodon* juveniles. Catacutan and Kanazawa (1985) showed that, the best growth of *Penaeus monodon* juvenile was obtained with lipid source containing high amounts of highly unsaturated fatty acids of (n-3) series in semi purified diet, at 10-11% level in their diet.

Sheen and D'Abramo (1991) while experimenting in *Macrobrachium rosenbergii* juveniles, tested 6 lipid levels 0 -12% with cod liver oil and corn oil in 2:1 ratio and observed significant reduction in weight when 10 or 12% lipid level was tried. Polyunsaturated fatty acids of linolenic (n-3) and linoleic (n-6) families, viz. 18:2 n-6, 18:3 n-3, 20:5 n-3, 22:6 n-3 have been recognised as very important nutrients for the growth of crustaceans (D'Abramo and Sheen, 1993).

Growth enhancing response to dietary oils containing comparatively high levels of C≥20 n-3 PUFA viz. shrimp head, anchovy, sardine, clam or cod liver oils, was observed by Sandifer and Joseph (1976) for *Macrobrachium rosenbergii*. A similar observation was made by Kanazawa et al., (1977), Guary et al., (1976) for *Penaeus japonicus*, by Read (1981) for *Penaeus indicus* and by Martin (1980) for *Palaemon serratus*. It was found that *Penaeus japonicus* and *Macrobrachium rosenbergii* species had the ability to synthesize saturated and monounsaturated fatty acids from 16:0 palmitic acid but these fatty acids are not in turn transformed into 18:2 n-6, 18:3 n-3, 20:5 n-3, or 22:6 n-3 (Kanzawa et al., 1979 and Reigh, and Stickney 1989).

Crustacaens do not synthesise sterols and therefore require a dietary source. Prior to 1989, research with semi purified diets containing a basal level of 0.12% cholesterol and 0.04% lecithin, had indicated that, a diet supplemented with 0.5 or 1% cholesterol or 5% lecithin, was not beneficial. D'Abramo and Daniels (1994) found that, depriving *Macrobrachium rosenbergii* of all sterols, caused death within 48 days. The cholesterol requirement was estimated as 0.3 and 0.6% dw of the diet. Levels above 0.6% either did not increase weight gain or decrease it. Substitution with 0.6% ergosterol or stigmasterol gave a lower survival and weight gain than 0.6% cholesterol. However, a

mixture of phytosterols (sitosterol, campesterol and dihydrobrassicasterol) was found to be as effective as cholesterol, the authors postulated that commercial diets may satisfy the sterol requirements of *Machrobrachium rosenbergii* with the inclusion of plant derived ingredients. This is in marked contrast to the marine shrimp which, being carnivorous appear to have a requirement exclusively for cholesterol.

By examining the effect of a protein source (Crab protein and casein) and 0,1 and 2% soyabean lecithin, in a 30 day trial with 0.8g *Macrobrachium rosenbergii* communally reared, Koshio et al., (1992) found that neither had any effect. The two alternative levels of soyabean lecithin showed no effect on communally reared prawns, but in a group reared individually, lecithin seemed to promote growth.

Using purified diets, containing various lipid sources and fatty acids, Teshima et al., (1992) found that, the highest weight gain in *Macrobrachium rosenbergii* was in the groups fed with soyabean oil plus linolenic acid 18:3 n-3 followed by those containing soyabean alone, pollack liver oil, corn oil and no lipid.

2:2:4 Minerals.

In animal nutrition, while protein, lipid and carbohydrates are required in major quantities, vitamins and

minerals are required in small quantities in the diet. Ali (1987) showed that minerals are very essential because, animals are not capable of synthesising them and should be supplied through, external source. Dietary mineral studies for prawns before 1990 were absent. The tendency was to treat their mineral requirements as similar to the marine shrimp.

Zimmer-mann et al., (1994) had demonstrated the important relationship between dietary calcium level and water hardness. As with most aquatic animals, shrimps and prawns can absorb or excrete minerals directly from or to aquatic environment, through their gills and body surface. Therefore, the dietary requirements of minerals largely depend on the mineral concentration of water in which, they are reared.

Das (1993) observed that, the ash content of prawn was as high as 15.9 and 21.3% of the dry weight, suggesting that, mineral nutrition may be important for the overall animal health and their well being.

According to Kitabayashi et al., (1971) the best growth rates were achieved, with diets for *Panaeus japonicus*, when supplementary levels of 1.04% phosphorous and 1.24% calcium were added. Inclusion of a mineral premix at 5% level in a casein based diet for *Penaeus aztcus*, gave 18% increase in

biomass (Sick et al., 1972) and no increase was seen when the premix was omitted.

Deshimaru and Kuroki (1974) using a casein egg albumin diet for *Penaeus japonicus* reported that the highest growth increment was noted, when the Ca:P ratio was maintained at 0.76:1. Shewbart et al., (1973) postulated that, Ca, K, Na and chloride requirements for *Penaeus aztecus* might be satisfied through osmotic regulation. Phosphorous however, may be essential in the diet because it is present in large quantities in shrimp, but not in sea water.

Kanazawa (1982) and Ali (1987) reported that, the optimum levels of minerals in the *Penaeus japonicus* juveniles are, 1% calcium, 1% phosphorous, 0.9% potassium, 0.3% magnesium and 0.006% copper.

2:2:5 Vitamins.

Knowledge of the vitamin requirements of crustaceans in general, is very limited, particularly if one is referring to those candidate species identified for commercial culture (Walker, 1988). The composition and levels of several vitamin premixes used in studies with Penaeus and Macrobrachium species were reviewed by New (1976 and 1995).

Little was known about the dietary vitamin and mineral requirements of prawns up to 1989, except that, vitamin C deficiency symptoms, including failure to moult, had been described. Significant growth rate reduction had been demonstrated by the omission of dietary pyridoxine.

The leaching of water soluble vitamins, even in apparently well bound feeds, is significant and it is this, rather than intrinsic vitamin deficiencies, which may cause problems. Both the calcium salt of ascorbyl 2 monophosphate and ascorbyl palmitate, appear to be effective sources of vitamin C, in juvenile prawn feeds (D'abramo and Reed 1983). Survival decreased, with the decreasing levels of ascorbic acid and maximum weight gain was observed, when the apparent available ascorbic acid level was between 50 and 100mg/kg diet, as reviewed by New (1995).

The present understanding of the qualitative vitamin requirements of Penaeid shrimps, has been based almost entirely on the studies on *Penaeus japonicus*, which is considered as the best documented species, observed by Kanazawa et al., (1976) and Deshimaru and Kuroki (1976). Chen (1993) found that, shrimps have much higher vitamin requirements than the other aquatic animals, such as fish and this variation is suggested to have caused by the differences in their feeding behaviour, size, growth rate, environmental factors and nutrient interrelationships. Since

shrimp is a slow feeder, prolonged suspension of feed in water, increases the leaching of nutrients. Hence, a higher vitamin requirement is needed for crustaceans, than for fish. Vitamins are essential for normal growth, maintenance and reproduction. The metabolic functions of vitamins in shrimps and prawns had been discussed by Paul Raj (1993).

In crustaceans, vitamin C deficiency, leads to the inhibition of alkaline phosphatase activity, resulting in, poor chitin synthesis (Paul Raj, 1993). Vitamin C is required for hydroxylation of collagen. Kitabayashi et al., (1971) observed accelerated growth in *Penaeus japonicus*, fed with diet containing vitamin C. The optimum level was found to be 0.22%. Deshimanu and Kuroki (1976) have shown that, juveniles of *Penaeus japonicus* require, 3000-10,000mg of vitamin C, 600mg of choline, 2000-4000mg of inositol, 60-120mg of thiamine and 120mg of pyridoxine per kilogram of feed.

Shiau and Jan (1992) found that *Penaeus monodon* requires 2000mg of vitamin/kg of feed, which is closer but slightly lower to the optimum suggested by Kitabayashi et al., (1971) but much lower than the optimum requirement suggested by Deshimaru and Kuroki (1976) for *Penaeus japonicus*.

One factor that may be involved in the apparent difference in dietary vitamin C requirement may be, the omnivorous nature of *Penaeus monodon* compared to *Penaeus japonicus* (Chen, 1993).

An important factor to be considered in the vitamin C requirement, is the source and its stability, during processing and subsequent storage. Ascorbic acid is the most sensitive vitamin to degradation, during feed processing and storage. As much as 80-100 % of the initial amount of supplemental L.ascorbic acid can be lost during processing and subsequent storage (Grant et al., 1989). L.ascorbyl 2 polyphosphate (APP) is relatively resistant to oxidation. When this form of vitamin C was used, the requirement of vitamin C for *Penaeus japonicus*, was found to be 100mg per kg of feed (Shigueno and Itoh, 1988), much lower than the requirement suggested by Deshimaru and Kuroki (1976) for the same species, using L.ascorbic acid.

Recently vitamin C requirement of *Penaeus vannamei* was determined as 120mg/kg of feed. He and Lawrence (1993), Catacutan and Cruz (1989) found that growth was poorest for treatments without vitamin supplement, inositol or choline deficient diet.

Limitations in our knowledge about nutrition of prawn underscore the need to continuously develop tests and apply new nutritional concepts. This is particularly true for the rapidly expanding

shrimp feed industry, when the feed formulations are presently based largely on assumptions and unknown growth factors, rather than nutritional science.

2:3 FOOD AND FEEDING

Food represents the largest single cost item in the intensive prawn production. The artificial feeds should be scientifically formulated meeting out the full requirements. At high stocking densities inadequate feeding leads to poor growth (Paul Raj, 1993 and Das, 1993).

A number of variables such as stage in growth, moulting, water quality, water temperature, feed stability, feed palatability, mode of presentation, bioavailability of protein and other nutrients, health of prawns, effect of natural feeds, feeding rates etc, synergistically affect the overall growth and feed utilisation (Paul Raj, 1987).

Presentation of feed in its most suitable physical form is the key to the most successful performance of the feed. Ali (1987) reported that the physical design of a feed should be in accordance with the feeding habits of the candidate animal and should not cause any impediment to its feeding activity.

Different types of feed may require further development to meet the varied needs of different species and sizes of larvae. Microencapsulated diet for larval and post larval diets have been

advocated by Meyers (1973), quoted by Kandaswami and Muthiah (1987). Several authors have reported very good growth and survival, by partial or complete replacement of live diets, with microencapsulated diets (MED). (Jones et al., 1987; Kurmaly et al., 1989; Amjad et al., 1993) and with microparticulated diets (Kanazawa et al., 1982; Galgani and Aquacop,1988; Ottogali, 1991) for rearing penaeid larvae both in the laboratory and in the hatcheries. Generally higher protein diets are fed during early post larval stages and the juvenile stages and the protein percentage is decreased during the grow out period (Lovell, 1989).

Paul Raj (1987) also reported that feeds containing a mixture of fish meal, squid meal, clam meal, mussel meal, crab meal, shrimp meal, shrimp head meal and squilla meal, are effectively utilised for shrimps. Tidewell et al., (1994) studied the effect of replacing all or part of the fish meal in a prawn feed with soyabean meal with DDGS. Replacing all or half of the original in 32% protein diets by 40% DDGS and a variable percentage of soyabean meal, had little effect on the average yield, survival, individual weight and feed conversion.

Jayalakshmy and Natarajan (1993) studied 4 different pelleted feeds prepared, using different plant matter for their growth, conversion efficiency and moulting in M.idella. Koshio et al, (1992) found that there was a general tendency for the FCR and PER of the prawn groups fed with diets containing soyabean protein to be better than

those including crab protein concentrate, even though the SBP has lower lysine and methionine levels, than either CPC or whole prawns

Law et al., (1985,1990) studied the digestibilities of low cost ingredients, such as fish meal, soyabean meal, copra cake, shrimp meal and wheat flour in 30% and 40% protein feeds by juvenile and adult *Macrobrachium rosenbergii.*

Mossmann et al., (1990) reported the use of earthworm as a protein source for the basic diet of the Malaysian prawn *Macrobracium rosenbergii*. Reed and D'Abramo (1989) evaluated two standard reference diets for the use in studies of nutrition. Das (1993) reported that, the main objective to prepare formula feed is to combine several selected ingredients proportionately, so as to make a balanced feed, which ensures good growth and optimum production. Formulation of the feed is mainly based on, fulfilling the protein and energy levels required for prawns.

Lovell (1989) described that, shrimps are territorial and do not swim great distances to get food. Thus, it is important to distribute the feed, uniformly over the pond and as they are very slow eaters and feed, more or less continuously, a multiple daily feeding is desirable. According to Lim and Pascual (1979) the optimum feeding frequency of *Penaeus monodon* juveniles, was three times a day.

Webster et al., (1992) showed that, soyabean meal or less expensive plant protein, is considered to be the most nutritious and is currently used as the major protein source in many fish diets, to partially or totally replace fish meal. Cheutama , (1992) studied the addition of soyabean meal paste, shrimp meal, anchovy head meal, brewers yeast meal and shrimp into diets up to 45%, 40%, 60% and 34%, to replace fish meal.

Teshima et al., (1993) showed that, the prawn *Penaeus japonicus* was successfully reared with a microparticulate diet, without using any live food. The larvae prawn requires, dietary sources of sterols and some phospholipids as indispensable nutrients, for normal growth and survival.

Hartnoll and Sallama(1992) reared the shrimp *Palaemon elegans,* with 35% protein content from different sources, like shrimp muscle, soyabean meal, ground dried glycimeri's and an equal mixture of the three. Bhasker and Ali (1984) formulated six purified diets., using casein, starch, fish and ground nut oil mixture 1:1 ratio, vitamins, minerals and other additives with the protein content, varying from 20 to 70%, to study the protein requirement of post larvae.

Ali (1982) formulated four purified diets, using casein, gelatin, starch, fish oil, ground nut oil, vitamins, minerals and cellulose. The carbohydrate in the diet was increased from 10 to

40% and he studied the effect of carbohydrate level on growth, survival and food conversion of prawn *Penaeus indicus*.

Shiau and Chou (1991) conducted experiments with two pelleted and one extruded diets, compounded with the animal proteins from fish meal, shrimp waste meal, squid offal meal in combination with, plant proteins from soyabean meal and groundnut oil cake, to juvenile *Penaeus indicus*. The growth was compared with the prawn, fed with clam and squilla meat.

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

Sick and Andrews (1973) observed higher growth rate for *Penaeus duorarum*, with shrimp meal diet, the casein and maize gluten meal, but showed, a poor growth response to soyabean meal and fish meal. Forster and Gabbot (1971) reported that, in *Palaemon serratus*, the assimilation efficiency for shrimp meal, was poorer than that of casein, gelatin, egg albumin, freeze dried egg, mussel mantle, fish meal, maize gluten meal, ground nut meal and bacterial protein. Balazs et al., (1973) observed no benefit, when shrimp meal was used along with Tuna and soyabean meal at 25 and 35% protein level.

Akiyama et al., (1988), Aquacop et al., (1989) reported poor protein digestibility of shrimp meal compared to the other protein meals in different species. Cruz Suarez et al., (1993) observed a positive dose response relationship in the growth rate of *Penaeus vannamei,* when shrimp meal was used at 18% level with the other sources. Shrimp meal is high in crude protein content and several amino acids and it is a good source for fatty acids. Forster (1976), Sandifer and Joseph (1976), Raman et al., (1982) and Pascal and Destajo (1979) observed that, shrimp meal as a single source of protein give a poor rate of growth in *Penaeus indicus* and *Penaeus monodon.*

Akiyama et al., (1992) found that commercial shrimp feed usually contained 5 to 15% shrimp meal. Durairaj et al., (1992) noted an improved growth rate when prawns in manured ponds, were fed with a pelleted feed containing shrimp head meal 20% and fish meal 10% compared to the conventional feed of 1:2:1 ground nut oil cake, rice bran and thrash fish.

Ali (1982) used squilla protein in the formulated feed of *Penaeus indicus* after coagulating and drying the protein. This was compared with the other compounded feeds based on clam meal, prawn waste, fish meal and a control (fresh clam meat). The best growth response was observed in the mantis shrimp

based diet, closely followed by clam meal diet. But the feed efficiency was better for clam meal based diet.

Later Ali and Mohammed (1985) tried four combinations of prawn waste and mantis shrimp in the formulated feed for *Penaeus indicus*. The best growth response and feed efficiency were noted when 25% prawn waste and 35% mantis shrimp were used together in the feed.

Silk worm pupae is yet another source of protein which seems to be potential for shrimp and prawn feed. Ali (1988) explored the possibility of incorporating silk worm pupae, as a protein source, in the formulated feeds for, *Penaeus indicus* juveniles. But the result showed that, feeding with silk worm pupae diet, resulted in low digestibility, protein efficiency ratio and biological value for, *Penaeus indicus*. He attributed it, to the high chitin content of silk worm pupae.

Ravishankar and Keshavanath (1988) found that *Macrobrachium rosenbergii* utilised, feed pellets containing silk worm pupae plus shrimp waste, more efficiently and gave a higher specific growth rate, than the diets containing fish meal, silk worm pupae alone or silk worm pupae plus clam meat.

Unnikrishnan et al., (1992) was able to substitute extracted silk worm pupae for extracted clam meal (both 66% protein) as 61% of a semipurified diet for *Macrobrachium rosenbergii* post larvae, without any detrimental effect on the survival, growth rate or PER. This confirms the ability of *Macrobrachium rosenbergii,* to utilise chitin via chitinoblastic bacteria in the intestinal tract. According to Hanson and Goodwin (1977) penaeids appear to have two chitinase systems, one in hepatopancreas and the other centred around the chitinoblastic bacteria, in the digestive gland. Several studies have reported, measurable levels of chitin degrading enzymes in the alimentary tract of various crustaceans (Chandramohan and Thomas, 1984; Lynn, 1990; Bath et al., 1990). In addition, it is commonly observed that shrimps and prawns consume their exuviae following ecdysis. These observations suggest that, crustaceans may be able to utilise the dietary chitin.

Vaitheswaram and Ali (1986) reported that, glucosamine and chitin, have a growth promoting effect in *Penaeus indicus*. It may also be seen that. the purified diets proposed for the use in nutritional studies with shrimps and prawns, contain 0.5% glucosamine (Kanazawa et al., 1970 and Kanazawa 1982).

Among algal powders, spirulina was found to promote growth in *Penaeus japonicus* (Cuzon et al., 1981). Zimmermann

(1991) demonstrated that dry sugarcane yeast, a byproduct of alcohol production from sugarcane, could be used at the inclusion levels up to, 20% in a 30% protein grow out feed, with acceptable results. Beef liver was found to be superior to squid mantle, cod muscle or cooked chicken egg when fed as 20% (DM) of the diet (Heinen and Mensi, 1991). Dried clam meat, as principal protein source, gave better result in terms of growth and survival. compared to a diet based on fish meal, for *Macrobrachium rosenbergii*, reported by Sherief (1989).

2:4 DIGESTIVE ENZYME ACTIVITY

A considerable amount of literature has accumulated on the digestive enzymes of the decapod crustaceans. Studies of crustacean digestive enzymes have been reviewed by Vonk (1960), Hartenstein (1964), Huggins and Munday (1968), Vanweel (1970), Devillez (1975), Wickins (1976), Gibson and Barker (1979) and Dall and Moriarty (1983).

According to Mclaughlin (1983) and Vonk (1960), the crustacean gut is mostly straight consisting of three main divisions, foregut, midgut and hindgut and its accompanying glands ceca and diverticula. The foregut may be a simple passage way or highly complex chambered structure provided with triturating, straining and filtering mechanisms (Mclaughlin, 1983 and Vijayakumaran, 1987). The digestion and absorption of nutrients are carried out inside the

tubules of the digestive gland. The digestive gland is a pair of bilobbed glands lying on either side of the gut and opens at or near the foregut and secretes digestive enzymes (Dall and Moriarty, 1983; Vijayakumaran, 1987).

Knowledge of the types and activities of crustacean digestive enzymes is meagre, although only a few enzymes have been characterised in detail (Dall and Moriarty, 1983). Digestive enzymes are secreted into the tubule by either the F-cells or the early B-cells. There is general agreement that the R-cells have absorptive and storage functions (Bay, 1992). Enzymes are secreted and passed into the foregut, while the chyme from the filter press enters the tubules, where the final stages of digestion and absorption occur.

Digestion involves a series of processes in the digestive tract and this is accomplished by a combination of mechanical and enzymatic processes (Hepher, 1988). Storage, trituration and digestion of food take place in the proventriculus and are completed in the midgut. In order to digest food, they require a full complement of digestive enzymes, proteases, amylases, and carbohydrases (Bay, 1992). Extensive studies on the gut structure of *Macrobrachium rosenbergii* (Deru, 1990) and *Palaemon elegans rathke* (Abubaker, 1991) revealed that, the early larvae, lacked the anterior midgut diverticula, which forms the main site for digestive enzyme production, during early stages in penaeids. The hepatopancreas is

also relatively small during early zoeal stages, but the size of this organ increases drastically after stage Z-5,6. Therefore it might be expected that a considerable increase in digestive enzyme activity levels may coincide with the expansion of hepatopancreas after stage Z-5, when they are able to survive on, artificial diets (Deru, 1990). Kamarudin et al., (1994) studied larval digestive enzymes in *Macrobrachium rosenbergii* and concluded that the larvae should have enough digestive enzymes, to digest artificial diets even during larval stages.

2:4:1 Proteolytic Digestive Enzymes

Proteolytic digestive enzymes are of two major types, Endopeptidases and Exopeptidases. Among the endopeptidases, trypsin, chymotrypsin, cathepsin and collagenase are peptidyl peptide hydrolases while the exopeptidases include, alpha amino acyl peptide hydrolases (aminopeptidases), peptidyl amino acid hydrolases (carboxypeptidase) and dipeptide hydrolases (dipeptidases) (Hemambika, 1989)

Proteolytic activity in gastric juice or hepatopancreas of decapods, was first demonstrated in Scyler 1876 quoted by Homar using casein, albumin, peptone, gelatin or fibrin as substrates. Protease activity from extracts of the stomach and hepatopancreas of the European lobster, *Homarus gammarus*, has been examined and compared by Glass and Stark (1994).

The growth, survival, digestive enzyme activity and biochemical composition of *Penaeus japonicus* larvae and post larvae were measured under three feeding regimes by Rodriguez et al., (1994). Ontogenic changes of proteases of *Penaeus monodon* from different larval stages to adult were investigated by Fanglee Shing et al., (1992) and found that the total protease activity was low during nauplius and zoea but peaked up, in mysis. The relationship between protein level and protein size and digestive protease enzyme activities of the marine shrimp *Penaeus vannamei boone* were investigated by Lee et al., (1984). A series of experiments were conducted on growth, survival, digestive enzyme activity and body protein content of *Penaeus japonicus* larvae reared on live and artificial diet by Levay et al., (1993).

The activities of trypsin like enzyme and pepsin in different stages of the larvae of prawn *Penaeus chinensis* under three different stages were different, as studied by Liu yumei et al., (1991). The effect of exogenous digestive enzymes on the growth, survival and midgut gland protease and amylase activities of the early post larvae *Penaeus monodon*, were studied by Chen and Kuo(1990) Protease in grass shrimp *Peneaus mondon* digestive tract were extracted by Jiang et al., (1991). Shukun (1987) studied the digestive enzymes of the prawn *Penaeus orientalis* and the adaptation of the digestive enzymes to diet composition showed that, the activities of proteinase and amylase can be affected and can respond to the diet,

the prawn, fed in about 5 to 8 days. In another study he found that the specific activities of caseinase, trypsin, carboxy peptidase A and B, alpha amylase, maltase and lipase in the hepatopancreas of the prawn *Penaeus orientalis* are far greater than those of chymotrypsin, beta glucosidase and beta galactosidase.

Lovett and Felder (1990) studied the ontogenetic changes in enzyme distribution and midgut function in the developmental stages of *Penaeus setiferus*, crustacea, decopoda, penaeidae and found that acid phosphatase and esterase activities were present in all gut tissue, at all stages. He also found that in all developmental stages, activities were present for trypsin, carboxypeptidases A and B, amylase and non specific esterase, none for pepsin or lipase were detected.

Glass et al., (1989) studied the digestive proteases in five marine species and compared them by biochemical methods. Galagani ., (1985 and 1983) studied the digestive proteases from the midgut gland of *Penaeus japonicus* in relation to temperature and the effect of environmental factors on digestive enzyme activities of crustaceans.

Maugle et al., (1982) studied the effect of short necked clam diets on shrimp growth and digestive enzyme activities of *Penaeus japonicus* and in another study he observed the

characteristics of the primary digestive enzymes fed for 30 days on a compounded dry diet containing 60% protein.

Galagani (1983) studied the proteolytic enzymes of hepatopancreas of six penaeid shrimps. Das et al., (1987) studied the comparitive activity of some digestive enzymes in fry and adult of a mullet *Liza parsia*. Buddington and Doroshev (1986) observed the digestive enzyme compliment of white sturgeon.

Ghosh (1985) studied the digestive enzymes of the Indian feather back *Notopterus chitala* in relation to its food habits. Clark et al., (1985) studied the metabolism in marine flat fish and protein digestion in Doversole. Onishi et al., (1976), and Overnell (1973) studied the changes in digestive enzyme levels in carp after feeding and its response of protease and amylase to twice a day of feeding and the digestive enzymes of the pyloric caeca and of their associated mesentry in the cod *Gadus morhua*. Brockerhoff et al., (1970) studied the digestive enzymes of the American lobster *Homarus americanus* and Hoyle (1973) studied digestive enzyme secretion after dietary variation in the American lobster *Homarus americanus*. [illegible]atra and [illegible] Ray (1987) studied the influence of dietary protein source on the protease activity, protein synthesis and certain biochemical composition of the flesh in *Anabas testudinius* for sixty days and the levels of protease activity in the hepatopancreas, stomach and intestine were recorded.

2:4:2 Amylase Activity

Among the carbohydrases which have been identified from Decapoda, amylase is found in most species. Blandamer and Beechey (1966) identified the enzyme from carcinus maenas as an amylase.

Alpha amylase has also been isolated and characterised from the isopod *Asellus aquatics* by Robson (1979). For the complete digestion of starch and glycogen, atleast two other types of enzymes are necessary viz either an oligo alpha 1,6 glucosidase or a debranching enzyme and a maltase (alpha 1,4 glucosidase). Alpha 1,4 glucosidase activity has been found in all crustaceans that have been investigated (Vanweel, 1970; Kristensen, 1972; Brun and Wojlowicz, 1976).

Kulkarni et al., (1979) investigated the amylase activity of the digestve system in marine penaeid prawns *Para penaeopsis hardwickii* and *Penaeus stylifera* and found that maximum amylase activity was found in the midgut gland, where as it decreased in the foregut and hindgut parts. Karunakaran (1977) studied the amylase activity of the extract of the different parts of the alimentary canal and hepatopancreas of the prawn, *Penaeus indicus* and *Metapenaeus monoceros*. Villasante et al., (1993) studied the crude extract from the Brown shrimp *Penaeus californiensis* and showed that considerable amylase activity was found at pH 6.5 to 8.0 with optimum pH at around 7.5 and temperature between 30 to 40°C.

Tolluec et al., (1992) measured the amylase activity and indicated that the two populations of F cells may be derived at the same cell type.

Alpha amylase activity has been demonstrated in all crustaceans and it has been isolated from *Homarus americanus* and in its acidic pH optimum at 5.2 (Dall and Moriarty, 1983). In *Penaeus japonicus* the enzyme alpha amylase has been identified to digest the complex carbohydrates, starch, or glycogen. (Maugle et al., 1982). Kitamikado and Tachino (1960) found that, amylolytic activity in young rainbow trout is quite high and increases as the fish grows reaching a peak at a weight of 100gm. Decreasing amylolytic activity with age was also found by Morishita et al., (19

Das et al., (1987) found that, in fry, amylase activity is a little less as compared to that of adults. Onishi et al., (1976), showed that, amylase activity reached a maximum at 5 to 7.5 hours although it decreased temporarily after each feeding.

2:4:3 Lipase Activity

The digestion of lipids has received less attention than protein digestion in crustacea. Lipase and esterase activities have been demonstrated in many crustaceans (Vanweel, 1970). Lipase has been partly purified from the foregut fluid of the lobster *Homarus americanus* and shown to have a molecular weight of 43000, which

is similar to that of hog pancreatic lipase. Optimum activity occured around pH 7 (Brockerhoff et al., 1970). Lipolytic enzymes and carboxylic ester hydrolases are of wide spread occurance in the decapoda. Early reports of lipolytic activity include those of Hoppe Scyler (1876), Yonge (1924) and Reddy (1938). True lipases which act only on ester water interface and preferentially hydrolyse the outer ester links, have been demonstrated only from *Homarus americanus*. The action of lipase in lobster foregut fluid towards triolein, is similar to that of vertebrate pancreatic lipase. In that, it seems to specifically hydrolyse the alpha linkage (Brockerhoff and Hoyle 1967). They have also shown that, radioactive labelled triacyl glycerol is absorbed with most of the label in the beta position intact. Thus fats are probably digested and absorbed as a mixture of free fatty acids, mono and diacyl glycerols.

Lipolytic activity has been found in extracts of digestive organs of many fish (Hepher, 1988). Lipases have been investigated in only three Penaeid species and all by, Lee and Lawrence in 1982. Lipases and esterases activities have been demonstrated in many crustaceans (Van Weel, 1970). Gopalakrishnan (1972) working with hepatopancreatic extracts of *Penaeus indicus* detected the presence of lipase using fresh cows milk. Hoyle (1973) has recorded lipase activity in the American lobster *Homarus americanus*. Trellu and Ciccaldi (1977) stated that, there was no true lipase in *Palemon serratus*, although they did find two esterases.

Lee et al., (1980), in the quantitative analysis of digestive enzymes of the fresh water prawn *Macrobrachium rosenbergii* could detect high esterase activity and only a trace of lipase activity. Phadate and Srikar (1987) studied the effect on growth and digestive protease, amylase and lipase activity of three species of carps. They were given three formulated feeds rich in protein, carbohydrate and lipid rich foods and found that protease activity was the highest in all the three species and carbohydrate rich feed had a slightly stimulating effect and lipid rich feed had a marked negative effect on the enzymatic activity.

2:5 NUCLEIC ACID COMPOSITION

Quantitative analysis of nucleic acids, provides a relatively simple means of estimating recent growth rates. The primary functions of Ribonucleic acid (RNA) involve protein synthesis. Deoxyribonucleic acid (DNA) is the primary carrier of genetic information and has been used as an index of cell number or biomass (Hansen, 1969; Regnault and Luquet, 1974).

The RNA-DNA ratio is an index of the amount of protein synthetic machinery per cell. Correlations between RNA concentration or RNA-DNA ratio and growth rate have been observed for a wide variety of organisms (Kennel and Magasanik, 1962; Bulow, 1970; Sutcliffe, 1970).

A number of indexes have been proposed for monitoring growth, feeding, reproduction, energy storage and conditions of fish populations. RNA-DNA ratios (RNA/DNA) have been demonstrated as reliable indicators of growth rate on experimental and natural populations of fish (Bulow, 1970; Nasiri, 1972; Haines, 1973; Sable, 1974; Spigarelli and Smith, 1976; Bulow et al., 1978).

Recent work has indicated, the usefulness of nucleic acid determinations, for the prediction of growth rate and the measurement of biomass production of marine phytoplankton and zooplankton (Sutcliffe, 1965; Hansen et al., 1968). In addition to the potential use in predicting and estimating growth and production, RNA and DNA analysis may be invaluable in many problematical areas requiring information as to whether, growth was occurring at the time of collection.

Buckley (1979) reported that, the RNA-DNA ratio appears to be a useful index, of nutritional status in larval Atlantic cod (Gadus morhua) and may be useful in determining, if cod larvae, were in a period of rapid or slow growth at the time of capture.

The ratio of RNA to DNA, has been related to both long term and recent growth in, adult fresh water fish. Haines in

1973, demonstrated a significant positive correlation between the population growth over a 15m period and the RNA-DNA ratio of muscle tissue, from small mouth bass *Micropterus dolomieui* and Carp *Cyprinus carpio.*

Bulow (1970,1971) reported that RNA-DNA ratios were very sensitive, to changes in feeding levels and could be used as an indicator of the recent growth rates on adult golden shiners *Notemigonus crysoleucas*. Buckley (1979) found that, the RNA-DNA ratio was useful for the diagnosis of the starving conditions in Winter Flounder *Pseudopleuronectes americanus* larvae.

Protein synthesis activity, in skeletal muscle tissue, is sensitive to alterations in the dietary composition and food intake. The content of ribosomal RNA (r RNA) in rat skeletal to incorporate amino acids into protein in vitro, is affected by protein starvation, amino acid depletion and the biological value of dietary nitrogen source (Decken and Omstedt, 1970,1972; Omstedt et al., 1973; Omstedt and Decken, 1972, 1974).

Buckley in 1984 reported that the RNA-DNA ratio analysis offers new possibilities in understanding the larval growth and mortality and their environmental variability.

Some laboratory studies have demonstrated relations between food availability and larval RNA-DNA ratio and growth rate of larval fish (Buckley 1979, 1980, 1981, 1982; Buckley et al., 1984).

2:6 WATER QUALITY PARAMETERS

Without proper water quality maintenance, one cannot get a good shrimp production. It affects the growth of fish and shrimp to a large extent (Hepher, 1988; Sundaraj, 1994). Various physical factors and chemical reactions, influence the water quality, to a great extent.

According to Chatterjee (1993), water is the primary requisite for the existence and growth of aquatic animals and in the absence of dissolved substances, water is unable to support aquatic life. Thus the presence of various substances depending, on their amount, alter the quality of water considerably and influence the biological production, in aquatic environments.

Temperature, nitrogenous compounds, dissolved oxygen, pH, hardness, ionic concentration, dissolved metals and salinity are the main physio-chemical components, which play important roles in the growth, metamorphosis and survival of larvae. Suitable ranges of these factors, contribute greatly to the successful seed production (Rao, 1993).

Water remains in contact with, both soil and atmosphere and also due to the presence of various dissolved substances, the water quality is modified considerably and thereby affect the biological production, in aquatic environment.

2:6:1 DISSOLVED OXYGEN

Determination of the metabolic rates, by measuring oxygen consumption is of prime importance in assessing the energy expenditure of an animal. Oxygen consumption of crustaceans, has been studied by several workers (Subramanyam, 1957 and 1962; Rao, 1958; Kutty, 1967; Kutty et al., 1971; Lakshminarayana and Kutty, 1982)g reported reduced oxygen consumption under hypoxic condition.

The concentration of oxygen in water may affect the growth of fish and crustaceans, either through their appetite and food consumption or through their food utilization (Hepher, 1988). Dissolved oxygen is indispensable, for the respiration of all forms of life, for aerobic decomposition and also for oxidation of various inorganic compounds. The dissolved oxygen content of water if more than 5.0mg/l at early morning hours, is considered favourable for a healthy fish and prawn growth and in no case the value should drop below 3.0mg/l (Chatterjee, 1993).

Of all the dissolved gases, oxygen plays the most important role in determining the potential biological quality of water, used in the rearing operations. It is essential for respiration, helps the

breakdown of organic detritus and enables the completion of biochemical pathways (Wickins, 1982). With dissolved oxygen levels less than 1mg/l, shrimps die, if exposed for prolonged time. At levels 1 to 4mg/l poor growth occurs, levels 5mg\l to saturation is best for growth and super saturation is harmful as gas bubble disease, may occur (Sunder Raj, 1994).

The dissolved oxygen values 6.1 to 7.0mg/l were also within the range required for optimum growth in the prawn (Smith et al., 1976, 1978, 1981; Subramanyam, 1987). New (1976) suggested 75% saturation of dissolved oxygen, as ideal for nutrition studies on the fresh water prawn. New and Singholka (1985) also reported an oxygen concentration of 75% saturation, as a provisional value, for Macrobrachium ponds.

2:6:2 pH

pH or the negative exponent of hydrogen ion concentration of water, is influenced by the soil and is an indicator to the environmental condition of the pond. However the lower and upper critical limits for pH for fish remain 4.0 and 11.0 respectively and in the range of 7.0 to 8.5 is considered ideal (Chatterjee, 1994; New and Singholka, 1985; and Sandifer and Smith, 1985).

Under normal conditions, the pH of a brackish water vary between 7 and 9, since phytoplankton use the carbon dioxide during

day light for the production process through photosynthesis, and the pH of water increases in the day hours. On the contrary, during night no carbon dioxide is utilised. Instead, all the organisms living in the pond release carbon dioxide, as a result of respiration (Sundaraj, 1994).

Cripps and Nakamura (1970); 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 *Macrobrachium rosenbergii*. Natividad (1982) reported that in Philippine rivers, *Macrobrachium rosenbergii* prefers, a wide range of pH 4.0 to 8.5. New (1976) also recommended a pH range of 7 to 8.5 for nutrition studies on *Macrobrachium rosenbergii*.

2:6:3 TEMPERATURE

Like all other life processes, growth of fish and crustaceans, is affected by temperature. It increases, with the increase in temperature upto an optimum, above which, growth decreases, until the upper lethal temperature is reached (Hepher, 1988). Solar radiation striking at the water surface, provides both heat and light energy required for both metabolic and physiological activities of aquatic organisms. The range of 25°C to 30°C is considered, favourably good for fish and prawn growth (Chatterjee, 1993). Prawns undergo stress, when the temperature is below 22°C and above 35°C and when the dissolved oxygen is below 2ppm (Rogers and Fast, 1988).

According to Manojnakra (1993), if the environment does not improve, prawns may get infected by germs, swim in a disoriented way to the surface or die due to exhaustion. If the temperature falls below 28°C, the metabolism reduces and also the active behaviour and growth rate. Below 20°C the prawns will take less feed. Prawns cannot tolerate, a temperature less than 13°C (Nakra, 1993).

2:6:4 NITRITE

Nitrogen is a basic and primary constituent of protein and chlorophyll, and is required to stimulate primary production in aquatic environment. The available nitrogen contents, vary from less than 25.0 mg/100gm to more than 75mg/100gm in most fish pond soils, while in the range of 25 to 50mg/100gm is considered favourable, for average production (Chatterjee, 1993).

The concentration of amino acid nitrogen in water, is considered as an index of pollution and in unpolluted water, the concentration does not exceed above 0.5mg/l. Both nitrite and unionised ammonia, are considered toxic to fish. Under normal condition, nitrite nitrogen does not occur in pond water, except in polluted water, while unionised ammonia, considered toxic, exists in water depending on the temperature and pH of the water and remains within the tolerance limit, under normal conditions (Chatterjee, 1993). According to Upadhyay (1993), nitrite toxicity can be controlled by the addition of chloride ions, such as sodium chloride and salt and by adopting correct stocking policies and proper biofilteration.

2:6:5 AMMONIA

Ammonia excretion rates in prawns, were also studied by many researchers (Wickens, 1976; Nelson et al., 1977; Stern and Cohen, 1982). Wickens (1976) reported that, an ammonia excretion of 0.25 to 0.85mgN/g/day for juvenile *Macrobrachium rosenbergii* is found.

Sevenfold increase in ammonia excretion rate, was found in heavily fed prawn, than in starved ones (Nelson et al; 1977). Stern and Cohen (1982) reported an increased ammonia excretion, during intermoult stages than premoult stages. Gopal (1986) reported less ammonia excretion rate for *Penaeus indicus* juveniles, fed with animal protein source than with plant protein source. Concentration of ammonia, nitrite or other adverse compounds in water, nutritional deficiencies, hormone applications and other factors, can also affect, the rate of metabolism in fish (Lovell, 1989).

Chakraborty et al (1993) studied the effect of ammonia at different pH on *Penaeus japonicus* post larvae and found that, ammonia was in the toxic form, as the pH was higher than 8.2. Hewitt and Iving (1990), studied the diets containing 30, 40 or 50% protein and were assessed for their effect on the pre and post feeding oxygen consumption and ammonia production, of juvenile *Penaeus esculentus*.

Oxygen consumption, ammonia excretion and random activity in relation to ambient oxygen, have been investigated by Lakshmi Narayana and Kutty (1982) in marine prawn *Penaeus semisulcatus*, in fresh water *Macrobrachium malcomsoni* and in a fresh water crab. Dall and Smith (1986) studied the oxygen consumption and NH3-N excretion in, fed and starved tiger prawns, *Penaeus esculentus*.

Ammonia is the primary excretory product of fishes. But if it is present in high concentrations, it will slow down growth rates. Wickens (1976) estimated the lethal limits of the nitrogenous compounds for prawns. The maximum acceptable limit of unionised ammonia was reported to be 0.1ppm NH3-N. Blooms of algae cause oxygen depletion during night, while toxic ammonia levels go up, following the collapse of the algal populations.

2:7 NUTRITIONAL EVALUATION METHODS

The dietary formulation of a nutritionally balanced diet, to meet the requirements, is one of the major aspects, in the development of aquaculture (Ray and Patra, 1989). The nutritional usefulness of a given food material, is variable between and within a species and is dependent on a host of other factors (Kapoor et al., 1975).

Digestibility studies on natural fish populations are very few (Bowen, 1981; De Silva et al., 1984, De silva, 1985). Halver

(1976) found that ingredients and finished diets can be evaluated, by a variety of chemical and biological tests. These tests are used, to check the accuracy of the manufacturing process of a feed with a desired composition, to measure the nutrient loss during manufacture and storage, to predict the nutritional value of a particular formulation, to detect oxidative rancidity and to measure the nutritional or feeding value of a formulation.

Sedgwick (1979) studied the effect of ration size and feeding frequency on the growth and food conversion of juvenile *Penaeus merguiensis* and found that, the conversion efficiency declined with the increase in weight and ration size. Food intake and ration size are inversely proportional to the feeding frequency and feeding rates of shrimps have been reported to range from 3 to 20% wet weight of the total biomass of animals, per day (Kanazawa et al., 1970).

Venkataramiah et al., (1975) studied the effect of protein level and vegetable matter on growth and food conversion efficiency, of brown shrimp. Deshimaru et al., (1978), in order to determine the optimum level of dietary protein for *Penaeus japonicus*, the growth and feed efficiency of prawns were examined, by using purified diets, containing 2 to 66.2% protein.

Catacutan (1991) studied the digestibility of four isonitrogenous practical diets 40% crude protein, containing different levels 5%, 15%, 25%, 35%, of gelatinised bread flour, as carbohydrate source, for *Penaeus monodon*. Mathew and Jayaprakash (1993) showed that, variation in the protein levels, considerably affected the survival, growth and conversion efficiency of *Penaeus indicus*. Goswami and Goswami (1982) with beef liver in diet, had reported the feed conversion ratio values of 1.54 and 1.64 with 33.58% and 42.24% protein content respectively, for *Penaeus indicus*. By altering the composition of compounded diet used in the study, FCR values could be bettered, to obtain similar values as above or even much better ones.

Forster (1976) had reported a FCR change of 1.2 to 2.6, for growth from 1.6gm to 18.35gm with *Penaeus japonicus*, fed on fresh clam meat. FCR has been preferred by some researchers, since it is positively correlated with, growth and PER (Boonyaratpalin, 1989).

Sambasivan et al., (1982) working on *Penaeus indicus* observed high conversion efficiency and growth rates in diets having 60% protein and the highest survival with 50% protein. The highest survival of 94.33% and conversion efficiency of 40.6% were obtained, with compound diet having 40% protein content. Sumitra et al.,

(19) had stressed the importance of using natural foods, to obtain greater food conversion efficiency.

Fujinaga (1963) reported FCR, of more than 10 for kuruma shrimp, fed on natural foods. Sick et al., (1972) using semi purified pelleted diets found that, FCR was reduced by nearly half of that reported for penaeids foods. Forster (1976) suggested 2.1 as good FCR target, to be achieved.

Ali (1982) reported the highest PER at 20.5% protein and Millikin et al., (1980) had noted that best FCR, PER and growth were obtained with 40% protein level. Eldin and Corliss (1976) had indicated that, factors other than protein, may be important in the growth of penaeids. Colvin (1976) had noted a decrease in protein utilization, with an increase in dietary protein.

Ali (1988) had reported a range of true digestibility from 35.57% to 71.08% on *Penaeus indicus* juveniles weighing 0.07 gm, fed with different protein sources. Elis et al., (1987) had reported protein digestibilities of 93 to 99% in *Macrobrachium rosenbergii*.

The capability of penaeid larvae, to feed solely on artificial diets, has been attributed to their short gastro evacuation time and high digestive enzyme activity during their early stages (Jones and Kurmaly, 1987; Abubaker and Jones, 1992).James et al., 1992

evaluated spirulina fusiforms as a protein source in the diet of the post larvae of *Macrobrachium rosenbergii* and found that spirulina has a high protein digestibility coefficient as that of casein diet.

According to Colvin (1976), protein sources that are deficient in essential nutrients, produce less efficient FCR. Ali (1982) while evaluating the nutritional quality of various protein sources, obtained the best feed efficiency for clam based diet for *Penaeus indicus* juveniles. Later it was found that, clam meal based diet produced the best feed conversion in *Penaeus indicus* (Ali, 1988). Similarly Josekutty (1991) found that clam meal based diet had given the best FCR among the various sources tested in *Penaeus monodon*. Deshimaru and Shigueno (1972) observed an increase in FCE with an increase in dietary protein concentration. Similar trends in improvement of FCE with an increase in dietary protein concentration up to a certain level and a decline in FCE, when the dietary protein concentration increased further were also reported in *Penaeus aztecus* by Venkataramiah et al; (1975), in *Penaeus japonicus* by Deshimaru and Yone (1978), in *Penaeus monodon* by Alava and Lim (1983) and in *Penaeus indicus* by Gopal (1986) and Ali (1988).

Millikin et al., (1980), Boonyaratpalin and New (1982) reported an inverse relationship between the dietary protein level and the PER in *Macrobrachium rosenbergii*. In *Penaeus indicus*, Colvin (1976) found a decline in the efficiency of protein utilization with

successive increase in the dietary protein level. Hajra et al., (1986) observed a steady increase in PER value, concomitant with an increase in total carbohydrate, in the diet. Ali (1988) observed higher efficiency of protein utilization in *Penaeus indicus* at lower dietary protein levels. The efficiency decreased as the protein level increased. However Ali (1982) noticed the highest PER values at an intermediate level of protein tested at 20.5%.

In *Penaeus monodon*, Alava and Lim (1983) observed an increase in PER with an increase in dietary protein level up to 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 up to 30% in *Penaeus indicus*. A further increase in dietary protein, resulted in the decrease in PER and the lowest value was obtained, when the protein level reached 60%.

Chapter 3

THE EFFECT OF DIETARY PROTEIN CONCENTRATION ON DIGESTIVE ENZYME ACTIVITY

SECTION A

3:1 CHANGES IN THE DIGESTIVE ENZYME ACTIVITY AFTER FEEDING

3:1:1 Introduction

Work on the response of digestive enzyme activity to feed ingestion, has elucidated the relationship between tropical digestive processes and feeding frequency in fishes. Onishi et al., (1973,1976) investigated the sequence of digestive enzyme levels in *Cyprinus Carpio* after feeding. Their results have shown that, there is a five hour delay between food ingestion and peak digestive enzyme activity. Takii et al., (1985) observed changes in the digestive enzyme activities in eels, *Anquilla japonica,* after feeding and found that, protease activities in its intestinal content reached a peak after five hours, while amylase had a smaller response time. Hecht and Walters (1987) determined the activities of gastric protease and pancreatic protease and amylase at specific intervals after feeding. Unfed fish showed no specific changes in the digestive enzyme activities but fish, which were fed, responded with increased digestive enzymes. Protease activities in digestive tissues changed significantly during the maturation process. The fish has higher enzyme activities in early maturation and lower in the later stages until maturity

Studies relating to the secretary response time of digestive enzymes in *Macrobrachium rosenbergii* are lacking. Investigations into the above aspects, would help to establish a better understanding of its digestive abilities, which in turn, can help in adopting correct feeding frequencies in commercial aquaculture.

3:1:2 Materials And Methods

3.1.2.1 Sample Selection

Macrobrachium rosenbergii post larvae were collected from the hatchery and maintained in the cylindrical fibre glass tanks, having a diameter of 55cm and a height of 35cm, to reach the juvenile stage. The water used was drawn from the fresh water distribution system of the hatchery. A depth of about 20cm water was maintained throughout the experiment. Aeration was provided to individual tanks through P.V.C tubes and diffusion stones, from the air distribution system of the hatchery. A gentle air supply was maintained uniformly throughout the experimental period, using air regulators except during feeding, cleaning of tanks, water changing and removal of leftover feed and faecal matter.

3:1:2:2 Feed Formulation

The feed was prepared consisting of clam meat, as a protein source, at a protein level of 35%. The compounding of feed was done, with mineral mix, and sun flower oil to give a final level

of 8% fat, 10% mineral, and 1% cholesterol, as given in table 1. The dry powdered feeds were mixed together and kneaded well with water to produce small granule size consistency. The mixture was then steam cooked and then particulated after mixing with vitamin mix and egg albumin binder, steam dried at 50 °C and made into particulated feed to give an average size of 0.01mm. This was stored in plastic containers at 4 °C in refrigerator prior to feeding.

3.1.2.3 Proximate Analysis

The proximate analysis of the diet was performed upon finely ground sample, as described in the Methods of Analysis by the Association of Official Analytical Chemists (AOAC, 1984).

The water content of a feed was determined gravimetrically by drying a sample in a drying oven at 105°C until a constant weight was reached. The protein content of the sample was determined by the Kjeldhal digestion and distillation method in which, the nitrogen content obtained, was converted into protein content using a conversion factor 6.25. Fat was extracted by Soxhlet extraction method using, diethyl ether (AOAC, 1984). For the estimation of crude fibre, the fat free material was boiled with a dilute acid solution, followed by dilute alkaline solution. From the residue obtained, the weight of the inorganic residue (ash) was subtracted to get the fibre content, as suggested by Pearson (1976).

Ash was determined gravimetrically, following combustion in a muffle furnace at 550°C for five to six hours (AOAC, 1984). NFE which include the simple sugars, compound sugars and soluble polysaccharides such as starch, was calculated by the difference as given by Hastings (1976).

3.1.2.4 Sampling of Experimental Animals

Groups of fifty juvenile animals were selected at random and transferred into three fibre glass tanks of the experimental system and acclimatized with a basal diet, containing 35% protein, on *ad libitum* basis. Before the experiment, the animals were starved for 24 hours. A sample of 5 animals in triplicate, were sampled for digestive enzyme analysis. The remaining animals were given the basal feed, at 3% of the body weight. The sampling of five animals each, in triplicate, was done at time intervals of 1hr, 2hrs, 3hrs, 4hrs, 5hrs, 6hrs, 7hrs and 9hrs, after giving the feed. The animals sampled, were immediately kept under chilled condition.

3.1.2.5 Preparation Of Enzyme Extract

The preparation of enzyme extract from the hepatopancreas of the sampled animals, was done according to the method of Overnell (1973). The tissue was homogenised with 0.005 M Tris buffer (pH 8.0) containing 0.15 M NaCl, 0.02 M $CaCl_2$ and 0.1% Triton X-100 at 4°C, using a high speed tissue homogeniser. The resulting homogenate (1/10 w/v), was centrifuged at 15,000 rpm

for 30 minutes at 4°C . The supernatant obtained, was used in the enzyme assays.

3.1.2.5.1 Estimation Of Protease Activity

Total protease activity was assayed according to Kunitz (1947). The extract was added to 1% casein, and 0.1 M citrate phosphate buffer (pH 7.0) followed by incubation at 37 °C, for one hour. The reaction was terminated by addition of 5% trichloro acetic acid. This was kept at 20°C for 30 minutes and centrifuged. The amount of liberated tyrosine was measured directly at 280 nm, in a UV visible spectrophotometer. The total protein content of the extract was determined according to the method of Lowry et al ., (1951).

The total enzyme activity was expressed, as milligram of tyrosine, liberated in one hour per gram of wet tissue (enzyme units per gram tissue) and the specific activity, as microgram tyrosine liberated per minute per milligram protein (enzyme units per milligram protein).

3.1.2.5.2 Estimation Of Amylase Activity

Amylase activity was assayed, according to Bernfield (1955), in which, the increase in the reducing power of a buffered starch solution was measured, with 3,5 dinitro salicylic acid at 540 nm. The assay medium consisted of soluble starch as substrate in 0.02 M phosphate buffer pH 6.9 containing 0.0067 M NaCl and 1 ml of enzyme extract. This was incubated for 30 minutes at the room

temperature. The reaction was interrupted by the addition of 2 ml of dinitrosalicylic acid reagent and heating for five minutes in a boiling water bath. The samples were cooled, diluted with distilled water and the absorbance was measured at 540 nm.

Total amylase activity was expressed as milligram of maltose liberated in 30 minutes per gram wet tissue (EU/gm tissue) and specific activity as, milligram of maltose liberated in 30 minutes per milligram protein (EU/mg protein).

3.1.2.5.3 Estimation Of Lipase Activity

Lipase activity was measured by pH changes of olive oil emulsions, according to Tietz et al., (1959). One part of a stabilised olive oil emulsion and two parts of the enzyme extract and tris hydrochloric acid were used to give a buffer concentration of 0.005 M, pH 8 . After incubating for 90 minutes, the mixture was titrated back to pH 8. This was compared with a blank, containing no substrate.

The total lipase activity was expressed as, volume of 0.02 M NaOH used in 90 minutes per gram wet tissue and specific activity as, volume of 0.02 M NaOH used in 90 minutes per milligram protein.

3.1.2.6 Statistical Analysis

Means, t-tests, standard deviations (SD) of the means, standard errors of the means (SEM) were calculated. The values

reported are the statistical means of units of enzyme activity from each treatment and the associated SEM.

3.1.3 Results

Table 1 gives the details of the feed formulation and proximate analysis of diet. The sequential changes of protease and amylase activity in the hepatopancreas of the juvenile *Macrobrachium rosenbergii* were recorded at different time intervals after giving the feed, the results of which are presented in table 2 and figures 1, 2 and 3

3.1.3.1 Protease

Table 2, shows the changes in protease and amylase activities in the hepatopancreas of *Macrobrachium rosenbergii* after feeding. The mean value with standard deviation is presented. From the table it is found that the total activity of protease is slightly higher in 0-day, compared to the value at one hour of feeding. . After that a gradual increase is observed and at 5 to 6 hours, the maximum enzyme activity is seen.(Fig 1) Protease specific activity also shows a similar pattern. The maximum protease specific activity is shown at 5 hours, after which, a decrease in the activity is seen at 7 to 9 hours (Fig2). The ratio of the total activity to body weight shows that at 5 to 6 hours, maximum enzyme activity is attained (Fig3). When the total protease activity is expressed per unit body weight a more distinct curve is obtained as compared to total protease activity.

Table 1 : Composition of the experimental diet containing 35% protein level

Ingredients	gm/100g dry diet
Clam meat	91
Cellulose	0.5
Vitamin mix*	3
Cholesterol	1
Egg Albumin	2
Mineral mix*	2.5
Proximate composition (%) of clam meat used in feed formulation	
Moisture	8.62
Protein	35
Fat	11
Fibre	0.88
Ash	7.5
NFE	37
E (Kcal/100g)	387

Vitamin mix * mg/kg dry diet (Supplivite M) Thiamine monochloride 45; Riboflavin 30; nicotinamide 100; pyridoxine hydrochloride 20; calcium-d-pantothenate 50; biotin 0.1; cyanocobalamine 0.01; choline chloride 400; ascorbic acid 400; inert carrier α cellulose 1955 mg.

Mineral mix * g/kg dry diet (USP Salt mixture)

Table 2: Changes in the protease and amylase activities in the hepatopancreas of Macrobrachium rosenbergii after feeding

	Protease			Amylase		
Time after feeding (hours)	Total activity (mg tyrosine/ hr/g tissue	Specific activity (μg tyrosine/min/ mg protein	Ratio of total activity to body weight.	Total activity (mg maltose/30 min/g tissue	Specific activity (mg maltose/30 min/mg protein	Ratio of total activity to body weight
0	14.237±0.213	13.308±0.675	10.459	21.380±0.529	1.222±0.077	15.660
1	13.153±0.057	9.342±1.592	9.0089	20.962±0.017	0.886±0.145	14.360
2	13.641±2.855	12.384±4.834	10.310	21.485±0.126	0.906±0.196	16.240
3	14.300±1.562	13.500±0.350	13.763	21.514±0.554	1.075±0.045	20.710
4	16.497±3.121	16.525±0.225	16.834	21.777±0.048	1.136±0.144	22.220
5	18.280±0.144	21.925±3.842	17.662	22.998±0.861	1.866±0.0024	22.220
6	21.211±0.186	18.092±0.692	18.631	23.485±0.126	1.934±0.055	20.620
7	17.118±1.022	9.992±0.309	11.404	19.694±0.584	0.703±0.032	13.120
9	12.080±0.733	7.342±0.159	6.829	18.350±1.213	0.477±0.013	10.373

Each value is a mean ±s.d (n=3) for each treatement and (n=3) for each assay.

Fig 1. Changes in total activity of
Proteases and amylases after feeding

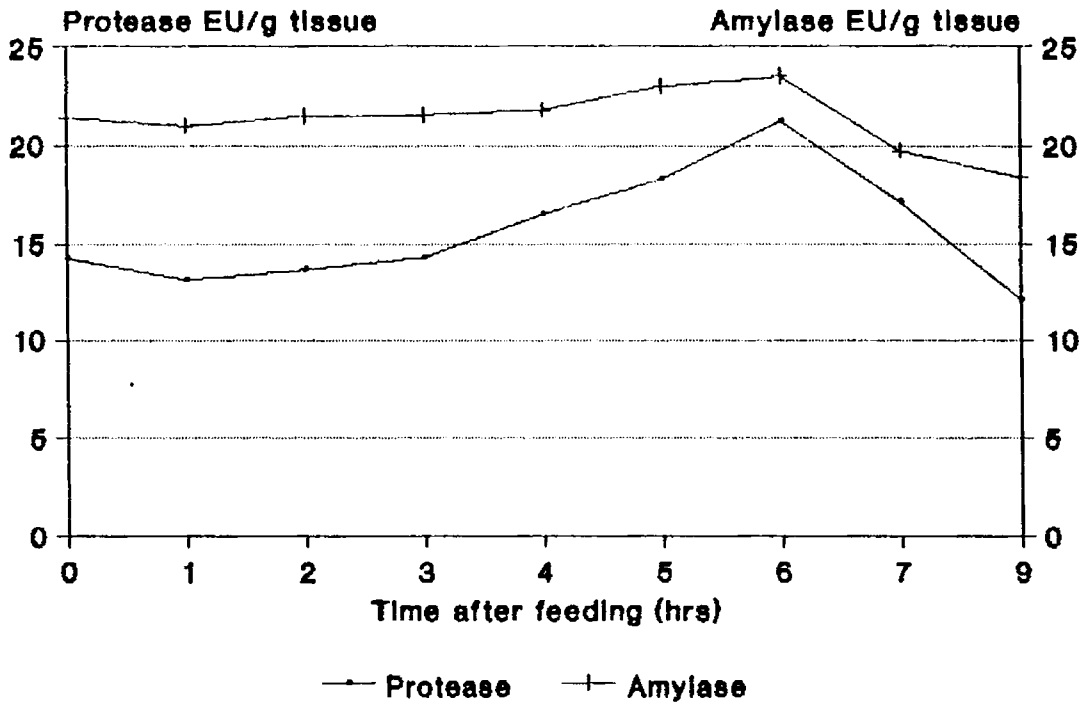


Fig 2. Changes in specific activity of
protease and amylase after feeding

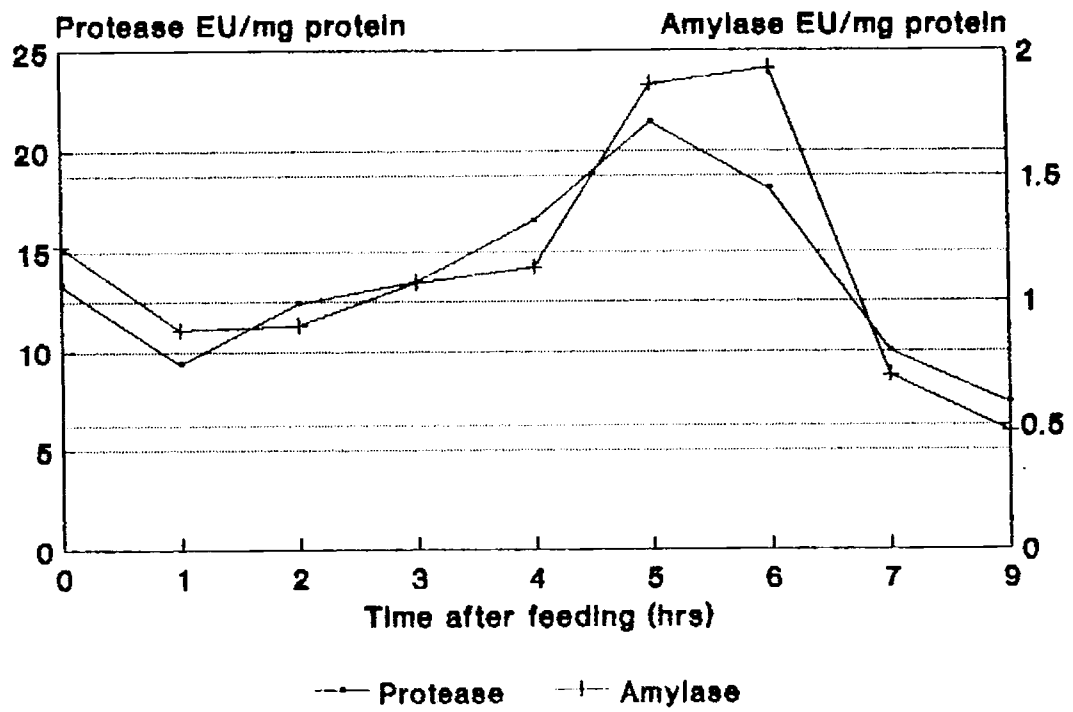
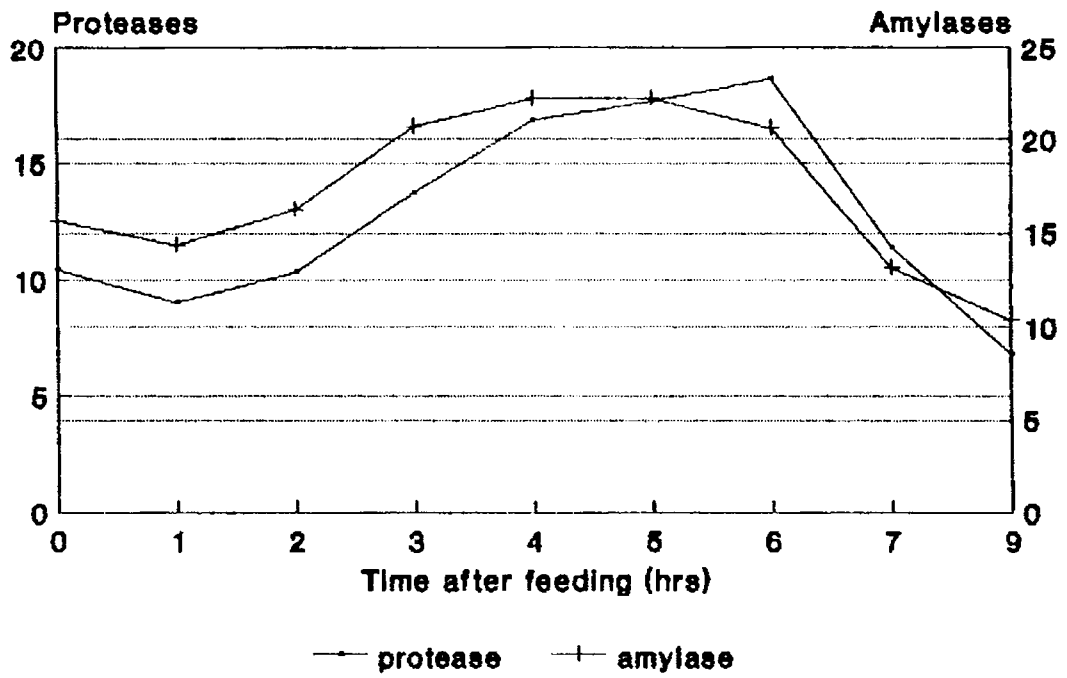


Fig 3. Ratio of total enzyme activity of Protease and amylases to body weight after feeding



3.1.3.2 Amylase

The total amylase activity, amylase specific activity and the ratio of amylase total activity to body weight are presented in table 2. It shows a slight increase in the amylase total activity from one to six hours. After that, the activity shows a decline(.Fig1) Amylase specific activity also shows a uniform increase and reaches its maximum at five to six hours(Fig2). From Fig3 it is seen that amylase total activity per g b.wt after an initial dip at one hour, increases further and remains high during 3 to 6 hours. After that it shows a decline. The total protease activity is expressed as milligram tyrosine per hour per gram tissue and protease specific activity as microgram tyrosine per minute per milligram protein under the assay conditions.

Amylase total activity is expressed as milligram maltose formed in 30 minutes per gram tissue and specific activity as milligram maltose formed in 30 minutes per milligram protein under the assay conditions.

3.1.4 Discussion

In the present study, the protease and amylase activities in the hepatopancreas of *Macrobrachium rosenbergii* showed the same type of pattern reaching a peak at 5 to 6 hours, after feeding. After one hour, both protease and amylase enzyme activity showed a decline and after that, it gradually increased and reached a peak at 5 to 6. hours, after feeding and subsequently decreased quite rapidly to reach the pre feeding level at 12 hours, after feeding.

The increase in the activity, after feeding, despite the dilution caused by the arrival of food, might be due to an increased secretion from the tissue into the lumen.

The protease specific activity decreased sharply at one hour after feeding and gradually increased to the maximum level at five to six hours and then declined again to reach the pre feeding level, at nine hours. The temporary decrease in protease activity at one hour after feeding may be due to the dilution by the ingested food and an inadequate rate of enzyme secretion. The highest activity attained at five to six hours after feeding, indicates that the enzyme is secreted continuously after feeding to counteract the dilution caused by the food. It seems therefore that the protease activities in the hepatopancreas of *Macrobrachium rosenbergii* were attained at more or less constant levels and the temporary decrease after feeding is caused only by the dilution effect. and the enzyme secreted into the lumen from the intestinal tissue or the adjoining pancreatic tissue is diffused almost throughout the length of the intestine (Ghosh et al., 1987). The studies of Lan and Pan (1993) showed that, due to feed ingestion, maximal organ weight was reached in 1 hour after feeding, for the foregut and five hours after feeding, for the midgut where the highest protease was observed in the whole digestive tract of Grass shrimp.

In the present study, it is observed that, there is a time lag between, the increase in the secretion of the enzymes and the progress of the digestion. It can be persumed that, the enzyme activities reached their maximum, when the absorption of the digested food had already set in. This also agrees with the finding of Onishi et al., (1976).

In the present study, protease activity recorded a decline after 5 to 6 hours of feeding in the hepatopancreas. The decline in the enzyme activity indicates that, little or no more enzyme is secreted in the stomach and that the enzymes are partially inactivated.

Onishi et al., (1973) observed that, in Carp, the amylase activity in the intestinal content, increased gradually, after feeding, to reach a maximum at five hours. They also studied the response of digestive enzyme levels, to twice a day feeding and found that, even in such cases the amylase activity in the intestinal content of Carp, attained its maximum at 5 to 7.5 hours, after feeding. The amylase activity seemed to decrease temporarily just after feeding. They suggested that, this might be due to the loss of enzyme excreted with the undigested residue of the previous day. The same suggestion may also hold good for the present study as such a decrease was observed immediately after feeding In the eel, *Anguilla japonica*, the amylase activity of the intestinal content showed slight increase from 3 to 5

hours after feeding, but maintained a significantly low level, compared with that in the tissue (Takii et al., 1985).

In the Catfish *Clarias gariepenus*, the amylase activity of the intestinal contents after feeding also displayed similar sequential changes to that of the present investigation (Uys et al., 1987). Thus, the sequential changes in the amylase in the hepatopancreas of *Macrobrachium rosenbergii*, after different hours of feeding, exhibit striking similarities with the other groups of fish investigated.

Compared to the total activity changes after feeding, the changes in the ratio of total activity to body weight is found to be more significant, as evident from the Fig3. This can be explained by the fact that when total activities calculated per gram tissue, the weight of gut contents also is included, resulting in dilution of the enzyme. But when the activity of the enzymes, protease and amylase are expressed as Eu per unit body weight of the animal (ratio of total activity to body weight), this dilution effect get minimised, thus showing a very pronounced change at different periods of sampling .Here also, the maximum response of the enzymes to feed is shown at 4-6 hours after feeding.

Hecht and Walters (1987), studied changes in the activities in gastric protease and pancreatic protease and amylase at specific intervals, after feeding. Unfed fish showed, no significant changes in the digestive enzyme activities, but fish which were fed, responded

with increased digestive enzyme levels. Onishi et al., (1976) suggested that there is a daily rhythm in digestive enzyme activity in Carp, since no change in enzyme activity was observed in unfed group of *Lizaparsia*. It appears that, digestive enzyme activity cycles are induced by feed intake and this agrees with the present study.

3.2 EFFECT OF DIETARY PROTEIN CONCENTRATION ON DIGESTIVE ENZYME ACTIVITY

Section B

3.2.1 Introduction

A few studies on the digestive enzymes have been reported in *Macrobrachium rosenbergii*, and they have helped in understanding the larval digestive processes better (Lee et al., 1980, Tsai et al., 1987). But still, to date no attempt is made and no information is available on the response of digestive enzymes in the juvenile *Macrobrachium rosenbergii* to compounded feed ration, containing varying concentration of protein. Hence an attempt is made in the present work, to study the changes of digestive enzyme activity in juvenile *Macrobrachium rosenbergii* viz, the protease, amylase and lipase at varying dietary protein concentrations.

Until recently, most investigations concerning the digestive enzymes of crustaceans, have been qualitative and focused on the comparative aspects of digestion. As a result, several reviews (Vanweel, 1970; Gibson and Barker, 1979; Dall and Moriarty, 1983) dealing with the comparative physiology of digestion have been published, but all contained only very little quantitative information. Since crustaceans have now been evaluated and considered for commercial culture, the changes in enzyme activities during the life

cycle and adaptation to new diets, need to be examined quantitatively (Lee et al., 1980, Maugle et al., 1982).

The principal objective of this research is to obtain information concerning the proteolytic enzymes present and the changes in the activities that occur, in the digestive tract of the fresh water shrimp *Macrobrachium rosenbergii* in their juvenile stage and their response to diet during a growth period of three months. In addition to that, the data are compared with other nutritional information, such as growth, feed digestibility which are simultaneously measured for the same populations of shrimps, the results of which are presented later in chapter 6. The secondary objective is to use this new information, to evaluate the formulation of a prepared diet to be used for crustacean aquaculture.

3.2.2 Materials and Methods.

3.2.2.1 Diet Formulation and Feeding Regime

The experiment was started with 50 animals each in fibre glass tanks, of 100 litre capacity. Prawns were fed with the test diets, for one week to acclimate them to the feed. At the end of the acclimation period, the animals in each tank were counted and weighed.

Table 3 : Formulation and proximate analysis of eperimental diets containing different levels of protein.

Ingredients :	**g/100g dry diet**				
Protein % :	**10**	**20**	**30**	**35**	**45**
Constituent	**Diet A**	**Diet B**	**Diet C**	**Diet D**	**Diet E**
Clam meat	19.59	48.13	76.48	91	91
Caesein	-	-	-	-	10
Sun flower oil	7.86	4.72	1.60	-	-
Mineral mix*	7.86	5.72	3.59	2.5	2.5
Starch	26.42	15.86	5.37	-	-
Cellulose	32.27	19.57	6.96	0.5	0.5
Vitamin mix*	3	3	3	3	3
Cholesterol	1	1	1	1	1
Egg albumin	2	2	2	2	2
E/kcal/100g	38.27	327	367	387	427

Proximate compostion (%) of clam meat

components	
Protein	35
Fat	11
Ash	7.5
Fibre	0.88
Moisture	8.62
NFE	37
E (Kcal/100g)	387

Vitamin mix * mg/kg dry diet ; Mineral mix * g/kg dry diet }Composition as given in table 1

The animals were then given a formulated micro particulated diet, in different protein levels, using clam meat with vitamins, minerals, cholesterol, a filter cellulose and a binder starch coated with egg albumin. The protein levels and the percentage composition of the diets are shown in Table 3. The entire diet consisted of a fat content of 11%, minerals 10%, carbohydrate 37%, vitamin mix 3%, egg albumin 2% and cholesterol 1%. They were packed in air tight containers and kept in a refrigerator. The proximate analysis of the feeds was done according to the methods already mentioned.

Each diet was fed to triplicate groups of animals, at a daily rate of 3% of their live weight, for 3 months. The lengths and weights of the animals were monitored, at 0 day, 15 days, 30 days, 60 days and 90 days of feeding.

The animals were sampled for enzyme assay at 5-6 hours after giving the test diet. The sampling was done at various periods of their growth viz. 0-day, 15-days, 30-days, 60-days and 90-days as already mentioned.

3.2.2.2 Enzyme Assay

The animals sampled were weighed and immediately chilled in ice. The hepatopancreas of the sampled animals were weighed and extracted the enzymes. The assay of protease, amylase and lipase enzymes were done, as detailed in chapter 3.1.2.5. The

total protein content in the enzyme extract was determined according to the method adopted by, Lowry et al., (1951).

The total protease activity was expressed as milligram of tyrosine liberated in one hour per gram of wet tissue (enzyme units per gram tissue) and protease specific activity as microgram tyrosine formed, per minute per milligram protein (enzyme units per milligram protein).

The total amylase activity was expressed as milligram of maltose liberated in 30 minutes per gram wet tissue (EU/gm tissue) and specific activity as, milligram of maltose liberated in 30 minutes per milligram protein (EU/mg protein).

The total lipase activity was expressed as, volume of 0.02M NaOH used in 90 minutes per gram wet tissue and specific activity as, volume of 0.02M NaOH used in 90 minutes per milligram protein (EU/mg protein).

3.2.2.3 Statistical Analysis

The feeding experiments were designed on the basis of completely randomised design. The results obtained from the experiments, were subjected to analysis of variance (ANOVA), as per the method of Snedecor and Cochran (1968) and the treatment means were compared by Duncans multiple range test (Steel and Torrie, 1980). An F-test was performed to determine if a difference

between the treatment means existed. If the value was found to be significant, the data was analysed by a least significant difference (LSD) test. All the possible difference between the means of each treatment were computed and compared to LSD. If the absolute value of the difference 'd' was greater than LSD, the difference was found to be significant at $p<0.05$.

3.2.3 Results

3.2.3.1 Protease

Two way analysis of variance (ANOVA) was carried out, for the total protease activity at different dietary protein levels and sampling periods. It is presented in Table 4a. This table shows that there is significant difference between various dietary protein concentrations on their protease activity. The critical difference between protein concentrations and periods is 1.115. The 35% and 45% protein concentrations shows the highest protease activity, and among these, the maximum protease activity is shown at 35% (Fig 4a) protein level. There is no difference in the protease enzyme activity, between 20% and 30% protein levels. The enzyme activity shows difference at each time intervals. The protease activity was the highest on the 60th day, followed by the 30th day. Both protein concentrations and period show significance at 1% level ($p<0.01$).

The protease specific activity is presented in Table 4b. A pair wise comparison is observed and it is found that, 35% and 45% protein concentration, exhibit a higher protease specific activity and the highest activity is shown at 35% protein concentration. The protease specific activity at different dietary protein levels and at various periods of growth, is given in Fig 4b. The period also shows difference in the enzyme specific activity and the highest specific activity is found at, 60 days followed by 30 days. Both protein concentrations and periods show significance at 1% level ($p<0.01$).

The ratio of total protease activity to body weight is presented in Table 4c and Fig 4c. A uniform pattern of variation in the activity is observed at, 30%, 35% and 45%, and among these the highest ratio of total protease activity is observed at, 35% level. A difference in protease activity is seen at different days. As the body weight increases, the enzyme activity per unit body weight shows a decline. Here, the early period shows a highest enzyme activity, compared to the other days. The protein concentrations and periods show significance at 1% level ($p<0.01$).

From all these results it is observed that, at 35% protein concentration the highest protease activity is seen, followed by 45% and 30% protein concentration and period 30 to 60 days shows the maximum protease activity.

Table 4a-c. Analysis of variance (ANOVA) of protease enzyme activity at different protein levels and periods

4a. Total protease activity

Source	Sum of squares	Degrees of freedom	Mean sum of squares	F-value
levels	126.6996	4	31.6749	45.8014**
periods	31.8031	4	7.9508	11.4967**
error	11.0651	16	0.69160	
total	169.5679	24		

CD= 1.1151

levels mean = 9.6440 11.8580 12.7980 15.7860 15.2340

periods mean = 67.7300 65.2700 68.3400 70.6800 54.5800

4b. Protease specific activity

Source	Sum of squares	Degrees of freedom	Mean sum of squares	F-value
levels	82.4927	4	20.6232	6.6126**
periods	292.3454	4	73.0863	23.4344**
error	49.9002	16	3.1188	
total	424.7383	24		

CD = 2.3678

levels mean = 11.2034 11.7966 12.9040 16.2580 14.2680

periods mean = 46.6500 45.8100 78.2200 87.7000 73.700

4c. Ratio of total protease activity to body weight

Source	Sum of squares	Degrees of freedom	Mean sum of squares	F-value
levels	385.7109	4	96.4277	10.1922**
periods	2213.6296	4	553.4074	58.4942**
error	151.3743	16	9.4609	
total	2750.7148	24		

CD= 4.1240

levels mean = 24.4840 32.8460 36.0800 39.3040 38.2180

periods mean = 249.5300 196.1900 166.2400 155.6100 107.0900

** *significant at $p<0.01$.*

Fig 4a.Changes in Total protease activity at different protein levels and periods

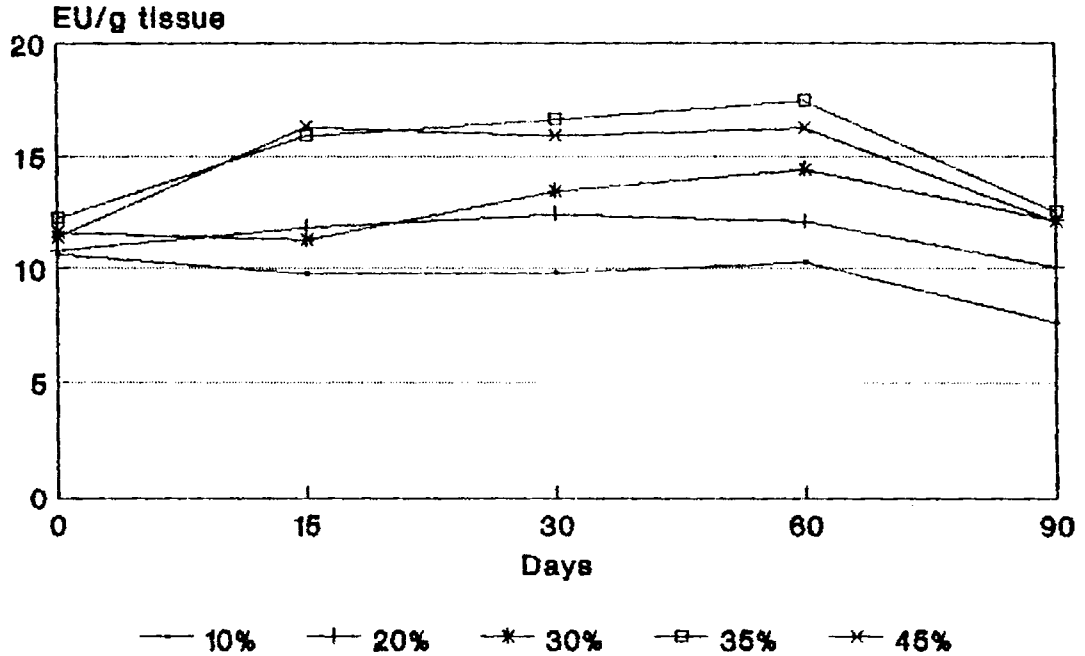


Fig 4b. Changes in protease specific activity at different protein levels and periods

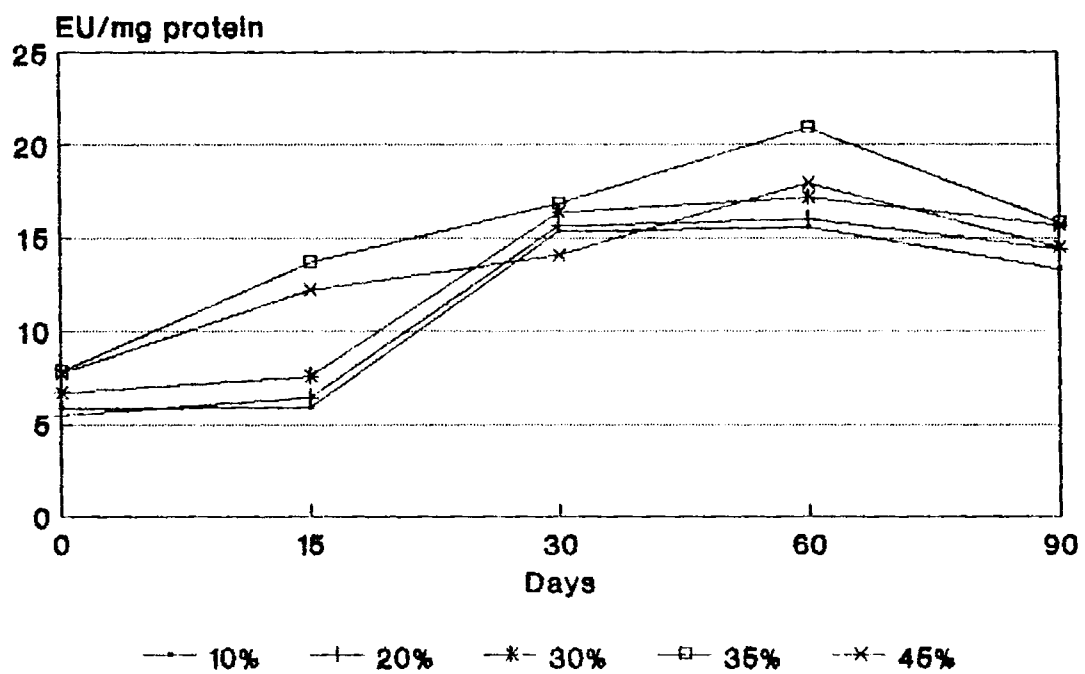
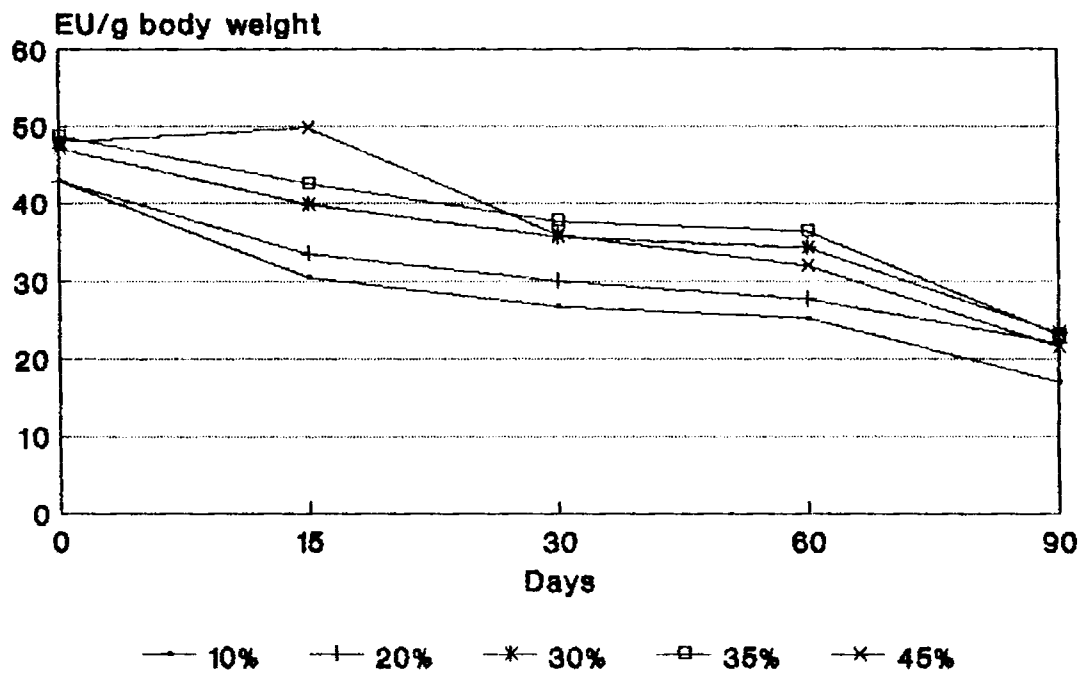


Fig 4c. Changes in the ratio of total protease activity to body weight at different protein levels and periods



3.2.3.2 Amylase

Two way analysis of variance (ANOVA) was carried out for the total amylase activity. It is presented in Table 5a and Fig 5a and 30%, 35% and 45% dietary protein concentrations exhibit high amylase activity, and among these the 35% protein concentration shows the highest total amylase activity. The period also shows difference in the total enzyme activity and at 60 days the maximum total amylase activity is seen, followed by 30 days. The dietary protein concentrations and periods show, significance at 1% level ($p<0.01$).

Table 5b shows the amylase specific activity at different protein concentrations and periods. Here it shows that, between 20%, 30%, 35% and 45% protein concentrations, there is no significant difference. The maximum increase in enzyme activity is observed at, 35% protein concentration. A distinction in the enzyme specific activity is observed for 35% protein level at 60 days, followed by 30 days (Fig 5b). Amylase specific activity at different protein concentrations and different periods shows significant difference at 1% level ($p<0.01$).

Table 5c shows a variation of the total amylase activity to body weight. The 30% protein concentration shows the highest value, followed by 35% in total amylase activity to body weight. The period also shows difference in the enzyme activity to body

Table 5a-c. Analysis of variance (anova) of amylase enzyme activity at different dietary protein levels and periods

a. Total amylase activity

Source	Sum of squares	Degrees of freedom	Mean sum of squares	F-value
levels	649.0524	4	162.2631	15.7122**
periods	5013.0835	4	1253.2709	121.3563**
error	165.2352	16	10.3272	
total	5827.3711	24		

CD = 4.3089

evels mean =	41.2560	46.5360	53.6720	55.2320	51.1820
eriods mean =	109.4700	268.7100	290.8300	305.7500	264.6300

b. Amylase specific activity

Source	Sum of squares	Degrees of freedom	Mean sum of squares	F-value
levels	6.3779	4	1.5945	6.0410**
periods	50.8068	4	12.7017	48.1232**
error	4.2231	16	0.2639	
total	61.4078	24		

CD = 0.6888

levels mean =	2.9056	3.4824	3.7270	4.3950	4.0374
periods mean =	7.4390	13.9670	22.9960	27.8210	20.5140

c. Ratio of total amylase activity to body weight

Source	Sum of squares	Degrees of freedom	Mean sum of squares	F-value
levels	3412.3875	4	853.0969	4.0867*
periods	21174.8125	4	5293.7031	25.3594**
error	3339.9563	16	208.7473	
total	27927.1563	24		

CD = 19.372

levels mean =	113.6160	118.4500	146.2580	129.2680	118.6620
periods mean =	404.5800	816.5600	713.9900	873.0100	523.1300

**significant at p<0.05 ; ** significant at p<0.01.*

Fig 5a. Changes in total amylase activity at different protein levels and periods

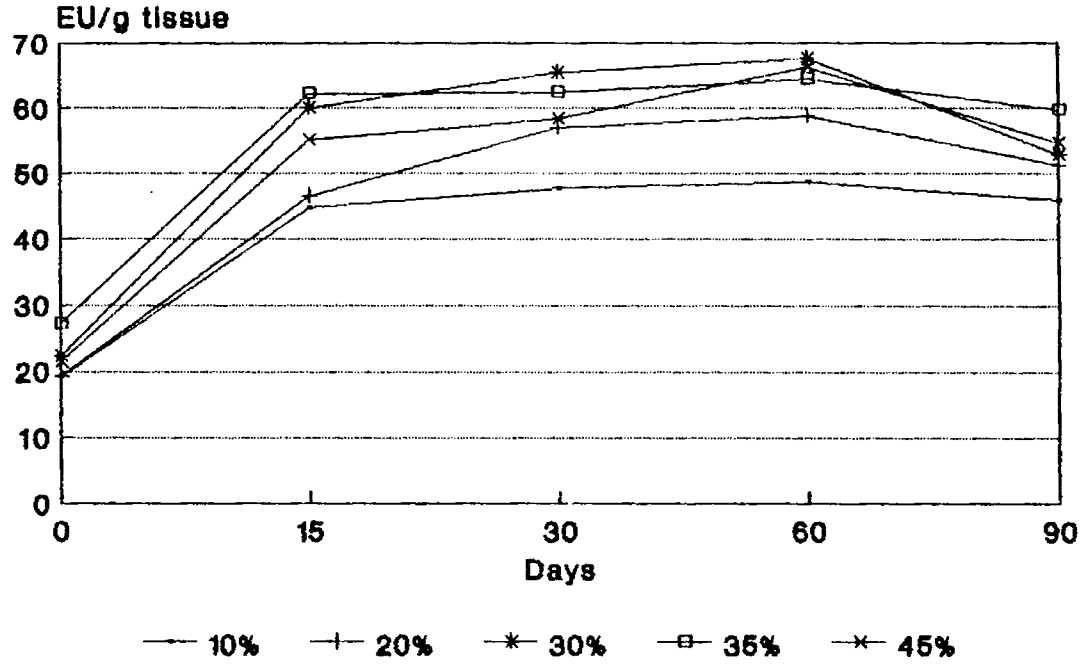


Fig 5b. Changes in amylase specific activity at different protein levels and periods

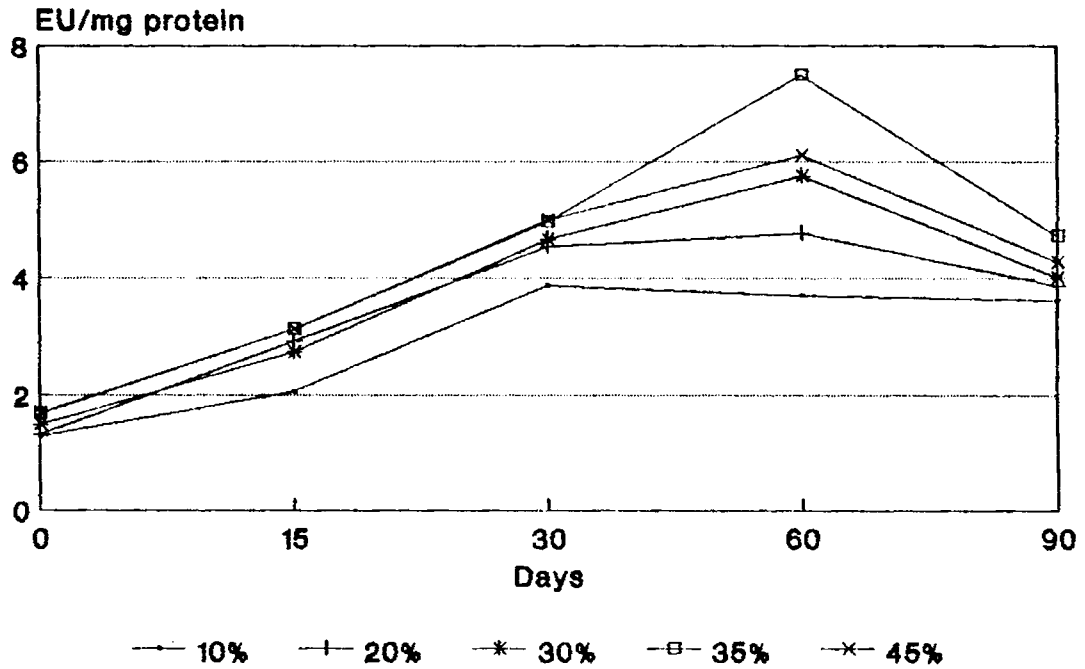
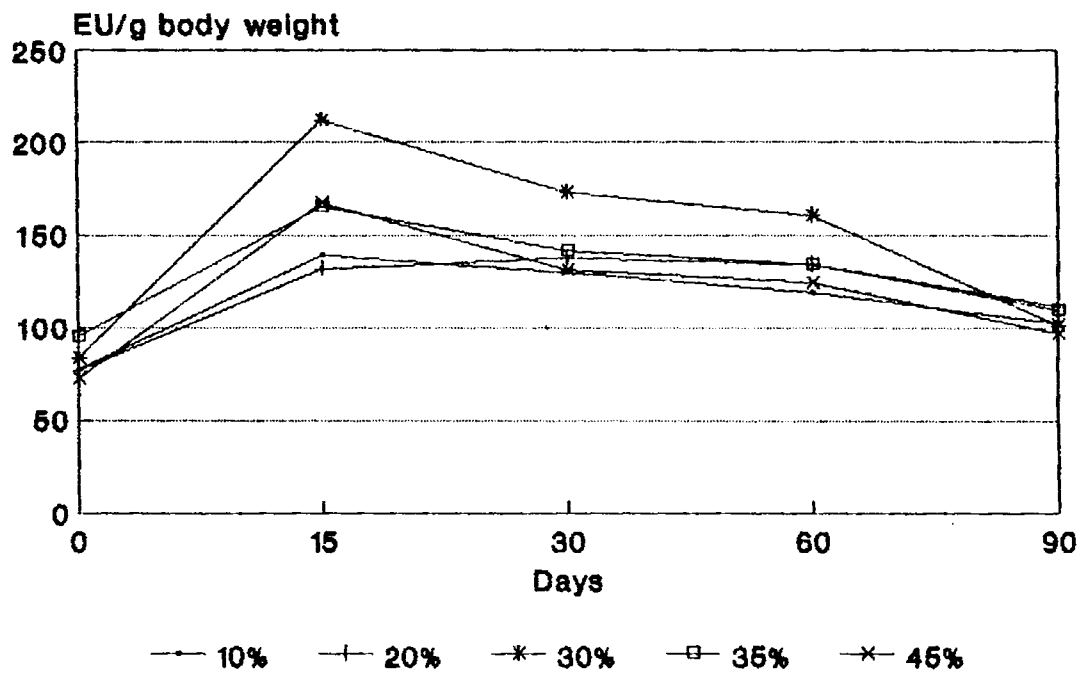


Fig 5c. Changes in the ratio of total amylase activity to body weight at different protein levels and periods



weight as evident from the Fig. 5c. At 15 days the maximum enzyme activity per gram body weight is observed, followed by a decline, afterwards. Both protein concentration and periods show significance at, 5% level ($p<0.05$).

3.2.3.3 Lipase

Two way analysis of variance (ANOVA) was carried out for the total lipase activity, lipase specific activity, ratio of total lipase activity to body weight and they are presented in Table 6a-c and in Fig.6a-c.

It is found that the maximum total lipase activity is found at 35% dietary protein concentration, followed by 15% and then 30% protein concentration. The total lipase activity shows a progressive increase at all levels, upto 60 days. Both protein and period show significance, at 5% level.

The lipase specific activity is different at different protein concentration. The maximum lipase specific activity is observed at 35% protein concentration, followed by 30%. The protein concentration and periods are significant ($p<0.01$).

Table 6c shows the total lipase activity per gram body weight. The initial stages (ie. 0 to 15 days) show the highest lipase activity. It is found that as the body weight increases, there is a decrease in total enzyme activity.

Table 6a-c. Analysis of variance (ANOVA) of lipase enzyme activity at different dietary protein levels and periods

6a. Total lipase activity

Source	Sum of squares	Degrees of freedom	Mean sum of squares	F-value
levels	7.7904	4	1.9476	9.3701*
periods	12.3703	4	3.0926	14.8788*
error	3.3256	16	0.2079	
total	23.4863	24		

CD = 0.6119

levels mean	= 3.6400	3.9400	4.4000	5.2000	4.7600
periods mean	= 16.8000	18.7000	24.0000	25.7000	24.5000

6b. Lipase specific activity

Source	Sum of squares	Degrees of freedom	Mean sum of squares	F-value
levels	0.0998	4	0.0250	22.2842**
periods	0.6417	4	0.1604	143.2352**
error	0.0179	16	0.0011	
total	0.7595	24		

CD = 0.04494

levels mean =	0.3033	0.3349	0.4038	0.4840	0.3525
periods mean =	0.99474	0.8957	2.3728	2.8375	2.3388

6c. Ratio of total lipase activity to body weight

Sources	Sum of squares	Degrees of freedom	Mean sum of squares	F-value
levels	15.4499	4	3.8625	3.5514*
periods	22.5092	4	5.6273	5.1742**
error	17.4013	16	1.0876	
total	55.3604	24		

CD = 1.3984

levels mean =	10.3500	10.5060	12.1380	12.2220	11.2260
periods mean =	62.1200	56.6200	58.7600	56.9100	47.8000

** significant at p<0.05; ** significant at p<0.01*

Fig 6a. Changes in total lipase activity at different protein levels and periods

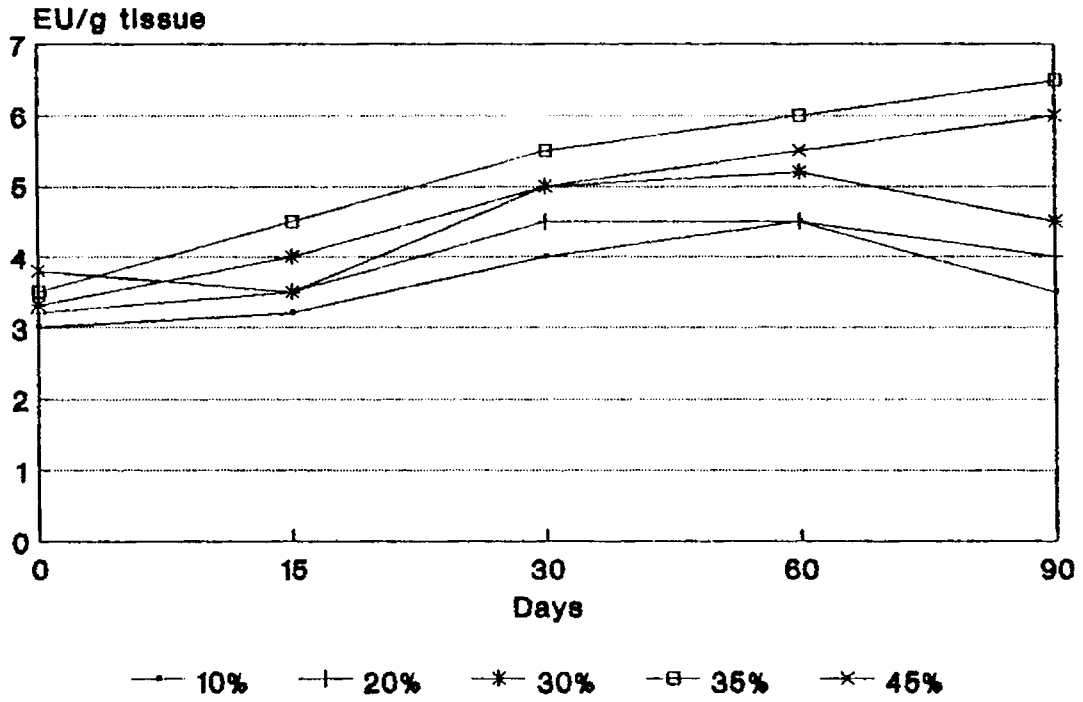


Fig 6b. Changes in lipase specific activity at different protein levels and periods

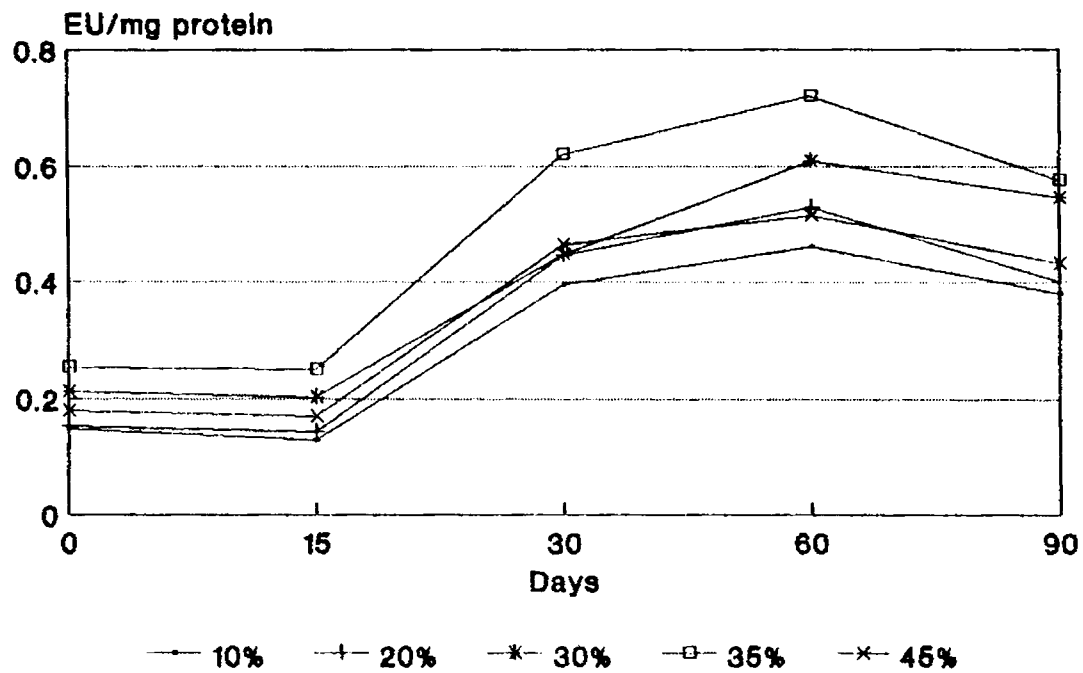
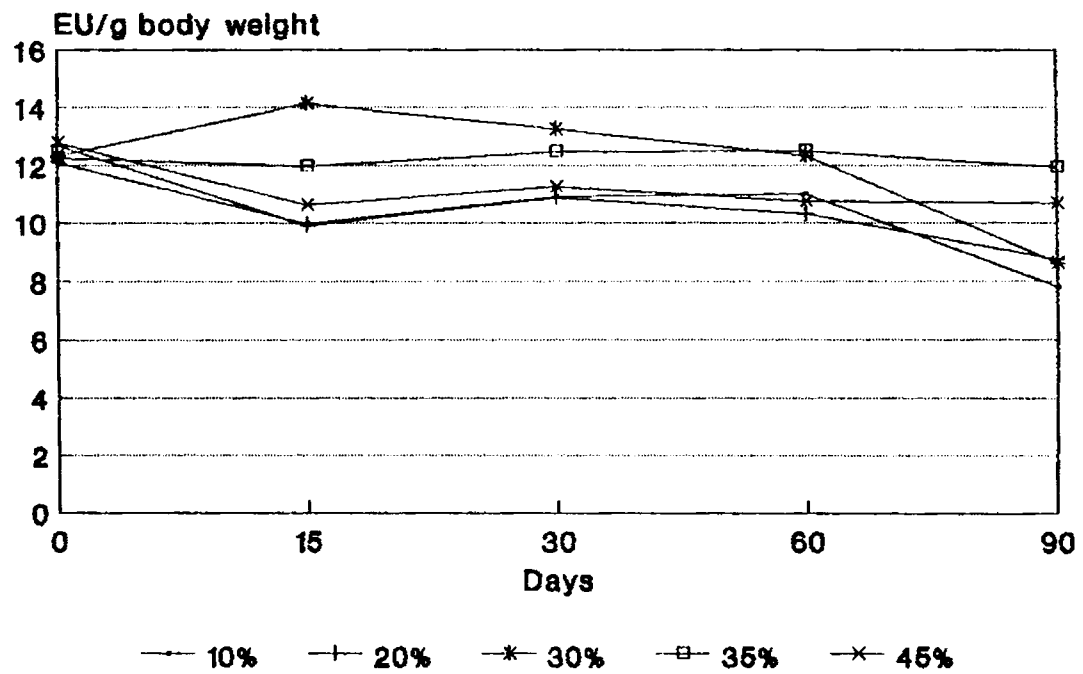


Fig 6c. Changes in the ratio of total lipase activity to body weight at different protein levels and periods



From the corresponding figures it is found that the enzyme activity increases gradually from 30% to 45% of dietary protein concentration and then shows, a slight decline. Also, 15 to 60 days of feeding trial shows the maximum enzyme activity. As the body weight increases gradually, the enzyme activity shows a declining pattern, when the total enzyme activity is expressed per unit body weight.

3.2.4 Discussion

In the present study, juvenile prawns were fed on a compounded diet containing varying protein concentrations and the effect of these protein levels on digestive enzyme activity, such as protease, amylase and lipase in the hepatopancreas of *Macrobrachium rosenbergii* was studied. In these studies it was found that the highest enzyme activity was attained at 30 to 35% protein level. Both specific activity and the ratio of total enzyme activity to body weight, were also studied and it was observed that, when the body weight increased, there was a proportionate decrease in the enzyme activity per unit body weight.

3.2.4.1 Protease

Several authors have reported the changes in the proteolytic enzyme activity in different fishes and crustaceans. Smith et al., (1987) observed that the size of the shrimp, the source and the level of protein concentration in the diet,

all affected the proteolytic activity to a certain degree in *Panaeus vannamei*. Shrimps fed with 30 % protein, displayed the highest enzyme activities. The work of Gopal (1986) and Ali (1988) showed that, an increase in the protein concentration in the diet, improved the final percentage survival of the post larvae and juveniles of *Macrobrachium rosenbergii*. Thus in the post larvae, the best survival rate of 92% was obtained at a dietary protein concentration of 30 to 40 %.

Smith et al., (198?) was able to draw an inverse correlation between the level of purified protein in the 2:1 animal plant ratio diet and growth for small and medium sized shrimps. The total enzyme activities clearly reflected the differences associated with the different protein concentration, while the differences in specific activities were usually, very little. Therefore, the concentration of enzymes in the digestive tract changed, in relation to the mass (wet weight) of the digestive tract (total activity). But, these changes were not as high in respect to the soluble protein in the digestive tract (specific activity) (Lee et al., 1984). In the present sudy also, the variations in the total enzyme activity of prawns, fed on different dietary protein levels, is observed to be more significant than the specific activity at different stages of growth. To minimise the effect of the change in the mass of digestive tract after feeding on the

total enzyme activity, it was also expressed per unit body weight.

The presence of high protease and comparatively low carbohydrase activities in the secretions of white sturgeon and other carnivorous species (Fish, 1955) and even in herbivorous and omnivorous fish, (Nagase, 1964) suggested that, fishes are more adapted to high protein and low carbohydrate diets.

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 into energy. Bages and Solane (1981) also found the need for the incorporation of enough carbohydrate to compensate for the high quantity protein in the diet, for *Penaeus monodon*. The decline in the growth rate, associated with an excess of dietary protein concentration, may also be attributed to the increased rate of catabolism of protein, for energy production. The amount of nitrogen excreted by the unit weight of the juveniles, is found to be influenced by the dietary level of proteins. A high positive correlation between the dietary protein concentration and the nitrogen excretion mg N/100g diet, was observed in the case of juvenile prawns. It indicates an increase in the level of catabolism of protein with an increase in the dietary protein concentration.

A large number of proteinases and peptidases are present in crustacea. Among the peptidases, carboxy peptidase, acrylamidase and a dipeptidase have been reported in cray fish *Aztecus aztecus* (Vijayakumaran, 1987). Das et al., (1987) investigated and found that in fry and adult of *Liza parsia*, protease, amylase and lipase are present in different regions in the alimentary canal and liver. In fry, the protease activity is comparatively higher, while amylase activity is a little less, compared to that of the adults. According to Kamarudin et al., (1994), the ontogenetic digestive enzyme activity in developing *Macrobrachium rosenbergii* larvae seem to coincide with that of the hepatopancreas development.

Lee et al., (1980) reported that, starvation had produced a general depression in the total activity of the individual protease enzymes, but had less effect on the specific activities in *Penaeus vannamei* boone. Dietary protein concentrations had a significant effect on the growth of post larvae and juveniles of *Macrobrachium rosenbergii*. The present study had also showed that, the 35% protein level had exhibited the highest enzyme activity in the juveniles. The growth rate improved, as the dietary protein level increased up to a certain, optimum level. A further increase in the protein level resulted in a decline in the growth rate of the animal. These findings quite interestingly indicate that, there is no significant added advantage.

in feeding the juvenile *Macrobrachium rosenbergii* with diets having 45% protein concentration, over the one with 35% protein concentration. These observations are important from the point of view of commercial aquaculture.

3.2.4.2 Amylase

During the post larval development, amylase activity increased steadily although it altered the enzyme activity. Diet did not appear to be the primary factor or the cause for the ontogenetic change in the digestive enzyme activity. Houdt (1982), showed that amylase and protease activities were seen during the larval development of the three species of crustacea decapoda. *In Palaemon serratus*, *Macrobrachium rosenbergii* and *Penaeus japonicus* the increase in the activity, is greater for amylase, than for proteases. These changes appear mainly at the end of the nauplius and mysis stages in the Penaeus, and during the mysis stage, in the *Palaemon* and *Macrobrachium rosenbergii*. They are related to the modification of nutrition and to the appearance of the hormonal control. The starch levels in the diets significantly influence the activity of amylase. A linear increase in amylase was observed with the starch level in the diet upto the optimum of 20% starch in the diet and beyond that level, the activity was greatly reduced (Hemambika, 1989).

The high amylase activity indicates that, starch is readily hydrolysed by *Macrobrachium rosenbergii* and probably plays an important role in energy metabolism. Tyagi and Prakash (1967) have

demonstrated high specific activities for several carbohydrases including amylase, in the prawn *Macrobrachium dayanam*. The prawns *Penaeus indicus* and *Metapenaeus monoceros* exhibited the highest amylase activity on diets composed of, 3 to 3.5% starch (Karunakaran and Dhage, 1977).

In the present study it was found that, the amylase activity was highest at 35% protein level in the hepatopancreas of the prawns, fed on experimental diets containing optimum level of carbohydrates. At a high level of protein, at 45%, the amylase activity had decreased, but it was statistically significant at 5% level. When the body weight increased, amylase activity showed a decline. Both specific and total enzyme activity showed the same pattern. From these observations, it is clear that, the maximum amylase enzyme activity in *Macrobrachium rosenbergii* juvenile is at, 30 to 35% protein level and at a feeding period of 15 to 60 days.

3.2.4.3 Lipase

The lipase activity is present in the digestive tract of most fishes (Lee et al , 1984). In the experimental fish, which received 12 to 14% dietary lipid, the lipase concentrations were found to be low in the alimentary canal contents (Buddington, et al 1986, Buddington and Doroshov, 1986). The lipase concentrations are at the highest during the larval feeding phase, but declined after the fish had metamorphosised into the juvenile

stage. The shift in the activity is related to the changes, in the natural feeding habits.

A high lipolytic activity during the larval feeding phase may represent an adaptation to the high dietary lipid levels and would enhance the utilization of the wax esters, which are less digestible (Patton et al., 1975) but can constitute, significant proportion of zooplankton lipid (Lee et al., 1971). There was no demonstratable lipase activity with olive oil substrate. It is suggested that, the fat droplets can pass through the mucosa and therefore lipid digestion could be intracellular, not requiring a soluble phase (Overnell, 1973).

In the present study it was found that the lipase enzyme activity was higher, at 30 to 35% protein level and then declined thereafter. The feed consisting of 11% fat was sufficient for the maximum growth, which was observed at a period of 15 to 60 days. This explains that, during the above period, the animals were more active than in the later stages because, throughout the experimental period, they were consuming the same feed. After 60 days their growth was retarded. From the figures also, the same results were observed.

The above findings suggested that the protein concentration had a pronounced influence on the growth of the juvenile *Macrobrachium rosenbergii*. The protease, amylase, lipase

total, specific, and total activity to body weight showed that at 30 to 35% protein concentration, the maximum enzyme activity was observed. When the body weight increases the enzyme activity shows a decrease in pattern.

Table 7a-c Matrix of correlation coefficient among digestive enzyme activity at different periods

Results

The present study has focused on the extend of variation in the enzyme activity of juvenile Macrobrachium rosenbergii at different days such as 15, 30 and 60.

The matrix of correlation (Snedecor and Cochran, 1967) relating to the various enzyme activities is given in Table 7 a, b and c.

Table 7a

15 Days

	pc 1	tpa 2	psa 5	tpb 8	taa 11	asa 14	tab 17
pc 1	1						
tpa 2	0.8398						
psa 5	0.8897*	0.9585*					
tpb 8	0.9561*	0.7437	0.8264				
taa 11	0.8384	0.7434	0.8489	0.9325*			
asa 14	0.7425	0.7311	0.6083	0.7355	0.6161		
tab 17	0.5100	0.2444	0.1368	0.5051	0.2269	0.7842	

Table 7b

30 Days

	pc 1	tpa 3	psa 6	tpb 9	taa 12	asa 15	tab 18
pc 1	1						
tpa 3	0.1978	-1					
psa 6	0.9281*	0.0889	1				
tpb 9	0.8962*	0.2504	0.9375*	1			
taa 12	0.9383*	0.0418	0.9682*	0.9228*	-1		
asa 15	0.3698	0.7382	0.4992	0.6964	0.5646	1	
tab 18	0.1389	0.5544	0.1023	0.4235	0.2033	0.8255	1

Table - 7c

	60 days						
	pc 1	tpa 4	psa 7	tpb 10	taa 13	asa 16	tab 19
pc 1	1						
tpa 4	0.6787						
psa 7	0.9170*	0.9103*					
tpb 10	0.7657	0.8690	0.9184*				
taa 13	0.8180	0.9551*	0.9764*	0.9472*			
asa 16	0.8744	0.5969	0.8392	0.8673	0.8033		
tab 19	0.1850	0.1201	0.2375	0.5578	0.3050	0.6282	

**significant at $p<0.05$;*
pc = protein concentration; tpa = total protease activity; psa = protease specific activity; tpb = ratio of total protease activity to body weight; taa = total amylase activity; asa = amylase specific activity; tab = ratio of total amylase activity to body weight.

It is evident from the table that there is a correlation that exists between the enzyme activity and the protein concentration at different days. The protease enzyme activity, protease specific activity and protease enzyme activity to body weight shows a correlation with protein concentration at 15 days, 30 days and 60 days and it is significant at 5% level. The amylase enzyme activity, amylase specific activity, total amylase activity to body weight shows a correlation with protein concentration at 15, 30 and 60 days and it is also significant at 5% level.

Chapter 4

EFFECT OF PROTEIN SOURCES ON DIGESTIVE ENZYME ACTIVITY

4.1 INTRODUCTION

Protein is the most important nutrient component for growth. They perform an essential role in the structure and functioning of animals, such as, maintenance, rebuilding new tissue for growth, formation of hormones, enzymes and other biologically important substances and also as a source of energy. It has been found that, a mixture of animal proteins gives a better growth than a mixture of plant proteins or any proteins tested alone (Dupree, 1967). The fairly good growth rate of the post larvae and juveniles of *Macrobrachium rosenbergii* is due to the well balanced mixture of amino acids in the diets obtained by a proper mixing of protein from various sources.

Most of the plant proteins have shown poor growth rate in the prawns when used individually, excepting in the cases of a few (Kanazawa et al., 1970; Deshimanu and Shigueno, 1972). Patra and Ray (1988) have observed a higher protease activity in the hepatopancreas, stomach and intestine of fish, fed with animal source of protein. Gopal (1986) reported that, prawns showed variations in body composition, depending on the quality of the protein in the diets. He observed a high protein content in prawns fed on protein from animal source and attributed it to the higher biological value of the animal protein.

The optimum protein level required for the maximum weight gain and digestive enzyme activity were arrived at, from the results obtained in the previous experiments. The aim of the present study is to determine the digestive enzymatic response to different protein diets and to evaluate, to what extent that would cause the varied changes during a 12 week feeding trial.

The protein sources selected for the preparation of the diets were clam meat meal, shrimp meal, silk worm pupae meal, squilla meal, groundnut oil cake meal, and algae meal, at an optimum protein concentration.

4.2 MATERIALS AND METHODS

4.2.1 Preparation of diets

The diets were prepared using different protein sources at 35% protein level. The proximate composition of the feed and the raw materials was determined as per the methods given in Chapter 3.1.2. The feed was formulated with 35% protein, 11% fat, 10% mineral, 3% vitamin mix and 1% cholesterol. The protein concentration was adjusted at 35% protein with casein. The steps involved in the formulation of the feed are given below:

* Procurement of quality ingredients and additives.

* Storing after processing and proper labelling.

* Preserving labile ingredients. e.g. oils, vitamins in a cool place.

* Accurate weighing of the ingredients (ground and unground in proportions required for the selected formula.

* Preparing vitamin and mineral premixes

* Grinding of unground materials

* Microgrinding

* Screening

* Mixing

* Cooking / Pelleting

* Cooling / drying

* Crumbling

* Screening particle segregation

* Packing

* Storage

4.2.1 The Processing Of The Raw Materials Of The Feed

Clam, Prawn, Silkworm pupae, Squilla, Groundnut oil cake were used for the preparation of the feed. The protein sources used for the feed formulation were processed following a uniform method in order to avoid variations in the quality during the processing.

Clam meal

Live clam *Villorita cyprinoids* were collected from the local market. They were steamed in an autoclave for 15 minutes. The meat was then separated and dried in an electric drier for 12 hours at 60°C

Prawn meal

Small sized shrimps were collected from the local market. They were then steamed in an autoclave for 15 minutes and dried in an electric drier for 12 hours at 60°C .

Silk worm pupae meal

Live pupae of silk worm *Bombyxmori*, used for sericulture at the cocoon peeling centre, Pattanakkad, Alleppey (Dist.), Kerala., were brought in a carton. They were steamed for 15 minutes in an autoclave and dried for 12 hours in an electric drier at 60°C and then partially deoiled by the solvent extraction method (Unnikrishnan et al., 1993).

Squilla meal

Whole squilla *Orato squilla nepa* were collected from the Fisheries Harbour, Kochi, washed thoroughly and then steamed in an autoclave for 15 minutes and dried in the electric drier for 24 hours at 60°C.

Groundnut oil cake meal

The Ground nut oil cake was purchased from the local market, dried in an electric drier for 24 hours at 60°C and then powdered.

Algae meal

Algae was collected from the local pond and dried in an electric drier for 12 hours and then powdered.

Compounding of the feeds

The feed was prepared, consisting of clam meal, prawn meal, silkworm pupae meal, squilla meal, ground nut oil cake meal and algae meal, at a protein level of 35%. The compounding of the feed was done with mineral mix,sunflower oil, cellulose and starch, to give a final level of 11% fat,10% mineral and 1% cholesterol. The dry powdered ingredients were weighed and mixed together and kneaded well with water to produce small granule size consistency .The mixture was steam cooked for 30 minutes and then particulated after mixing with vitamin mix and egg albumin binder, steam dried at 60°C and made in to particulated feed to give an average size of 0.01mm. The particulated feeds are stored in air tight containers, in refrigerator.

4.2.2 The feeding regime and sampling

The experiment was started with 50 animals, each in 18 fibre glass tanks of 100 litre capacity They were fed with the test diet for one week to acclimatise them to the feed. Water quality was maintained

through out the experiment. Aeration was provided. The dissolved oxygen, pH, temperature, nitrite and ammonia were maintained at the optimum level.

The animals were then given the particulated feed. everyday and after 24 hours of feed ration, the residual feeds were collected. The feeds were given on the adlibitum basis. The length, weight, general health and survival of the juvenile prawns were all monitored at 0 days, 15 days, 30 days, 60 days and 90 days. Protease, amylase and lipase activities were also monitored. The details of sampling, enzyme extraction and the assay of the enzymes are all detailed in Chapter 3.

4.2.3 Statistical analysis

The feeding experiments were designed on the basis of completely randomised design. The results obtained from the experiments were subjected to analysis of variance (ANOVA) as per the method of Snedcor and Cochran (1967) and the treatments means were compared by Duncan's multiple range test (Steel and Torrie, 1980). The F test was performed to determine if a difference between these treatments existed. If the value was found to be significant, the data was analysed by a Least Significant Difference (LSD) test. All the possible difference between the means of each treatments were computed and compared to LSD. If the absolute value of the difference 'd' was greater than LSD, the difference was found to be significant at $p<0.05$.

4.3 RESULTS

The details of the formulation of experimental feeds from different protein sources and the proximate composition of the raw ingredients are presented in Table 8. The results of the protease, amylase and lipase activity in the hepatopancreas of *Macrobrachium rosenbergii* juveniles fed on the experimental diet containing various protein ingredients are presented in Fig. 7, 8 and 9. The ANOVA of the results are given in Tables 9, 10 and 11.

4.3.1 Protease activity

The different diets used in the study were, clam meat, shrimp meat. silkworm pupae, squilla, groundnut oil cake and algae which were prepared from the protein sources, are represented as diet A, B, C, D, E and F respectively. The period represents the different days of sampling during the experiment. All the diet formulations contain 35% protein level

Anova Table 9a shows, the total protease activity with different protein diets, on different days of growth. Here, shrimp meal (Diet B) shows, the maximum total protease activity, followed by diet C, which contains silkworm pupae meal.. But the sources and periods means are not very significant.

Anova Table 9b shows the protease specific activity in prawns, fed on different protein diets. It is found that, there is not much variation in the specific activity of the enzyme at different

Table 8 : Formulation and proximate analysis of experimental diets from different protein sources.

Ingredients. **Protein (%) 35**			**g/100g Dry Diet**			
Sources	**Diet A**	**Diet B**	**Diet C**	**Diet D**	**Diet E**	**Diet F**
Protein	78.652	72.165	73.684	88.832	82.742	100
Sun flower Oil	-	2.66	2.14	2.13	1.5	6.84
Mineral mix*	2.5	-	-	-	-	-
Vitamin mix*	3	3	3	3	3	3
cholesterol	1	1	1	1	1	1
Egg albumin	2	2	2	2	2	2
Cellulose	3.468	1	1	1	1	1
Starch	9.38	18.175	17.176	2.038	8.758	-

Proximate composition of different protein sources

Source	**Diet A**	**Diet B**	**Diet C**	**Diet D**	**Diet E**	**Diet F**
Moisture	8.5	8.2	8.45	6.95	8.65	5.28
Protein	44.5	48.5	47.5	39.4	42.3	28
Fat	11	8.34	8.86	8.87	9.50	4.16
Fibre	0.88	7.26	6.42	6.35	9.65	1.5
Ash	7.5	24.36	21.94	25.70	13.34	10.80
NFE	27.62	3.34	6.83	12.73	16.56	50.34
E/Kcal/ 100g	387	282	297	174	321	351

Diet A : Clam Meal, Diet B : Prawn Meal, Diet C : Silkworm Pupae Meal,
Diet D : Squilla Meal, Diet E : Ground Nut Oil Cake Meal and Diet F : Algae Meal
Mineral Mix* g/kg dry diet; Vitamin Mix* mg/kg dry diet } Composition as given in table 1

Table 9a-b. Analysis of variance (ANOVA) of protease enzyme activity at different protein sources and periods

9a. Total protease activity

Sources	Sum of squares	Degrees of freedom	Mean sum of squares	F - value
sources.	10.0144	5	2.0029	2.5289
periods	6.6343	4	1.6586	2.0942
error	15.8396	20	0.7920	
total	32.4883	29		

	CD = 1.0718			CD = 1.1740		
sources. mean =	9.7940	10.4080	10.3960	9.1100	10.1360	8.9700
periods. mean =	54.3700	60.8200	62.2500	59.6800	56.9500	

9b. Protease specific activity

Sources	Sum of squares	Degrees of freedom	Mean sum of squares	F-value
sources.	10.9815	5	2.1963	1.0011
periods.	88.0216	4	22.0054	10.0302**
error	43.8782	20	2.1939	
total	142.8813	29		

	CD = 1.7839			CD = 1.9542		
sources. mean =	13.5140	13.6940	13.3760	14.5020	14.1440	12.5860
periods. mean =	76.2200	77.5600	96.3900	90.7500	68.1600	

***significant at p<0.01.*

Fig 7a. Changes in total protease activity at different dietary protein sources.

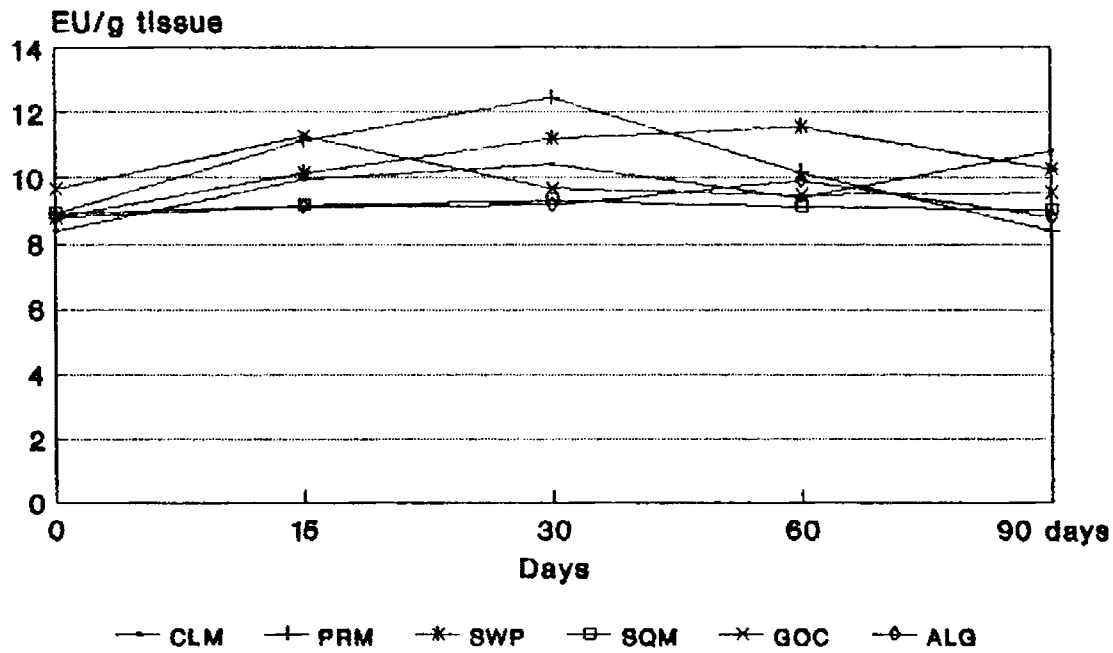
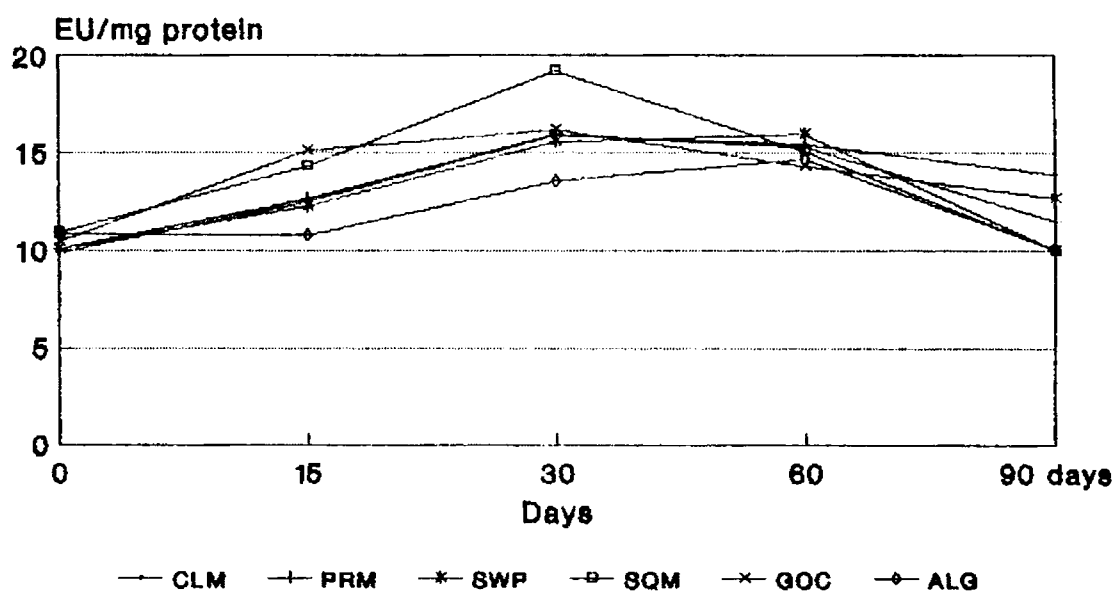


Fig 7b. Changes in protease specific activity at different dietary protein sources



CLM-clam meal, PRM-prawn meal
SWP-silkworm pupae meal, SQM-squilla meal, GOC-groundnut meal, ALG-algae meal

protein sources. Diet D which contains squilla meal shows, maximum activity, especially at 30 days of feeding. Algae meal Diet F shows the least enzyme activity throughout the culture period. In periods also, the maximum enzyme activity is shown at 30th day. The different days show significance at 5% level.

Figures 7a and 7b represents the total enzyme activity and specific activity for different diets at different days of sampling and they show not much variation between each other. The growth periods of 30 to 60 days show the maximum total and specific activity.

4.3.2 Amylase activity

Fig. 8a and Anova Table 10a show the total amylase activity in different protein diets. The maximum enzyme activity is seen on a diet which contains clam meat meal, at 15 days. This is followed by the silk worm pupae diet and the shrimp meal diet. Their difference is not significant. The enzyme activity shows maximum at 30 days, irrespective of the protein sources and the difference is significant at 5% level.

Fig. 8b and Anova Table 10b show the amylase specific activity in different protein diets at different periods. The highest enzyme activity is observed in diet D, which contains the squilla meal. It is followed by diet E which contains groundnut oil cake meal and diet A which contains clam meal. The period also shows significance at

Table 10a-b. Analysis of variance (ANOVA) of amylase enzyme activity at different protein sources and periods

10a. Total amylase activity

Sources	Sum of squares	Degrees of freedom	Mean sum of squares	F - value
repln.	139.3813	5	27.8763	1.47771
trmt.	514.1927	4	128.5482	6.8116**
error	377.4417	20	18.8721	
total	1031.0157	29		

			CD = 5.2319		CD = 5.7313	
sources mean =	51.8960	50.8500	51.3560	50.5060	50.4600	45.3860
periods mean =	269.4000	271.6100	326.1300	312.8700	322.2600	

10b. Amylase specific activity

Sources	Sum of squares	Degrees of freedom	Mean sum of squares	F - value
sources	8.2995	5	1.6599	3.5585*
periods	24.5514	4	6.1379	13.1583**
error	9.3292	20	0.4665	
total	42.1801	29		

			CD = 0.8225		CD = 0.9012	
sources mean =	4.7996	4.5472	4.2526	5.5992	4.9642	3.9590
periods mean =	23.5420	23.8930	34.1360	35.3660	23.6720	

* *Significant at p<0.05* ; ** *Significant at p<0.01.*

Fig 8a. Changes in the total amylase activity at different dietary protein sources

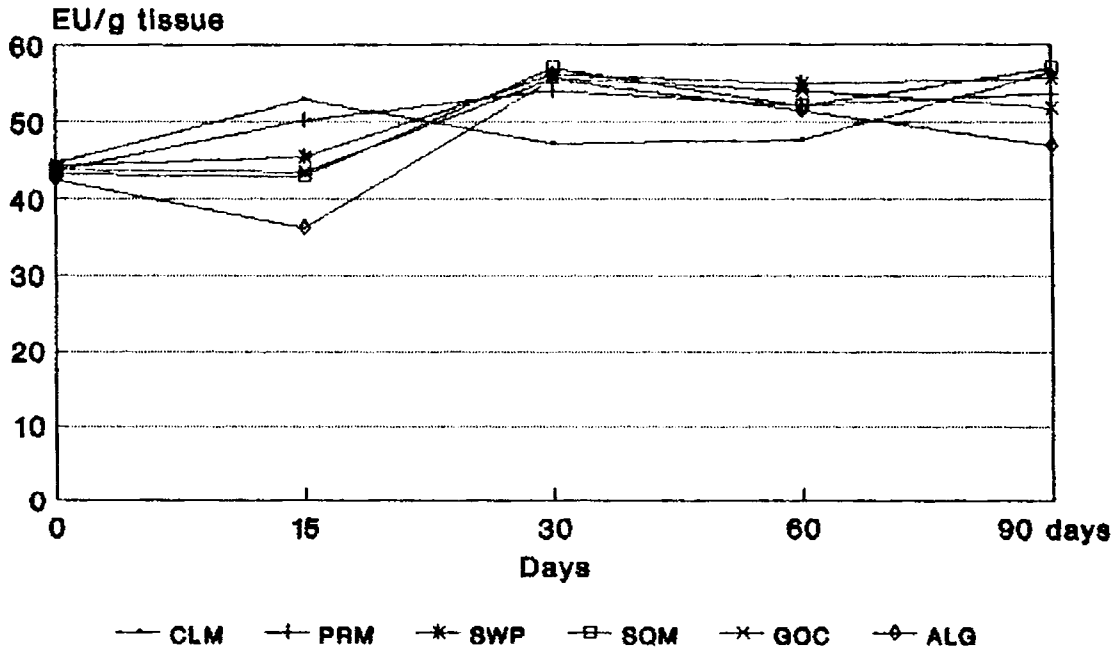
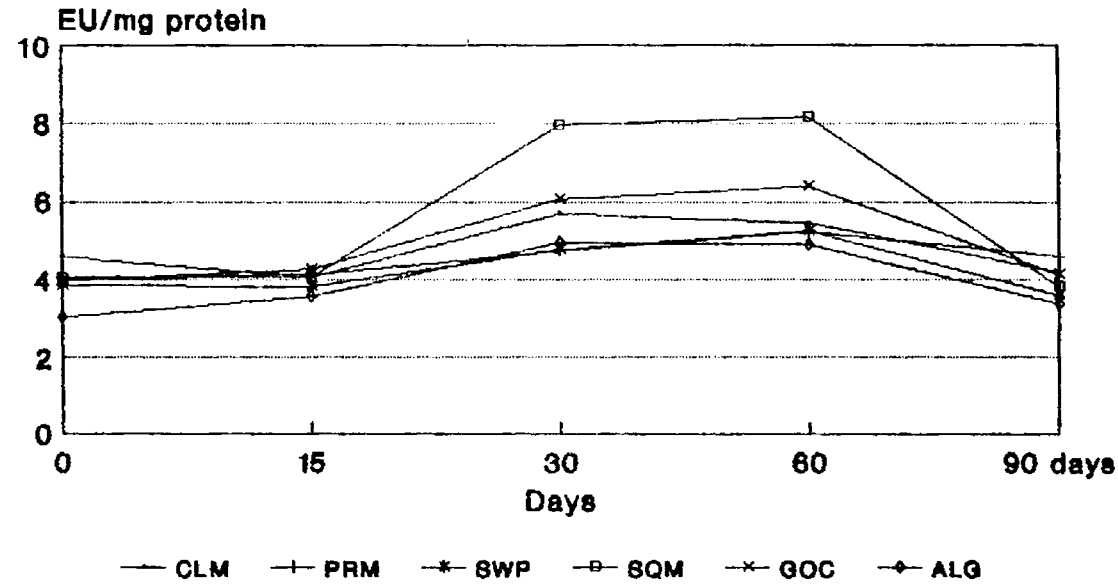


Fig 8b. Changes in amylase specific activity at different dietary protein sources



CLM-clam meal, PRM-prawn meal, SWP-silkworm pupae meal, SQM-squilla meal, GOC-groundnut meal, ALG-algae meal

5% level. Maximum enzyme activity is seen at 60 days. At 90 days the activity has decreased.

From these observations, it is clear that, amylase activity is maximum in squilla meal diet and clam meal diet, though their difference is not statistically significant. The period at 30 days shows the best activity.

In Figures 8a and 8b, the graphical representation of total amylase activity and specific activity respectively, are given at different protein sources.

4.3.3 Lipase activity

Anova Table 11a shows the total lipase activity to different protein sources at different periods. Here the maximum enzyme activity is attained in the diet D which contain squilla meal. It is followed by silk worm pupae diet (diet C). Clam meal and shrimp meal show the next total lipase enzyme activity. This is because of the type of fat content in these particular diets. The activity is statistically significant at 5% level. The above changes are represented graphically in Fig.9a. The period showed a maximum activity at 60 days, followed by 30 days and it is significant at 5% level.

Anova Table 11b shows the lipase specific activity. Here the maximum enzyme activity is shown at diet D which contains squilla meal followed by groundnut oil cake meal diet. The activity is

Table 11a-b Analysis of variance(ANOVA) of lipase enzyme activity at different protein sources and periods.

11a. Total lipase activity

Sources	Sum of squares	Degrees of freedom	Mean sum of squares	F - value
sources	11.0416	5	2.2083	11.0413**
periods	11.8719	4	2.9680	14.8395**
error	4.0001	20	0.2000	
total	26.9136	29		

			CD = 0.5386		CD = 0.5899	
sources. mean = 5.7000	5.7000	5.9000	6.5000	5.0600	4.6000	
periods. mean = 30.5000	29.5000	33.5000	40.3000	33.5000		

11b. Lipase specific activity

Sources	Sum of Squares	Degrees of Freedom	Mean Sum of Squares	F - Value
sources.	0.0657	5	0.0131	4.1517*
periods.	0.3942	4	0.0985	31.1321**
error	0.0633	20	0.0032	
total	0.5232	29		

			CD = 0.0679		CD = 0.0743	
sources. mean = 0.4194	0.4408	0.4814	0.5552	0.5121	0.4428	
periods. mean = 1.8897	2.3814	3.4673	3.7631	2.7574		

**significant at p<0.05; ** significant at p<0.01.*

Fig 9a. Changes in total lipase activity at different dietary protein sources

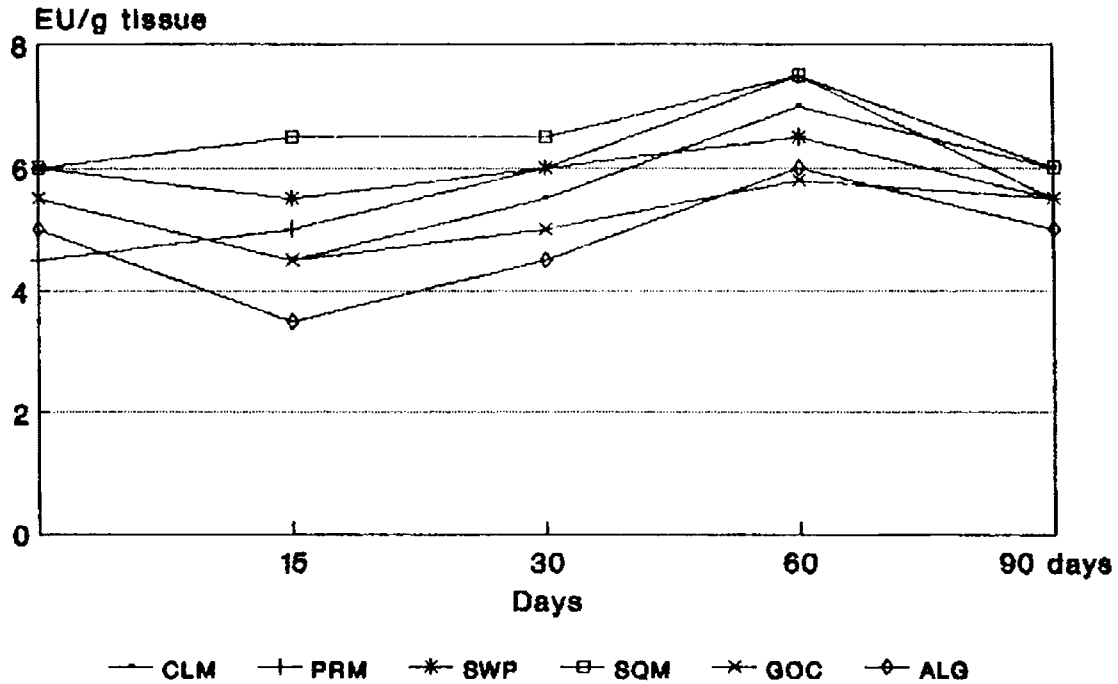
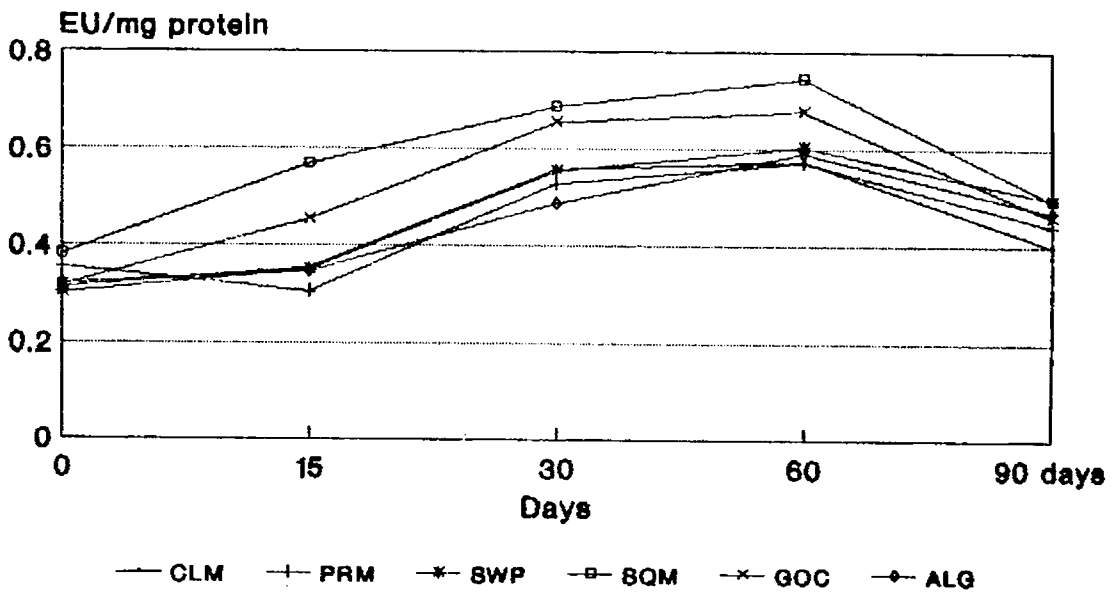


Fig 9b. Changes in lipase specific activity at different dietary protein sources



CLM-clam meal, PRM-prawn meal, SWP-silkworm pupae meal, SQM- squilla meal, GOC-groundnut meal, ALG-algae meal

significant at 5% level. Among the sampling days, the highest enzyme activity is observed at 60 days followed by 30 days and this is statistically significant at 5% level. The results are given graphcally in Fig. 9b.

All these results show that, the squilla meal diet, shrimp meal diet and clam meal diet show the maximum lipase activity during the stages at 15 to 60 days of growth. The plant protein, groundnut oil cake and algae meal show low enzyme activity.

4.4 DISCUSSION

The results of the present study have revealed the possibility of a successful use of clam meal, prawn meal, silk worm pupae meal, squilla meal, groundnut oil cake meal and algae meal in commercial diets for the fresh water prawn *Macrobrachium rosenbergii* juvenile prawn.

In the present study animals which were given shrimp meal and squilla meal show the maximum enzyme activity. The exoskeleton is primarily chitin and it has limited the nutritional value. Thus the visceral organ in the head section is the most valuable, nutritionally. According to Lovell (1988), crude protein analysis of shrimp waste meal has to be accounted for the nitrogen in chitin and the nitrogen in chitin accounts for about 10 to 15% of the total nitrogen. In addition to the supply of

proteins, shrimp waste meal is another source of n-3 fatty acids, cholesterol and astaxanthin. It is highly palatable and may serve as an attractant in feeds for fish and crustaceans.

4.4.1 Protease Activity

Grossman et al., (1943 and 1944) had conducted a series of experiments using lab rats, which revealed a positive relationship between high protein diets and protease activities. The same positive relationship was described for high carbohydrate diets and amylase activity. Prosser and Vanweel (1958) and Vanweel (19[illegible]), while investigating the effects of dietary composition on the giant African snail *Achatura fulica* found that, a continued high protein diet caused a suppression of proteolytic activities. Thus, the effect of the diet on enzyme activities may be variable. The fact that the former study was on vertebrates and the latter was on invertebrates, may be the reason for the conflicting results.

In the present study it was found that there is a relationship between protein diets and enzyme activities. The shrimp meal based diet, squilla meal diet and clam meat showed higher protease and amylase activities, than the diet containing plant protein such as groundnut oil cake meal and algae meal. The protein content of all the above diets were fixed at 35%. But in the case of lipase, a higher activity is found in the diet containing groundnut oil cake. But all animal protein show a

higher activity during the 30 days and 60 days of the growth period.

Some scientists, while investigating the amylase and proteinases of decapoda, discovered a higher protease activity in omnivores than in carnivores (Yonge, 1937; Reed, [illegible]; Sather, 1969). It is now generally accepted that, the high protease activity is characteristic of carnivorous organisms. The inverse is true for herbivores while detritivores and omnivores fall in a more intermediate position.

The hepatopancreas of *Macrobrachium rosenbergii* contains enzymes, capable of hydrolysing a wide variety of protein. Murthy (1977) using *Macrobrachium lamerrei* detected trypsin, aminopeptidase, leucine aminopeptidase, and dipeptidase, however chymotrypsin was not detected.

Digestive enzyme activities vary, during larval growth (Ceccaldi, 1978). According to him almost all the marine larvae usually begin to eat phytoplankton and later zooplankton. Vanwormhoudt (1973) and Vanwormhoudt et al (1980) have observed marked changes in the activity during the metamorphosis of zoeal stages to mysis in post larval stages of *Palaemon Serratus* and *Penaeus japonicus*.

The adults, getting prepared for the maturation and propagation of the species, show greater protease activities, demonstrating their preferences for carnivores type of diet (Hemambika, 1989) which agrees with the present observation, where animal protein showed a uniform increase in the protease activity. Results of the various studies indicate that, digestive enzyme activity changes with the stages of the animal growth, while amylases show the maximum activity in the juvenile stage.

This statement agrees with the present study and it is found that in the juvenile stage, the maximum activity is seen during the 30 to 60 days after feeding and then show a decline at 90 days because, the same type of diet is not properly utilised by the animal. Clam meat has been reported as a good source of protein for shrimps and prawns by many scientists (Deshimanu, 1981; Ali, 1982; 1988; Goswami and Goswami, 1982; Kungvankij et al., 1986; Akiyama, et al 1988; Aquacop et al., 1989). Earlier, scientists had demonstrated that the protease activity was related to the protein quantity in the diets (Kawai and Ikeda, 1973; Albertini Berhaut, 1978; Hofer, et al 1985).

4.4.2 Amylase activity

The high amylase activity indicates that, starch is readily hydrolysed by *Macrobrachium rosenbergii* and possibly plays an important role in energy metabolism. Tyagi and Prakash

(1967) have demonstrated high specific activities for several carbohydrases including amylase in the prawn *Macrobrachium dayanam*. Biddle (1977) observed that the ability of prawns to digest carbohydrases, varied with species, but complex carbohydrates in the diet, resulted in a better growth than simple sugar. In growth experiments with *Penaeus setiferus*, an increased starch levels at 30% , reduce the protein requirement, while maintaining the adequate growth (Andrews et al., 1972). The addition of glucose from 20 to 40% to the diet, resulted in a reduced growth, suggesting that glucose was quickly but inefficiently utilised, whereas the sugars in complex carbohydrates were utilised slowly and more efficiently.

In the present study it is found that the highest amylase specific activity is exhibited when the animals were fed with a diet containing squilla meal, clam meal and groundnut oil cake having 35% level protein content followed by silk worm pupae and shrimp meal diet. The variation in the observed enzyme activity may be due to the difference in the type of carbohydrates present in the different feed ingredients.

4.4.3 The Lipase Activity

In the present study there is obvious gradient of activity of lipase enzymes. The prawn *Macrobrachium lamarrei* display a high esterase activity and a moderate lipase activity (Murthy, 1977). Van weel (1970) has proposed that, esterases

rather than lipases are primarily responsible for the hydrolysis of fats in crustaceans. Lipase activity is rather low in post larval and juvenile groups but a two fold increase in activity is recorded in the adult group when compared to the juveniles. (Hemambika, 1989). Similar conclusion can be drawn from the present study that, the total enzyme activity showed a progressive increase from the juvenile to the later stages of adulthood.

Phadate and Srikar (1987) found that protease activity was at the highest in all the three species of carps, when they were fed on protein rich food. The carbohydrate rich feed had a slightly stimulating effect, while lipid rich feed had marked negative effect on the enzymatic activity. During the starvation period there was a decrease in the activity accompanied by stagnation in growth. He also observed that in most cases definite trends in enzyme activities were discernible only after 15 days of feeding, which indicated the time required for metabolic adjustments to the feeds.

Kawai and Ikeda (1972 and 1973) had observed definite trend in enzyme activities in trouts after 10 days and in carps after 7 days, of the commencement of feeding.

From all these observations it is clear that different protein sources have a great influence on the digestive enzyme

activity. In the present experiment, the animal protein showed a higher activity at 35% protein level compared to plant protein. The variation between them were not found to be statistically significant. The difference in the various enzyme activities during the different culture periods may be due to the fact that, the efficiency of the animal to hydrolyse and assimilate the various complex nutrients in the feeds selected, decreases towards the adult stage compared to the early juvenile period.

Chapter 5

NUCLEIC ACID CONTENT IN THE MUSCLE OF MACROBRACHIUM ROSENBERGII FED WITH DIFFERENT PROTEIN LEVELS AND PROTEIN SOURCES

5.1 INTRODUCTION

Protein synthesis in animal tissue is a complex process which involves, deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and ribosomes. DNA, the chromosomal component of cells, carries the genetic information in the cell and transmits inherited characteristics from one generation to the next (Lovell, 1984). In crustacea, growth is an increase in the dry weight of the body which generally occurs in the periods between moults, when the absorbed water is gradually replaced by protein. DNA controls the development of the organism by controlling the formation of RNA (Thomas, 1993).

Growth in terms of accumulation of protein is always accompanied by high turn over rate of RNA concentration which is a prime factor of protein synthetic machinery. Since the DNA content per cell is generally constant, the RNA/DNA ratio reflects the growth (Buckley, 1984). Growth is generally faster in the early phases of the life cycle of the animals (Dagg and Page, 1972). Therefore small animals with high RNA/DNA ratio are expected to grow at a faster rate than those with a lower ratio. Maintenance of this high RNA/DNA ratio and the continuity of

the growth at a faster rate also depend on the availability of sufficient food (Buckley, 1979).

In the present study, an attempt has been made to detect the changes in DNA, RNA and the protein content in the muscle during the development of *Macrobrachium rosenbergii* juveniles fed on variable dietary protein levels and protein sources in order to investigate the use of RNA: DNA ratio, as a growth assessing parameter.

5.2 MATERIALS AND METHODS

5.2.1 Preparation Of Experimental Diets And Feeding Trial

To produce a growth differential test of the RNA-DNA technique, two controlled laboratory feeding experiments were conducted. In both experiments 3 months old *Macrobrachium rosenbergii* were fed on the experimental diet, at approximately 3% of the total wet body weight of prawn per day, until they got acclimatised to the laboratory conditions.

In the first experiment, prawns were fed with a feed at different protein levels of 10%, 20%, 30%, 35%, and 45%, the diet composition of which are given in Chapter 3 (3.2.2.1).

In the second experiment, prawns were fed with the formulated feeds from different protein sources such as clam

meal, shrimp meal, silk worm pupae meal, squilla meal, groundnut oil cake meal, and algae meal, at 35% protein level, as described in Chapter 4. The diet composition of the feed and proximate analysis of the feed ingredients are given in Chapter (4.2.1). The prawns were fed once daily. The excess of feed and faecal matter were siphoned from the tanks between the feedings. The exchange of water was carried out, approximately twice or thrice in a week. The average water temperature and dissolved oxygen during the experimental period were 27°C to 28°C and 6.4 to 7.0 ppm respectively. Sampling of animals was done at 0 day,and at 15 days, 30 days, 60 days, and 90 days of feeding. Five animals each were selected in triplicate from each treatment and the DNA, RNA and protein content were determined.

5.2.2 Estimation Of Muscle Protein And Nucleic Acid

A weighed quantity of muscle tissue was homogenised with distilled water and was treated with 10 % cold trichloroacetic acid, to precipitate the proteins. The homogenate was centrifuged at 5000 rpm for about 15 minutes and then the supernatant was discarded. The process was repeated for the complete removal of acid soluble compounds. The residue was treated with 95% ethanol twice and with solvent ether twice. After discarding the solvent, the residual defatted mass was dried to remove solvent traces. The dried fat free sample was used for estimating protein and nucleic acid content.

Protein was estimated by the method of Lowry et al., (1951) using bovine serum albumin as the standard. RNA was extracted and estimated by the method of Schneider (1957). A calibration curve was prepared using purified yeast RNA as the standard. DNA was extracted by the method of Webb and Levy (1955) and estimated using the technique of Ashwell (1957). The calibration curve was prepared using calf thymus DNA as the standard. RNA : DNA ratio was calculated by dividing RNA to DNA value.

Growth was determined by the method described by Halver (1976). The initial and final weight of the animals under different treatments were monitored at different days of sampling. Specific growth rate is calculated using the formula,

$$\text{Specific growth rate} = 100\ (\log_e w_2 - \log_e w_1)/(t_2 - t_1)$$

where, w_2 = final weight

w_1 = Initial weight

t_2 = final time

t_1 = Initial time

5.2.3 Statistical Analysis

The feeding experiments were designed on the basis of completely randomised design. The results obtained from the

experiments, were subjected to analysis of variance ANOVA as per the method of Snedecor and Cochran (1967) and the treatmeants means were compared by Duncan's multiple range test (Steel and Torrie, 1980). An F test was performed to determine if the difference between the treatment means, existed. If the value was found to be significant the data was analysed by a least significant difference test (LSD). All the possible difference between the means of each treatment, were computed and compared to LSD. If the absolute value of the difference (d) was greater than LSD the difference was found to be significant at $p<0.05$.

5.3 RESULTS

In this experiment, the results of RNA-DNA content, and protein in the muscle tissue, RNA : DNA ratio in prawns fed on diet at different protein levels and protein sources at different growth periods are presented. The relation of RNA-DNA ratio to different days, and weight of animals are also presented.

5.3.1 Effect of different dietary protein levels

DNA content

Anova Table 12a and Fig 10a shows the DNA content, in relation to different protein concentration.. From this, it appears that a 35% protein level, shows the minimum value of DNA at all the sampling days followed by 30% protein level. The DNA content which shows a slightly higher in the group fed on 10% protein

Table 12a. Analysis of variance (ANOVA) of DNA content at different protein levels and periods.

Sources	Sum of squares	Degrees of freedom	Mean sum of squares	F - value
levels	38513.7500	4	9628.4375	4.8700**
periods	37111.0508	4	9277.7627	4.6926*
error	31633.6992	16	1977.1062	
mean	107258.5	24		

CD = 59.6184

levels. mean =	263.1940	247.4340	179.2400	160.1518	203.4260
periods. mean =	804.4690	1039.9900	1026.3501	997.2900	1399.1300

Table 12b. Analysis of variance (ANOVA) of RNA content at different protein levels and periods.

Sources	Sum of Squares	Degrees of Freedom	Mean Sum of Squares	F - Value
levels.	26481.6504	4	6620.4126	6.5545**
periods.	5710.9502	4	1427.7375	1.4135
error	16160.8994	16	1010.0562	
total	48353.5000	24		

CD=42.6126

levels mean=	212.2340	228.1920	271.8320	302.7600	238.3180
periods mean=	1353.5900	1292.4500	1254.8601	1242.2500	1123.5300

***significant at p<0.01; *significant at p<0.05.*

Fig 10a. Changes in DNA content in the muscle tissue of M.rosenbergii fed with different levels of protein.

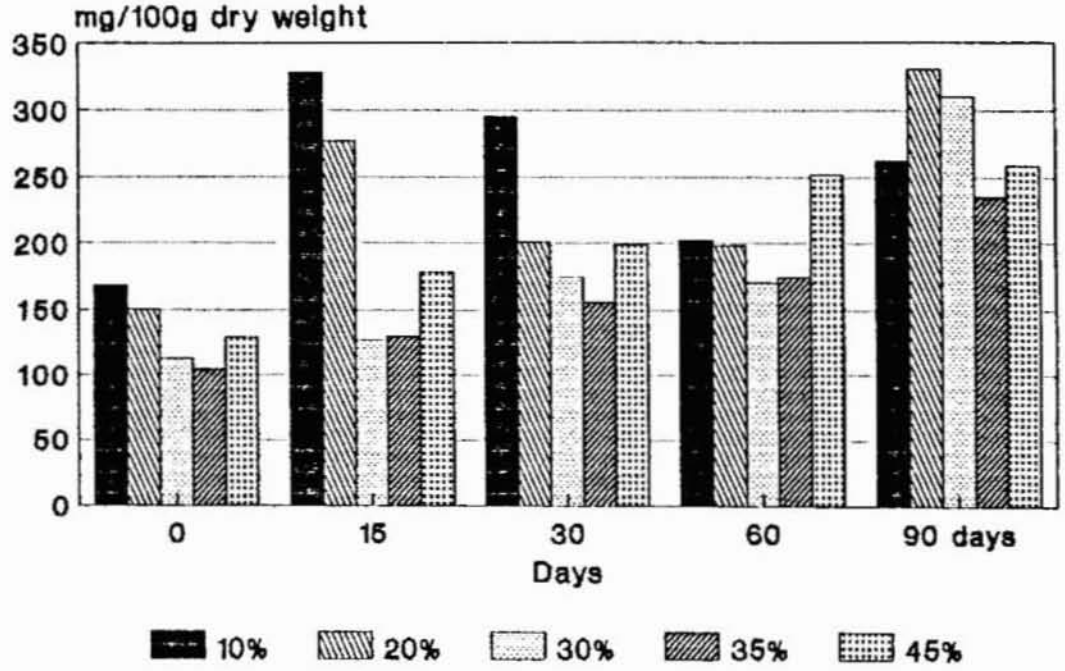


Fig 10b. Changes in RNA content in the muscle tissue of M.rosenbergii fed with different levels of protein.

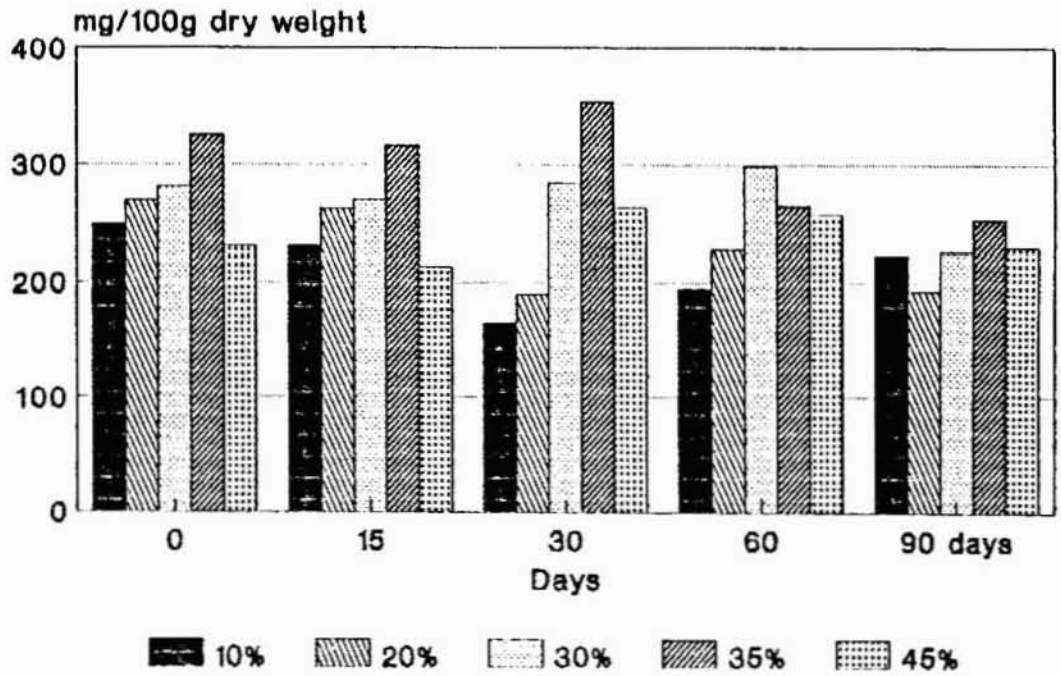
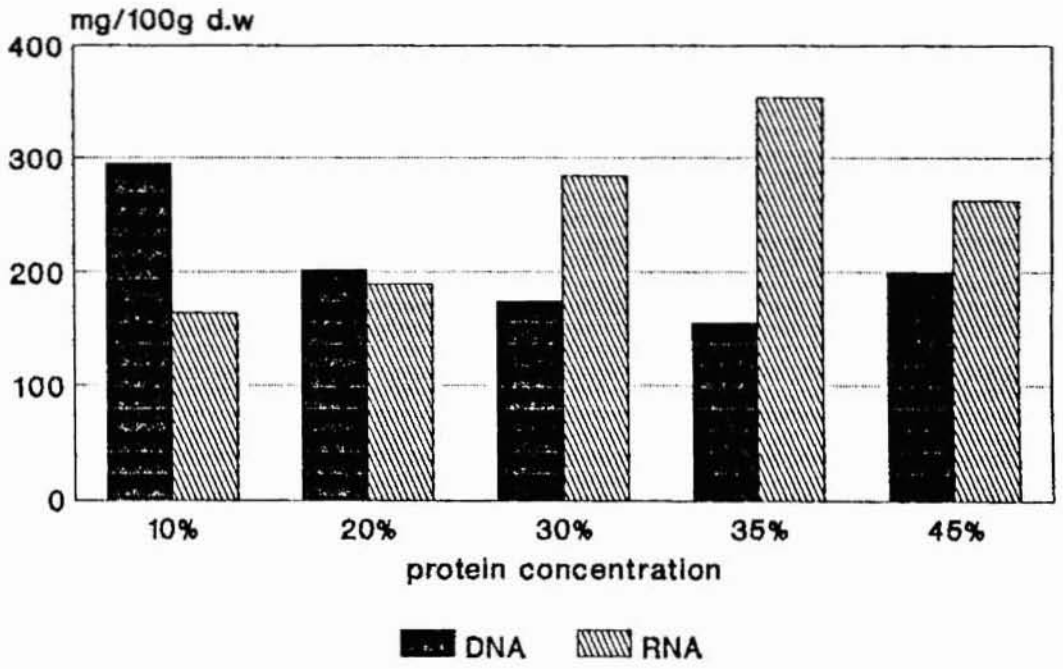


Fig 10c. Variation in the DNA and RNA content in the muscle tissue at 30 days of feeding



diet. The quantity of DNA however, showed a high value at 45% protein level. The period also shows variation in DNA content and the maximum DNA value was obtained at 90 days for all the protein levels. Both periods and treatments are statistically significant at 5% level ($p<0.05$). From this it is clear that the DNA value shows a low profile at 35% and 30% protein levels, compared to other dietary protein levels (Fig 10a).

RNA Content

Anova Table 12b shows the RNA content in relation to different protein concentration. The levels are statistically significant at 5% level ($p<0.05$). The Fig 10b. shows that there is a linear increase in the RNA content with increase in the dietary protein level upto 35%. On further increase of dietary protein levels, RNA content decreased. A similar pattern of RNA variation is observed at all the sampling days upto 60 days and the changes being most pronounced at 30 days of feeding trial. Towards 90 days of feeding, the RNA content of the muscle protein remains at a low level irrespective of the different dietary protein levels.

From the above results it is clear that the RNA value show a maximum at 35% protein level and DNA value show an inverse pattern at 35% protein level. Both the values are most pronounced at 5% level. Such a variation in RNA and DNA content at various protein levels is significant after feeding the experimental diet for 30 days and this is depicted in Fig 10c.

RNA : DNA ratio

The initial and final weight of the animals fed on different protein levels were monitored at different stages of growth. From this the SGR% and average weight gain were calculated. It is found that there is a uniform increase in body weight with the increase in dietary protein levels. Fig 12a to d, and 13a to d describes the variation of RNA\DNA ratio to the average weight gain% and SGR% respectively.

Anova Table 14a shows the RNA:DNA ratio with different dietary protein levels. The results show that at 35% dietary protein level, the maximum RNA-DNA ratio is seen whereas 45% dietary protein level shows a lesser value. The period also shows maximum RNA-DNA ratio at the early stages compared to the later stages. Both levels and periods show significance at 1% level ($p<0.01$). Fig 14a-d represents the changes in RNA-DNA ratio and muscle protein content at varying protein levels and varying growth periods. The lowest RNA-DNA ratio is at 10% protein level and highest at 35% protein. The variation in protein content also shows variation from 15 to 60 days.

5.3.2 Effect of dietary protein sources

The results of the variations in DNA, RNA content and RNA : DNA ratio with different dietary protein sources at different days of feeding are presented here.

DNA Content

In Table 13a, it is seen that the different protein sources have direct influence on DNA content, in the muscle. The DNA level is low in the shrimp meal feed, compared to the plant protein diets during the initial stages of feeding. But the values are not significant. But after 30 days of feeding, both animals and plant proteins show similar patterns (Fig 11a).

RNA Content

Anova Table 13b shows the RNA content of the muscle using different protein sources. The highest RNA value is seen in groundnut oil cake meal diet and shrimp meal diet and squilla meal diet.

The treatment also show that at 60 days, the maximum RNA content is obtained followed by 30 days. The sampling days are significant at 5% level. At 90 days the RNA values in all treatments are uniformly at a low value (Fig 11b) while DNA content remains at a high value.

RNA:DNA Ratio

Anova Table 14b shows the RNA : DNA ratio at different protein sources. Here also the RNA : DNA ratio is the highest with the shrimp meal diet followed by clam meal diet and squilla meal diet. The treatment is also higher at the 60th day. Fig 15a-d shows the RNA-DNA ratio with muscle protein

Table 13a. Analysis of variance (ANOVA) of DNA content at different protein sources and periods.

Sources	Sum of squares	Degrees of freedom	Mean Sum of squares	F - value
sources	13005.2002	5	2601.0400	1.1977
periods	24847.8340	4	6211.9585	2.8604
error.	43433.7148	20	2171.6858	
total	81286.749	29		

CD = 56.1245 CD = 61.4813

sources. mean = 209.3740 179.0520 200.4880 201.1900 246.5240 221.8820

periods. mean = 984.8000 1196.7100 1306.7700 1286.4500 1517.8201

Table 13b. Analysis of variance (ANOVA) of RNA content at different protein sources and periods.

Sources	Sum of squares	Degrees of freedom	Mean sum of squares	F - value
sources	22993.4258	5	4598.6851	1.7264
periods	91405.9609	4	22851.4902	8.5787**
error	53275.2383	20	2663.7620	
total	167674.625	29		

CD = 62.1586 CD = 68.0914

sources. mean = 263.3000 260.8760 226.2340 257.6480 311.4700 231.6240

periods mean = 1621.8000 1474.7599 1622.2000 2033.4899 1003.5100

** *significant at p<0.01.*

Table 14a. Analysis of variance (ANOVA) of RNA-DNA ratio of different protein levels and periods.

Sources	Sum of squares	Degrees of freedom	Mean sum of squares	F - value
levels	3.161068	4	0.790267	6.2764**
periods	5.601647	4	1.400412	11.1223**
error	2.014561	16	0.125910	
total	10.777276	24		

CD = 0.4757

levels. mean =	1.9280	1.4816	1.3424	1.2794	0.8228
periods. mean =	0.8320	0.9578	1.7422	2.0812	1.2410

Table 14b. Analysis of variance (ANOVA) of RNA-DNA ratio at different protein sources and periods.

Sources	Sum of Squares	Degrees of Freedom	Mean Sum of Squares	F - Value
sources.	4.145004	4	1.036251	8.2895**
periods.	0.560627	5	0.112125	0.8969
error	2.500149	20	0.125007	
total	7.20578	29		

CD = 0.4664 CD = 0.4258

sources. mean =	1.7012	1.2712	1.2847	1.6448	0.6592	
periods. mean =	1.3890	1.4994	1.2298	1.3482	1.3438	1.0630

***significant at p<0.01;*

Fig 11a. Changes in DNA content in the muscle tissue of M.rosenbergii fed with different protein sources

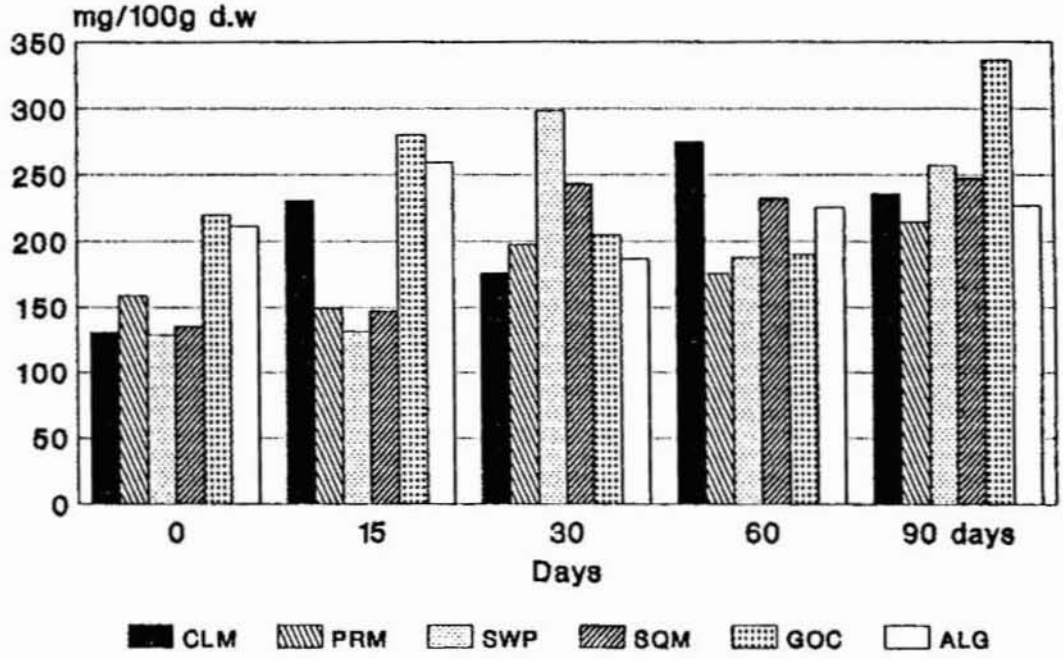
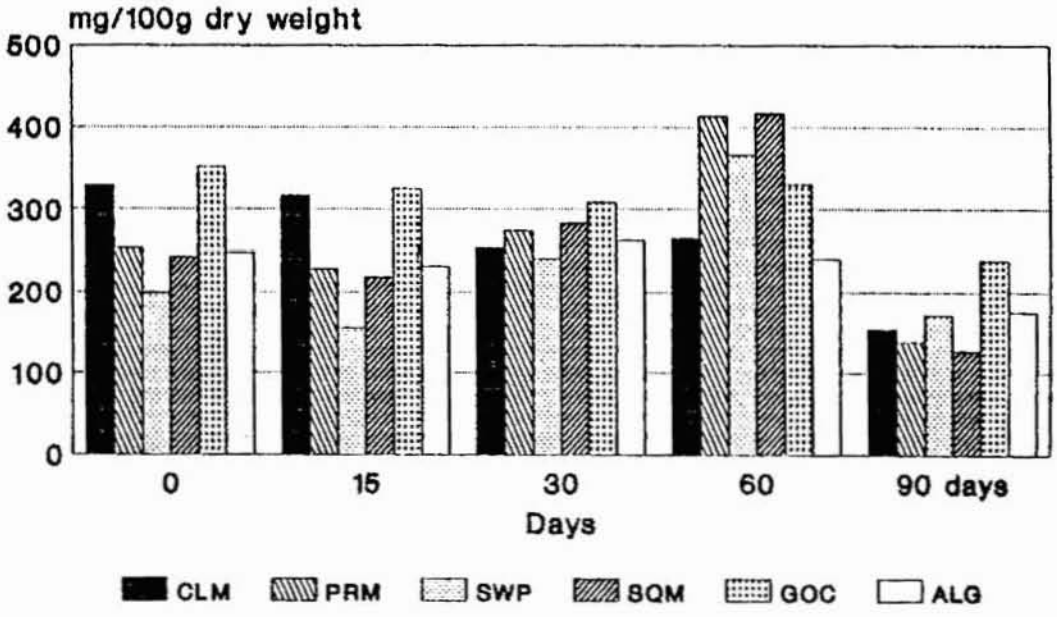
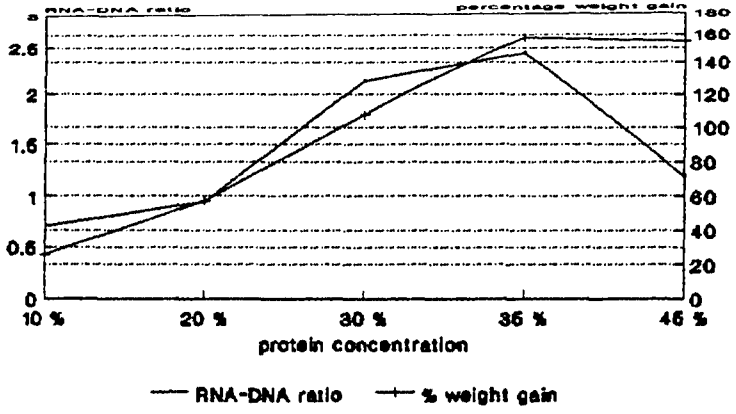


Fig 11b. Changes in RNA content in the muscle tissue of M.rosenbergii fed with different protein sources



CLM-clam meal, PRM-prawn meal, SWP-silkworm pupae meal, SOM-squilla meal, GOC-groundnut meal, ALG-algae meal



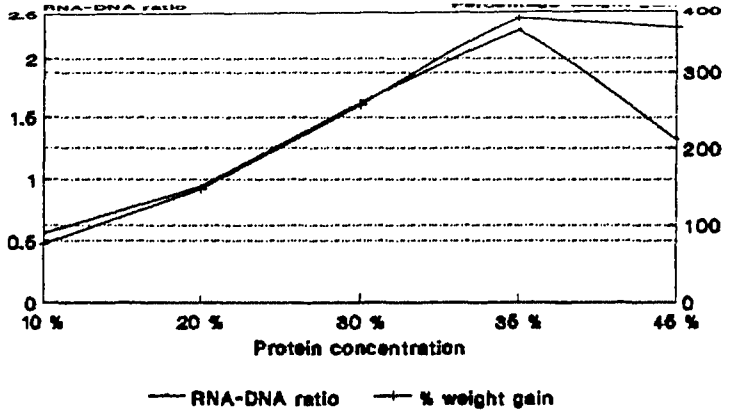


Fig 12c. 60 days

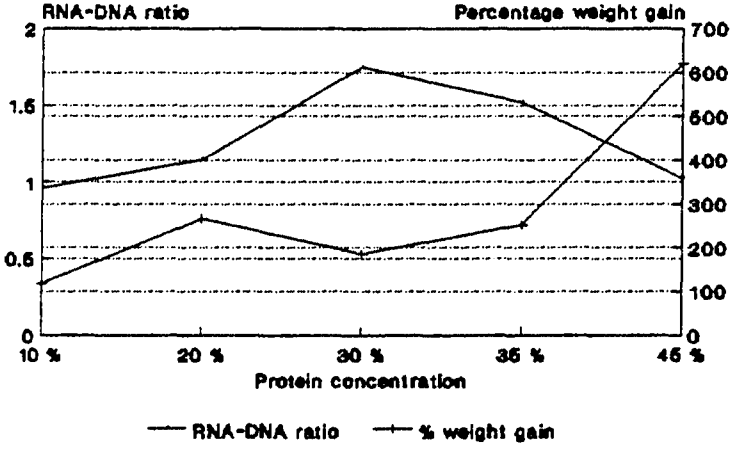


Fig 12d. 90 days

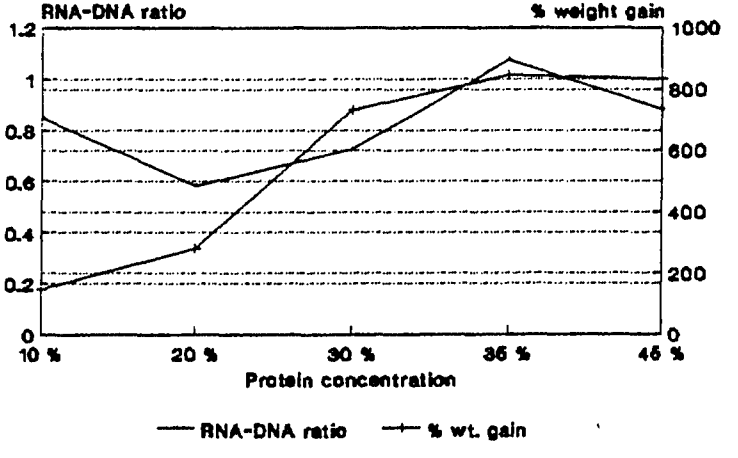
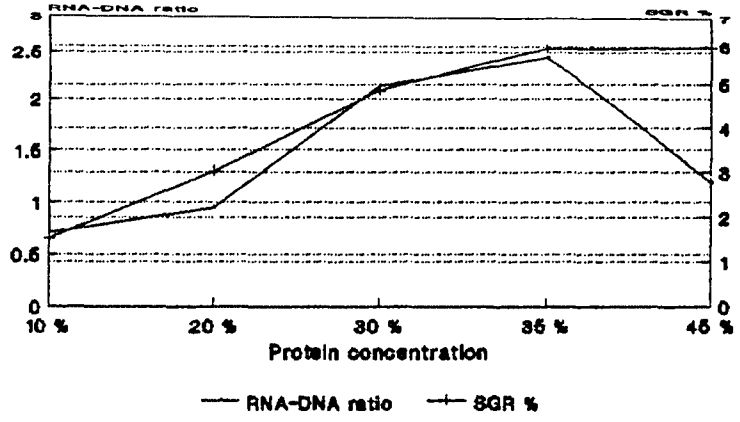


Fig 12a-d Average weight gain percentage to RNA-DNA ratio at different dietary protein levels at different periods.



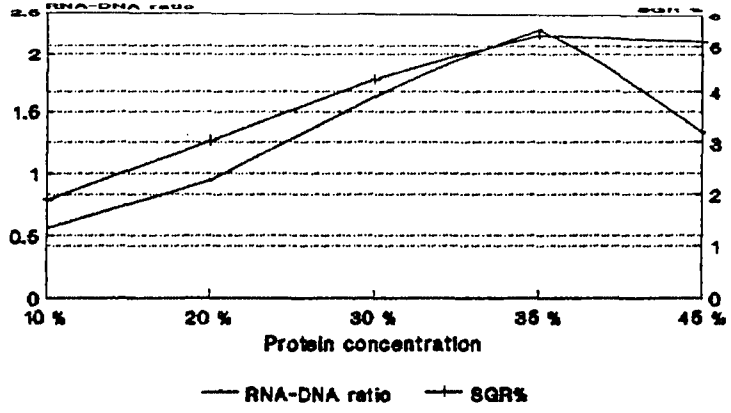


Fig 13c. 60 days

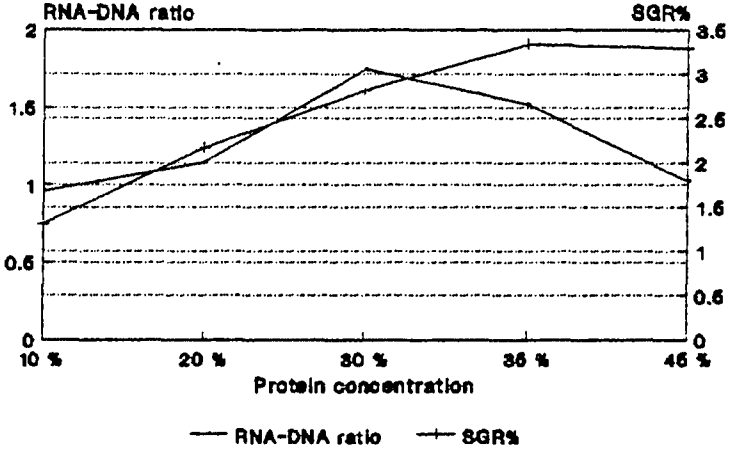


Fig 13d. 90 days

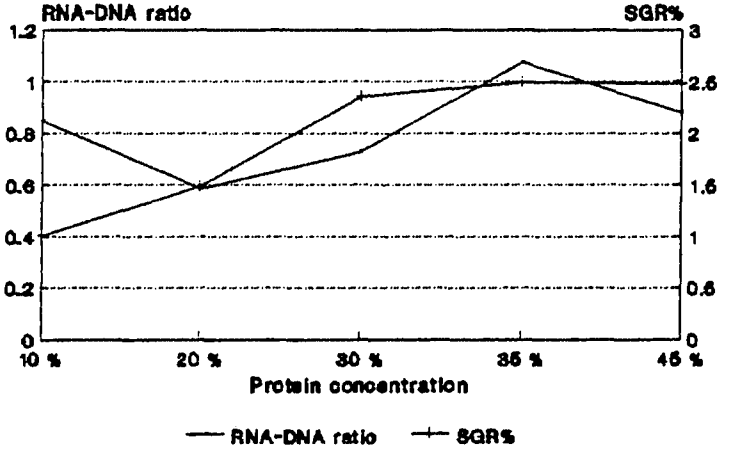
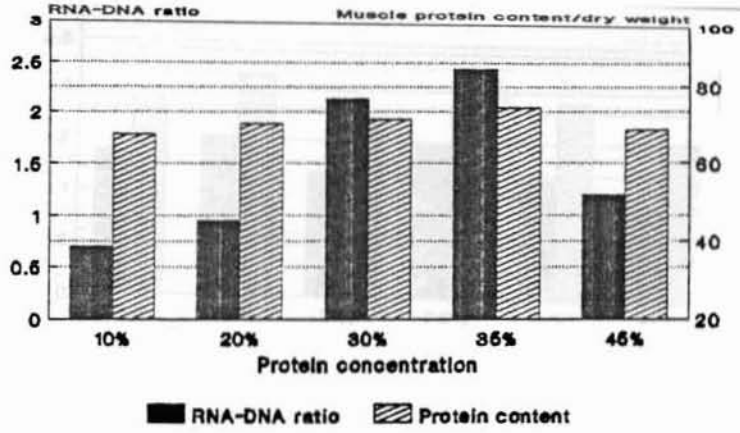


Fig 13a-d Specific growth rate percentage to RNA-DNA ratio at different dietary protein levels at different periods.



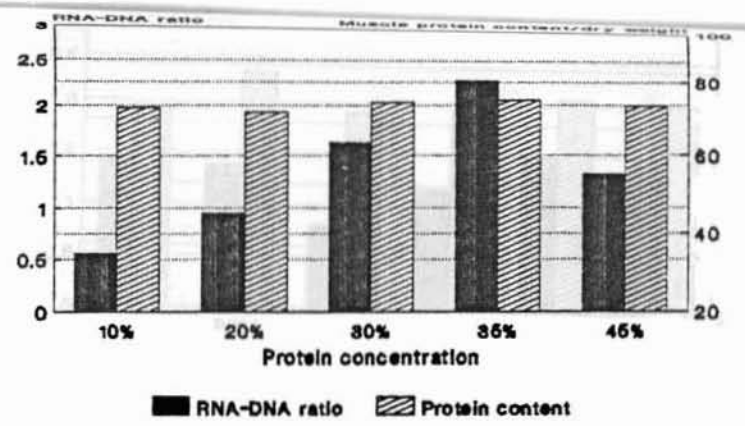


Fig 14c. 60 days.

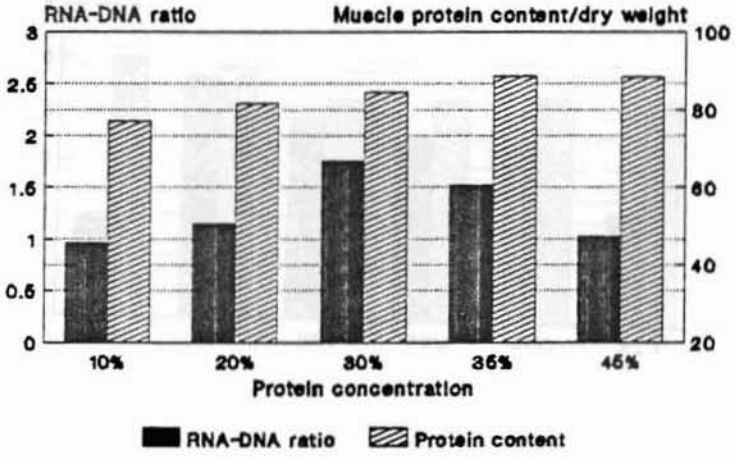


Fig 14d. 90 days

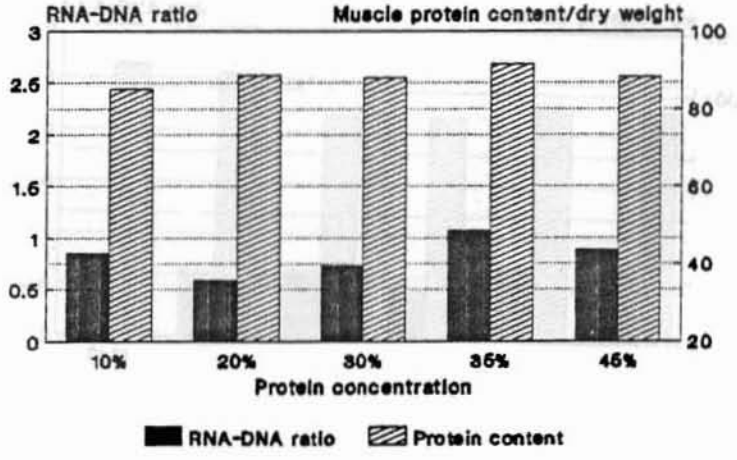
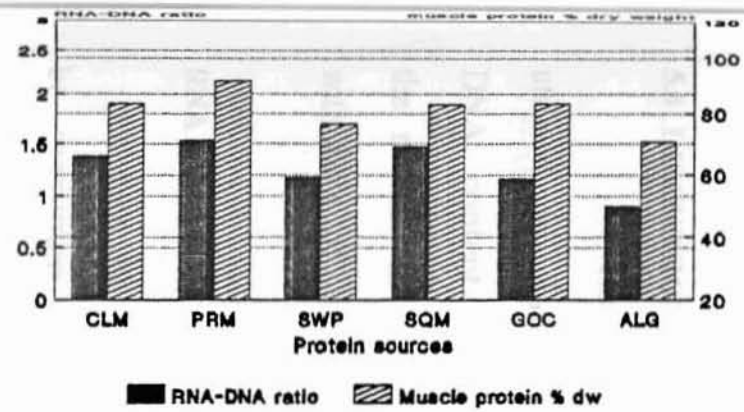


Fig 14a-d Variation in RNA-DNA ratio and protein content in muscle tissue of prawn fed with different dietary protein levels at different periods



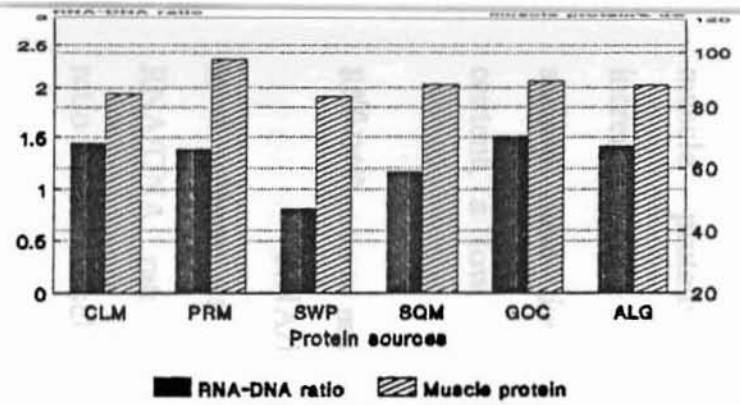


Fig 15c. 60 days

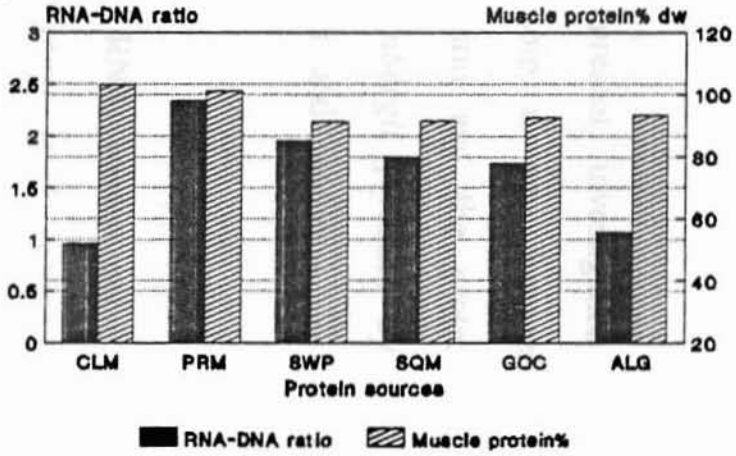


Fig 15d. 90 days

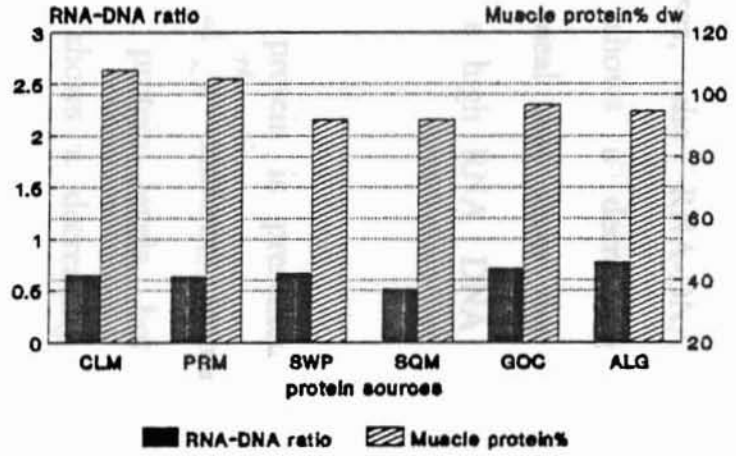


Fig 15a-d Changes in RNA-DNA ratio and muscle protein content of prawns fed with different protein sources at different days.

content at different sources. The results showed that when the muscle protein content increases, the RNA-DNA ratio also increases and at 90 days, it shows a decreasing pattern. The shrimp meal diet and the clam meal diet show the highest RNA content, a low DNA content and a high RNA : DNA ratio.

RNA/DNA ratio to muscle protein

RNA/DNA to muscle protein is presented in fig 14a to d. Here it is observed that there is a uniform increase in the RNA/DNA ratio content. At 45% protein levels, both RNA/DNA ratio and muscle protein content shows a decreasing trend. These changes are both distinct at 15 to 30 days of feeding while at 90 days a stagnant level is reached.

5.4 DISCUSSION

The results of the present investigation are mainly utilised to find out the relationship if any, between the RNA, DNA content, the RNA : DNA ratio and the protein content in the muscle of *Macrobrachium rosenbergii* juveniles by feeing them with diets of different protein levels and sources.

RNA content

In the present study, RNA concentration is found to be maximum at 35% dietary protein level compared to 10 to 30% and 45% protein. Upto 35% dietary protein level a steady increase in muscle RNA content is observed and thereafter, a steady

decline is noticed with the further increase in the dietary protein. The increase in RNA concentration appears to be the result of a more efficient utilization upto 35% of crude protein intake, leading subsequently to an increased protein synthesis (Khan and Jafri, 1991).

A fall in muscle protein and RNA concentration beyond 35% dietary protein intake, strengthens the already established fact that, there are limits to the amount of protein that a fish can convert to its body material (Love, 1980). Sutcliffs (1965), Dagg and Page (1972) found increased amount of RNA content during the exponential growth phase of the *Artemia salina* but they did not find any significant change thereafter. The present findings of RNA concentration agrees with the above observations . An increase in the level of RNA is necessary for an active protein synthesis and that was found to be mainly responsible for the growth of fish (Bulow, 1970;Mustafa and Jafri, 1977).

The extent of the growth increase, measured in terms of specific growth rate of the fish, declined beyond the 35% dietary protein level. Any factor that prevents or slows growth, is known to be reflected by a reduced RNA concentration (Buckley, 1979, 1982 ,1984; Martin , 1980).

DNA

Bulow (1970) while estimating RNA-DNA ratio in relation to the growth rate of fish, found that there was a slight decrease in the DNA content with an increased growth and a slight increase in the DNA content with an increased weight loss. He further explained that this change was probably due to the changes in the cytoplasmic volume. With food deprivation, other cellular constituents are metabolised and DNA is preserved (Leslie, 1955).

The present findings of decrease in DNA levels with the increase in protein levels, agree with the observations of Bulow (1970) and Dagg and Littlepage (1972) but, no inverse correlation is seen, with an increased protein level like RNA, as there are many fluctuations in the DNA values. It may be noted that, the animals maintain a general tendency to conserve DNA, with an increase in the size, most of the time.

DNA carries the genetic material in each cell and is present in the nucleus, in fixed quantities (Love, 1980). It is considered as an index of cell numbers, contributing to the unit weight of the tissue. In a weight losing fish, the size of the cells decreases. Thus the number of cells contributing to the unit weight of the tissue, increases, enhancing the number of nuclei and this will contribute to an increase in the DNA content. In a weight

gaining fish, on the other hand, the DNA content becomes diluted, with a larger volume of cells per unit weight. The variation in the DNA content on diets containing low, optimum and high dietary protein, can be explained on the basis of the above findings

RNA-DNA Ratio & Muscle Protein Content

The RNA-DNA ratio is considered as a sensitive measure for the growth rate of fish (Love, 1980; Buckley, 1979). This concept is based on the fact that, the quantity of RNA varies directly with the activity of the protein synthesis, in tissues undergoing a faster growth, while the amount of DNA per cell, remains constant within the species. Thus the ratio of RNA to DNA which is indicative of the amount of RNA per cell is usually a more accurate index of protein synthetic activity than the RNA concentration alone, because the ratio is not affected by the differences in the cell numbers.

There are some indications that size may affect the relation between the RNA-DNA ratio, and the temperature and the growth rate in older fish (Buckley, 1982). Large individuals with the same RNA-DNA ratio may grow at a slower rate than the smaller individuals. Also, due to the increasing energy reserves, the macromolecular composition of the larger individuals may require a longer time to change in response to the changes in the food availability. RNA-DNA ratio analysis will continue to provide

information on the growth rate and condition of the larval fish and shellfish in their active growth phase.

The direct positive relationship between the RNA-DNA ratio and the growth rate has also been observed for adult golden Shiners (Bulow, 1970), in small and mouthbass and carp (Haines, 1973) and in the muscle of catfish (Khan and Jafri, 1991).Here the data indicates that prawns, with high RNA-DNA ratio at 35% dietary protein level will more actively synthesise and accumulate protein than prawns with a low RNA-DNA ratio receiving either a low or a very high protein diet. This is also true with regard to the growth period. The initial stages of growth show higher RNA-DNA ratio compared to the later stages of growth. The RNA-DNA ratio was higher in the juvenile *Macrobrachium rosenbergii* fed on diets containing shrimp meal, clam meal and squilla meal, compared to the other sources. The period also shows significant variation and at 60th day, it shows the highest RNA-DNA ratio.

From the present findings, a direct correlation is observed between specific growth rate and RNA-DNA ratio emphasising the usefulness of RNA:DNA ratio as an effective index for monitoring growth and protein deposition.

Chapter 6

EFFECT OF DIETARY PROTEIN LEVELS AND SOURCES ON GROWTH AND FOOD CONVERSION OF MACROBRACHIUM ROSENBERGII JUVENILES

SECTION A

6.1 INTRODUCTION

A knowledge of the digestibility of ingredients in a formulated diet is required, to maximise the economic benefit of least cost feed formulation. In a practical feeding situation, diet digestibility can be affected by factors unrelated to the diet, such as the environmental conditions, the fish health and the feeding practices. Growth rate is related to the food consumption, apart from the nutritional quality of the food (Sullivan and Reigh, 1995; Segar and Roe, 1975). Inadequate protein in the diet, results in a reduction or cessation of growth and a loss of weight due to the withdrawal of protein from less vital tissues, to maintain the functions of more vital tissues (Wilson, 1989).

In the present study, an attempt has been made to study the effect of dietary protein levels and sources on growth such as weight and length, survival and food conversion, and also the Specific growth rate (SGR), Feed conversion ratio (FCR), Feed conversion efficiency (FCE), Protein efficiency ratio (PER), Protein digestibility coefficient (PDC), Apparent digestibility coefficient (ADC) and the productive protein value (PPV) of

Macrobrachium rosenbergii, by giving them different diets. The study also describes the relationship between all these parameters to growth and then finally, arrive at a conclusion that, there is a correlation between all these factors. In order to ascertain the requirement values, a second order polynomial and regression equations are established between the dietary protein levels tested and the mean value. On the basis of all these factors, Correlation coefficient matrix between the results from the previous chapters with growth parameters in *Macrobrachium rosenbergii*, is also reported.

6.2 MATERIALS AND METHODS

The experiment was started with 50 animals, each in 20 fibre glass tanks of 100 litre capacity. Prawns were fed with the test diets for 1 week to acclimate them to the feed. The animals were then given a formulated micro particulated diets in different protein levels as detailed in Chapter 3.2.2.1.

In the second experiment, feeds were prepared using different protein sources at 35% protein level. The proximate composition of the feed and raw materials, was estimated as per the methods given in Chapter 4.2.1. The feed was formulated with 35% protein, 11% fat, 10% mineral, 3% vitamin mix and 1% cholesterol. Clam meal, shrimp meal, silk worm pupae meal, squilla meal, groundnut oil cake meal and algae meal were used for the preparation of the feed and the processing methods are also given in Chapter 4.2.1. The feeding regime and sampling are done, as discussed in the earlier chapters.

6.2.1 Water stability of feeds.

The particulated feed were tested for water stability following the method of Hastings (1964). Water stability was determined after one hour, 2 hrs, 3 hrs, 4hrs, 5 hrs, 6 hrs and 12 hours of the immersion in water. Each feed was tested in triplicate for their stability. It was calculated according to the percentage of the dry matter obtained after immersing the feeds in water over varying durations.

6.2.2 Water quality parameters.

The water quality was maintained throughout the experiment. The pH, temperature, dissolved oxygen, nitrite and ammonia were determined according to the method suggested by Water quality management in aquaculture (CMFRI special publication No. 22, 1985)

Water pH was measured by using a pH meter. Temperature was measured by using a thermometer.

The dissolved oxygen content was measured by the Winkler method. It consisted of fixing oxygen through oxidation of a precipitate of manganous hydroxide to manganous dioxide which in acid medium liberated Mn2+ ions and which in turn oxidise iodide ions. The iodine thus formed was titrated against thiosulphate. The oxygen content is then expressed in mg oxygen per litre of water (Anon, 1983).

Organic nitrogen content was estimated acording to the method suggested by Strickland and Parsons (1968).

Ammoniacal nitrogen content was determined by the calorimetric method given by Koroliff using indophenol blue in an oxidising medium. Ammonia and phenol form a coloured complex with an absorbance at 630 nm (Anon, 1983).

Nitrite nitrogen content was measured calorimetrically by the formation of diazocompounds in the presence of sulphanilamide which gave a coloured complex with N - naphthyl Ethylene Diamine. The absorbance was measured at 543 nm (Anon, 1983; Strickland and Parsons, 1968).

6.2.3 Nutritional evaluation

Growth parameters such as weight, general health and survival of juvenile *Macrobrachium rosenbergii* at 0 day, 15 days, 30 days, 60 days and 90 days were monitored.

Survival rate

During the course of each experiment, the mortality of prawns was noted down and the percentage of the survival rates was calculated at the end of each experiment.

$$\text{Survival Rate Percentage} = \frac{\text{initial number - final number}}{\text{initial number}} \times 100$$

The growth rate

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

$$SGR\% = \frac{(\log_e \text{final wt } w_2 - \log_e \text{initial wt } w_1) \times 100}{T2 - T1}$$

Where w_1 and w_2 are initial and final weights and t_1 and t_2 represent different periods respectively.

The instantaneous percentage growth of the prawn in unit weight, was also determined (IPGU) and average weight gain was also calculated.

Evaluation parameters

At the end of each experiment, the data of feed supplied, the residual feed, and faecal nitrogen were monitored. The Feed Conversion Ratio (FCR), and the feed conversion efficiency (FCE), were calculated according to Venkataramiah et al., (1975), Halver (1976). The protein efficiency ratio (PER), was done according to NAS/NRC (1963), and the Protein Digestibility Coefficient (PDC), Apparent Digestibility

Coefficient (ADC), and the Productive Protein Value (PPV) were calculated as described by Halver (1976), and by Furakawa and Tsukahara (1966).

The initial and the final tissue protein content of the prawns were determined by estimating the total nitrogen as per kjeldhal's method (protein=N x 6.25). The following equations were used to calculate the different evaluation methods.

$$\text{Food conversion ratio (FCR)} = \frac{\text{Food intake (dry weight)}}{\text{Wet body weight gain}}$$

$$\text{Food conversion efficiency (FCE)} = \frac{\text{Wet weight gain}}{\text{Feed intake (Dry weight)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Gain in weight}}{\text{Protein intake in food}}$$

$$\text{Protein digestibility coefficient (PDC)} = \frac{\text{Protein intake-Faecal protein}}{\text{Protein intake}} \times 100$$

$$\text{Apparent digestibility coefficient (ADC)} = \frac{\text{Total food consumed-Total excreta produced}}{\text{Protein intake}} \times 100$$

$$\text{Productive protein value (PPV)} = \text{Protein gain} = \frac{(\text{final body protein} - \text{Initial body protein}) \times 100}{\text{Protein intake}}$$

6.2.4 THE STATISTICAL DESIGN AND ANALYSIS

The feeding experiments were designed on the basis of, a completely randomised design The results obtained from the experiments were analysed using students t test and analysis of variance (ANOVA).

An F test was performed to determine, if there ~~was~~ existed a difference between treatments and means . If the value was found to be significant, the data was analysed by a Least significant difference test (LSD). All possible differences between the means of each treatments were computed and compared to LSD. If the absolute value of the difference 'd' was greater than LSD the difference was found to be significant at $p<0.05$.

6.3 RESULTS

In this Chapter, the results of the water quality parameters, the water stability, the growth rate, the survival rate, and the nutritional evaluation methods are discussed.

6.3.1 Water stability parameters

The results of the water stability studies on different formulated feeds, from various protein sources are given in Table 15. The stability of feeds, is influenced by different factors like, feed composition,

Table 15 Water stability of formulated feeds with percentage of dry matter obtained after immersing the feeds in water over varying durations.

Table 15. Water stability of feeds at different hours.

Sources	Hours								
	0	1	2	3	4	5	6	12	24
CLM	100	98 ± 0.98	96 ± 0.96	85.6± 0.856	80± 0.8	76± 0.76	70± 0.72	56± 0.56	50± 0.54
PRM	100	97± 0.97	93± 0.93	83± 0.83	82± 0.82	70± 0.71	64± 0.64	59.6± 0.596	57± 0.57
SWP	100	96± 0.96	90± 0.91	82± 0.82	81± 0.81	60± 0.63	56± 0.56	50± 0.51	48± 0.48
SQM	100	96.4± 0.964	94.4± 0.944	85.6± 0.856	80.2± 0.802	70± 0.75	60± 0.64	56± 0.56	50± 0.5
GOC	100	95± 0.95	93± 0.93	83± 0.83	80± 0.8	62± 0.62	60.4± 0.604	51.2± 0.512	46± 0.46
ALG	100	94.2± 0.942	90± 0.9	82.2± 0.822	80.4± 0.804	6 4± 0.064	62± 0.62	45± 0.45	40± 0.4

CLM: Clam meat; PRM: Prawn meat; SWP: Silkworm pupae meal; GOC: Groundnut oil cake meal; ALG: Algae meal.

nature of ingredients, type of processing and moisture content. In the present study the highest water stability is recorded in the early hours, from 1 to 6 hours. After that, a slight disintegration of the feed is seen. But there is not much variation seen between different feeds tested.

6.3.2 Water quality parameters.

The results of the study show that, the pH value vary, between 7 to 8.5, at different protein levels and protein sources. The water temperature is important for a good growth and survival of the prawn and in the present study, it varies between 26 to 32°C.

Dissolved oxygen is the most critical factor, in aquaculture. The main factors influencing the presence of oxygen in water are temperature, salinity and atmospheric pressure. The dissolved oxygen in the present study, varies between 6 to 8.5 mg per litre, through out the experiment, at different protein levels and protein sources.

In natural water and culture systems, nitrite is present only in low concentration. In the present study the nitrite value ranges from 0.026 to 0.04 mg per litre at different protein levels and sources.

The tolerance of aquatic organisms to ammonia, varies with species, physiological conditions and environmental factors. A safe level of 0.05 mg per litre of ammonia, can be taken for fishes. In the

Fig 16a. Changes in the ammonia content in the water at different hours after feeding with different protein levels

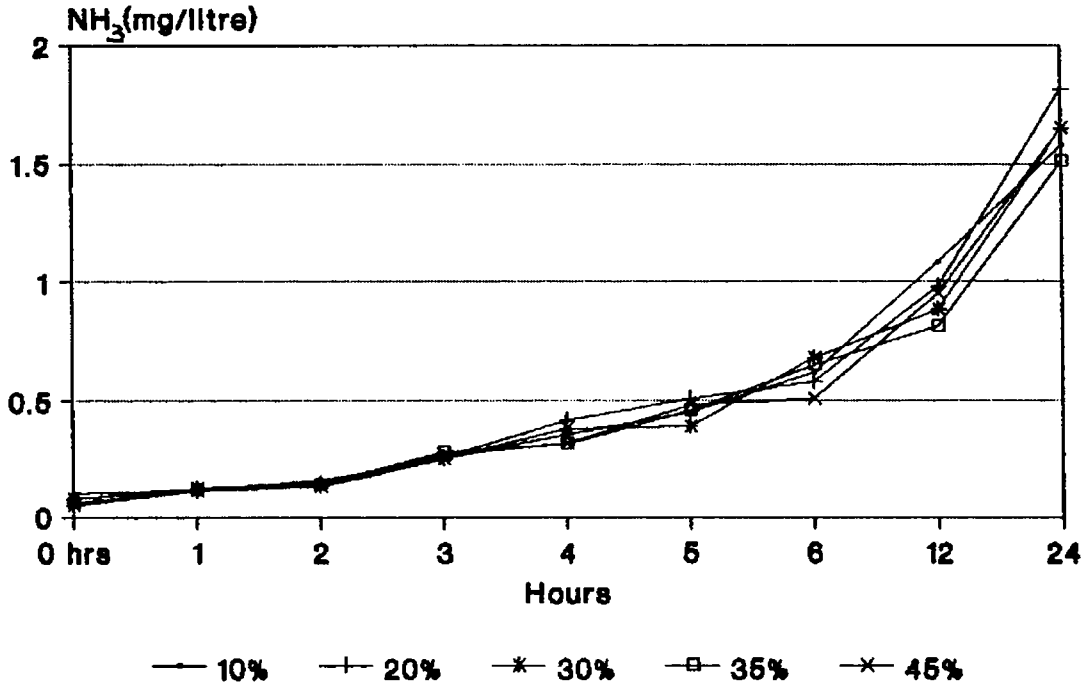
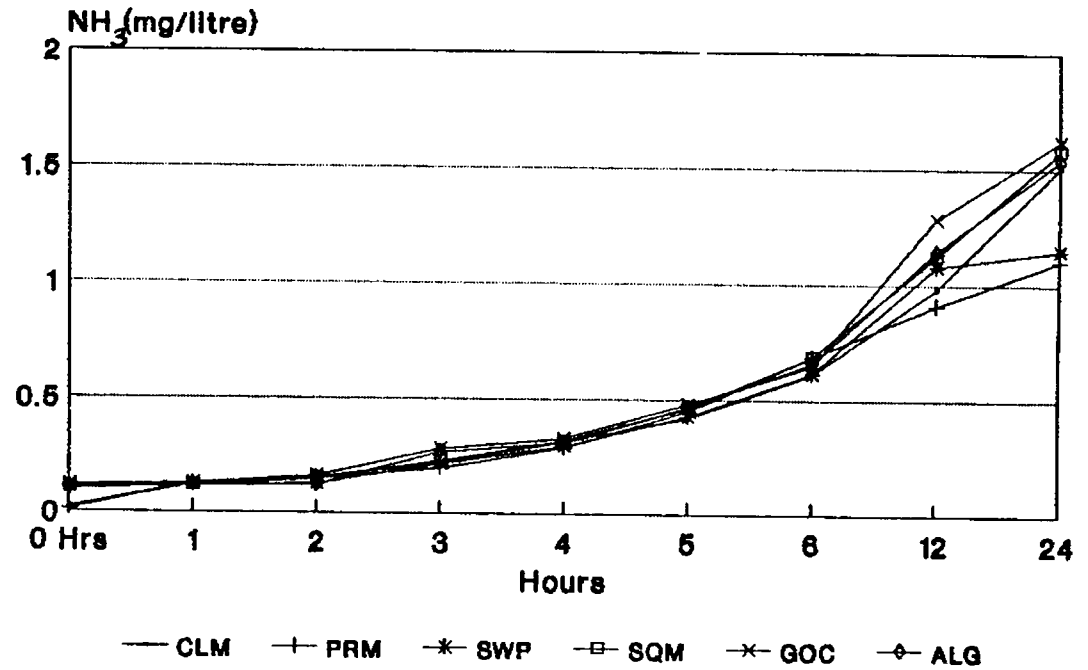


Fig 16b. Changes in the ammonia content in the water at different hours after feeding with different protein sources



present study, the ammonia content ranges from 0.03 to 0.45 mg per litre, at different hours (upto 5 hrs). The results show that, the ammonia content increases gradually, when the time increases. The variation in the ammonia content in the water at different hours with a given feed of different protein levels and sources are presented in Fig. 16a and 16b respectively. From the graph it is seen that there is a uniform increase in the ammonia content at different hours.

6.3.3 Nutritional evaluation at different protein levels

The results of the suvival rate show that at different protein levels and sources, the survival rate, ranges from 95 to 98%. This is due to the frequent exchange of water, and the good quality of feed used, for the entire study.

Anova of the results of the specific growth rate %, the instantaneous growth % and the average weight gain% are given in Table 16a-16c.

Anova Table 16a and Fig.17a shows the specific growth rate at different protein levels. It shows that, as the protein level increases from 10 to 45%, the specific growth rate also increases. At 35% protein level show the maximum increase and thereafter the value shows a decline. The value ranges from 1.4319 to 4.2612 and it shows significance at 5% level. The period also shows significance and at 15 to 30 days the maximum growth is seen.

Anova Table 16b shows the instantaneous growth %. The value ranges from 0.7833 to 4.7253 and at 35 to 45% shows the maximum increase in weight. At 30 days of feeding the maximum growth is attained.

Anova Table 16c shows the average weight gain. Here the maximum growth is attained at 45% protein level, followed by 35% protein level and the value ranges from 0.387 to 2.3178 A uniform increase in weight is observed throughout the study. The values are significant at 5% level ($p<0.05$).

Anova Table 17a shows, the FCR value. The lowest value is attained at 35% protein level, followed by 45%. The value ranges from 7.003 to 1.2748. The values are significant at 5% level. The maximum FCR is seen at 60 to 90 days.

Anova Table 17b shows the feed conversion efficiency (FCE) at different protein levels. The values range from 0.1605 to 0.8800. The maximum FCE is found at 35% protein level, followed by 45% and the values are significant at 5% level. The values at different culture days range from 0.3240 to 0.7580. The maximum FCE is shown at 90 days, followed by 60 days.

Anova Table 17c shows the protein efficiency ratio (PER) at different protein levels. The PER ranges from 1.6038 to 2.5145. The

Table 16a-c. Analysis of variance (ANOVA) of growth parameters of Macrobrachium rosenbergii fed on different protein levels

16a. Specific growth rate percentage

Source	Sum of squares	Degrees of freedom	Mean sum of squares	F-Value
levels	24.0899	4	6.0225	17.5840**
periods	17.6906	3	5.8969	17.2172**
error	4.1099	12	0.3425	
total	45.8904	19		

			SE= 0.3701		SE = 0.4138	
levels mean =	1.4319	2.4242	3.5704	4.2612	4.2164
periods mean =	4.2615	3.9129	2.5832	1.9655	

16b. Instantaneous growth percentage at different protein levels

Source	Sum of squares	Degrees of freedom	Mean sum of squares	F - Value
levels	50.4245	4	12.6061	19.4319**
periods	15.0741	3	5.0247	7.7454**
error	7.7848	12	0.6487	
total	73.2834	19		

			SE = 0.5094		SE = 0.5695	
levels mean =	0.7833	1.5002	3.4665	4.7253	4.4998
periods mean =	2.8148	4.4519	2.5068	2.2066	

16c. Average weight gain at different protein levels

Source	Sum of squares	Degrees of freedom	Mean sum of squares	F - Value
levels	11.6916	4	2.9229	9.5109**
periods	12.2485	3	4.0828	13.2853**
error	3.6878	12	0.3073	
total	27.6279	19		

			SE = 0.3506		SE = 0.3920	
levels mean =	0.3870	0.7902	1.6177	2.3178	2.2100
periods mean =	0.4222	1.0900	1.8420	2.5040	

***significant at p<0.0122 ; *significant at p<0.05*

Table 17a-f. Analysis of Variance (ANOVA) of nutritional evaluation studies of *Macrobrachium rosenbergii* fed on different dietary protein levels

Table 17a. Food conversion ratio

Sources	Sum of squares	Degrees of freedom	Mean sum of squares	F - value
levels	93.331910	4	23.334980	17.8324**
periods	22.294280	3	7.431427	5.6790*
error	15.702850	12	1.308571	
total	131.32904	19		

	SE = 0.7235	SE = 0.8089			
levels. mean =	7.0033	3.1940	1.7112	1.2748	1.3645
periods. mean =	4.6996	2.6652	2.1484	2.1250	

Table 17b. Food conversion efficiency

Sources	Sum of squares	Degrees of freedom	Mean sum of squares	F - value
levels	1.560581	4	0.390145	26.9961**
periods	0.554054	3	0.184685	12.7793**
error	0.173423	12	0.014452	
total	2.288058	19		

	SE = 0.0760	SE = 0.0850			
levels mean	= 0.1605	0.3410	0.6605	0.8800	0.8265
periods. mean =	0.3240	0.5278	0.6850	0.7580	

Table 17c. Protein efficiency ratio

Sources	Sum of squares	Degrees of freedom	Mean sum of squares	F - value
levels	2.286789	4	0.571697	9.1004**
periods	6.334862	3	2.111621	33.6133**
error	0.753853	12	0.062821	
total	9.375504	19		

	SE = 0.1585	SE = 0.1772			
levels mean	= 1.6038	1.7058	2.2012	2.5145	1.8366
periods mean =	1.1116	1.8432	2.3689	2.5658	

**significant at p<0.05; **significant at p<0.01*

Table 17d. Protein digestibility coefficient

Sources	Sum of squares	Degrees of freedom	Mean sum of squares	F - value
levels	44.656250	4	11.164060	0.5874
periods	549.828100	4	137.457000	7.2324*
error	304.093800	16	19.005860	
total	898.57815	24		

SE = 2.7572 SE = 2.7572

levels mean =	78.6580	77.4680	79.9180	80.9280	80.8820
periods mean =	81.2080	84.6880	83.5560	72.2580	76.1440

Table 17e. Apparent digestibility coefficient

Sources	Sum of squares	Degrees of freedom	Mean Sum of squares	F - value
levels	46.906250	4	11.726560	0.5950
periods	542.875000	4	135.718800	6.8865*
error	315.328100	16	19.708010	
total	905.10935	24		

CD = 5.9523

levels mean =	78.7040	77.3440	79.9480	80.9120	80.8840
periods. mean =	81.1300	84.6980	83.5460	72.3980	76.0200

17f. Productive protein value

Sources	Sum of squares	Degrees of freedom	Mean sum of squares	F - Value
levels	3658.8910	4	914.7227	3.8543**
periods	3530.5620	3	1176.8540	4.9589**
error	2847.8670	12	237.3223	
total	10037.32	19		

SE = 9.7431 SE = 10.8932

levels mean =	46.9375	23.7025	30.1650	15.2325	7.3875
periods mean=	42.9360	31.6120	14.1040	10.0880	

**significant at p<0.05; **significant at p<0.01*

highest PER value shows at 35% protein level, followed by 30% and at 45% protein concentration the PER shows a decline. The values are significant at 5% level. The period also shows that the PER gradually increases and the values range from 1.1116 to 2.5658, the 90th day, shows in the maximum PER.

Anova Table 17d shows the protein digestibility coefficient PDC at different protein levels. The PDC values range from 77.6580 to 80.9280. The highest PDC is seen at 35% protein level, followed by 45% protein. level. But the values are not significant. The 15th day shows maximum PDC, followed by 30 days.

Anova Table 17e shows the apparent digestibility coefficient (ADC). Here the values show only slight variation among different treatments, the maximum ADC being at 35% protein level followed by 45% protein. The highest ADC shows a significant increase at 15 days.

Anova Table 17f shows the productive protein value (PPV) at different protein levels. The values range from 7.3875 to 46.9375. The highest PPV shows at 30% protein level. During the initial stages, the protein is utilised more quickly than during the later periods and the values are significant at 5% level. The period also shows significance. The values range from 10.0880 to 42.9360. At 15th day and 30th day show the maximum PPV, compared to the other periods. In the later stage a decline in PPV is seen.

Fig.17a Changes in specific growth rate percentage at different protein levels.

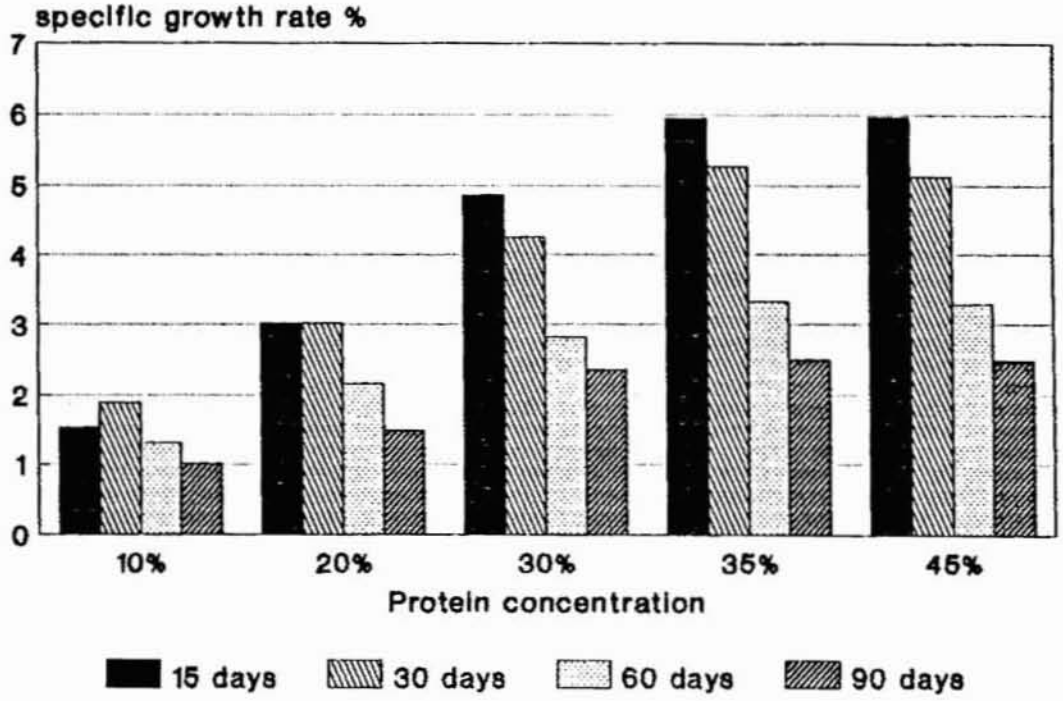
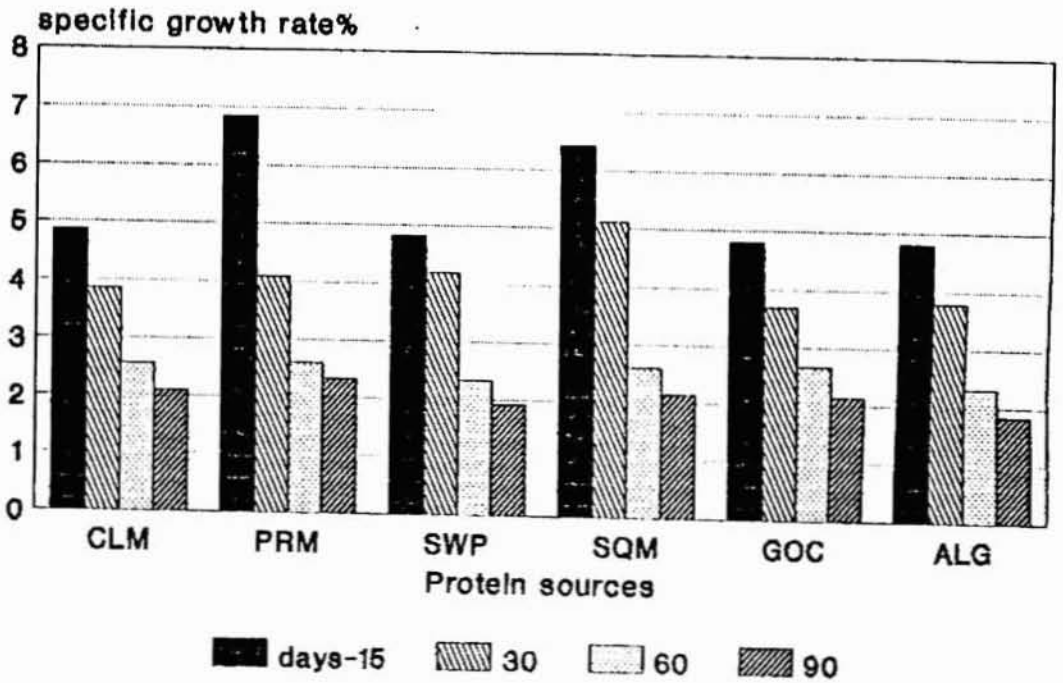


Fig.17b Changes in specific growth rate percentage at different protein sources



From all these nutritional evaluation studies, the level 30% to 35% dietary protein shows the best SGR, FCR, FCE, PER, PDC, ADC and PPV and the period 15 to 30 days shows, the maximum conversion. There is a relationship between the growth and these various parameters.

6.3.4 Nutritional evaluation at different dietary protein sources

Anova Table 18a shows the SGR at different protein sources, and the graphical presentation of the same is given in Fig.17b. The squilla meal diet shows the maximum specific growth rate %, followed by the shrimp meal diet and the clam meal diet. The values are significant at 5% level. The period 15 to 30 days shows maximum SGR.

Anova Table 19 a-f shows the various nutritional evaluation parameters on feeding *Macrobrachium rosenbergii* with diet containing different protein sources. The shrimp meal diet and squilla meal diet show the maximum FCR. The values range from 1.2760 to 2.4273, and these values show significant at 5% level. At 90 days the maximum FCR is seen compared to the other periods.

Anova Table 19b shows the FCE at different protein sources. The values show variation and the shrimp meal diet shows the maximum FCE, followed by the squilla meal diet and the clam meal diet. At 0 to 90 days a uniform increase in FCE, is seen. Both sources and periods show significance at 5% level.

Table 18a-c Analysis of Variance (ANOVA) of different growth parameters of Macrobrachium rosenbergii fed on different protein sources at different periods.

18a. Specific growth rate percentage

Sources	Sum of squares	Degrees of freedom	Mean sum of squares	F - Value
Sources	2.899	5	0.509	2.716
Periods	42.511	3	14.170	66.361
Error	3.203	15	0.214	
Total	48.613	23		

	SE = 0.267			SE = 0.327		
sources mean = 3.347	3.97	3.322	4.073	3.326	3.862	
periods mean = 5.425	4.13	2.516	2.078			

18b. Instantaneous growth rate Percentage

Source	Sum of Squares	Degrees of freedom	Mean sum of squares	F - Value
Sources	2060.82	5	412.164	0.780
periods	31555.15	3	10518.38	19.892
Error	7931.586	15	528.772	
Total	41547.556	23		

	SE = 13.2762		SE = 16.26		
sources mean = 61.951	77.806	58.077	78.335	63.619	51.188
periods mean = 127.587	54.089	37.102	43.872		

18c. Average weight gain percenrage

Sources	Sum of squares	Degrees of freedom	Mean Sum Of Squares	F - Value
Sources	44604.5	5	8920.901	3.486
Periods	591475.3	3	197158.4	77.0361
Error	38389.5	15	2559.3	
total	674469.3	23		

	SE = 29.2079			SE = 35.772		
sources mean = 311.965	377.611	282.907	373.690	328.314	260.688	
periods mean = 127.587	248.997	354.125	555.286			

significant at $p < 0.05$

Anova Table 19c shows the PER at different protein sources. The highest PER is seen with the shrimp meal diet, followed by the squilla meal diet and the clam meal diet. The period also shows a uniform increase during 15 to 90 days. Both sources and periods show significance at 5% level.

Anova Table 19d shows the PDC at different protein sources. Here the groundnut oil cake meal and algae meal show the highest PDC, followed by the squilla meal diet. The period at 15 days shows the highest PDC. The values are not significant.

Anova Table 19e shows the ADC at different protein sources. The algae meal and the groundnut oil cake meal diet show the highest ADC, followed by the squilla meal diet. This may be due to the higher absorption of the plant materials, rather than the animal protein. But the values are significant at 5% level. The 15 to 30 days show the maximum ADC compared to the other periods, but the values are not significant.

Anova Table 19f shows the PPV at different protein sources. Here the shrimp meal diet shows maximum PPV, followed by the algae meal and the clam meal diet. The values are significant at 5% level. At 15 to 30 days show the maximum PPV, compared to the other periods.

Table 19a -f. Analysis of variance (ANOVA) of nutritional evaluation studies of Macrobrachiumn rosenbergii fed on different dietary protein sources.

19a. Food conversion ratio.

Source	Sum of squares	Degrees of freedom	Mean sum of squares	F - Value
sources	3.2238	5	0.6448	7.9912**
periods	3.9390	3	1.3130	16.2738**
error	1.2102	15	0.0807	
total	8.373	23		

	SE = 0.1640		SE=0.2009			
sources mean =	1.7723	1.2760	1.9578	1.5117	1.9780	2.4273
periods mean =	2.4600	1.8038	1.6723	1.3458		

19b. Food conversion efficiency

Sources	Sum of squares	Degrees of freedom	Mean sum of squares	F - Value
sources	0.3429	5	0.0686	6.9866**
periods	0.3459	3	0.1153	11.7470**
error	0.1472	15	0.0098	
total	0.836	23		

	SE = 0.0572		SE = 0.0701			
sources mean =	0.6060	0.8075	0.5380	0.6865	0.5615	0.4270
periods mean =	0.4465	0.5752	0.6133	0.7827		

19c. Protein efficiency ratio

Sources	Sum of squares	Degrees of freedom	Mean sum of squares	F - Value
sources	2.8941	5	0.5788	7.7491**
periods	2.8120	3	0.9373	12.5488**
error	1.1204	15	0.0747	
total	6.8265	23		

	SE = 0.1578		SE = 0.1933			
sources mean =	1.7313	2.3323	1.5375	1.9615	1.6038	1.2258
periods mean =	1.2755	1.6600	1.7560	2.2365		

19d. Protein digestibility coefficient.

Sources	Sum of squares	Degrees of freedom	Mean sum of squares	F - Value
Sources	211.2031	5	42.2406	2.7050
periods	118.1094	4	29.5274	1.8909
error	312.3125	20	15.6156	
total	641.625	29		

	SE = 2.2815	SE = 2.4993			
sources mean = 76.0480	76.7400	76.7360	79.7440	80.8320	83.4080
periods mean = 81.0017	80.6767	79.2150	75.5067	78.1900	

19e. Apparent digestibility coefficient

Sources	Sum of squares	Degrees of freedom	Mean sum of squares	F - Value
sources	206.6875	5	41.3375	2.7684*
periods	111.0313	4	27.7578	1.8589
error	298.6406	20	14.9320	
total	616.3594	29		

	SE = 2.2310	SE = 2.4439			
sources mean = 76.0460	76.7600	76.9280	79.8000	80.7820	83.4160
periods mean = 81.0467	80.6583	79.1783	75.6983	78.1950	

19f. Productive protein value

Sources	Sum of Squares	Degrees of freedom	Mean sum of squares	F - Value
sources	3661.4970	5	732.2995	2.9789**
periods	3921.7310	3	1307.2440	5.3176**
error	3687.4820	15	245.8321	
total	11270.71	23		

	SE = 9.0523	SE = 11.0868			
sources mean = 19.3175	44.9925	11.4000	7.1100	15.0125	25.1775
periods mean = 40.4017	22.5950	11.8900	7.1200		

**significant at p<0.05; ** significant at p<0.01*

From all these observations it is found that the shrimp meal, the squilla meal and the clam meal diet show the highest SGR, FCR, FCE, and PER. The PDC, ADC and PPV are higher at plant protein sources. The period also shows variation and at 15 to 60 days show the maximum SGR, FCR, FCE, and PER. The PDC, ADC, and PPV also show the highest conversion at 15 to 30 days. From this it is clear that during the early stages, the conversion of food material, is faster than the later stages. The animal proteins show the highest variation compared to the plant proteins.

6.4 DISCUSSION

In the present study it is found that the specific growth rate, the food conversion ratio, the food conversion efficiency, the protein efficiency ratio, the protein digestibility coefficient, the apparent digestibility coefficient, and the productive protein value, all had a relationship to the protein levels and protein sources. When the protein content increases, the conversion efficiency of the prawn *Macrobrachium rosenbergii* also increases. In the case of the protein sources, the animal proteins showed, a higher conversion efficiency than the plant proteins. The survival rate, water quality parameters of the culture systems and the water stability of the feeds prepared were also discussed.

Venkataramiah et al., (1975) observed that the mean growth measured by both length and weight, increased with an increase in the protein content of the rations. The protein levels between 22.5 to 40%,

were generally considered to be the optimum for the omnivorous penaeid shrimp. Forster (1978) and Koshio et al., (1993) found that the weight gain, the specific growth rate and the food conversion efficiency values for prawns fed on diets containing 21 and 31.4% protein, were significantly lower than those for prawns fed on diets containing 41.6, 50.3 and 60.7% protein contents. Digestion efficiencies for the dry matter and the protein ranged from 73 to 77% and 93 to 96% respectively.

Mohiuddin and Swain (1993) conducted feeding experiments with pelletised feeds, consisting of crude protein ranging between 21.8 to 39.2% with *Penaeus merguiensis* post larvae. The daily growth rate was found to be maximum of 6.32% and the overall feed efficiency was 2.3% when the prawn post larvae were fed with feed containing a combination of squid meal and earth worm meal.

Mathew and Jayaprakash (1993) found that, the feed containing 45% protein, gave the maximum growth rates and the survival rates. Ali (1994) conducted experiments with four purified proteins, albumin, caesin, fibrin and gelatin, and were evaluated for the juvenile *Penaeus indicus* and found that the albumin diet gave the highest growth rate, PER and low FCR and emerged as the best protein source. The excretion of nitrogen in faeces increased with dietary protein and reached a peak at 37% protein in the diet.

Sick et al (1972) found that the protein and the fat digestibility of *Penaeus orientalis*, were positively correlated with their protein levels in diet. The correlation between the starch digestibility and its content in the diet, was negative, when dietary starch content exceeded 20%. Hartnoll and Salama (1992) found that by giving different protein sources, with 35% protein content in *Palaemon elegans*, the shrimp meal diet produced, the highest growth, higher food consumption and better food conversion, than the mixed diet. The present study also shows similar observations with prawn meal diet. They also studied the growth and survival, when fed with two diets and found that the survival was better by feeding with the commercial pellets, and the growth was comparable for both the diets.

Tidewell et al., (1992) evaluated the distillers dried grains with solubles, as a protein source in the diets with 3 isonitrogenous diels, 35% protein each for the fresh water prawn *Macrobrachium rosenbergii*, and found that the average yield, survival, individual weight and feed conversion did not differ significantly during treatments. It appeared that DDGS, was a suitable ingredient for the use in practical diets at inclusion rates up to 40% of the total formulation. The present study also agrees that at 35% protein level, the shrimp meal feed shows the highest growth rate.

Bhaskar and Ali (1984) found that, in post larvae *Penaeus indicus*, the maximum growth with the diets containing 30% protein was

obtained and it was significantly different from the performances of the other diets, which contained 40% and 30 to 50% protein contents.

Ravishankar and Keshavanath (1988) studied the ability of *Macrobrachium rosenbergii*, to utilise the artificial pelleted feeds containing, fish meal, silkworm pupae, silkworm pupae with shrimp waste and swp with clam meal as the major protein source. The digestibility of protein, form shrimp waste was the highest while fat digestibility was better with fish meal. Protein and fat digestibility from pellets of silk worm pupae and clam meal were poor.

Sakaras et al (1987) studied the growth and survival rates of banana prawn, and the *Penaeus merguiensis* and were measured for a period of 16 weeks, using 4 types of pellets and found that the pellet number 1, which contained fish meal, rice bran, broken rice, soyabean meal, sea weed, shrimp shell and vitamin mix, gave the highest growth and survival rate.

Foster (1975) found that, the quality of protein in shrimp diets with proteins from different sources, had influenced growth rates.

An increase on the protein concentration in the diet improved the final percentage survival of the juveniles of the prawn. Thus in the juveniles of *Macrobrachium rosenbergii*, the best survival rate of 98%

was obtained, at a dietary protein level of 35%. The final percentage survival values of the juvenile *Macrobrachium rosenbergii* was obtained in the present study were relatively high, which may be attributed to the better balancing of nutrients in the experimental diets, the maintenance of water quality within the desirable range throughout the experimental period by the daily exchange of almost the entire quantity of the rearing water and also to the provision of artificial substrate in each rearing tank.

In the present study the growth rate at different protein levels showed that at 35% levels and 45% levels the maximum growth was obtained and at 15 to 60 days a difference was observed in the growth of the prawn when fed with diets having 10 to 45% proteins but the values were not significant.

In the juveniles, the protein levels below 30% and above 35%, resulted in a reduction in growth. The former might 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 the carbohydrate present in those diets. Feeding with diets containing 40% carbohydrate have been reported to result in the growth retardation in finfish (Kanazawa et al., 1979) while the optimum dietary carbohydrate for the prawn remains near 20% (Alava and Pascaul, 1987). He also found that a reduction in the growth of penaeus monodon occurred when dietary carbohydrate level exceeded 20%.

The observation that, a dietary protein level of 40 to 50% resulted in decreased growth rate, might be attributed to the presence of extra protein and the availability of low protein dietary energy. Wilson (1989) observed that, the combination of high dietary protein in the presence of low non protein energy, might force the crustaceans to deaminate sufficient portion of the protein and thus yielding the carbon fragments required for the cellular energy metabolism. As a result of this altered metabolism, the animal could be expected to show reduced growth rate and efficiencies. Balazs et al., (1973) suggested that a dietary protein concentration in excess of 35% may be required for maximum growth rate among juvenile *Macrobrachium rosenbergii*.

In the present study it was found that the protein level 30 to 35% was ideal for the growth of *Macrobrachium rosenbergii* juveniles. The efficiency with which an animal could convert food for the growth process was reflected in the ratios of food consumed, to the live weight it had gained. Thus the low food conversion ratios indicated a high efficiency in the food utilisation.

The present study was also conducted to evaluate the nutritional quality of different locally available sources of protein, viz the clam meal, the shrimp meal, the silk worm pupae meal, the squilla meal, the ground nut oil cake meal and the algae meal in the formulated feed of the juveniles of *Macrobrachium rosenbergii* based on

the survival rate growth, FCR, FCE, PER, PPV, PDC and the apparent digestibility.

High survival rate observed for *Macrobrachium rosenbergii* juveniles, fed with diets differing in protein sources suggested that, the different protein sources tested, did not show any variation with regard to their influence on the survival rate and could be incorporated in the diet without any deleterious effect on the rate of survival. Slight variations in the growth rate and in the feed efficiency were observed, when these diets were tested. These were also found to be not grossly different in their nutritional quality.

Among the different feeds tested in the present study, the diet based on shrimp meal, gave the highest growth rate followed by the squilla meal diet, the clam meal diet and the ground nut oil cake. The difference in the response was insignificant. Thus, these could be considered, more or less equivalent, as far as the quality of the protein was concerned.

Kanazawa et al., (1970) reported that fresh short necked clam gave a superior growth compared to the other compounded diet for *Penaeus japonicus*. Recently, Rao (1994), studied fresh clam meat along with mantle fluid and suggested that they could be incorporated along with the other low quality, locally available ingredient, as the fresh clam, serving as an attractive flavouring agent. Fom the

results of the present study, it could be seen that clam meat was superior in terms of growth, but feed efficiency and PER were found higher in shrimp meal diet, and squilla meal diet followed by clam meal diet. The better growth response of shrimp meal diet and squilla meal diet might be due to the use of purified protein which was obtained by boiling and drying of coagulated protein. Ali and Mohamed (1985) suggested the possibility of using a combination of shrimp and squilla meal in the practical feed for shrimps.

Venkataramiah et al., (1975) observed an increase in FCE, when vegetable matter was increased in the diet. However, diets with plant proteins were found to give relatively higher FCR and a lower FCE, than those with animal proteins. These findings agreed with the present study. Steffens (1981) reported that the PER values could be used to evaulate the quality of protein in the diet. Those with high PER values were of good quality protein and those with low PER values are of poor quality. In the present study the highest PER was obtained when the prawns were provided with a diet containing the shrimp meal diet and the squilla meal diet. It might be attributed to the better balancing of amino acid in the diet. The efficiency with which the protein was assimilated by prawns was most likely to be affected by the relative proportion of lipids and carbohydrates in the formula as well as the aminoacid composition of whatever proteins that were employed. (Hanson and Goodwin 1977).

The results of the digestibility studies showed that the Apparant digestibility of protein was not significantly affected by the animal or plant origin of the feed stuff. In general proteins of animal origin were found to be more digestibile, compared to that of plant origin. Fattah and Sayed (1991) found that the digestibility and utilisation of polysaccharides should be improved by cooking. A critical evaluation of the results of various experiments revealed the importance of evaulation of the quality of protein sources in the formulated feed of prawn, as the protein requirement was dependent on the source of protein.

In the present study, among the experimental diets, shrimp meal diet, was observed as a more quickly acceptable feed, compared to the other feeds. It indicated that it was better to incorporate shrimp meal in to prawn feed to serve as an attractant and also for setting better pigmentation. It contains a variety of feeding stimulants, chemoattractants, mainly amino acids and nucleotides, which enhanced the value of shrimp feed considerably.

Section B

Matrix of correlation coefficient Analysis

The correlation matrix of the changes in digestive enzyme activity to different growth parameters are presented in Table 20a-f. The study is focussed on the extent of variation in the enzyme activity with other growth parameters of juvenile Macrobrachium rosenbergii at different culture periods such as 15, 30, 60 and 90 days.

From the results it is observed that there exists a significant correlation between protease activity and RNA \DNA ratio at 15 to 30 days (Table 20a). Table 20b shows the matrix of correlation between the changes in protease activity with muscle protein of Macrobrachium rosenbergii fed with different protein concentration. The significant relationship exists between 60 days and 90 days.

Table 20c shows the linear relationship between total protease activity to average weight gain at different periods. There is a gradual increase in weight gain at different protein concentration at the 15th and the 30th day and the relationship is significant ($p<0.05$) When the protein level increases, the protease activity also increases and it is significant at 5% level.(Table 20d).

Table 20e shows the correlation between total protease activity and protein efficiency ratio at different periods. There is a relationship seen between protease activity and PER but it is not

Table 20a-f. Matrix of correlation of digestive enzyme activity with other growth parameters at different periods of feeding

20a-Changes in total protease activity with RNA-DNA ratio at different periods.

	tp1	tp 2	tp 3	rd 1	rd 2	rd 3	rd 4
tp 2	0.943*						
tp 3	0.903*	0.986*					
tp 4	0.752	0.922*	0.933*				
rd 1	0.431	0.679	0.761	0.800			
rd 2	0.672	0.854	0.901*	0.879*	0.954*		
rd 3	0.058	0.381	0.469	0.650	0.898*	0.745	
rd 4	0.604	0.546	0.616	0.322	0.483	0.587	0.095

20b-Changes in total protease activity to muscle protein at different periods

	tp 1	tp 2	tp 3	mp 1	mp 2	mp 3	mp 4
tp 2	0.943*						
tp 3	0.903*	0.986*					
tp 4	0.752	0.922*	0.933*				
mp 1	0.448	0.654	0.682	0.700			
mp 2	0.007	0.566	0.690	0.606	0.656		
mp 3	0.918*	0.993*	0.986*	0.951*	0.620	0.576	
mp 4	0.732	0.851	0.824	0.807	0.908*	0.485	0.811

** significant at $p<0.05$*

tp:total protease activity; rd:RNA-DNA ratio; mp:muscle protein content subscripts 1,2,3 & 4 represents the periods 15 days, 30 days, 60 days and 90 days respectively.

20c-Changes in protease activity to average weight gain

	tp 1	tp 2	tp 3	aw 1	aw 2	aw 3	aw 4
tp 2	0.943*						
tp 3	0.903*	0.986*					
tp 4	0.752	0.923*	0.933*				
aw 1	0.914*	0.981*	0.992*	0.927*			
aw 2	0.919*	0.984*	0.996*	0.921*	0.999*		
aw 3	0.765	0.629	0.550	0.486	0.634	0.616	
aw 4	0.803	0.928*	0.965*	0.954*	0.975*	0.969*	0.541

20d-Changes in protease activity to specific growth rate at different periods.

	tp 1	tp 2	tp 3	sgr 1	sgr 2	sgr 3	sgr 4
tp 2	0.943*						
tp 3	0.903*	0.986*					
tp 4	0.752	0.922*	0.933*				
sgr 1	0.869	0.971*	0.981*	0.968*			
sgr 2	0.889*	0.983*	0.990*	0.962*	0.998*		
sgr 3	0.873	0.978*	0.977*	0.977*	0.996*	0.996*	
sgr 4	0.641	0.856	0.904*	0.963*	0.914*	0.910*	0.915*

** significant at $p<0.05$*

tp:total protease activity; aw: average weight gain; sgr: specific growth rate subscripts 1,2,3&4 represents the periods 15 days, 30 days, 60 days and 90 days respectively

20e-Changes in total protease activity to PER at different periods.

	tp 1	tp 2	tp 3	per 1	per 2	per 3	per 4
tp 2	0.943*						
tp 3	0.903*	0.986*					
tp 4	0.752	0.922*	0.933*				
per 1	0.520	0.757	0.809	0.858			
per 2	0.554	0.666	0.716	0.583	0.845		
per 3	0.563	0.749	0.786	0.765	0.965*	0.942*	
per 4	0.398	0.642	0.744	0.772	0.934*	0.786	0.859

20f-Changes in total protease activity to FCE at different periods

	tp 1	tp 2	tp 3	fce 1	fce 2	fce 3	fce 4
tp 2	0.943*						
tp 3	0.903*	0.986*					
tp 4	0.752	0.922*	0.933*				
fce 1	0.869	0.971*	0.987*	0.963*			
fce 2	0.923*	0.988*	0.996*	0.927*	0.991*		
fce 3	0.901*	0.986*	0.995*	0.950*	0.997*	0.998*	
fce 4	0.803	0.934*	0.973*	0.956*	0.989*	0.969*	0.979*

**significant at $p<0.05$;*

tp:total protease activity at 15, 30, 60 and 90 days; per: protein efficiency ratio; fce: food conversion efficiency

subscripts 1,2,3&4 represents the periods 15, 30, 60 and 90 days respectively.

Table 21a-d. Second order polynomial regression equations between dietary protein concentration and growth parameters at different periods regression equations ($y=a+bx+cx^2$)

21a. Specific growth rate with protein concentration at different periods

Protein conc.	**15 days**	**30 days**	**60 days**	**90 days**
10%	1.3498	1.7569	1.2686	0.921
20%	3.4088	3.2879	2.2576	1.714
30%	4.9078	4.3989	2.9466	2.247
35%	5.4473	4.7969	3.1786	2.416
45%	6.1063	5.2779	3.4176	2.559

21b. Food conversion ratio with protein concentration at different periods

Protein conc.	15 days	30 days	60 days	90 days
10%	11.4761	5.9191	5.0782	5.0902
20%	5.6321	3.2131	2.5552	2.5732
30%	2.3881	1.6271	1.1322	1.1562
35%	1.7411	1.2541	0.8382	0.8602
45%	2.3971	1.3481	1.060	1.093

21c. Food conversion efficiency with protein concentration at different periods

Protein conc.	15 days	30 days	60 days	90 days
10%	0.0694	0.1323	0.1514	0.1145
20%	0.2664	0.3803	0.5154	0.5915
30%	0.4234	0.5683	0.7794	0.9285
35%	0.4869	0.6398	0.8739	1.0445
45%	0.5839	0.7378	0.9879	1.1715

21d. Protein efficiency ratio with protein concentration at different periods

Protein conc.	15 days	30 days	60 days	90 days
10%	0.7983	1.5799	1.8292	1.7017
20%	1.1253	1.8669	2.4072	2.5917
30%	1.2523	1.9939	2.6252	2.9817
35%	1.2408	1.9974	2.5992	2.9892
45%	1.0678	1.8844	2.2772	2.6292

statistically significant. There exists a correlation between total protease activity and food conversion efficiency at different periods. Here the enzyme activity shows a close correlation with food conversion efficiency at different periods and it is statistically significant ($p<0.05$) as presentedin Table 20f.

From all these observations it is seen that the digestive enzyme activity is closely correlated between protein concentration at different periods. Also the total protease activity is shown to be positively correlated with most of the growth parameters analysed, emphasizing its significance in the evaluation of feed formulation.

Regression Analysis

Second order polynomial regression equations ($y=a+bx+cx^2$) was established between the dietary protein concentrations and the corresponding growth of the juvenile *Macrobrachium rosenbergii*. The curvilinear regressions were fitted to the individual data of the protein concentration against SGR, FCE and PER as shown in fig 18a-d. From the regression curve, it is observed that, different growth parameters such as SGR, FCE and PER show an increasing pattern with different protein concentration.

Fig.18a Second order polynomial relation
of the SGR of the juvenile M.rosenbergii
and dietary protein concentration

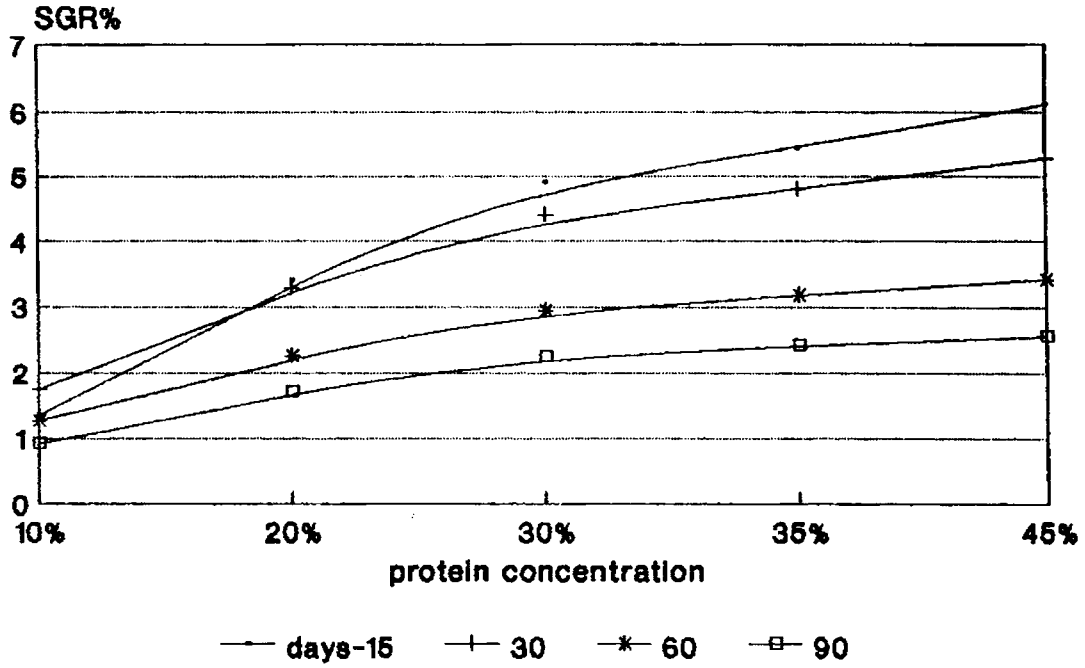


Fig.18b Second order polynomial relation
of the FCR of the juvenile M.rosenbergii
and dietary protein concentration

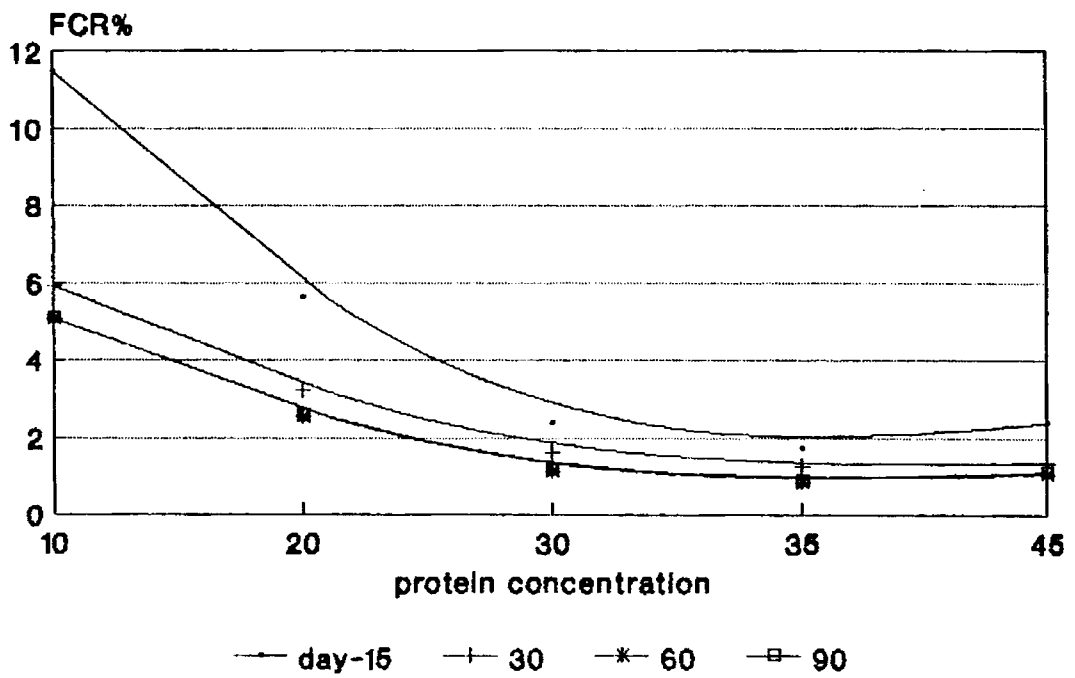


Fig.18c Second order polynomial relation
of the FCE of the juvenile M.rosenbergii
at diff. dietary protein concentration

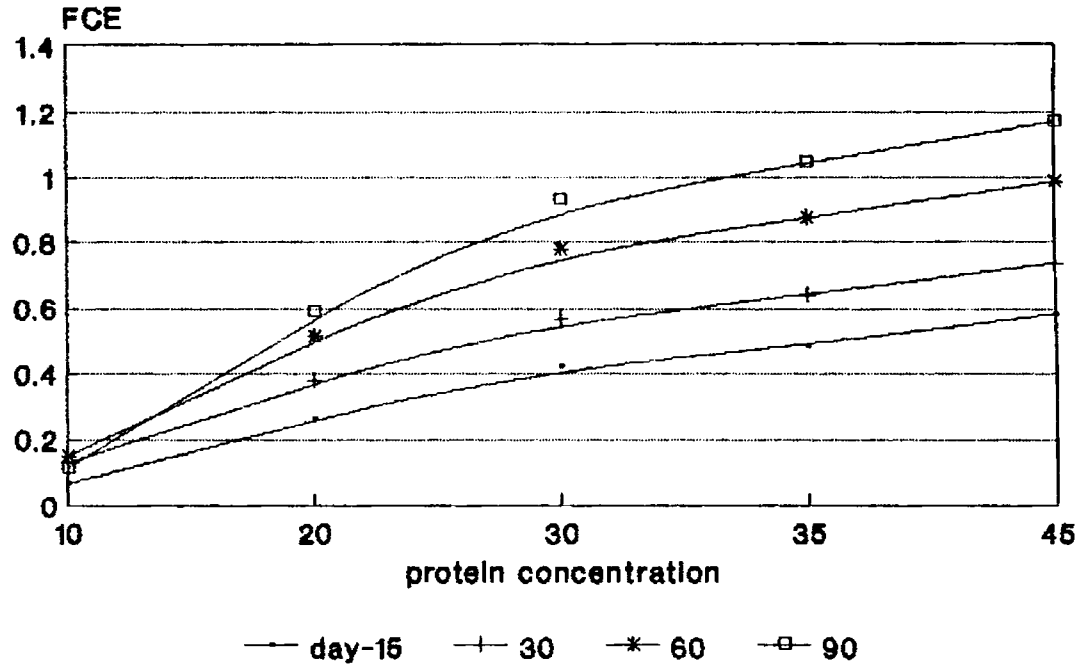
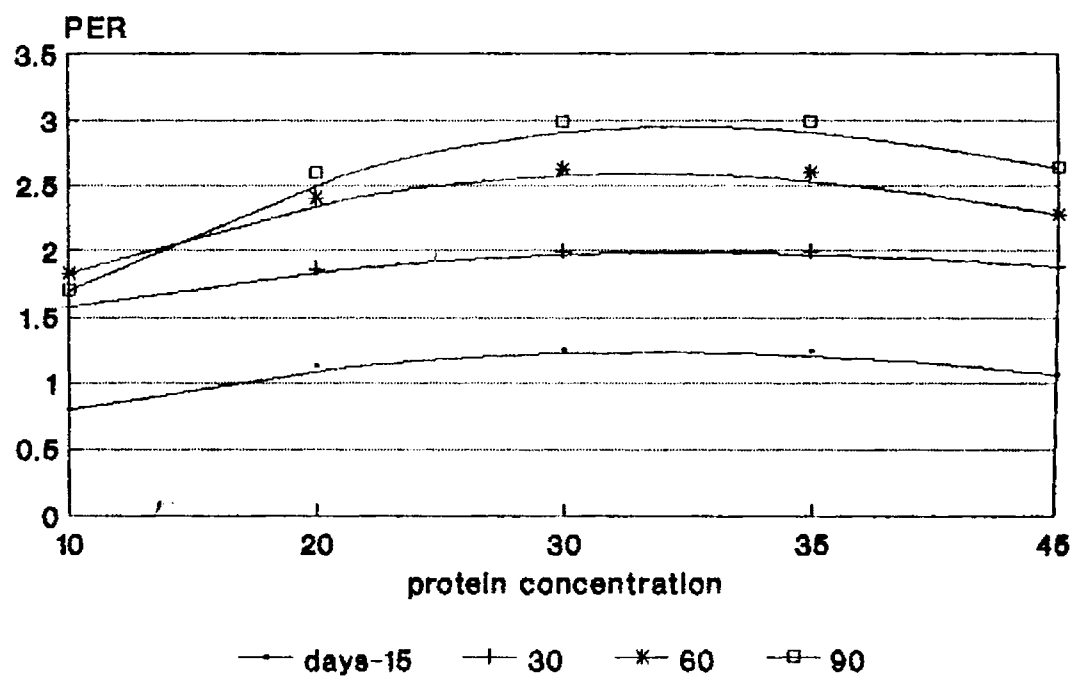


Fig.18d Second order polynomial relation
of the PER of the juvenile M.rosenbergii
at diff. dietary protein concentration



Chapter 7

SUMMARY AND CONCLUSION

The subject matter of the thesis relates to the investigations concerning the digestive enzyme activity of juvenile *Macrobrachium rosenbergii* fed on experimental diets containing different protein concentrations and protein sources. The thesis is presented in six chapters comprising of a general introduction, review of literature, digestive enzyme activity at different protein concentrations and sources, nucleic acid composition and the nutritional evaluation methods used for the growth study.

The general introduction embodies the present status of the culture and the various aspects relating to the growth of *Macrobrachium rosenbergii*. It also explains the main objectives and scope of the study. The second chapter provides a detailed review of the literature on the culture, nutritional requirements and food and feeding of *Macrobrachium rosenbergii*. The importance of nucleic acid composition, water quality parameters of culture systems and the nutritional evaluation methods adopted were also reviewed in detail.

The first experiment was done to determine the changes in the protease and amylase activity in the hepatopancreas of the animals, at different hours, after feeding a basal diet using clam meat at an optimum level of protein. The proximate composition of the feed was determined and a micro particulated feed was prepared. Samples were collected in triplicate and a proper culture system was maintained through out the study, with gentle air supply and the removal of water and faecal matter

frequently. The water quality parameters such as dissolved oxygen, pH, temperature, nitrite, and ammonia was maintained at an optimum level.

The second experiment was conducted to determine the changes in protease, amylase and lipase activity, by giving graded levels of protein consisting of 10, 20, 30, 35 and 45% protein. The effect of the various levels of protein on the survival and growth rate were considered for a period of 15, 30, 60 and 90 days to arrive at an optimum level of dietary protein.

The third experiment was planned with the results of the above study and it was conducted to determine the changes in protease, amylase and lipase activity on feeding diets containing certain locally available sources of protein which have the potential for its use in commercial shrimp diets viz., clam meal, prawn meal, silkworm pupae meal, squilla meal, ground nut oil cake meal and algae meal at 35% protein concentration. This was undertaken to fix the best protein sources to be incorporated in practical diets. The effect of various protein sources in the digestive enzyme activity such as proteases, amylases and lipases at different periods of the feeding trial such as 0 day, 15, 30, 60 and 90 days were monitored.

The experiment was repeated by giving the experimental diets with different protein concentrations and sources to the juvenile prawns and determined the RNA-and DNA content of the muscle tissue and the relationship of RNA- DNA ratio to muscle protein content and growth parameters. The water quality parameters such as temperature, pH, dissolved

oxygen, nitrite and ammonia monitored during the culture period, were found to be within a suitable range and did not affect the growth of the prawns adversely.

The effect of the various levels and sources of dietary protein on the survival, growth rate, food intake, food conversion efficiency, protein digestibility coefficient, protein efficiency ratio, apparent digestibility coefficient and productive protein value were also monitored during the feeding trial. The data was statistically analysed and presented.

The salient findings of the various experiments are summarised as follows:

1. The results of digestive enzyme activity in the hepatopancreas after feeding, at different hours (such as 0 to 9 hours) showed that the changes in total and specific activity of protease and amylase were maximum at 5 to 6 hours after feeding. After that, the enzyme activity shows a declining pattern.

2(a). The protein inclusion had a pronounced influence on the growth of the juvenile of the prawn. The changes in protease total activity, specific activity and the ratio of total protease activity to body weight showed that there is a uniform increase in enzyme activity with increasing dietary protein concentrations. The enzyme activity was maximum at 35% protein level followed by 30%. When protein concentration increased beyond 35%, the enzyme activity showed a declining pattern. A culture period of 15 to 60 days showed the maximum enzyme activity.

(b). The amylase total activity, specific activity and ratio of total activity to body weight also showed a similar pattern. As the body weight increases, the enzyme activity decreases. The results were statistically significant at 1% level ($p< 0.01$).

(c). The lipase enzyme activity increases gradually and at 30 to 35% protein level showed the maximum activity. In the early period at 15 to 30 days, the maximum enzyme activity was observed.

3. Substitution of protein, both animal and plant protein sources at 35% protein in the diet of juvenile *Macrobrachium rosenbergii* revealed that the enzyme activity was maximum with a diet based on shrimp meal and squilla meal followed by clam meal at 15 to 60 days. The amylase and lipase activity also showed maximum value with a diet based on shrimp meal and squilla meal. In the case of lipase activity, ground nut oil cake meal attained the maximum enzyme activity. The results showed significance at 1% level ($p<0.01$). Substitution of protein above 35% resulted in a gradual decline in enzyme activity. The other sources of proteins also showed equally good results. But the results were not statistically significant.

4. Protein synthesis is accompanied by an increase in RNA and decrease in DNA concentration. The DNA content of the muscle decreased with increase in protein level and the RNA content and the RNA\DNA ratio showed a progressive increase with protein level upto 35%. The maximum change in the DNA and RNA content in the muscle and RNA\DNA ratio was shown in the juvenile *Macrobrachium rosenbergii* fed on diet containing shrimp meal and squilla meal diet. Thus the

RNA\DNA ratio could be used as a more sensitive tool for investigating the effectiveness of an experimental diet in promoting growth compared to conventional growth assessing parameters.

5. On the basis of the observations on the growth and digestive enzyme activity of the juvenile *Macrobrachium rosenbergii* in response to the various dietary protein concentrations, it was suggested that a protein ration of 30 to 35% was ideal for the growth and optimum utilisation. In prawns receiving protein rations equal or below the optimum levels, the tissue protein gain, growth rate, digestive enzyme activity and protein concentration in the diet were linearly related. The portion of the dietary protein digested decreased with increasing protein ration above 35%, as evident from the pattern of digestive enzyme generation during the feeding trial.

The specific growth rate of the prawn showed a curvilinear relationship with the dietary protein concentration. The growth increased as the dietary protein concentration increased, upto an optimum level of 30 to 35%. A further increase in protein intake resulted in an apparent but statistically not significant reduction in the growth. By establishing second order polynominal relationship between the growth rate and dietary protein concentration and by employing the differential calculation method, the protein concentration which resulted in maximum growth were determined to be 30 to 35% level. Dietary protein levels had little influence on the food intake per day per unit weight of the juvenile *Macrobrachium rosenbergii*. The feed conversion efficiency was also influenced by the dietary protein concentration and the changes were similar to that specific

growth rate. The dietary protein concentration and the PER were negatively linearly correlated with higher dietary protein levels.

The apparent digestibility of protein improved as the dietary protein concentration increased. The protein concentration had a significant effect on the protein digestibility coefficient and productive protein value. The plant proteins such as groundnut oil cake meal and algae meal showed significantly high digestibility coefficients indicating that, the plant protein sources could be effectively utilised by the animal, but the efficiency ratio was better in prawns fed on animal protein.

On the basis of the observation on growth performance and digestive enzyme activity, there exist a correlation between protease and amylase activity to growth rate, RNA/ DNA ratio, feed conversion efficiency, protein efficiency ratio and muscle protein content. All these parameters are interrelated. Upto an optimum dietary protein level, the protein concentration, protein conversion efficiency, RNA content, and RNA\DNA ratio of the muscle are linearly related. The protein requirement to achieve maximum growth decreased with increasing animal size. Smaller shrimps exhibited higher digestive enzyme activity when fed on animal proteins like prawn meal, squilla meal and clam meal, while towards the later stages of growth they exhibited similar but decreased enzyme activity in response to all types of diets. Hence, proteins which are generally considered the most expensive macronutrient of prawn feeds, could be made available to prawns through least - cost effective feeds prepared from locally available raw ingredients of both animal and plant origin. In the present study, the changes in digestive enzyme activity at

different stages of growth, and adaptation to new diets of the juvenile *Macrobrachium rosenbergii* have been studied quantitatively and this can serve as an effective index for feed evaluation.

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