GROWTH AND SURVIVAL OF *PENAEUS MONODON* FABRICIUS JUVENILES FED ON DIET AT DIFFERENT LEVELS OF REPLACEMENT OF CLAM MEAT WITH DE - OILED SILKWORM PUPAE

BY

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THESIS

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DECLARATION

I hereby declare that this thesis entitled " GROWTH AND SURVIVAL OF *PENAEUS MONODON* FABRICIUS JUVENILES FED ON DIET AT DIFFERENT LEVELS OF REPLACEMENT OF CLAM MEAT WITH DE - OILED SILKWORM PUPAE " is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree , diploma , associateship, fellowship or other similar title of any other University or Society.

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CERTIFICATE

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1. INTRODUCTION

Successful and sustainable aquaculture of finfish and crustaceans (prawns, lobsters and crabs) depend upon the provision of nutritionally adequate, environment-friendly and economically viable artificial feeds. The feed is normally the largest single item in the running of a shrimp farm and supplementary feed costs normally range from 40 - 60% of the total operational costs in intensive farming operations (Anon, 1983). The suitability and cost effectiveness of the ration are of paramount importance for the commercial success of culture of any species (New, 1976). Formulation of a balanced feed containing low cost protein ingredients can bring down the cost of supplementary feed to a great extent. Hence for the development of sound semi-intensive and intensive farming of finfish and shellfish, development of an economically viable and biologically efficient diet with cheap locally available ingredients are highly warranted.

Unlike natural food, artificial diets are not subjected to seasonal variations in supply and can be manufactured under strict quality control. As shrimp feeds become better formulated in accordance with the research findings of balanced nutrient requirements and texturised to improve acceptability, they are likely to show improved utilisation.

As in fishes, crustaceans also show preferential use of proteins over carbohydrates. Because of this, and the fact that proteins in the compounded feed form the most expensive item, studies on evaluation of cheaper protein sources as ingredients for supplementary feeds have received much attention in recent years. In this regard, a variety of natural feed sources are currently being utilised the world over. As against the highly nutritious practical feeds used in the developed countries, which make use of high quality feed ingredients, developing countries relay on mostly nutritionally poor quality raw materials for feed formulation.

In India, detailed research on penaeid shrimp nutrition has been taken up only recently when commercial culture of shrimps gained momentum. One of the areas of active research has been the feed formulation technology for the penaeids and suitable feeds are being developed both for the larvae in hatcheries and the juveniles in grow-out systems. The types of feeds used at present include clam meal, mussel, squid, squilla meal, fish meal, trash fish, animal flesh, silkworm pupae, bone meal, liver, blood meal, eggs, meat offals, oil cakes, rice bran, wheat bran, grains, cattle feeds, different types of pelleted compounded feeds etc. Many of the feeds are inadequate to meet the nutritional requirements of shrimps. Also they may not be always available in sufficient quantities. Moreover, the exorbitant cost of these feed stuffs and pelleted feeds is another hindrance for its large scale use in shrimp rearing. Hence formulation of a low cost, nutritionally balanced shrimp feed containing adequate levels of protein with locally available cheap ingredients or by substitution and/or replacement, either partially or fully the costly ingredients with cheap locally available ingredients is essential.

Silk worm pupae which is a highly proteinaceous, low cost by-product of silk industry has been used as an important protein source in the commercial feeds of fishes and prawns in countries like Japan and China.

Several experiments have been done in India regarding the possibility of utilisation of silk worm pupae in the diet of Common carp (Jayachandran and Paul Raj, 1976;1977), Catla, Rohu (Jayaram and Shetty, 1980), Mullets (Liao, 1981), *Tor khudree (Srikanth, 1986)*, *Macrobrachium rosenbergii* (Unnikrishnan *et al.*, 1991), *Heteropneustes fossilis* (Hossain *et al.*, 1993), *Puntius gonionotus* (Mahata *et al.*, 1994), *Clarias batrachus* (Habib *et al.*, 1994), *Macrobrachium malcomsonii* (Das *et al.*, 1995). Most of the studies on the utilisation of silkworm pupae in the diets have been done in fishes and prawns, except the ones done in *Penaeus indicus* (Ali,1982; 1992). More studies on the use of silkworm pupae in the diets of shrimps are required.

Since the black tiger prawn *Penaeus monodon* is the most extensively cultured crustacean in the countries of South East Asia, due to its fast growth rate, capacity for adaptation to various culture systems and efficient response to compounded feeds (AQUACOP, 1976; Alava and Lim, 1983) and as it is an ideal species for large scale farming in estuarine and brackish-water ecosystems, acceptance of de-oiled silkworm pupae by the species is worth to be evaluated.

In the present study, it is proposed to evaluate the efficiency of de-oiled silkworm pupae as a protein source in the diet of *Penaeus monodon* juveniles at various levels of replacement of clam meat.

2. REVIEW OF LITERATURE

2.1. Nutrition of shrimps

The major, and exhaustive review on caridean and penaeid nutrition is that of New (1976). Since then commercial farming of these species has rapidly increased, prompting continued research efforts around the world. Less wide ranging articles have also been produced by Wickins (1976), Biddle *et al.* (1977), Kanazawa (1981), Muthu *et al.* (1982), Corbin *et al.* (1983), Piedad-Pascual (1983), Sick and Millikin (1983), Kanazawa (1985), Teles (1986), Tacon (1987) and Lio and Liu (1990). New (1980) has provided a bibilography covering the majority of papers published till then.

In terms of nutritional research, the most widely studied species have probably been the sub-tropical marine shrimp, *Penaeus japonicus* (Bate,1888) and the freshwater prawn, *Macrobrachium rosenbergii* (De Man,1879). This may be due to their establishment as suitable species for commercial aquaculture as early as the 1960s. More recently, rapid developments in commercial culture of other species such as *Penaeus monodon* (Fabricius,1798) in south- east Asia, *Penaeus vannamei* (Bonne,1931) and *Penaeus stylirostris* (Stimpson,1874) in Ecuador and *Penaeus chinensis* (Osbeck,1765) in China have also taken place and these developments have necessitated an expansion of nutritional research to a wider range of species.

The feeding habits of penaeids in the wild have been reviewed by Dall *et al.* (1990). Whilst several studies have defined the natural prey items of prawns and shrimps

(Marte,1980; Thomas,1980; Chong and Sasikumar, 1981; Robertson,1988; Wassenberg and Hill,1993), there is relatively little information available on the biochemical composition of the natural diet. Two exceptions are the studies of Moriarty and Barclay (1981) and Dall *et al.* (1991). Data of this type can provide valuable base-lines for artificial diet formulation.

2.1.1. Proteins

Among the nutrients, protein is the most important, as it forms the major growth factor in animal tissues. It is the major as well as the most expensive component in shrimp diets. Greater emphasis was given for understanding the protein requirement and for determining the optimum protein levels in the diet of different species (Kanazawa *et al.*, 1971; Deshimaru and Shigueno, 1972; Balaz *et al.*, 1973; Foster and Beard, 1973; Deshimaru and Kuroki, 1975; Colvin, 1976; Khannapa, 1979; Kanazawa *et al.*, 1981; Ali, 1982; Alava and Lim, 1983; Teshima and Kanazawa, 1984; Bautista, 1986; Ali, 1988; Shiau *et al.*, 1991). These studies indicate that protein requirement of different species of penaeid prawns range from 15 to 80 %.

2.1.1.1. Quantitave protein requirements

Balazs *et al.* (1973) have reported the optimum protein level for *Penaeus japonicus* as 40 %. Deshimaru and Kuroki (1974) obtained best growth for *Penaeus japonicus* with a diet containing 50 % protein. Deshimaru and Yone (1978) have shown that the protein requirement of *Penaeus japonicus* with mean weight 0.8 g for optimum growth and food conversion efficiency as 52 to 57 % using a diet based on casein and egg albumin (9:1). Teshima and Kanazawa (1984) have demonstrated that *Penaeus japonicus* showed best growth with 45-55 % casein. Koshio *et al.* (1993) have reported the optimum protein level for *Penaeus japonicus* as 42 % when fed with crab protein based diet. Colvin (1976) has reported the optimum protein level for *Penaeus indicus* as 43 %. Ali (1982) has reported good growth and survival of *Penaeus indicus* when fed with diets containing total protein levels of around 34 %. But in a second experiment, highest average daily weight increase and best food conversion ratio were obtained with crude protein level of 42.9 %. Sambasivam *et al.* (1982) reported that juveniles of *Penaeus indicus* had a protein requirement above 60 % on the basis of growth and conversion efficiency, but the survival was higher when the diet contained 50 % protein. Ali, in yet another experiment reported best growth with 29 % casein diet (Ali, 1988). Bhasker and Ali (1984) and Udayakamura and Ponniah (1988) found that early post larvae and juvenile *Penaeus indicus* required 40 % casein in a defined diet for optimum growth, while Gopal and Raj (1990) reported the requirement as between 35 and 37.5 %.

Bages and Sloane (1981) have demonstrated that *Penaeus monodon* showed best growth with a diet containing 35 % casein whereas Alava and Lim (1983) have found that 40 % protein (fish and shrimp meal with casein as the variable component) gave the best growth and conversion efficiency ratio for *Penaeus monodon* juveniles. Bautista (1986) has obtained best growth and survival for *Penaeus monodon* with 40-50 % protein (casein and gelatin based diet) in the presence of 20 % carbohydrate and 5-10 % lipid. Nezaki *et al.* (1986) have found that 55 % protein with 15 % carbohydrate in grow-out diets gave the best growth for *Penaeus monodon*. However, according to them, when the carbohydrate content is increased to 25 %, a 45 % protein diet can give result comparable to those diets containing 55 % protein. Shiau and Chou (1991) have obtained best growth of *Penaeus monodon* with 36-40 % casein.

In *Penaeus merguiensis* the protein requirement was reported to be 50-55 % in a casein based diet (AQUACOP, 1978), while Sedgwick (1979) has reported the protein requirement to be 34-42 % using mussel meal based diet. In *Penaeus aztecus* the protein requirement has been reported to be 23-31 % (Shewbart *et al.*, 1973). Balazs *et al.* (1973) has obtained good growth and survival of *Penaeus aztecus* with 25 % protein. Venkataramiah *et al.* (1975) have reported an optimum protein level of 40 % for *Penaeus aztecus*. Fenucci and Zein Eldin (1976) have obtained the optimum protein level for *Penaeus aztecus* as 36.5 % using squid mantle meal as the protein source. The protein requirement for *Penaeus setiferus* was shown to be 28-32 % (Andrews *et al.*, 1972). Colvin and Brand (1977) have shown the protein requirement for *Penaeus californiensis* to be 31 %, for *Penaeus vannamei* to be 30 % and for *Penaeus stylirostris* to be 30-35 %. Cousin *et al.* (1991) found that optimum protein level for *Penaeus vannamei* was close to 30 % of diet. Kanazawa *et al.* (1981) reported that *Metapenaeus monoceros* required 55 % casein in the diet for maximum weight gain and 60 % for optimum survival. Thus previous studies show that there is uncertainity to a certain extent as to the quantitative dietary protein requirements of shrimps. The wide range in optimum protein requirement noted in these studies , could mainly be attributed to the widely varying protein sources employed, the stage of experimental animals used and variations in the experimental procedures followed, including unstandardized way of expressing the proximate composition of test diets.

The variation in protein requirement in the previous studies may also be attributed to various factors such as differences in biological value of protein sources which again depends on the amino acid composition of protein (Harper,1981; Kies,1981), composition of other dietary components,viz. fat and carbohydrate ratio (Andrews *et al.*,1972; Teshima and Kanazawa,1984) and amount of organic salts present in the diet (Deshimaru and Kuroki,1974; New,1976). Variation in environmental factors, stages in the life history of test species and differences in feeding habits also contribute to a certain extent to the differences in protein requirement. Although most studies have utilized juvenile animals because of their high growth rates, recent work has emphasised age-related changes in protein requirements. Colvin and Brand (1977) suggested that the optimum dietary protein level for early post-larvae of *Penaeus californiensis* and *Penaeus stylirostris* was 44 %, but it declined to less than 30 % in the juveniles. Bhaskar and Ali (1984) found that in *Penaeus indicus* reared on casein based diets, the optimum protein level was 40 % for PL1 to PL10 and 30 % for PL27 to PL42. In contrast, Khannapa (1977) has reported that the protein requirement of *Penaeus monodon* increased with age from 30 % to 40 % (PL10 and one month old juveniles respectively) fed a diet containing rice bran and fish meal. Yaquan and Wenjuan (1986) described a similar increase for *Penaeus chinensis*. Non-protein energy is yet another factor that influence the optimum protein requirement. The combination of high dietary proteins in the presence of low levels of non protein energy can force the crustacean to deaminate significant proportion of the protein, thus yielding carbon fragments required for cellular energy metabolism (Hanson and Goodwin, !977).

2.1.1.2. Amino acids

Since animo acids are building blocks of protein, their profile in the protein source greatly determines the efficiency of their utilization. Protein requirement is significantly affected by the quality of dietary protein which in turn is dependent on the level, balance and bio-availability of external amino acids.

2.1.2.1. Qualitative and quantitative requirements

Shewbart *et al.* (1972) investigated the amino acid requirement of *Penaeus aztecus*. Miyajima *et al.* (1977) determined the essential amino acids for *Macrobrachium ohione* to be arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine and valine by incorporation of [U-C^{14} glucose]. Coloso and Cruz (1980) established similar results for juvenile *Penaeus monodon* by injection of [U-C^{14}] acetate and this pattern for essential amino acids has been extended to *Penaeus japonicus* by Kanazawa and Teshima (1981). In addition Kanazawa and Teshima (1981) were able to confirm the essentiality of tryptophan. Dall and Smith (1987) have emphasized that dietary

Table. 1 Essential amino acid ratios for various shirmp and prawn species and a variety of protein sources commonly üseed in feeds

	Analyses of animals						Analyses of dietary proteins				
	Macrobrachium rosenbergii	*P. japonicus*		*P. monodon*		*P. aztecus*					
Tissue	tail muscle	whole body	larvae	whole body		tail muscle	Clam	White fish meal		Squid meal	Soybean meal
Reference	1	2	3	4	5	6	7	3	8	6	6
Arginine	20.7	20.5	14.3	16.7	16.0	15.3	9.4	14.2	13.5	15.3	13.8
Histidine	4.6	4.5	4.6	3.8	5.1	4.6	5.7	4.5	4.6	4.5	7.0
Isoleucine	7.1	7.7	8.6	6.5	10.3	8.6	8.1	8.6	9.2	8.6	9.8
Leucine	14.8	14.9	14.5	15.6	13.7	14.6	17.6	14.4	14.7	15.7	15.7
Lysine	17.3	17.1	17.0	16.1	14.0	14.4	11.0	16.9	15.3	15.9	12.7
Methionine [9]	6.4	6.4	5.0	6.5	6.5	5.3	6.2	5.0	5.5	5.7	3.1
Phenylalanine	7.4	7.2	7.6	8.7	9.9	8.1	10.3	7.7	7.7	6.9	11.1
Tyrosine [10]	6.6	6.7	10.3	8.0	ND	7.4	5.0	7.2	6.1	6.3	5.9
Threonine	7.7	7.7	9.2	8.0	6.7	7.7	9.7	9.4	8.6	8.4	8.3
Tryptophan	ND	ND	ND	1.1	7.2	2.1	1.8	ND	2.1	2.0	0.1
Valine	7.4	7.3	9.7	6.3	9.8	9.7	9.2	9.8	10.1	8.7	9.6
Cystine	ND	ND	2.2	2.7	ND	2.2	6.0	2.3	2.6	2.0	2.6

1 Farmanfarmaian and Lauterio (1980) (1.4g animals). 2 Farmanfarmaian and Lauterio (1980) (21.0 g animals). 3 Deshimaru and Shigeno (1972). 4 Deshimaru (1982). 5 Teshima *et al.* (1986a). 6 Dy-Penaflorida (1989). 7 Shewbart *et al.* (1972). 8 NRC (1983).

9 The requirement for methionine is frequently calculated as the sum of the requirement for methionine and cystine since conversion of the former to the latter may be possible in shrimp and prawns.

10 The requirement for tyrosine is frequently calculated as the sum of the requirement for tyrosine and phenylalanine as conversion of the former to the latter may be possible in shrimp and prawns.

sources of amino acids regarded as non- essential may be required if they have a high rate of metabolic turnover. The requirement for amino acids may also vary with moult cycle and age. In addition, environmental salinity may have an influence, since it has been shown that free amino acids, such as proline, have an osmoregulatory role (Fair and Sick, 1982; Claybrook,1983; Mc Coid *et al.*, 1984; Smith and Dall, 1991). Wilson (1984), has shown that, the closer the essential amino acid pattern of the diet to that of the cultured species, the more effective is the diet for its growth.

The quantitative requirements for essential amino acids have traditionally been determined by feeding graded levels of each amino acid in purified form within a defined diet so as to elicit a dose response curve. This approach has been used with finfish (Ketola,1982) but has failed with crustaceans owing to their poor utilization of crystalline amino acids. Deshimaru (1976,1982) has shown that the incorporation of dietary amino acids in the free form into shrimp muscle is significantly lower than for amino acids derived from whole protein.

Despite poor results of using crystalline amino acids, some workers have claimed success in supplementing amino acid deficient protein sources with amino acids in the free form. Farmanfarmaian and Lauterio (1979, 1980) reported that supplimenting and repelleting a commercial ration with 1 % crystalline arginine, phenylalanine, leucine or isoleucine gave an increased relative growth / convertion index when fed to *Macrobrachium rosenbergii*. Growth of *Penaeus japonicus* was enhanced when diets were supplimented with L-lysine (to give a total level of 6.6 %), but the addition of L-arginine had no effect (Hew and Cuzon, 1982). Fernandez *et al.* (1995) obtained better growth rate, FCR, PER, assimilation, metabolism, gross growth efficiency (GGE) and daily growth rate (DGR) for *Penaeus indicus* fed on diet based on squid meal with the synthetic crystalline amino acid mixture of arginine, lysine, methionine and tryptophan incorporated at 0.6 % level. Fox *et al.* (1995) reported that the apparent requirement of lysine by *Penaeus vannamei* juveniles fed diets containing 45 % crude protein was 4.67 % of the protein and that fed with diet containing 35 % crude protein supplemented with wheat gluten and with L-lysine Hcl was 4.49 and 5.19 % of the protein respectively.

Since there is no precise data on amino acid requirements, the most effective method of providing a balanced dietary amino acid profile is to combine various defined protein sources with the aim of matching the profile found in shrimp muscle (Deshimaru and Shigeno,1972; Poh 1985). Teshima *et al.* (1993) obtained good growth and high survival of *Penaeus japonicus* when reared on diets with essential amino acid profile simulated to those of larval whole body proteins. Some low molecular weight nitrogen compounds such as peptides (Ala-Gly-Gly, Ala-Val,(Gly-Gly-Gly) and betaine had a growth promoting effect. Some workers have compared the profile of all the amino acids but most have used the essential amino acid ratios (E/A defined as the essential amino acid/totalamino acids X 100). Dall and Smith (1987) reported that *Penaeus esculentus* could synthezise the non-essential amino acid,proline, but relatively slowly. Variations in the amino acid profiles of shrimps do occur with the age of the animal. Dy Penaflorida (1989) found that zoeal *Penaeus monodon* had significantly higher alanine, phenylalanine and tyrosine content but lower arginine, glutamic acid, methionine and tryptophan levels compared with juvenile and adult shrimps. Cystine,glycine, serine and histidine were significantly higher in juvenile than in the adult. There was a consistent increase in the levels of arginine, glutamic acid and tryptophan in the muscle with age. These changes may be reflected in changes in dietary amino acid requirements, but direct evidence for this is currently lacking.

2.1.2. Protein sources used in supplementary feeds

Several workers have attempted to define optimum protein requirements using diets containing purified protein sources such as vitamin-free casein. It is, however, reported that casein is a poor protein source for Crustacèa (Sick and Andrews, 1973; Lim *et al.*, 1979; Boghen and Castell, 1981; Deshimaru, 1982) and the high protein requirements quoted in some of the above studies may be the result of qualitative inadequacy of the protein source.

New (1976) has recommended the replacement of casein with a more satisfactory reference protein for future nutritional research. Recently, efforts have been directed towards this goal (Boghen *at el.*, 1982;Castell *et al.*,1989; Reed and D'Abramo, 1989; Castell, 1990). Both crab and squid have been suggested as possible sources and methods for the purification of their proteins partially evaluated. Although crab protein has been used in nutritional trials (Sheen and D'Abramo, 1991),a source of purified material sufficient to supply the demands of researchers world-wide is not currently available (Castell, 1990).

Based on origin of the protein sources used for compounding feeds can be broadly divided into plant protein sources and animal protein sources.

2.1.2.1. Plant protein sources

Feed stuffs of vegetable origin are, as a whole, lower in protein content compared to those of animal origin. In addition, the presence of high amuonts of carbohydrates, fibre and other organic molecules such as glucoses, phytates and cyclopropanes in these sources, cause problems to the nutritionist that are generally not encountered with sources of animal origin (Spinelli *et al.*, 1979). Apart from amino acid profile which are often imbalanced, endogenous antinutritional factors limit the use of plant feed stuffs in aquafeeds at high levels (Tacon and Jackson, 1985).

Hanson and Goodwin (1977) reported that most plant proteins yielded poor growth rates in shrimps when used individually excepting a few like soyabean meal (Akiyama,1989), wheat gluten (Desimaru and Shigueno,1972) and peanut meal (Gopal,1986). The improved growth rates produced by some of the plant protein sources have been attributed to their higher polysaccharide contents compared to monosaccharides (Forster and Gabbot, 1971; Kitabhayashi *et al.*,!971; Andrews *et al.*,1972; Sick and Andrews,1973).

Kanazawa *et al.* (1970) reported that soyabean meal is the best protein source among plant proteins for *Penaeus japonicus*. Deshimaru and Shigueno (1972) observed that the amino acid profile of the prawns is similar to that of soyabean to a large degree which may be the reason for higher growth rate in prawns fed with soyabean meal. But when compared to animal sources it is on the lower side. Sick and Andrews (1973) also made the same observation in *Penaeus duorarum*.

Venkataramiah *et al.* (1975) showed that plant material is an essential part of shrimp diet and found that it specifically improves feed conversion efficiency and survival in *Penaeus aztecus*. Zein-Eldin and Corliss (1976) used ricebran and soy flour as the major protein sources for rearing *Penaeus aztecus*. Viola *et al.* (1981) reported that soyabean meal can successfully replace fish meal in the diet of fish. Gopal (1986) has reported higher growth and survival for *Penaeus indicus* juveniles fed on groundnut oil cake based feed. High growth rate and conversion efficiency were reported when mangrove foliage was incorporated in the diet of *Penaeus indicus* juveniles (Sambasivan and Krishnamurthy, 1986). Galgani *et al.* (1988) found that growth of *Penaeus vannamei* was suppressed when the level of soyabean meal in the diet was increased from 0 to 40 %. Lim and Dominy (1990) reported that soyabean meal could be included at upto 43 % of the diet for *Penaeus vannamei*. However, higher levels of incorporation reduced growth rates. Soyabean meal has been used as a protein source in the diet of *Penaeus monodon* by Josekutty and Susheela (1991). But they obtained lower growth rate in comparison to animal protein sources. Unnikrishnan (1992) reported that *Penaeus indicus* exhibited best preference for detritus from *Rhizophora apiculata*, followed by *Chromolaena odorota*, *Pistia stratiotes* and paddy detritus. Briggs *et al.*(1996) showed that diets containing seaweed *Gracilaria* meal upto 10 % inclusion had no significant effect on water stability (after 12 hrs),*Penaeus monodon* juvenile performance or carcass composition compared with control diet lacking seaweed. It is suggested that with the low cost and increasing availablity of *Gracilaria*, it may be a suitable ingredient for low level inclusion into formulated shrimp feeds.

2.1.2.2. Animal protein sources

For the feed formulation of shrimps, it is found that animal protein sources are better than plant protein sources. It is also found that among the animal protein sources, proteins of marine origin are superior than those of fresh water or terrestrial origin, not only due to their amino acid profile but also due to the better composition of their unsaturated fatty acids essential for shrimps (Cowey and Sargent, 1972) as well as higher ash content (Boghen and Castell, 1981). A variety of animal protein sources and the substitution effect of one source with another in the formulated feeds of many species of shrimps and prawns, have been evaluated by various workers.

2.1.2.2.1. Fish meal

Fish meal is one of the most common ingredients used in the commercial shrimp feed, possibly due to its large scale availability. Fish meal is reported to be a good source of protein with essential aminoacids and has high biological value. Fish meal made from good quality whole fish that is properly processed is the best quality protein source commonly available for the preparation of aquafeeds. Fish meal prepared from whole fish contains 60-80 % protein and is a rich source of energy and minerals and is highly digestible and palatable for most species of shrimp. It is high in available lysine and methionine, the two amino acids most deficient in plant feed stuffs. Marine fish meal contains 1-1.25 % n-3 fatty acids, which are essential for most species of shrimps.

But, although fish meal is a high quality protein source for finfishes, it seems to have lower nutritional value for shrimps and prawns,especially when used as a sole protein source. Deshimaru and Shigueno (1972) attributed this to the shortage of phenylalanine and the basic amino acids (arginine, histidine and lysine) in fish meal. Generally, the essential amino acid profile of the protein of the animal's body closely approximates its dietary requirements. Fish meal normally does not provide all the essential amino acids to the required level and is found to be generally poor in threonine, phenylalanine,arginine and histidine (Lovel, 1989).

Deshimaru and Shigueno (1972) observed that diets prepared with fish meal were inferior to short necked clam for *Penaeus japonicus*. Colvin (1976) and Ahmad Ali (1982) obtained poor results with fish meal based diets in *Penaeus indicus*.

Fish meal made from wastes of fish processing and canning plants is lower in quality and quantity in respect to protein. The freshness of raw material and the method of drying affect the quality of fish meal.Vacuum dried and steam dried fish meals are recommended for shrimp feeds (Akiyama *et al.*, 1992). Though fish meal is reported to be a poor source of protein , it is one of the most common ingredients in commercial shrimp feeds at 10-40 % inclusion level (Akiyama *et al.*, 1992). The high cost and variable quality of fish meals combined with localized shortages have led to studies for their replacement with cheaper, more readily available ingredients. Che,Utama; Che,Musa *(1992)* showed that the addition of soyabean meal, paste: shrimp meal,anchovy head meal, brewer's yeast meal and shrimp meal in the diets upto the levels of 45%,60%,34% and 34% respectively to replace fish meal did not give significant difference on survival rate and growth rate of *Penaeus monodon* post larvae.

2.1.2.2.2. Crustaceans

Shrimp meal is another commonly used protein source in the formulation of shrimp feeds. The better results reported with shrimp meal may be due to its similarity in amino acid profile with the body protein of shrimp, although, contradictory results have also been reported.

Sick and Andrews (1973) obtained higher growth and survival in *Penaeus duorarum* fed on diets with shrimp meal. Forster (1976), reported that prawn waste contain several essential amino acids which induce high growth rate in prawns. Joseph and Meyers (1975), reported that prawn head oil contain polyunsaturated fatty acids essential for crustaceans. Pascual and Destajo (1978) reported that shrimp head meal is one of the most promising protein sources in the diet of *Penaeus monodon*. However, the same authors observed slow growth and low survival of post-larvae of *Penaeus monodon* fed with diet prepared exclusively from shrimp head meal and concluded that diet prepared from shrimp head alone is not sufficient to produce good growth and survival.

Shrimp meal as a single protein source has been reported to result poor rate of growth in *Penaeus monodon* (Pascual and Destajo, 1979) and *Penaeus indicus* (Raman *et*

Table. 2 Potential dietary components for inclusion in shrimp and prawn diets as alternative to fish meal

Feedstuff	Reference
Acacia meal	AQUACOP (1976), New (1980b)
Blood meal	Perry and Tarver (1984), Tacon and Jackson (1985)
Cassava	New and Singholka (1982)
Copra (coconut) meal	AQUACOP (1976), Piedad-Pascual (1983), Perry and Tarver (1984)
Corn gluten or meal	Piedad Pascual (1983), Tacon and Jackson (1985)
Corn silage	Moore and Stanley (1982)
Cow pea (de-hulled)	Eusebio (1991)
Earthworm meal	Piedad- Pascual (1983), Garcia (1990)
Groundnut cake	Ravishanker and Keshavanath (1986)
Ipil-ipil leaf meal	Piedad- Pascual (1983), Vogt *et al.* (1986)
Meat and bone meal	Corbin *et al.* (1983), Tacon and Jackson (1985)
Poultry faeces, by-products and feathermeal	New and Singholka (1982), Tacon and Jackson (1985)
Prawn processing waste	New (1980b), Wood (1982)
Rice and rice by-products	New and Singholka (1982), Piedad-Pascual (1983)
Slaughter house waste	Goswami and Goswami (19820
Soybean meal	New (1980b), Corbin *et al.* (1983), Piedad-Pascual (1983), Tacon and Jackson (1985), Akiyama (1989), Piedad-Pascual *et al.* (1990) Lim and Dominy (1990), Lim and Dominy (1992)
Tapioca	New and Singholka (1982)
Mangrove leaves, Chromolaen a leaves and Pistia	Unnikrishnan (1992)

***al*, 1982). The growth promoting effect of shrimp head meal for *P. indicus* may be due to the presence of higher levels of calcium, phosphorus and chitin present in it (Ali, 1982). Commercial shrimp feed usually contain 5-15 % shrimp** meal. Meyers (1981) recommended that not more than 25% shrimp head meal should be included in diets to prevent weakening of the pellets caused by the high ash and fibre levels in the meal. Fox *et al.* (1994) reported that a diet with 31% solar- dried or oven dried shrimp head meal plus sufficient fish meal generated an isoproteinaceous diet which performed significantly better in terms of final weight gain, FCR and production compared with 54% fish meal based diet.

An expensive and locally available potential crustacean source of protein is the stomatopods-*Oratosquilla nepa*, the mantis shrimp. Ali and Mohamed (1985),observed that out of the four combinations of prawn waste and mantis shrimp tried in the formulated feed for *Penaeus indicus*, best growth and feed efficiency were noted with 25% prawn waste and 35% mantis shrimp.

2.1.2.2.3. Molluscs

Fresh clam meat was conventionally used as feed for prawns by Kanazawa *et al.* (1970) who reported that fresh short necked clam *Tapes philippinarum* gave superior growth rates for *Penaeus japonicus* compared to compounded diet. Deshimaru and Shigueno (1972) attributed the superiority of short necked clam to the similarity of its amino acid profile with that of *Penaeus japonicus*. Forster and Beard (1973) obtained similar results in *Palaemon serratus* with fresh mussel meat. Ali (1982) obtained high increase in live weight and good FCR using clam meat powder feed for *Penaeus indicus*. He reported poor performance and heavy mortality with fresh clam meat, *Sunetta scripta* used as control diet for *Penaeus indicus*. Vellegas (1978) obtained that growth and survival of *Penaeus monodon* larvae fed with *Tapes* was only next to that of compounded diet. Josekutty and Susheela (1991) obtained higher percentage weight gain, lowest FCR and 100% survival for *Penaeus monodon* fed on diet containing clam meat as major protein source. Reghunathan and Nair (1993) reported that black clam *Villorita cyprinoides* is a good source of dietary protein for *Penaeus indicus*.

Several studies have demonstrated the high nutritional value of squid meal as a protein source viz., *Penaeus japonicus* (Deshimaru and Shigueno, 1972), *Penaeus monodon* (Cruz-Ricque and AQUACOP, 1987), *Penaeus indicus* (Cruz-Ricque *et al.*, 1987). Che, Utama.;Che, Musa (1992) reported that diet which contained 10% squid head meal gave the best effect on average wet and dry body weight.

Cruz-Ricque *et al.* (1987) reported that supplementation of semi-defined diets with a squid protein fraction resulted in significant increases in the growth of *Penaeus stylirostris* and *Penaeus vannamei*, even with only 1.5 % squid protein in the diet. Cruz-Suarez *et al.* (1987) obtained substantial improvements in weight gain with levels of squid protein upto 6%. They demonstrated that the growth promoting effect of squid protein is due to an increase in metabolic activity and protein synthesis per cell, since it tends to induce hypertrophy of cells and not hyperplasy. Squid meal is also an excellent attractant and levels in commercial feeds usually ranges from 2 to 10 %. Anilkumar (1994) obtained highest growth rate for *M. rosenbergii* with clam meal and squid based diets, and intermediate growth response with squilla and shrimp head based diets.

2.1.2.3. Mixed protein sources

Deshimaru and Shigueno (1972) and Conklin *et al.* (1977) have demonstrated that a mixture of two or more protein sources in the diet show better growth than single protein source. New (1976) reported that relatively higher amount of animal proteins than plant protein sources gave better results in the mixed diets. The improved performance of mixed diet is mainly because of the fact that neither animal protein source nor plant protein source can provide all the essential amino acids in adequate levels. While the plant protein sources are deficient in lysine, the animal protein sources are deficient in sulphur containing amino acids. The deficiency may be overcome by mixing both the sources of protein (Shigueno, 1972).

Zein-Eldin and Corliss (1976) reported that in the mixed diet, animal and plant source ingredients are to be included in correct proportions to obtain better growth and survival of penaeid shrimps, since interactions of various dietary components affect quality or palatability of the feed. Boghen and Castell (1980) reported that mixture of shrimp meal and fish meal were found to meet the protein needs of juvenile lobsters. Colvin and Brand (1977) found that growth performance of *Penaeus californiensis* improved when soyabean protein was included in the diet along with menhaden and shrimp meal. Gopal (1986) reported higher growth rates in *Penaeus indicus* juveniles fed on diets containing both animal and plant protein sources than when they were used individually. Pascual (1988) reported higher growth rate in *Penaeus monodon* juveniles when 10% Ipil-ipil leaf (*Leucaena leucocephala*) meal was incorporated in a diet containing animal protein source. For juvenile *Penaeus esculentus*, a practical type diet containing 40% protein (fish meal,squid meal and soy isolate) was reported to give optimum growth and the most efficient level of muscle protein retention (Hewitt and Irving, 1990; Hewitt, 1992). Piedad-Pascual *et al.* (1990) obtained good growth of *Penaeus monodon* using 55% soyabean meal + 15% shrimp meal. Unnikrishnan (1992) reported that plant detritus could be used for substituting upto 50% of the animal protein source in a standard shrimp diet, while a diet containing 10% plant detritus fared even better than a diet which was totally devoid of detrital protein. Dy-Penaflorida (1995) reported that papaya (*Carica papaya L.*) leaf meal can partially replace (10%) animal protein in shrimp diets and serve as a source of exogenous proteolytic enzyme.

2.1.2.4. Single cell protein sources

Single cell proteins(SCP) are a group of unconventional protein sources, which include a wide range of algae, fungi (including yeast) and bacteria, that can be used in shrimp diets as a supplementary protein source. The greatest research efforts has been centred on the use of marine yeast SCP for feeding aquatic organism. Fisher (1894) separated red and white yeast from the Atlantic ocean (marine yeast) and identified them as *Torula* sp. and *Mycoderma* sp. Kawano and Ohsawa (1971) started the mass culture of marine strains of *Saccharomyces* sp. and tested its practical applications in mariculture. Furukawa *et al.* (1973) demonstrated the utilization of marine yeast for feeding the larvae of *Penaeus japonicus*. Tiews *et al.* (1979) reported that alkane yeast (*Candida lipolitica*) was found to produce equivalent growth to fish meal based ration in rainbow trout. Atack and Matty (1979) found reduced weight gain and feed efficiency for alkane yeast compared to fish meal ration in rainbow trout. A combination of *Turulopsis utilis* and *Endomycopsis fubuliger* (Symba yeast) and mould *Pekilomyces varioti* has been successfully used to replace 50% of the fish meal protein in the diet of Atlantic Salmon (Bergstorm, 1979). Jose and Josekutty (1995) reported that gain in weight obtained with marine yeast based diet was almost comparable to that of prawn meal based diet for *Penaeus monodon* and that marine yeast can be incorporated into penaeid larval feeds and its performance is often better than conventional feeds. High amount of PUFAs, vitamins and minerals present in marine yeast make it an ideal protein source in shrimp feed.

Atack *et al.* (1979) reported that bacterial SCP was found to have higher nutritive value for common carp than alkane yeast SCP, when used as sole source of dietary proteins. It was reported that bacterium *Methylophilus methylotrophus*, methanol bacteria SCP can replace up to 75% of the fish meal protein in salmonid ration (Beck *et al.*,1979; Spinelli *et al.*, 1979; Tacon *et al.*, 1983) and up to 50% of fish meal protein in tilapia production diet (Viola and Arieli, 1984). Stanley and Jones (1976) reported the direct use of dried algal meal in compounded feeds of fish and prawn. Dried algae SCP, in general, has been found to have a lower food value for fish than either yeast SCP, bacterial SCP or fish meal (Atack and Matty,1979). Studies of Hepher *et al.* (1979) with common carp and Appler and Jauncey (1983) with *Oreochromis nilotica* indicate that certain dried algal meals have potential to replace fish meal in practical food rations. Ali (1992) obtained better growth, NPU and Biological Value (BV) in *Penaeus indicus* using Spirulina.

2.1.2.5. Other sources

Silkworm pupae is another dietary source of protein which seems to have potential to be incorporated into shrimp feeds. Most of the studies on utilization of silkworm pupae as dietary protein source has been carried out in fishes. (Jeyachandran and Paulraj, 1976; Jayaram and Shetty, 1981; Nandeesha *et al.*, 1989; Srikanth *et al.*,1989; Mahata *et al.*, 1994). Ravishankar and Keshavanath (1988) reported that the artificial pelleted feed containing silkworm pupae plus shrimp waste (SW) gave the best growth for *M.rosenbergii*. The digestibility of protein from SW was the highest, indicating better utilization of pellet SW by *M. rosenbergii*. Unnikrishnan *et al.* (1991) studied the possibility of utilizing silkworm pupae as a protein source in the feed of *M. rosenbergii*. No significant differences were found either in weight gain or survival rates of prawns reared on silkworm pupae and clam meal diets. The protein efficiency ratios of the two diets were comparable. They reported that protein present in silkworm pupae is as good as that present in clam meal. Ali (1992) reported that feeding *Penaeus indicus* juveniles with silkworm pupae diet resulted in low digestability, NPU, PER and BV, indicating that silkworm pupae is a poor protein source for *Penaeus indicus* diet. He attributed it to the high chitin content of silkworm pupae. Das *et al.* (1995) obtained poor growth performance with silkworm pupae based diet in *M. malcomsonii.*

Various unconventional sources of protein like blood meal, slaughter house wastes, Whale meal, meat and bone meal etc. have been tried by many workers in the formulated feeds with variable success. Goswami and Goswami (1982) reported that good FCR could be obtained by rearing *Penaeus indicus* on diets containing slaughter house waste. It is pointed out that slaughter house waste and meat and bone meal can be cost effectively used as partial substitute to marine protein in shrimp feeds. Josekutty (1991) found that slaughter house waste meal based diet as superior to diets based on prawn meal, soyabean meal and SCP but inferior to clam meal for *Penaeus monodon.* Akiyama *et al.* (1992) reported that blood meal is not commonly used in commercial feeds and when used, the level should not exceed 7% of the diet.

2.1.3. Lipids

2.1.3.1. Fatty acid requirements

Lipids are important in the diet as a source of energy, essential fatty acids, sterols, phospholipids, as carriers of fat - soluble vitamins (Vitamin A,C,D and K) and in lipid transport. A lipid level of below 10% was found to be adequate in shrimp diets (Andrews *et al.*, 1972; Forster and Beard, 1973). New (1976) concluded that high levels of (n-3) fatty acids were beneficial to shrimps. The optimal level of linolenic acid, 18 : 3(n-3) was thought to be 1-2%. It has been confirmed by the use of radiotracers that shrimps are unable to efficiently synthesize 18 : 3(n-3) or 18 : 2(n-6) *de novo* (Kanazawa *et al.*, 1979a., Kanazawa *et al.*, 1979b). It has been demonstrated that penaeids have a limited ability to elongate and desaturate 18 : 3(n-3) and 18 : 2(n-6) to longer chain PUFAs (Kanazawa *et al.*, 1979d; Kayama *et al.*,1980). Colvin (1976) reported that when *Penaeus indicus* were fed on diets containing seed oils, rich in 18 : 2(n-6) or 18 : 3(n-3), these fatty acids were deposited from diet into body tissues. Read (1981) found that although addition of 2% 18 : 3(n-3) or 18 : 2(n-6) to a purified diet promoted the growth of *Penaeus indicus*, addition of fish oil containing (n-3) HUFAs had a greater effect. Catacutan (1991a) also found that fatty acid profiles of *Penaeus monodon* fed various lipid sources tended to reflect the dietary input.

The growth of *Penaeus japonicus* was enhanced when compounded diets were supplemented with oils containing 18 : 3(n-3), 20 : 5(n-3) and 22 : 6(n-3) (Guary *et al.*, 1976). Using casein based diets, Kanazawa *et al.* (1977) studied the effect of supplementing 18 : 2(n-6) and 18 : 3(n-3) on the growth of *Penaeus japonicus*. Weight gain was enhanced by a diet containing 1% 18 : 2(n-6) or 1% 18 : 3(n-3), the latter being slightly more effective. The best growth was attained with a diet containing 5% pollack residual oil.

Table. 3 Fatty acid profiles (as % of total lipid) from shrimp and prawns

	Marine			Freshwater	
	Penaeus aztecus wild ,whole	*Penaeus indicus* wild	*Penaeus japonicus* wild, whole	*Macrobrachium rosenbergii* cultivated, whole	
Reference	1	2	3	4	5
Fatty acid					
13:0	-	-	0.2	-	-
14:0	-	1.1	2.4	1.1	-
14:1	-	-	0.5	-	-
15:0	-	-	2.6	-	-
15:1	-	-	0.5	-	-
16:0	17.6	15.5	15.4	15.2	26.0
16:1	13.5	7.5	6.9	1.3	6.4
16 PUFA	-	-	1.7	-	-
17:0	-	2.2	2.0	-	-
17:1	-	0.9	-	-	-
18:0	9.3	8.2	6.5	8.3	9.8
18:1 n-9	14.9	12.8	9.0	19.7	28.8
18:2 n-6	2.9	4.3	2.0	24.2	16.3
18:3 n-6	2.6	-	-	-	0.7
18:3 n-3	1.5	1.0	0.4	2.0	1.9
18:4 n-3	-	-	2.0	-	-
20:1 n-9	-	1.4	7.9	-	-
20:2 n-6	1.7	-	1.2	1.6	1.0
20:3 n-6	-	-	0.8	-	0.1
20:4 n-6	6.4	8.7	3.3	3.2	2.7
20:4 n-3	-	-	1.8	-	-
20:5 n-3	15.5	11.2	13.1	12.1	3.7
22:1 n-11	-	1.1	-	-	-
22:3 n-6	-	-	0.4	-	-
22:4 n-6	0.8	-	2.2	-	0.2
22:5 n-6	1.2	-	-	-	0.2
22:5 n-3	1.5	1.9	3.0	-	0.2
22:6 n-3	10.3	11.0	7.6	5.0	2.1
24:1	-	-	-	-	-
24:4?	-	-	4.0	-	-
24:5?	-	-	0.3	-	-
Total n-3	28.8	11.2	27.9	19.1	7.9
Total n-6	13.0	8.7	9.9	29.0	20.4
n-3/n-6	2.2	1.3	2.8	0.7	0.4

1 Chanmugan *et al.* (1983), 2 Read (1981), 3 Guary *et al.* (1974), 4 Sandifer and Joseph (1976), 5 Chanmugan *et al.* (1983).

In several trials the best growth and survival occurred with diets containing pollack liver oil or short necked clam lipids suggesting that a range of fatty acids are required for optimum growth (Kanazawa *et al.*, 1977a; Kanazawa *et al.* 1977b; Kanazawa *et al.* 1979d; Kanazawa *et al.*, 1979f). Deshimaru *et al.* (1979) concluded that a mixture of pollack liver oil and soyabean oil, in a ratio between 1:1, produced optimum growth in *Penaeus japonicus* juveniles when incorporated at 6% into a casein / egg albumin based diet.

Crustacean requirements for fatty acids of the (n-6) series have not been studied in depth. Dietary inputs of 20:4 (n-6) are the major source of this fatty acid in shrimp since it has been shown that the level of *de novo* synthesis of this fatty acid in *Penaeus setiferus* is low (Lilly and Bottino, 1981). Analysis of several shrimp and prawn species indicates that their tissue lipids, especially phospholipids, contain relatively higher levels of 20:4 (n-6) eg. 11-14% in muscle polar lipid from *Penaeus monodon* (O'Leary and Matthews, 1990).

2.1.3.2. Dietary lipid levels

The inclusion of lipids in diets for prawns and shrimp is designed to satisfy their essential fatty acid requirements and to supply energy. Deshimaru *et al.* (1979) found that 6% dietary lipid, comprising pollack liver oil and soyabean oil in a 1:1 ratio, was optimal for juvenile *Penaeus japonicus*. Read (1981) showed that the growth of juvenile *Penaeus indicus* were enhanced when a commercial type ration (containing 2.9% lipid) was supplemented with 3% of a mixture of sunflower and fish oil (2:1 by volume). Deshimaru

Table 4 Some references reporting suitable lipid sources and levels of inclusion in diets for various shrimp and prawn species

Species	Lipid source	Level (%)	Reference
M. rosenbergii	Commercial ration + shrimp head oil	10.3	Sandifer and Joseph (1976)
	Neutral lipid	5 - 7	Biddle *et al.* (1977)
	Menhaden oil	8.7 - 9.8	Clifford and Brick (1978, 1979)
	Menhaden + Corn oil	10.5 - 11.6	Millikin *et al.* (1980)
	Commercial ration	3 - 10	Corbin *et al.* (1983)
	Capelin oil + Lecithin	14.7	Hilton *et al.* (1984)
	Cod liver oil + corn oil (2:1)	2 - 10	Sheen and D' Abramo (1991)
Penaeids	Total	6 - 7	NRC (1983)
P. japonicus	Shrimp and fish meal + pollock liver oil and soybean oil	8.8	Deshimaru and Shigeno (1972)
	Cod liver oil and soybean oil	6.0	Deshimaru and Kuroki (1974)
	Sardine or clam oil	4.0	Guary *et al.* (1976a)
	Tapes lipid	8.0	Kanazawa *et al.* (1977b0
	Pollock liver oil and soybean oil (1 : 1)	6.0	Deshimaru *et al.* (1979)
	Pollock liver oil and lecithin	8.0	Kanazawa *et al.* (1979e)
P. monodon	Cod liver oil	7.0	Alava and Lim (1983)
		4.3 - 10.5	Deshimaru *et al.* (1985)
		12.0	Catacutan (1991a)
P. merguiensis	Cod liver oil	7.4	Sedgwick (1979b)
P. indicus	Fish meal + sunflower oil	5.0	Colvin (1976b)
	Commercial ration + sunflower and fish oil	5.9	Read (1981)
Metapenaeus monoceros	Pollock liver oil	8.5	Kanazawa *et al.* (1981)

et al. (1985) reported that there was no relationship between total dietary lipid level (from 4.3-10.5%) and growth in *Penaeus monodon.* A wide range of dietary lipid levels (2-10% of cod liver oil / corn oil 2:1 W/V) were satisfactory for juvenile *M. rosenbergii*, but growth was poor on a lipid free diet (Sheen and D'Abramo, 1991).

Data on quantitative lipid requirements of shrimps are complicated by the fact that growth is affected by the fatty acid composition of the diet, its protein : lipid : carbohydrate balance and to some extend the lipid classes present in the feed. Excessive dietary lipid has an adverse effect on prawn and shrimp growth and survival (Bautista, 1986). Lipid contents of juvenile and larval shrimps are often elevated (O'Leary and Matthews, 1990; Clark *et al.*, 1990). The inability of shrimps to tolerate high levels of dietary lipid is probably a reflection of the low lipid levels in their natural diet (Dall *et al.*, 1991) and the low lipid levels found in their tissues.

2.1.3.3. Sterols

Sterol metabolism in Crustacea has been reviewed by Teshima (1982). Teshima *et al.* (1983) reported that ergosterol and 24-methlenecholesterol were almost as effective as cholesterol in promoting the growth and survival of *Penaeus japonicus* larvae.Survival was reduced when dietary cholesterol level was increased to 5%. Despite the presence of relatively large amounts of sterols (cholesterol) in crustacean tissues *(Penaeus esculentus,* total lipid from abdominal muscle contains 23% cholesterol (Chandumpai *et al.*, 1991)) they are incapable of *de novo* synthesis of sterols.

The optimal cholesterol level for *Penaeus kerathurus* was 1% in terms of a combined consideration of growth and mortality (Bianchini,1985) and for *Penaeus pencillatus* the optimum was 0.5% (Chen and Jenn,1991). Briggs *et al.*(1988) found that there was no advantage in supplementing cholesterol above the residual 0.12% level of a semi-purified diet when feeding juvenile *M. rosenbergii*. Teshima *et al.* (1989) found that weight gain and feed conversion efficiency of *Penaeus japonicus* were reduced when cholesterol was partially replaced with sitosterol (to give total dietary sterol levels of 0.5%). Mahesh (1996) reported that supplimentation of lecithin at a level of 2.5% in the diet can accelerate growth and improve FCR during the early post larval phase of *M. rosenbergii.*

2.1.4. Carbohydrates

In shrimps, carbohydrates are important in energy production, chitin synthesis and non-essential fatty acid synthesis. In shrimp diets, carbohydrates are useful for their energy value, protein sparing function and also for their binding properties. New (1976) concluded that polysaccharides or disaccharides were utilised more efficiently by shrimps than monosaccharides. In addition, starches have superior binding qualities compared with monosaccharides, thus improving stability. Several studies have demonstrated the adverse effects of including glucose at levels of more than 10% in shrimp diets (Deshimaru and Yone, 1978; Abdel-Rahman *et al.,* 1979; Alava and Pascul, 1987; Diaz and Nakagawa, 1990). Abdel Rahman *et al.* (1979) found that the best growth rates for juvenile *P. japonicus* were obtained with diets containing 19.5% maltose followed by 19.5% sucrose.

Ali (1982) reported that optimum level of dietary carbohydrate appeared to be greater than 40% but the best feed conversion ratio was with 30% starch. Alava and Pascual (1987) found that diets containing trehalose and sucrose performed better for *P. monodon* than those fed glucose and a dietary sugar level of 20% appeared to be optimal. For *P. monodon* juveniles, varying the level of bread flour between 0% and 35% in isonitrogenous, practical type diets did not significantly enhance growth (Catacutan, 1991). Anilkumar (1994) found that protein level in the diet of *M. rosenbegrii* could be lowered from 35% to 30% by increasing the carbohydrate level from 20% to 30%. Ali (1996) reported that shrimps (*P. indicus*) are capable of handling significant quantities of dietary carbohydrate and have a preference for carbohydrate as energy source in the diet over lipid at a given protein level and this indicates the importance of dietary carbohydrate in reducing cost of practical feeds.

2.1.4.1. Fibre

Little information is available on the effects of fibre in shrimp diets. Several studies have noted that the inclusion of cellulose as a non-digestible filler,can reduce the palatability of the diet. However, Fair *et al* (1980) reported that there was no detrimental effect in including upto 30% cellulose in diets for *M. rosenbergii* and that levels of 5% and 20% actually stimulated growth. In contrast, Diaz *et al.* (1988) found that *Macrobrachium* fed with a diet containing 20% cellulose exhibited poor growth and depressed locomotive activity. Enhanced survival of *P. monodon* was obtained when fed with *Nagas graminea* as opposed to commercial pellet (Primavera and Gacutan, 1989). A combination of plant and animal material enhanced growth when compared with those fed on animal based feed alone (McTigue and Zimmerman, 1991). Chitin may be present in commerical shrimp rations as a consequence of including shrimp meal in the diet. In laboratory trials, juvenile *P. monodon* did not appear to be able to utilize dietary chitin (Fox, 1993). Chitin (0.5%) or its precursor glucosamine (0.8%) when included in the diet, has shown to improve growth and feed efficiency in shrimps. The inclusion of high levels (>25%) of shrimp meal in pellets will also reduce pellet stability and elevate ash content (Meyers, 1981). Vinod and Susheela (1997) incorporated chitin and its derivative chitosan and glucosamine at levels of 0.25, 0.5, 0.75 and 1 mg per 100g in the diets of *P. monodon* and found that glucosamine at 28 mg/100g diet gave maximum growth rate.

2.1.5. Vitamins

New (1976) stated that few qualitative but no quantitative studies have been carried out on the vitamin requirements of prawns and shrimps but that it was a normal practise to add vitamin supplements to shrimp rations on the assumption that they are required. Of the 15 vitamins listed by the National Research Council (NRC) as routine additives for diets (NRC, 1983), the quantitative requirements for only five have been assessed in shrimps.

Deshimaru and Kuroki (1976) reported that inclusion of ascorbic acid in a purified diet for *P. japonicus* led to low growth and high mortality. In contrast, Lightner *et al.* (1977 , 1979) and Magarelli *et al.* (1979) identified a dietary requirement of 0.1% ascorbic acid in purified diets for *P. californiensis* and *P. stylirostris*. Kanazawa *et al.* (1976) found that there was definite requirement for choline chloride of 600mg/kg diet. Deshimaru and Kuroki (1979) estimated the dietary thiamin requirement of *P. japonicus* to be between 60 and 120 mg/kg diet. Deshimaru and Kuroki (1979) reported that choline supplementation was not essential in diets for *P. japonicus* but all the shrimp in this trial exhibited poor growth. Shigueno and Itoh (1988) reported that Mg-L-ascorbyl-2-phosphate can be used as an effective dietary source of vitamin C. Chen *et al.* (1991) recommended 13-14 mg/kg diet of thiamin for *P. monodon*. Shiau (1994) found that optimum dietary niacin requirement for maximum growth of *P. monodon* is 7.18 mg/kg diet. It is a common practice to include a vitamin pre-mix in shrimp diets (Akiyama and Dominy, 1989). NRC (1983) indicated that excess levels should be incorporated since losses due to leaching, particularly of the water soluble vitamins, and oxidation may occur. Trino *et al.* (1992) reported that although growth was reduced for *P. monodon* post-larvae fed with vitamin deficient diet, the reductions were not statistically significant. Piedad-Pascual and Catacutan (1990) found that suvival of juvenile *P. monodon* was significantly reduced if vitamin and mineral supplements were not added to the diet. Utama-Che-Musa-Che (1991) obtained the highest average wet body weight for *P. monodon* post-larvae fed with diets added with 2.2% vitamin C and also with 0.5% choline chloride. Lawrence *et al.* (1993) estimated vitamin E requirement of *P. vannamei* based on growth as 99 mg/kg diet. They found that vitamin E was an effective anti-oxidant and levels of 25 and 100 mg/kg diet were required for suppressing ascorbic acid stimulated lipid peroxidation and growth reduction of shrimp resulting from vitamin E deficiency. Shiau *et al.* (1994) reported that optimum dietary vitamin K requirement for *P. chinensis* is about 185 mg/kg diet. Gijo (1996) reported that growth, survival, FCR, moulting rate and whole body ascorbic acid content were significantly affected by dietary vitamin C in *M. rosenbergii* and the optimum dietary level was found to be 150 mg CVC-F90 (a hydrogenated vegetable oil form of vitamin C) per kg dry diet.

2.1.6. Minerals

New (1976) reported that relatively little work had been conducted on the mineral requirements of prawns and shrimps. It was recommended to include a mineral premix in the diets for both research and commercial rearing, since it provides a source of potassium, calcium, phoshorus, chloride, magnesium, iron and manganese.

The utilisation of minerals is especially important for Crustacea because of their role in strengthening the exoskeleton (Conklin, 1982). The mineral content of the carapace can be affected by the levels of minerals in the diet, as demonstrated by Davis *et al.* (1992).

Huner and Colvin (1977) examined the effect of rearing *P. californiensis* on diets with varied calcium : phosphorus ratios. Shrimps fed diets with a Ca/P ratio greater than 2.42:1 exhibited poor growth. Kanazawa *et al.* (1984) found that the requirement of calcium and phosphorus by juvenile *P. japonicus* was inter-related. The best growth was achieved on a diet containing 1-2% Ca/P in the ratio of 1:1.

Table 5 Vitamin requirements of shrimps

Vitamin	Species	Dietary level (mg/ kg diet)	Notes	Reference
Thiamin (Vit. B_1)	*P. japonicus*	60	Based on growth	Deshimaru and Kuroki (1979)
		120	Based on body thiamin content	Deshimaru and Kuroki (1979)
	P. monodon	14	Based on haemolymph thiamin content	Chen *et al.* (1991)
Pyridoxine (Vit B_6)	*P. japonicus*	120	Based on growth and body pyridoxine content	Deshimaru and Kuroki (1979)
Choline.	*P. japonicus*	600	Based on growth	Kanazawa *et al.* (1976c)
	P. japonicus	<150	Growth of all test animals were poor	Deshimaru and Kuroki (1979)
Inositol.	*P. japonicus*	>4000	Deficiency caused high mortality	Deshimaru and Kuroki (1976)
	P.japonicus	2000	Based on growth	Kanazawa *et al.* (1976c)
Ascorbic acid (Vit C)	*P. japonicus*	0	Inclusion led to poor growth and high mortality	Deshimaru and Kuroki (1976)
	P. japonicus	10000	Based on growth	Guary *et al.* (1976b)
	P. stylirostris	1000	Deficiency led to' Black death' syndrome	Lightner *et al.* (1979)
	P.californiensis			Magarelli *et al.* (1979)

Dietary requirement for the following vitamins have not been investigated for shrimps and prawns:
Riboflavin (Vit B_2), pantothenic acid, nicotinic acid (niacin), biotin, folic acid, cyanocobalamin (Vit B_{12}),retinol (Vit A_1), cholecalciferol (Vit D_3) and menadione (Vit K_2) :
Phylloquinone (Vit K_1) may be antagonistic to some crustacean species whilst Tocopherols (Vit E) are normally included in diets as antioxidants.

Supplemental magnesium has been reported to be undesirable for juvenile *P. japonicus* (Deshimaru and Yone, 1978). In contrast, Kanazawa *et al.* (1984) recommended that supplementary magnesium should be supplied at 0.1-0.5%. The copper requirement of juvenile *P. vannamei* has been found to be satisfied at 34 mg/kg diet. Kanazawa *et al.* (1984) reported that the addition of copper and manganese had little effect on growth. Copper deficient diets caused reduced growth, enlarged hearts and depressed copper levels in the haemolymph, carapace and midgut gland (Davis *et al.*, 1993). Zhao *et al.* (1993) reported that the optimum content of $CuSO_4$ in fish meal - soybean meal diets was 20 mg/kg and the survival rate and growth rate decreased with the increase in copper additions. For *P. japonicus* the addition of iron above 0.014% reduced growth (Kanazawa *et al.*, 1984). The non-essentiality of supplemental iron has been further confirmed for *P. vannamei* by Davis *et al.* (1992). A dietary potassium level of 2% or between 0.9% and 1.8% has been recommended for shrimps (Deshimaru and Yone, 1978; Kanazawa *et al.*, 1984). The active accumulation of calcium from sea-water by penaeids has been demonstrated, but the uptake of other minerals by shrimp has not been investigated (Deshimaru *et al.*, 1978; Dall and Smith, 1981). Li *et al.* (1995) reported that the highest increase in body weight and digestibility was seen in *P. chinensis* fed with diets added with carbosan ($30x10^{-6}$) and methionine mineral chelate ($30x10^{-6}$) in organic forms. Saju (1996) reported that phosphorus level in the diet and Ca:P ratio significantly influenced FCR, PER, whole body phosphorus and whole body calcium content of *M. rosenbergii* and he suggested an optimum phosphorus level of 1.8% for *M. rosenbergii*.

2.1.7. Pigments

The occurrence of carotenoid pigments and the biochemical pathways involved in their metabolism have been reviewed by Goodwin (1984), Davies (1985) and Matsumo and Hirao (1989). Astaxanthin is usually the predominant carotenoid present in shrimp tissues. In wild *P. japonicus* this pigment contributed 54.1-60.8% of the total carotenoids, but significant amounts of phoenicoxanthin (16.5-22.9%) and ß-carotene (20.0-22.0%) were also detected (Ha *et al.*, 1985).

In *P. japonicus*, astaxanthin may be synthesised *in vivo* from dietary ß-carotene and other carotenoid pigments (Tanaka *et al.*, 1976). Whether the carotenoids have metabolic role other than their chromatic role is unclear (Tacon, 1981; Ghidalia, 1985; Bendich, 1989). Yamada *et al.* (1990) demonstrated that the inclusion of astaxanthin in shrimp diets led to better pigmentation of the animals. The incorporation of shrimp meal or oil can provide a good dietary source of astaxanthin. Certain commercial diets also include canthaxanthin since this carotenoid pigment is a registered feed additive. It has been noted that shrimps reared in semi-intensive systems, with access to benthic algae, frequently show more colouration than those reared solely on commercial rations (Howell and Matthews, 1991). In an alternative strategy to supplement artificial diets with algae, it has been found to be effective when diet of *P. monodon* was supplemented at 3% level with *Spirulina* for one month prior to harvest. *Spirulina,* a blue-green algae, available commercially in dried form, contains significant quantities of zeaxanthin which can be converted to astaxanthin by the shrimp (Liao *et al.*, 1993).

2.1.8. Palatability, feed attractants and anti-nutritional factors.

New (1976) stressed the importance of palatability and the physical structure of diets for prawns and shrimps. It was concluded that moist diets were in general preferable to dry pellets and the role of attractants in increasing feed consumption was noted. For practical diet formulation, the use of locally available binders is recommended. Goswami and Goswami (1979, 1982) found that wheat flour at 35% is the most suitable binder, whilst gum acacia and gram flour led to pellets which rapidly disintegrated in water. The stability of pellets may also vary in relation to the non-binding ingredients. Lim and Dominy (1990) reported that the water stability of pellets was inversely related to the level of soybean meal they contained, despite inclusion of binders.

Supplementary feed attractants and stimulants may be incorporated into shrimp and prawn rations. Attractants and stimulants are generally low molecular weight compounds such as L-amino acids, nucleotides or quarternary ammonium compounds. Deshimaru and Yone (1978) found that food intake in *P. japonicus* was stimulated by the addition of 1.5% glycine, an amino acid mixture based on clam extract, taurine, alanine or serine, in decreasing order of effectiveness. Murai *et al.* (1981) stated that supplementation of a compounded diet with 2% glycine or with mussel extract significantly increased its attractability for *P. monodon*. For *M. rosenbergii* behavioural responses were noted to taurine, glycine, arginine, betaine and trimethylamine, when the chemical concentration was between 10^{-5} and 10^{-8} M (Harpaz *et al.*, 1987). Trimethylamine hydrochloride has also been reported to be effective as an attractant (Costa- Pierce and Laws, 1985; Diaz and

Nakagawa, 1990). Several commercial attractants available now may enhance feed utilization when included in the diet at 1 or 2% (Ung and Junilla,1989). Betaine is a powerful attractant and inducer of feeding behaviour in *M. rosenbergii* at levels as low as $1x10^{-4}$ M (Harpaz and Steiner, 1990). Hartai *et al.* (1993) reported that feed attractants, taurine, yeast extract and a mixture of amino acids designed to mimic a clam extract promoted good growth performance in *P. monodon.* Dy Penaflorida (1996) reported that *P. monodon* fed with soyabean meal enhanced with 1% Finn Stim (FS), a chemo attractant had a significantly higher weight gain, as it may have caused faster feed consumption resulting in less feed disintegration and nutrient loss. It could also have acted as a stimulant and counteracted the palatability problem.

NRC (1983) refers to 19 anti-nutritional factors which occur in feed stuffs commonly used in aquaculture. Soyabean meal may contain phytic acid and trypsin inhibitors, linseed meal contains a pyridoxine antagonist and cottonseed meal contains gossypol. In addition, man-made contaminants and heavy metals may cause problems. Anti-nutritional factors can often be inactivated or removed by cooking or processing the raw material. Eusebio (1991) found that dehulling leguminous seeds improved their digestibility for shrimp by reducing the levels of protein inhibitors. The leaves of ipil-ipil (*Leucaena leucocephala*) contain a toxic amino acid mimosine. Araneda(1990) showed that shrimps fed with *L. leucocephala* meal showed a lower growth and higher FCR than the control diet. However, soaking the leaves in water for 24 hrs. will remove up to 70% of this amino acid and it can be incorporated into shrimp diets (Vogt *et al.*, 1986). Vitamin E (alpha tocopherol) or a synthetic anti- oxidant such as butylated hydroxy-toluene or butylated hydroxyanisole can be included with the lipid supplement in compounded diets to reduce auto oxidation (NRC, 1983).

Substances like hormones, antibiotics etc are added to shrimp diets to promote growth of shrimps. Sambhu and Jayaprakas (1994) reported that *P. indicus* juveniles fed with hormone diets, testosterone propionate (TP), diethyl stilbesterol (DES) and human chorionic gonadotropin (HCG) showed better growth than control diet, except in case of HCG (20ppm) which inhibited growth. The diet containing a combination of HCG (10ppm) and TP (1.5ppm) showed superior growth, better feed consumption, FCR, assimilation, protein and lipid digestibility. Cruz-Suarez *et al.* (1993) reported that shrimp fed Virginiamycin 80, 100 and 200 ppm showed better growth rates than those fed the control and 50ppm. Boby and Susheela (1994) reported superior growth of *M.rosenbergii* when antibiotic, oxytetracycline was incorporated in the diet at 10 mg / 100 g. Mustafa and Nakagawa (1995) reported that algae meal used as an additive in fish feed raised the growth rate and feed efficiency and improved protein assimilation, lipid metabolism, liver function stress response, disease resistance and carcass quality and this indicate that algae might be essential for efficacious utilization of nutrients in fish. Galindo Reyes *et al.* (1995) reported that the results obtained when insulin-like peptides isolated from hepatopancreas of lobster *Panulirus gracilis*, as a growth factor for juvenile *P. vannamei* indicate that shrimp fed with diets supplemented with these compounds improve weight gain, with respect to animals given the same diets supplemented with human insulin or with out supplement.

3. MATERIALS AND METHODS

The present study is aimed at finding the growth and survival of *Penaeus monodon* juveniles fed with diet at different levels of replacement of clam meat with de-oiled silkworm pupae. The experiment was carried out at the College of Fisheries, Panangad for a period of 6 weeks from 25-05-1997 to 09-07-1997.

3.1. Experimental rearing facilities

The experiment was conducted in the wet laboratory of Department of Aquaculture,College of Fisheries, Panangad. Circular flat bottom fibre glass tanks of capacity 83 litres, diameter 55 cm, height 35 cm, rim width 3 cm, thickness 4 mm and colour aquamarine were used for stocking the experimental animals.

The tanks were kept inside the wet laboratory which had provision for subdued light penetration. Brackish water was pumped from the backwaters behind the college campus. The salinity was adjusted to 20 ppt. This water was filtered twice using bolting silk and was stored in four fibre reinforced plastic (FRP) tanks, each having 2 ton capacity. This water was used to fill the experimental tanks upto 3/4th level. Gentle aeration was given throughout the experimental period in the tanks via diffuser stones from a Roots air blower and was maintained uniformly in all the tanks by means of control valves.

Uniform sized earthern tiles were provided as artificial substrata in each tank to reduce cannibalism among prawns. The tiles were placed in a slanting position over small piece of stones.

3.2. Experimental animals

Penaeus monodon juveniles were procured from West Coast Hatchery, Anthakaranazhi, Cherthala. They were transported to the wet laboratory in oxygen filled polythylene bags of 8 litre capacity, three fourth with fresh filtered seawater of 25 ppt salinity, under minimum stress. In the laboratory they were first acclimatised to saline water of 20 ppt and then they were stocked in 1 ton FRP tanks filled with 20 ppt saline water and provided with gentle aeration. The shrimp juveniles were acclimatised to tray feeding. During this period the shrimp juveniles were fed with raw clam meat.

3.3. Preparation of experimental diets

3.3.1. Feed ingredients

The main feed ingredients used were clam meat, silkworm pupae, groundnut oil cake, tapioca powder, cholesterol, starch, cellulose, salt mixture and vitamin mixture. Among these, clam meat, groundnut oil cake and tapioca powder were procured from local market. Silkworm pupae was obtained from 'Sahredaya Silk Centre', Angamaly. Minerals were supplemented through salt mixture USP IX (SISCO RESEARCH LABORATORIES PVT. LTD, BOMBAY). Vitamins were supplemented through Supplevite-M (SARABHAI CHEMICALS, BOMBAY). Cholesterol, potato starch and cellulose were obtained from the Department of Processing Technology, College of Fisheries.

Protein sources used in the diets were clam meat and/or de-oiled silkworm pupae. Bages and Sloane (1981) identified the protein requirement of *P. monodon* to be 35%. Nezaki *et al* (1986) reported that when carbohydrate content is increased to 25%, a 45% protein diet gave the results comparable to those diets containing 55% protein. Shiau and Chou (1991) recommended 36-40% protein level in the diet of *P.* monodon. Hence in the present study isonitrogenous diets containing 35% protein were prepared.

Catacutan and Kanazawa (1985) showed that 10 to 11% lipid in the diet gave best growth in *Penaeus monodon* juveniles. As clam meat contains 10.8% fat (Josekutty *et al.*,1991) and de-oiled silkworm pupae contains 7.07 % fat (Srikanth *et al.*,1986), no extra fat was added in the diets.

Penaeid prawns generally utilise disaccharides and polysaccharides better than monosaccharides (Cowey and Forster, 1971; Forster and Gabbot, 1971; Sick and Andrews, 1973; Ahmad Ali, 1982; Alava and Pascual, 1987). As potato starch is available in pure form and is a good source of carbohydrate it was incorporated in the diets.

Crustaceans require cholosterol for their normal growth and survival (Castell *et al.*, 1975) and are incapable of *de novo* synthesis of cholosterol from simple sugars (Teshima and Kanazawa,1971;Kanazawa *et al.*,1971). Hence cholosterol was incorporated in the diets at 1% level.

Vitamins and minerals are important for regulating body processes in shrimps. Vitamin B is necessary for proper utilization of proteins, carbohydrates and fats, while vitamin A and C are important in building resistance to infection. Vitamin D together with minerals calcium and phosphorus is necessary for the formation of exoskeleton (Fisher,1960; Kanazawa *et al.*,1970; Kitayama *et al.*, 1971; Forster and Gabbot, 1971; Forster and Beard,1973; Balaz s *et al.*,1974). Calcium and phosphorous rations are important in the diets of *Penaeus monodon* and found a 1:1 ratio to be effective in hardening of the exoskelton and preventing soft shell disease. Appropiate levels of vitamins and minerals were incorporated in the diets. Tapioca starch was used as the binder in the preparation of pelleted feeds.

Clam meal was prepared by drying raw clam meat at 60°C for overnight and the dried clam meat was finely powdered and sieved. The raw silkworm pupae was put in boiled water for 20 minutes and pressed in a screw press to remove excess fat so as to obtain de-oiled silkworm pupae, as silkworm pupae contain 35% fat (Unnikrishnan *et al.*,1991) which is not desirable in shrimp feeds. All the ingredients were finely powdered, sieved through a 250 μ sieve and stored in air tight containers.

3.3.2. Proximate composition of feed ingredients

Proximate composition of feed ingredients was analysed prior to feed formulation.

The moisture level was estimated by heating the sample to 105°C for 30 minutes and then drying at 65° C till a constant weight was obtained, following the method of Boyd (1979). The crude protein content was estimated by Microkjeldahl's method (AOAC,1984). The nitrogen content was multiplied by the factor 6.25, to arrive at crude protein content. Crude fat was extracted using petroleum ether (B.P.40^{0}C to 60°C) in a Soxhlet extraction apparatus for 16 hours. The ash content was determined by burning the sample at 550°C + 10°C for 6 hours in a muffle furnace. The carbohydrate content (nitrogen free extract, NFE) was found out by Hastings (1976) difference method.

NFE = 100 - (% crude protein on dry weight basis + % crude fat on dry weight basis + % ash)

3.3.3. Formulation and processing of test diets

Six types of isonitrogenous pelleted feeds were formulated maintaining their protein level at 35%.

Table. 6. Proportion of ingredients (clam meal and de-oiled silkworm pupae) used for the preparation of different pelleted feeds

Ingredients	Feeds 1	2	3	4	5	6
Clam meal (%)	100	80	60	40	20	0
Silkworm pupae (%)	0	20	40	60	80	100

Table 6 gives the proportion of ingredients (clam meal and de-oiled silkworm pupae) used for the preparation of different pelleted feeds. Each type of pelleted feed was prepared by mixing separately accurately weighed quantity of ingredients. The mixture was hand kneaded with sufficient water (1:1.25 W/V) to get soft dough. The dough was cooked for 30 minutes in an autoclave at ambient pressure. The cooked dough was cooled, salt and vitamin mixture were then added, mixed, pelletized through a hand pelletiser having a die of 3mm diameter. The extruded pellets were collected in an enamel tray and dried at 60°C overnight in a hot air oven to a moisture level less than 10%. The pellets were then broken into small pieces, packed in air tight containers separately and stored at 4°C in a refrigerator until use.

3.3.4. Proximate composition of formulated feeds.

The proximate analysis of formulated feeds were carried out immediately after

Table 7 Percentage composition of different ingredients used in the preparation of pelleted feeds.

Ingredients	Feeds (g/100g feed)					
	1	2	3	4	5	6
Clam meat	49.06	36.79	32.77	16.38	12.26	0
Silkworm pupae (de-oiled)	0	11.40	15.23	30.47	34.21	45.61
Groundnut oil cake	20.00	20.00	20.00	20.00	20.00	20.00
Tapioca	10.00	10.00	10.00	10.00	10.00	10.00
Starch	12.44	13.31	13.50	14.65	15.03	15.89
Cellulose	2.00	2.00	2.00	2.00	2.00	2.00
Cholesterol	1.00	1.00	1.00	1.00	1.00	1.00
Mineral mix *	2.50	2.50	2.50	2.50	2.50	2.50
Vitamin mix **	3.00	3.00	3.00	3.00	3.00	3.00

* Mineral mix (Salt mixture USP IX - SISCO Research Lab. Pvt. Ltd., Bombay)

** Vitamin mix (Supplevite - M , Sarabhai Chemicals , Bombay) their preparation. Methodology employed was the same as that of ingredients.

3.4. Water stability

(% dry matter obtained after exposing pellets in water for 6 hours) was determined by the method of Jayaram and Shetty (1981).

3.5. Experimental design and procedure

The shrimp juveniles were starved for a day prior to the experiment. Healthy, well pigmented, active and uniform sized juveniles were collected randomly from the nursery tanks where they were initially stocked and each of them was blotted dry carefully between the folds of filter paper and weighed individually in an electronic balance to the nearest milligram. The initial weight of the shrimp juveniles ranged from 0.1616 to 0.2084 g. Ten numbers of weighed juveniles were then transferred to each tank selected at random.

For each treatment of feed three replicates were maintained. So in the total, eighteen, circular fibre glass tanks of 83 litre capacity, each with ten numbers of animals were used for the study. Treatments were allocated to each experimental unit by random allocation method following completely randomized design technique. The duration of the experiment was 6 weeks.

The shrimps were provided with the feed *ad libitum* daily in the evening in petridishes kept at the bottom of the tank close to the substratum provided. The size of the feed was adjusted according to the size of the animal. Every day before feeding, left over feed was recovered gently and petridishes cleaned thoroughly. The recovered left over feed was dried in an oven at 60°C to a constant weight. During morning hours bottom and sides of the tanks were scrubbed well to prevent algal growth and excreta accumulation. Dead animals found were removed and weighed. The water in the rearing tank was exchanged twice in a week to maintain water quality. During the experimental period shrimps were subjected to growth assessment every fortnight. At the end of the feeding study shrimps were starved for one day and number in each tank counted and weighed separately.

The body protein content of the shrimps at the beginning of the experiment and at the end of the rearing period was also estimated under all the treatments.

3.6. Determination of body protein

To determine the initial body protein, before the start of the experiment about 5 shrimp juveniles were weighed and left for drying in an oven at 40°C for 48 hours. Dried prawns were then weighed and the initial body protein content was estimated using Microkjeldahl's method (AOAC, 1984). Similarly, the final body protein content of shrimps in each treatment was estimated using the same procedure.

3.7. Monitoring of water quality parameters

Physicochemical parameters of water in the rearing tanks were measured by the following methods.

1. Temperature - With bulb thermometer having an accuracy of 0.1°C
2. pH - Using Universal pH indicator solution.
3. Dissolved Oxygen - Standard Winkler method

Temperature, pH and D.O were monitered once in a week (Water quality management in aquaculture, CMFRI Sp. Publ.,No.22)

3.8. Evaluation criteria

Parameters like growth (weight gain), specific growth rate (SGR), percentage survival, Food Conversion Ratio (FCR), Protein Efficiency Ratio (PER) and Productive Protein Value (PPV) were determined (Halver, 1976) in order to study the growth and survival of *Penaeus monodon* fed on diets at different levels of replacement of clam meat with de-oiled silkworm pupae.

3.8.1. Net weight gain

This gives the increase in weight of shrimps during the experimental period when fed with different test diets. Net weight gain was calculated using the following formula:

Net weight gain = Final weight - Initial weight

3.8.2. Percentage growth

The percentage growth of the animal was calculated using the following formula:

$$\text{Growth } \% = \frac{\text{Final measurement - Initial measurement}}{\text{Initial measurement}} \times 100$$

3.8.3. Specific Growth Rate (SGR)

Specific growth rate (SGR) was calculated as

$$SGR = \frac{\log_e W2 - \log_e W1}{T2 - T1} \times 100$$

Where W1 - Weight at time T1

W2 - Weight at time T2

The calculated value gives the average percentage increase in body weight per day over 42 days.

3.8.4. Survival Rate

The survival rate of the shrimps is expressed in terms of percentage. This was calculated as follows:

$$\text{Survival \%} = \frac{\text{Initial number} - \text{Final number}}{\text{Initial number}} \times 100$$

3.8.5. Food Conversion Ratio (FCR)

This refers to the ability with which an animal can convert the feed consumed into edible and other products (Devendra, 1989).

FCR gives an idea about the amount of feed required to produce unit increase in weight of prawn. It is the commonly used index to measure the efficiency of different diets used in the trial.

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

3.8.6. Protein Efficiency Ratio (PER)

Protein efficiency ratio is defined as the weight gain per unit intake of protein (Paulraj, 1982). It was estimated using the following formula:

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

3.8.7. Productive Protein Value (PPV)

Productive protein vaule (PPV) gives the measurement of body protein deposition in the prawns with unit amount of protein consumed.

$$PPV\ (\%) = \frac{\text{Final body protein} - \text{Initial body protein}}{\text{Protein consumed}} \times 100$$

3.9. Statistical analysis

All indices such as growth, specific growth rate, food conversion ratio, protien efficiency ratio and productive protein value were subjected to comparison using analysis of variance (ANOVA) and pair wise comparison was performed whereever found necessary by using least significant difference based on 't' test (Snedecor and Cochran, 1968).

4. RESULTS

The results of the experiment carried out to determine the growth and survival of *Penaeus monodon* fed on diets having different levels of replacement of clam meat with de-oiled silkworm pupae are detailed below. The treatments, T_1, represent the diet with 100%clam meal and 0% de-oiled silkworm pupae; T_2, diet with 80% clam meal and 20% de-oiled silkworm pupae; T_3, diet with 60% clam meal and 40% de-oiled silkworm pupae; T_4, diet with 40% clam meal and 60% de-oiled silkworm pupae; T_5, diet with 20% clam meal and 80% de-oiled silkworm pupae and T_6, diet with 0% clam meal and 100% de-oiled silkworm pupae.

4.1. Proximate composition of feed ingredients and formulated feeds

4.1.1. Proximate composition of feed ingredients

The proximate composition of the ingredients used in the formulation of experimental diets is presented in the Table 8.

The moisture content of the ingredients viz; clam meal, de-oiled silkworm pupae, groundnut oil cake and tapioca ranged from 9.12% to 10.57%, the maximum being in groundnut oil cake (10.57%) and minimum in clam meal (9.12%).

Table 8 Proximate composition of the ingredients used in feed formulation. *

Ingredients	Moisture (%)	Crude protein (%)	Crude fat (N-free extract) (%)	Carbo-hydrate (%)	Ash (%)	Calorie content (K.cal/g)
Clam meal	9.12	53.22	9.4	19.47	8.79	3.75
De-oiled silkworm pupae	0.31	57.2	25.22	1.05	8.32	4.59
Groundnut oil cake	10.57	44.26	7.6	30.14	7.43	3.66
Tapioca powder	10.21	7.12	0.8	79.77	2.1	3.54

* Average of three values

The moisture content of the ingredients viz; clam meal, de-oiled silkworm pupae, groundnut oil cake and tapioca ranged from 9.12% to 10.57%, the maximum being in groundnut oil cake (10.57%) and minimum in clam meal (9.12%).

The highest crude protein content was recorded in de-oiled silkworm pupae (57.2%), while it was lowest in tapioca powder (7.12%). The percentage of crude protein of clam meal and groundnut oil cake were 53.22% and 44.26% respectively.

The crude fat content of the ingredients, ranged between 25.219% (de-oiled silkworm pupae) and 0.8% (tapioca powder). The crude fat content of clam meal was 9.4% and that of groundnut oil cake was 7.6%.

Tapioca powder contained the highest carbohydrate content of 79.77% (nitrogen-free extract), whereas de-oiled silkworm pupae had the least amount (1.049%).

The ash content of the ingredients varied from 2.1% to 8.79%, the highest being in clam meal (8.79%) and the lowest in tapioca powder (2.1%). The caloric content of the feed ingredients ranged from 3.54 KCal./g (tapioca powder) to 4.59 KCal./g (de-oiled silkworm pupae).

4.1.2. Proximate composition of formulated pelleted feeds

4.1.2.1. Initial analysis

Data on the proximate composition of the formulated feeds are presented in Table 9. The moisture content of the six feeds ranged between 3% (Feed 4) and 8% (Feeds 3 and 5). The crude protein content was between 34.89% (Feed 6) and 35% (Feeds 1,3 and 4), while the fat content varied from 6.25% (Feed 1) to 9.85% (Feed 6). The percentage of nitrogen- free extract was highest in pellet 1 (46.75%) and lowest in pellet 3 (40.253%). The ash content ranged between 6% (Feed 6) and 8% (Feed 1). The caloric values of all the six diets were almost similar, ranging from 3.83 (Feed 1) to 4.06Kcal/g (Feed 4). Hence, the test diets were more or less isocaloric and isonitrogenous.

4.2. Water stability

The results of stability test for different feed pellets are presented in Table 10. The most stable feed at the end of six hours was pellet T_6 (89.34%), followed by pellet T_5 (88.8%). The lowest stability was observed for pellet T_1 (83.8%).

A decrease in average stability was observed in all the six feeds as time increases. The decrease in stability was maximum in pellet T_1 (1.6%) and minimum in pellet T_6 (0.76%).

Table 9 Proximate composition of formulated feeds

Parameters *	Diets @					
	1	2	3	4	5	6
Moisture (%)	4	5	8	3	8	5
Crude protein (%)	35	34.9	35	35	34.97	34.89
Crude fat (%)	6.25	9.07	9.25	9.38	9.46	9.85
Carbohydrate (%) (N-free extract)	46.75	43.53	40.25	45.6	40.57	44.26
Ash (%)	8	7.5	7.5	7	7	6
Calorific value (K.cal/g)	3.83	3.95	3.84	4.06	3.87	4.05

* Average of three values

@ Diet 1 - 100% clam meal,0% de-oiled silkworm pupae

Diet 2 - 80% clam meal, 20% de-oiled silkworm pupae

Diet 3 - 60% clam meal, 40% de-oiled silkworm pupae

Diet 4 - 40% clam meal, 60% de-oiled silkworm pupae

Diet 5 - 20% clam meat, 80% de-oiled silkworm pupae

Diet 6 - 0% clam meal, 100% de-oiled silkworm pupae

Table 10 Water stability (%) of pelleted feeds

Feeds	Hours					
	1	2	3	4	5	6
T_1	85.4	85	84.9	84.5	84.2	83.8
T_2	89.2	89.1	88.8	88.8	88.4	88.15
T_3	89.5	89.35	89.1	88.85	88.6	88.4
T_4	89.5	89.45	89.2	89.1	88.7	88.5
T_5	89.9	89.7	89.4	89.25	89.1	88.8
T_6	90.1	89.95	89.8	89.62	89.5	89.34

4.3. Evaluation of relative efficiencies of the different diets

4.3.1. Biological parameters

During the six weeks of experimental study with six different feeds each under three replications the following observations were made.

4.3.1.1. Growth

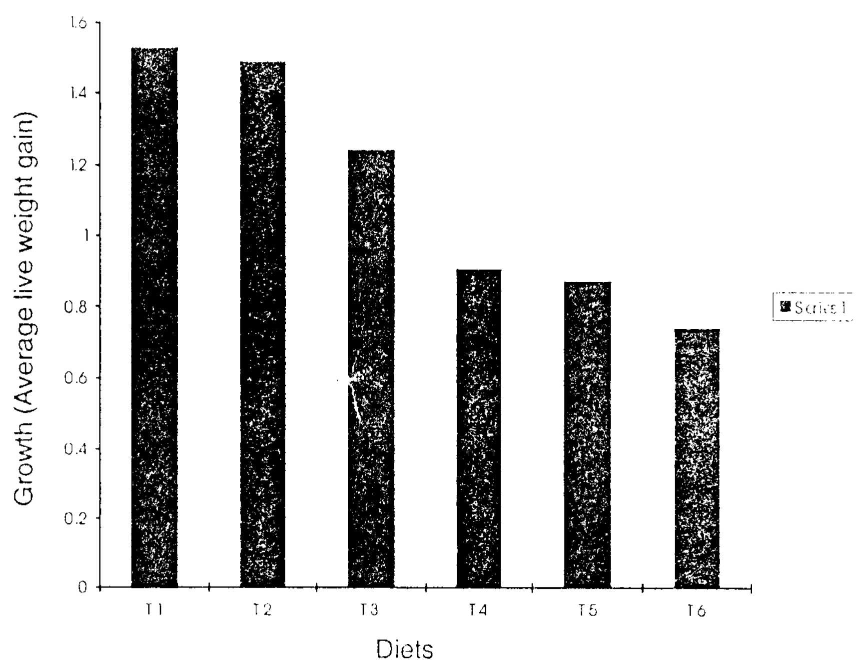
The data regarding the gain in weight of shimps fed with feeds containing different levels of clam meal and de-oiled silkworm pupae are presented in Table 11.

The average initial weights of shrimp juveniles used as test animals were 0.1923 g for T_1, 0.1893 g for T_2, 0.1791 g for T_3, 0.1756 g for T_4, 0.1914 g for T_5 and 0.1813 g for T_6. The average final weights were found to be 1.7899 g, 1.6779 g, 1.4138 g, 1.0805 g, 1.0622 g and 0.9164 g for T_1, T_2, T_3, T_4, T_5, and T_6 respectively. The net weight gain of the shrimp juveniles were different for different treatments. Maximum average weight gain (1.5282g) was observed for shrimp juveniles of treatment T_1 ie., those fed with diet 1 containing 100% clam meal and 0% de-oiled silkworm pupae, followed by those of treatment T_2 (1.4886g) ie; those fed with diet 2 containing 80% clam meal and 20% de-oiled silkworm pupae.

Table 11 Growth of *Penaeus monodon* juveniles fed on diet with different levels of replacement of clam meat with de- oiled silkworm pupae.

Treatment	Replication	Av. Initial Weight (g)	Av. Final Weight (g)	Gain in Weight (g)	Av.live Weight Gain(g)	%Weight Gain	Av.% Weight Gain
T_1	1	0.2084	1.6497	1.4413		691.60	
	2	0.1968	1.7689	1.5721	1.5282	798.83	787.95
	3	0.1799	1.7512	1.5713	±0.075	873.42	±91.4
T_2	1	0.1938	1.7876	1.5938		822.39	
	2	0.1770	1.6950	1.5180	1.4886	857.62	788.99
	3	0.1971	1.5511	1.3540	±0.123	686.96	±90.1
T_3	1	0.1920	1.4586	1.2666		651.69	
	2	0.1677	1.5155	1.3478	1.2346	803.69	689.51
	3	0.1777	1.2673	1.0896	±0.132	613.16	±100.74
T_4	1	0.1616	1.043	0.8814		545.42	
	2	0.1974	1.1448	0.9474	0.9048	479.93	517.53
	3	0.1680	1.0538	0.8858	±0.037	527.26	±33.81
T_5	1	0.1872	1.0012	0.8140		434.82	
	2	0.1912	0.9640	0.7728	0.8708	404.18	454.26
	3	0.1958	1.2214	1.0256	±0.136	523.79	±62.13
T_6	1	0.1806	0.9989	0.8183		453.10	
	2	0.1794	0.8317	0.6523	0.7350	363.60	405.31
	3	0.1840	0.9186	0.7346	±0.083	399.23	±45.06

Fig. 1.
Growth of *Penaeus monodon* juveniles fed on diet at different levels of replacement of clam meat with de-oiled silkworm pupae



The average weight gain of the shrimps which were fed on diets T_3, T_4, T_5 and T_6 were 1.2346g, 0.9048g, 0.8708g and 0.7350g respectively. The average percentage weight gain of different treatments ranged between 788.99% (T_2) and 405.31% (T_6).

Analysis of variance of the data (ANOVA TABLE 1) showed that there is significant difference ($P \leq 0.05$) between treatments. Comparison of treatment means based on critical difference showed that there is no significant difference ($P \geq 0.05$) in growth between treatments T_1 and T_2 and also between treatments T_4, T_5 and T_5 and T_6.

4.3.1.2. Specific growth rate

Table 12 and Fig. 2 give the specific growth rate (SGR) of shrimp juveniles from different treatments. The shrimp juveniles of treatment T2 had the highest SGR (5.1937), followed by those of T_1 (5.1908) and T_3 (4.8065). The lowest SGR (3.9753) was recorded for shrimps of treatment T_6.

Analysis of variance of the data (ANOVA TABLE 2) showed that there is significant difference ($P \leq 0.05$) between treatments. Comparison of treatment means based on critical difference showed that there is no significant difference ($P \geq 0.05$) in specific growth rate between treatments T_1 and T_2 and also between treatments T_4, T_5 and T_6.

ANOVA TABLE 1

Analysis of variance of the data on growth of *P.monodon* juveniles fed on diet at different levels of replacement of clam meat with de-oiled silkworm pupae

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F
Between diets	5	1.7121	0.3424	30.72 *
Error	12	0.1337	0.0111	
Total	17	1.8458		

Critical difference at 5 % level = 0.15

Comparision of treatment means based on critical difference.

Treatments	T_1	T_2	T_3	T_4	T_5	T_6
Means	1.5282	1.4886	1.2346	0.9048	0.8708	0.7350

(Underscored: T_1–T_2; T_4–T_5; T_5–T_6)

Underscored means are not significantly different

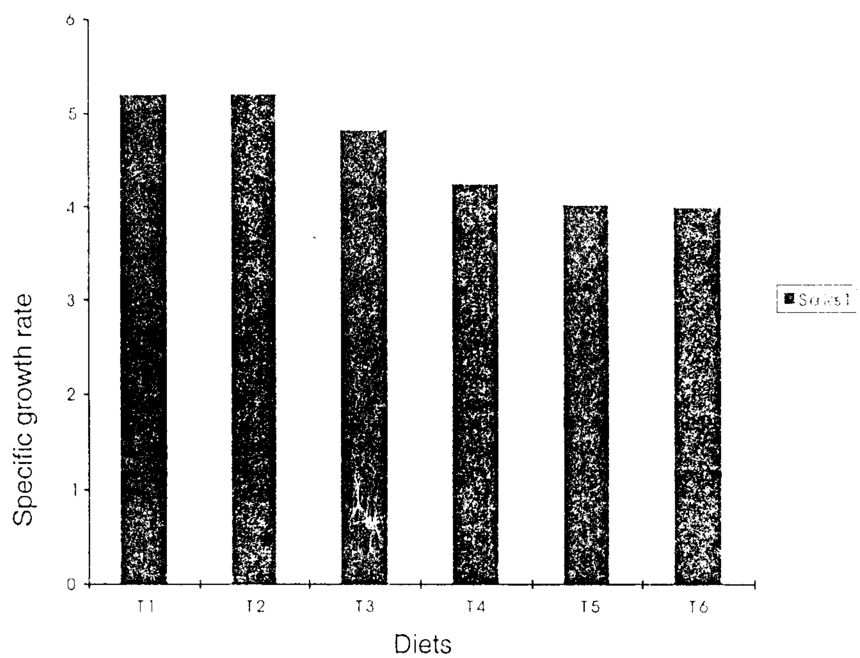
* Significantly different at 5 % level.

Table 12 Specific growth rate of *Penaeus monodon* juveniles fed on diet at different levels of replacement of clam meat with de-oiled silkworm pupae.

Treatment	Replication	Av.Initial Weight (g)	Av.Final Weight (g)	Specific Growth Rate	Mean ±S.E
T_1	1 2 3	0.2084 0.1968 0.1799	1.6497 1.7689 1.7512	4.9259 5.2283 5.4182	5.1908 ±0.25
T_2	1 2 3	0.1938 0.1770 0.1971	1.7876 1.6950 1.5511	5.29 5.3792 4.9119	5.1937 ±0.25
T_3	1 2 3	0.1920 0.1677 0.1777	1.4586 1.5155 1.2673	4.8279 5.2412 4.3503	4.8065 ±0.46
T_4	1 2 3	0.1616 0.1974 0.1680	1.043 1.1448 1.0538	4.3816 3.9983 4.2995	4.2264 ±0.20
T_5	1 2 3	0.1872 0.1912 0.1958	1.0012 0.9640 1.2214	3.9901 3.9024 4.0825	3.9916 ±0.10
T_6	1 2 3	0.1806 0.1794 0.1840	0.9989 0.8317 0.9186	4.0738 3.9065 3.9456	3.9753 ±0.09

Fig. 2.

Specific growth rate of *Penaeus monodon* juveniles fed on diet at different levels of replacement of clam meat with de-oiled silkworm pupae



4.3.1.3. Survival

The percentage survival values of the shrimp juveniles in the various treatments are given in Table 13; Fig. 3. Among the diets tested, the highest survival (93.33%) was recorded for treatment T_1, followed by treatments T_2, T_3 and T_4 (86.666%). The lowest survival percentage was observed for treatments T_5 and T_6 (83.333%).

Analysis of variance of the data (ANOVA TABLE 3) showed that there is no significant difference ($P \geq 0.05$) between treatments.

4.3.1.4. Food conversion ratio

The food conversion ratio (FCR) recorded for the various treatments are presented in Table 14; Fig. 4. The mean FCR values ranged from 2.5233 to 6.8933. Treatment T_1 gave the lowest FCR value (2.5233), followed by T_2 (2.5466). The average FCR values obtained for treatments T_3, T_4, T_5 and T_6 were 3.55, 5.1366, 5.7933 and 6.8933 (highest) respectively.

Analysis of variance of the data (ANOVA TABLE 4) showed that there is significant difference ($P \leq 0.05$) between treatments. Comparison of treatment means based on critical difference showed that there is no significant difference ($P \geq 0.05$) in food conversion ratio between treatments T_1 and T_2.

ANOVA TABLE 2

Analysis of variance of the data on specific growth rate of *P.monodon* juveniles fed on diet at different levels of replacement of clam meat with de-oiled silkworm pupae.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F
Between diets	5	4.9245	0.9849	15.59 *
Error	12	0.7583	0.0632	
Total	17	5.6828	0.0632	

Critical differences at 5 % level = 0.37

Comparision of treatment means based on critical difference.

Treatments	T_1	T_2	T_3	T_4	T_5	T_6
Means	5.1908	5.1937	4.8065	4.2264	3.9916	3.9753

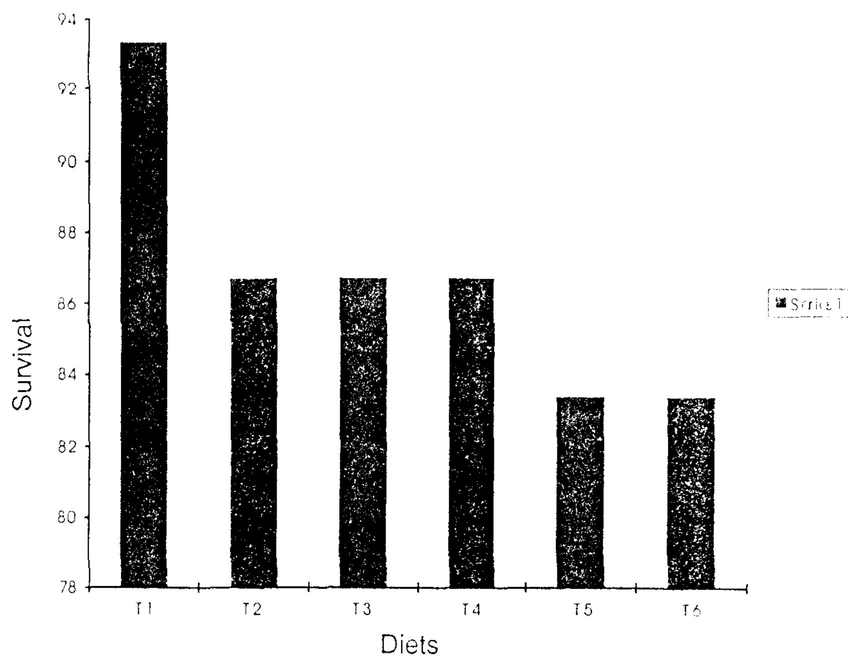
Under scored means are not significantly different.

* Significantly different at 5 % level.

Table 13 Percentage survival obtained in various treatments of *Penaeus monodon* juveniles fed on diet at different levels of replacement of clam meat with de-oiled silkworm pupae.

Treatment	Replication	% Survival	Mean ± S.E.
T_1	1 2 3	100 100 80	93.33 ±11.55
T_2	1 2 3	80 90 90	86.666 ±5.77
T_3	1 2 3	80 90 90	86.666 ±5.77
T_4	1 2 3	90 80 90	86.666 ±5.77
T_5	1 2 3	90 80 80	83.333 ±5.77
T_6	1 2 3	80 80 90	83.333 ±5.77

Fig. 3.
Survival of *Penaeus monodon* juveniles fed on diet at different levels of replacement of clam meat with de-oiled silkworm pupae



ANOVA TABLE 3

Analysis of variance of the data on percentage survival of *P. monodon* juveniles fed on diet at different levels of replacement of clam meat with de-oiled silkworm pupae.

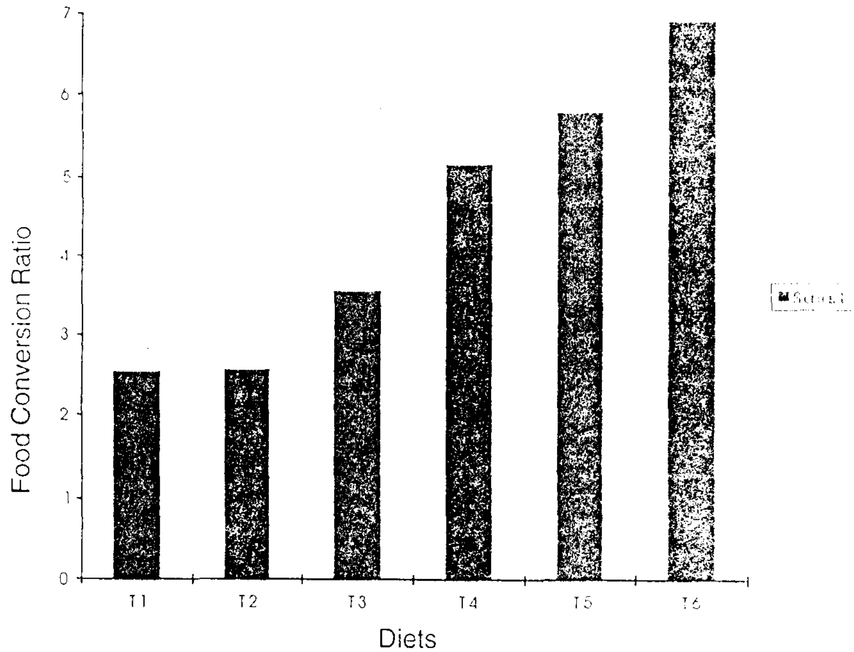
Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F
Between diets	5	200.00	40.00	0.80
Error	12	600.00	50.00	
Total	17	800.00		

Table 14 Food conversion ratio of *Penaeus monodon* juveniles fed on diet at different levels of replacement of clam meat with de-oiled silkworm pupae

Treatment	Replication	Av. Initial Biomass (g)	Av. Final Biomass (g)	Av.Growth Increment (g)	Av.Feed Consumed (g)	FCR	Mean ±S.E.
T_1	1	0.2084	1.6497	1.4413	3.8496	2.45	
	2	0.1968	1.7689	1.5721	4.0258	2.56	2.5233
	3	0.1799	1.7512	1.5713	3.6197	2.56	±0.06
T_2	1	0.1938	1.7876	1.5938	4.0323	2.53	
	2	0.1770	1.6950	1.5180	3.9772	2.62	2.5466
	3	0.1971	1.5511	1.3540	3.3715	2.49	±0.07
T_3	1	0.1920	1.4586	1.2666	4.5978	3.63	
	2	1.1677	1.5155	1.3478	4.6885	3.48	3.55
	3	1.1777	1.2673	1.0896	3.8578	3.54	±0.08
T_4	1	0.1616	1.043	0.8814	3.9573	4.49	
	2	0.1974	1.1448	0.9474	5.1471	5.43	5.1366
	3	0.1680	1.0538	0.8858	4.8634	5.49	±0.56
T_5	1	0.1872	1.0012	0.8140	4.9979	6.14	
	2	0.1912	0.9640	0.7728	4.8793	6.31	5.7933
	3	0.1958	1.2214	1.0256	5.0566	4.93	±0.75
T_6	1	0.1806	0.9989	0.8183	5.5975	6.84	
	2	0.1794	0.8317	0.6523	4.7954	7.35	6.8933
	3	0.1840	0.9186	0.7346	4.7721	6.49	±0.43

Fig. 4.

Food Conversion Ratio of *Penaeus monodon* juveniles fed on diet at different levels of replacement of clam meat with de-oiled silkworm pupae



4.3.1.5. Protein efficiency ratio

The protein efficiency ratio (PER) obtained for various treatments are shown in the Table 15; Fig. 5. The highest PER of 1.1398 was recorded for treatment T_1, followed by treatment T_2 (1.1255). The lowest PER of 0.4168 was for treatment T_6.

Analysis of variance of the data (ANOVA TABLE 5) showed that there is significant difference ($P \leq 0.05$) between treatments. Comparison of treatment means based on critical difference showed that there is no significant difference ($P \geq 0.05$) between treatments T_1 and T_2 and also between T_4 and T_5.

4.3.1.6. Productive protein value

Productive protein value (PPV) gives an indication of the protein deposition in the shrimp. The average initial body protein of the shrimp juveniles on a wet weight basis were 18.09% for T_1, 17.57% for T_2, 17.27% for T_3, 16.5% for T_4, 16.52% for T_5 and 15.95% for T_6. At the end of the experiment, there was a significant increase in the body protein with the amount of protein deposited in the muscle tissue differing significantly in various treatments. The average value of gain in body protein for different feeds were 5.07% for T_1 (highest), 3.45% for T_2, 2.1% for T_3, 0.84% for T_4, 0.78% for T_5 and 0.68% for T_6 (lowest).

ANOVA TABLE 4

Analysis of variance of the data on food conversion ratio of *P. monodon* juveniles fed on diet at different levels of replacement of clam meat with de-oiled silkworm pupae.

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	F
Between diets	5	49.1391	9.8278	54.50 *
Error	12	2.1639	0.1803	
Total	17	51.3030		

Critical difference at 5 % level = 0.62

Comparison of treatment means based on critical difference.

Treatments	T_1	T_2	T_3	T_4	T_5	T_6
Means	2.5233	2.5466	3.55	5.1366	5.7933	6.8933

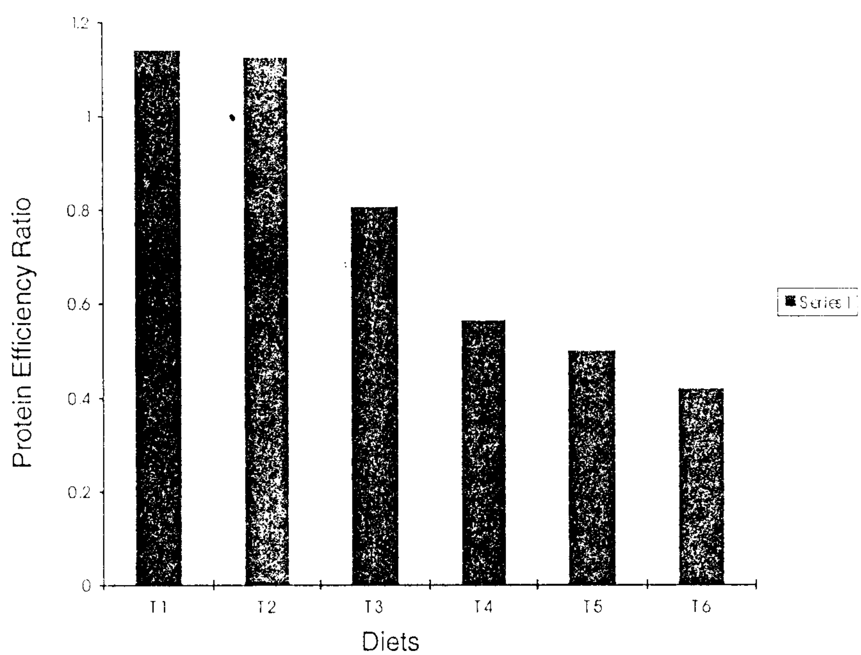
Under scored means are not significantly different.

* Significantly different at 5 % level.

Table 15 Protein efficiency ratio of *Penaeus monodon* juveniles fed on diet at different levels of replacement of clam meat with de-oiled silkworm pupae.

Treat ment	Repli cation	Av. Initial Weight (g)	Av. Final Weight (g)	Av. Live Weight Gain (g)	Av Weight of Food (Consumed) (g)	Av. Weight of Protein (Consumed) (g)	PER	Mean ±S.E.
T_1	1	0.2084	1.6497	1.4413	3.8496	1.2669	1.1376	
	2	0.1968	1.7689	1.5721	4.0258	1.4090	1.1157	1.1398
	3	0.1799	1.7512	1.5713	3.6197	1.3474	1.1661	±0.03
T_2	1	0.1938	1.7876	1.5938	4.0323	1.4073	1.1325	
	2	0.1770	1.6950	1.5180	3.9772	1.3880	1.0936	1.1255
	3	0.1971	1.5511	1.3540	3.3715	1.1767	1.1506	±0.03
T_3	1	0.1920	1.4586	1.2666	4.5978	1.6092	0.787	
	2	1.1677	1.5155	1.3478	4.6885	1.6409	0.8213	0.805
	3	0.1777	1.2673	1.0896	3.8578	1.3502	0.8069	±0.02
T_4	1	0.1616	1.043	0.8814	3.9573	1.3851	0.6363	
	2	0.1974	1.1448	0.9474	5.1471	1.8015	0.5258	0.5608
	3	0.1680	1.0538	0.8858	4.8634	1.7022	0.5203	±0.07
T_5	1	0.1872	1.0012	0.8140	4.9979	1.747	0.4659	
	2	0.1912	0.9640	0.7728	4.8793	1.706	0.4529	0.4996
	3	0.1958	1.2214	1.0256	5.0566	1.768	0.5800	±0.07
T_6	1	0.1806	0.9989	0.8183	5.5975	1.952	0.4192	
	2	0.1794	0.8317	0.6523	4.7954	1.673	0.3898	0.4168
	3	0.1840	0.9186	0.7346	4.7721	1.664	0.4414	±0.03

Fig. 5.
Protein Efficiency Ratio of *Penaeus monodon* juveniles fed on diet at different levels of replacement of clam meat with de-oiled silkworm pupae



ANOVA TABLE 5

Analysis of variance of the data on protein efficiency ratio of *P. monodon* juvenile fed on diet at different levels of replacement of clam meat with de-oiled silkworm pupae.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F
Between diets	5	1.516	0.3032	159.58 *
Error	12	0.023	0.019	
Total	17	1.539		

Critical difference at 5 % level = 0.063

Comparison of treatment means based on critical difference.

Treatments	T_1	T_2	T_3	T_4	T_5	T_6
Means	1.1398	1.1255	0.805	0.5608	0.4996	0.4168

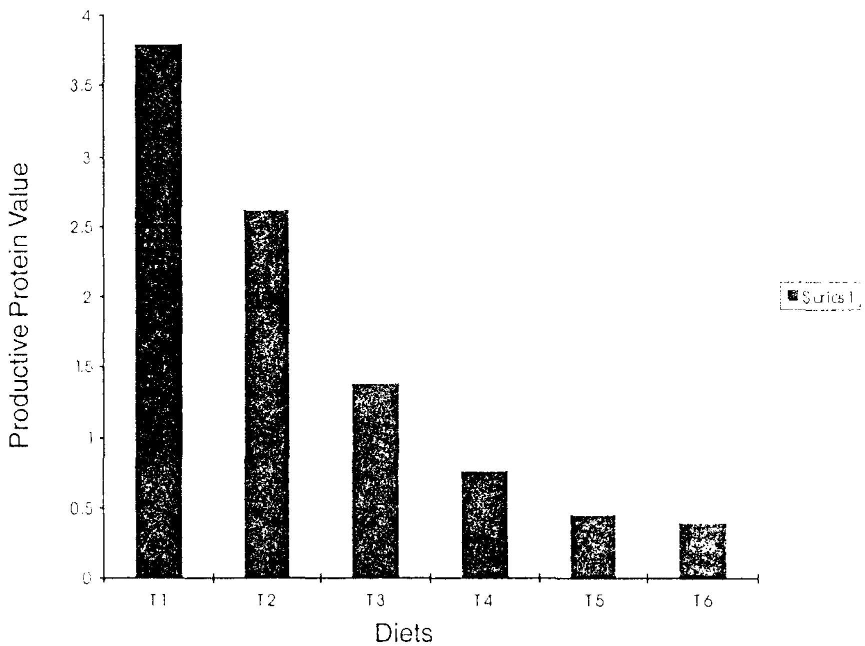
Under scored means are not significantly different.

* Significantly different at 5 % level.

Table 16 Productive protein value of *Penaeus monodon* juveniles fed on diet at different levels of replacement of clam meat with de-oiled silkworm pupae.

Treatment	Replication	Av. Initial Weight (g)	Av. Final Weight (g)	Initial Body Protein (g)	Final Body Protein (g)	Gain in Protein (g)	Av. Protein Consumed (g)	PPV	Mean ±S.E.
	1	0.2084	1.6497	0.1827	0.235	0.0523	1.2669	4.128	
T_1	2	0.1968	1.7689	0.1617	0.2109	0.0492	1.4090	3.4918	3.7917
	3	0.1799	1.7512	0.1983	0.2489	0.0506	1.3474	3.7553	±0.32
	1	0.1938	1.7876	0.1687	0.2036	0.0349	1.4073	2.47	
T_2	2	0.1770	1.6950	0.1683	0.2041	0.0358	1.3880	2.579	2.6149
	3	0.1971	1.5511	0.1902	0.2231	0.0329	1.1767	2.7959	±0.17
	1	0.1920	1.4586	0.1635	0.1863	0.0228	1.6092	1.4168	
T_3	2	0.1677	1.5155	0.1818	0.2003	0.0185	1.6409	1.1274	1.3820
	3	0.1777	1.2673	0.1728	0.1945	0.0217	1.3502	1.602	+0.24
	1	0.1616	1.043	0.1727	0.1897	0.0170	1.3851	1.22	
T_4	2	0.1974	1.1448	0.1658	0.1752	0.0094	1.8015	0.5217	0.7528
	3	0.1680	1.0538	0.1566	0.1654	0.0088	1.7022	0.5169	±0.40
	1	0.1872	1.0012	0.1694	0.1760	0.0066	1.747	0.3777	
T_5	2	0.1912	0.9640	0.1620	0.1701	0.0081	1.706	0.4747	0.4462
	3	0.1958	1.2214	0.1647	0.1733	0.0086	1.768	0.4864	±0.06
	1	0.1806	0.9989	0.1667	0.1734	0.0067	1.952	0.3432	
T_6	2	0.1794	0.8317	0.1458	0.1524	0.0066	1.673	0.3945	0.3881
	3	0.1840	0.9186	0.1661	0.1732	0.0071	1.664	0.4266	±0.04

Fig. 6 .
Productive Protein Value of *Penaeus monodon* juveniles fed on diet at different levels of replacement of clam meat with de-oiled silkworm pupae



ANOVA TABLE 6

Analysis of variance of the data on productive protein value of *P.monodon* juvenile fed on diet at different levels of replacement of clam meat with de-oiled silkworm pupae.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F
Between diets	5	28.2072	5.6414	94.97 *
Error	12	0.713	0.0594	
Total	17	28.9202		

Critical difference at 5 % level = 0.355

Comparison of treatment means based on critical difference.

Treatments	T_1	T_2	T_3	T_4	T_5	T_6
Means	3.7917	2.6149	1.382	<u>0.7528</u>	<u>0.4462</u>	0.3881

Under scored means are not significantly different.

* Significantly different at 5 % level.

There was a decrease in gain in body protein as the level of silkworm pupae increased. The PPV was highest for treatment T_1 (3.7917) and lowest for treatment T_6 (0.3881). Data on the PPV are given in Table 16; Fig. 6.

Analysis of variance of the data (ANOVA TABLE 6) showed that there is significant difference ($P \leq 0.05$) between treatments. Comparison of treatment means based on critical difference showed that there is no significant difference ($P \geq 0.05$) between treatments T_4 and T_5 and also between treatments T_5 and T_6.

4.3.2. Water quality parameters

4.3.2.1. Temperature

Table17 depicts the water temperature recorded from the experimental tanks at weekly intervals during the study period. The water temperature ranged between $24^{\circ}C$ and $28^{\circ}C$.

4.3.2.2. pH

Table 18 gives the details of variations observed in pH in the rearing tanks during the study period. p H of water was found to be alkaline in all the rearing tanks throughout the experiment, its range being 7.5 to 8.5.

Table 17 Water temperature (C^{O}) in the experimental tanks during the study period. (Mean ± S.D shown in paranthesis)

Treat-ment	Repli-cation	Weeks						
		0	1	2	3	4	5	6
T_1	1	28	27.5	27.5	26.5	26	25.5	24
	2	28	28	28	27.5	26.5	26	25
	3	28	27.5	26.5	26	25.5	25	25
		(8.0 ± 0)	(27.666± 0.24)	(27.333± 0.62)	(26.666± 0.62)	(26.333± 0.53)	(25.5± 0.41)	(24.666 ± 0.47)
T_2	1	28	28	28	27.5	26	25	24.5
	2	28	27.5	27.5	27	27	26	25
	3	28	28	27.5	27	26.5	26	24.5
		(28.0± 0)	(27.833± 0.24)	(27.666± 0.24)	(27.166± 0.24)	(26.5 ± 0.41)	(25.666±0.47)	(24.666 ± 0.24)
T_3	1	28	28	27	26.5	26	25	24
	2	28	28	27.5	26.5	25	24	23.5
	3	28	27.5	26.5	26.5	25.5	25	24.5
		(28.0± 0)	(27.833± 0.24)	(27.0 ± 0.41)	(26.5 ± 0)	(25.5 ± 0.41)	(24.666±0.47)	(24.0 ± 0.41)
T_4	1	28	28	27.5	26.5	25.5	24.5	24.5
	2	28	27.5	27.5	27	26	25	24
	3	28	27.5	26	26	25	25	24.5
		(28.0± 0)	(27.666± 0.24)	(27.0 ± 0.71)	(26.5 ± 0.41)	(25.5 ± 0.41)	(24.833±0.24)	(24.333 ± 0.24)
T_5	1	28	27.5	27.5	27	26	25.5	24
	2	28	28	27.5	27	26.5	25	24
	3	28	28	28	26.5	25	25	24
		(28.0± 0)	(27.833± 0.24)	(27.666± 0.24)	(26.833± 0.24)	(25.833± 0.62)	(25.166±0.24)	(24.0 ± 0)
T_6	1	28	27.5	28 27	26	25	24	
	2	28	27.5	27	26.5	25.5	24	24
	3	28	28	27.5	26.5	26	25	24
		(28.0± 0)	(27.666± 0.24)	(27.5 ± 0.41)	(26.666± 0.24)	(25.833± 0.24)	(24.666±0.47)	(24.0 ± 0)

Table 18 Fluctuation of pH in the experimental tanks during the experimental period (Mean ± SD shown in paranthesis)

Treat-ments	Repli-cation	Weeks						
		0	1	2	3	4	5	6
T_1	1	8.0	8.0	7.5	8.0	8.5	8.0	8.0
	2	8.0	8.0	8.0	8.5	8.0	7.5	7.5
	3	8.0	7.5	8.0	8.0	8.0	8.5	8.0
		(8.0 ± 0)	(7.833 ± 0.24)	(7.833 ± 0.24)	(8.166 ± 0.24)	(8.166 ± 0.24)	(8.0 ± 0.41)	(7.833±0.24)
T_2	1	8.0	7.5	7.5	8.0	8.0	8.5	8.0
	2	8.0	8.0	7.5	7.5	8.0	8.5	8.0
	3	8.0	7.5	8.0	8.5	8.0	7.5	8.0
		(8.0 ± 0)	(7.666 ± 0.24)	(7.666 ± 0.24)	(8.0 ± 0.41)	(8.0 ± 0)	(8.166 ± 0.47)	(8.0 ± 0)
T_3	1	8.0	8.0	8.5	8.0	7.5	8.0	7.5
	2	8.0	8.0	8.0	7.5	8.0	8.0	8.0
	3	8.0	8.0	8.0	8.0	8.5	8.5	8.0
		(8.0± 0)	(8.0 ± 0)	(8.166 ± 0.24)	(7.833 ± 0.24)	(8.0 ± 0.41)	(8.166± 0.24)	(7.833± 0.24)
T_4	1	8.0	7.5	8.0	8.0	8.5	8.5	8.0
	2	8.0	8.0	7.5	8.0	8.0	8.0	7.5
	3	8.0	8.0	8.0	7.5	8.0	7.5	8.0
		(8.0 ± 0)	(7.833 ± 0.24)	(7.833 ± 0.24)	(.833 ± 0.24)	(8.166 ± 0.24)	(8.0 ± 0.41)	(7.83 ± 0.24)
T_5	1	8.0	8.0	8.0	8.0	7.5	7.5	8.0
	2	8.0	8.5	8.0	8.5	8.0	7.5	7.5
	3	8.0	8.0	7.5	8.0	8.5	8.0	8.0
		(8.0 ± 0)	(8.166 ± 0.24)	(7.833 ± 0.24)	(8.166 ± 0.24)	(8.0 ± 0.41)	(7.666± 0.24)	(7.833±0.24)
T_6	1	8.0	8.0	8.0	8.5	8.0	7.5	8.0
	2	8.0	7.5	8.0	8.0	8.5	8.0	8.5
	3	8.0	8.0	8.5	8.0	7.5	8.0	8.0
		(8.0 ± 0)	(7.833 ± 0.24)	(8.166 ± 0.24)	(8.166 ± 0.24)	(8.0 ± 0.41)	(7.833±0.24)	(8.166±0.24)

4.3.2.3. Dissolved oxygen

The dissolved oxygen content of water recorded over the experimental period is shown in the Table 19. The average values in different treatments varied from 6.94 ppm to 7.7 ppm. Dissolved oxygen levels were found to remain almost constant during the study period, since, mild aeration was provided in the experimental tanks.

Table 19 Dissolved oxygen (ppm) in the experimental tanks during the study period. (Mean ± SD shown in paranthesis).

Treat-ment	Repli cation	Weeks						
		0	1	2	3	4	5	6
T_1	1	7.12	7.0	7.2	8.02	7.6	7.2	7.31
	2	7.0	6.82	7.5	7.5	7.0	6.79	7.1
	3	7.2 (7.11 ± 0.08)	7.1 (6.97 ± 0.12)	7.3 (7.33 ± 0.12)	6.95 (7.49 ± 0.44)	7.17 (7.26 ± 0.25)	8.2 (7.4 ± 0.59)	7.8 (7.4 ± 0.29)
T_2	1	7.13	7.01	7.5	7.6	7.1	7.4	6.95
	2	7.1	6.9	7.47	8.01	7.7	8.01	7.6
	3	7.0 (7.08 ± 0.06)	7.1 (7.0 ± 0.08)	7.4 (7.46 ± 0.04)	7.4 (7.67 ± 0.25)	7.0 (7.27 ± 0.31)	7.18 (7.53 ± 0.35)	7.42 (7.32 ± 0.27)
T_3	1	7.0	7.2	7.6	6.97	6.84	7.2	7.5
	2	6.9	7.1	7.33	7.4	7.3	7.47	7.1
	3	7.0 (6.97 ± 0.05)	7.2 (7.17 ± 0.05)	6.95 (7.29 ± 0.27)	7.1 (7.16 ± 0.18)	7.4 (7.18 ± 0.24)	7.0 (7.22 ± 0.19)	7.3 (7.3 ± 0.16)
T_4	1	7.2	7.0	7.43	7.2	7.4	7.3	7.1
	2	7.15	6.84	7.0	7.7	7.1	6.81	7.0
	3	6.9 (7.08 ± 0.13)	7.0 (6.95 ± 0.08)	7.52 (7.32 ± 0.23)	8.2 (7.7 ± 0.41)	7.9 (7.47 ± 0.29)	7.4 (7.17 ± 0.26)	6.85 (6.98 ± 0.1)
T_5	1	6.8	6.93	7.44	7.0	7.6	7.5	7.8
	2	7.03	7.2	7.63	7.67	7.4	6.85	7.19
	3	7.0 (6.94 ± 0.1)	7.0 (7.04 ± 0.11)	7.2 (7.57 ± 0.23)	7.24 (7.3 ± 0.28)	7.08 (7.36 ± 0.21)	6.9 (7.08 ± 0.3)	7.4 (7.46 ± 0.25)
T_6	1	7.1	7.1	7.51	7.8	7.3	7.5	7.5
	2	7.1	7.0	7.2	7.25	7.3	7.44	7.2
	3	6.9 (7.03 ± 0.09)	6.86 (6.99 ± 0.1)	7.5 (7.4 ± 0.14)	6.8 (7.28 ± 0.41)	7.11 (7.24 ± 0.09)	7.3 (7.41 ± 0.08)	7.48 (7.39 ± 0.14)

5. DISCUSSION

5.1. Proximate analysis of formulated pelleted feeds

The protein requirement of *Penaeus monodon* has been identified to be 35% by Bages and Sloane (1981). Bautista (1986) reported that 40 to 50% protein gave best growth and survival of *Penaeus monodon* in the presence of 20% carbohydrate and 5 to 10% lipid. Nezaki *et al.* (1986) found that 55% protein with 15% carbohydrate in grow-out diets gave the best growth for *P. monodon.* However, according to them, when the carbohydrate content is increased to 25%, a 45% protein diet gave results comparable to those diets containing 55% protein. Shiau and Chou (1991) recommended 36 - 40% protein level in the diet of *P. monodon*. Proximate analysis of the diets used in the present study revealed that they contained protein in the range of 34.89% - 35% which is almost the same as the optimum value suggested by previous workers.

The diets used in the present experiment contained carbohydrate in the range of 40.25% - 46.75%. Bages and Sloane (1981) and Alava and Pascual (1987) obtained higher growth rate in *P. monodon* with a diet containing 20% carbohydrate. Consequently Shiau and Peng (1991) who used 20% carbohydrate together with 40% protein obtained high growth rate for *P. monodon*. They observed that *P.monodon* may be able to use a relatively high content of carbohydrate in the diet. But Catacutan (1991) did not find any difference in growth of juvenile *P.monodon* fed isonitrogenous diets containing 5 - 35% carbohydrate. Nezaki *et al.* (1986) reported that by keeping carbohydrate content in the diet at 25%, lower level of protein (45%) could give satisfactory results in comparison to those diet containing a higher (55%) level of protein.

Andrews *et al.* (1972) and Forster and Beard (1973) reported that a lipid level of below 10% was adequate in shrimp diets. Bautista (1986) observed that excessive dietary lipid has an adverse effect on the growth and survival of prawns and shrimps. Akiyama (1989) recommended lipid levels of 6 - 7.5% in commercial shrimp diets, while Sheen and D'Abramo (1989) found similar level to be optimal for prawn as well. The lipid content of the diets used in the present study is in the range of 6.25% - 9.85%. The ash content was worked out to be varying between 6% - 8% in the experimental feeds. According to Forster and Gabbot (1971) shrimps can digest upto 30% of ash fraction when the ash content is as high as 15%.

5.2. Growth studies

5.2.1. Feeding experiment

In the present study, efficiency of de-oiled silkworm pupae in replacing clam meat at different levels in the diet for *Penaeus monodon* juveniles was tested based on growth, specific growth rate, survival, food conversion ratio, protein efficiency ratio and water stability of pelleted feeds.

5.2.1.1. Growth

Growth of *Penaeus monodon* juveniles in various treatments indicated that shrimp juveniles fed on diet T_1 containing 100% clam meal and 0% de-oiled silkworm pupae (*Bombyx mori*) gave the maximum average live weight gain, followed by, those fed with diet T_2 containing 80% clam meal and 20% de-oiled silkworm pupae. Statistically no significant difference ($P \geq 0.05$) was observed between treatments T_1 and T_2. The lowest growth performance was recorded in treatment T_6 ie; those fed with diet containing 0% clam meal and 100% de-oiled silkworm pupae. It was observed that with increase in de-oiled silkworm pupae content above 20% and decrease in clam meal content below 80%, the growth of *P. monodon* juveniles decreased significantly. The result indicate that clam meal can be replaced with de-oiled silkworm pupae only at 20% level in the diet for *P. monodon* juveniles.Most of the works regarding the use of silkworm pupae in the diets (either as a protein source or as a replacement / or substitute to other sources of protein used in the diets) have been done in fishes (Jeyachandran and Paul Raj, 1976; 1977; Jayaram and Shetty, 1981; Nandeesha *et al.*, 1989; Shyama and Keshavanath, 1993; Devaraj and Vijayakumaraswamy, 1994). Silkworm pupae has been used in the diets of palaemonids by Ravishankar and Keshavanath, 1988; Unnikrishnan *et al.*, 1991; Das *et al.*, 1995, while in *Penaeus indicus* it was tried by Ali (1982, 1992).

Jeyachandran and Paul Raj (1976, 1977) have reported satisfactory growth of common carp with pelleted feed containing silkworm pupae. They found that there is a positive correlation between the protein content of the feed and the daily increment in the weight of fish and also between the fat content of the feed and the daily weight increment of the fish. The proximate analysis of various ingredients of the pelleted feeds, in the present study showed that de-oiled silkworm pupae contained higher percentage crude protein (57.2%) than clam meal (53.22%), and it was seen to contain higher level of fat (25.219%) than clam meal (9.4%). In the present study, the second best growth rate observed for *P.monodon* juveniles fed with diet T_2 containing 80% clam meal and 20% de-oiled silkworm pupae, to diet T_1 with 100% clam meal and 0% de-oiled silkworm pupae (though statistically there was no significant difference ($P \geq 0.05$) between treatments T_1 and T_2) can be attributed to these factors. Tsushima (1978) reported that for common carp, 70% methanol extract of silkworm pupae was the most effective of the three methods of extraction tested. He attributed this to the attractive activity of silkworm pupae extract which evoked feeding behaviour. In the present study also better growth was observed in shrimp juveniles fed with diet T_2 containing 80% clam meal and 20% de-oiled silkworm pupae. Chowdhary and Srivastava (1978) found that a mixture of silkworm pupae, rice bran and Bengal gram flour was found to be superior to the traditionally used mixture of mustard oil cake and rice bran for common carp fingerlings. Jayaram and Shetty (1980) obtained good growth of Catla and common carp when fed on a pelleted feed containing 30% non - deoiled silkworm pupae as compared to fish meal based diet and attributed the same to the higher fat level of the test diet. According to them the better growth with silkworm pupae based feed was due to better utilization of the feed. They concluded that expensive fish meal may be effectively substituted by cheaper dried silkworm pupae in the formulation of pelleted feeds to rear Catla and Common carp. In a later experiment, they observed poor growth of rohu when fed a diet containing 30% silkworm pupa and attributed it to the low fat requirement of the fish (Jayaram and Shetty, 1981). Murofushi and Ina (1981) isolated feeding stimulants from dried silkworm pupae for the Sea Bream. But Akiyama *et al.* (1984) obtained best growth performance for chum salmon, *Oncorhynchus keta*, swim-up fry, when fed with fish meal diets supplemented with earthworm powder (5%), followed by krill meal (5%) than with silkworm pupae powder (5%) but, feed efficiency was found to be satisfactory with all the three supplements. Pezzato *et al.* (1984) reported that for *Oreochromis niloticus* substitution of meat and bone meal with silkworm chrysalis (*Bombyx mori*) meal at 0%, 33.3%, 66.6% and 100% showed no significant differences between treatments and concluded that chrysalis meal can substitute meat and bone meal with no depressive effect on the fingerling development. Venkatesh *et al.* (1985) suggested that silkworm pupae can be incorporated into the operational diet of *Clarias batrachus* with advantage.

Nandeesha *et al.* (1986) reported superior growth of rohu with silkworm faecal matter based pelleted feed. Srikanth (1986) reported better growth in *Tor khudree* with de-oiled silkworm pupae. According to Sharma *et al.* (1987) maximum growth was recorded for carps fed on gas slurry plus silkworm pupae meal. Nandeesha *et al.* (1988) reported better growth of catla- rohu hybrid with pellet containing 15% non-defatted silkworm pupae and10% fish meal than with fish meal diet alone. According to him though the synergistic effect of fish meal (10%) and pupa (15%) cannot be easily explained; it appears that apart from better acceptability, the balanced nutrient profile of the combination resulted in the faster growth. In the present study also, the combination of 80% clam meal and 20% de-oiled silkworm pupae has produced good growth almost similar to that obtained with 100%

calm meal and 0% de-oiled silkworm pupae ($P \geq 0.05$). In a later work, Nandeesha *et al.* (1989) obtained best growth of mahseer with de-oiled silkworm pupa pellet. But for Catla, Rohu and Common carp it induced only lower growth than the fish meal based diet whereas, for silver carp silkworm faecal matter based pellet induced the best growth (Nandeesha *et al.*,1989).They attributed this to the probable loss of attractants and appetite stimulants contained in silkworm pupa(Tsushima and Ina,1978) during the process of extraction of oil which would have adversely affected the intake and conversion of de-oiled silkworm pupa pellet resulting in the poor growth of carp species. But the cost of fish production was high with fish meal based diet as compared to de-oiled silkworm pupa based diet.

In the present study also, growth of *P. monodon* juveniles decreased with increase in de-oiled silkworm pupae content beyond 20% level. Non-defatted silkworm pupa (30%) diet without fish meal gave better growth of Common carp indicating that non- defatted pupà could replace fish meal in the diet of Common carp, without affecting growth and quality (Nandeesha *et al.*, 1989). They found that increased level of pupa incorporation led to proportionate increase in growth of common carp, indicating the possible presence of growth promoting factors in non-defatted pupa. In yet another study with Common carp Nandeesha *et al.* (1989) obtained best growth with fish meal based diet, followed by that containing 10% de-oiled pupa and 15% fish meal. According to them the better growth observed with 10% inclusion of de-oiled silkworm pupa with 15% fish meal than the other combinations, might be due to the balance of nutrients in this combination to some extent. They concluded that it is not advisable to use de-oiled pupa beyond 10% in commom carp diet. Though rich in protein, de-oiled pupa appears to have a different amino acid profile as compared to fish meal, apart from lacking appetite stimulant (Keshavanath, 1993). In the present study, the results indicated that increased percentage of inclusion of de-oiled silkworm pupae beyond 20%, decreased the growth of *P. monodon* juveniles.

Hossain (1992) reported that *Oreochromis mossambicus* might be able to utilise silkworm pupae and de-oiled silkworm pupae meal efficiently. Habib *et al.* (1993) reported that silkworm pupae meal is a good dietary source of protein for *Clarias batrachus* and that growth in fish fed with diet containing 100% silkworm pupae meal was similar to that of diet containing 100% fish meal. The economic analysis showed that the diet containing 100% silkworm pupae meal was the cheapest amongst all diets and it costed least to produce unit biomass of fish.Shyama and Keshavanath (1993) observed that 50% defatted silkworm pupa induced best growth of mahseer,*Tor khudree*.Hossain *et al.*(1993) reported that diets containing 50 and 75% silkworm pupae meal produced best growth performance while the fish meal based control diet produced the lowest growth in cat fish (*Heteropneustes fossilis*) fingerlings. They suggested that silkworm pupae meal can be used as a substitute for fish meal in fish feed and up to 75% of dietary protein could be replaced with silkworm pupae meal in cat fish without affecting growth. In *Puntius gonionotus* highest growth performance was observed in fish fed a diet replacing about 38% of total dietary protein by silkworm pupae meal (Mahata *et al.*, 1994). They observed that silkworm pupae meal at all levels of inclusion showed better growth performances than fish meal based control diet and the result demonstrated that silkworm pupae may be useful as a partial replacement of fish meal protein. Economic analysis of diets suggested the possibility of using silkworm pupae as an alternative source of protein in *Puntius gonionotus* feed.

Ravishankar and Keshavanath (1988) reported better growth of *Macrobrachium rosenbergii* with silkworm pupae plus shrimp waste pelleted feed than with clam meat, silkworm pupae and fish meal. Unnikrishnan *et al.* (1991) observed no significant difference in weight gain of *Macrobrachium rosenbergii* reared on semi- purified diet containing de-oiled dry silkworm pupae and dry clam meat as protein sources. They reported that protein present in silkworm pupae is as good as that present in clam meat for *M. rosenbergii*. No statistically significant difference ($P \geq 0.05$) in average weight gain was observed between treatments T_1 (100% clam meal and 0% de-oiled silkworm pupae) and T_2 (80% clam meal and 20% de-oiled silkworm pupae) in the present study also. Das *et al.* (1995) reported that silkworm pupae is a poor source of protein for *M. malcomsonii* compared to prawn meal and mussel meat. Anilkumar (1994) also reported poor growth of *M. rosenbergii* fed with de-oiled silkworm pupae diet, compared to clam meal diet.

Ali (1988) observed poor growth rates when silkworm pupae was used as protein source in *Penaeus indicus* juveniles. He attributed this to the high lipid content in silkworm pupae, which produce an off- flavour due to rancidity of lipid, making the feed not attractive or acceptable to the shrimp. In the present study, though silkworm pupae was de-oiled, there was a good content of lipid and this might have resulted in decreased growth of *P. monodon* juvenile fed with higher levels of de-oiled silkworm pupae diet. In a later study in 1992, Ali obtained only lower growth of *P. indicus* with silkworm pupae diet than with fish meal, clam meat, shrimp waste and mantis shrimp diets. According to him silkworm pupae protein is not as good as clam meat protein for *P. indicus*. He attributed this to the presence of high non-protein nitrogen (NPN) content in silk worm pupae. The chief NPN component in silkworm pupae is chitin. The reduced growth rate of *P.monodon* juveniles at higher levels of silkworm pupae in the diet observed in the present study may be attributed to the improper utilization of this protein source by the shrimp.

Most of the plant proteins have been shown to yield poor growth rates in shrimps, when used individually, excepting a few like soyabean meal, wheat gluten, peanut meal etc (Kanazawa *et al.*, 1970; Sick and Andrews, 1973; Deshimaru and Shigueno, 1972; Balazs *et al.*, 1973). These plant protein sources have been observed to promote superior growth rates when mixed with animal protein sources (Lee,1970).

For the feed formulation of shrimps, it is found that animal protein sources are better than plant protein sources. It has been reported that among the animal protein sources, proteins of marine origin are superior to fresh water or terrestrial origin, not only due to their amino acid profile but also due to the better composition of their unsaturated fatty acids essential for shrimps (Cowey and Sargent, 1972) as well as higher ash content (Boghen and Castell, 1981). Das *et al.*(1990) reported that proteins of marine origin have a relatively high proline and arginine content, which might account for the good growth of prawn fed with protein from these sources. In the present study also, diet T_1 with 100% clam meal and 0% de-oiled silkworm pupae produced the highest weight gain.

Fresh clam meat was conventionally used as feed for prawns by Kanazawa *et al.* (1970) who reported that fresh short- necked clam *Tapes philippinarum* gave superior growth rates for *P. japonicus* compared to compounded diet. Ali (1982) obtained high increase in live weight of *P. indicus* using clam meat powder feed. Deshimaru *et al.* (1985) demonstrated that fresh clam could produce superior growth, survival and feed efficiency compared to a series of compounded diets in *P. monodon* juveniles and stated that fresh short necked clam is rich in essential amino acids with high ratios of methionine and arginine to total amino acid content besides being rich in polar lipids and sterols. Josekutty and Susheela (1991) obtained superior growth of *P. monodon* fed on diet containing clam meat as the major protein source. Rao (1994) stated that fresh clam meat along with mantle fluid could be incorporated with other low quality locally available ingredients, the fresh clam serving as an attractive flavouring agent. In the present study also best growth was obtained in *P. monodon* juveniles fed with diet T_1 containing 100% clam meal and 0% de-oiled silkworm pupae followed by diet T_2 containing 80% clam meal and 20% de-oiled silkworm pupae.

5.2.1.2. Specific growth rate

Specific growth rate can be considered as an index of growth in the evaluation of diets. The results of the present study indicate that the highest specific growth rate was obtained with the diet T_2 with 80% clam meal and 20% de-oiled silkworm pupae, followed by diet T_1 with 100% clam meal and 0% de-oiled silkworm pupae, though statistically there was no significant difference ($P \geq 0.05$) between these two diets. The comparatively better specific growth rate recorded with these two diets indicates their better utilization and efficient conversion. It was observed that specific growth rate decreases with increase in the content of de-oiled silkworm pupae in the diet and the lowest specific growth rate was observed with diet T_6 with 0% clam meal and 100% de-oiled silkworm pupae which reflect its poor utilization. Venkatesh *et al.* (1985) reported lower specific growth rate for *Clarias batrachus* with dried silkworm pupae compared to Halver's diet and fish meal. Ravishankar and Keshavanath (1988) reported highest specific growth rate of 1.1923% per day for *M. rosenbergii* with pelleted feed containing silkworm pupae and shrimp waste. Nandeesha *et al.* (1989) obtained comparatively better specific growth rate of 3.86% for Common carp fed with diet containing 10% de-oiled silkworm pupae and 15% fish meal than with other combination, though it was lower than the control diet containing 100% fish meal. Mahata *et al.* (1994) observed higher specific growth rate for *Puntius gonionotus* fed a diet replacing about 38% of total dietary protein by silkworm pupae meal. Das *et al.* (1995) reported poorer specific growth rate with silkworm pupae diet than prawn meal in *M. rosenbergii*. In the present study, all levels of silkworm pupae tried except 20% level was seen to have produced lower specific growth rates.

5.2.1.3. Survival

In the experiment, maximum survival was observed in treatment T_1, where *Penaeus monodon* juveniles were fed with pelleted feed containing 100% clam meal and 0% de-oiled silkworm pupae.The average overall survival observed was 86.66%. Nandeesha *et al.*(1989) obtained an overall survival of 76% for carps fed on de-oiled silkworm pupae pelleted feed. But in yet another experiment, Nandeesha *et al.* (1989) obtained a survival of 93.33 to 100% for common carp fed with different levels of de-oiled silkworm pupae and fish meal feed. Unnikrishnan *et al.* (1991) obtained a survival of 72.5% for *M. rosenbergii* post larvae with de-oiled silkworm pupae diets which was lower than that obtained with clam meat diet (75%). Shyama *et al.* (1993) reported that survival was unaffected by dietary administration of de-fatted silkworm pupae in *Tor khudree*. Das *et al.* (1995) reported a lower survival of *M. malcomsonii* juveniles fed with diet containing silkworm pupae as compared to that containing mussel meat.

5.2.1.4. Food conversion ratio

Food conversion ratio (FCR) is the ratio of food consumed by the animal to the live weight it has gained. It indicates the efficiency with which an animal can convert food for the growth process. Thus, low food conversion ratios indicate high efficiency in food utilization. In the present study, the lowest food conversion ratio of 2.52 was obtained for diet T_1 containing 100% clam meal and 0% de-oiled silkworm pupae and the highest of 6.89 for diet T_6 with 0% clam meal and 100% de-oiled silkworm pupae.

Specific studies on feeds and nutrition of *P. monodon* conducted at Kakdwip fish farm by Rajyalakshmi *et al.* (1979) have reported a food conversion ratio ranging from 2.4 to 6 for various feeds prepared from fish meal, shrimp meal and squid meal as main components. Feeding trials conducted on *P. monodon* using a practical feed in the Philippines (SEAFDEC, 1981) have shown a food conversion ratio of 4.8. In a semi-intensive culture experiment with the same species (SEAFDEC, 1983), a commercial prawn feed with 45% protein and an experimental feed with 35% protein produced FCR of 3.4 to 4.6 and 6.1 respectively. Ali (1982) while evaluating the nutritional quality of various protein sources obtained best feed efficiency for clam based diet for *P.indicus* juveniles. Srikanth (1986) reported an absolute conversion rate of 4.3 with de-oiled silkworm pupae diet in *Tor khudree.*

Ravishankar and Keshavanath (1988) reported an absolute conversion rate of 11.31, 6.84 and 7.77 for pelleted feeds containing silkworm pupae, silkworm pupae plus shrimp waste and silkworm pupae plus clam meat respectively for *M. rosenbergii.* Nandeesha *et al.* (1989) obtained a food conversion ratio of 2.24 with de-oiled silkworm pupae pellet for carp. In a subsequent experiment, Nandeesha *et al.* (1989) reported food conversion ratios of 3.14 to 3.38 for diets containing different levels of non-defatted silkworm pupae in Common carp. Nandeesha *et al.* (1989), in yet another experiment with Common carp reported a FCR of 2.96 with diet containing 10% de-oiled silkworm pupae and remaining 90% fish meal which was higher than control diet (2.85) of fish meal. Unnikrishnan *et al.* (1991) reported a FCR of 3.2 with de-oiled silkworm pupae diet which was higher than clam meat diet (3.0) for *M. rosenbergii.* Josekutty (1991) found that clam meal based diet gave the best FCR among the various protein sources tested in *P. monodon.* Ali (1992) reported a higher FCR with silkworm pupae diet than with clam meat powder diet for *Penaeus indicus*. Hossain *et al.* (1993) reported better FCR with diets containing 50 and 75% silkworm pupae protein for *Heteropneustes fossilis* fingerlings. Mahata *et al.* (1994) observed better FCR in *Puntius gonionotus* fingerlings fed a diet replacing about 38% of total dietary protein by silkworm pupae meal. Vijayakumaraswamy and Devaraj (1994) reported a FCR of 3.19 with dried silkworm pupae based feed in *Catla catla*. Habib *et al.* (1994) obtained comparatively better FCR for *Clarias batrachus* fingerlings fed with diet in which fish meal was replaced with silkworm pupae meal at different levels. The superior FCR obtained for 100% clam meal diet in the present study is indicative of better utilisation of the diets which may be due to the better nutritive quality of clam meal compared to the de-oiled silkworm pupae used in the diets. Anilkumar (1994) reported lower FCR of 2.69 with clam meal diet than de-oiled silkworm pupae based diet (3.82) for *M. rosenbergii*. In the present study also, best FCR of 2.52 was obtained with diet T_1 containing 100% clam meal and 0% de-oiled silkworm pupae, followed by diet T_2 (2.5466) with 80% clam meal and 20% de-oiled silkworm pupae and the highest FCR of 6.8933 with diet T_6 containing 100% de-oiled silkworm pupae and 0% clam meal.

5.2.1.5. Protein efficiency ratio

The results of the present study indicate that *P.monodon* juvenile fed on pellets containing 100% clam meal and 0% de-oiled silkworm pupae were more efficient in converting dietary protein followed by those fed on pellets containing 80% clam meal and 20% de-oiled silkworm pupae and the least efficient are those fed with 0% clam meal and 100% de-oiled silkworm pupae pellet.

Steffens (1981) reported that the protein efficiency ratio (PER) values can be used to evaluate the quality of protein in the diet, those with high PER values, are of good quality protein and those with low PER values are of poor quality. Colvin (1976) reported a PER ratio of 0.49 - 0.95 in *P.indicus* when fed on feed containing different seed oils. Sedgwick (1979) obtained PER values in the range of 0.076 - 0.902 in *P. merguiensis* when fed on feeds based on freeze dried *Mytilus edulis*, wheat starch, cod liver oil etc.

Ali (1992) reported lower PER in *P. indicus* with silkworm pupae diet than the clam meat powder diet. Unnikrishnan *et al.* (1992) obtained comparatively lower PER value with de-oiled silkworm pupae diet than with clam meat diet in *M. rosenbergii.* Hossain *et al.* (1993) obtained comparatively higher PER with diets containing 50% and 75% silkworm pupae in *Heteropneustes fossilis*. Mahata *et al.* (1994) reported better PER in *Puntius gonionotus* fed a diet replacing about 35% of total dietary protein by silkworm pupae. Das *et al.* (1995) reported a higher PER of 0.9 with prawn meal diet than silkworm pupa diet in *M. malcomsonii.* The lower PER values obtained in the present study with the increase in de-oiled silkworm pupae content as compared to 100% clam meal diet may be attributed to the improper utilisation of protein present in de-oiled silkworm pupae which inturn reflects the importance of the source of protein used in the diets and the species tried.

5.2.1.6. Productive protein value

Productive protein value (PPV) is considered as an appropriate and simple measure of utilisation of dietary protein by organisms. Steffens (1981) used productive protein values for comparing the protein utilisation in Rainbow trout and Carp. Degani *et al.*

(1989) also used this index to study the protein utilisation by *Clarias gariepinus*. James *et al.* (1990) reported a PPV of 6.67 for *Spirulina* based diet and 9.42 for casein based diet for *M. rosenbergii* post larvae of size 3.85mg. The PPV reported by Steffens (1981) and Degani *et al.* (1989) in fishes is high compared to those reported in prawns.

In general, protein utilisation depends essentially on the species and size, environmental factors, protein quality and level of dietary protein. It is also seen to be influenced by utilizable dietary energy, kind of energy source and amount of feed (Steffens, 1981). The dependence of protein utilisation on fish size and other factors was also shown by De Silva *et al.* (1989).

In the present study, maximum PPV was obtained in *P. monodon* juveniles fed with diet T_1 containing 100% clam meal and 0% de-oiled silkworm pupae. It was observed that PPV decreases with increase in the content of de-oiled silkworm pupae in the diets. The result indicates a better utilisation of clam meat protein than de-oiled silkworm pupae protein present in the diets by *Penaeus monodon* juveniles.

5. 3. Water quality parameters

5.3.1. Temperature

A temperature range of 28 ± 2 °C has been found to be optimum for *Penaeus monodon* growth (Forster and Beard, 1974). Several workers have reported wide temperature tolerance for *P.monodon* (Liao, 1977; Sasai, 1981; Chen, 1985). Chakraborti *et al.* (1986) observed a temperature tolerance of 24° - 30.5° C for this species. The maximum temperature tolerance of *P.monodon* has been reported to be 35° C (Ravichandran *et al.*, 1982). The weekly range of temperature observed during the present experimental period was 24°C to 28°C. The values recorded are within the optimum range suggested for the growth of *P. monodon*. The temperature fluctuation was gradual and could be maintained uniformly throughout the experimental period since the tanks were housed indoors.

5.3.2. pH

Subramanyam (1973) has observed that *Penaeus monodon* requires slightly alkaline pH, the optimum range being 8.1 - 8.5. Chen (1985) also suggested a similar pH range for its rearing. Chakraborti *et al.* (1986) obtained maximum growth rate at pH 8.4 - 8.7. A pH range of 7.3 - 8.5 has been suggested to be suitable for nursery rearing of *P.monodon* by Parado - Estepa *et al.* (1990). Noor-Hamid *et al.* (1992) recommended a near neutral pH of 7.6 - 8.0 for faster growth. In the present experiment, the weekly range of pH value in the tanks was from 7.5 to 8.5. These values conform to those obtained in the previous studies and is within the optimum range.

5.3.3. Dissolved oxygen

Studies made by Liao and Huang (1975) and Chen (1985) revealed that the oxygen consumption of post larvae of *Penaeus monodon* decreased whenever dissolved oxygen fell below 3.8 and 4.0 ppm. Chakraborti *et al.* (1985) reported that the dissolved oxygen below 2.5ppm affected the growth and survival of *P. monodon*. Chakraborti *et al.* (1986) have suggested the optimum range to be 6.8 - 7.6 ppm for *P. monodon* though they can tolerate dissolved oxygen as low as 4.8 ppm. The weekly dissolved oxygen values in the experimental tanks ranged from 6.8 to 8.2 ppm, since mild aeration was provided in the tanks. These values were found to be optimum for the growth of *P. monodon* juveniles.

5.3.4. Salinity

In brackish water ponds in the Philippines, juveniles of *P.monodon* grew about 50 - 100 g at 10 - 20 ppt salinity (Mochizuki, 1979), while in Indonesia, Manik *et al.* (1979) recorded better growth and production of juveniles at 15 - 20 ppt. Sundararajan *et al.* (1979); Sebastian *et al.* (1980) and Ravichandran *et al.* (1982) also reported optimum growth of *P.monodon* at a salinity range of 4 - 20 ppt. Navas (1988) did not find any significant difference in growth of *P. monodon* post larvae reared at 4.5 and 15 ppt with those reared at 20 ppt. Accordingly the salinity maintained in the experimental tanks for rearing *P. monodon* juveniles levels 20 ppt is the optimal level for this species.

5.4. Water stability of pelleted feeds

Stability of compounded pellets is of utmost importance in an aquaculture system, as the efficiency of the pelleted feeds depend on the retension of nutritive compounds in the pelleted feeds for longer time without disintegration. Also this makes the compounded pelleted feeds more economical. The formulated pelleted feeds should remain stable for atleast one hour, so that they become available for aquatic organisms.

Hastings (1971) and Kainz (1977) reported that ingredient composition, nature of ingredients, type of processing and moisture content are known to influence the feed stability. In the present study, diet T_6 containing 0% clam meat and 100% de-oiled silkworm pupae was the most stable at the end of six hours (stability 89.34%), compared to the other feeds tested while the diet T_1 with 100% clam meal and 0% de-oiled silkworm pupae was the least stable (stability 83.8%) at the end of six hours. According to Stivers (1971) and Jayaram and Shetty (1981), the degree of stability is dependant on the gelatinization of starch content of the feed during cooking. Hastings (1971) has stated that higher fat content affects gelatinization thereby reducing the stability of the feed. Jayaram and Shetty (1981) suggested that higher fat content prevents water penetration, thus facilitating to retain the compactiveness of the feed for longer time. In the present study the water stability of the different feeds is in the order, feed $T_6 > T_5 > T_4 > T_3 > T_2 > T_1$ whereas the fat content of the feeds is in the order, feed $T_6 > T_5 > T_4 > T_3 > T_2 > T_1$ which shows a linear relationship of water stability with fat content.

Prawns being demersal, feed by grasping the feed pellets with pincer like appendages and masticate externally (Forster, 1971; Zein-Eldin and Meyers, 1973). Hence pellets with high water stability are likely to show better results. Too much stability is also not desirable because, the nutrients in them become unavailable to organisms owing to their bound form in addition to the higher production cost (Balazs *et al.*, 1973). The pelleted feeds tested during the present investigation showed satisfactory stability up to six hours and hence suitable as shrimp feeds.

6. SUMMARY

1. The objective of the present investigation is to evaluate the feasibility of replacing clam meat at various levels with de-oiled silkworm pupae in the diet of black tiger shrimp *Penaeus monodon* juveniles.

2. Proximate analysis of de-oiled silkworm pupae and clam meat showed that de-oiled silkworm pupae contained 57.2% crude protein whereas, clam meat contained 53.22% crude protein.

3. Six isonitrogenous test diets with 35% protein were prepared for the study. They were diet T_1 with 100% clam meal and 0% de-oiled silkworm pupae, diet T_2 with 80% clam meal and 20% de-oiled silkworm pupae, diet T_3 with 60% clam meal and 40% de-oiled silkworm pupae, diet T_4 with 40% clam meal and 60% de-oiled silkworm pupae, diet T_5 with 20% clam meal and 80% de-oiled silkworm pupae and diet T_6 with 0% clam meal and 100% de-oiled silkworm pupae. The other ingredients included groundnut oil cake, tapioca powder, starch, cholesterol, vitamins and minerals.

4. Proximate analysis of the formulated pelleted feeds revealed that crude protein content of the diets ranged between 34.89% and 35%, crude fat content ranged between 6.25% and 9.85%, carbohydrate content between 40.253% and 46.75%, ash content between 6% and 8% and the caloric content between 3.83 and 4.06 Kcal/g.

5. Water quality parameters in the experimental tanks were monitered at weekly intervals and the variations observed in the water quality parameters were found to be well within the tolerance limits of *Penaeus monodon* juveniles.

6. The pellet T_6 was found to be the most water stable (89.34%) at the end of six hours, followed by the pellet T_5 (88.8%) and the pellet T_1 was found to be the least water stable (83.8%).

7. At the end of 42 days of rearing, the highest growth rate of 1.5282g was recorded in *P. monodon* juveniles fed with diet T_1 containing 100% clam meal and 0% de-oiled silkworm pupae, followed by diet T_2 with 80% clam meal and 20% de-oiled silkworm pupae (1.4886g) and the lowest (0.7350g) with diet T_6 containing 0% clam meal and 100% silkworm pupae. No statistically significant difference ($P \geq 0.05$) was observed between treatments T_1 and T_2.

8. The highest survival of 93.33% was recorded in *P. monodon* juveniles fed with diet T_1 and the lowest (83.333%) with diets T_5 and T_6.

9. The lowest food conversion ratio (FCR) of 2.5233 was recorded in shrimp juveniles fed on diet T_1, followed by diet T_2 (2.5466) while the highest was observed for diet T_6 (6.8933). Statistically no significant difference ($P \geq 0.05$) was observed in FCRs of treatment T_1 and T_2.

10. Protein efficiency ratio was found to be highest in shrimp juveniles fed on diet T_1 (1.1398), followed by diet T_2 (1.1255) and the lowest with diet T_6 (0.4168).

11. Productive protein value was found to be highest in *P. monodon* juveniles fed on diet T_1 (3.7917), followed by diet T_2 (2.6149) and the lowest with diet T_6 (0.3881).

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ABSTRACT

The present study was conducted to evaluate the feasibility of replacing clam meat either partially or completely with de-oiled silkworm pupae in the diet of the black tiger shrimp, *Penaeus monodon* juveniles.

Proximate analysis of the ingredients used in the preparation of formulated pelleted feed showed that de-oiled silkworm pupae contained 57.2% crude protein, while clam meat contained 53.22% crude protein. Six test diets were prepared with different levels of inclusion of clam meal and de-oiled silkworm pupae; the other ingredients being groundnut oil cake, tapioca powder, starch, cholsterol, vitamins and minerals. Diet T_1 contained 100% clam meal and 0% de-oiled silkworm pupae, diet T_2 80% clam meal and 20% de-oiled silkworm pupae, diet T_3 60% clam meal and 40% de-oiled silkworm pupae, diet T_4 40% clam meal and 60% de-oiled silkworm pupae, diet T_5 20% clam meal and 80% de-oiled silkworm pupae and diet T_6 0% clam meal and 100% de-oiled silkworm pupae. All the feeds were isonitrogenous with crude protein content ranging between 34.89% and 35% and isocaloric with caloric value ranging from 3.83 to 4.06 Kcal/g. Water stability levels of the pelleted feeds were found to be satisfactory ranging from 83.8% (T_1) to 89.34% (T_6) at the end of six hours.

Penaeus monodon juveniles were reared for six weeks in experimental tanks. The water quality parameters were maintained well within the tolerance limits of *P. monodon* juveniles throughout the course of the study. The highest growth

(1.5282g) was recorded in shrimp juveniles fed with diet T_1, followed by diet T_2 (1.4886g) and the lowest with diet T_6 (0.7350g). Analysis of variance of the data showed that there is no significant difference ($P \geq 0.05$) between treatments T_1 and T_2. No statistically significant difference ($P \geq 0.05$) could be discerned in specific growth rate of shrimp juveniles of treatmentsT_1 and T_2. The highest survival (93.33%) was obtained in *P. monodon* juveniles fed with diet T_1 and lowest (83.333%) with diets T_5 and T_6.

The food conversion ratios (FCR) obtained with different feeds range from 2.52 to 6.89. The lowest FCR was recorded with diet T_1 (2.52) followed by diet T_2 (2.5466) and the lowest with diet T_6 (6.89). Statistically no significant difference ($P \geq 0.05$) in FCRs of treatments T_1 and T_2 was observed.

Protein efficiency ratio was found to be highest in shrimp juveniles fed on diet T_1 (1.1398) followed by diet T_2 (1.1255) and the lowest with diet T_6 (0.4168). No statistically significant difference ($P \geq 0.05$) was observed in protein efficiency ratios of treatment T_1 and T_2.

Productive protein value was found to be highest in shrimp juveniles fed on diet T_1 (3.7917) followed by diet T_2 (2.6149) and the lowest with diet T_6 (0.3881).