EVALUATION OF *SPIRULINA FUSIFORMIS* AS A PROTEIN SOURCE IN THE DIET OF *PENAEUS MONODON* FABRICIUS

By

TANK KETAN VALLABHDAS

B.F.Sc.

THESIS

Submitted in partial fulfillment of the requirement for the degree of

MASTER OF FISHERIES SCIENCE

Faculty of Fisheries

Kerala Agricultural University

2008

DEPARTMENT OF AQUACULTURE

COLLEGE OF FISHERIES

PANANGAD, COCHIN

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DECLARATION

I hereby declare that this thesis entitled "**EVALUATION OF *SPIRULINA FUSIFORMIS* AS A PROTEIN SOURCE IN THE DIET OF *PENAEUS MONODON* FABRICIUS**'' 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.

Date: Panangad
Place: 12-08-2008

TANK KETAN VALLABHDAS
(2006-14-103)

CERTIFICATE

Certified that this thesis entitled "**EVALUATION OF *SPIRULINA FUSIFORMIS* AS A PROTEIN SOURCE IN THE DIET OF *PENAEUS MONODON* FABRICIUS** '' is a record of research work done independently by **TANKE KETAN VALLABHDAS** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

Date: Panangad
Place: 12-08-2008

Dr.Thresiamma James
(Chairperson, Advisory Board),
Professor (Aquaculture),
College of Fisheries,
Panangad, Kochi.

ADVISORY COMMITTEE/EXAMINATION COMMITTEE

NAME AND DESIGNATION ***SIGNATURE***

CHAIRPERSON

Dr.Thresiamma James,
Professor,
Department of Aquaculture,
College of Fisheries,
Panangad, Kochi.

MEMBERS

Dr. C. Mohanakumaran Nair,
Professor and Head,
Department of Aquaculture,
College of Fisheries,
Panangad, Kochi.

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Associate Professor,
Department of Aquaculture,
College of Fisheries,
Panangad, Kochi.

Dr. Lizy Behanan,
Professor (Retd),
Department of Processing Technology,
College of Fisheries,
Panangad, Kochi.

EXTERNAL EXAMINER
Dr. Aneykutty Joseph,
Professor,
Department of Marine Biology, Microbiology and Biochemistry,
Cochin University of Science and Technology,
Cochin

DEDICATED TO

MY PARENTS

&

Dr. THRESIAMMA JAMES

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1. INTRODUCTION

The giant tiger shrimp *Penaeus monodon* Fabricius is one of the most prominent farmed crustaceans in international trade and has featured significantly in the aquaculture in many developing countries in Asia. This trend is expected to continue in the foreseeable future. Apart from its high market potential, it is ideal for intensive cultivation because of its adaptability to different culture systems, rapid growth, ready availability of seed and positive response to supplementary feeding.

Efficient feed management is the major criteria for the successful crop since feed can account for over 60% of the production cost in intensive systems (Akiyama *et al*., 1992). For successful shrimp farming it is necessary to develop a feed which is efficient and economic. This requires the understanding of the nutritional requirement in terms of protein, energy, lipids, carbohydrates, vitamins and minerals.

Feed processing technology has also been developed indigenously. Farmers involved in extensive farming generally use either farm made or commercially available indigenous feed. Some of the progressive farmers are also using feed imported from South East Asian countries. The major problem faced by the indigenous technology is non-availability of quality ingredients. Feed and labour are the major components of the cost of cultured shrimp (Lawrence and Lee, 1997). The cost of the feed mainly depends upon the cost of the protein source. The most commonly used protein source is fish meal. The locally available fish meal is very often of inferior quality. Consequently, many feed manufacturing companies import fish meal for incorporating in their feed. Therefore, both price and availability of raw material continue to be a challenge (Singh *et al.*, 2007).

Owing to the high cost, fluctuating quality and uncertain availability, a variety of feed ingredients that can be used as alternatives for fish meal are being assessed in order to attain sustainable aquaculture in the current millennium. One of the approaches being made is to reduce the fishmeal proportion in shrimp feed with suitable plant protein sources and thereby reduce the cost of feed. Among the various alternative plant protein sources, algae are receiving increasing attention as possible protein source for fish/shrimp diets (Venkataraman, 1980). The

most commonly mass cultured algae which have been evaluated as protein source for fish feed are unicellular microalgae such as *Chlorella, Scenedesmus, Spirulina* and *Chaetoceros*.

Of all the algal forms, the blue green algae *Spirulina* has been exhaustively and extensively tested by scientists around the world, and is found to be one of the most powerful and well-balanced source of nutrition available on the planet (Sethi and Naik, 2007). It has the highest protein content of 60-65 % and is rich in vitamins, minerals, fatty acids (gamma-linolenic acid (GLA)) and antioxidant pigments such as carotenoids (Belay *et al*., 1996). *Spirulina* feed is of better quality, possessing improved flavour and results in firmer flesh, bright skin colour and increased survival rate (Mustafa and Nakagawa, 1995). Unlike the other algae, the blue green algae in general and *Spirulina* in particular are unique in that they are highly digestible and thus do not require special processing (Richmond, 1989). Several authors have mentioned that this microalga has an important role in metabolic, antioxidant, respiratory and immunological activities of aquatic organisms (Mustafa *et al..,* 1994; Miyasaki *et al.,* 1995; Gabaudam, 1998; Takeuchi *et al.,* 2002; Venkataraman, 2003). More recently, there has been renewed interest in the therapeutic effects of *Spirulina* as a "probiotic" or booster for the immune response system in fish and shrimp (www.brineshrimpdirect.com).

Nevertheless, only very few studies have been conducted on the use of *Spirulina* as a protein source in the diet of shrimp/prawn (Chow and Woo, 1990; James *et al*., 1990; Watanabe *et al*., 1990; Shripatrao, 2002; Jaime *et al.,* 2004 and Ceballos *et al*., 2005). Therefore, in the present study, an attempt has been made to find out the feasibility of using *Spirulina fusiformis* meal as a source of protein for black tiger prawn *Penaeus monodon* and also to evaluate the formulated diet through growth trials employing the postlarvae of *Penaeus monodon* to determine the optimum level of substitution.

2. REVIEW OF LITERATURE

2.1 Nutritional requirements of *Penaeus monodon*

The first exhaustive review on nutritional requirements of shrimp and prawn was made by New (1976). Feeding trials were also conducted in the laboratory using *Fenneropenaeus indicus* (Colvin, 1976), *Marsupenaeus japonicus* (Teshima *et al*. 1986; Liao, 1998), *Litopenaeus setiferus* (Lee and Lawrence, 1985) and *Litopenaeus vannamei* (Velasco *et al*., 2000; Brito *et al*., 2001). Dietary requirements for different penaeid species have been studied by various authors (Pascual, 1989; Chen, 1993; Millamena, *et al*., 1997; Shiau, 1998; Smith *et al.*, 1999; Ali *et al.*, 2000). Jones (1998) reported that the absolute nutritional requirement of penaeid can only be identified when a water stable formulated diet is accepted, ingested, digested and assimilated at comparable level of diets.

2.1.1. Protein

Growth rates of penaeid shrimp are closely related to the quality of the diet mainly to the protein source employed. Protein is the indispensable nutrient for growth and maintenance of all animals. It is typically the most expensive macronutrient in shrimp feeds and consequently their determination of the optimal protein level is important for formulation of cost effective diets (Velasco *et al*., 2000). Therefore, the research efforts have focused on identifying effective sources and their optimal inclusion levels in the diet (Colvin 1976; Liao and Liu, 1989; Conklin, 2003).

2.1.1.1 Quantitative protein requirements

Animals, unlike plants cannot synthesize protein from inorganic nitrogen. Therefore, dietary protein is essential for all animals including shrimp. Many of the studies on the protein requirement of shrimp have yielded various results. Deshimaru and Yone (1978) have demonstrated that *Marsupenaeus japonicus* showed best growth with a diet containing 52-57% protein whereas Paulraj (1993) reported best growth on 45-50% protein content. In case of *Litopenaeus vannamei*, Pedrazzoli *et*

al. (1998) obtained best growth with 30-40% protein level. On the other hand Velasco *et al.* (2000) reported comparable growth in postlarvae with protein levels as low as 18-20%. Wu and Dong (2002) have indicated the optimum protein level for *Fenneropenaeus chinensis* as 45%.

The optimum protein requirement of *Fenneropenaeus indicus* has been reported as 35-50% (Colvin, 1976; Mathew and Jayaprakash, 1990; Boonyaratpalin, 1998). Sambavisam *et al.*, (1982) recorded optimum protein requirement values for *Fenneropenaeus indicus* to be 50-60% whereas Paulraj (1993) reported highest growth at 35-40% protein level. Sahadevan (1994) reported that growth of *Fenneropenaeus indicus* was optimum at the protein level of 40% in the diet.

According to many workers the protein level required by *Penaeus monodon* postlarvae were between 35 to 50% (Bages and Sloane, 1981; Godfred-Ponraj, *et al*. 1990; Mathew and Jayaprakash, 1990; Chen, *et al.*, 1992; Paulraj, 1993). Protein levels of starter, grower and finisher feed are 40-45%, 38-40% and 35-38% respectively in feeds prepared by CIBA (Ali, 2006). Studies at SEAFDEC AQD indicated a need for 40% protein in the diet of *Penaeus monodon* growout rearing (Shiau *et al*., 1991).

The diversity of optimum protein levels for crustaceans is probably an account of a variety of factors *viz*., discrepancy in food habits, age of specimen, salinity, water temperature and energy level of diet. The variation in protein requirement was also due to differences in biological value of protein sources, composition of protein (Harper, 1981; Kies, 1981), composition of other dietary components, *viz.,* fat and carbohydrate (Teshima and Kanazawa, 1984).

Among the dry feeds tested, maximum growth rate was obtained with 40% protein pellets (Shiau *et al*., 1991). As the dietary protein was increased to 50%, 60%, 70% and 80% there was corresponding decrease in growth rate and survival rate (Venkatramiah *et al.*, 1975). Burford *et al.* (2004) used 3 experimental diets with 30%, 35% and 40% protein level. They found that shrimp growth rate was statistically higher in the 35% and 40% diet. In general, for optimum growth of *Penaeus monodon* a protein level of about 40% in the diet was reported by

Sahadevan (1994). Therefore, the present study has been conducted using 40% protein in the diet of *Penaeus monodon*.

2.1.2. Amino acids

Since amino acids are the 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 bioavailability of natural amino acids. Shrimp are unable to synthesize the 10 essential amino acids (EAA) in the diet. These amino acids such as arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine are generally considered essential for animals (Conklin, 2003).

The optimum dietary requirements of *Penaeus monodon* for essential amino acids, in percent of the diet, were: 0.8% histidine, 1.01% isoleucine, 1.7% leucine, 1.4% phenylalanine and 0.5% tryptophan (Millamena *et al*., 1999), 2.08% lysine, 1.85% arginine (Millamena *et al*., 1998), methionine 1.3% and cystine 0.41% (Millamena *et al*., 1996). Expressed as percent of the dietary protein, the requirement values are: 2.2% histidine, 2.7% isoleucine, 4.3% leucine, 3.7% phenylalanine and 0.5% tryptophan (Millamena *et al*., 1999), 5.2% lysine, 5.3% arginine (Millamena *et al*., 1998), methionine 2.4% and cystine 1.1% (Millamena *et al*., 1996).

The assessment of essential amino acid requirements (expressed as percentage of protein) for *Penaeus monodon* are: 3.71% arginine, 0.69% histidine, 0.61% isoleucine, 1.036% leucine, 3.16% lysine, 1.26% methionine, 1.70% phenylalanine, 1.61% threonine, 0.51% tyrosine and 0.6% valine (Alagarswami and Ali, 2000). On the other hand the values reported by Lawrence (2004) for *Penaeus monodon,* were: 5.3% arginine, 2.2% histidine, 2.7% isoleucine, 4.3% leucine, 5.2% lysine, 2.4% methionine, 3.7% phenylalanine, 3.5% threonine, 0.5% tryptophan and 3.7% valine.

Dy-Penaflorida (1989) found that cystine, glycine, serine and histidine were significantly higher in juveniles than in adults. The amino acid composition of the

shrimp muscle is used to provide guidance values in with feed formulation. The ill effects of feeds without balanced amino acid or with poor quality protein are manifested as slow growth, low protein efficiency and higher dietary protein requirement (Mitra *et al.*, 2005).

2.1.3. Lipids and fatty acids

Lipids are a source of essential fatty acids, sterols, phospholipids and fat-soluble vitamins. Lipids are also an important source of metabolic energy. Since shrimp do not utilize carbohydrates particularly well, lipids are often used in rations as a key source of energy. This reduces the protein denaturation (removal of nitrogen from amino acids) for use as energy. For shrimp, optimum levels of lipid inclusion were between 5% and 8% of the diet (D'Abramo, 1997). Recommended lipid levels for commercial shrimp feed range from 6% to 7.5% and a maximum level of 10% was suggested (Akiyama *et al*., 1991). Higher lipid levels reduced the growth of shrimp (Gonzalez-Felix *et al*., 2002).

As is the case with protein, shrimp do not have a specific need for lipid, but rather they require specific fatty acids, cholesterol and perhaps phospholipids. Growth experiment of early post larval *Penaeus monodon* with alginate-encapsulated diets indicated the requirement for HUFA to be 0.5-1 % of the diet (Chen, 1993). However, excessive dietary n-3 HUFA may lead to detrimental effects on both the growth and survival of postlarvae. Dietary lipid requirement of *Penaeus monodon* is 3.5-8% and essential fatty acid requirement (in percentage) were: Linoleic acid (18:2n-6) 0.05%, Linolenic acid (18:3n-3) 0.10%, Eicosapentaneoic acid (20:5n-3) 0.01%, Docosahexanoic acid (22:6n-3) 0.005% and Lecithin 0.1-0.2% (Alagarswami and Ali, 2000).

Differing from most animals, marine shrimp have only limited ability to transform PUFAs into HUFAs (Kanazawa and Teshima, 1977). Gonzales-Felix and Perez-Velazquez (2002) suggested that this species was able to meet its essential fatty acid requirements with highly unsaturated fatty acids from either the n-3 or n-6 family of fatty acids. Teshima *et al*. (1992) analyzed the requirements of essential fatty acid by sparing of EPA and DHA by linolenic acid. There may also be a need for a balance between n-6 and n-3 fatty acids in the diet (Xu *et al*., 1993).

Merican and Shim (1997) suggested a 1.44% fatty acid requirement for *Penaeus monodon*. Deering *et al*. (1997) have suggested, based on their studies with *Penaeus monodon,* that to improve dietary formulation, EFA levels should be considered as a proportion of total fatty acids in the diet and not simply as a percentage of the total diet. The combination of vegetable oil and fish oils generally produces superior results, but experimental results were still contradictory (Lim and Akiyama, 1995).

2.1.4. Sterols

Cholesterol is an important animal sterol which occurs free or in combination with fatty acids in all cells and blood. It serves as a precursor of a number of compounds, such as sex hormones, adrenal corticoids, bile acids and vitamin D. Most animals can synthesize sterols from acetate, but crustaceans have shown to be incapable of *de novo* sterol synthesis from acetate (Teshima and Kanazawa, 1971). Therefore, dietary cholesterol is considered to be essential for good growth and survival of crustaceans. It has been found to be most effective at dietary inclusion level of 0.25% to 0.50% for crustaceans (Paulraj, 1993).

The interaction between cholesterol and phosphatidylcholine on weight gain of *Penaeus penicillatus* and *Penaeus monodon* has been investigated by Chen and Jenn (1991) and Chen (1993) respectively. Both studies used three level of dietary cholesterol (0, 0.5 and 1%). These workers concluded that 0.5% dietary cholesterol required for good growth of *Penaeus penicillatus* and *Penaeus monodon*.

The optimum dietary cholesterol level of 0.5% is not difficult to achieve in multiingredient diets (New, 1976). The effect of dietary cholesterol on the growth and survival of juvenile tiger shrimp (*Penaeus monodon*) was tested using semi-purified diets by Sheen *et al*. (1994). There was no significant difference in weight gain of *Penaeus monodon* fed the diet containing 0.2-0.8% cholesterol. However, the diet containing 1% cholesterol had an adverse or toxic effect on *Penaeus monodon.*

2.1.5. Phospholipids

The deficiency of phospholipid in the diet significantly reduced the weight gain and feed efficiency (Teshima *et al*., 1982). Deshimaru *et al.* (1985) suggested that both polar-lipids and sterols may be essential ingredients in the diet of *Penaeus monodon* and of *Marsupenaeus japonicus.* Paulraj, (1993) reported that shrimp required a source of phospholipids rich in phosphatidylcholine with same quantity of phosphatidylethanolamine. Shrimp require inositol for normal growth, moulting, metamorphosis and maturation.

A requirement for dietary phospholipids particularly phosphatidylcholine has been demonstrated in various species, including larval (Teshima *et al*., 1982) and post larval *Marsupenaeus japonicus* (Teshima *et al*., 1986) and *Penaeus monodon* (Chen, 1993). Inclusion levels of dietary phospholipids reported for various species of penaeids ranged from 0.84% to 1.25% (Chen, 1993). Soybean lecithin at dietary level of 1-2% is observed to promoted growth in shrimp (Paulraj, 1993)

2.1.6. Carbohydrates

Carbohydrate is a cheap natural source of energy. Digestible carbohydrates, at optimum levels spare protein for growth, especially in omnivorous and herbivorous species. Carbohydrates are used mainly for their energy value and also to spare protein from being used for energy. Hence, both fats and carbohydrates are known as protein spares. Disaccharides, trehalose, and sucrose are utilized better by shrimp. Starches provide energy and also are useful for their binding properties in the diet. Around 25% nitrogen-free extract or carbohydrates excluding fiber was suggested in the diet of *Penaeus monodon* juveniles (Paulraj, 1993).

Pascual *et al*. (1983) fed diets containing maltose, sucrose, dextrin, molasses, cassava starch, corn starch or sago palm starch to *Penaeus monodon* at levels of either 10% or 40% and found no correlation between survival and relative complexity of carbohydrates. Alava and Pascual (1987) fed diets containing either 10 or 30% trehalose, sucrose or glucose to *Penaeus monodon*. The author furthermore demonstrated that those shrimp fed diets containing trehalose and sucrose led to higher weight gains and lower mortality than those fed the glucose diet.

Shiau and Peng (1992) investigated the utilization of different carbohydrate sources and the possible sparing effect of dietary protein by carbohydrate for *Penaeus monodon* reared in sea water. In their study, three dietary protein levels (40, 35, 30%) and three carbohydrates levels (20, 25 and 30%) from three carbohydrate sources (glucose, dextrin, and starch) were tested. Results indicated that shrimp fed starch or dextrin had significantly higher weight gain, feed efficiency ratio, protein efficiency ratio and survival than those fed with glucose. It also appears that starch has a better protein sparing effect than dextrin or glucose. Accordingly, the required dietary protein level for *Penaeus monodon* is lower if starch, instead of glucose or dextrin, is used as the carbohydrate source.

For *Penaeus monodon,* Pascual *et al.* (1983) found that the highest survival rate (56%) was obtained in juveniles fed with diet containing 10 % sucrose. He inferred that sucrose is the best source of carbohydrate for *Penaeus monodon.* However, at higher levels energy utilization tends to be less efficient, resulting in poor digestibility. An optimum dietary sugar level for *Penaeus monodon* was therefore considered to be 20 % (Bautista 1986; Alava and Pascual, 1987). Paulraj (1993) reported a starch level of 35-40% in feeds for semi-intensive culture.

2.1.7. Vitamins

Vitamins are important in regulating body processes. The B vitamins are necessary for proper utilization of proteins, carbohydrates, and fats. Vitamin A and C are important in building resistance to diseases. Vitamin D together with minerals like calcium and phosphorus is necessary for the formation of exoskeleton or shell. All the nutrients are so interrelated that they have to be incorporated in proper amount in the diet to be efficiently utilized by the shrimp (Pascual, 1989).

D'Abramo and Sheen (1994) estimated the dietary vitamin C requirement of *Penaeus monodon* is to be approximately 100 mg/kg. Shiau and Hwang (1994) indicated that the adequate dietary cholecalciferol (vitamin D) concentration for growing *Penaeus monodon* is 0.1 mg/kg. Vitamin E requirement of juvenile grass shrimp *Penaeus monodon* was found to be 200 mg/kg (Lee and Shiau, 2004). Shiau and Liu (1994) reported that adequate dietary vitamin K concentration in growing *Penaeus monodon* was 30–40 mg/kg diet. Other vitamin requirements were:

thiamine-13.14 mg (Chen *et al*., 1991), riboflavin 22.5 mg (Chen and Hwang, 1992), pyridoxine 30-50 mg (Paulraj, 1993), pantothenic acid 50-100 mg (Paulraj, 1993), niacin 7.2 mg (Shiau and Hwang, 1994), folic acid 2-8 mg (Lung, 1991), biotin-1 mg (Paulraj, 1993) and vitamin B_{12} 0.02-1 mg (Paulraj, 1993) per kg of diet.

Dietary vitamin requirement of *Penaeus monodon* per kg of diet, were: thiamine 120 mg, riboflavin 40 mg, pyridoxine 120 mg, pantothenic acid 100 mg, niacin 150 mg, folic acid 5 mg, biotin 1.0 mg, vitamin B_{12} <0.1 mg, choline 600 mg, inositol 2000 mg, vitamin C plain 261.5 mg, vitamin C protected 40.25 mg, vitamin D 0.025-0.05 mg, vitamin E 200 mg, vitamin K 40 mg and vitamin A 3-6 mg (Alagarswami and Ali, 2000). In general, vitamin requirement for *Penaeus monodon* for starter, grower and finisher feed was 0.5-2 g/kg of feed (Paulraj, 1993).

2.1.8. Minerals

Investigation on mineral requirements of shrimp is very limited (Shiau, 1998). Alagarswami and Ali (2000) reported in detail the dietary mineral requirement of *Penaeus monodon.* It was found to be calcium 20-20.5%, phosphorus 1.2-1.4%, potassium 0.7-0.9%, magnesium 0.08-0.15%, iron 60-80 mg, zinc 80-100 mg, manganese 40-50 mg, copper 8-10 mg, cobalt 0.8-1 mg, iodine 4-5 mg, chromium 0.6-0.8 mg and selenium 0.17-0.21 mg in kg of diet.

2.2 Recent progress in the use of processed microalgae in aquaculture

Research to date has focused largely on the use of processed microalgae as a sole or major component of aquaculture diets. However, considerable potential exists to use microalgae to fulfill specific nutritional requirements (Sommer *et al.*, 1992). Microalgal feeds are currently used in relatively small amounts in aquaculture, mainly for the production of larvae and juvenile shellfish and finfish, as well as for raising zooplankton required for feeding juvenile animals (Benemann, 1992). Mass cultured algal biomass has been tested as a food source for a number of aquaculture animals because of its low cost and convenience. Nutritional studies using species of *Spirulina, Chlorella, Scenedesmus* and other mass produced algae with respect to the

culture of molluscs, crustacean, rotifer and fish culture indicated that microalgae could be an effective dietary component provided processing, diet formulation and representation requirements are met (Sommer *et al.*, 1992).

Benemann (1992) emphasized the potential large-scale application of microalgae in the cultivation of *Haematococcus* for the production of the carotenoid astaxanthin. In the long term, microalgae biomass high in lipids (omega-3 fatty acids) may be developed as substitutes for fish oil based aquaculture feeds. In shrimp ponds, the indigenous algal blooms supply a part of the dietary requirements of the animals. The development of cultivation technologies for such microalgae would allow the onshore production of fin fish and shellfish, with greatly improved product quality and safety. He also opined that, the blue-green alga *Spirulina* can be used in substantial amounts as a fish and shrimp feed with scope for market expansion if production costs could be reduced. A separate algal production system could feed the shrimps and minimize the need for supplementary feed (Benemann, 1992).

2.3. Chemical composition of *Spirulina*

2.3.1. Amino acids and fatty acids

Switzer (1980) studied amino acid composition of *Spirulina* in detail and reported that it is generally well balanced but is low in sulphar containing amino acid and tryptophan. Amino acid analysis of spray-dried *Spirulina* in g/(16gN)$^{-1}$ was isoleucine 5.7 g, leucine 8.7 g, lysine 5.1 g, methionine 2.6 g, phenylalanine 5.0 g, threonine 5.4 g, tryptophane 1.5 g, valine 7.5 g, alanine 7.9 g, arginine 7.6 g, aspartic acid 9.1 g, cystine 0.9 g, glutamic acid 12.7 g, glycine 4.8 g, histidine 1.5 g, proline 4.1 g, serine 5.3 g and tyrosine 4.6 g (Switzer, 1980). From the nutritional stand point, the most important fatty acid components are linolenic (C_{18}) acid and γ–linolenic acid, which in *Spirulina* were 1.24% and 1.04% of dry weight, respectively (De Pauw *et al.*, 1984).

2.3.2. Carbohydrates

Quillet (1975) analyzed the carbohydrates of *Spirulina* in detail. These constitute approximately 15% of the dry matter and hydrolysis yielded glucose,

levulose, sucrose, glycerol and several polyols. Cholesterol and β-sitosterol have been isolated and identified in *Spirulina maxima* (Nadal, 1971).

2.3.3. Vitamins and Minerals

Switzer (1980) reported that the vitamin content of *Spirulina* per kg of body weight as follows: provitamin A 1700 mg; Cyanocobalamine (B_{12}) 1.6 mg; Vitamin B_5 11 mg; Folic acid 0.5 mg; inositol 350 mg; Niacin (B_3) 118 mg; Pyridoxine(B_6) 3 mg; Thiamine (B_1) 55 mg and Tocopherol (E) 190 mg. Mineral content of *Spirulina* is also high and it contains the various minerals (g/100 g dry algae) like calcium 0.36 g; phosphorus 0.88 g; iron 0.53 g; sodium 0.05 g; chlorine 0.46 g and others 7.40 g (Venkataraman, 1983).

2.3.4. Pigments

Pigments in *Spirulina* belong to three classes: a) Chlorophyll a, comprising 1.7% of the organic cell weight. b) Carotenoids and xanthophylls, which comprise approximately 0.5% of the organic weight (Tornabene *et al.*, 1985). C) Two phycobiliproteins; c-phycocyanin and allophycocyanin, which normally comprise about 20% of cellular protein are quantitively the dominant pigments in *Spirulina*. Astaxanthin was shown to be the predominant carotenoid associated with the body colour of the black tiger prawn *Penaeus monodon* (Howell and Matthew, 1991).

2.4. Nutritive role of *Spirulina*

Sethi and Naik (2007) reported that *Spirulina* is nature's richest source of protein (60-72%) and it provides all essential and non-essential amino acids, vitamins like A, B_1, B_2, B_6, C, D, E, F and H along with an array of trace elements. *Spirulina* is also a good source of beta-carotene, gamma-linolenic acid and Vitamin B_{12}. Blue green algae, in general, and *Spirulina* in particular, are unique in that it has no cellulose in its cell walls, being composed of soft mucopolysaccharides. This makes it easily digestible and assimilable and therefore does not require special processing (Becker and Venkataraman, 1982).

As feed additives, dried algae improve growth, feed efficiency, carcass quality, and physiological response to stress and disease in several species of fish (Mustafa and Nakagawa, 1995). An evaluation was made of the benefits of *Spirulina* in the fish and shrimp culture industry such as better growth rates, improved quality colouration, better survival rates, reduced medication requirement and reduced waste in the effluent (Henson, 1990; Sethi and Naik, 2007). It has also been found recently to have application in the preparation of biofertilizer, cattle feed, fuel and in the making of cosmetics (Sethi and Naik, 2007).

2.5. Usage and potential of *Spirulina* in aquaculture

Among the algae employed in commercial aquaculture, the cyanobacterium *Spirulina* (Arthrospira) probably has the broadest range of application (Belay *et al*., 1996). Reports on the utilization of *Spirulina* in aquaculture dates back to the seventies, when the commercial mass cultivation of these microalgae was still in its early stage. Already in the 1976, studies were performed by Stanley and Jones (1996) on the effect of feeding *Spirulina* to big mouth buffalo (*Ictiobus cyprinellus*) and blue tilapia (*Tilapia aurea*). At a daily feeding rate of 29 g of algae per kg body weight, the authors obtained a daily growth rate of 14 g/kg of body weight and a FCR of 2.0. In contrast, feeding *Spirogyra* to grass carp (*Ctenopharyngodon idella*) resulted in very poor growth and FCR of about 10. These findings led to the conclusion that an aquaculture system based on filamentous green algae and grass carp is less efficient than one based on *Spirulina* and tilapia or bigmouth buffalo.

Successful substitution of formulated diets upto 50% *Spirulina* was reported by Santiago *et al.* (1989) for the growth of milkfish fry (*Chanos chanos*). Ayyappan (1991) evaluated the potential of *Spirulina* as feed for carp fry, in rural development in Central India, on six different carp species including silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idella*) and common carp (*Cyprinus carpio*). The fish were fed with either a control diet composed of a mixture of groundnut oilcake and rice bran, or with the addition of 10% *Spirulina* to this mixture; grass carp and common carp in ponds were also studied by feeding them live algae. In almost all trials, addition of *Spirulina* to the diet resulted in a

better performance of the fish. Manju (1994) also evaluated the *Spirulina fusiformis* as a protein source in the diet of *Etroplus suratensis*.

Nandeesha *et al.* (1998) fed common carp a series of diets in which different amounts of fish meal protein were replaced by *Spirulina platensis* (25%, 50%, 75% and 100%). They demonstrated that even 100% replacement by alga had no negative effects on final weight, specific growth rate, feed conversion ratio and protein efficiency ratio, not on any other parameter including organoleptic quality. On comparing the nutritive potential of soyabean, *Spirulina* meal and chicken offal meal as replacement for fish meal in the feed of the silver sea bream (*Rhabdosargus sarga*). *Spirulina* was found to be utilized more effectively than other two diets (El-Sayed, 1994). *Spirulina* is employed as a supplement in regular diets, not only in fish rearing, but also in shrimp farming. The production of the giant fresh water prawn (*Macrobrachium rosenbergii*), significantly improved by dietary inclusion of *Spirulina* meal. Survival and feed utilization upto a supplementation level of 20% in the feed improved, probably due to enhancement of protein utilization (James *et al*., 1990; Nakagawa & Gomez-Diaz, 1995).

2.6. Use of *Spirulina* for shrimp

Relatively little information is available on the use of processed microalgae for other crustaceans (Lim *et al.* 1979). He compared spray-dried *Spirulina* sp. powder, casein, shrimp meal and squid meal as a protein source for postlarval *Penaeus monodon*. Tanaka (1978) tested diets containing 10% *Spirulina* as a source of pigment for the tiger prawn *Marsupenaeus japonicus*. He concluded that shrimp grown on algal powder had more intense colouration and higher pigment levels. A preliminary study on the evaluation of casein, shrimp meal, squid meal and *Spirulina* meal as a protein source for postlarvae of *Penaeus monodon* Fabricius and found that the *Spirulina* based diet gave better growth than other test diets (Lim, *et al.*, 1978). Tsai (1981) reported that weight gains of *Marsupenaeus japonicus* was more satisfactory with feed containing *Spirulina platensis* and fish meal as a source of protein. He observed that the weight gain of the juvenile shrimp was more satisfactory with fish meal and *Spirulina platensis* than on *S. platensis* alone.

Ali (1992) evaluated five animal protein sources, namely clam meat powder, fish meal, mantis shrimp, shrimp waste and silkworm pupae; three plant protein sources, coconut cake, gingely cake, groundnut cake; and the single cell protein *Spirulina* were evaluated for formulating isonitrogenous diets for the shrimp *Fenneropenaeus indicus*. Results revealed that groundnut oilcake and *Spirulina* produced significantly higher growth than coconut and gingely cakes. However, the net protein utilization (NPU) and biological value (BV) of *Spirulina* were significantly higher than that of groundnut cake. D'Souza *et al.* (2000) studied that centrifuged micro algal concentrates as diets for *Penaeus monodon* postlarvae and found that centrifuged concentrates could be as effective as fresh algae when fed in conjunction with the artificial diet.

Spirulina flakes boost shrimp growth (Anon, 1999). In the shrimp farming industry though many feed additives have been utilized *Spirulina* is the only microalgae additive which demonstrates benefits to growers that offset the initial cost and provide significant cost/performance ratio. *Spirulina* is the single most effective natural source of pigmentation for cultured *Penaeus monodon. Spirulina* significantly enhances the pigmentation of *Penaeus monodon* and increases the economic value (Lorenz, 1998; Puanglap, 2000).

Chuntapa *et al.* (2003) studied water quality control using *Spirulina platensis* in shrimp culture tanks. Hemtanon *et al.* (2005) reported the application of *Spirulina platensis* for prevention of white spot syndrome virus in postlarvae and juvenile black tiger shrimp (*Penaeus monodon*). Soong (1980) reported that *Spirulina* powder was an excellent source of food for shrimp larvae. Jaime *et al.* (2004) evaluated the effect of *Spirulina platensis* meal as a feed additive on growth, survival and development in *Litopenaeus schmitti* shrimp larvae and indicated that 5% *Spirulina* promoted better performance.

3. MATERIALS AND METHODS

The experiment was conducted to evaluate *Spirulina fusiformis* as a protein source in the diet of *Penaeus monodon* postlarvae. Dietary evaluation of the formulated diet through growth trials was done to determine the optimum level of substitution. The experiment was conducted at the Department of Aquaculture, College of Fisheries, Panangad, Cochin over a period of 60 days.

3.1. Preparation of the feed

3.1.1. Feed ingredients

Six experimental diets (T1, T2, T3, T4, T5 and T6) were prepared for *Penaeus monodon* postlarvae at same level of protein 40% and different percentage of *Spirulina viz.,* 0%, 5%, 10%, 20%, 30% and 40%. Control diet (T1) was prepared by using clam meat, wheat bran, groundnut oil cake, tapioca flour, cod liver oil and vitamin-mineral mixture. Different test diets (T2 to T6) were prepared by replacing clam meat with *Spirulina*. The ingredient proportions of the experimental diets are given in the Table 1. The composition of Supplevite-M, the vitamin mineral mix incorporated in the feed is presented in Table 2.

Plate 1 ***Spirulina fusiformis***

Table 1. Ingredient composition (g/100g) of experimental diets fed to *Penaeus monodon* postlarvae

Treatment/ ingredient	T1 (0%S)	T2 (5%S)	T3 (10%S)	T4 (20%S)	T5 (30%S)	T6 (40%S)
Spirulina meal	-----	5	10	20	30	40
clam meat	40	35	30	20	10	00
Wheat bran	12.5	14	14.5	15	15.5	17
GOC	39.5	38	37	35	33.5	32
Tapioca flour	6	6	6.5	8	9	9
Cod liver oil	1	1	1	1	1	1
Vitamin & Mineral mix	1	1	1	1	1	1
TOTAL	100	100	100	100	100	100

S-*Spirulina fusiformis*

Table 2. Composition of *Supplevite-M (Vitamin mineral concentrate).

Each 250 mg provides	Quantity
Vitamin A	500,000 IU
Vitamin D3	100,000 IU
Vitamin B2	0.2g
Vitamin E	75 units
Vitamin K	0.1g
Calcium pantothenate	0.25g
Nicotinamide	1g
VitaminB12	0.6 g
Choline chloride	15g
Calcium	75g
Manganese	2.75g
Iodine	0.1g
Iron	0.75 g
Zinc	1.5g
Copper	0.2g
Cobalt	0.045g

***Supplevite-M: Sarabhai Chemicals, Mumbai**

3.1.2. Processing of the feed ingredients

Various ingredients used for feed formulation were processed as follows:

All the ingredients for formulation of the feeds were procured locally except *Spirulina* powder which was brought from Aqua world, Chennai. Meat of the black clam *Villorita cyprinoides* was washed, dried and powdered. The other ingredients namely wheat bran, groundnut oil cake and tapioca flour were dried, powdered and sieved through 250µ mesh. The powdered ingredients were stored separately in air tight plastic bottles and kept in a refrigerator along with other ingredients till they were used for feed preparation.

3.1.3 Proximate composition of feed ingredients

Proximate composition of all the feed ingredients was analyzed prior to feed formulation following AOAC (1990) methods.

Moisture content%	:	Drying the sample at 105ºC till a constant weight was arrived
Ash content %	:	Ashing the sample at 100±10ºC for 6 hours in a muffle furnace.
Crude fat %	:	Solvent extraction using petroleum ether (BP 40-60ºC) in a soxhlet extraction apparatus for 6 hours.
Crude protein%	:	Microkjeldhal method (AOAC, 1990).
Crude fibre%	:	Pearson method (1976).
Nitrogen free extract %	:	100-(%protein+%lipid+%ash+%fiber+%moisture) By difference method (Hastings, 1976).

3.1.4. Formulation and processing of experimental diets.

Six types of pelleted feeds were formulated fixing their protein level at 40%. The experimental feeds were prepared separately by mixing required quantity of ingredients. The respective ingredients were weighed accurately in an electronic balance and all the ingredients except Supplevite-M and cod liver oil was mixed well with sufficient water to make smooth dough. The dough was transferred to a vessel and steamed for 20 minutes in a pressure cooker. The steamed dough was cooled under fan. Cod liver oil and vitamin-mineral mixture were added to this and mixed well. Then it was extruded through a hand pelletizer and dried in an oven at 60ºC to a moisture content of less than 12%. After drying, the pellets were broken into small pieces and packed in airtight plastic bottles.

3.1.5. Proximate composition of experimental diets

Proximate composition of the experimental diets was analyzed to evaluate the nutrient status. Methodology employed was the same as that of the ingredients for analysis (AOAC, 1990).

3.1.6. Water stability of experimental diet

Water stability of feeds was determined using the percentage of dry matter recovered after immersing the pellets in water for 1, 2, 4 and 6 hours interval. (Jayram and Shetty, 1981).

3.2. Experimental animals

The postlarvae of *Penaeus monodon* were obtained from a single female hatch were procured from Government Hatchery (ADAK), Varkala and were transported to the College hatchery in oxygen filled polyethylene bags. Five hundred postlarvae were acclimatized at 20 ppt into an oval, round bottomed fiber glass tank of 1.2 ton capacity. Gentle aeration was provided using air diffuser stones. The postlarvae were fed *ad libitum* with granulated artificial feed having clam meat as the chief source of protein. Leftover feed and waste were removed daily by siphoning and 25% of water was renewed every 2 days. Postlarvae of uniform size collected from tank were used

for the present experiment. Ten postlarvae were randomly distributed to all tanks after recording their initial length and weight.

3.3 Experimental set up

The experiment was carried out in the Department of Aquaculture, College of Fisheries, Panangad, roofed with translucent polyvinylchloride (PVC) sheets for moderate light conditions. Circular, flat bottomed fiber glass tanks with the following specifications were used for the experiments.

Capacity of the tank	:	83litres
Diameter	:	55cm
Height	:	35cm
Rim width	:	3cm
Thickness of wall	:	4mm
Colour	:	Aquamarine

Clear saline water filtered through a close meshed nylon bolting silk was used for filling the tanks upto ¾th level. Gentle aeration was given throughout the experimental period in the tanks via diffuser stones from Root Air Blower and was maintained uniformly in all tanks by means of control valves. Small PVC pipes were kept at the bottom of the tank for providing shelter to the animals.

3.4. Experimental design and procedure

Flat bottomed circular fiber glass tanks were used for the experiment. Two hundred and forty numbers of healthy uniform sized postlarvae each having an average weight of 0.04 g and average length 1.8 cm were selected from the population of 500 numbers. The experiment was conducted in a Completely Randomized Design with 6 treatments and 4 replications each. 10 numbers each were randomly distributed in twenty four experimental tanks. Before the commencement of feeding with the experimental diet, the postlarvae were conditioned with the control diet for 5 days and they were acclimatized to tray feeding. Shrimps from each tank were collected and individual length and weight of each animal recorded separately.

Each treatment group of animals was initially fed at 10% of total body weight Shiau *et al.,* 1991). During the experiment, the amount of diet given was progressively changed and adjusted according to the appetite of the shrimp by checking the bottom of the tanks for excess feed remaining after feeding. Petri dishes were kept at the bottom of the tank close to the substratum provided. Every day before offering the feed, leftover feed and faecal matter were collected and dried at 60 ºC for estimation of FCR and crude digestibility. Petri dishes were cleaned thoroughly before next feeding. The tanks were cleared of left over feed and faecal matter daily before feeding. Water quality was maintained by regular replenishment as required.

Sampling was done at fortnightly intervals. At each sampling all the shrimps stocked were taken and measured length and weight. The quantum of feed given was adjusted based on the increased weight.

Carcass protein content of the shrimps were analyzed initially and also on termination of the feeding trial following standard methods the same as that for analysis of the ingredients.

3.5. Determination of body protein

The initial and final body protein was estimated by using Microkjeldhal's method (AOAC, 1990).

3.6. Water quality parameters

During the experiment water quality parameters such as temperature, pH, Dissolved oxygen, ammonia and alkalinity were checked by the following methods.

Temperature	:	DO Testing Kit (AQUASOL)
pH	:	By using universal indicator solution
Dissolved oxygen	:	DO Testing Kit (AQUASOL)
Ammonia	:	Ammonia Testing Kit (BIOSOL)
Alkalinity	:	Alkalinity Testing Kit (BIOSOL)

All the water quality parameters were measured once in a week.

3.7. Evaluation indices

The parameters evaluated are growth(Gain in length and weight), Specific growth rate (SGR), Percentage survival, Food Conversion Ratio (FCR), Food Conversion Efficiency (FCE), Protein efficiency ratio (PER), protein digestibility coefficient and proximate composition of experimental animals.

3.7.1. Gain in weight

It gives the increase in the weight of shrimp during the experimental period. It was calculated by using the formula.

Gain in weight (g) = Final weight (g) – Initial weight (g)

3.7.2. Gain in standard length

It gives the increase in the length of shrimp during the experimental period. It was calculated by using the formula.

Gain in standard length (cm) = Final weight (cm)–Initial weight (cm)

3.7.3. Growth

The growth is assessed in terms of weight gain over a stipulated period.

$$\%\text{Weight gain} = \frac{\text{Final Weight (g)} - \text{Initial Weight (g)}}{\text{Initial Weight (g)}} \times 100$$

3.7.4. Survival rate

It is expressed in terms of percentage

$$\text{Percentage Survival} = \frac{\text{Final Number}}{\text{Initial Number}} \times 100$$

3.7.5. Specific growth rate (SGR)

The value can be used to compare growth on a daily basis as:

$$\text{SGR} = \frac{\ln \text{Final weight (g)} - \ln \text{Initial weight(g)}}{\text{Time interval in days}} \times 100$$

3.7.6. Food conversion ratio (FCR)

This term is useful to compare the ability of feed formulation to support weight gains. It is calculated as:

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

3.7.7. Food Conversion Efficiency (FCE)

Food conversion efficiency is the wet gain of animal from one unit of food consumed and this was calculated using the following formula.

$$\%\text{FCE} = \frac{\text{Wet weight gain of shrimp (g)}}{\text{Dry weight of food comsumed (g)}} \times 100$$

3.7.8. Protein efficiency ratio (PER)

This is a useful method to compare protein sources in a single experiment. Higher the PER value indicate better the feed value. It is calculated as:

$$\text{PER} = \frac{\text{Increase in the mass of animal produced (wet wt.)}}{\text{Mass of protein in feed (dry wt.)}}$$

3.7.9. Protein digestibility coefficient

Protein digestibility was expressed as apparent digestibility of protein, calculated employing the following formula:

$$\text{Protein digestibility coefficient} = \frac{\text{Quantity of protein consumed} - \text{Quantity of protein in the faeces}}{\text{Quantity of protein consumed}} \times 100$$

3.8. Statistical analysis

The experiment was carried out by using the Completely Randomized Design (CRD). The data pertaining to biological parameters were statistically analyzed. The means of the treatments were compared using one way ANOVA ($P<0.05$). Multiple comparisons were carried out using Tukey's HSD test (Rangaswami, 2002).

4. RESULTS

The efficacy of *Spirulina fusiformis* as a partial substitution for clam meat in the diet of *Penaeus monodon* postlarvae was evaluated over a period of 60 days. The results of the experiment are presented below. The treatments, T1 represents the diet with 40% clam meat and 0% *Spirulina* powder; T2, represents the diet with 35% clam meat and 5% *Spirulina* powder; T3, represents the diet with 30% clam meat and 10% *Spirulina* powder; T4, represents the diet with 20% clam meat and 20% *Spirulina* powder; T5, represents the diet with 10 % clam meat and 30% *Spirulina* powder and T6, represents the diet with 0% clam meat and 40% *Spirulina* powder.

4.1. Proximate composition of the feed ingredients and the formulated pelleted feeds.

4.1.1. Feed ingredients

The proximate composition of the ingredients used in the formulation of experimental diets is presented in Table 3.

The moisture content of the feed ingredients *viz.*, *Spirulina fusiformis*, clam meat, wheat bran, groundnut oil cake and tapioca flour ranged from 5 to 10%, the maximum being in wheat bran (10%) and minimum in *Spirulina* (5%).

The highest percentage of crude protein content was recorded in *Spirulina* (60), while it was lowest in tapioca flour (3.75). The crude protein of clam meat, groundnut oil cake and wheat bran were 53%, 42% and 16.25% respectively.

Tapioca flour contained the highest carbohydrate content of 82.65% (Nitrogen free extract) whereas *Spirulina* powder had the least amount (17%).

The ash content of the ingredient varied form 1.5% to 10%, the highest obtained in *Spirulina* powder (10%) and the lowest in tapioca flour (1.5%).

TABLE 3. Proximate composition of the ingredients used in the formulation of feeds.

Ingredients	Moisture %	Crude protein %	Crude fat %	Ash %	Crude fibre %	Nitrogen free extract %
Spirulina Powder	5	60	6	10	2	17
Clam meat	7.8	53	8.9	8.5	3.9	17.9
Groundnut oil cake	6	42	7.3	1.7	13.0	30
Wheat bran	10	16.25	2.6	3.5	12.2	55.45
Tapioca flour	8.8	3.75	1.3	1.5	2.0	82.65

4.1.2. Formulated pelleted feeds

The proximate composition of the formulated feeds is presented in Table 4. The moisture content of the six feeds varied between 3.8% (T6) to 7% (T1 and T4) whereas crude protein content ranged from 40% (T3) to 42% (T2 and T6). The crude fat content ranged between 3.39% (T6) and 7.89% (T2) while the percentage of nitrogen free extract was highest in T6 (41.42) and lowest in feed T2 (32.59%). Crude fibre range between 0.39% (T6) and 0.86% (T1). The ash content ranged from 9% (T5 and T6) to 12% (T1).

4.1.3. Water stability of feed

The results of test for different feed pellets are presented in Table5. Among the diets, the maximum water stability was for the T4 (76%), followed by T3 (75%). The lowest stability was observed for diet T6 (53%). A decrease in average percentage stability was observed in all six feeds with time.

TABLE 4. Proximate composition of test diets used in the experiment

Test Diets	Moisture %	Crude protein %	Crude fat %	Ash %	Crude fibre %	Nitrogen free extract %
T1	7	41	5.57	12	0.86	33.57
T2	6.8	42	7.89	10	0.72	32.59
T3	5.6	40	6.35	11	0.54	36.51
T4	7	40.16	5.93	10.5	0.48	35.93
T5	6	41.13	3.98	9	0.46	39.43
T6	3.8	42	3.39	9	0.39	41.42

TABLE 5. Percentage water stability of experimental diets

Feed	1hr	2hr	4hr	6hr
T1	83	72	64	58
T2	88	75	68	60
T3	88	82	81	75
T4	92	83	81	76
T5	86	77	73	65
T6	83	73	63	53

4.2. Efficiency of test diets

4.2.1. Growth

Data regarding average live weight gain of prawns fed on the experimental feeds are presented in Table 6. Comparison of growth of *Penaeus monodon* on different diets is shown in Plate 2.

4.2.1.1. Weight

The gain in weight of shrimp fed with different levels of clam meat and *Spirulina* are presented in Table 6. Shrimp postlarvae with a uniform weight of 0.04g were used for the experiment. A maximum weight gain (2.374 g) was observed in T4 and minimum in growth rate for T6 (0.451 g). The average percentage weight gain of different treatment ranged between 1114.18% (T6) and 5890.36% (T4).

Analysis of variance (Table 6.1) shows the average weight gain of the shrimp juveniles was different for different treatments. Graphical representation of Growth in weight of *Penaeus monodon* postlarvae fed the experimental diets are shown in Fig. 1.

4.2.1.2. Length

The gain in standard length of shrimp fed with different levels of clam meat and *Spirulina* are presented in Table 7. Maximum length gain of 5.78 cm was obtained for feed T4 and the minimum was 2.36 cm for feed T6. The average percentage length gain of different treatments ranged between 134.75% (T6) and 296.35% (T4).

Graphical representation of gain in standard length of *Penaeus monodon* postlarvae fed the experimental diets are shown in Fig. 2. Analysis of variance (Table 7.1) shows that the average length gain was different for different treatments.

TABLE 6. Gain in weight of *Penaeus monodon* postlarvae fed on different experimental diets.

Treatment	Replication	Average Initial Weight (g)	Average Final Weight (g)	Average Weight Gain (g)	Average gain in weight(g) Mean±SE	% weight gain	Average % weight gain Mean±SE
T1	1	0.043	1.060	1.017	1.015± 0.0025	2342.17	2422.13± 68.00
	2	0.042	1.058	1.016		2407.11	
	3	0.042	1.054	1.012		2432.59	
	4	0.041	1.058	1.018		2506.65	
T2	1	0.040	1.468	1.428	1.425± 0.0334	3543.18	3502.47± 111.19
	2	0.042	1.446	1.404		3359.33	
	3	0.040	1.437	1.397		3484.04	
	4	0.041	1.512	1.471		3623.32	
T3	1	0.040	2.233	2.193	2.196± 0.0293	5469.33	5431.35± 65.59
	2	0.041	2.277	2.236		5494.10	
	3	0.040	2.206	2.166		5416.00	
	4	0.041	2.227	2.187		5345.97	
T4	1	0.041	2.446	2.405	2.374± 0.0505	5939.26	5890.36± 105.38
	2	0.041	2.453	2.412		5825.36	
	3	0.040	2.342	2.302		5783.17	
	4	0.040	2.415	2.375		6013.67	
T5	1	0.039	1.208	1.169	1.164± 0.0229	2996.15	2894.68± 105.49
	2	0.041	1.177	1.135		2748.67	
	3	0.040	1.203	1.163		2936.62	
	4	0.041	1.232	1.191		2897.26	
T6	1	0.041	0.504	0.463	0.451± 0.0194	1140.76	1114.18± 54.24
	2	0.040	0.510	0.470		1172.18	
	3	0.041	0.469	0.428		1047.62	
	4	0.040	0.481	0.441		1096.16	

TABLE 6.1. ANOVA table for the comparison of mean weight.

Source of variation	SS	DF	MSS	F value	P value
Treatment	10.711	5	2.142	2369.87**	0.000
Error	0.016	18	0.001		
Total	10.0727	23			

**Statistically significant ($p<0.01$).

TABLE 6.2. Tukey's HSD test

Treatment	Growth (gm)
T1	1.015 [e]
T2	1.425 [c]
T3	2.196 [b]
T4	2.374[a]
T5	1.164 [d]
T6	0.451 [f]

*abcdef, Means in a column with the same superscript are not significantly different ($p>0.05$).

*Multiple comparison as per Tukey's test.

*Mean Growth in terms of weight in homogenous subsets are displayed (n=4).

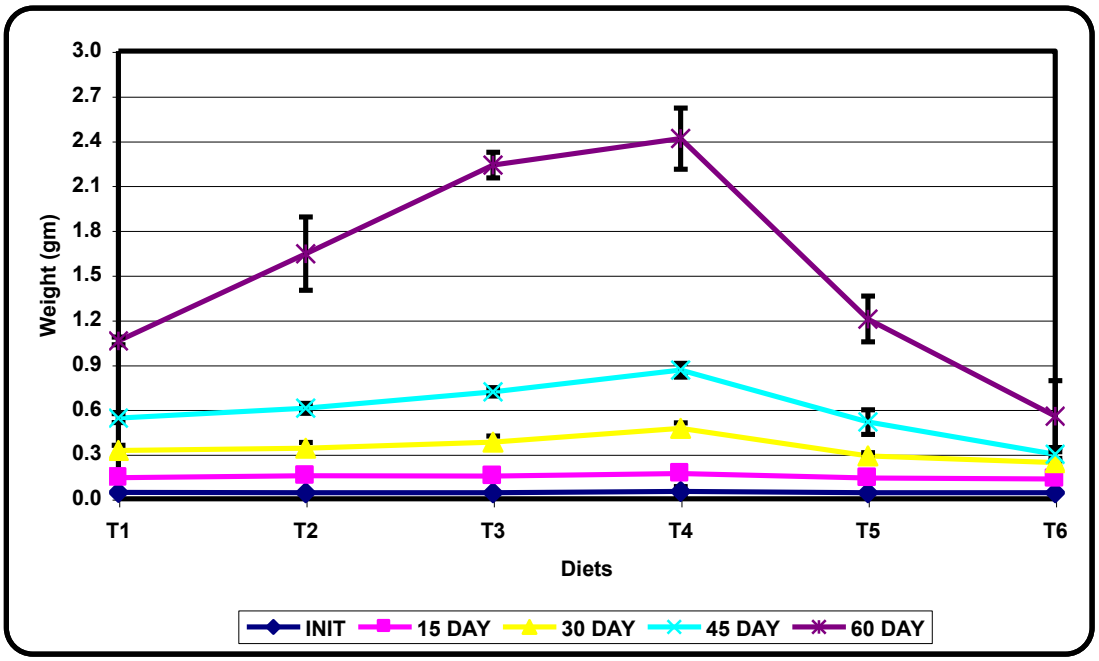

Fig.1. Gain in weight of *Penaeus monodon* postlarvae fed the experimental diets

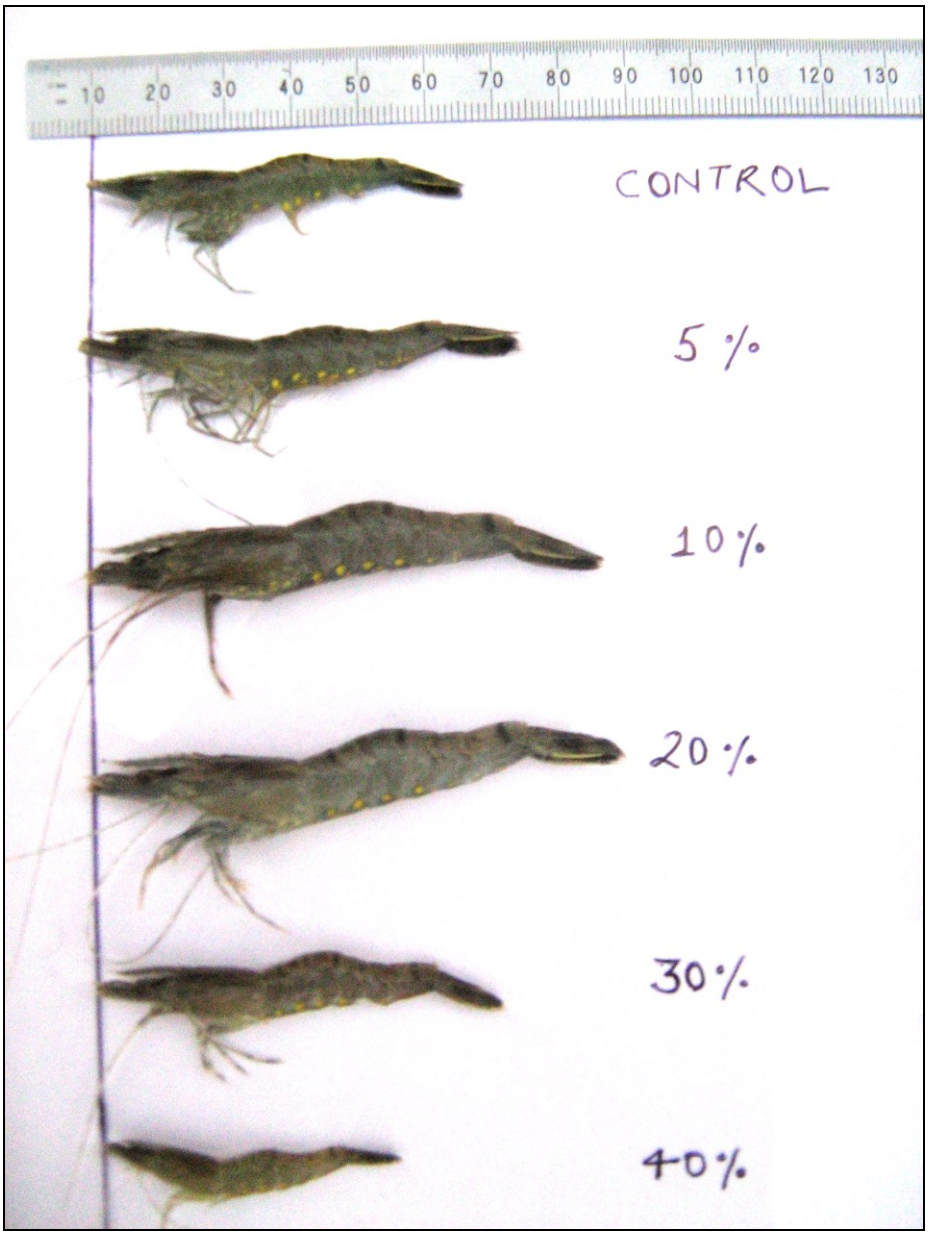

Plate 2. Comparison of growth of *Penaeus monodon* on different diets

TABLE 7. Gain in standard length of *Penaeus monodon* postlarvae fed on different experimental diets.

Treatment	Replication	Average initial length (cm)	Average final length (cm)	Average length gain (cm)	Average gain in length(cm) Mean±SE	% Length gain	Average % length gain Mean±SE
T1	1	1.92	5.49	3.57	3.61± 0.0547	185.94	194.05± 6.0404
	2	1.85	5.44	3.59		194.05	
	3	1.87	5.46	3.59		191.74	
	4	1.84	5.53	3.69		200.54	
T2	1	1.85	6.05	4.20	4.26± 0.0774	227.03	224.08± 2.8783
	2	1.91	6.19	4.28		224.08	
	3	1.88	6.07	4.19		222.87	
	4	1.90	6.26	4.36		229.24	
T3	1	1.86	7.10	5.24	5.23± 0.0562	281.72	280.42± 3.5377
	2	1.89	7.19	5.30		280.42	
	3	1.86	7.03	5.17		277.96	
	4	1.90	7.10	5.20		273.68	
T4	1	1.89	7.65	5.76	5.78± 0.0656	304.76	296.35± 10.8352
	2	1.92	7.61	5.69		296.35	
	3	1.84	7.68	5.84		317.39	
	4	1.82	7.63	5.81		319.23	
T5	1	1.82	5.98	4.16	3.99± 0.1187	228.30	203.95± 10.6449
	2	1.90	5.78	3.88		203.95	
	3	1.83	5.79	3.96		216.26	
	4	1.90	5.86	3.96		208.55	
T6	1	1.88	4.24	2.36	2.36± 0.0938	125.40	134.75± 6.3368
	2	1.85	4.34	2.49		134.75	
	3	1.87	4.20	2.33		124.60	
	4	1.90	4.17	2.27		119.55	

TABLE 7.1 ANOVA table for the comparison of mean weight.

Source of variation	SS	DF	MSS	F value	P value
Treatment	29.233	5	5.847	886.403**	0.000
Error	0.119	18	0.007		
Total	29.352	23			

** Statistically significant ($p<0.01$).

TABLE 7.2 Tukey's HSD test

Treatment	Length (cm)
T1	3.61 [e]
T2	4.26 [c]
T3	5.23 [b]
T4	5.78[a]
T5	3.99 [d]
T6	2.36 [f]

*[abcdef,] Means in a column with the same superscript are not significantly different($p>0.05$).

*Multiple comparison as per Tukey's HSD test.

*Mean Growth in terms of length in homogenous subsets are displayed (n=4).

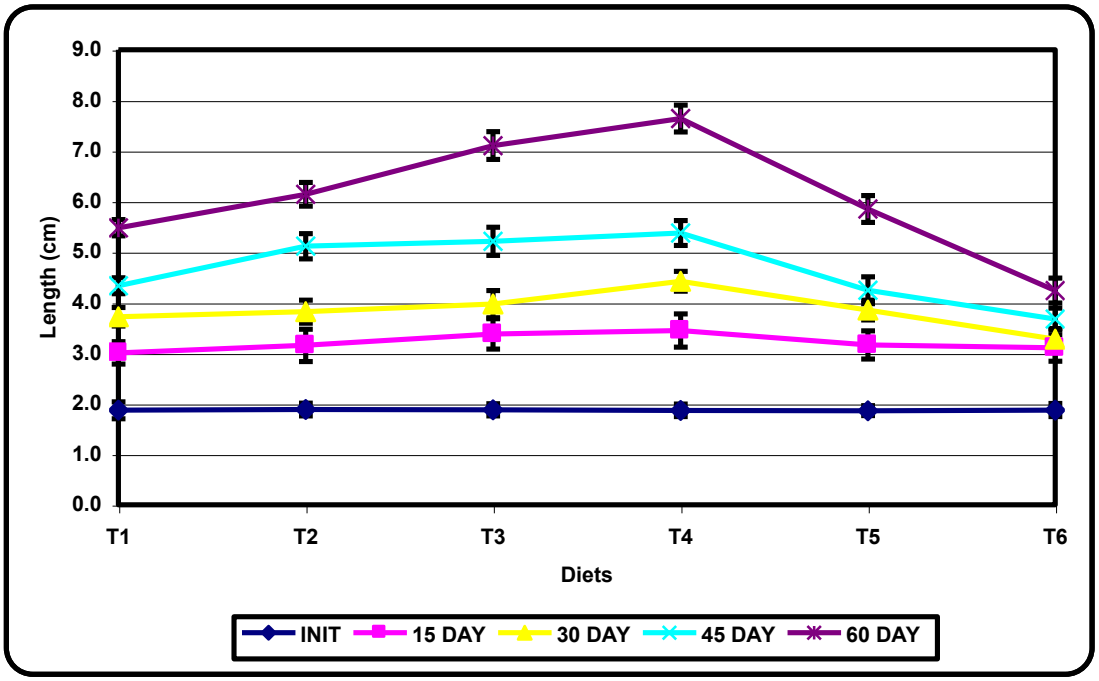

Fig.2. Gain in standard length of *Penaeus monodon* postlarvae fed the experimental diets

4.2.2. Specific growth rate

Table 8 gives the specific growth rate of shrimp postlarvae fed different experimental diets. The shrimp juveniles of treatment T4 had the highest specific growth rate (6.82), followed by those of T3 (6.69) and T2 (5.97). The lowest SGR (4.16) was recorded for shrimp of treatment T6.

Analysis of variance of SGR values shows significant difference ($p<0.05$) between treatments (Table 8.1). Graphical representation of specific growth rate of *Penaeus monodon* postlarvae fed the experimental diets are shown in Fig. 3.

4.2.3. Survival

The percentage survival values of the shrimp juveniles in the various treatments are given the Table 9 and Fig. 4. Among the diets tested, the average highest survival (97.5 %) was recorded for treatment T3 and T4, followed by T1 (95.0), T2 (95.0) and T5 (77.5). The lowest percentage survival was observed for treatment T6 (75.0 %).

Analysis of variance shows (Table 9.1) shows there is no significant difference ($p<0.05$) in survival rate for juveniles on feed T1, T2, T3 and T4.

TABLE 8. Specific growth rate (SGR) of *Penaeus monodon* fed on different experimental diets.

Treatment	Replication	Average Initial Weight (g)	Average Final Weight (g)	SGR %	Average SGR (%) Mean±SE
T1	1	0.043	1.060	5.33	5.38± 0.0449
	2	0.042	1.058	5.37	
	3	0.042	1.054	5.39	
	4	0.041	1.058	5.43	
T2	1	0.040	1.468	5.99	5.97± 0.0517
	2	0.042	1.446	5.91	
	3	0.040	1.437	5.97	
	4	0.041	1.512	6.03	
T3	1	0.040	2.233	6.70	6.69± 0.0198
	2	0.041	2.277	6.71	
	3	0.040	2.206	6.68	
	4	0.041	2.227	6.66	
T4	1	0.041	2.446	6.83	6.82± 0.0293
	2	0.041	2.453	6.80	
	3	0.040	2.342	6.79	
	4	0.040	2.415	6.86	
T5	1	0.039	1.208	5.72	5.66± 0.0593
	2	0.041	1.177	5.58	
	3	0.040	1.203	5.69	
	4	0.041	1.232	5.67	
T6	1	0.041	0.504	4.20	4.16± 0.0748
	2	0.040	0.510	4.24	
	3	0.041	0.469	4.07	
	4	0.040	0.481	4.14	

TABLE 8.1 ANOVA table for the comparison of mean SGR.

Source of variation	SS	DF	MSS	F value	P value
Treatment	18.980	5	3.796	1512.164**	0.000
Error	0.045	18	0.003		
Total	19.025	23			

** Stastically significant ($p<0.01$).

Table 8.2 Tukey's HSD test

Treatment	SGR (%)
T1	5.38 [e]
T2	5.97 [c]
T3	6.69 [b]
T4	6.82[a]
T5	5.67 [d]
T6	4.16 [f]

*[abcdef,] Means in a column with the same superscript are not significantly different($p>0.05$).

*Multiple comparison as per Tukey's HSD test.

*Mean SGR in homogenous subsets are displayed (n=4).

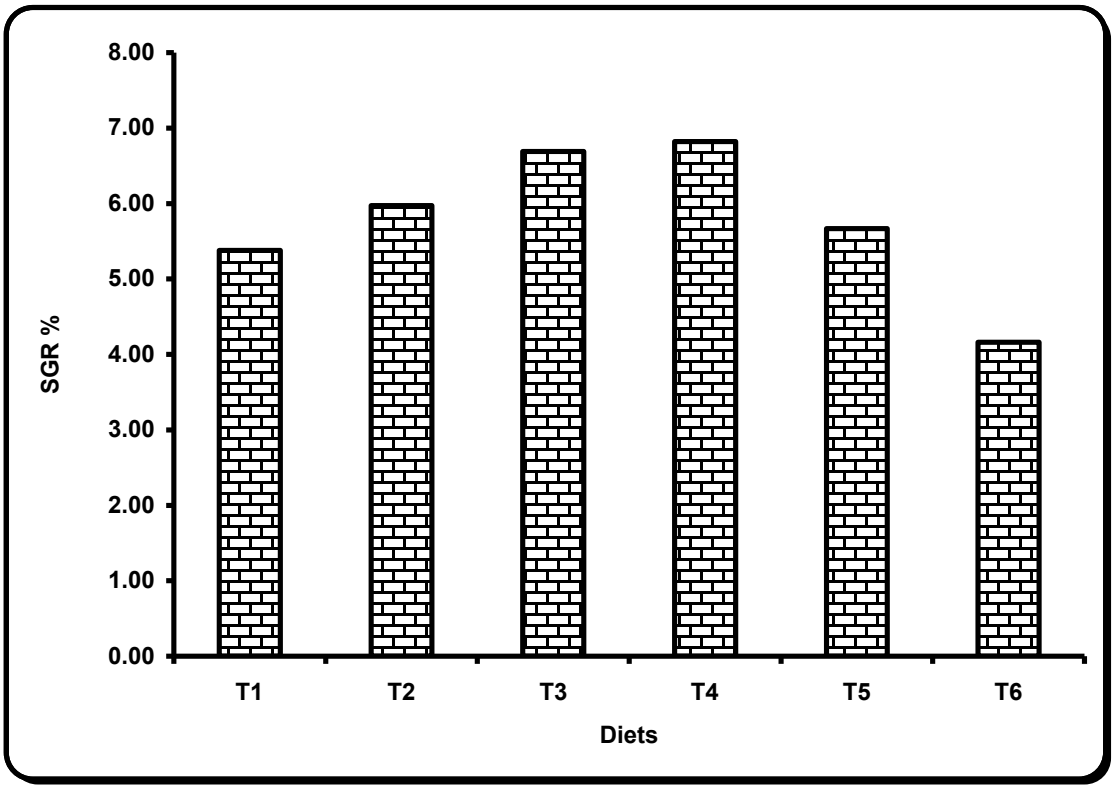

Fig.3. Specific Growth Rate of *Penaeus monodon* postlarvae fed the experimental diets

TABLE 9. Percentage survival of *Penaeus monodon* postlarvae fed on different experimental diets.

Treatment	Replication	Initial No.	Final No.	% survival	Average % survival Mean± SE
T1	1	10	9	90	95.0± 5.77
	2	10	10	100	
	3	10	9	90	
	4	10	10	100	
T2	1	10	10	100	95.0± 5.77
	2	10	9	90	
	3	10	10	100	
	4	10	9	90	
T3	1	10	10	100	97.5± 5.00
	2	10	10	100	
	3	10	10	100	
	4	10	9	90	
T4	1	10	10	100	97.5± 5.00
	2	10	10	100	
	3	10	9	90	
	4	10	10	100	
T5	1	10	8	80	77.5± 5.00
	2	10	8	80	
	3	10	7	70	
	4	10	8	80	
T6	1	10	8	80	75.0± 5.77
	2	10	7	70	
	3	10	8	80	
	4	10	7	70	

TABLE 9.1 ANOVA table for the comparison of mean percentage survival.

Source of variation	SS	DF	MSS	F value	P value
Treatment	2704.532	5	540.906	7.681**	0.001
Error	1267.507	18	70.417		
Total	3972.039	23			

**Statistically significant ($p<0.01$).

*Data subjected to angular transformation.

TABLE 9.2 Tukey's HSD test

Treatment	Percentage survival
T1	95.0 [a]
T2	95.0 [a]
T3	97.5 [a]
T4	97.5 [a]
T5	77.5 [c]
T6	75.0 [c]

*[ab] Means in a column with the same superscript are not significantly different ($p>0.05$).

*Multiple comparison as per Tukey's HSD test.

*Mean survival in homogenous subsets are displayed (n=4).

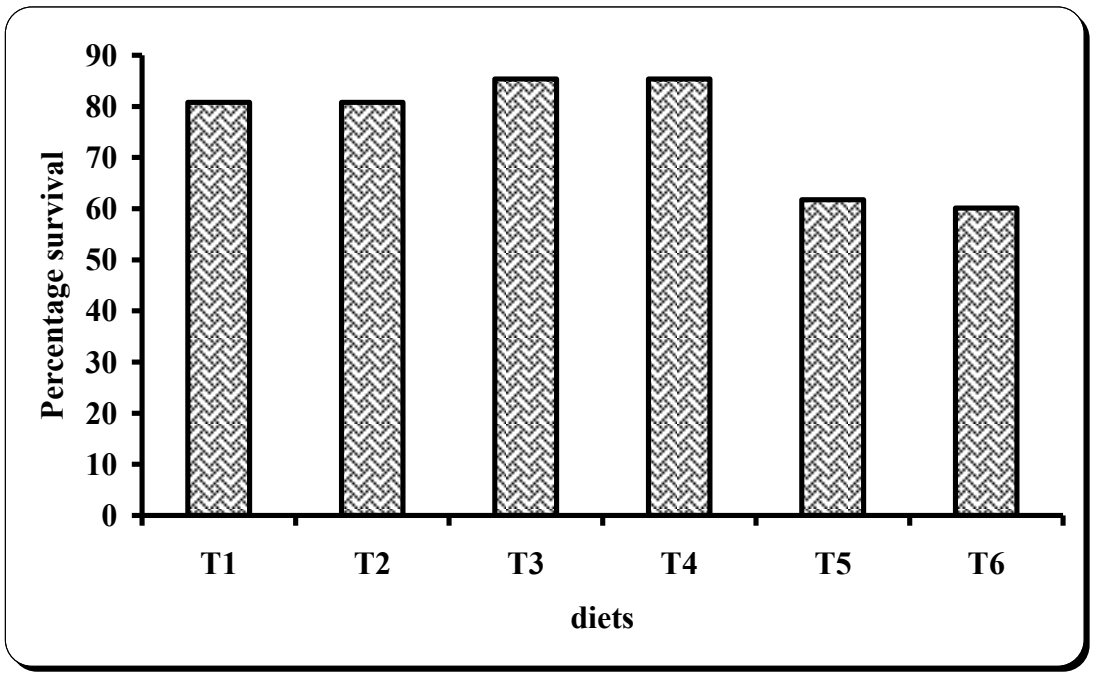

Fig.4. Percentage survival of *Penaeus monodon* postlarvae fed the experimental diets

4.2.4. Food conversion ratio

The food conversion ratio recorded for the various treatments are presented in Table 10 and Fig. 5. The mean FCR ranged from 1.49 to 3.86. Treatment T4 gave the lowest FCR value (1.49), followed by T3 (1.69). The average FCR value obtained in T5, T2, T1, and T6 were 1.93, 1.96, 2.41 and 3.86.

Analysis of variance (Table 10.1) shows that the FCR for feeds T2 and T5 is the same and all other feed FCR differ significantly. The best FCR value is for feed T4 (1.49).

4.2.5. Food conversion efficiency

The food conversion efficiency recorded for the various treatments are presented in Table 11. The mean FCE ranged from 25.82% to 66.43%. Feed T4 gave the highest FCE value (66.43%) and least value for T6 (25.82%).

Graphical representation of food conversion efficiency of *Penaeus monodon* postlarvae fed the experimental diets are shown in Fig. 6. Analysis of variance (Table 11.1) shows that the FCE for feeds T2 and T5 is the same and all other feed FCE differ significantly.

4.2.6. Protein efficiency ratio

The PER obtained for various treatments are shown in the Table 12. The highest PER of 1.65 was recorded for treatment T4, following by T3 (1.53). The lowest PER of 0.61 was for treatment T6.

Analysis of variance (Table 12.1) for PER indicated significant difference between feeds T1, T3, T4 and T6. Graphical representation of Protein efficiency ratio of *Penaeus monodon* fed the experimental diets is shown in Fig. 7.

TABLE 10. Food Conversion Ratio (FCR) of *Penaeus monodon* postlarvae fed on different experimental diets.

Treatment	Replication	Average Weight Gain (g)	Average food consumed (g)	FCR	Average FCR Mean ± SE
T1	1	1.017	2.53	2.49	2.41± 0.0725
	2	1.016	2.49	2.45	
	3	1.012	2.36	2.33	
	4	1.018	2.41	2.37	
T2	1	1.428	2.76	1.93	1.96± 0.0170
	2	1.404	2.76	1.97	
	3	1.397	2.73	1.95	
	4	1.471	2.90	1.97	
T3	1	2.193	3.83	1.75	1.69± 0.0633
	2	2.236	3.64	1.63	
	3	2.166	3.79	1.75	
	4	2.187	3.61	1.65	
T4	1	2.405	3.56	1.48	1.49± 0.0271
	2	2.412	3.63	1.51	
	3	2.302	3.48	1.51	
	4	2.375	3.45	1.45	
T5	1	1.169	2.29	1.96	1.93± 0.0712
	2	1.135	2.21	1.95	
	3	1.163	2.32	2.00	
	4	1.191	2.18	1.83	
T6	1	0.463	1.78	3.84	3.86± 0.0285
	2	0.470	1.82	3.87	
	3	0.428	1.67	3.90	
	4	0.441	1.69	3.84	

TABLE 10.1 ANOVA table for the comparison of mean FCR.

Source of variation	SS	DF	MSS	F value	P value
Treatment	14.792	5	2.958	1097.918**	0.000
Error	0.049	18	0.003		
Total	18.840	23			

**Statistically significant ($p<0.01$).

TABLE 10.2 Tukey's HSD test

Treatment	FCR
T1	2.41 [b]
T2	1.96 [c]
T3	1.69 [d]
T4	1.49 [e]
T5	1.93 [c]
T6	3.86[a]

*abcde, Means in a column with the same superscript are not significantly different($p>0.05$).

*Multiple comparison as per Tukey's HSD test.

*Mean FCR in homogenous subsets are displayed (n=4).

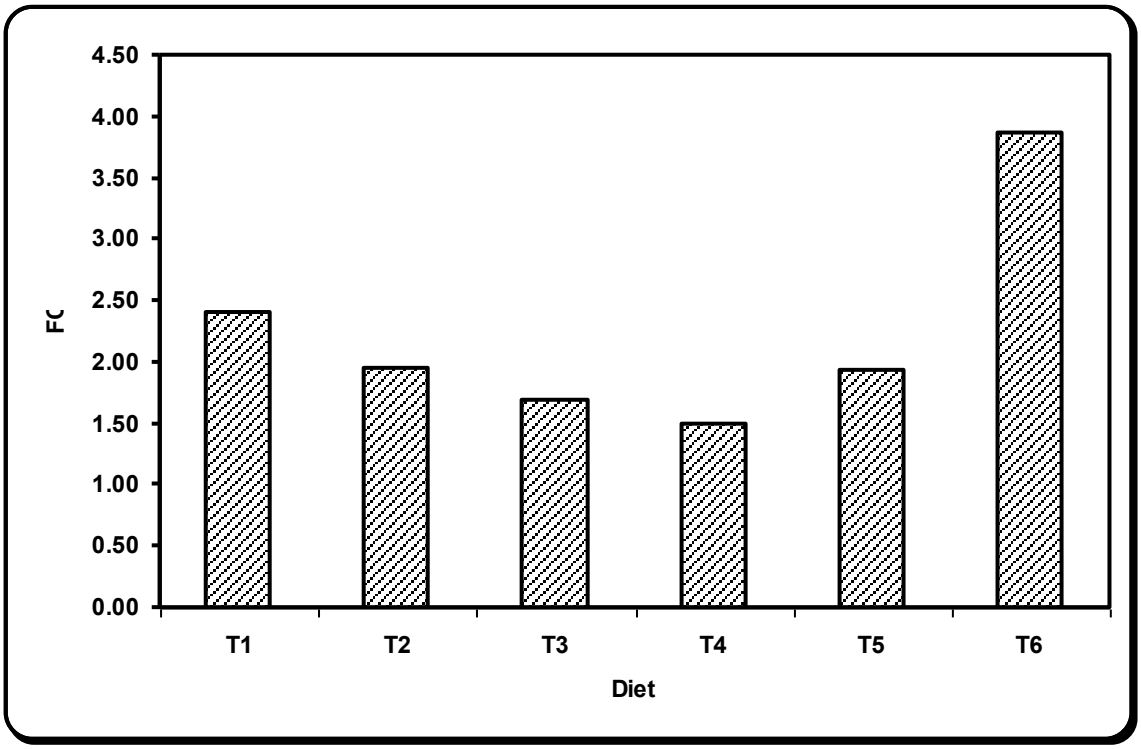

Fig.5. Food conversion ratio of *Penaeus monodon* postlarvae fed with the experimental diets

Table11. Food Conversion Efficiency (FCE) of *Penaeus monodon* postlarvae fed on different experimental diets.

Treatment	Replication	Average Weight Gain (g)	Average food consumed (g)	FCE %	Average FCE (%) Mean ± SE
T1	1	1.016	2.53	40.17	40.79± 1.2485
	2	1.015	2.49	40.79	
	3	1.012	2.36	42.87	
	4	1.017	2.41	42.22	
T2	1	1.427	2.76	51.73	50.87± 0.4456
	2	1.404	2.76	50.87	
	3	1.397	2.73	51.17	
	4	1.471	2.9	50.72	
T3	1	2.193	3.83	57.26	61.43± 2.2152
	2	2.236	3.64	61.43	
	3	2.166	3.79	57.16	
	4	2.186	3.61	60.56	
T4	1	2.405	3.56	67.56	66.43± 1.2325
	2	2.411	3.63	66.43	
	3	2.301	3.48	66.14	
	4	2.375	3.45	68.85	
T5	1	1.168	2.29	51.02	51.36± 1.9629
	2	1.135	2.21	51.36	
	3	1.162	2.32	50.12	
	4	1.190	2.18	54.62	
T6	1	0.463	1.78	26.01	25.82± 0.1906
	2	0.470	1.82	25.82	
	3	0.428	1.67	25.65	
	4	0.440	1.69	26.07	

TABLE 11.1 ANOVA table for the comparison of mean FCE.

Source of variation	SS	DF	MSS	F value	P value
Treatment	4144.317	5	828.863	411.923**	0.000
Error	36.219	18	2.012		
Total	4180.536	23			

**Statistically significant ($p<0.01$).

TABLE 11.2 Tukey's HSD test

Treatment	FCE (%)
T1	40.79 [d]
T2	50.87 [c]
T3	61.43 [b]
T4	66.43[a]
T5	51.36 [c]
T6	25.82 [e]

*abcde, Means in a column with the same superscript are not significantly different($p>0.05$).

*Multiple comparison as per Tukey's HSD test.

*Mean FCE in homogenous subsets are displayed (n=4).

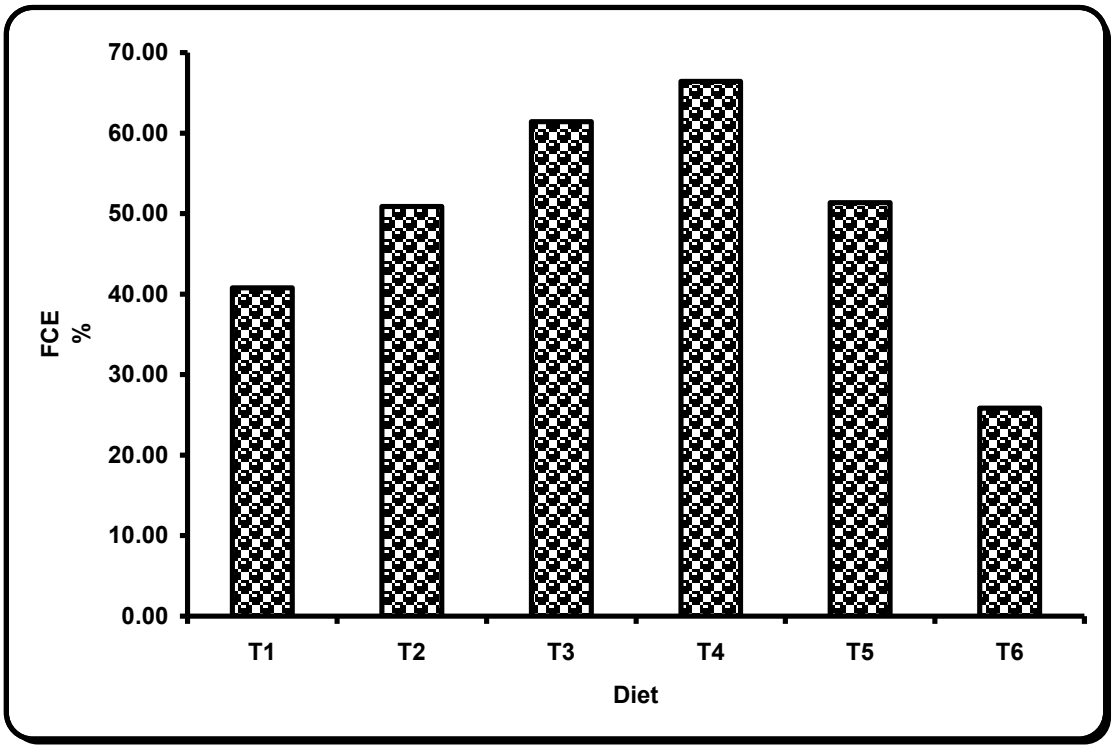

Fig.6. Food conversion efficiency of *Penaeus monodon* postlarvae fed the experimental diets

TABLE 12. Protein Efficiency Ratio (PER) of *Penaeus monodon* postlarvae fed on different experimental diets.

Treatment	Replication	Average Weight Gain (g)	Protein intake (g)	PER	Average PER Mean±SE
T1	1	1.016	1.037	0.97	0.99± 0.0305
	2	1.015	1.020	0.99	
	3	1.012	0.967	1.04	
	4	1.017	0.988	1.03	
T2	1	1.427	1.159	1.23	1.21± 0.0106
	2	1.404	1.159	1.21	
	3	1.397	1.146	1.21	
	4	1.471	1.218	1.20	
T3	1	2.193	1.532	1.43	1.53± 0.0554
	2	2.236	1.456	1.53	
	3	2.166	1.516	1.42	
	4	2.186	1.444	1.51	
T4	1	2.405	1.429	1.68	1.65± 0.0307
	2	2.411	1.457	1.65	
	3	2.301	1.397	1.64	
	4	2.375	1.3855	1.71	
T5	1	1.168	0.941	1.24	1.24± 0.0477
	2	1.135	0.909	1.24	
	3	1.162	0.954	1.21	
	4	1.190	0.896	1.32	
T6	1	0.463	0.747	0.61	0.61± 0.0045
	2	0.470	0.764	0.61	
	3	0.428	0.701	0.61	
	4	0.440	0.709	0.62	

TABLE 12.1 ANOVA table for the comparison of mean PER.

Source of variation	SS	DF	MSS	F value	P value
Treatment	2.724	5	0.545	444.712**	0.000
Error	0.022	18	0.001		
Total	2.746	23			

**Statistically significant ($p<0.01$).

TABLE 12.2 Tukey's HSD test

Treatment	PER
T1	0.99 [d]
T2	1.21 [c]
T3	1.53 [b]
T4	1.65[a]
T5	1.24 [c]
T6	0.61 [e]

*abcde, Means in a column with the same superscript are not significantly different($p>0.05$).

*Multiple comparison as per Tukey's HSD test.

*Mean PER in homogenous subsets are displayed (n=4).

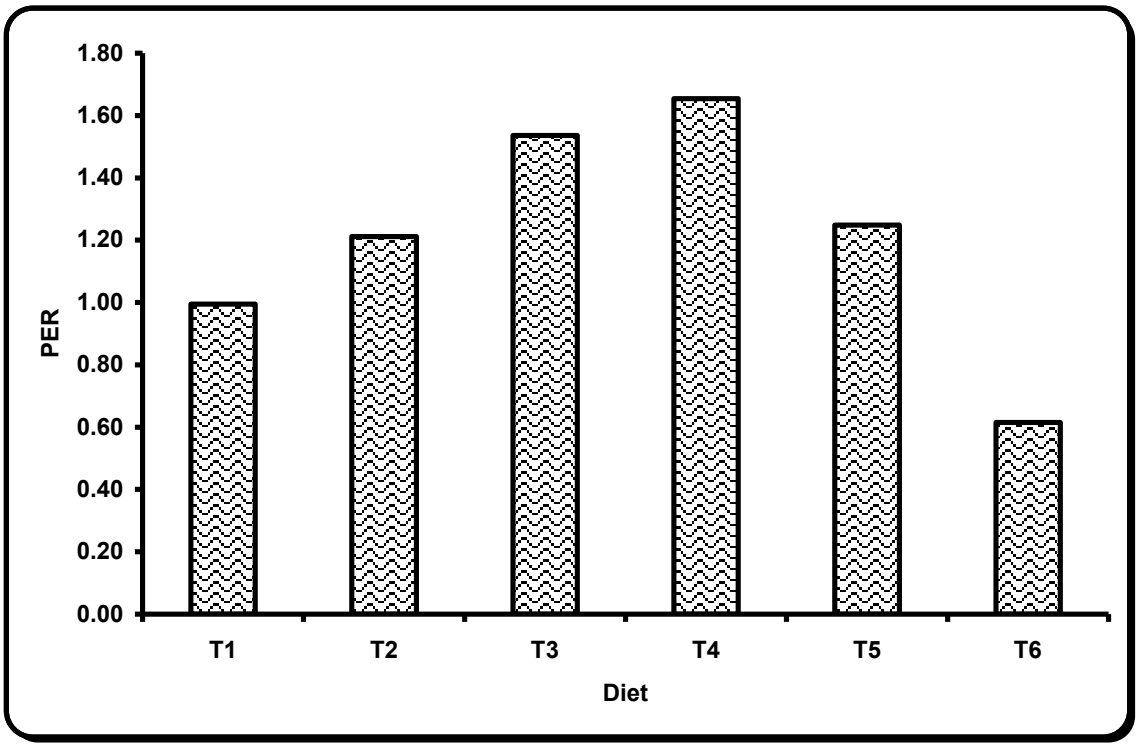

Fig.7. Protein efficiency ratio of *Penaeus monodon* fed the experimental diets

4.2.7. Protein digestibility coefficient

Protein digestibility coefficients of various treatments are given in Table 13. The highest values was recorded for feed T4 (90.17%) and least for T6 (59.30%). The protein digestibility coefficient obtained in various treatments are graphically presented in Fig. 8

4.3. Determination of body protein

4.3.1. Initial body protein of experimental animals

The initial protein content of the animal was estimated as 18.75 % of the body weight.

4.3.2. Final body protein of experimental animals

Final body protein of *Penaeus monodon* postlarvae were evaluated after the experimental period of 60 days and the data are 23.05, 23.25, 23.33, 23.56, 23.14 and 21.01 for treatment T1, T2, T3, T4, T5 and T6 respectively. Graphical representation of final body protein content is shown in Fig. 9.

TABLE 13. Protein digestibility coefficient (PDC) of *Penaeus monodon* postlarvae fed on different experimental diets.

Treatment	Average protein consumed (g)	Average protein in faeces (g)	PDC %
T1	1.020	0.267	73.80
T2	1.131	0.178	84.21
T3	1.570	0.162	89.68
T4	1.459	0.143	90.17
T5	0.938	0.274	70.81
T6	0.729	0.297	59.30

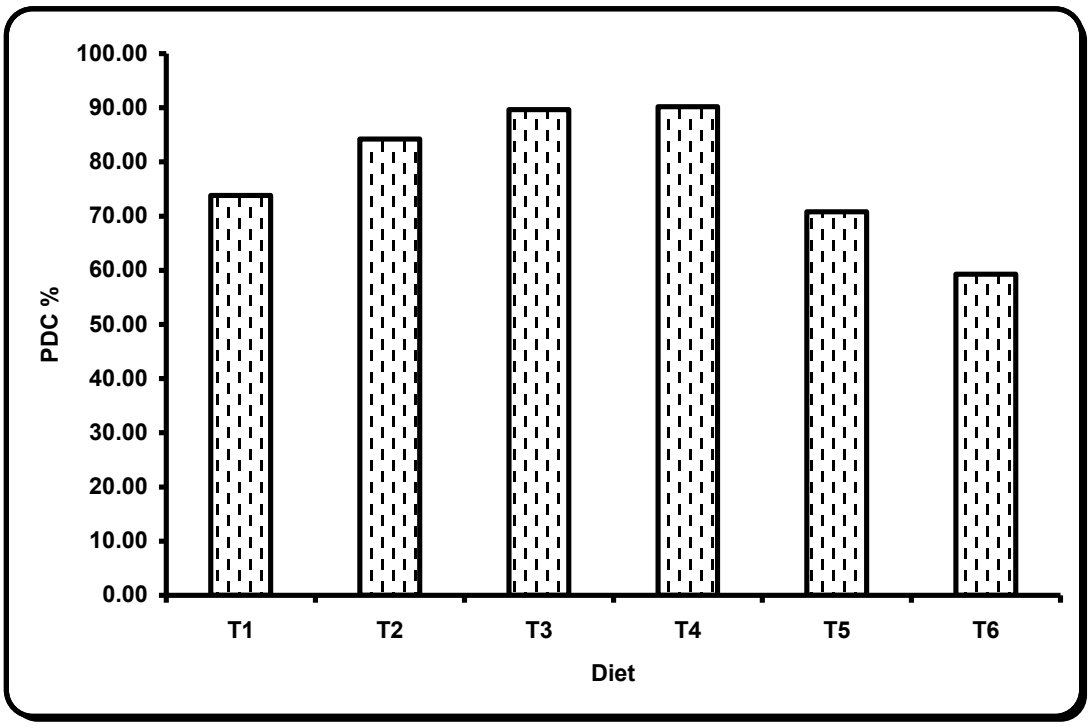

Fig.8. Protein digestibility of *Penaeus monodon* fed the experimental diets

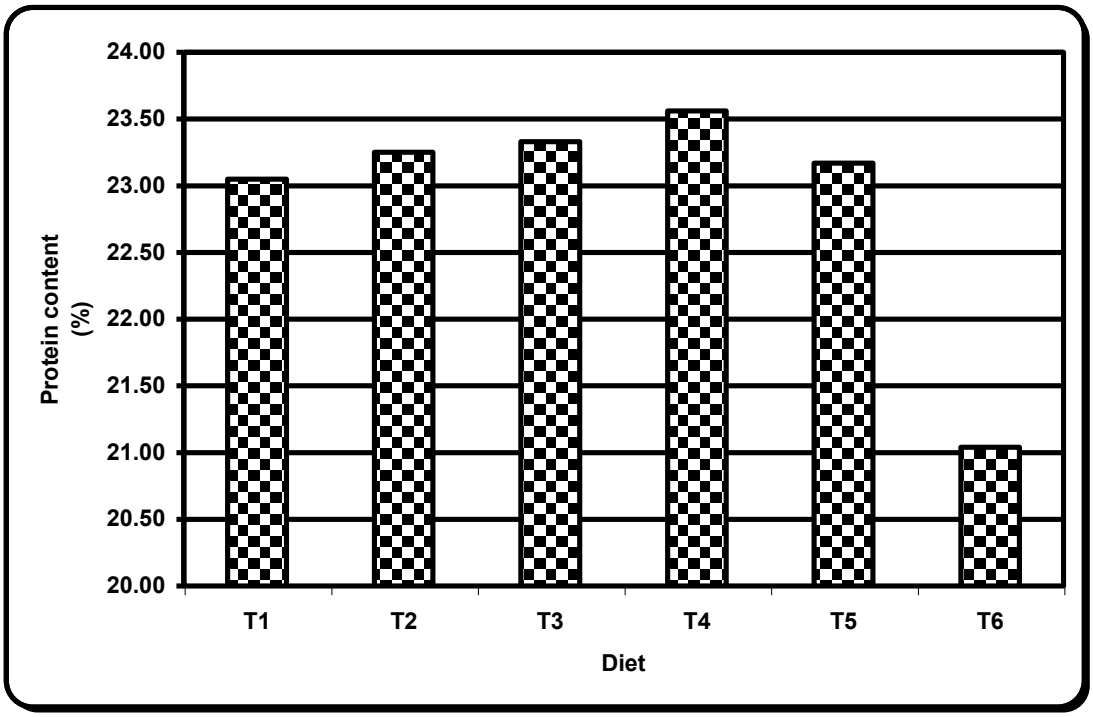

Fig.9.Final protein content of body of *Penaeus monodon* at the end of the experiment

4.4. Water quality parameters

4.4.1. Water temperature

Table 14 depicts the water temperature recorded from experimental tanks at weekly interval during the study period. Minimum temperature recorded was 26.9ºc and maximum temperature was32.1ºc. Weekly mean temperature values ranged from 28.5 to 30.4ºc.

4.4.2. pH

Table 15 gives the variation in pH in the rearing tanks during the study period. Minimum and maximum pH observed during the study period was 7 and 8 respectively. Weekly mean pH values ranged from 7.6 to 7.9.

4.4.3. Dissolved oxygen

Range of dissolved oxygen (DO) in the experimental tanks is given in Table 16. A minimum of 5 ppm and a maximum of 7.8ppm were obtained during the study period. Weekly mean values ranged from 6 to 7.3ppm.

4.4.4. Total alkalinity

The alkalinity of water recorded over the experimental period shown in the table 17. A minimum of 80ppm and a maximum of 110ppm were observed during the study period. Weekly mean values ranged from 80 to 103.3ppm.

4.4.5. Ammonia

Ranges of ammonia in the experimental tanks are given in Table 18. A minimum of 0.01mg/l and a maximum of 0.03mg/l were observed during the study period. Weekly mean values ranged from 0.01 to 0.09.

Table 14. Water temperature (^{0}C) in the experimental tanks during the study period

Time / Parameter	Week								
Temperature	1	2	3	4	5	6	7	8	9
Mean ±SE	28.6± 0.00	30.4± 0.41	28.5± 0.45	28.5± 0.54	30.4± 0.41	30.4± 0.41	28.5± 0.45	28.6± 0.49	28.5± 0.45
Range	28.6-28.6	30.1-32.1	27.8-29.7	26.9-29.7	30.1-32.1	31.1-32.1	27.9-28.7	27.7-29.7	27.8-29.9

Table 15. pH of water in the experimental tanks during the study period

Time / Parameter	Week								
pH	1	2	3	4	5	6	7	8	9
Mean ±SE	7.8± 0.28	7.7± 0.33	7.7± 0.29	7.9± 0.21	7.7± 0.25	7.6± 0.30	7.6± 0.30	7.6± 0.28	7.8± 0.24
Range	7-8	7-8	7-8	7.5-8	7.5-8	7-8	7-8	7-8	7.5-8

Table 16. Dissolved oxygen (ppm) of water in the experimental tanks during the study period

Time / Parameter	Week								
DO	1	2	3	4	5	6	7	8	9
Mean ±SE	6.0± 0.00	7.2± 0.31	6.1± 0.87	6.1± 0.86	7.3± 0.33	7.3± 0.33	6.1± 0.87	6.2± 0.80	6.1± 0.87
Range	6-6	6.7-7.8	5.1-7.8	5-7.8	6.6-7.8	6.7-7.7	5.3-7.8	5-7.3	5.6-7.8

Table 17. Total alkalinity (ppm) of water in the experimental tanks during the study period

Time / Parameter	Week								
Alkalinity	1	2	3	4	5	6	7	8	9
Mean ±SE	80.0± 0.00	88.9± 2.14	91.2± 5.14	103.3± 8.26	81.6± 4.28	84.1± 9.70	84.5± 7.86	85.4± 5.11	89.5± 11.66
Range	80-80	85-90	80-100	90-110	80-90	80-110	70-90	80-90	80-110

Table 18. Ammonia (ppm) of water in the experimental tanks during the study period

Time / Parameter	Week								
Ammonia	1	2	3	4	5	6	7	8	9
Mean ±SE	0.00± 0.00	0.01± 0.006	0.05± 0.006	0.09± 0.008	0.01± 0.007	0.08± 0.008	0.06± 0.005	0.07± 0.007	0.01± 0.005
Range	0-0	0-0.01	0-0.03	0-0.03	0-0.03	0-0.02	0-0.02	0-0.02	0.1-0.02

5. DISCUSSION

5.1. Proximate analysis of formulated feeds

Proximate composition of experimental diets was analyzed to evaluate the nutrient status. The protein requirement of *Penaeus monodon* has been identified by various authors in the range of 35% to 50% (Bages and Sloane, 1981; Godfred-Ponraj *et al*., 1990; Mathew and Jayaprakash, 1990; Chen, 1993; Paulraj, 1993). Burford *et al.* (2004) found that the shrimp growth rate was statistically higher in the diet containing 35% and 40% protein. Among the dry food type tested, maximum growth was obtained with 40% protein in the diet (Shiau *et al*., 1991). Proximate analysis of the diets in the present study revealed that they contained crude protein in the range of 40-42%, which falls within the levels of protein in the feeds suggested by previous investigators.

The experimental diets used in the present study contained carbohydrate in the range of 32.59%-41.42%. New (1976) reported that the shrimp and prawns appear to utilize complex carbohydrates more efficiently than simple ones. Alava and Pascual (1987) obtained high growth rate in *Penaeus monodon* with diets containing 20% carbohydrate. But Catacutan (1991) did not find any difference in growth of juvenile shrimp fed isonitrogenous diets containing 5%-35% carbohydrate. Paulraj (1993) suggested the range of 35-40% for semi-intensive shrimp culture.

Akiyama *et al.* (1992) and D'Abramo (1997) reported that the recommended lipid level for commercial shrimp feed ranged from 5% to 8%. Gonzalez-Felix *et al.* (2002) reported that higher lipid levels above 10% reduced the growth of shrimp. The lipid content of the diet used in the present study was in the range of 3.39-7.89% which is almost same as that of the optimum value reported. The ash content was worked out to be varying in between 9-12 % (Table 4).

5.2. Water stability of formulated diets

Water stability was found to be good in the diet containing 10% and 20% *Spirulina* after 6 hours with retention over 75%. The higher water stability of the

feeds, obtained in the present study may be due to the better gelatinization of starch (Table 5).

5.3. Evaluation of efficiency of experimental diets

The present study was carried out to evaluate the nutritional quality of the *Spirulina* based diets through growth trials employing postlarvae of *Penaeus monodon*. Evaluation efficiency of the diets was based on growth (gain in length and weight), specific growth rate, survival, FCR, FCE, PER and protein digestibility coefficient. Initial as well as final carcass protein composition was also conducted to evaluate the test diets.

5.3.1. Growth

Among the different formulated diets tested in the present study, the treatment T4 with 20 % inclusion level of *Spirulina* gave superior growth performance in terms of length (gain in length 5.78cm)and weight (Gain in weight 2.374g) than other experimental diets tested individually (Table 6, 7). Further increase in the algal protein content in the diets significantly decreased the growth. The lowest growth was observed in the diet T6 with *Spirulina* content 40%. The result indicated that *Spirulina* can replace clam meat upto 20% level in the diet of *Penaeus monodon* postlarvae

Though a number of reports on replacement of fish meal with non-conventional plant feed stuffs are available (De Silva and Gunasekara, 1989; Ng and Wee, 1989 and Watanabe *et al*., 1990) it appears that for most non-conventional plant feed stuffs, the maximum recommended level of inclusion appears to be between 20% and 30% the of diets. Reports of fish meal replacement from practical fish and prawn diets with a single cell protein *Spirulina* were also made by Chow and Woo (1990), El-Sayed (1994), Manju (1994), Watanabe *et al.* (1990) and Nakagawa and Gomez-Diaz (1995). The maximum recommended levels of inclusion in all the above reports also in the range 20-35%.

Chow and Woo (1990) recommended 20% replacement of fishmeal with *Spirulina* in the diet for *Oreochromis mossambicus*. *Spirulina* was also studied as a

feed supplement for the fresh water prawn *Macrobrachium rosenbergii* (James *et al.,* 1990). They suggested that *Spirulina fusiformis* protein cannot be used as a sole protein source but can be effectively used as a supplementary protein source in the diet of *M. rosenbergii.* A similar observation was also made by Jaime *et al.* (2004) for *L. schmitti* larvae fed on *Spirulina platensis* based diet.

Ingthamjitr (1989) opined that the considerable amount of linolenic and (18:3 n3) in *Spirulina* may play an important role on larval shrimp growth. Tsai (1981) reported in case of the postlarvae of *Marsupenaeus japonicus* that the weight gain of shrimp was more satisfactory with fish meal and *Spirulina platensis* than on *Spirulina platensis* alone. In view of the above as well as the results obtained from the present experiment it can be concluded that *Spirulina fusiformis* protein cannot be used as a sole protein source but it can be used as supplementary protein in the diet of postlarvae of *Penaeus monodon*.

Early works on shrimp and prawn by Deshimaru and Shigueno (1972), New (1976) and Conklin *et al.* (1977) have shown that a mixture of two or more protein sources invariably gave better growth than single protein source. The improved performance of the mixed diet was mainly due to the fact that single protein source may not be able to provide all the essential amino acids in adequate levels and the deficiency may be overcome by mixing a number of protein sources so as to formulate a diet which closely meets the amino acid requirement of the test species (Boonyaratpalin and Chittiwan, 2003).

5.3.2. Specific growth rate

Specific growth rate can be considered as an index of growth in the evaluation of diets since it is a more refined and improved growth index than absolute weight gain or percentage growth rate (Hepher, 1988). The results of the present study indicated the highest SGR with the diet T4 containing *Spirulina* inclusion level of 20%, closely followed by T3 with 10% inclusion level (Table 8). However, the diet T1 (control) produced significantly lower SGR than the former two diets. The diets T6 with the *Spirulina* inclusion of 40% produced the lowest SGR, the value being lower than that in the control (T1). By complete replacement of

clam meat, comparatively less growth rate was obtained suggesting that some amount of animal protein in the diet may be essential for optimum growth in shrimp.

Manju (1994) reported the *Spirulina* at the level of 35% was optimum for obtaining maximum SGR in *Etroplus suratensis*. Matty and Smith (1978) also evaluated *Spirulina* as the sole source of protein for rainbow trout and reported that 35% protein diet produced highest SGR.

The SGR obtained in the present study indicated that diets with lower inclusion levels of *Spirulina* (10% and 20%) enhanced specific growth rate (6.69% and 6.82%) whereas diet with higher inclusion level (40%) of *Spirulina* significantly depressed growth performance (4.16%) in *Penaeus monodon* postlarvae. The results showed that the inclusion of plant protein, derived from single cell protein *Spirulina*, upto 20% inclusion level had beneficial effects on the growth performance of *Penaeus monodon* postlarvae.

5.3.3. Survival

In the present study, the postlarval shrimp in all the six treatments in the present study showed fairly good survival rates ranging from 75% to 97.5% (Table 9). The results indicated that upto 20% inclusion level did not produce much variation on survival rates. New (1976) in his review on the nutritional studies of shrimp and prawns has opined that mortalities in nutritional studies are rare unless the diet is grossly deficient in nutrients.

Neeraja (1998) found that the juvenile prawn showed fairly good survival rates ranging from 86.67% to 96.67%, suggesting that the different protein sources tested either individually or in various combinations did not produce much variation on survival rates. Survival was satisfactory for *M. rosenbergii* with *S. fusiformis* (James *et al*. 1990). Lu *et al.* (2002) also reported that juvenile tilapia fed *Spirulina* grew efficiently and showed a higher survival rate of 95%. Barbirto *et al.* (2006) reported a survival of (82-87%) in *Litopenaeus schmitti* larvae with the diet based on *Spirulina* and *Chaetoceros mulleri*. Hemanton *et al.* (2001) obtained a survival rate of 56% when fed *Penaeus monodon* with *Spirulina*. Nevertheless, high survival rate (75-

97.5%) was obtained in the present study. Shripatrao (2002) reported highest survival in *Penaeus monodon* at 30% inclusion level.

On comparing with the above the survival percentage (95-97.5%) obtained in the present study was much higher at lower inclusion levels of *Spirulina*. Even though there was no evident feed related mortality, the reduction in survival rate might be due to handling stress during sampling. The high survival of postlarvae observed in the present study could be attributed to the availability of nutritionally rich *Spirulina fusiformis* diet with high HUFA content.

5.3.4. Food conversion ratio and food conversion efficiency

Food conversion ratio is the ability with which an animal can convert food for the growth process and is reflected in the ratios of food consumed to the live weight it has gained (Mathew and Jayaprakash, 1990). It indicates the efficiency with which an animal can convert food for the growth process whereas food conversion efficiency is the ratio of live weight gained by animal to the feed it has consumed. Thus, low FCR indicates high food conversion efficiency or in other words, better food utilization. In the present study, lowest food conversion ratio and highest food conversion efficiency were registered for T4 followed by T3 diets with *Spirulina* level of 20% and 10% respectively (Table 10, 11). Control diet (T1) with clam meat alone gave FCR higher than the former two (Table 10). Diets T2 and T5 were not seen to be significantly different. However, when *Spirulina* level increased to 40% FCR showed the highest value and FCE showed lowest value, suggesting poor utilization and assimilation of feed. Similar observation was made by Shripatrao (2002).

Indrajasmine (1992) used plant protein (40 to 48%) and obtained FCR ranging between 1.46 to 2.25 for *Fenneropenaeus indicus*. James *et al.* (1990) reported a higher FCR of 4.97 in *M. rosenbergii* postlarvae fed with solar dried *Spirulina* as a protein source.

The lowest FCR and highest FCE values 1.49 and 66.43 obtained in the present study indicated the higher efficiency of food utilization of the diet containing 20% *Spirulina*. Harpaz and Schmalbach (1986) also attributed the better efficiency of the

diet supplemented with plant protein sources like *Spirulina*, to the better availability of nutrients like vitamin C to the shrimp.

According to Colvin (1976), protein source that is deficient in essential nutrients in adequate quantities produces less efficient feed conversion ratio. In the present study, the better food conversion ratios obtained for diets containing 10% and 20% level of inclusion of *Spirulina* might be due to the fact that even at lower diet intake level, combination of animal and plant protein sources provided essential nutrients in required proportion for better growth of shrimp.

5.3.5. Protein efficiency ratio

Protein efficiency ratio (PER) is used to evaluate the quality of dietary protein, those with high protein efficiency ratio can be considered as better quality and those with low values as poor quality. In the present study it was found that, *Penaeus monodon* postlarvae fed on diets containing 20% *Spirulina* was more efficient in converting dietary protein with PER of 1.67, followed by those fed on diets with 10 % *Spirulina* with PER of 1.48 (Table 12). There was no significant difference ($p<0.05$) in PER between these two diets. Control diet (T1) gave lower PER value compared to the former two diets. The diet T2 was not significantly different from T5.

Manju (1994) reported a PER of 1.12 to 2.31 in *Etroplus suratensis* fed a *Spirulina* based diet. James *et al.* (1990) found a PER value of 0.36 in *M. rosenbergii* postlarvae fed with *Spirulina fusiformis* based diet. On the contrary results of the present study showed higher PER values (0.62-1.67) with *Spirulina* based diets, of which the diet T4 (with 20% *Spirulina*) exhibited highest PER value (1.67).

De Silva and Davy (1990) opined that for optimum growth of shrimp, it is important to have a balance between dietary protein and energy ratio in diets and the ratio may differ according to the type of ingredients. The protein energy ratio lower or higher than optimum may lead to the reduction in growth of shrimp (Akiyama *et al.*, 1992).

In view of the above, it can be inferred that the diets (T3 and T4) containing low *Spirulina* inclusion levels of 10% and 20% have the required protein energy levels in the diets, yielded best results. This formulation therefore could be used to reduce the feed cost and enhance protein conversion for *Penaeus monodon*.

5.3.6. Protein digestibility coefficient

The digestibility obtained for the test diet T3 (89.68) and T4 (90.17) was considerably high, when compared to the other test diets (T1, T2, T5 and T6) indicating that incorporation of *Spirulina fusiformis* beyond a level of 30% impairs digestibility (Table 13). Better food conversion ratio and protein efficiency ratio of a diet result from better protein digestibility and absorption. The higher values obtained for the above two parameters for diets T3 and T4 directly imply that the protein digestibility and absorption were significantly better for these diets.

Jaime *et al.* (2004) reported that protein digestibility of 92 % for *Spirulina* fed to *Litopenaeus schmitti* larvae. In the present study also protein digestibility coefficient obtained for *Penaeus monodon* fed with *Spirulina* based diets was considerably high. This high digestibility attributed to the absence of cellulose and the presence of mucopeptide and muramic acid in the cell wall of this blue green alga (Venkataraman, 1983).

5.4 Determination of body protein

Protein analysis of experimental animals was done initially and on termination of the study (Fig. 9). From the result it was clear that shrimp fed with a diet containing optimum level of inclusion of *Spirulina* showed proper utilization of feed and protein. The level of inclusion of *Spirulina* significantly influenced the protein composition of body of shrimp.

The results showed that protein content of the test animals increased with increase of *Spirulina* in the diet and protein reached the maximum at 20% inclusion of *Spirulina* followed by T3 and T2. Lu *et al*. (2002) also reported similar increase of protein in the body of *Oreochromis mossambicus* fed with *Spirulina* based diets. It is thus clear from the present study that higher protein content of the *Spirulina* is

responsible for the higher content of protein in the body of *Penaeus monodon* postlarvae.

5.5. Water quality parameters

5.5.1. Temperature

A temperature range of 28±2^{0}C has been found to be optimum for *Penaeus monodon* (Foster and Beard, 1974). Several workers reported wide range of temperature tolerance for *Penaeus monodon* (Liao, 1977; Sasai, 1981). Chakraborti *et al.* (1986) observed a temperature range 24-35^{0}C for this species. The maximum temperature tolerance of *Penaeus monodon* has been reported to be 35^{0}C (Ravichandran *et al*., 1982). The weekly range of the present study was 26.9 to 32.1 (Table 14). The values recorded are within optimum range suggested for the growth of *Penaeus monodon*. The temperature fluctuation was gradual and could be maintained uniformly throughout the experimental period since the tanks were housed indoors.

5.5.2. pH

A range of 7.3-8.5 was suggested to be suitable for nursery rearing of *Penaeus monodon* by Parado-Estepa *et al*. (1990). Noor-Hamid *et al*. (1992) recommended he neutral pH for faster growth. In the present study, the weekly range of pH value in the tanks was from 7.5 to 8. These values conform to those obtained in the previous studies and within optimum range (Table 15).

5.5.3. Dissolved oxygen

Chakraborti *et al.* (1986) have suggested the optimum range to be between 6.8-7.6 ppm for *Penaeus monodon* though they can tolerate DO as low as 4.8 ppm. The weekly DO values in the experimental tank ranged from 5 to 7.8 ppm (Table 16), since mild aeration was provided in the tanks. These values were found to be optimum for the growth of *Penaeus monodon* postlarvae.

5.5.4. Total alkalinity

Waters of low alkalinity are poorly buffered against fluctuations in pH and consequently rapid reduction in pH occurs when carbon dioxide levels goes down. Ayyappan and Rao (2000) reported tolerable range of alkalinity as 40 to 150 ppm. Total alkalinity values recorded in the present study ranged from 80 to 110 ppm which was in this tolerable limit (Table 17).

5.5.5. Ammonia

In the present study the ammonia concentration ranged between 0.01 ppm to 0.03 ppm. Growth of *Penaeus monodon* was found impaired at unionized ammonia concentration of 0.01 ppm to 0.5 ppm (Smith *et al.,* 2002). The concentrations of ammonia recorded in the present study were within these limits (Table 18).

6. SUMMARY

In the present study, the efficacy of pelleted feeds formulated by replacing clam meat with *Spirulina fusiformis* was evaluated. Clam meat was progressively replaced by *Spirulina fusiformis* in the diets fed to *Penaeus monodon* postlarvae at various inclusion levels. A comparison has also been made between control diet and diets containing *Spirulina fusiformis* at various inclusion levels.

In the present study, postlarvae of *Penaeus monodon* having an average weight 0.04g were used as experimental animals. The experiment was conducted in Completely Randomized Design with six treatments and four replications each, for a period of 60 days.

Proximate analysis of various feed ingredients used in the formulation of test diets showed that *Spirulina* contained highest crude protein of 60% followed by clam meat (53%) and groundnut oil cake (42%).

Six isonitrogenous (40% protein) test diets were prepared for the study. They were diet T1 with clam meat + GOC + tapioca flour+ Wheat bran + cod liver oil + vitamin mineral mix, diets T2, T3, T4, T5 and T6 where clam meat was progressively replaced with 5%, 10%, 20%, 30% and 40% *Spirulina* respectively.

The feed preparation method used in the present study was simple, rapid and relatively inexpensive leading to acceptable levels of water stability.

Proximate analysis of the formulated diets showed that crude protein content of the diets ranged between 40% and 42% while the crude fat content ranged between 1.3% and 8.9%.

The variations in water quality parameters over the experimental period were found to be well within the tolerable limits for the optimum growth of *Penaeus monodon* postlarvae.

Various evaluation indices *viz*., gain in weight, specific growth rate, percentage survival, FCR, FCE, PER, protein digestibility coefficient and carcass protein composition of the test animals were determined.

On termination of 60 days rearing, better growth rates were recorded in *Penaeus monodon* postlarvae fed the diet with 20% of *Spirulina*.

Specific growth rate was highest in shrimp postlarvae fed with 20 % inclusion of *Spirulina* powder.

Higher percentage of survival of *Penaeus monodon* postlarvae was found with inclusion level upto 20%. Further increase in the inclusion level significantly decreased the survival.

Low food conversion values were obtained in shrimp fed on the diets with 20% and 10% level of inclusion of *Spirulina fusiformis* over the control diet without *Spirulina fusiformis*.

High protein efficiency ratios were recorded with diets T4 (20% *Spirulina*) and T3 (10% *Spirulina*) than T1 without *Spirulina fusiformis*.

7. REFERENCES

A.O.A.C. 1990. *Methods of Analysis. Association of Official Analytical Chemists*. 15th edition, Arlington, VA., p.72.

Akiyama, D.M., Dominy, W.G. and Lawrence, A.L., 1991. Penaeid shrimp nutrition for commercial feed industry. *Proceedings of the Aquaculture Feed Processing and Nutrition Workshop, September 19-25, 1991* (Eds. Akiyama, D.M., Tan, R.K.M.). American Soyabean Association, Singapore, Thailand and Indonesia, pp. 80-98.

Akiyama, D.M., Dominy, W.G. and Lawrence, A.L. 1992. Penaeid shrimp nutrition. *Marine Shrimp Culture: Principle and Practices* (eds. Fast, A.L. and Lester, L.J.), Elsevier Science Publishing Inc., New York, pp. 535-568.

Alagarswami, K. and Ali, S.A. 2000. Indigenous feeds for shrimp farming-Development and assessment in comparison with imported feeds. *Aquaculture Feed and Health* (eds. John, G. and Ninawe, A.S.), Biotechnology Consortium India Ltd., New Delhi, p. 56-66.

Alava, V.R. and Pascual, F.P. 1987. Carbohydrate requirement of *Penaeus monodon* Fabricius juveniles. *Aquaculture* 61: 211-217.

Ali, S.A. 1992. Evaluation of some animal and plant protein sources in the diet of shrimp *Penaeus indicus*. *Asian fisheries sci.* 5(3): 277-289.

Ali, S.A. 2006. *Indigenous Shrimp Feed Technology by CIBA*. CIBA Technlogy Series-1, Central institute of Brackish water Aquaculture, Chennai, p. 8.

Ali, S.A., Gopal, C. and Ramana, J.V. 2000. *Shrimp Feed Processing and Production Technology*. CIBA, Chennai, p. 17.

Anon. 1999. *Spirulina* flakes boost shrimp. *Fish Farming Int.* 26(5): 32

Ayyappan, S. 1991. Potential of *Spirulina* as a feed supplement for carp fry. *Spirulina, Ecology, Taxonomy, Technology and application* (eds. Sheshadri,

C.V. and Jeeja Bai), proceeding of national symposium on *Spirulina*, 1992. MCRC, Madras pp. 171-172.

Ayyappan, S. and Rao, J. 2000. Hatchery technology of Giant freshwater prawn, *Macrobrachium rosenbergii*. *Fishing Chimes* 19 (10 &11): 112-121.

Bages, M. and Sloane, L. 1981. Effect of dietary protein and starch level on growth and survival of *Penaeus monodon* (Fabricius) postlarvae. *Aquaculture* 25: 117-128.

Barbirto J., Ceballos, J., Liamas, A.H., Galano, T.G. and Villarreal, H. 2006. Substitution of *Chaetoceros muelleri* by *Spirulina platensis* meal in diets for *Litopenaeus schmitti* larvae. *Aquaculture* 260(1-4): 215-220.

Bautista M.N., 1986. The response of *Penaeus monodon* juveniles to varying protein/energy ratios in test diets. *Aquaculture* 53**:** 229-242.

Becker, E.W. and Venkataraman, L.V. 1982. *Biotechnology and exploitation of Algae: The Indian approach.* German Agency for Technical cooperation, Eschborn, p.140.

Belay, A., Kato, T. and Ota, Y. 1996. *Spirulina* (Arthrospira): Potential application as an animal feed supplement. *J. Appl. Phycol*ogy 8: 303-311.

Benemann, J.R. 1992. Microalgae aquaculture feeds. *J. of Appl. Phycology* 4(3):12-13.

Boonyaratpalin, M. 1998. Nutrition on *Penaeus merguiensis* and *Fenneropenaeus indicus*. *Rev. in Fisheries Sci*. 6: 69-78.

Boonyaratpalin, M. and Chittiwan, V. 2003. *Macrobrachium* Nutrition: food and feeding. *Fresh water prawns: Advances in biology, aquaculture and marketing* (eds. Nair, C.M, Namboodiri, D.D., Jose, S., Sankaran, T.M., Jayachandran, K. and Salin, K.R). Allied Publishers. New Delhi, pp. 521-534.

Brito, R., Rosas, C., Chimal, M.E. and Gaxiola, G. 2001. Effect of different diets on growth and digestive enzyme activity in *Litopenaeus vannamei* (Boone, 1931) early postlarvae. *Aquaculture Res.* 32: 257-266.

Burford M.A., Smith D.M., Tabrett S.J., Coman F.E., Thompson P.J., Barclay M.C. and Toscas P.J. 2004. The effect of dietary protein on the growth and survival of the shrimp, *Penaeus monodon* in outdoor tanks. *Aquaculture Nutr.* 10:15-23.

Catacutan, M.R. 1991. Apparent digestibility of diets with various carbohydrate levels and the growth response *Penaeus monodon*. *Aquaculture* 95: 89-96.

Ceballos, B.J., Villarreal, H., Garcia, T., Perez-Jar, L., and Alfonso, E. 2005. Effect of *Spirulina platensis* meal as a feed additive on growth, survival and development in *Litopenaeus schmitti* shrimp larvae. *Rev. Invest. Mar*. 26(3): 235-241.

Chakraborti, R.K., Holder, D.D., Das, N.K., Mandal, S.K. and Bhowmik, M.L. 1986. Growth of *Penaeus monodon* Fabricius under different environmental conditions. *Aquaculture* 51: 189-194.

Chen, H.Y. 1993. Requirements of marine shrimp, *Penaeus monodon* juveniles for phosphatidycholine and cholesterol. *Aquaculture* 109: 165-176.

Chen, H.Y. and Hwang, G. 1992. Estimation of the dietary riboflavin required to maximize tissue riboflavin concentration in juvenile shrimp (*Penaeus monodon*). *J. Nutr*. 122: 2474-2478.

Chen, H.Y. and Jenn, J.S. 1991. Combined effect of dietary phosphatidylcholine and cholesterol on the growth, survival and body lipid composition of marine shrimp, *Penaeus penicillatus*. *Aquaculture* 96: 167-178.

Chen, H.Y., Leu, Y.T. and Roelants, I. 1992. Quantification of arginine requirements of juvenile marine shrimp, *Penaeus monodon*, using microencapsulated arginine. *Marine Biol.* 114: 229-233.

Chen, H.Y., Wu, F.C. and Tang, S.Y. 1991. Thiamin requirement of juvenile shrimp (*Penaeus monodon). J. Nutr.* 121: 1984-1989.

Chow, C.Y. and Woo, N.Y.S. 1990. Bioenergetics studies on an omnivorous fish *Oreochromis mossambicus*. *Evaluation of the utilization of Spirulina algae in feed* (eds. Hirano, R. and Hanyu, I.). Proceeding of the 2nd Asian Fisheries Forum, 1990. The Asian Fisheries Society; Manila, pp.291-294.

Chuntapa, B., Powtongsook, S. and Menasveta, P. 2003. Water quality control using *Spirulina platensis* in shrimp culture tanks. *Aquaculture* 220(1-4): 355-366.

Colvin, P.M. 1976. Nutritional studies on penaeid prawns: protein requirement in compounded diets for juvenile *Penaeus indicus. Aquaculture* 7: 315-326.

Conklin, D.E. 2003. *Use of soybean meal in the diet of marine shrimp. United soybean board and American soybean association.* Technical paper, Department of Animal Science, University of California, Davis, p. 144.

Conklin, D.E., Devers, K. and Bordner, C.E. 1977. Development of artificial diets for lobster *H. americanus. Proc. Ann. Meeting Wld. Maricult. Soc*. 8: 841-855.

D'Abramo, L.R. and Sheen, S.S. 1994. Nutritional requirements, feed formulation, and feeding practices for intensive culture of the fresh water prawn *Macrobrachium rosenbergii. Rev. in Fisheries Sci*. 21: 1-21.

D'Abramo, L.R. 1997. *Triacylglycerols and fatty acids. Crustacean Nutrition,* Vol. 6 (eds. D'Abramo, L.R., Conklin, D.E. and Akiyama, D.M.). World Aquaculture Society, Baton Rouge, Louisiana, pp. 235.

D'Souza, F.M.L., Leccossois, D., Heasman, M.P., Diemar, J.A., Jackson, C. and Pendrey, R.C. 2000. Evaluation of centrifuged microalgae concentrates as diets for *Penaeus monodon* Fabricius larvae. *Aquaculture Res.* 31: 661-670.

De Silva, S.S. and Gunasekara, R.M. 1989. Effect of protein level and amount of plant ingredients (*Phaseolus aureus*) incorporated into feed on consumptions,

growth performance and carcass composition of *Oreochromis niloticus* fry. *Aquaculture* 80: 121-123.

De Pauw, N., Morales, J. and Persoone, G. 1984. Mass culture of microalgae in aquaculture system: progress and constrains. *Hydrobiogia* 116/117: 121-134.

De Silva, S.S. and Davy, F.B. 1990. Fish nutrition research for semi-intensive culture system in tropics–some strategies for the future. *J. Wld. Aquaculture Soc*. 17:37-43

Deering, M.J., Fielder, D.R. and Hewitt, D.R. 1997. Growth and fatty acid composition of juvenile leader prawns, *Penaeus monodon*, fed different lipids. *Aquaculture* 151: 131-141.

Deshimaru, O. and Shigueno, K. 1972. Introduction to artificial diet for prawn, *Penaeus japonicus*. *Aquaculture* 1:115-133.

Deshimaru, O and Yone, Y. 1978. Optimum level of dietary protein for prawn. *Bull. Japanese Soc. of Sci. Fisheries* 44: 1395-1397.

Deshimaru, O., Kuroki, K. and Mazid, M.A. 1985**.** Nutritional quality of compounded diets for prawn *Penaeus monodon. Bull. Japanese Soc. of Sci. Fisheries,* 51(6): 1037-1044.

Dy-Penaflorida, V. 1989. An evaluation of indigenous protein sources as potential component in the diet formulation for tiger prawn, *Penaeus monodon* using essential amino acid index (EAAI). *Aquaculture* 83:319-330.

El-Sayed, A.F.M. 1994. Evaluation of Soybeans meal, *Spirulina* meal and chicken offal meal as a protein source for silver sea bream (*Rhabdosargus sarba*) fingerlings. *Aquaculture* 127: 169-176.

Foster, J.R.M. and Beard, T.W. 1974. Experiments to assess the suitability of nine species of prawns for intensive cultivation. *Aquaculture* 3: 355-368.

Gabaudam, J. 1998. Dietary astaxanthin improves production yield in shrimp farming. *Fishing Chimes* 18: 37-39.

Godfred-Ponraj, J., Kannupandi, T. and Sriraman, K. 1990. Evaluation of growth parameters of the prawn, *Penaeus monodon* (Fabricius) fed on different diets. *Proceedings of the National Seminar on Aquaculture Development in India-Problem and Prospectus, November, 27-29, 1990*. University of Kerala, Trivendram, pp. 217-224.

Gonzales-Felix, M.L. and Perez-Velazquez, M. 2002. Current status of lipid nutrition of Pacific white shrimp, *Litopenaeus vannamei.Proceeding of Advaces en Nutrición Acuícola VI. Memorias del VI Simposium Internacional de Nutrcion Acuicola Cancun* (eds. Cruz-Suarez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Gaxiola-Cortes, M.G. and Simoes, N.), Quintana Roo, Mexico, pp. 78-85.

Gonzalez-Felix, M.L., Gatlin, D.M., Lawrence, A.L. and Perez-Velazquez, M. 2002. Effect of various dietary lipid levels on quantitative essential fatty acid requirements of juvenile Pacific white shrimp *Litopenaeus vannamei. J. of the Wld. Aquaculture Soc.* 33: 330-340.

Harpaz, S. and Schmalbach, E.A. 1986. Improved growth and health of the Malaysian prawn, *Macrobrachium rosenbergii* by addition of fresh leaves to the artificial diets. *Aquaculture* 55(2): 81-85.

Harper, H.A. 1981. Some direction in the evaluation of protein quality. *J. Agric. Chem*. 29: 425-429.

Hastings, W.E. 1976. Fish nutrition and fish feed manufacture. *FAO Tech.Conf.on Aquaculture, Japan* FIR:AQ/Conf/76/R.,23 p.13

Hemtanon, P., Sataporn, D., Vira, J. and Opas, T. 2001. Study on using *Spirulina platensis* for nursing black tiger shrimp postlarvae. *Proceedings of the 3rd National Symposium on Marine Shrimp, November 8-9, 2001*. Queen Sirikit National Convention Center, Bangkok, p.121

Hematanon, P., Direkbusarakom, S. and Bunyaviwat, V. 2005. Application of *Spirulina platensis* for prevention of white spot syndrome virus in postlarvae

and juvenile black tiger shrimp (*Penaeus monodon*). *Songklanakarin J. of Sci. and Technol.* 27(1): 253-263.

Henson, R.H. 1990. *Spirulina* algae improve Japanese fish feeds. *Aquaculture mag.* 16(6): 38-43.

Hepher, B. 1988. *Nutrition of pond fishes*. Cambridge University Press, Cambridge, p.388.

Howell, B.K. and Matthew, A.D. 1991. The carotenoid of wild and blue disease affected farmed tiger shrimp (*Penaeus monodon*) Fabricius. *Comp. Biochem. Physiol*. 98B: 375-379.

Indrajasmine, G. 1992. Effect of non-conventional feed ingredients on biochemical composition and growth in the Indian white Prawn *Penaeus indicus*, Thesis submitted to Manonmanian Sundaranar University, Thirunelveli, p.102.

Ingthamjitr, S. 1989. Use of *Spirulina* of *Penaeus monodon* larvae, Masters thesis, Asian Institute of Technology, Bangkok, p.86.

Jaime C.B., Villarreal-Colmenares, H., Garcia-Galano, T., Civera-cerecedo, R. and Gaxiola- cortes, G. 2004. Effect of *Spirulina platensis* meal as feed additive on growth, survival and development in *Litopenaeus schmitti* shrimp larvae (perez-Farfante and Kensley, 1997). *Proceeding of advance en Nutricion Acuicola VII, memorials del VII simposium International de Nitricion Acuicola*, *November 16-19, 2004* (eds. cruz-suarez, L. E., Ricque Marie, D., Nieto Lopez, M.G., Villarreal, D., Scholz U.and Gonzalez, M.). Hermosillo, Sonora, Mexico, pp. 617-635.

James, T., Shrief, P.M., Nair, C.M. and Thampy, D.M. 1990. Evaluation of *Spirulina fusiformis* as a protein source in the diet of the postlarvae of *Macrobrachium rosenbergii*. (eds. Silas, E.G.). *Proceedings of the National Symposium on Freshwater prawns (Macrobrachium sp.).* Kerala Agricultural University, Kochi, pp. 234-237.

Jayaram M.G. and shetty, H.P.C. 1981. Studies on growth rates of catla, rohu and

common carp fed on different formulated diets. *Mysore J. Agri. Sci.* 14: 598-606.

Jones, D.A. 1998. Crustacean larval microparticulated diets. *Rev. in Fisheries Sci*. 6(1-2): 41-54.

Kanazawa, A. and Teshima, S. 1977. Biosynthesis of fatty acids from acetate in the prawn, *Penaeus japonicus. Mem. of the Fac. of Fisheries, Kagoshima Univ.* 26: 49-53.

Kies, C. 1981. Bioavailability a factor in protein quality. *J. Agric. Fd. Chem*. 29: 435-490.

Lawrence, A. 2004. Recent Advances in shrimp nutrition. *Aqua feeds: Formulation and Beyond* 1(2): 25-28.

Lawrence, A.L. and Lee, P.G. 1997. Research in Americas. *Crustacean nutrition* (eds. D'Abramo, L.R., Concklin, D.E. and Akiyama, D.M.). World Aquaculture Society, Baton Rouge, USA, pp. 566-587.

Lee, P.G. and Lawrence, A.L. 1985. Effects of diet and size on growth, feed digestibility and digestive enzyme activities of the marine shrimp*, Penaeus setiferus* Linnaeus. *J. Wld. Maricult. Soc.* 16: 275-278.

Lee, M.H. and Shiau, S.Y. 2004. Vitamin E requirements of juvenile grass shrimp, *Penaeus monodon*, and effects on non-specific immune system. *Fish Shell Fish Immunology* 16(4): 475-485.

Liao, I.C. 1977. A culture study on grass prawn *Penaeus monodon* in Taiwan the patterns, the problems and prospects. *J. Fisheries Soc., Taiwan*, 5(2): 11-29.

Liao, I.C. 1998. History, present status and prospectus of prawn culture in Taiwan. *Proceeding of shrimp 88*, *January 26-28, 1998*. INFOFISH, Bangkok, pp. 195-213.

Liao, I.C. and Liu, F.G. 1989. A brief review on nutritional studies of *Penaeus monodon*. *J. of Adv. in trop. Aquaculture* 9: 355-380.

Lim, C., Suraniranat, P. and Platon, R. 1978. A preliminary study on the evaluation of casein, shrimp meal, squid meal and *Spirulina* meal as a protein source of *Penaeus monodon* (Fabricius) postlarvae. *Quarterly Report on the SEAFDEC AQD* 2(2): 13-18.

Lim, C., Suraniranat, P. and Platon, R. 1979. Evaluation of various protein sources for *Penaeus monodon* postlarvae. *Kalikasan, Philipp. J. Biol.* 8(1): 29-36.

Lim, C. and Akiyama, D.M. 1995. Nutrient requirements of penaeid shrimp. *Nutrition and utilization technology in aquaculture* (eds. Sessa, D.J. and Lim, C.), AOCS Press, Champaign, Illinois, pp. 39.

Lorenz, R.T. 1998. *A review of Spirulina as a carotenoid and vitamin source for cultured shrimp. Spirulina* Pacifica Technical Bullerin No. 50. Cyanotech Corporation, Tokyo, p. 24.

Lu, J., Yoshizaki, G., Sakai, R. Takeuchi, T. and Satoh, S. 2002. Effect of growth and body composition of juvenile tilapia *Oreochromis niloticus* fed raw *Spirulina*. *Fisheries Soc.* 68(1) 34-40.

Lung, C.Q. 1991. Vitamin B_{12} and folic acid requirement of *Penaeus monodon* and Vitamin B_{12} requirement of tilapia (*Oreochromis niloticus* x *O. aureus*). Masters thesis, National Taiwan Ocean University, Keelung, p.143.

Manju, K.G., 1994. Culture of *Spirulina fusiformis* and its evaluation as a protein source in the diet of *Etroplus suratensis*. Masters thesis, Kerala Agricultural University, Trichur, p.146.

Mathew, A. and Jayaprakash, V. 1990. Role of dietary protein on the survival, growth and conversion efficiency of the white shrimp, *Feneropeneaus indicus*. *Proceeding of the National Seminar on Aquaculture Development in India- Problem and Prospects*, *November 27-29, 1990* (eds. Edwards, M.). University Of Kerala, Trivendram, pp. 209-216.

Matty, A.J. and Smith, P. 1978. Evaluation of yeast, bacterium and alga as a protein source for rainbow trout: Effect of protein level on growth, gross conversion efficiency and protein conversion efficiency. *Aquaculture* 14: 235-246.

Merican, Z.O. and Shim, K.F. 1997. Quantitative requirements of linolenic and docosahexaenoic acid for juvenile *Penaeus monodon. Aquaculture* 157: 277-295.

Millamena, O.M., Bautista-Teruel, M.N. and Kanazawa, A. 1996. Methionine requirement of juvenile tiger shrimp *Penaeus monodon* Fabricius. *Aquaculture* 143: 403 - 410.

Millamena, O.M., Bautista-Teruel, M.N., Reyes, O.S. and Kanazawa, A. 1997. Threonine requirement of juvenile marine shrimp *Penaeus monodon*. *Aquaculture* 151: 9 - 14.

Millamena, O.M., Bautista-Teruel, M.N., Reyes, O.S. and Kanazawa, A. 1998. Requirements of juvenile marine shrimp, *Penaeus monodon* (Fabricius) for lysine and arginine. *Aquaculture* 164:95-104.

Millamena, O.M., Bautista-Teruel, M.B., Kanazawa, A. and Teshima, S. 1999. Quantitative dietary requirements of post-larval tiger shrimp, *Penaeus monodon*, for histidine, isoleucine, leucine, phenylalanine, and tryptophan. *Aquaculture* 179: 169-179.

Mitra, G., Mukhopadhyay, P.K.and Chattopadhyay, D.N.2005. Nutrition and feeding in Fresh water prawn (*Macrobrachium rosenbergii*) farming. *Aquafeeds: Formulation and Beyond* 2 (1):17-19.

Miyasaki, T., Sato, M., Yoshinaka, R. and Sakaguchi, M. 1995. Effect of vitamin C on lipid and carnitine metabolism in rainbow trout. *Fisheries Sci.* 61, 501-506.

Mustafa, M.G. and Nakagawa, H. 1995. A review: Dietary benefits of algae as an additive in fish feed. *Isr. J. Aquaculture* 47: 155-162.

Mustafa, M.G., Umino, T. and Nakagawa, H. 1994. The effect of *Spirulina* feeding on muscle protein deposition in red sea bream, *Pagrus* major. *J. Appl. Ichthyology* 10: 141-145.

Nadal, N.G.M. 1971. Sterols of *Spirulina* maxima. *Phytochem.* 10: 2537-2538.

Nakagawa, H. and Gomez-Diaz, G. 1995. Usefulness of *Spirulina* Meal as feed additive for giant freshwater prawn, *Macrobrachium rosenbergii*. *Suisanzoshoku* 43:521-526.

Nandeesha, M.C., Gangadhar, B., Varghese, T.J. and Keshavanath, P. 1998. Effect of feeding *Spirulina platensis* on the growth, proximate composition and organoleptic quality of common carp, *cyprinus carpeo* L. *Aquaculture Res.* 29: 305-312.

Neeraja, T. 1998. Growth response of giant freshwater prawn *M .rosenbergii* juveniles to feed based on combined animal protein sources. Masters thesis, Kerala Agricultural University, Trichur, pp.101.

New, M.B. 1976. A review of dietary studies with shrimp and prawns. *Aquaculture* 9: 101-144.

Ng, W.K. and Wee, K.L. 1989. The nutritive value of cassava leaf meal in pelleted feed for Nile tilapia. *Aquaculture* 89: 45-48.

Noor-Hamid, S., Fartes, R.D. and Parado-Estepa, F.D. 1992. Effect of pH and ammonia on survival and growth of early stages of *Penaeus monodon* Fabricius. *Aquaculture* 125: 67-72.

Parado-Estepa, F.D., Quinitio, E.T. and Borlongan, E.L. 1990. *Prawn hatchery operation*. *SEAFDEC Extension Mannual* 19: 14-15.

Pascual, F.P. 1989. *Nutrition and feeding of Penaeus monodon*, third edition, SEAFDEC AQD, Tigbauan, Iloilo, pp. 24.

Pascual F.P., Coloso, R.M and Tamse, C.T. 1983**.** Survival and some histological changes in *Penaeus monodon* Fabricius juveniles fed various carbohydrates. *Aquaculture,* 31**:** 169-180.

Paulraj, R. 1993. *Handbook of aquafarming: Aquaculture feed*. MPEDA, Cochin, p. 93.

Pearson, 1976. *The Chemical Analysis of Food*, First edition, Churchill, London, p. 381.

Pedrazzoli, A., Molina, C., Montoya, N., Townsend, S. Leohing, A., Parades, Y. and Calderon, J. 1998. Recent advanason Nutrition Research on *Litopenaeus vannamei* in Equador. *Rev. in Fisheries Sci.* 6: 1-2.

Puanglap, N. 2000. An application of *Spirulina* sp As a source of carotenoid pigment for the tiger prawn *Penaeus monodon* Fabricius diet. Masters thesis. Kasetsart University, Bangkok, p. 112.

Quillet, M. 1975. Recherche sur les substanances glucidiques elabores par les spirulines. *Annales de la Nutrition et de l'Alimentation* 29:553-561.

Rangaswami, R. 2002. *A Textbook of Agriculture Statistics*. New Age International (P) Limited, New Delhi, pp. 496.

Ravichandran, P., Verghese, P.V., Karmaker, H.C. and Rajyalakshme, T. 1982. Effects of stocking density and size of *Penaeus monodon* Fabricius postlarvae in nursery rearing experiments. *Proc. Symp. Coastal Aquaculture Mar. Biol. Assn.* 1982*,* India 1: 412-423.

Richmond, A. 1989. *Spirulina. Micro-algal Biotechnology*, Second edition (eds. Borowitzka, M. And Borowitzka, L.J.), Cambridge University Press, Cambridge, pp. 85-121.

Sahadevan, P. 1994. A good quality feed for shrimp: some notes for the farmers. *Fishing Chimes* 26(11): 16-18.

Sambavisam, S., Subramanian, P. and Krishnamurthy, K. 1982. Observation of growth and conversion efficiency in the prawn *Fenneropenaeus indicus* fed on different protein levels. *Symp. Ser. Marine Biol. Assoc. India* 6: 406-409.

Santiago, C.B., Pantastico, J.B., Baldia, S.F. and Reyes, O.S. 1989. Milkfish (*Chanos chanos*) fingerling production in freshwater ponds with the use of natural and artificial feeds. *Aquaculture* 170: 307-318.

Sasai, K. 1981. A better experience in grass prawn culture. *Fish. Cult.* 18(1): 63-67.

Sethi, S.N. and Naik, G.B. 2007. Wonder gift of nature: *Spirulina*. *Fishing Chimes* 27(3): 16-17.

Sheen, S.S., Liu, P.C., Che, J.C. 1994. Cholesterol requirement of tiger shrimp (*Penaeus monodon*). *Aquaculture* 125:131-137.

Shiau, S.Y. 1998. Nutrient requirement of penaeid shrimp. *Aquaculture* 164: 77-93.

Shiau, S.Y., Kwok, C.C. and Chou, B.S. 1991. Optimum dietary protein level of *Penaeus monodon* reared in seawater and brackishwater. *Nippon Suisan Gakkaishi* 57: 711-716.

Shiau, S.Y. and Hwang, S.Y. 1994. Dietary folic acid requirement determined for grass shrimp, *Penaeus monodon*. *J. of Nutr.* 124(1): 245-250.

Shiau, S.Y. and Liu, J.S. 1994. Quantifying the Vitamin K Requirement of Juvenile Marine Shrimp (*Penaeus monodon*) with Menadione. *J. of Nutr.* 124 (2): 277-282.

Shiau, S.Y. and Peng, C.Y. 1992. Utilization of different carbohydrates at different dietary protein level in grass prawn, *Penaeus monodon*, reared in sea water. *Aquaculture* 101: 241-250.

Shripatrao, J.V. 2002. Biomodulation of non-specific immune response in the tiger shrimp *Penaeus monodon* Fabricius with *Spirulina* incorporated diet. Masters thesis, Kerala Agricultural University, Trichur, p.99.

Singh, S.K., Singh, K.M. and Prashant, K. 2007. Preparation and application of feed. *Fishing Chimes* 26(11): 16-18.

Smith, D.M., Burford, M.A., Tarbrett, S.J. and Ward, L. 2002. The effect of feeding frequency on water quality and growth of the black tiger shrimp (*Penaeus monodon*). *Aquaculture* 207: 125-136.

Smith, D.M., Tonks, M.L., Tabrett, S.J. and Williams, K.C. 1999. A reference diet for nutritional studies for the giant tiger prawn *Penaeus monodon*. *Aquaculture Nutr.* 5: 33-39.

Sommer, T.R., Potts, W.T. and Morrissy, N.M. 1992. Recent progress in the use of processed microalgae in aquaculture. *J. of Appl. Phycology* 4(3):54-58.

Soong, P., 1980. Production and development of Chlorella and *Spirulina* in Taiwan. *Algae Biomass* (eds. Shelef, G. & Soeder, C. J.), Elsevier/ North-Holland, Amsterdam pp. 97-113.

Stanley, C.G. and Jones, J.B. 1996. Feeding algae to fish. *Aquaculture* 7: 219-223.

Switzer, L. 1980. *Spirulina- The Whole Food Revolution.* Bantam Books, New York, p. 39.

Takeuchi, T., Lu, J. Yoshizaki, G. and Satoh, S. 2002. Effect on the growth and body composition of juvenile tilapia *Oreochromis niloticus* fed raw *Spirulina*. *Fisheries Sci*. 68: 34-40

Tanaka, Y. 1978. Comparative biochemical studies on carotenoid in aquatic animals. *Mem. Fac. Fish., Kagoshima Univ* . 27: 355-422 .

Teshima, S. and Kanazawa, A. 1971. Biosynthesis of sterols in the lobster, *Panulirus japonicus*, the prawn, *Marsupenaeus japonicus*, and the crab, *Portunus trituberculatus*. *Comparative Biochem. Physiol*. 38B: 597-602.

Teshima, S. and Kanazawa, A. 1984. Effect of protein, lipid and Carbohydrate level in purified diets on growth and survival rates of the prawn larvae. *Bull. Japanese Soc. Sci. Fisheries* 50:1709-1715.

Teshima, S.I., Kanazawa, A., Hitotsumatsu, K.I., Kim, K.S., Oshida, K., Koshio, S. 1992. Tissue uptake and bioconversion of ecosapentaenoic acid and phosphatidylcholine in prawns, *Penaeus* and *Macrobrachium*. *Comparative Biochem. and Physiol.* 102B: 885-890.

Teshima S., Kanazawa, A. and Kakuta, Y. 1986**.** Effects of dietary phospholipids on growth and body composition of the juvenile prawn. *Bull. Japanese Soc. Sci. Fish.,* 52 (1**):** 155-158.

Teshima S., Kanazawa, A., Sasada, H. and Kawasaki, M. 1982**.** Requirements of the larval prawn, *Penaeus japonicus* for cholesterol and soybean phospholipids. *Mem. of the Fac. of Fisheries, Kagoshima Univ.,* 31**:** 193-199.

Tornabene, T.G., Bourne, T.F. Raziuddin, S. and Ben-Amoz, A. 1985. Lipid and lypopolysaccharide constituents of cyanobacterium *Spirulina platensis* (Cyanophyceae, Nostacles). *Marine Ecol.* 22, 121-125.

Tsai, P.H. 1981. Evaluation of *Spirulina platensis* as a protein source in fish and shrimp nutrition. *Aquaculture* 2(3): 32-40.

Velasco, M., Lawrence, A.L. Castile, F.L. and Obaldo, L.G. 2000. *Dietary protein requirement for Litopenaeus vannamei. Proceeding of Advance en Nutrición Acuícola*, *November 19-22, 2000* (eds. Cruz-suarez, L.E., Risqué-Marie, D., Tapia-Salazar, M., Olvera-Novova, M.A. and Civera-Cerecedo, R.). Merida Yucutan, Mexico, pp. 181-192.

Venkataraman, L.V. 1980. Algae for feed and food. *A critical appraisal based on Indian expieriences*. Proceeding of National Workshop on Algal systems. Indian society of Biotechnology, New Delhi, India, pp. 83-134.

Venkataraman, L.V. 1983. *A monograph on Spirulina platensis: Biotechnology and application.* Department of Science and Technology, Mysore, p.100.

Venkataraman, L.V. 2003. *Spirulina* as a source of immunostimulants and growth

factor in aquaculture systems. *Aquaculture medicine* (eds. Singh, I.S.B., Pai, S.S., Philip, R. and Mohandas, A.). Centre for fish disease diagnosis and management, school of environment studies, Cochin University Of Science And Technology, Cochin, Kerala, pp. 174-177.

Venkatramiah, A., Lakshmi, G.J. and Gunter, G. 1975. Effect of protein level and vegetable matter on growth and food conversion efficiency of brown shrimp. *Aquaculture* 9: 115-125.

Watanabe, T., Liao, W., Takeuchi, T. and Yamamoto, H. 1990. Effect of dietary *Spirulina* supplement on growth performance and flesh lipids of cultured striped jack. *J.Tokyo Univ. Fish*. 77: 231-239.

Wu, L. and Dong, S. 2002. Effect of protein restriction with subsequent realimentation on growth performance of juvenile Chinese shrimp (*Fenneropenaeus chinesis*). *Aquaculture* 210: 343-358.

www.brineshrimpdirect.com/Fact-Sheet-c88.html

Xu, X., Ji, W., Castell, J.D. and O'Dor, R. 1993. The nutritional value of dietary n-3 and n-6 fatty acids for the Chinese prawn (*Penaeus chinensis*). *Aquaculture* 118: 277-285.

8. ABSTRACT

The blue green alga *Spirulina fusiformis* has been accepted as an ideal food organism since it has a high protein content of 60-65 %, besides being rich in vitamins, minerals, fatty acids (gamma-linolenic acid (GLA)) and antioxidant pigments such as carotenoids. The present study is aimed at finding out the feasibility of using *Spirulina fusiformis* as a protein source in the diet for black tiger shrimp *Penaeus monodon* (Fabricius). Six isonitrogenous test diets T1 to T6 were prepared with 40% crude protein. Clam meat was replaced with *Spirulina* at an inclusion level of 5%, 10%, 20%, 30% and 40%, and the substitution effect was compared with a control diet in which clam meat was the sole source of protein. The study was conducted for a period of 60 days employing *Penaeus monodon* postlarvae (0.04 g avg. weight) with six treatments and four replications. The postlarvae were stocked at the rate of 10 numbers per tank. On completion of the experiment the specific growth rate, food conversion ratio and protein efficiency ratio were superior in shrimp postlarvae fed with the test diet containing 20% *Spirulina.* The diet T4 (20% *Spirulina*) led to significantly higher growth response ($p>0.05$) than in other diets. Further increase in the *Spirulina* inclusion levels significantly decreased the growth and feeding performance. The highest SGR (6.82%) and PER (1.67) were recorded with diet T4 and lowest SGR (4.16%) and PER (0.62) were obtained with the dietT6. The percentage survival was significantly higher (97.5%) in T3 (10% *Spirulina*) and T4 (20% *Spirulina*). The best FCR was recorded for 20% *Spirulina* (1.49). The results suggest that *Spirulina fusiformis* cannot serve as the sole protein source in the diet of *Penaeus monodon* postlarvae but can be effectively used as a supplementary protein source.

EVALUATION OF *SPIRULINA FUSIFORMIS* AS A PROTEIN SOURCE IN THE DIET OF *PENAEUS MONODON* FABRICIUS.

By

TANK KETAN VALLABHDAS,
B.F.Sc

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