

**GROWTH AND PROTEIN UTILIZATION IN THE
GREEN TIGER PRAWN, *Penaeus semisulcatus*
REARED ON VARYING PROTEIN : ENERGY RATIOS**

DISSERTATION SUBMITTED BY

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IN PARTIAL FULFILMENT FOR THE DEGREE OF
MASTER OF FISHERIES SCIENCE (MARICULTURE)
OF THE
CENTRAL INSTITUTE OF FISHERIES EDUCATION
(DEEMED UNIVERSITY)

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POST GRADUATE PROGRAMME IN MARICULTURE
CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
COCHIN - 682 014

JULY 1999



**Dedicated
To
My
Parents & Teachers**

CERTIFICATE

Certified that the dissertation entitled " **Growth and Protein utilization in the Green Tiger Prawn, *Penaeus semisulcatus* reared on varying protein : Energy ratios**", is a bonafide record of work done by Mr.Rama Manga Babu, M.H.L.N.B, under our guidance at the Central Marine Fisheries Research Institute during the tenure of his M.F.Sc. (Mariculture) programme (1997-1999) and that it has not previously formed the basis for the award of any other degree, diploma or other similar titles or for any publication.



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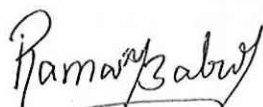


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DECLARATION

I hereby declare that this dissertation entitled "**Growth and Protein utilization in the Green Tiger Prawn, *Penaeus semisulcatus* reared on varying Protein: Energy ratios**" is based on my research work and has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles of recognition.

Place : Kochi


(Rama Manga Babu M.H.L.N.B)

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सारांश

हरा पूलि झींग पेनियस सेमिसलकेटस के किशोरों में छः आहारी प्रोटीन और परिणमी ऊर्जा के मिश्रण अनुपात का पोषणिक मूल्यांकन किया गया, परिक्षण किए गए मिश्रणों में प्रोटीन (%) और परिणमी ऊर्जा का अनुपात प्रति 100 ग्रा. आहार में किलोकलोरी के क्रम में इस प्रकार था - 35:280, 35:300, 35:320, 40:280, 40:300 और 40:320. प्रोटीन और ऊर्जा का अनुपात (मि.ग्रा.प्रोटीन / किलोकलोरी) छः खाध्यों में क्रमशः - 126.06, 113.30, 110.38, 139.63, 128.18 और 124.51 थे।

प्रोटीन और परिणमी ऊर्जा के मिश्रण 3 एवं 4 में बढ़ती [संबंधित बढ़ती दर (आर.जी.आर.) विशेष बढ़ती दर (एस.जी.आर.), खाध्य परिणमी क्षमता (एफ.सी.ई) और प्रोटीन क्षमता दर (पी.ई.आर)] और प्रोटीन पाच्यता की अच्छी प्रतिक्रिया और समरूपता देखी गई। इस से यह स्पष्ट होता है कि खाध्य 3 में 40 किलोकलोरी प्रोटीनेतर ऊर्जा की वृद्धि होने से प्रोटीन में 5% बचाव हो पाया है।

पेनियस सेमिसलकेटस में अनुकूलतम प्रोटीन एवं ऊर्जा अनुपात (मिलिग्राम प्रोटीन / किलोकलोरी) 110.38 और 139.63 देखा गया।

ACKNOWLEDGEMENTS

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At the onset I express my heart felt gratitude to Dr. G. Maheswarudu, Major Advisor & Chairman and Shri. P. Vijayagopal ,Co-Chairman of my advisory committee respectively, for suggesting this topic and for their able, inspiring guidance, encouragement and patience all through my work and my study.

Thanks are due to Dr. A.C.C. Victor, office-in-charge, Mandapam Regional Centre of Central Marine Fisheries Research Institute, and Member of my Advisory Committee, for all the necessary facilities provided during my stay at Mandapam.

I am very grateful to The Director, Central Marine Fisheries Research Institute and Dr.C.Suseelan, officer-in-charge, postgraduate programme in Mariculture, for all the facilities provided during this Master in Fisheries Science programme.

I am very thankful to Dr. Manpal Sridhar and Dr.G. Sudhakara Rao, Head of the Crustacean Fisheries Division for their timely advises; encouragement and support provided all through my study at C.M.F.R.I.

My heartiest gratitude to Mr.S. Nanda Kumar Rao, of P.N.P.Division, for familiarizing us with the laboratory techniques and for the enthusing and brotherly company provided all through my endeavor.

The help rendered by the staff of PGPM, C.M.F.R.I is highly acknowledged.

My regards are to Ms. Josleen Jose, Scientist, for being a constant source of inspiration during my work at Mandapam.

This work would not have been possible, if not for the help of Mr.M.R.Arputharaj and Mr S. Mohan, Senior Technical Assistants, the staff of the Shrimp Hatchery, Sri.S.Ramakrishnan, Sri.A.Vairamani, Sri Sadiq, Sri.A.Ramakrishnan and the staff of Marine Fish Farm Sri. Tirupathi and Mr. Muruganandan.

I shall be failing in my efforts, if I do not acknowledge the support and affection of Mr.S. Sathianesan,Assistant Librarian, at the Mandapam Regional Centre of C.M.F.R.I.

The help rendered by Mr. T.V. Sathianandan, Scientist, in the statistical analysis of the results is highly acknowledged.

Thanks are due to Mr. S. Renjith, SRF for his timely advises, Mr. Roopaknath, Sadu Hari, Ms. Jyothi Menon ,and all my seniors and juniors for their Co-operation.

My heartiest wishes are to my classmates, Binu Varghese, Sunil Lambade, Radhika, Madhavi, Unnikrishnan, Prabhakar, Ashok Reddy and Sandeep.

I convey my deep sense of gratitude to Indian Council of Agricultural Research, New Delhi, for the Institutional Fellowship awarded during the tenure of this M.F.Sc. Course.

Lastly, I am very much indebted to the support, encouragement, admiration, and what not.. Provided by Amma, Naana, My Brother Choudhary and loving Sister Durga.

My thanks are to M/s South tower Communication Centre for their help in the preparation of this dissertation.

INTRODUCTION

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Growth of human population has lead to an increased search for methods of producing animal protein other than those of terrestrial livestock production and capture fisheries. Both of these face constraints in production. The potential of aquaculture as a method to augment animal protein production is thus of more interest, than ever, leading to rapid increases in production and development of new technologies.

In the recent years, food production worldwide through aquaculture has become increasingly important. During the past decade, there has been a tremendous development in shrimp farming, often overshadowing many other commercially important aquatic species. This trend is expected to continue in the foreseeable future. Reasons for this include the high demand for shrimp in the world market, the unpredictability and high cost of harvesting shrimp from the wild, and the recent development and improvement of technologies in the areas of breeding, culture management, nutrition, disease control and engineering.

Penaeid shrimps are the most important and extensively cultured crustaceans all over the world. Apart from their great demand and high market potential, they are ideal for intensive cultivation because of their adaptability to different culture systems, rapid growth, availability of seed

through reproduction in captivity and positive response to supplemental feeding.

Feeds represent the major expense, often accounting for over 50-70% of the total variable operating costs in aquaculture. Thus, development of feeds that are efficient and economical is fundamental to successful shrimp farming. This requires the understanding of nutritional requirements in terms of protein, energy, lipids, carbohydrates, vitamins and minerals.

Significant information is now available on the basic nutritional requirements of crustaceans (Fox *et al.*, 1994). Crustaceans like other animals need protein in the form of essential aminoacids for maintenance, growth and reproduction. The protein requirement of aquatic animals is apparently high, which is a consequence of the low energy requirements of ectothermic animals (Bowen, 1987). The natural aquatic food web (i.e., scarcity of carbohydrates and abundance of lipids and protein) is probably responsible for the common trend of aquatic organisms to use protein as an energy source. (Guillaume, 1991). This trend explains the positive effect of dietary protein towards improving growth. In addition to their structural and metabolic roles, lipids have higher calorific value than proteins or carbohydrates on a weight to weight basis. They can be catabolized for energy and exert a protein sparing effect.

Although, no specific dietary requirement of carbohydrates for crustaceans and finfish has been reported, reduced growth rates have been noted when various finfish species are fed diets lacking a carbohydrate source (NRC 1983). They serve as precursors of various metabolic intermediates necessary for growth, like non-essential aminoacids, nucleic acids. Though they have a lower calorific value than proteins and lipids, they are generally cheaper in economic terms and can reduce the amounts of dietary protein needed for supplying energy.

Many of the studies have attempted to define optimum levels for dietary protein, carbohydrates or lipids in a single factor experiments, although these dietary components interact.

Shrimp require energy for maintenance, growth and reproduction. Utilization of the dietary component for energy is affected by its dietary level, the ability of the animal to catabolize that substrate and the availability of the other dietary energy sources (Capuzzo, 1982). Lipids, carbohydrates and proteins can all be used for energy by prawns and shrimps (Lim and Persyn, 1989).

Crustaceans in common with finfish are able to derive more net energy from the catabolism of proteins than mammals because they do not have to maintain a constant body temperature, they exert less energy in maintaining their posture and because nitrogenous wastes are excreted

mostly as ammonia rather than uric acid or urea. (NRC 1983, Cho and Kaushik, 1985).

Protein metabolism is in a state of dynamic equilibrium with energy metabolism, so evaluation of optimum dietary protein level must consider the amount of non-protein energy in the diet (Clifford and Brick, 1979). Diets consisting excessive protein will be economically wasteful (Capuzzo, 1982). So, it is desirable to provide as much energy as possible as non-protein energy (lipids and carbohydrates). In this situation, diets containing excessive non-protein sources may lead to the animals ingesting insufficient protein for growth. This follows the fact that crustaceans, like fish eat to satisfy their energy requirements (Sedgwick, 1979.)

It would be economically disadvantageous if expensive protein is used as an energy source, which otherwise can be used for growth. Thus, the provision of energy from non-protein sources is important. This is termed as protein sparing. If optimal levels of non-protein energy are available in the diet, the protein level in the diet could be reduced.

Therefore, the correct dietary protein and energy balance is very essential for efficient feed formulation, indicating the importance of protein to energy ratios. This ratio is expressed as the gram or milligram of crude protein or digestible protein per mega Joules(MJ) or kilo calories of digestible energy(DE) or metabolizable energy.

Protein to energy ratios have been worked out for certain penaeid species. The present study is carried to arrive at an optimum protein to energy ratio for green tiger prawn, *Penaeus semisulcatus* .de Haan.

The culture of *Penaeus semisulcatus* is done in Kuwait, Israel, Bahrain, Taiwan and Malaysia (Liao and Chao, 1987, Seidman and Issar; 1988). It has been informally evaluated to be the most savoury "gourmet" among the penaeid shrimps. (Liao and Chao,1987). Its prospects as a candidate for mariculture has been highlighted by Maheswarudu, *et al.*, (1994)(1995) and (1997). The above mentioned authors have stressed the need for formulating a suitable feed for *Penaeus semisulcatus*, as there is no formulated feed for this species till date.

The present study is an attempt to elucidate appropriate protein:energy ratios in diets for attaining maximum growth with a view to formulate suitable diets for *Penaeus semisulcatus*.

REVIEW OF LITERATURE

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Significant information is now available about the basic nutritional requirements of commercially cultured crustaceans. However, information regarding the nutrient utilization of non-protein dietary components as energy sources is sparse. Information on energy utilization and requirements is essential for developing cost-effective diets, because energy must be supplied in sufficient amounts, so that protein is almost exclusively used for tissue synthesis.

Crustaceans cannot tolerate lipid levels greater than 10% in their diet; leaving carbohydrates as ideal non-protein energy sources. However, a high level of glucose or monosaccharides in general, is not tolerated well by crustaceans. It appears imperative to find out suitable non protein energy sources so as to define appropriate protein:energy ratios in feeds..

A few results of relevant work published on the investigations related to P/E ratios in crustaceans are reviewed here.

Andrews *et al.*, (1972) studied the effects of dietary protein, carbohydrates and lipids on the growth and survival of juvenile *Penaeus setiferus* and concluded that 28-32% was the optimum protein level and the addition of supplemental lipids resulted in reduction of growth and survival.

This indicated that lipids can not and polysaccharides alone can be ideal protein spacers.

AQUACOP (1976), estimated that a total dietary energy content of 3.3 kcal/gm was required for optimal growth of *Penaeus monodon* fed a diet containing 40% protein.

Clifford & Brick (1978) and (1979) measured oxygen consumption, carbon dioxide excretion and nitrogen excretion of *Macrobrachium rosenbergii*, fed diets with variable protein, lipid and carbohydrate contents and indicated that protein sparing was maximized when the dietary lipid: carbohydrate was 1:4 and optimal dietary protein level for growth was calculated to be 25%.

Sedgwick(1979) while assessing the requirement of juvenile *Penaeus merguensis* for dietary protein and energy concluded that the rate of food consumption is related to the energy content of the diet and that the protein level required to support maximum growth and optimum protein conversion efficiency are energy dependent. There was some evidence for the decline in the dietary protein requirement from 51%-34%, if the dietary energy was maintained at 2.9-4.4 kcal/gram by increasing the level of carbohydrates.

Bages and Sloane(1981) studied the effects of dietary protein and starch levels on the growth and survival of *Penaeus monodon* postlarvae and concluded that protein/starch ratio had an effect on the survival and growth and that a minimum supply of carbohydrates was necessary to assure a metabolic energy pool to allow the shrimps to utilize the protein in the diet efficiently. An excess of carbohydrates was found to be an obstacle in the development of postlarvae since the quantity of ingested protein was insufficient.

Alava and Lim (1983) reported in *Penaeus monodon* juveniles that the optimum protein level of 40% and the energy content of 3.68 kcal/gm diet corresponding to a protein:energy ratio of 112.5mg/kcal was optimal.

Teshima and Kanazawa (1984) demonstrated that the effects of altering the dietary protein level on the growth and survival of *Penaeus japonicus* larvae were related to the carbohydrate content of the feed and highest survival was on a diet containing 45% protein (casein) and 25% carbohydrate (type unspecified).

Bautista (1986) reported that for *Penaeus monodon* juveniles the protein content of the diet could be reduced from 50% to 40% while maintaining an energy level of 330 kcal/100gm diet. A protein sparing effect was confirmed, but inclusion of lipid at more than 15% or sucrose (used to adjust the dietary physiological energy levels) at more than 20% led to

reduced growth and histological abnormalities. It was added that complex dietary carbohydrates yield better growth than either glucose or sucrose and had a better protein sparing effect.

El-Dakour (1986) fed a range of diets containing various protein (45.0 to 55.0%) and energy levels (3.20kcal/gm to 3.68kcal/gm) to *Penaeus semisulcatus* and concluded that shrimp fed diets containing higher dietary protein levels generally showed faster growth and better survival rates. Growth and survival rates increased proportionally with increasing dietary protein content up to 51% and protein: energy ratio of 1:0.304.

Alava and Pascual (1987) reported that for *Penaeus monodon* juveniles semipurified diet containing trehalose at 20% level was very effective and that the digestible energy content of 4.0kcal/gm of diet corresponding to a protein: energy ratio of 114 (mg protein/kcal) was found to promote the most rapid growth.

Hajra *et al.*, (1988) reported in *Penaeus monodon* juveniles that at constant protein level of 46%, weight gain, feed efficiency and protein utilization increased with the increase in dietary energy level up to 412.60 kcal/100gm diet (protein: energy = 112.2) and not beyond that. Digestible energy from dietary carbohydrate was seen to exert a transient protein sparing action.

Dall *et al.*, (1991) concluded that the inability of prawns and shrimps to tolerate high levels of dietary lipid may be probably due to a reflection of the low lipid levels in the natural diet, and the low lipid levels found in their tissues.

Shiau and Chou (1991) reported that in *Penaeus monodon* dietary protein level could be reduced from 40% to 36% while maintaining metabolizable energy level of around 330 k cal/100gm. This suggests that protein was spared by carbohydrate (dextrin here) as calorific requirements were met, thus permitting more efficient utilization of protein.

Diaz-Herrera *et al.*, (1992) found that *Macrobrachium rosenbergii* reared on a commercial ration obtained most of its energy from carbohydrates and lipid in the diet. Consequently, protein was used efficiently by inclusion of optimum levels of carbohydrates and lipids.

Shiau and Peng (1992) investigated the utilization of different carbohydrate sources and the possible substitution of carbohydrates for dietary protein for *Penaeus monodon* and concluded that prawns fed starch and dextrin gave significantly better results than glucose. Protein sparing action of starch was observed as inclusion of 30% starch caused a reduction in dietary protein requirement from 40% to 30%.

Koshio *et al.*, (1993) have reported that the dietary protein requirement for *Penaeus japonicus* could be reduced from 60% to 42%, by increasing the levels of suitable non-protein energy sources. The diet used consisted of 42% of highly digestible protein source, 15% carbohydrates and 8% lipids.

Gopakumar (1996) while studying the essential aminoacid requirement of *Penaeus semisulcatus* tested diets containing six protein levels of 20% ,25%, 30%, 35%, 40%, and 45% to arrive at an optimum protein level. The weight gains were 2.4%, 3.6%, 7.8%, 10.1%, 11.9%, and 8.6% respectively. Eventhough, a protein level of 40% appeared optimum in his work on the basis of weight gain ,he did not discern between 35%, and 40% protein in the diet suggesting a range of 35%-40%.

Ameeri and Cruz (1998) studied the effect of sand substrate on the growth, feed conversion ratio (FCR) and survival rate of *Penaeus semisulcatus* juveniles. Shrimp growth rate with sand substrate was significantly faster (75.75%) and FCR was significantly better (37.61%) than those reared with out sand.

Reports on protein, energy interactions in crustaceans are scanty. A thorough understanding of dietary protein/energy ratios in shrimps is presently lacking. *Penaeus semisulcatus*, in particular is a species on which very little information is available regarding the nutritional requirements.

The present investigation was designed to arrive at the optimum protein: energy ratios in the green tiger prawn, *Peneaus semisulcatus*.

MATERIALS AND METHODS

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The experiment was conducted in the backyard shrimp hatchery at the Regional Centre of Central Marine Fisheries Research Institute, Mandapam.

I Facilities and Experimental Animals

For the present study juveniles of *Penaeus semisulcatus* were collected from Thallumadi operators (local shore seine) in the Palk Bay and acclimatized to the hatchery conditions for one week prior to the start of the experiment. During the acclimatization, juveniles were fed with a commercial shrimp feed of a reputed company (38% crude protein) at 10% of the biomass per day.

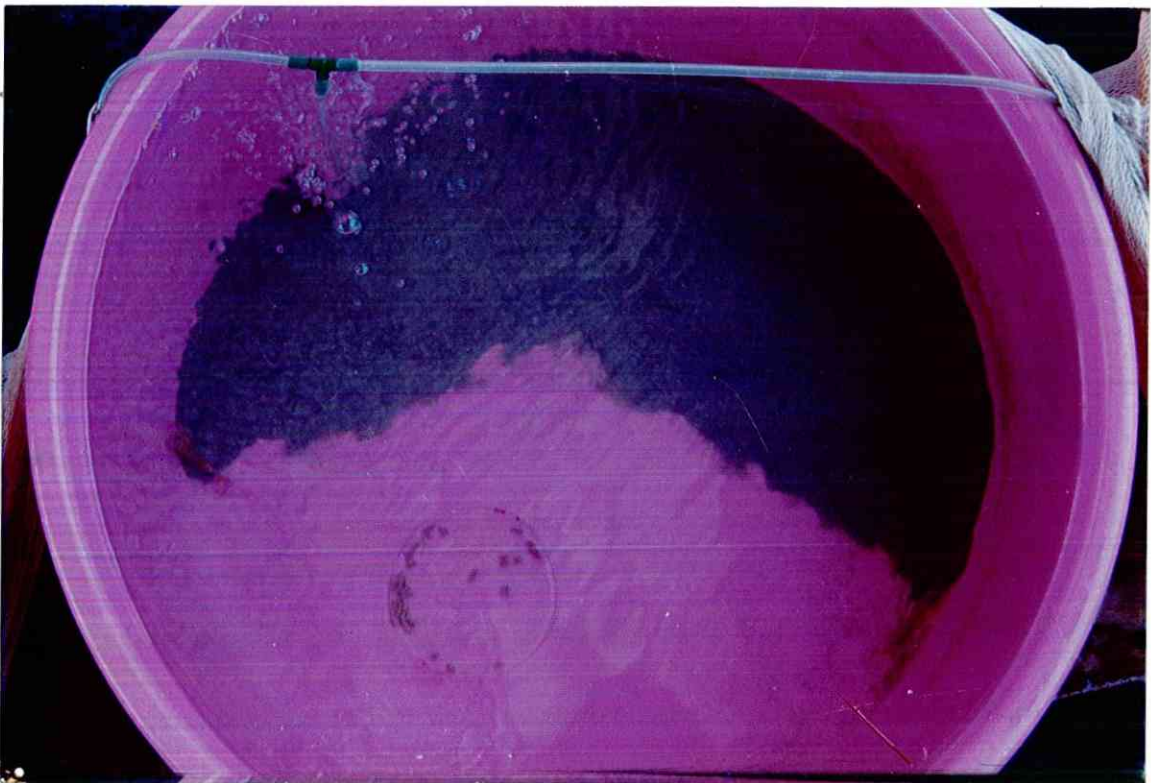
Totally six feeds of different compositions were tested with these juveniles for a period of one month to evaluate the performance in terms of growth, protein digestibility and gross body composition.

Each treatment had two replicates and five juveniles with initial mean length of 57.77 mm, With total biomass of 8 to 9grams per replication, was selected and introduced into 40litre capacity circular plastic troughs. Since, *Penaeus semisulcatus* is a highly burrowing species; sandy

PLATE 1 - Experimental Setup



PLATE 2 - Inner view of the Experimental trough



substratum for a height of 5 cm was provided to cover 1/2nd of the bottom of each trough.

Continuous aeration was provided for each trough. Seawater that was filtered through sand bed was used during the experimental period.

During the experimental period water temperature ranged from 27.5 - 29 degrees C; and salinity varied between 35-35.5 ppt; pH from 7.6 to 8.2 and The dissolved oxygen level was maintained above 4.0 mg/l by continuous aeration.

II Experimental diets:-

Two dietary protein levels 40% and 35% were used in the study .For each protein level digestible energy (DE) content of 280, 300 and 320 kcal/100gram diet (with protein: energy ratios of 126.06, 113.30, 110.38,139.63, 128.18 and 124.51 respectively) were incorporated to formulate six semipurified experimental diets.

In order to simplify the interpretations, as few ingredients as possible were used in the formulation. The ingredients used were Albumin (Sigma.,USA), shrimp meal, clam meal, groundnut oil cake flour, tapioca flour (as a carbohydrate source for adjusting the energy level). Cod liver oil, cholesterol, soyalecithin, vitamin mix, mineral mix (Salt mixture USP XIV,

Sisco Research laboratories, Mumbai, India) and cellulose (Hi-media laboratories, Mumbai) as a filler.

The proximate composition of the ingredients i.e., moisture, crude protein (from total quantity nitrogen using micro kjeldahl method and converting to total protein by multiplying with a factor 6.25) ether extract, crude fibre, crude ash, acid insoluble ash and nitrogen free extractives (NFE) were estimated using the Official Methods of Analytical Chemists [AOAC (1990)].

The energy content was calculated based on the factors suggested by ADCP (1983) for digestible energy (DE) of fish feeds.

They are 4.25 kcal g^{-1} for animal protein

8.0 kcal g^{-1} for Fats.

3.0 kcal g^{-1} for Non leguminous Carbohydrates.

$4.0 \text{ } 2.0 \text{ kCal g}^{-1}$ for leguminous Carbohydrates.

The proximate composition of the ingredients is given in table I.

The Six experimental feeds for present study were formulated according to the ingredient composition given in table II.

Table 1. Proximate composition of the feed ingredients used in the experimental diets.

Composition (%) ▶ Ingredient ▼	DM	CP	EE	CF	Ash	AIA	NFE
Albumin	100	94	-	-	4.5	-	1.5
Shrimp meal	95.86	66.89	3.69	-	19.44	1.35	5.84
Clam meal	94.54	60.76	13.82	-	5.82	1.17	14.119
Ground nut oil cake	96.51	49.53	8.41	3.58	6.88	1.74	28.09
Tapioca flour	89.13	3.18	0.978	1.992	1.983	0.28	80.99

DM = Dry Matter

CP = NX6.25

EE = Ether extract

CF = Crude fibre

NFE = Nitrogen Free extractives

Table II. Ingredient composition of the Experimental diets

Ingredients (%)	35% Protein			40% Protein		
	Feed I	Feed II	Feed III	Feed IV	Feed V	Feed VI
Albumin	29	29	29	35	35	35
Shrimp meal	5	5	5	5	5	5
Clam meal	2	2	2	2	2	2
GNOC	5	5	5	5	5	5
Tapioca flour	29	36	44	20	28	36
Cod Liver oil	6	6	6	6	6	6
Vitamin Mix	2	2	2	2	2	2
Mineral Mix	3	3	3	3	3	3
Cholesterol	1	1	1	1	1	1
Soyalecithin	1	1	1	1	1	1
Cellulose	17	10	2	20	12	4
Total	100	100	100	100	100	100
Digestible energy k cal/ 100gm	283.18	300.99	322.61	283.88	304.78	325.81
P/E (mg Protein/kcal)	124.00	117.00	110.60	143.00	134.00	126.00

Vitamin Mix:

Vitamin	Quantity (mg)
Thiamine mononitrate	60.0
Vitamin B2	60.0
Pyridoxine hydrochloride	18.0
Nicotinamide	600.0
Ca pantothenate	300.0
Folic acid	9000.0 micro gm
Vitamin B12	90.0 micro gm
Vitamin C (Ascorbic acid)	500.0
Choline chloride	1200.0
Ionosital	4000.0

Table III. Proximate compositions, DE levels and P/E ratios of the experimental diets.

Ingredients (%)	35% Protein			40% Protein		
	Feed I	Feed II	Feed III	Feed IV	Feed V	Feed VI
DM	93.84	94.30	92.31	94.17	92.61	93.06
CP	35.39	33.94	35.33	39.40	38.89	39.87
EE	8.08	7.39	7.02	8.51	8.00	7.56
Ash	6.35	6.43	6.31	6.36	6.31	6.68
AIA	0.27	0.33	0.25	0.23	0.25	0.26
CF	21.20	13.96	5.16	24.09	14.23	8.36
NFE	22.82	32.58	38.49	15.81	25.18	30.59
Digestible energy kcal/100gm	280.72	299.55	320.06	282.16	303.40	320.21
P/E (mg Protein/kcal)	126.06	113.30	110.38	139.63	128.18	124.51

III Preparation of the Experimental feeds:-

The ingredients were weighed in required proportions. Tapioca flour was gelatinized by boiling with 30ml of distilled water and then all other ingredients were mixed homogeneously, to get a dough of desired consistency by adding the required quantity of water. Then the dough was extruded through a 2mm die using a hand pelletiser. The pellets were dried in an oven at 60 degrees Celsius for 12 hours to reduce the moisture content of feeds to approximately 7%. The dried pellets were then broken in to small pieces and stored in airtight plastic containers. The proximate composition of feeds is given in table III.

IV. Management: -

The study consisted of six treatments with two replications. The shrimps were fed with the experimental diet at 10% of the biomass once a day in the evening hours i.e., between 1700-18:00 hours. Attempts to feed half of the ration during day time i.e., between 9:00-10:00 hrs proved futile as the animals were found burying in to the sandy substratum.

Every morning faeces was collected from each trough, oven dried and stored in glass vials for further analysis. Excess feed from each trough was also collected, dried and weighed.

Daily 100% water exchange was done every morning during which the bottom sand was briskly washed to avoid any decomposition of debris.

Water quality parameters such as temperature, salinity and pH were monitored at 10:00 hrs daily.

The feed trail was terminated after 30 days and final size and biomass of the shrimps from each treatment were recorded. All the animals from each treatment (also initial) were dried in an oven at 60 degrees Celsius for 24 hrs for subsequent analysis. The samples from each treatment were ground and homogenized. They were then analyzed for crude protein and crude fat (AOAC, 1990) The faecal matter for each treatment were also pooled and crude protein was estimated to calculate the protein digestibility.

The parameters such as relative growth rate (RGR), Specific growth rate (SGR), feed conversion efficiency (FCE), Protein efficiency ratio (PER) and protein digestibility were worked out using the following formulae.

$$\text{RGR} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

$$\text{SGR} = \frac{\text{Ln Final weight} - \text{Ln Initial weight}}{\text{Number of days}} \times 100$$

$$\text{FCE} = \frac{\text{Biomass at the end of the experiment} \times 100}{\text{Feed given}}$$

$$\text{PER} = \frac{\text{Wet weight gain (gm)}}{\text{Dry weight of protein consumed (gm)}}$$

$$\text{Protein Digestibility} = \frac{\text{protein in feed intake} - \text{protein in faeces} \times 100}{\text{protein in feed intake}}$$

V. Statistical Analysis

To test the effects of different protein and energy levels and their interaction on growth a 2x3 factorial analysis was carried out. To compare the means of significant factors and interactions, Students-Neuman Keuls Range test was made (Das and Giri, 1979).

RESULTS

RESULTS

The experiment was terminated after 30 days and a survival rate of 100% was recorded in all the six treatments.

The responses viz., RGR, SGR, FCE, PER and protein digestibility recorded were presented in Table IV and figures 1,2,3,4 and 5.

Relative Growth Rate (RGR)

In terms of RGR feeds III and IV were the highest indicating the superiority of protein: energy levels 35:320 and 40:280 over the other treatments. However, the main effects viz., protein (%) and DE (kcal/100g) were not significant statistically. Significant interaction between protein and energy could be observed ($P < 0.05$). Means when compared using SNK (Student-Newman and Keuls) procedure indicated 8 subsets as shown in table V.

Specific Growth Rate (SGR)

SGR also showed that feeds III and IV were superior with a value of 0.53. Following the trend observed in the case of RGR, the main effects were not significant and interaction between protein and energy levels was significant ($P < 0.05$). While comparing the means (table V) 7 subsets similar to RGR were obtained.

Feed Conversion Efficiency (FCE)

FCE of 5.30 was the maximum obtained by feeding feed IV followed by 5.05 obtained with feed III. FCE also followed the trends recorded for RGR and SGR. However, in the case of FCE, both the main effects and interaction was found to be significant ($P<0.05$).

Protein Efficiency Ratio (PER)

PER values were 0.14 for feed III and 0.13 for feed IV respectively (Table IV). Statistically, the main effect viz., protein was not significant, whereas DE level was found to influence PER significantly ($P<0.05$). Following RGR, SGR and FCE, interaction between protein and energy was significant in the case of PER also. When the means were compared larger homogeneous subsets were obtained (Table V).

Protein Digestibility (PD)

Significantly higher protein digestibilities were registered in the case of feeds III and IV respectively ($P<0.05$). Digestibility of protein was 79.85% and 81.34% for the aforementioned feeds and the main effects and interactions were found to be significant statistically ($p<0.05$). Six homogeneous subsets were observed in the case of protein digestibility as shown table V.

Body Composition

At the termination of the experiment, shrimp fed on feed I had higher crude protein composition (66.04%) followed by feed III and feed IV which had an identical crude protein composition of 63.09% and 62.35% respectively as shown in Table VI. The ether extract (%) was highest in shrimp reared on feed VI, (5.46 %) followed by 5.12% for feed III, 4.94% for feed II and 4.44% for feed IV.

The overall picture which emerges is that feeds III and IV are at par in terms of RGR, SGR, FCE, PER and protein digestibility which shall be perused and discussed in the light of relevant reports in the ensuing discussion.

Table IV. Growth, SGR, FCE, PER and PD in *Penaeus semisulcatus* juveniles fed semipurified diet with varying levels of Protein and digestible energy.

	Initial Bio mass (gm)	Final Bio mass (gm)	Relative Growth Rate (RGR)	Specific Growth Rate (SGR)	Food Conversion Efficiency (FCE)	Protein Efficiency Ratio (PER)	Protein Digestibility (%) (PD)
Feed I	8.95	9.39	4.92	0.16	1.50	0.04	60.00
Feed II	8.39	9.51	13.35	0.42	3.85	0.11	55.61
Feed III	8.55	10.03	17.24	0.53	5.05	0.14	79.85
Feed IV	8.70	10.19	17.14	0.53	5.30	0.13	81.34
Feed V	8.70	9.73	11.73	0.37	4.03	0.10	66.37
Feed VI	8.98	9.82	9.55	0.30	3.90	0.10	82.68
Main effects							
Protein Level			NS	NS	S	NS	S
Energy Level			NS	NS	S	S	S
2. Way Interactions							
Protein Level			S	S	S	S	S
Energy Level							

$$RGR = \frac{\text{Final Wt.} - \text{Initial Wt.}}{\text{Initial Wt.}} \times 100$$

$$PER = \frac{\text{Wet Weight gain (gm)}}{\text{Dry Weight of Protein fed (gm)}}$$

$$SGR = \frac{\text{Ln Final Wt.} - \text{Ln Initial Wt.}}{\text{Number of Days}} \times 100$$

$$\text{Protein Digestibility} = \frac{\text{Protein in feed} - \text{Protein in faeces}}{\text{Protein in feed}} \times 100$$

$$FCE = \frac{\text{Bio mass at the end of Experiment}}{\text{Feed Consumed}}$$

NS-Not Significant ($P > 0.05$)

S-Significant ($P < 0.05$)

Table V. Homogeneous subsets of Protein level : Digestible Energy level Interactions

	Relative Growth Rate (RGR)	Specific Growth Rate (SGR)	Food Conversion Efficiency (FCE)	Protein Efficiency Ratio (PER)	Protein Digestibility (%) (PD)
1	35:300 35:320 40:280	35:300 35:320 40:280	35:300 40:300 40:320	35:300 35:320 40:280 40:300 40:320	35:320 40:280 40:320
2	35:300 40:300 40:320	35:300 40:300 40:320	35:300 40:320	35:300 40:280 40:300 40:320	35:320 40:280
3	35:300 40:300	35:300 40:300	40:300 40:320	35:300 40:300 40:320	35:280
4	40:300 40:320	35:280	40:280 35:320	40:300 40:320	35:300
5	35:280	35:300	35:280	35:280	35:320
6	35:300	35:320	35:300	40:320	40:300
7	40:280	40:280	35:320	-	-
8	40:320	-	40:300	-	-

Table VI. Body composition of *Penaeus semisulcatus* juveniles fed six combinations of protein: energy.

	Crude Protein (%)	Ether Extract (%)
Initial	40.96	2.27
Feed I	66.04	4.28
Feed II	61.95	4.94
Feed III	63.09	5.12
Feed IV	62.35	4.44
Feed V	59.95	4.36
Feed VI	61.83	5.46

Fig.1 RGR in *Penaeus semisulcatus* fed six combinations of protein:energy

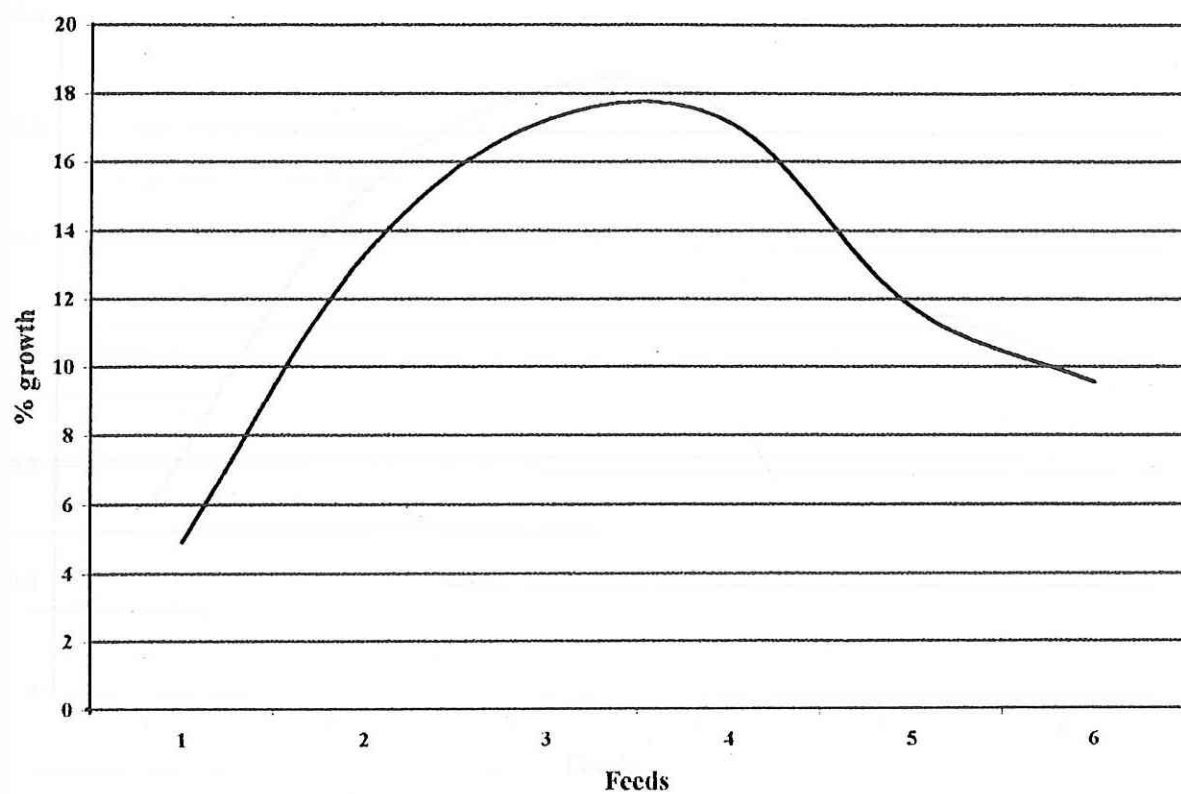


Fig. 2 SGR in *Penaeus semisulcatus* fed six combinations of protein:energy

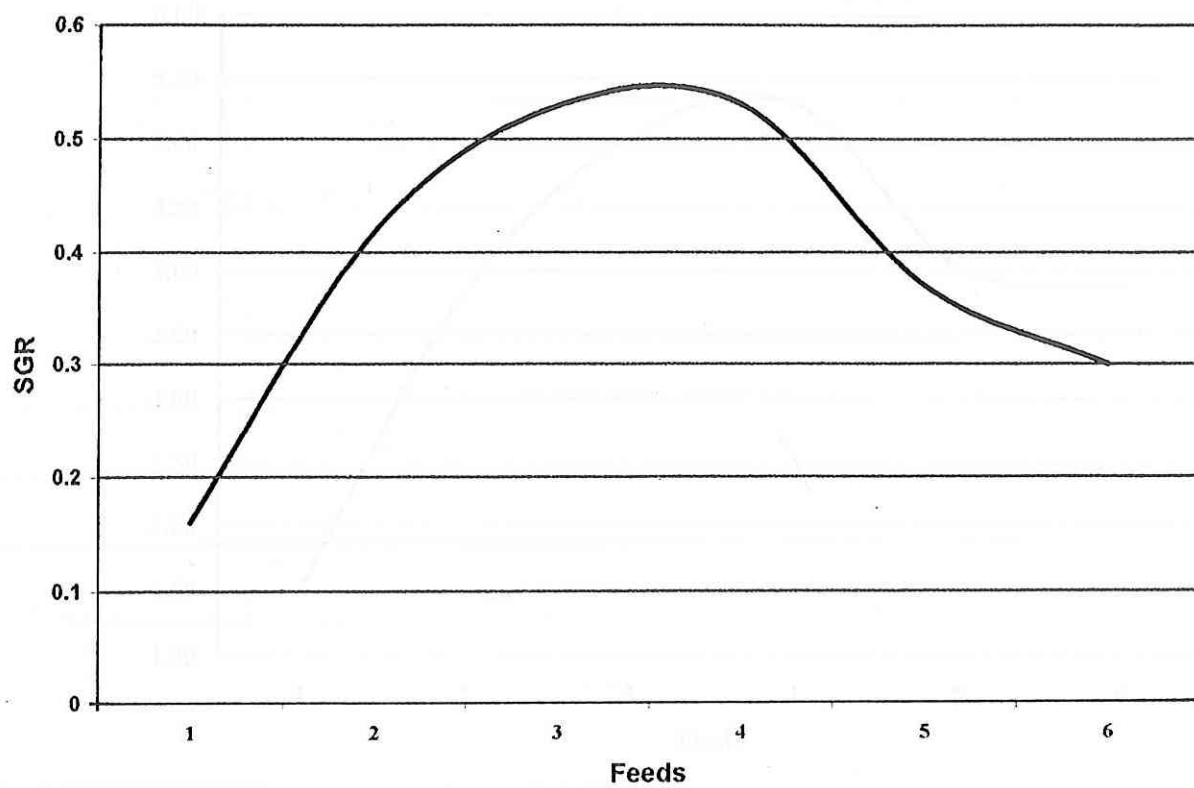


Fig.3 FCE in *Penaeus semisulcatus* fed six combinations of protein:energy

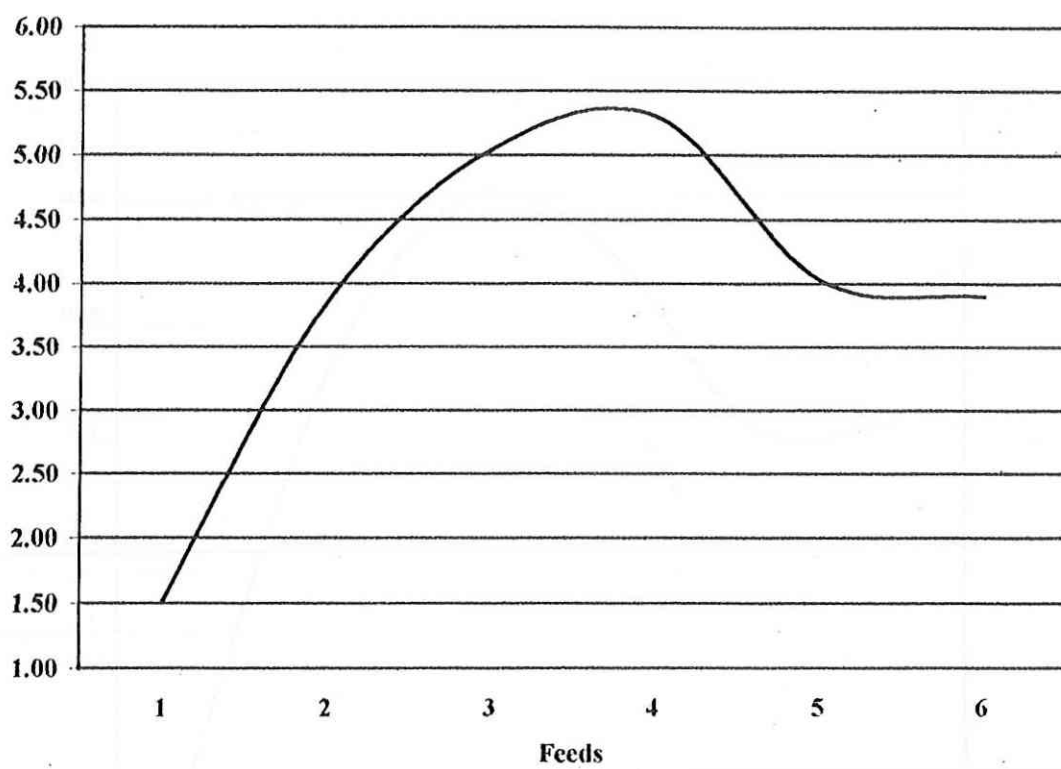


Fig.4 PER in *Penaeus semisulcatus* fed six combinations of protein:energy

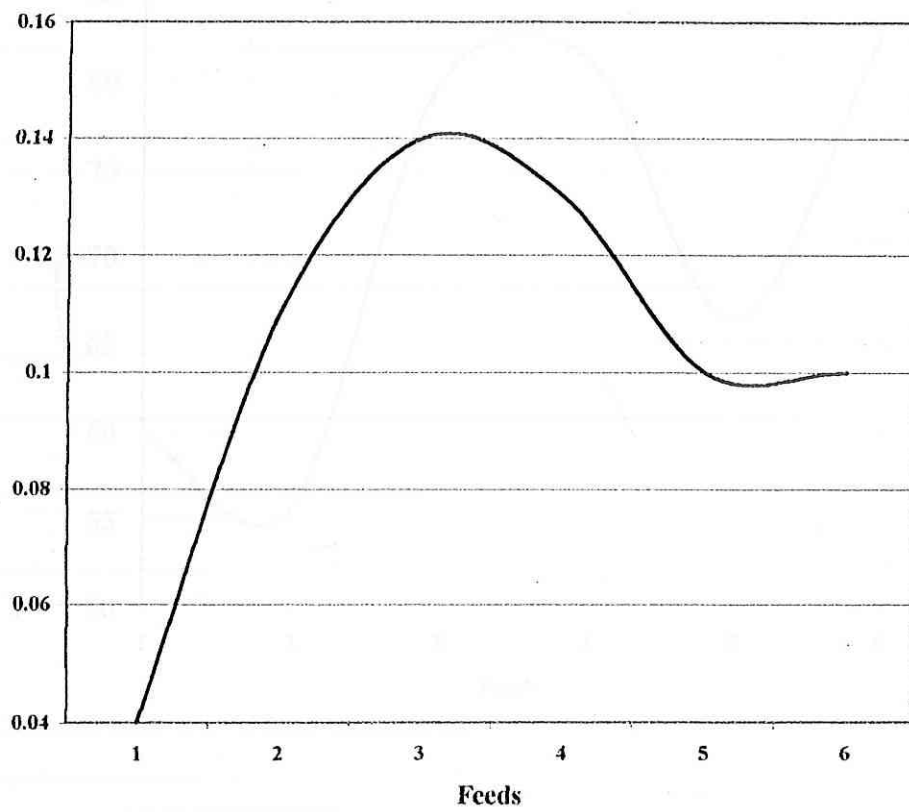


Fig.5 Protein digestibility in *Penaeus semisulcatus* fed six combinations of protein:energy

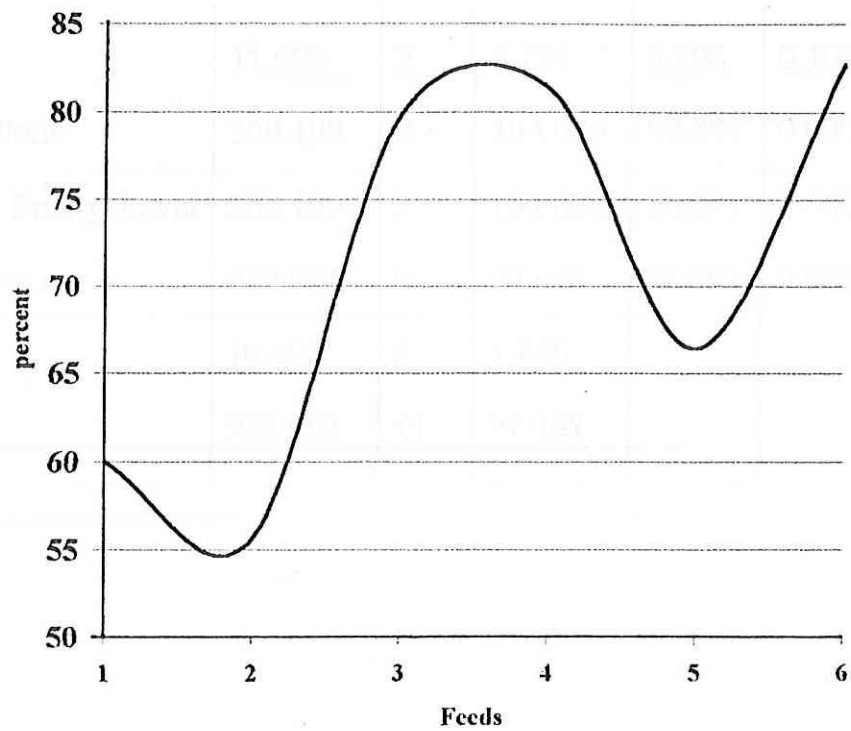


Table VII. Analysis of Variance Relative Growth Rate

Level of significance $P < 0.05$

Significant : **

Source of Variation	Sum of squares	DF	Mean Square	F	Significance of F
Main effects	14.326	3	4.775	2.737	0.136
Protein Level	2.817	1	2.817	1.614	0.251
Energy Level	11.509	2	5.754	3.298	0.108
2-way interactions	208.199	2	104.099	59.661	0.000**
Protein Level, Energy Level	208.199	2	104.099	59.661	0.000**
Explained	222.524	5	44.505	25.507	0.001
Residual	10.469	6	1.745		
Total	232.993	11	21.181		

Table VIII. Analysis of Variance

Specific Growth Rate

Level of Significance $P < 0.05$

Significant : **

Source of Variation	Sum of squares	DF	Mean Square	F	Significance of F
Main effects	0.014	3	0.005	2.968	0.119
Protein Level	0.003	1	0.003	1.774	0.231
Energy Level	0.011	2	0.005	3.564	0.095
2-way interactions	0.187	2	0.093	61.299	0.000**
Protein Level, Energy Level	0.187	2	0.093	61.299	0.000**
Explained	0.200	5	0.040	26.300	0.001
Residual	0.009	6	0.002		
Total	0.209	11	0.019		

Table IX . Analysis of Variance
Feed Conversion Efficiency

Level of Significance $P < 0.05$
Significance: **

Source of Variation	Sum of squares	DF	Mean Square	F	Significance of F
Main effects	4.988	3	1.663	19.996	0.002
Protein Level	2.676	1	2.676	32.189	0.001 **
Energy Level	2.311	2	1.156	13.900	0.006
2-way interactions	13.120	2	6.560	78.902	0.000
Protein Level, Energy Level	13.120	2	6.560	78.902	0.000
Explained	18.107	5	3.621	43.558	0.000
Residual	0.499	6	0.083		
Total	18.606	11	1.691		

Table X . Analysis of Variance
Protein Efficiency Ratio

Level of Significance $P < 0.05$

** : Significance

Source of Variation	Sum of squares	DF	Mean Square	F	Significance of F
Main effects	0.003	3	0.001	11.881	0.006
Protein Level	0.000	1	0.000	4.347	0.082
Energy Level	0.002	2	0.001	15.648	0.004**
2-way interactions	0.010	2	0.005	63.131	0.000
Protein Level, Energy Level	0.010	2	0.005	63.131	0.000**
Explained	0.012	5	0.002	32.381	0.000
Residual	0.001	6	0.0001667		
Total	0.013	11	0.001		

Table XI. Analysis of Variance
Protein Digestibility

Level of Significance $P < 0.05$

**: Significance

Source of Variation	Sum of squares	DF	Mean Square	F	Significance of F
Main effects	1228.989	3	409.663	365.967	0.000
Protein Level	406.574	1	406.574	363.207	0.000**
Energy Level	822.415	2	411.208	367.347	0.000**
2-way interactions	172.572	2	86.286	77.082	0.000
Protein Level, Energy Level	172.572	2	86.286	77.082	0.000**
Explained	1401.561	5	280.312	250.413	0.000
Residual	6.716	6	1.119		
Total	1408.278	11	128.025		

DISCUSSION

DISCUSSION

In this section the results obtained shall be discussed in detail.

1. **Diet design:** The feed ingredients used were albumin, cellulose (purified ingredients), shrimp meal, clam meal, ground nut oil cake, tapioca flour, cod liver oil, cholesterol, lecithin (soya), vitamin and mineral mixtures.

In the absence of any published information from India regarding the nutritional requirements of this species, the work reported by Gopakumar (1996) was used as the basis. He opined that a diet containing 35-40% protein as the optimum requirement to sustain a maximum growth of 11.9%. Hence, both the levels 35% and 40% protein was taken as the levels of protein to be tested. El-Dakour (1986) had tested different protein levels (45-55%) and energy levels (320 kcal/100gm – 368 kcal/100gm) for *Penaeus semisulcatus* and reported that growth and survival increased with increasing dietary protein content up to 51%. However, lower levels of protein reported by Gopakumar (1996) and minimum energy level reported by El-Dakour (1986) were combined to fix the protein: energy levels in this study, because protein: energy interaction and transient protein sparing if any, had to be examined. The variation in the levels of protein was achieved by varying the levels of albumin. Further DE levels of 280, 300 and 320kcal/100gm were obtained for the aforementioned levels of protein by adjusting the levels of tapioca flour and cellulose as

shown in table II. There were only minor variations in the analyzed values as shown in table III. This aspect of the diet design ascertained the fact that the intended combinations of protein %: energy (kcal/100gm) were only available to the experimental animals.

2. Growth

Growth observed as gain in bio mass in this short-term, time bound experiment with *P.semisulcatus* was 0.44gm with feed I, 1.12g with feed II, 1.48gm with feed III, 1.49gm with feed IV, 1.03gm with feed V and 0.84gm with feed VI in 30 days of experimental rearing and the corresponding RGR's were 4.92, 13.35, 17.24, 17.14, 11.73 and 9.55 respectively (Table IV). Growth of *Penaeus semisulcatus* reported by Seidman and Issar (1988) and Maheswarudu *et al.*, (1994) are not comparable here because the aforementioned reports are on their growth under on-farm conditions. Hence, Gopakumar (1996) is the only report available for comparison of growth under controlled conditions of culture and the RGR's reported by him with 35% protein and 40% protein were found to be lower. This may be because of the difference in the initial bio mass of the shrimps used in this investigation, which is <2gm; where as shrimps of 2-3g size were used by Gopakumar (1996). However, the optimum range of protein in the diet reported by Gopakumar (1996) holds good in this study also. As depicted in figs. 1 and 2 and table IV, the animals fed with 35% protein

and 40% protein recorded maximum growth. Significant interaction with DE levels ($p < 0.05$) is the salient aspect in this investigation (table V). Implications here are that a protein sparing of 5% is achievable by increasing the level of non-protein dietary constituents (table II). When the interaction means between protein and energy levels were compared (table V); the first subset includes the protein: DE combinations of 35:300, 35:320 and 40:280 indicating that there is no significant variation among these three treatments in terms of RGR. It could be inferred at this juncture that growth achieved using a feed (feed IV) containing 40% protein and 280kcal of DE is also achievable using a feed (feed III) containing 35% protein and 320kcal of DE. Thus with an increase of 24% of non-protein energy (using tapioca flour in this study) a reduction in the protein level by 5% can be resorted to without any negative impact on the growth of the animal. Such reports in other species like *P. merguensis* (Sedgwick, 1979), *P. monodon* (Hajra *et al.*, 1988), Shiau and Chou (1991) are available. However, in *P. semisulcatus*, this study appears to be first in terms of arriving at a protein sparing action. Though, El-Dakour (1986) has studied the effects of varying dietary protein and energy levels, he has not stated about the protein sparing action.

Specific Growth Rate

The SGR'S reported for feed II and feed IV are the same (0.53), which supports the aforementioned finding that feed III and feed IV resulted in similar growth response. When the SGR means are compared to obtain the interaction protein and energy levels (table V), the first subset includes the protein (%): DE combinations of 35:300, 35:320 and 40:280, indicating that there is no significant variation among these treatments in terms of SGR. It can be inferred that SGR obtained by feeding feed IV containing 40% protein and 280 kcal of DE can be obtained even by using feed III, containing 35% protein and 320kcal of DE. SGR's thus confirm the inference regarding RGR.

Food Conversion Efficiency (FCE)

The FCE is maximum for feed IV, however the FCE of feed III is also close to that of feed IV indicating that there is no difference between both these feeds. The fourth subset of the interaction means include protein (%): DE combinations of 40:280 and 35:320 indicating that there is no significant variation among these two treatments in terms of FCE. Hence, feed III and feed IV are similar in their responses in terms of conversion of nutrients.

Protein Efficiency Ratio (PER)

PER is maximum for feed III followed closely by feed IV, but the PER's obtained by feed II, V and VI are also similar approaching that of feed III and feed IV. The first subset of the interaction means of protein and energy levels include the protein and energy combinations of 35:300, 35:320, 40:280, 40:300, 40:320 corresponding to feed II, III, IV, V and VI respectively indicating that there is no significant difference among these five treatments in terms of PER. Since, PER is considered to be a reflection of the amino acid balance of the feed, it can be inferred here that the feeds tested in the experiment have a balanced amino acid profile. The lower PER obtained with feed I may be due to the catabolism of protein for energy due to the insufficient non-protein energy in the feed.

Protein Digestibility

The protein digestibilities show a similar trend (table V) in all the feeds indicating that the six feeds are similar in their protein digestibilities. The interaction means shows six homogeneous subsets (Table V) involving all the six combinations indicating that there is no significant difference in their protein digestibilities among the six treatments. Feed III and Feed IV had similar protein digestibilities of 79.85% and 81.34% respectively. The slight increase of protein digestibility in Feed IV may be due to its higher

protein content (40%). Decrease in digestibility in the case of feeds II and V as evident in fig.5 could not be explained. Since total collection of faeces was resorted to, errors in estimation can not be ruled out.

The body composition of the shrimps at the termination of the experiment shows identical crude protein (%) composition for feed III and feed IV, although maximum crude protein of 66.04 was obtained with feed I. The Ether Extract (%) is maximum for shrimp reared on feed VI (5.46%). The values were not subjected to statistical analysis because the samples were pooled. The variations however, were minor (Table VI) to be explained or discussed.

SUMMARY

SUMMARY

1. The Experiment was conducted at the Regional Centre of Central Marine Fisheries Research Institute, Mandapam.
2. Six semipurified diets of different protein (%): digestible energy (DE) (kcal/100gm diet) were tested on *Penaeus semisulcatus* juveniles to arrive at an optimum protein: energy ratio.
3. The six feeds had protein (%): DE (kcal/100gm diet) combinations of 35:280, 35:300, 35:320, 40:280, 40:300 and 40:320 respectively.
4. The protein: energy ratios of the six feeds were 126.06, 113.30, 110.38, 139.63, 128.18 and 124.51 (mg protein/ kcal) respectively.
5. The *Penaeus semisulcatus* juveniles were collected from Thallumadi; (local shore seine) operators in the Palk Bay and acclimatized to the laboratory conditions for one week prior to the experiment.
6. Each treatment was carried out in two replicates. Five juveniles with an initial mean length of 57.77mm with total biomass of 8 to 9 grams per replication were studied.
7. The experiment was conducted for 30 days. The responses were recorded in terms of growth (Relative Growth Rate, Specific Growth Rate, Feed

Conversion Efficiency, and Protein Efficiency Ratio) protein digestibility and gross body composition.

8. The best and identical results were obtained with feed III and feed IV, indicating that these two feeds are similar in their performance.
9. It could be concluded that the growth obtained with feed IV could be also obtained with feed III by increasing the non-protein energy by 40k cal/100gm. This clearly indicated that the protein sparing of 5% could be achieved by increasing the major non-protein dietary constituent, which was tapioca flour in this investigation.
10. The optimum protein: DE ratios required in the feeds designed for *Peneaus semisulcatus* juveniles (<2g.) were determined to be 35:320 and 40: 280 and the corresponding P/E ratios (mg protein/ kcal) were 110.38 and 139.63.

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