NUTRITIONAL RESPONSES IN INDIAN WHITE SHRIMP *Fenneropenaeus indicus* TO VARYING PROTEIN: ENERGY COMBINATIONS IN COMPOUNDED ARTIFICIAL FEEDS

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By

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Certificate

This is to certify that this thesis entitled **'Nutritional responses in Indian white shrimp *Fenneropenaeus indicus* to varying protein: energy combinations in compounded artificial feeds'** is an authentic record of research work carried out by **Mr. P. Vijayagopal,** under my scientific supervision and guidance at Central Marine Fisheries Research Institute (CMFRI), Cochin in partial fulfilment of the requirements for the award of the degree of **Doctor of Philosophy** of the Cochin University of Science and Technology (CUSAT) under the Faculty of Marine Sciences, and no part thereof has been presented for the award of any other degree, diploma or associateship in any University.

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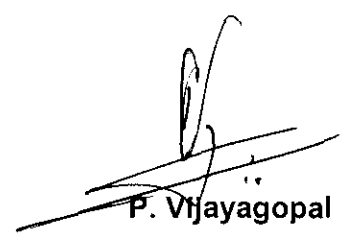
Declaration

I, P. Vijayagopal, do hereby declare that this thesis entitled **'Nutritional responses in Indian white shrimp *Fenneropenaeus indicus* to varying protein: energy combinations in compounded artificial feeds'** is a genuine record of the research work done by me under the scientific supervision of Dr. Babu Philip, Professor and former Head of the Department, Department of Marine Biology, Microbiology & Biochemistry, Cochin University of Science and Technology, and has not previously formed the basis for award of any degree, diploma or associateship in any University.



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To my late father

for all the love

Contents

Abbreviations and Acronyms

ADCP	Aquaculture development and coordination programme
ADE	Apparent digestible energy
ADMD	Apparent dry matter digestibility
AIA	Acid insoluble ash
ALD	Apparent lipid digestibility
APD	Apparent protein digestibility
AQUACOP	Aquaculture Research Centre of the Pacific, Tahiti, French Polynesia
CF	Crude fiber
CIBA	Central Institute of Brackishwater Aquaculture
CMFRI	Central Marine Fisheries Research Institute
CP	Crude protein
DE	Digestible energy
DM	Dry matter
E/P	Energy/protein
EE	Ether extract or crude fat
FAO	Food and Agriculture Organisation
FCE	Food conversion efficiency
FCR	Food conversion ration
FER	Feed efficiency ratio
GE	Gross energy
GE	Gross energy
GLP	Good laboratory practice
GMP	Good manufacturing practice
L: C	Lipid: carbohydrate
ME	Metabolisable energy
MT	Metric tonnes
NFE	Nitrogen free extract
NRC	National Research Council
P/E	Protein/energy
PER	Protein efficiency ratio
PNPD	Physiology Nutrition and Pathology Division
RGR	Relative growth rate
SGR	Specific growth rate

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Preface

Shrimp[1] mariculture is the major aquaculture activity in India despite the challenges it poses. Even though 75 – 85% of it comes from low input extensive farming systems (Rosenberry 1999), India maintains a moderate sixth position in the production of farmed shrimp (114670 MT) with a positive growth of 41.4 % over 1998-1999 figures (FAO 2001). The remaining 10 – 20% of the farmed shrimp production is from semi-intensive and 5% from intensive production systems (Tacon 2002). Awareness and use of nutrient inputs thus appears to be limited to this 25% of the farming systems. The cost of feeds and feeding (23-56%) followed by seed cost (10-22%) according to an estimate by Ling *et al.* (1997), is the major expenditure in the operation that requires, research and refinement to be acceptable to the cost conscious aquafarmer for augmenting production.

The approach to reducing cost of production in aquaculture in general and mariculture in particular is focused on minimising the cost of feeds. Reduction in the inclusion of costly animal protein sources, mainly of marine origin is an area, which is incessantly worked upon. Definition of species-specific requirements, scientific rationing and unravelling of animal-specific requirement of nutrients are some of the other key areas of work. Improving the bioavailability of nutrients with biotechnological interventions is another frontline in aquatic nutrition research.

[1] The common names shrimp and prawn are applied to different species in different parts of the world. According to a convention by the United Nation's Food and Agriculture Organization (FAO), the term shrimp refers to marine and brackish-water forms of Penaeidae and Palaemonidae, while fresh-water forms of Palaemonids are called prawn.

Identification of anti-nutritional factors, their amelioration and laying down of safety standards regarding their use is nascent in shrimp mariculture. Currently farming system intensification with biosecurity, vertical integration of inputs, organic farming etc., are adding new dimensions to this sector.

Focus of nutritional requirement studies have shifted from definition of absolute requirements in terms of protein, lipid and carbohydrate to definition of more precise nutritional requirements in terms of amino acids and fatty acids. Application of these findings has become easier now with linear programming software's available for feed formulation.

Nutrient interactions and interrelationships cannot be ignored in nutrition research. Among the macronutrient interaction mechanisms studied calorie protein interaction is the first to be taken up in any animal either terrestrial or aquatic. Shrimp is no exception in this regard. Thus research in crustacean nutrition began in the laboratories of Dr. Kanazawa and Dr. Provasoli in the 1960s in Japan (Kagoshima University) and United States (Yale University) respectively. Dr. Kanazawa focused on development of test diets by modifying his own diets designed for silkworm, to study the absolute macronutrient requirements in *Penaeus japonicus*. His effort was with a vision to support the commercially successful shrimp mariculture in Japan then. Dr. Provasoli, motivated by his success in defining the nutrient requirements in the culture media for freshwater and marine algae, extended his work to define the nutrient requirements of certain crustaceans like *Artemia* and *Moina*, which consumed algae. Some of the first descriptions of macronutrient and micronutrient interactions came from him.

Today even with an ever-growing shrimp mariculture industry led by Asian countries commercial aquaculture is dependent upon empirically formulated commercial feeds. Farming system crashes leading to heavy economic losses have led adoption of `good farming practices´ similar to good manufacturing practice (GMP) and good laboratory practice (GLP) standards followed in Europe and Americas. Organic aquaculture similar to organic agriculture is also in place today because there is a growing awareness regarding the long term benefits of its consumption coupled with a significant growth in the market segment for such produce.

It is in this context an investigation of this nature was taken up with the broad aim of definition of gross energy requirements in a shrimp abundant in Indian waters viz. *Fenneropenaeus indicus*[2], the Indian white shrimp. Interaction of protein in the feeds with energy, and how best energy can be utilised to spare protein without affecting the animals' growth and health, the possibility of cost reduction and effect of energy as a variable in shrimp feeds and its impact on growth are the two major facets in which the knowledge advanced through this investigation can be applied.

[2] Synonymous to *Penaeus indicus*

INTRODUCTION

CHAPTER - I

INTRODUCTION

Shrimp nutrition research really started off in the 1960s. Researches on location-specific problems both applied and basic are innumerable. Although majority of crustacean aquaculture operations are conducted within earthen-pond farming systems (New 1995; Rosenberry 1993) almost all published information on nutrient requirements in crustaceans is derived from laboratory or indoor tank based feeding trials. This according to Tacon and Akiyama (1997) has been due to a variety of reasons, including 1) the higher economic cost of conducting feeding trials within outdoor experimental ponds, 2) the difficulty of readily quantifying the contribution of natural food organisms in the overall nutritional budget of pond raised crustaceans, 3) the often large variability of results obtained from superficially identical outdoor ponds or pens, and 4) the general reluctance of the conventional laboratory based nutritionist to work under outdoor field conditions (for a review, please see Tacon 1995).

According to Tacon (2002) the shrimp farming sector currently consumes 470,386 MT of fish meal and 36,184 MT of fish oil within compound aquafeeds (dry basis) or the equivalent of 2,351,930 MT of fish (pelagic fish live weight equivalent) for the total global production of 1,130,737 MT of farmed shrimp in 1999; this is equivalent to the consumption of 2.08 kg of fish for every 1.0 kg of shrimp produced.

The mean fishmeal and fish oil content of shrimp aquafeeds in 1999 was estimated to be 26% and 2%, respectively.

The mean food conversion efficiency of shrimp aquafeeds was 2.0 in 1999, with 2.0 kg of shrimp feed (dry basis) being consumed for each 1.0 kg of shrimp biomass harvested (wet basis). This feed efficiency is equivalent to a shrimp

nutrient utilization efficiency of about 25% the remainder being lost to the surrounding aquatic environment.

At present the majority of shrimp aquafeeds used by farmers are nutritionally over-formulated as complete diets (DeVresse, 1995 and DeVresse, 2000) irrespective of the farming system, shrimp stocking density employed and natural food available and no practical guidelines exist concerning good on-farm feed manufacture and on-farm feed management practices.

Shrimp feeds available commercially in the Asian region are reported to be 'over-formulated' in the absence of accurate information regarding nutrient density in feeds used under different farming systems, viz., extensive, semi-intensive and intensive (Tacon 2002). Thus, relevance of laboratory based nutritional evaluation for nutrient requirements in shrimp is only in the context of 20-25% semi-intensive and intensive shrimp farms in the region (Rosenberry, 1999). However, energy requirement in shrimp feeds is still an area where even laboratory-based investigations are scant.

Fenneropenaeus indicus formerly *Penaeus indicus* (Perez-Farfante and Kensley, 1997)* popularly known as the Indian white shrimp is a major alternative species farmed and is ranked eighth contributor to the world production of farmed shrimp (FAO, 2001)**.

*Suggested new names for shrimp (Perez-Farfante and Kensely, 1997)

Old Name	**New Name**
Penaeus vannamei	*Litopenaeus vannmei*
Penaeus stylirostris	*Litopenaeus stylirostris*
Penaeus chinensis	*Fenneropenaeus chinensis*
Penaeus japonicus	*Marsupenaeus japonicus*
Penaeus schimitti	*Litopenaeus schimitti*
Penaeus setiferus	*Litopenaeus setiferus*
Penaeus occidentalis	*Litopenaeus occidentalis*

Old Name	**New Name** (Contd.)
Penaeus brasiliensis	*Farfantepenaeus brasiliensis*
Penaeus aztecus	*Farfantepenaeus aztecus*
Penaeus californiensis	*Farfantepenaeus californiensis*
Penaeus duorarum	*Farnfantepenaeus duorarum*
Penaeus noitalis	*Farnfantepenaeus notialis*
Penaeus subtilis	*Farnfantepenaeus subtilis*
Penaeus paulensis	*Farnfantepenaeus paulensis*
Penaeus merguiensis	*Fenneropenaeus merguiensis*
Penaeus pencillatus	*Fenneropenaeus pencillatus*

No name change

Penaeus monodon, P. esculentus and P. semisulcatus

**Total world production of farmed shrimp in 1999, by weight (FAO 2001).

Shrimp species	Production (MT)	Change 1998–99 (%)
Giant tiger prawn *Penaeus monodon*	575,842	+3.9
Whiteleg shrimp *Penaeus vannamei*	187,224	-5.6
Fleshy prawn *Penaeus chinensis*	171,972	+19.5
Penaeid shrimp *Penaeus* spp (spp not given)	95,634	+20.2
Banana prawn *Penaeus merguiensis*	53,109	+7.5
Metapenaeid shrimp *Metapenaeus* spp	22,421	+1.0
Blue shrimp *Penaeus stylirostris*	12,390	-22.1
Indian white prawn *Penaeus indicus*	7,043	+13.7
Kuruma prawn *Penaeus japonicus*	2,359	-6.6
Southern white shrimp *Penaeus schmitti*	1,364	-21.3
Natantian decapods *Natantia*	904	+175.0
Akiami paste shrimp *Acetes japonicus*	270	+2.3
Redtail prawn *Penaeus penicillatus*	107	-21.9
Palaemonid shrimp, spp. Not given	98	-39.9
Total	**1,130,737**	**+5.2**

CMFRI initially and CIBA subsequently, researched on the nutrition of this species of shrimp, addressing the absolute nutrient requirements both at macro and micro levels. However, macronutrient interaction studies were limited to Ali (1990 and 1996) and Hamid (1998). In *P. monodon* a couple studies of starting from AQUACOP (1977) to Chuntapa *et al.* (1999) is limited to not more than twenty reports in all.

This investigation is presented in six chapters. Chapter II deals with the review of literature, which contains only the reports, which are relevant to shrimp. However, reports dealing with the associated factors which directly or indirectly influences the protein: energy interaction are also included. Materials and methods are presented in the III Chapter. Results and discussion are dealt with in Chapters IV and V respectively. Chapter VI is summary and conclusions, followed by references.

CHAPTER - II

REVIEW OF LITERATURE

The importance of balance between dietary energy and protein was understood at the beginning of the century in the nutrition of human and farm animals. The ratio has been expressed as either percent of total dietary energy arising from protein or the energy/protein ratio. Protein/energy ratio (P/E) is somewhat analogous to protein content. In this thesis it is defined as mg crude protein or digestible protein per kilocalorie (kcal) and energy is expressed as kcal $100g^{-1}$. Values in joules (J) found in all the reports for the sake of uniformity have been converted to kilocalories (kcal). A brief review of the systems of expression of units in vogue is as follows.

2.1 Traditional Systems

The traditional measurement systems measured mechanical energy and work with "mechanically" derived units, while special thermal units were used to measure heat energy. Accordingly, the **Btu** (British thermal unit) was defined as: the quantity of heat that must be added to 1 lb of water to raise its temperature 1°F (in Canada, 60–61°F). Similarly, the kilocalorie is the quantity of heat needed to raise the temperature of 1 kg of water by 1°C, at its point of maximum density (4°C).

2.2 International System

The International System of Units (SI) has one common unit for work and energy — the joule (J), which measures all forms of energy and work, whether the discipline is mechanical, thermal, electrical, chemical or nuclear. Work is the expenditure or receipt of some form of energy. Energy is the capacity for doing work. The joule (J) is defined as the work done when the point of application of a force of one Newton is displaced a distance of one meter in the direction of the force. In symbolic language, the formula is J = N•m. The unit is named after James Prescott Joule, English, (1818–1889).

2.3 Historical Note

SI is the official abbreviation — in all languages — for the International System of Units (Système International d'Unitès), adopted in 1960 by the 11th General Conference on Weights and Measures. New units such as the Newton (N), Pascal (Pa), and Joule (J) were adopted. These units will gradually replace the traditional units for force, pressure, energy, work, etc. However, literature originating from the Americas and Europe is found to use J and reports from UK and Asia prefer kcal for which following conversion factors are routinely used.

Change:	To:	Multiply By:
Kilograms	Pounds	2.205
Pounds	Kilograms	0.454
Calorie	Joule (J)	4.185
Joule	Calorie (cal)	0.239
g/MJ	g/Mcal	4.185
g/Mcal	g/MJ	0.239

Capuzzo (1983) reviewed the information available on the effects of dietary energy on growth, body composition and feed efficiency in *Homarus, Macrobrachium* and *Penaeus* genera. This was the last review of the general method of partitioning of ingested food energy into various measurable energetic fates with particular reference to crustaceans. In its simplest form, and following the terminology suggested by the U.S. National Research Council (NRC 1981), the energy-partitioning budget of any growing animal is expressed as: IE = FE + HE + WE + RE. The intake of dietary energy (IE) is balanced by the sum of undigested energy lost to the animal through faeces (FE) plus catabolic wastes (WE) and the remaining energy available for use by the animal. Available energy for use consists of combination of the total heat production (HE), as a result of both the metabolic and behavioural activities, and the net energy gain which is channelled into growth or recovered energy (RE). While crustaceans use energy in the same fashion as other animals that have been studied, some characteristics unique to crustaceans appear at several levels of this partition scheme. Digestibility and

related faecal energy loss (FE) may differ among carnivorous, herbivorous and scavenger species. Waste energy (WE) losses arising from metabolism, primarily through urine and the gill excretions will be similar to those determined or calculated for ammonotelic fish rather than those for higher vertebrates. Heat energy (HE) will include energy losses associated with moulting, the shedding of the exoskeleton, which has been estimated by several investigators (Logan and Epifano, 1978; Capuzzo 1983; Khmeleva and Gobulev 1986).

2.4 Energy values of nutrients for crustaceans

To balance the energy level of crustacean diets appropriately, estimation of the energy value of dietary nutrients is necessary. In the absence of empirically determined values, the energy level of a diet can be estimated from gross energy (GE) values of the carbohydrate, fat and protein level in each of the feed ingredients. However, using gross energies of these macronutrients may be misleading due to incomplete digestion. Consequently, apparent digestible energy (ADE) values are better than GE values for estimating the biological value of nutrients. Using the standard National Research Council (NRC) terminology: IE – FE = ADE. FE includes not only undigested material that was never assimilated but also some energy from tissue products produced by the animal as well as products of bacterial action in the animals' gut. Therefore, ADE is slightly different (lower) than true digestible energy (TDE). A more accurate measure of usable dietary energy takes into account the other source of energy loss WE. Thus, available or metabolic energy (ME) is ADE – WE = ME. By using the three equations it is evident that ME = HE + RE. To estimate the ME of different nutrients, average values were compiled and standard estimates established for the various classes of nutrients in feeds (NRC, 1981). These estimates, termed physiological fuel values (pfv's), are routinely used in calculating the energy content of formulated feeds. The pfv's of 4, 9 and 4 kcal g^{-1} for carbohydrate, lipid and protein respectively (Brody, 1964) were obtained using arbitrary digestibilities and assuming the end product of protein catabolism to be urea. These assumptions are not applicable to crustaceans.

Brett and Groves (1979) stated that 4.78 kcal g^{-1} was more realistic digestible energy variable for protein in finfish. They derived this value from calculation of metabolism in finfish as well as crustaceans. Brett and Groves (1979) proposed focusing on ME values rather than on total nutrient levels. Although the value of 4.8 kcal g^{-1} can be used for minor nitrogenous compounds, as demonstrated in the study of Le Gal (1987), Elliot and Davidson (1975) still considered 4.8 kcal g^{-1} to be a GE value more appropriate than the pfv of protein (4 kcal g^{-1}) for mammals.

The values recommended by aquaculture coordination and development programme (ADCP), 1983 of FAO that are used in this study, are as follows:

Nutrient	**Gross energy (GE) kcal g^{-1}**	**Digestible energy (DE) kcal g^{-1}**	
Protein	5.5	Animal protein	4.25
		Vegetable protein	3.8
Fat	9.1		8.0
Carbohydrate	4.1	Animal carbohydrate	3.0
		Vegetable carbohydrate	2.0

2.5 Energy requirements in *Fenneropenaeus indicus*

Colvin (1976) reported that substitution of protein by potato starch, involving only a small change in caloric value (4.8 – 4.7 kcal g^{-1}) did not affect growth in *P. indicus* in spite of the reduction of protein from 53.1 to 42.8% in the first report on evaluation of protein requirement in this species.

Ali (1990) assessing the relative efficiencies of different lipids and lipid levels in the diet of *Penaeus indicus* tested four lipids viz. cod liver oil, prawn head oil, sardine oil and soybean lecithin at 60 g kg^{-1} level in a purified diet. Diets with prawn head oil and a mixed lipid consisting of all the four lipids in equal proportions registered significantly higher growth (P <0.01) and food conversion ratio (FCR). Using this lipid mixture and starch in the ratio 1:7, the calorific value of the diet was varied

from 271.68 kcal $100g^{-1}$ to 462.43 kcal $100g^{-1}$, keeping the protein constant (400 g kg^{-1}). Feeding experiments conducted with these diets have shown that the growth of shrimps increased and the FCR improved with the increase in dietary energy. The diet having 414.72 kcal $100g^{-1}$ recorded the highest growth and least FCR. A further increase in the dietary energy is reported to have no beneficial effect on the growth or FCR.

Ali (1996) in a set of three experiments reported the propensity of *P. indicus* to utilise carbohydrates to spare proteins. Keeping protein (350 g kg^{-1}) and lipid (70 g kg^{-1}) levels constant, he observed that an energy level (DE) of 348 kcal $100g^{-1}$ to be appropriate where the level of carbohydrate was 225 g kg^{-1}. In the next experiment with lipid (70 g kg^{-1}) levels kept constant and allowing protein and carbohydrate levels to vary he reported than a DE level of 399.4 kcal $100g^{-1}$ to be appropriate where the protein and carbohydrate levels were 219 and 534 g kg^{-1} respectively. When protein (350 g kg^{-1}) was kept constant and lipid and carbohydrate levels were allowed to vary, the optimum DE was 392.4 kcal $100g^{-1}$ where the lipid and carbohydrate levels were 70 and 332 g kg^{-1} respectively.

Hamid (1998) reported three nutritional evaluations with *P. indicus* in which the levels of protein (g kg^{-1}) and GE (kcal $100g^{-1}$) tested were 350: 380, 420, 460; 400: 380, 420, 460 and 450: 380, 420, 460 respectively in a 3x3 factorial experiment. In animals weighing <1g an optimum could not be delineated because, 450:460 combination of protein and energy registered the maximum growth. In animals of 1-5g a lowering of protein and GE was reported where the optimum combination was 400:420. A further lowering of protein and energy requirement was also reported in animals of 5-10g where the best combination was 350:380 which was not an optimum because levels/nutrient density below this was not tested. These results are also summarised in Table shown below. Hamid (1998) in another set of three experiments interestingly reported that *P. indicus* below 1g could utilise 120g kg^{-1} lipids in concert with 450g kg^{-1} proteins. In the animals weighing 1-5g optimum was reported to be 400:90 and in animals weighing 5-10g the maximum growth was at 350:60. These three experiments were also inconclusive as the former three experiments because, levels above 450:120 and below 350:360 in the sizes <1g and 5-10g were not tested.

Spp.	S ‰	I_W g	CP	L: C	Energy levels tested	Opt. Energy kcal100g^{-1}	P/E ratios tested	Opt. P/E ratio	Reference
P. indicus	15	0.075 w	40	1:7	272-462	415	87-147	96	Ali 1990
P. indicus	17	0.010 d	35	1:3	286-470	348	85-140	101	Ali 1996
P. indicus	17	0.010 d	22	1:8	379-401	399	55-135	55	Ali 1996
P. indicus	17	0.010 d	35	1:5	358-419	392	112-125	89	Ali 1996
P. indicus	25	< 1 w	45	1:11	380-460	460	79-112	95	Hamid 1998
P. indicus	25	1 – 5 w	40	1:8	380-460	420	79-112	95	Hamid 1998
P. indicus	25	5-10 w	35	1:6	380-460	380	79-112	95	Hamid 1998

S = salinity, I_W = Initial weight, w = wet weight, d = dry weight CP = crude protein, L: C = lipid: carbohydrate, Opt. = optimum

2.6 Energy requirements in *Penaeus monodon*

AQUACOP (1977) estimated that a total dietary energy content of 330 kcal $100g^{-1}$ was required for optimal growth of *P. monodon* growth with a diet containing 400 g kg^{-1} protein.

Bautista (1986) investigating on the response of *P. monodon* to varying protein/energy ratios in test diets reported the results of two sets of factorial experiments conducted for 8 weeks to determine the response of juveniles (average weights 0.60 $\pm$ 0.16 g and 0.80 $\pm$ 0.05 g) to diets containing various protein/energy ratios. The first experiment used casein as the sole source of protein, while the other used a combination of 70%: 30% casein: gelatin for its protein source. A two fold increase in the body weight was achieved for shrimps fed on diet combinations of 400-500 g kg^{-1} protein, 50-100 g kg^{-1} lipid and 200 g kg^{-1} carbohydrate with energy values of 285-370 kcal $100g^{-1}$, regardless of the protein source used. Reduction in protein content of the diet from 500 to 400 g kg^{-1} while maintaining the total energy level at 330 kcal $100g^{-1}$ resulted in a non-significant decrease in growth. The inclusion of 150 g kg^{-1} lipid in diet produced adverse affects in the animal while sucrose levels beyond 200 g kg^{-1} resulted in decreased growth rate. An increase in energy level, at constant dietary protein level, resulted in improved utilisation of protein and feed conversion efficiency. Survival of the prawn was higher with diets containing casein and gelatin as the protein source than with those containing casein as the sole source of protein. Both, Bautista (1986) and Shiau and Peng (1992) concluded that a protein: energy ratio of 125 mg protein $kcal^{-1}$ is optimal for *P. monodon* growth.

Hajra *et al.* (1988) indicating a transient protein sparing action exerted by digestible energy from dietary carbohydrate reported that, at 460 g kg^{-1} protein, weight gain, feed efficiency and protein utilization increased with increase in dietary energy level up to 412.60 kcal $100g^{-1}$ (P/E 112.2) in a 21-day study in near fresh water conditions (3.5 – 4.5 ‰ salinity).

Shiau and Chou (1991) testing two protein levels 360 and 400 g kg^{-1} and six energy levels 280, 300, 320, 340, 360 and 380 kcal $100g^{-1}$, reported that the weight gain, FCR and protein gain of shrimp improved as dietary energy level was raised up to around 330 kcal/100g when 360 g kg^{-1} protein diet was fed and up to around 320 kcal $100g^{-1}$ when 400 g kg^{-1} protein diet was fed. Further elevation in dietary energy level of the diet had no beneficial effect on either levels of protein. At a salinity 32-34 ‰, they opined that at 400 g kg^{-1} protein and 320 kcal $100g^{-1}$ to be the optimum (P/E = 125) and at 360 g kg^{-1} protein energy level of 330 kcal $100g^{-1}$ (P/E = 110) to be the optimum implying protein sparing of 4%.

Chuntapa *et al.* (1999), reported optimal lipid: carbohydrate and protein: energy ratios in semi-purified diets for *P. monodon* Fabricius juveniles. Two experiments were performed and reported using completely randomised designs in semi-closed recirculating water systems. Juveniles of 0.4- 0.8 g in weight and 4.0 to 5.5 cm in length stocked at a density of 80 individuals m^{-2} were fed semi-purified diets. The first experiment determined optimal lipid: carbohydrate ratios: 40:390, 70:320, 90:250, 140:180 and 160:120 (g kg^{-1} wt/wt). The lipid: carbohydrate ratio of 70:320 gave the highest growth rate (P <0.05), while survival rates of shrimp in all other diet groups were similar but less. Thus, optimal lipid: carbohydrate ratio for the juvenile tiger shrimp was 1:4.6. In the second experiment, optimal protein: energy (P: E) ratio was studied using five protein levels (250, 300, 350 and 400 and 450 g kg^{-1}) with a fixed lipid: carbohydrate ratio of 1:4.6. Nine diets containing energy content (203-459 $kcal^{-1}$ 100g) with a protein: energy ratio (63-171 mg protein $kcal^{-1}$) was formulated. Shrimp fed the diet containing 330-440 g kg^{-1} protein and an energy content of 223-371 kcal $100g^{-1}$ had a significantly higher growth rate than those fed the other diets (P <0.05). A regression analysis indicated than an optimal P: E ratio for optimal growth and survival of juvenile tiger shrimp was 146-150 mg protein $kcal^{-1}$. This diet contained 330-440 g kg^{-1} protein and had an optimal energy of 263-331 kcal $100g^{-1}$.

Available data of P/E in *P. monodon*, which was compiled, by Cuzon and Guillaume (1997) is updated and Tabled below.

S‰	Coefficient values protein: lipid: CHO	P/E mg/kcal	Initial weight g	CP %	EE%	CHO%	Source of CHO	Reference
37	4:9:4(DE)	117-154	1.0	30-55	7	18	Gl. St.	AQUACOP 1997
37	4:9:4(DE)	130-153	1.0	40	2-10	14-20	Wh.	AQUACOP 1997
37	4:9:4(DE)	112-145	1.0	35-45	4-9	18-24	Wh.	AQUACOP 1997
32-34	4.5:8:3.3(ME)	71-127	1.3	25-60	7	8-43	Dx.	Alava and Lim 1983
32	4:9:4(DE)	90-204	0.6	30-50	5-15	0-20	Su.	Bautista 1986
32-34	5:9:4	112	0.6	45	10	10-30	Gl. Su. Tre.	Alava and Pascual 1987
4	4:9:4(DE)	94-121	0.5	46	9-14	26-31	Co. Mo.	Hajra *et al.* 1988
40	5:9:4(ME)	100-140	0.8	36-40	9	5-30	Dx.	Shiau and Chou 1991
32-34	5:9:4	83-116	0.5	30-40	4-5	20-30	Glu. Dx. St.	Shiau and Peng 1992
23-25	4:9:4	63-171	0.4-0.5	26-45	3-11	13.6-49.9	Co.	Chuntapa *et al.* 1999

S=salinity, CHO=carbohydrate, Gl.=glucose, St.=starch, Wh.=wheat, Dx.=dextrin, Su.=sucrose, Tr.=trehalose, Co.=corn, Mo.=molasses

2.7 Energy requirements in penaeids other than *P. monodon* and *F. indicus*

Sedgwick (1979) assessed the requirement of juvenile *Penaeus merguiensis* for dietary protein and energy was in growth trials by using rations based on freeze-dried *Mytilus edulis* meal. Evidence was obtained to indicate that the rate of food consumption in this shrimp is related to the energy content of the diet. Protein level required to support maximum growth and optimum protein conversion efficiency were reported to be energy dependent. Optimum protein levels were estimated in the range 340-420 g kg^{-1} for diets of energy content 290-440 kcal $100g^{-1}$.

Cousin *et al.* (1992) and Koshio *et al.* (1993) studied other penaeid species. These studies differ from those with *P. monodon* because; practical diets containing crab meal (Koshio *et al.*, 1993) or casein and crab meal Cousin *et al.* (1992) were used. Results for these penaeids confirm a protein sparing effect of carbohydrate, and suggest differences in protein requirements; 320 –350 g kg^{-1} for *P. vannamei* and *P. setiferus*, Cousin *et al.* (1992) and 420 g kg^{-1} for *P. japonicus* (Koshio *et al.* 1993). By increasing the level of non-protein energy sources, the protein requirement of *P. japonicus* was reduced from 600 – 420 g kg^{-1}. The optimal dietary P/E values of other penaeid species are similar to those of *P. monodon* and *P. vannamei* (84 mg protein $kcal^{-1}$) and if this value is exceeded, a growth depression results.

P. japonicus a carnivorous species with a presumed high dietary protein requirement (Deshimaru and Shigueno, 1972) grows on a 420 g kg^{-1} protein diet containing a highly digestible protein source, 150 g kg^{-1} carbohydrate, 80g kg^{-1} lipids. *P. japonicus* reaches a plateau in growth expressed as specific growth rate (SGR), beyond its optimal level of dietary protein. *P. merguiensis* which requires a dietary protein level similar to that of *P. japonicus* is able to grow at equivalent levels when fed diets containing less dietary protein, provided that a non protein energy source is provided. Collectively these studies suggest that an increase in dietary energy tends to increase the performance when a diet low in protein is fed.

Species	S ‰	CP:EE:NFE	P/E mg/ kcal	In. wt. g	CP%	EE%	NFE %	CHO source	Reference
P. merguiensis	37	5.55:9.45:4.2 (GE)	37-111	0.2	16-50	1-17	6-50	Wh. +St.	Sedgwick 1979
P. vannamei	37	4:8:3.8	80-120	1.0	25-30	6	20-40	Sa.	Cousin *et al.* 1993
P. japonicus	37	5.65:9.45:4.1 (DE)	90-120	0.4	20-60	3-14	5-24	De.	Koshio *et al. 1992*

S=salinity, CP=crude protein, EE=ether extract, NFE=nitrogen free extract, In.Wt.=initial weight, CHO=carbohydrate, Wh.=wheat, St.=starch, Sa.=saccharose, De.=dextrin

2.8 Effect of dietary protein and energy levels on other physiological and biochemical indices in shrimp

Rosas *et al.* (2001) reported the effect of dietary protein and energy levels on growth, oxygen consumption, haemolymph and digestive gland carbohydrates, nitrogen excretion and osmotic pressure of *Litopenaeus vannamei* (Boone) and *Litopenaeus setiferus* (Linne) juveniles. Influence of protein and energy levels on growth rate, survival, pre- and post-prandial oxygen consumption, ammonia excretion, haemolymph glucose (HG), glycogen in digestive gland and osmotic pressure (OP) in white shrimp *L. vannamei* and *L. setiferus* was studied. Diets containing high quality protein at a P/E ratio of 67, 109 and 151 were fed at 20% of the shrimp body weight of two sizes: <1 g and > 1 g. Both species showed an optimum P/E ratio of 151 (330-440 g kg^{-1} protein and 60 – 230 g kg^{-1} carbohydrate). In both experiments, the growth rate of *L. vannamei* was 2-3 times that observed in *L. setiferus*. Routine oxygen consumption and apparent heat increment (AHI) of *L. setiferus* was two times higher than that observed in *L. vannamei* juveniles, which could indicate that *L. setiferus* has a higher metabolic rate. The overall results showed that juveniles of > 1 g of both the species are less dependant of P/E ratio than juveniles of < 1 g. *L. vannamei* is indicated to be the most tolerant species with a high capacity to use a wide range of dietary P/E ratios for growth, which they attribute to lower energy requirements. *L. setiferus* is reported to have a lower capacity to accept different P/E in spite of its capacity to accept a high carbohydrate level. They stressed upon to take note of the importance of these species-specific physiological and nutritional differences in commercial culture.

Guzman *et al.* (2001) investigated the effect of dietary protein and energy content on the activity of digestive enzymes (total proteinases, trypsin, chymotrypsin, α-amylase and lipase), growth and survival of *L. setiferus* under controlled conditions. There was a clear relationship between the diet fed and the post larval growth and survival. Highest weight gain (2110± 96.7%) was obtained with a 400 g kg^{-1} protein and low energy diet (332 kcal 100 g^{-1}). The optimum P/E ratio estimated was 120 mg protein $kcal^{-1}$. Good survival was

obtained with low energy diets containing between 200 and 400 g kg^{-1} protein. Higher values for total proteinases, trypsin and α-amylase were obtained with low energy, 400 g kg^{-1} protein diet. Chymotryptic activity was considerably lower than that of other proteinases and lipase activity was too low to be reliably measured with the turbidometric method employed. Total proteinase activity was significantly lower than in experimentally grown post larvae. The α-amylase activity was at least two orders of magnitude higher in wild post larvae than in animals fed with the best experimental diet. Protein requirement was related to total energy content of the diet; best growth and digestive enzyme activity coincide with low energy, 400 g kg^{-1} protein diet. They opined that dietary carbohydrates could not spare protein because growth rates obtained with diets containing 200-300 g kg^{-1} protein (337 and 226 g kg^{-1} dextrin respectively) were significantly lowered.

2.9 Carbohydrate utilisation in shrimp

Since the level of lipid in shrimp diets cannot exceed 120 g kg^{-1} the choice of energy yielding nutrients excluding protein and lipid gets limited to carbohydrate. The status of knowledge essential for this work is summarised here. Cousin (1995) opined that energy retention in shrimp is more efficient in a higher protein diet than a low one because amino acids not used for protein synthesis were more efficiently used as energy source than dietary glucose. In *L. sylirostris* he showed that energy retention was less efficient in higher protein diet than low one as shown in the following Table.

Energy retention and wheat starch level (Cousin, 1995)

Wheat starch %	Protein %	Energy retention %
30	35	19
25	45	17
17	50	15
11	55	14

In *L. vannamei* also, he showed the same trend that was lower (10-14%) compared to *L. stylirostris*. Shiau (1997) reviewed the work done in crustaceans extensively and Tabled the carbohydrate utilization by penaeid shrimp as shown in the following Table.

Carbohydrate utilization by penaeid shrimp (Shiau, 1997)

Carbohydrate source	% Tested	Species	Results	Reference
Glucose/starch	0, 20, 30, 40	*P.setiferus*	>utilization = starch	Andrews *et al.* (1972)
Glucose, starch	10, 40	*P.duorarum*	>Utilization = starch	Sick& Andrews (1973)
Glycogen, glucose Dextrin, glucose, Sucrose	10	*P.japonicus*	>weight gain = sucrose, >feed efficiency = starch < utilization = glucose	Deshimaru & Yone (1978)
Glucose, starch, Dextrin, potato Starch, glycogen, Galactose, fructose, Sucrose, maltose	19.5	*P.japonicus*	<utilization = monosaccharides (glucose, galactose)	Abdel- Rahaman *et al.* (1979)
Maltose, sucrose Dextrin, molasses, Cassava starch, Corn starch, sago, Palm starch	10, 40	*P.monodon*	>survival = sucrose	Pascual (1983)
Trehalose, sucrose Glucose	10, 20, 30	*P.monodon*	>utilization = trehalose and sucrose utilized	Alava & Pascual (1987)
Wheat flour; straight First grade clear Second grade clear	35	*P.monodon*	no difference	Shiau *et al.* (1991)
Gelatinised bread flour	5, 15, 25, 35	*P.monodon*	<weight gain and conversion ratio = 35%	Catacutan(1991)
Glucose, dextrin, Starch	20, 25, 30	*P.monodon*	>utilization = starch or dextrin	Shiau and Peng (1992)

> = highest, < = lowest

Recently, Cuzon *et al.* (2000) in their review examined the carbohydrate utilisation by shrimp and the biochemical mechanisms involved in carbohydrate metabolism. Their conclusions were – digestibility of carbohydrate in shrimp varied according to flour type, botanical origin of starch and inclusion level. Native starch was digested as well as pre-cooked starch. Best results were attained with standard wheat starch.

Levels of glucose in plasma varied according to the botanical origin of starch in the diet (Cousin, 1995). For starch levels in feed contributing up to 45% of available energy, no negative effect on growth was seen. Increasing the amount of starch from 0 – 400 g kg^{-1} FCR was not affected. At low inclusion levels (ca. 30g kg^{-1}), starch promoted growth with a lowering in nitrogen excretion. Protein retention, PER, growth etc., depends on an optimal energy balance between protein and fat (Cousin, 1995), keeping the carbohydrate content enough for metabolic needs. Nature of starch fed had some correlation with the variations in hepatopancreatic glycogen. At 350 g kg^{-1} inclusion of amylose rich starch provided the lowest glycogen content in hepatopancreas; where as pre-cooked starch gave the highest hepatopancreatic glycogen values. Glycogen concentrations in muscle are very low and probably not affected by starch content in feed. Shrimps are equipped with digestive enzymes, which facilitate a large range of carbohydrate digestion. As in fishes, shrimp utilizes energy derived from protein better than energy derived from any other nutrient. Thus, the difficulty pointed out is to maintain optimal growth by balancing the P/E ratio including as much carbohydrates as possible.

On perusal of the literature on the subject in shrimp in general and *F. indicus* in particular it is evident that expensive protein inclusion in shrimp feeds can be reduced with a concomitant increase in the non-protein energy yielding constituents. Reduction in the inclusion of expensive proteins in feeds being the major application. GE, DE is used interchangeably due to the absence of experimental baseline data on DE and ME in shrimp. Ranges of protein and energy tested many at times are found to be insufficient to deduce the optima. Taking all these factors into consideration the present investigation was designed to delineate the P/E ratios in *F. indicus* early juveniles (< 1 g) in size with fixed level of protein and varying levels of lipid and carbohydrate under controlled conditions of culture. From the experimental data theoretical optima are also worked out.

MATERIALS AND METHODS

CHAPTER - III

MATERIALS AND METHODS

The feed material procurement, analysis and nutritional evaluations in this research were done in the Nutrition Laboratory of CMFRI and Marine Hatchery complex of CMFRI, Cochin. In total, one experiment of 28 days duration and six experiments of 42 days duration were conducted with different diet designs. The first experiment (A) conducted with feeds containing only natural and location specific feedstuffs is described first. The remaining six experiments that are similar are elaborated next as (B) 1 - 6.

III.1 Shrimp and experimental culture conditions – Experiment A

A feeding trial for 28 days was conducted with early juveniles of *Fenneropenaeus indicus* of one brood procured from MPEDA Hatchery, Vallarpadom, Cochin. Shrimp of mean average weight 0.43 $\pm$ 0.03 g (0.38 g – 0.48 g) were segregated into 18 groups of 10 animals each and were stocked in non-toxic plastic tubs (50 cm dia. x 25 cm h; 45-liter water volume) equivalent to a calculated shrimp density of 50 m^{-2} bottom surface area, in triplicate. After acclimatization and conditioning of the experimental animals for a period of three days, initial weights were recorded using an electronic balance. Seawater trucked from Manassery Beach, Cochin, stored in concrete tanks was used for the experiment. Aged seawater drawn through a biological filter and stored in 1-ton fibreglass tanks was diluted to 25‰ and used through out the experiment. All the experimental units received 30% water exchange daily and 100% exchange on weekends. All the plastic tubs were scrubbed clean weekly with minimum disturbance to the experimental animals to check plankton growth. Aeration was provided through a single air-stone inserted though the aperture on circular transparent lid. Water temperature, dissolved oxygen, pH and salinity measurements were made weekly (Table 1).

Table 1. Environmental conditions of culture containers in Experiment A

Parameter	Week 1	Week 2	Week 3	Week4
Temperature °C	28.4	28.9	29.0	28.8
Dissolved Oxygen (mg L^{-1})	5.5	6.1	5.8	5.9
pH	8.4	8.2	8.1	8.2
Salinity (g L^{-1})	25.2	25.0	25.4	25.5

III.2 Diets and feeding protocol - Experiment A

Six experimental diets were formulated using natural feed ingredients available locally. The proximate chemical compositions of these feed ingredients were determined prior to the experimental diet design (Table 2). The ingredient composition of the experimental diets is shown in Table 3. Ascending levels of protein were obtained (Table 4) in the experimental feeds formulated by varying the major proteinaceous ingredients viz., fish meal (dried, unsalted anchovies), shrimp meal (dried *Parapenaeopsis stylifera*), deoiled groundnut oil cake and clam meal (*Villorita cyprinoidis*). Varying the inclusion of oil and tapioca flour varied energy levels.

Table 2. Proximate chemical composition of feed ingredients (Exp.A) (As fed basis)

Ingredients	DM	OM	CP	CF	EE	NFE	Ash	AIA
Fish meal	84.06	68.51	61.75	-	5.39	16.83	15.55	0.90
Shrimp meal	89.06	61.17	37.98	11.00	2.83	20.31	27.87	0.31
GNOC	92.78	85.37	49.07	3.57	6.70	33.25	7.41	0.48
Tapioca flour	89.95	88.68	1.72	1.42	0.49	95.09	1.28	0.16
Clam meal	94.33	86.69	52.60	-	10.63	28.46	7.64	2.57

DM= Drymatter, OM=Organic matter, CP=Crude protein, CF= Crude fiber, EE=Ether extract, NFE= Nitrogen free extractives, AIA= Acid insoluble ash.

Table 3. Ingredient composition of the experimental diets (g kg^{-1}) (Exp.A)

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Fish meal	-	150	-	160	200	200
Shrimp meal	200	-	-	260	200	200
GNOC	200	150	300	260	200	200
Tapioca flour	440	420	330	170	100	50
Clam meal	60	180	270	50	200	250
Oil[1]	60	40	20	20	40	60
CMC	-	20	40	40	20	-
Cholesterol	5	5	5	5	5	5
Vitamin mixture[2]	10	10	10	10	10	10
Mineral mixture[3]	20	20	20	20	20	20
Cr_2O_3	5	5	5	5	5	5

[1]Codliver oil and groundnut oil mixed in the ratio 1:1

[2]Contains Vitamin B_1 - 10 mg; Vitamin B_2 - 10 mg; Vitamin B_6 – 3 mg; Nicotinamide – 110mg;Calcium pantothenate – 50 mg; Folic acid - 1500 mcg; Vitamin B_{12} – 15 mcg ; Vitamin C – 50 mg ; Choline chloride – 1200mg and Inositol – 4000 mg

[3]Salt mixture USP XIV from M/s Sisco Research Laboratories, Mumbai.

Table 4. Proximate chemical composition of the experimental diets (% on dry matter basis) and their gross energy content (Exp.A)

	Diet Nos.					
Proximate principles	1	2	3	4	5	6
CP	22.43	31.99	35.71	43.28	47.65	52.68
EE	6.2	7.74	7.34	4.26	9.31	10.69
CF	3.14	1.41	1.76	3.4	2.61	2.19
NFE	57.05	50.06	47.03	31.92	25.61	20.18
Ash	11.18	8.8	8.16	17.64	14.82	14.26
AIA	0.64	1.41	1.42	0.36	0.74	1.17
GE kcal 100 g^{-1}*	413.69	451.63	438.17	407.68	429.97	470.83
DE kcal 100g^{-1}**	259.0275	297.9975	304.5475	281.86	328.2125	349.77
P/E ratio	54.22	70.83	81.5	106.16	110.82	111.89
E/P	18.44	14.12	12.27	9.42	9.02	8.94
L:C ratio	1:9.2	1:6.5	1:6.4	1:7.5	1:2.8	1:1.9
L:C (% weight)	6:57	8:50	7:47	4:32	9:26	11:20
NFE + EE	63.25	57.8	54.33	36.18	28.22	30.87

*Analysed values for protein, EE and NFE multiplied by 5.5, 9.1 and 4.1kcal g^{-1} respectively (ADCP1983)

**Analysed values for animal protein x 4.25, vegetable protein x 3.8, EE x 8, animal NFE x 3 and vegetable NFE x 2 kcal g^{-1} respectively (ADCP 1983)

All the ingredients were pulverized and sieved through 200 μ mesh to obtain uniform particle size. The dry ingredients except tapioca flour and carboxymethylcellulose (CMC) were weighed and mixed well and blended with oil manually. Tapioca flour and CMC were gelatinised in 200 ml water and subsequently mixed with other ingredients into thick dough. The dough was so formed that its consistency was soft enough to facilitate manual pelletization using a kitchen noodle maker. Moist noodles were made using a 2 mm (dia.) die and dried in a hot air oven at constant temperature (65± 2°C). The dry pellets were then crumbled and stored in airtight containers for subsequent chemical analyses and feeding.

The gross energy (GE) values were calculated from the values reported by ADCP (1983) i.e., 5.5 kcal g^{-1} for protein, 4.1 kcal g^{-1} for carbohydrate (excluding crude fibre) and 9.1 kcal g^{-1} for fat. Thus, six known protein: energy combinations formed the treatments tested in shrimps. The P/E i.e., mg protein $kcal^{-1}$ of the experimental diets was also calculated. Chromic oxide was incorporated at 0.5% level in all the feeds for estimating the apparent dry matter digestibility (ADMD) and apparent protein digestibility (APD).

Feeding was started at the rate of 15% of the body weight during the acclimatization period and the rate of feeding was decreased to 10% of the body weight, which was the level at which minimum feed residues were observed. Feeding was carried out at the rate of 10% of the body weight at 10.00 h and 17.00 h daily in two divided doses of 40% in the morning and 60% in the evening. The tubs were cleaned before each feeding daily throughout the experimental duration. Faecal strands and leftover feed from each tub were siphoned out and collected daily with the help of a thin tube and bolting silk and rinsed with distilled water to remove traces of adhering salts. Feed residue and faecal output were quantified and dried in a hot air oven at 55±2°C and pooled for analyses.

Growth was measured as biomass gain $shrimp^{-1}$ (g), relative growth rate (RGR) and specific growth rate (SGR); protein efficiency ratio (PER), food conversion ratio (FCR) and survival % was also estimated. Apparent dry matter digestibility

(ADMD) and apparent protein digestibility (APD) were calculated using the formula, apparent digestibility coefficient (ADC) = 100 – 100 (% Cr_2O_3 in feed / % Cr_2O_3 in faeces) x (% nutrient in faeces/ % nutrient in feed).

III.3 Chemical analyses of diets and water – Experiment A

Feed ingredients, experimental feeds and faeces were analysed for their proximate chemical compositions according to A.O.A.C. (1990). Chromic oxide (Cr_2O_3) was estimated according to Furukawa and Tsukahara (1966). Seawater was analysed according to the standard methods of Strickland and Parsons (1972).

III.4 Statistics - Experiment A

Comparison of means was carried out through analysis of variance (ANOVA) of the data according to Snedecor and Cochran (1973) using SPSS software. To estimate the optimum levels of protein and GE second-degree polynomials were fitted.

III.5 Shrimp and experimental culture conditions – Experiments (B 1-6)

Shrimp post larvae from a single brood were procured separately for each experiment from M/s SS Hatchery, Kodungallur, Cochin. The post larvae were reared in the wet laboratory to mean average weight 0.040 – 0.050g using a commercial post larval feed. The animals were hand sorted and weighed individually and stocked in the culture units (circular Perspex tanks of 50 cm dia. x 25 cm h; 45-liter water volume) at the rate of 15 animals (Photograph of the experimental set-up in the next page). The calculated densities of shrimp in these experimental units equal 75 m^{-2}, in triplicate. Seawater diluted to 25‰ was used in all the experiments. Unlike experiment A, 90% water exchange was done in all the experimental units daily and 100% water exchange and scrubbing of the tubs were done weekly. Sampling of seawater for analysis for pH, D.O. and salinity was reduced to fortnightly intervals due to the absence of marked fluctuations. Temperature was recorded daily.

III.6 Diets and feeding protocol - Experiments (B 1-6)

Six experiments performed were by using a uniform diet design. For each experiment the protein content in the diets were 250, 300, 350, 400, 450 and 500 g kg^{-1}. GE levels varied from 280 kcal $100g^{-1}$ to 450 kcal $100g^{-1}$. All the feeds contained a common ingredient mixture (CIM). By varying mainly the content of CIM and starch (tapioca flour) content the variations protein and GE and thereby DE was brought about. Wherever, desirable variation in energy was not obtained lipid levels were adjusted to obtain them. Experiment B-1 was conducted with diets containing a CIM, which had a lower nutrient density (Table 9), compared to the experiments B 2-6 because fishmeal and albumin used in the former experiment were lower in their protein and energy contents (Table 14 and 15). In diets where tapioca flour was less than 100g kg^{-1} or avoided, carboxymethylcellulose (CMC) was used as a binder. Cellulose was used as the filler. CIM was blended separately. Tapioca flour and CMC were gelatinised in water and CIM and cellulose were mixed and blended to form the dough for hand pelleting using a kitchen noodle maker with a 2 mm die. The pellets were air dried first and oven-dried at 55°C, crumbled, crushed using a food mixer and sieved through 0.5 mm and stored in airtight containers in a refrigerator and used. Experiment-wise, the composition feed ingredients used; CIM, and the ingredient composition of the experimental diets are shown in Tables 8, 9, 10, 11, 14, 15, 16, 17, 20, 21, 24, 25, 28, 29, 32 and 33 respectively.

Table 8. Proximate composition of the natural and purified feed ingredients used for experimental diet compounding (Exp.B-1)

	DM	CP	EE	CF	NFE	Ash	AIA
Fish meal	98.28	70.58	3.09	0.93	0.36	23.32	11.52
Shrimp meal	92.51	67.45	3.29	0.00	5.27	16.50	4.39
Clam meal	94.37	59.79	13.01	0.00	15.10	6.47	1.94
GNOC	94.55	43.75	8.13	5.49	30.10	7.08	2.36
Tapioca flour	87.18	2.82	0.29	1.79	80.26	2.02	0.10
Cellulose	93.80	0.65	0.28	92.56	0.00	0.31	0.00
Albumin	92.91	80.50	0.00	0.00	5.97	6.44	0.00

Table 9. Ingredient composition, proximate analysis and calculated values of gross energy (GE) and digestible energy (DE) in common ingredient mixture (CIM) (Exp. B-1)

CIM	g kg^{-1}	CP	EE	CF	NFE	Ash	AIA
Fish meal	50	3.53	0.15	0.00	0.02	1.17	0.58
Shrimp meal	50	3.37	0.16	0.00	0.26	0.83	0.22
Clam meal	50	2.99	0.65	0.00	0.76	0.32	0.10
GNOC	50	2.19	0.41	0.27	1.51	0.35	0.12
Oil[1]	90		9.00				
Albumin	710	57.16			4.24	4.57	
Calculated	1000	69.23	10.38	0.27	6.78	7.24	1.01
Analysed		68.25	10.52	0.32	7.02	7.52	1.10
GE kcal 100 g^{-1}		380.78	94.42		28.78		503.99
DE kcal 100 g^{-1}		290.06	84.16		14.04		388.26

[1]As in experiment A (Table 3).

GE and DE calculated according to ADCP (1983) as shown in Table 4.

Table 10. Ingredient composition of the experimental feeds (g kg^{-1}) (Exp. B-1)

Ingredients	Feed 1	Feed 2	Feed 3	Feed 4	Feed 5	Feed 6	Feed 7	Feed 8
CIM	350	350	350	350	350	350	350	350
Cellulose	300	250	190	130	70	10	0	0
Tapioca flour	300	350	410	470	530	590	570	540
Oil[1]	0	0	0	0	0	0	30	60
Lecithin	5	5	5	5	5	5	5	5
Cholesterol	5	5	5	5	5	5	5	5
Mineral mixture[2]	20	20	20	20	20	20	20	20
Vitamin mixture[3]	20	20	20	20	20	20	20	20

[1]As in experiment A (Table 3)

[2] U.S.P. XIV (1950) Salt mixture M/s Sisco Research Laboratories, Mumbai. As required in the various biological test diets listed U.S.P. XIV p.789. % Composition: Calcium carbonate 6.86000, Calcium citrate 30.83000, Calcium phosphate monobasic 11.28000, Magnesium sulphate.$7H_2O$ 3.83000, Manganese carbonate 3.52000, Potassium chloride 12.47000, Dipotassium phosphate 21.88000, Sodium chloride 7.71000, Copper sulphate.$5H_2O$ 0.00777, Ferric citrate (16-17% Fe) 1.52815, Manganese sulphate.H_2O 0.02008,Potassium aluminium sulphate 0.00923, Potassium Iodide 0.00405, Sodium flouride 0.05070.

[3]According to recommended levels of vitamins for shrimp by Conklin (1997)
Vitamin premix to supply mg or IU kg^{-1} diet. Thiamin 60 mg, Riboflavin 25 mg, Niacin 40 mg, Pyridoxine 50 mg, Pantothenic acid 75 mg, Biotin 1 mg, Folic acid 10 mg, Cyanocobalamin 0.2 mg, choline 600 mg, Myo-inositol 400 mg, Ascorbic acid polyphosphate 200 mg, Retinol 5000 IU, Vitamin E 100 mg, Vitamin D_3 0.1 mg and Vitamin K 5 mg.

Table 11. Nutrient composition of the experimental diets (% on dry matter basis) and their energy contents and ratios of non-protein energy yielding nutrients (Exp. B-1)

Proximate principles	Diet Nos.							
	1	2	3	4	5	6	7	8
DM	90.06	89.73	89.34	88.94	88.54	88.14	88.46	88.85
CP	24.93	25.04	25.17	25.30	25.43	25.56	25.49	25.41
EE	4.85	4.85	4.85	4.85	4.86	4.86	7.85	10.84
NFE	26.54	30.55	35.36	40.18	44.99	49.81	48.21	45.80
Ash	5.33	5.42	5.52	5.62	5.72	5.83	5.78	5.72
AIA	0.42	0.42	0.43	0.43	0.44	0.44	0.44	0.44
GE kcal $100g^{-1}$	290.06	307.12	327.58	348.05	368.51	388.98	409.27	426.16
DE kcal $100g^{-1}$	197.84	206.33	216.52	226.71	236.90	247.09	267.54	286.30
P/E ratio	85.94	81.52	76.83	72.68	69.00	65.70	62.29	59.63
E/P ratio	11.64	12.27	13.02	13.76	14.49	15.22	16.05	16.77
L: C ratio	1:5.5	1:6.3	1:7.28	1:8.28	1:9.27	1:10.26	1:6.1	1:4.2
L: C (% weight)	5:27	5:31	5:35	5:40	5:45	5:50	8:48	11:46
EE+NFE	31.39	35.40	40.22	45.03	49.85	54.67	56.05	56.64

GE and DE calculated according to ADCP (1983) as shown in Table 4.

Table14 . Proximate composition of the natural and purified feed ingredients used for experimental diet compounding (EXPERIMENTS B 2-6)

	DM	CP	EE	CF	NFE	Ash	AIA
Fish meal	95.16	68.50	8.49	0.00	0.61	17.56	2.71
Shrimp meal	92.51	67.45	3.29	0.00	5.27	16.50	4.39
Clam meal	94.37	59.79	13.01	0.00	15.10	6.47	1.94
GNOC	94.55	43.75	8.13	5.49	30.10	7.08	2.36
Tapioca flour	87.18	2.82	0.29	1.79	80.26	2.02	0.10
Cellulose	93.80	0.65	0.28	92.56	0.00	0.31	0.00
Albumin	100.00	94.00	0.00	0.00	1.50	4.50	0.00

Table 15. Ingredient composition, proximate analysis and calculated values of gross energy (GE) and digestible energy (DE) in common ingredient mixture (CIM). (EXPERIMENTS B 2-6)

Ingredients	g kg^{-1}	CP	EE	CF	NFE	Ash	AIA
Fish meal	50	3.43	0.42	0.00	0.03	0.88	0.14
Shrimp meal	50	3.37	0.16	0.00	0.26	0.83	0.22
Clam meal	50	2.99	0.65	0.00	0.76	0.32	0.10
GNOC	50	2.19	0.41	0.27	1.51	0.35	0.12
Oil[1]	90	0.00	9.00	0.00	0.00	0.00	0.00
Albumin	710	66.74	0.00	0.00	1.07	3.20	0.00
Calculated	1000	78.71	10.65	0.27	3.62	5.58	0.57
Analysed		73.02	11.21	0.44	2.59	6.65	0.54
GE kcal $100g^{-1}$*		401.61	102.01		10.62		514.24
DE kcal $100g^{-1}$**		310.34	89.68		5.18		405.20

[1] As in experiment A (Table 3)
GE and DE calculated according to ADCP (1983) as shown in Table 4.

Table 16. Ingredient composition of the experimental feeds (g kg^{-1}) (Exp. B-2)

Ingredients	Feed 1	Feed 2	Feed 3	Feed 4	Feed 5	Feed 6	Feed 7	Feed 8
CIM	400	400	400	400	400	400	390	390
Tapioca flour	210	270	330	390	450	500	550	530
Cellulose	340	280	220	160	100	50	0	10
Oil[1]	0	0	0	0	0	0	10	20
Lecithin	5	5	5	5	5	5	5	5
Cholesterol	5	5	5	5	5	5	5	5
Mineral mixture[2]	20	20	20	20	20	20	20	20
Vitamin mixture[3]	20	20	20	20	20	20	20	20

[1]As in Experiment A (Table3)
[2]As in Exp. B 1 Table 10
[3]As in Exp. B 1 Table 10

Table 17. Proximate chemical composition of the experimental diets (% on dry matter basis) and their energy contents and ratios of non-protein energy yielding nutrients (Exp. B-2)

Nutrients and energy	Diet Nos.							
	1	2	3	4	5	6	7	8
DM	89.12	90.61	90.22	89.83	89.44	89.11	88.84	91.03
CP	30.01	30.15	30.28	30.41	30.54	30.65	30.03	29.98
EE	5.63	5.64	5.64	5.64	5.64	5.64	6.53	9.53
NFE	17.89	22.71	27.52	32.34	37.15	41.17	45.15	43.55
Ash	5.18	5.29	5.39	5.50	5.60	5.69	5.70	5.67
AIA	0.24	0.24	0.25	0.26	0.26	0.27	0.27	0.26
GE kcal $100g^{-1}$	289.67	310.26	330.72	351.19	371.66	388.71	409.72	430.14
DE kcal $100g^{-1}$	208.39	218.68	228.87	239.06	249.25	257.74	270.18	290.73
P/E ratio	103.59	97.18	91.56	86.60	82.18	78.85	73.29	69.70
E/P ratio	9.65	10.29	10.92	11.55	12.17	12.68	13.64	14.35
L:C ratio	1:3.1	1:4.0	1:4.9	1:5.7	1:6.6	1:7.3	1:6.9	1:4.6
L:C (% weight)	6:18	6:23	6:28	6:32	6:37	6:41	7:45	10:44
EE+NFE	23.53	28.35	33.16	37.98	42.80	46.81	51.68	53.08

GE and DE calculated according to ADCP (1983) as shown in Table 4.

Table 20. Ingredient composition of the experimental feeds (g kg^{-1}) (Exp. B-3)

Ingredients	Feed 1	Feed 2	Feed 3	Feed 4	Feed 5	Feed 6	Feed 7	Feed 8
CIM	480	480	480	480	480	470	470	470
Tapioca flour	90	150	210	270	330	400	450	440
Cellulose	360	320	260	200	140	80	30	20
CMC	20	0	0	0	0	0	0	0
Oil[1]	0	0	0	0	0	0	0	20
Lecithin	5	5	5	5	5	5	5	5
Cholesterol	5	5	5	5	5	5	5	5
Mineral mixture[2]	20	20	20	20	20	20	20	20
Vitamin mixture[3]	20	20	20	20	20	20	20	20

[1]As in Experiment A (Table3)
[2]As in Exp. B 1 Table 10
[3]As in Exp. B 1 Table 10

Table 21. Proximate chemical composition of the experimental diets (% on dry matter basis) and their energy contents and ratios of non-protein energy yielding nutrients (Exp. B-3)

Nutrients and energy	Diet Nos.							
	1	2	3	4	5	6	7	8
DM	89.96	91.44	91.05	90.66	90.27	89.81	89.48	87.79
CP	35.54	35.68	35.81	35.94	36.07	35.50	35.61	35.56
EE	6.51	6.51	6.51	6.52	6.52	6.41	6.41	8.40
NFE	8.47	13.28	18.10	22.91	27.73	33.32	37.33	36.53
Ash	5.49	5.59	5.70	5.80	5.90	5.96	6.04	6.01
AIA	0.27	0.27	0.28	0.29	0.29	0.29	0.30	0.30
GE kcal $100g^{-1}$	289.39	309.98	330.44	350.91	371.37	390.17	407.22	421.77
DE kcal $100g^{-1}$	220.03	230.32	240.51	250.70	260.89	268.77	277.26	291.36
P/E ratio	122.80	115.11	108.37	102.42	97.13	90.98	87.44	84.31
E/P ratio	8.14	8.69	9.23	9.76	10.30	10.99	11.44	11.86
L:C ratio	1:1.30	1:2.04	1:2.78	1:3.52	1:4.26	1:5.20	1:5.83	1:4.35
L:C (% weight)	7:8	7:13	7:18	7:23	7:27	6:33	6:37	8:37
EE+NFE	14.97	19.80	24.61	29.43	34.24	39.73	43.74	44.93

GE and DE calculated according to ADCP (1983) as shown in Table 4.

Table 24. Ingredient composition of the experimental feeds (g kg^{-1}) (Exp. B-4)

Ingredients	Feed 1	Feed 2	Feed 3	Feed 4	Feed 5	Feed 6	Feed 7	Feed 8
CIM	540	540	540	540	540	540	540	540
Tapioca flour	20	60	120	180	240	300	360	380
Cellulose	370	330	290	230	170	110	50	30
Oil[1]	0	0	0	0	0	0	0	0
CMC	20	20	0	0	0	0	0	0
Lecithin	5	5	5	5	5	5	5	5
Cholesterol	5	5	5	5	5	5	5	5
Mineral mixture[2]	20	20	20	20	20	20	20	20
Vitamin mixture[3]	20	20	20	20	20	20	20	20

[1]As in Experiment A (Table 3)
[2]As in Exp. B 1 Table 10
[3]As in Exp. B 1 Table 10

Table 25. Proximate chemical composition of the experimental diets (% on dry matter basis) and their energy contents and ratios of non-protein energy yielding nutrients (Exp. B-4)

Nutrients and energy	Diet Nos							
	1	2	3	4	5	6	7	8
DM	92.33	92.07	91.68	91.29	90.90	90.51	90.11	89.98
CP	39.74	39.83	39.96	40.09	40.22	40.35	40.48	40.52
EE	7.17	7.17	7.17	7.17	7.17	7.17	7.17	7.17
NFE	3.00	6.21	11.03	15.85	20.66	25.48	30.29	31.90
Ash	5.75	5.82	5.92	6.03	6.13	6.23	6.33	6.37
AIA	0.29	0.30	0.30	0.31	0.32	0.32	0.33	0.33
GE kcal $100g^{-1}$	296.12	309.77	330.23	350.70	371.16	391.63	412.09	418.91
DE kcal $100g^{-1}$	232.25	239.05	249.24	259.42	269.61	279.80	289.99	293.39
P/E ratio	134.20	128.57	121.00	114.31	108.36	103.03	98.23	96.73
E/P ratio	7.45	7.78	8.26	8.75	9.23	9.71	10.18	10.34
L:C ratio	1:0.42	1:0.87	1:1.54	1:2.21	1:2.88	1:3.55	1:4.22	1:4.45
L:C (% weight)	7:3	7:6	7:11	7:16	7:21	7:25	7:30	7:32
EE+NFE	10.17	13.38	18.20	23.02	27.83	32.65	37.46	39.07

GE and DE calculated according to ADCP (1983) as shown in Table 4.

Table 28. Ingredient composition of the experimental feeds (g kg^{-1}) (Exp.B-5)

Ingredients	Feed 1	Feed 2	Feed 3	Feed 4	Feed 5	Feed 6	Feed 7	Feed 8
CIM	610	610	610	610	610	610	610	610
Tapioca flour	0	50	80	110	140	170	200	280
Cellulose	320	270	240	230	200	170	140	60
Oil[1]	0	0	0	0	0	0	0	0
CMC	20	20	20	0	0	0	0	0
Lecithin	5	5	5	5	5	5	5	5
Cholesterol	5	5	5	5	5	5	5	5
Mineral mixture[2]	20	20	20	20	20	20	20	20
Vitamin mixture[3]	20	20	20	20	20	20	20	20

[1]As in Experiment A (Table 3)
[2]As in Exp. B 1 Table 10
[3]As in Exp. B 1 Table 10

Table 29. Proximate chemical composition of the experimental diets (% on dry matter basis) and their energy contents and ratios of non-protein energy yielding nutrients (Exp. B-5)

	Diet Nos							
Nutrients and energy	1	2	3	4	5	6	7	8
DM	92.51	92.18	91.98	91.79	91.59	91.40	91.20	90.68
CP	44.76	44.87	44.94	45.00	45.07	45.13	45.20	45.37
EE	7.93	7.93	7.93	7.93	7.93	7.94	7.94	7.94
NFE	1.58	5.59	8.00	10.41	12.82	15.22	17.63	24.05
Ash	6.16	6.25	6.30	6.35	6.40	6.45	6.50	6.64
AIA	0.33	0.33	0.34	0.34	0.34	0.35	0.35	0.36
GE kcal $100g^{-1}$	324.87	341.92	352.16	362.39	372.62	382.85	393.09	420.37
DE kcal $100g^{-1}$	256.87	265.36	270.46	275.55	280.64	285.74	290.83	304.42
P/E ratio	137.79	131.23	127.60	124.18	120.95	117.88	114.98	107.93
E/P ratio	7.26	7.62	7.84	8.05	8.27	8.48	8.70	9.27
L:C ratio	1:0.20	1:0.70	1:1.00	1:1.31	1:1.62	1:1.92	1:2.22	1:3.03
L:C (% weight)	8:2	8:6	8:8	8:10	8:13	8:15	8:18	8:24
EE+NFE	9.51	13.53	15.93	18.34	20.75	23.16	25.57	31.99

GE and DE calculated according to ADCP (1983) as shown in Table 4.

Table 32. Ingredient composition and proximate composition of the experimental feeds (g kg^{-1}) (Exp. B-6)

Ingredients	Feed 1	Feed 2	Feed 3	Feed 4	Feed 5	Feed 6	Feed 7	Feed 8
CIM	680	680	680	680	680	680	680	680
Tapioca flour	0	50	80	110	140	170	200	270
Cellulose	250	200	170	160	130	100	70	0
Oil[1]	0	0	0	0	0	0	0	0
CMC	20	20	20	0	0	0	0	0
Lecithin	5	5	5	5	5	5	5	5
Cholesterol	5	5	5	5	5	5	5	5
Mineral mixture[2]	20	20	20	20	20	20	20	20
Vitamin mixture[3]	20	20	20	20	20	20	20	20

[1]As in Experiment A (Table 3)
[2]As in Exp. B 1 Table 10
[3]As in Exp. B 1 Table 10

Table 33. Proximate chemical composition of the experimental diets (% on dry matter basis) and their energy contents and ratios of non-protein energy yielding nutrients (Exp. B- 6)

Nutrients and energy	Diet Nos. 1	2	3	4	5	6	7	8
DM	90.68	92.23	92.03	91.83	91.64	91.44	91.25	90.79
CP	49.82	49.94	50.00	50.07	50.13	50.20	50.26	50.42
EE	8.69	8.70	8.70	8.70	8.70	8.70	8.70	8.70
NFE	1.76	5.77	8.18	10.59	13.00	15.41	17.81	23.43
Ash	6.60	6.69	6.74	6.79	6.85	6.90	6.95	7.07
AIA	0.37	0.37	0.38	0.38	0.38	0.38	0.39	0.39
GE kcal $100g^{-1}$	360.31	377.49	387.72	397.96	408.19	418.42	428.65	452.53
DE kcal $100g^{-1}$	284.78	293.37	298.47	303.56	308.66	313.75	318.85	330.74
P/E ratio	138.26	132.29	128.96	125.81	122.82	119.97	117.26	111.41
E/P ratio	7.23	7.56	7.75	7.95	8.14	8.34	8.53	8.98
L:C ratio	1:0.20	1:0.70	1:1.00	1:1.22	1:1.49	1:1.77	1:2.05	1:2.69
L:C (% weight)	9:2	9:6	9:8	9:11	9:13	9:15	9:18	9:23
EE+NFE	10.45	14.47	16.88	19.29	21.70	24.11	26.51	32.13

GE and DE calculated according to ADCP (1983) as shown in Table 4.

Feeding was carried out at the rate of 15% of the body weight in two doses. Pre-weighed petri dishes containing 40% of the feed was provided at 10:00 h and 60% was provided at 16:00 h. Feed residue and faecal matter was removed daily prior to water-exchange. Feeding rates were adjusted based on daily observations to compensate mortality if any, and reduce feed residues to minimum. Daily record of mortality was also maintained. On termination of the experiment shrimps were weighed and dried and pooled treatment wise for chemical analyses.

Growth was measured as biomass gain shrimp^{-1} (g), absolute growth rate (AGR), relative growth rate (RGR) and specific growth rate (SGR). Protein efficiency ratio (PER), food conversion ratio (FCR), food conversion efficiency (FCE) and survival % were also estimated.

III.7 Chemical analyses of diets, water and shrimp - Experiments (B 1-6)

Feed ingredients, CIM and all experimental feeds were analysed for their proximate chemical compositions according to A.O.A.C. (1990). GE and DE were calculated using the conversion factor according to ADCP (1983). Seawater was analysed according to the standard methods of Strickland and Parsons (1972). Shrimps dried and pooled treatment wise were analysed for moisture, CP and EE and ash.

III.4 Statistics - Experiments (B 1-6)

Comparison of means and analysis of variance (ANOVA) of the data was done according to Snedecor and Cochran (1973) using SPSS software. To estimate the optimum levels of protein and GE, second-degree polynomials of the form $y = a + bx + cx^2$ were fitted.

RESULTS