STUDIES ON THE EVALUATION OF DIFFERENT SOURCES OF PROTEINS, CARBOHYDRATES AND MINERAL REQUIREMENTS FOR JUVENILE PENAEID PRAWN *PENAEUS INDICUS* H. MILNE EDWARDS

THESIS SUBMITTED
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BY

SYED AHAMAD ALI, M.Sc.

CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
COCHIN - 682 031
DECEMBER, 1988

CERTIFICATE

This is to certify that the thesis entitled 'Studies on the evaluation of different sources of proteins, carbohydrates and mineral requirements for juvenile Penaeid Prawn *Penaeus indicus* H.Milne Edwards' is the bonafide record of the work carried out by Sri.SYED AHAMAD ALI under my guidance and supervision and that no part thereof has been presented for any other degree.

P.VEDAVYASA RAO
Scientist - S3
Central Marine Fisheries
Research Institute
Dr.Salim Ali Road
Cochin - 682 031.

Cochin

December, 1988

DECLARATION

I hereby declare that this thesis entitled 'Studies on the evaluation of different sources of proteins, carbohydrates and mineral requirements for juvenile Penaeid Prawn *Penaeus indicus* H. Milne Edwards', has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.

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(SYED AHAMAD ALI)

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PREFACE

Prawns and shrimps occupy an important place in the marine Fisheries of India. The present prawn production of the country, contributed by penaeid and non-penaeid prawns, is of the order of about 0.2 million tonnes annually. The penaeid prawns, forming about 62% of the total marine prawn catch, greatly influence not only the prawn production of the country, but also the sustained growth and development of the marine products export trade. The intense exploitation of the penaeid prawn resources over the time and space has resulted in near stagnation or declining trend in their production in recent years. This situation has lead to an urgent need to develop the prawn culture in the coastal waters to augment the production and to an awareness to change over the prevailing traditional prawn culture practice to the more beneficial system of culture entailing selected fast growing species, supplementary feeding and effective water management. With the advent of hatchery technology for production of penaeid prawn seed and other technological advancements, the prawn culture fisheries is now witnessing a rapid growth in several regions including India.

Feed is one of the major inputs in the hatchery production of prawn seed and their subsequent culture in the grow-out ponds to marketable size. Among the different types of feed, the development of nutritionally balanced compounded formula feed has gained considerable attention due to its distinct advantage of preparation and mass production using low-cost ingredients and its use off-the-shelf wherever and whenever required. In fact, this aspect has been given top priority

in the aquaculture programmes.

A comprehensive knowledge of the nutritional requirements and related aspects of the candidate species selected for culture and of the characteristics of the food sources used in the formulation and preparation of the compounded diet, is an essential prerequisite for evolving balanced feeds. Over the past 20 years, there has been considerable progress in the study of dietary nutrient requirements of fishes and shellfishes including prawns. Several compounded feeds using a variety of conventional and non-conventional ingredients and having different levels of protein, lipid, carbohydrate, vitamins and minerals have been developed and some of them are being used in the semi-intensive and intensive culture of prawns abroad. As the efficacy of the compounded feed, among other factors, depends greatly on the judicious manipulation of the selected ingredients and since the cost of feed plays a significant role in the economics of the overall prawn culture operation, the search for more suitable and economical food sources and their evaluation *vis-a-vis* the nutrient profile, nutritional and growth requirements of the cultured species is still continuing vigorously.

In India, directed research on penaeid prawn nutrition was taken up only recently when the aquaculture of prawns gained momentum. One of the important penaeid prawns sought for culture and has great potential is *Penaeus indicus*, H.Milne Edwards. The Central Marine Fisheries Research Institute working on different aspects of culture of this species over the past one and half decades, has developed a hatchery technology

for mass production of its seed and has suggested several improvements on its farming in the grow-out systems. One of the areas of active research in this direction has been on the nutrition of the species with a view to develop suitable feed not only for hatchery production of seed, but also in the field culture. As part of this investigation, the present study, on the evaluation of different protein and carbohydrate sources and mineral requirements for the juvenile P. indicus was taken up and the results obtained are embodied in the thesis.

The thesis is parted in four chapters. In the first chapter, the evaluation of four purified proteins, albumen (egg), casein, fibrin (blood) and gelatin and nine natural protein sources - five animal materials (clam meat, fish meal, mantis shrimp, prawn waste and silkworm pupa), four plant materials (coconut cake, gingelly cake, groundnut cake, single cell protein Spirulina) for the juveniles of P. indicus, is presented. These evaluations are carried out employing the standard methods of nutritional biochemistry by determining the digestibility, biological value (BV), net protein utilization (NPU), protein efficiency ratio (PER) and growth.

In the second chapter, seven different sources of carbohydrates - three monosaccharides (fructose, galactose, glucose), two disaccharides (maltose, sucrose) and two polysaccharides (glycogen, starch) were evaluated in the diet of P. indicus. The effect of carbohydrate level in the diet on digestibility, growth, food conversion ratio and survival were

investigated and discussed. The role of cellulose in the diet of prawn was elucidated.

The third chapter contains the results of the studies on the requirement of six minerals (calcium, phosphorous, copper, zinc, magnesium and manganese) in the diet of P. indicus. The requirement of each of the minerals was determined by not only measuring the growth, food conversion ratio and survival but also by investigating the relationship between the dietary levels and body levels of each mineral.

Based on the information obtained in the present study, a purified diet and a practical feed were formulated, prepared and fed to P. indicus in long term feeding experiments in the laboratory, and the results were compared with those of a conventional prawn feed. The prospects of using the purified diet as a basal diet for nutritional studies on prawns in this region and the practical feed for the culture of penaeid prawns are discussed in the fourth chapter.

Nutritional research on the prawn P. indicus was initiated by the author by studying the relative efficiencies of some proteins and the effect of protein (Ahamad Ali, 1982a), carbohydrate (Ahamad Ali, 1982b) levels in the diet on growth, food conversion ratio (FCR) and survival. Subsequently, different sources of lipids were evaluated and the role of vitamin and mineral mixtures in the feed of the same prawn were studied. The relative efficiencies of different binding materials in preparing water stable feed pellets were investigated (Ahamad Ali, 1986). Using the experience gained in the field of nutirtion,

feed formulation and feed preparation techniques, the author evolved certain compounded formula feeds with locally available feed ingredients for feeding the larvae (Mohamed *et al.*, 1983), Post larvae (Ahamad Ali and Sivadas, 1983) and juveniles (Ahamad Ali and Mohamed, 1985) of *P. indicus*. However, gaps still existed in the knowledge, especially on the carbohydrate, minerals and protein nutrition of this prawn and these aspects have been taken up for investigation in the present study.

As envisaged, the results obtained in the present study have provided valuable information on the missing links in the protein, carbohydrate and mineral nutrition of penaeid prawns in general and of *P. indicus* in particular. The data are immensely useful in the selection of a better protein source for formulating suitable and economical compounded feeds for use in feeding the prawns on large scale culture. The investigations on carbohydrate nutrition have great practical utility in formulating high efficiency - low cost practical feeds. The information obtained on mineral requirements would go a long way in preparing nutritionally more balanced feeds, thus contributing to the establishment and promotion of an organised prawn culture industry in the country.

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(SYED AHAMAD ALI)

INTRODUCTION

The first documented studies on fish nutrition date back to early Nineteen thirties. It was however, only after the second World War, the field attracted greater attention as an area of separate investigation or as part of the overall studies on the biology of fishes. In the earlier years, the methods commonly employed in animal husbandry studies were also applied to study the food and feeding habits of fishes. These methods though posed considerable difficulties in providing a comprehensive information on different aspects of nutrition in fishes that are poikilotherms and inhabit the dynamic aquatic environment unlike the homeothermic land animals, enabled to gather valuable data on the food and feeding habits, particularly of the commercially exploited fishes. In the capture fisheries, the implications of these studies thus helped to explain the distribution pattern, growth and fluctuations of the exploited fish resources.

With the advancement and standardisation of methods in nutritional and physiological investigations, introduction of biochemical analysis and exposition of the relation between the environment and the fish and the predator-prey relationship, a wealth of information on qualitative and quantitative aspects of food consumed by the fishes, the digestion process, energy utilisation at various trophic levels and nutrient requirements, was accumulated between 1950 and 1970, and formed the subject of excellent reviews by Winberg (1956), Cowey and Sargent (1972), Halver (1972) and others. Robertson (1945), Hynes (1950),

and later, Nose (1963, 1967a), Ogino and Chen (1973a,b), Ogino, Kakino and Chen (1973) and Schneider and Flatt (1975) perfected, standardised and developed new methodologies/techniques in fish nutrition studies.

Fish and shellfish nutrition received tremendous impetus with the active development of aquaculture all over the world during the past one and half decades. As a result, enormous literature is now available not only on the dietary requirements of fishes and shellfishes, digestion and bioenergetics, but also on the larval nutrition, feed formulation and feed technology. Synthesising these works, several reviews are also now available, the most important among these being the 'Bioenergetics and growth' in the series of 'Fish Physiology' edited by Hoar Randall (1978); National Research Council (1977, 1981, 1983); Castell et al. (1981); Millikin (1982) and Tytler and Calow (1985). Realising the importance of a comprehensive knowledge of fish nutrition, particularly in the context of development of aquaculture under controlled conditions, a series of Symposia, Workshops and Task Force have been organised during this period. The noteworthy of these are the EIFC - Symposium on Finfish Nutrition and Feed Technology held in Hamberg in 1978 (Halver and Tiews, 1979), World Mariculture Society Nutrition Task Force established in 1970 to 'co-ordinate the Society's role in nutritional Science' (Conklin and Beck, 1979) and the Asian Fish Nutrition Workshop held in Singapore in 1983 (Cho, et al., 1985). The publication and the Proceedings of these Symposia/ Workshops reviewed the different aspects of fish and shellfish nutrition, its status, constraints encountered, methodological approach and the strategies for future development.

Among shellfishes, crustaceans that include the familiar forms such as shrimps, prawns, lobsters and crabs, occupy an important place both in the capture and culture fisheries of many nations in the world. Inhabiting diversified ecosystems, crustaceans feed on a variety of material which vary from microorganisms in microcrustaceans, to detritus and a range of animal and plant matter in larger crustaceans. Armoured with a diversity of external appendages and mouth parts, but with a rather simple alimentary system, they are equipped to ingest, digest and assimilate protein, lipids, carbohydrates and other nutrients required for their growth, survival and reproduction. Since, crustaceans excrete nitrogenous waste products in the form of ammonia, they are known as 'ammonotelic' animals.

A perusal of literature on crustacean nutrition reveals that the bulk of the information on food and feeding habits, nutritional requirements and digestive physiology is derived from the larger crustaceans belonging to Decapoda, although appreciable data are also available on the lower crustacean groups such as amphipods, isopods and cirripedes. Marshall and Orr (1960), Vonk (1960) and Fisher (1960) reviewed the available works upto 1960 on feeding and nutrition, digestion and metabolism and vitamins respectively in the 'Physiology of Crustacea' volumes edited by Waterman (1960). Over the last 20 years which paralleled the growth and development of aquaculture of decopod crustaceans, the nutritional science of Crustacea has grown considerably and reviewed by New (1976, 1980); Zein-Eldin and Meyers (1973); Kinne (1977); Conklin (1980); Dall and Moriarty (1983) and by Grahame (1983).

Among decapods, prawns are of vital economic importance, being intensively exploited from the wild by over 20 countries including India and widely cultivated in tropical and subtropical regions. The warm-water penaeid prawns (Order, Decapoda, Sub-order, Dendrobranchiata; Superfamily, Penaeiodea; Family, Penaeidae) constitute the most commercially important group. Most of the penaeid prawns breed in the sea and the eggs hatch out as free swimming planktonic naupliar larvae. Passing through different larval stages such as protozoea and mysis, the postlarvae enter into shallow inshore waters or estuaries wherever available, and develop further into juvenile stage. The juveniles or sub-adults after certain period of growth in these ecosystems, migrate offshore for further growth and spawning. The food, feeding mechanism and behaviour of penaeid prawns are found to vary with the different life stages. Thus the first larval stage, namely the nauplius, does not feed and lives by utilising the internal yolk. The protozoea larvae feed mainly on the available phytoplankton of approximately 3 to 10 micron size. In the mysis stage, the particulate food of about ten times the size of the food of protozoea and in the postlarval stage, still larger size particulate food available in the water table are ingested. As the prawn grows, it gradually changes to different modes of feeding described as omnivorous, scavanger, detritus or carnivorous feeders, depending on the species by different workers. The structure of the mouth parts and feeding appendages play a significant role in the food selection, collection and feeding behaviour.

As in the case of fishes, most of the earlier works on food and feeding habits of penaeid prawns were based on the gut

content analysis which provided information on the qualitative and quantitative aspects of the food constituents of the species studied (Gopalakrishnan,1952; Williams,1955, 1958; Ikematsu, 1955; George,1959, 1974; Hall,1962; Dall,1967; Thomas,1972,1980; Kuttyamma,1973; and Wickins,1976). These studies by and large, related to juvenile and adult prawns feeding in the wild on a community level and found much application in their capture fisheries rather than in the culture. Similarly, the knowledge on larval nutrition at that time had been grossly inadequate. The successful rearing of Penaeus japonicus by Hudinaga (Fujinaga) in 1942, the technological advancements made in the physiological and biochemical studies and the realisation of great growth potential of aquaculture of prawns,stimulated intensive interest in penaeid prawn nutrition and a good deal of work cameforth during the past 20 years from several laboratories in the world. Compiling these information, New in 1976 presented an excellent review of the literature available on dietary studies with prawns and shrimps. This was followed by another comprehensive review by Kinne (1977). Biddle (1977) described the various aspects of nutrition in freshwater prawns. Besides, the books published by Shigueno (1975 and 1978), Chen (1976) Imai (1977), Hanson and Goodwin (1977) and Stickney (1979) treated some aspects of nutrition of the candidate species dealt with by them. Subsequently, New (1980) compiled a bibliography of prawn and shrimp nutrition and Pruder et al. (1983) compiled studies on penaeid nutrition. More recently, Kanazawa (1984) presented the recent advances made in penaeid prawn nutrition at the First International Conference on the .

Culture of Penaeid prawns/shrimps held at Iloilocity, Philippines.

One of the areas which received considerable attention, ever since the report of Hudinaga (1942) on rearing of P. japonicus on the diatom, Skeletonema costatum, has been the search for suitable live food organisms to rear the larval stages. The works carried out by Fujinaga and Miyamura (1962), Cook and Murphy (1969), Liao and Huang (1972), Thomas et al. (1976a , 1976b), AQUACOP (1978), Platon (1978), Beard et al. (1977), New (1979), Kurata and Shigueno (1979) and Muthu (1982) identified several species of diatoms and other live food organisms which could be advantageously employed for feeding penaeid larvae and postlarvae. Parallel to these studies, attempts were also made on large scale culture of microalgae and zooplankton by several investigations (Ukeles, 1976; Shaw Watson, 1979; Kinne, 1977; Kahan, 1982; DePauw and Pruder 1981; Sorgeloos, 1981 ; Nellen, 1981) to meet the requirements of hatchery production of larvae.

While the endeavours in the selection and mass production of live food organisms have been progressing in one front, attempts have been made in the other front to replace the live food organisms, as their large scale production posed constraints due to wide fluctuation in the yield, contamination by unwanted species and considerable cost of production, with artificial diet (Subrahmanyam and Oppenheimer, 1969; Kanazawa et al.,1970; Forster and Gabbott, 1971; Cowey and Forster, 1971; Hirata et al., 1975; Shigueno, 1975; Sick et al., 1972; Kitabhayashi et al., 1971 a,b,c,d; AQUACOP, 1978; Villegas and Kanazawa,1980; Villegas et al.,1980; Alikunhi et al., 1980, 1982; Usha Goswami and

Goswami, 1979, 1982; Raman et al.,1982; Mohammed Sultan et al., 1982; Ahamad Ali,1982a ; Mohamed et al., 1983, Ahamad Ali and Mohamed, 1985). The results of these investigations have shown the feasibility of using different types of artificial diets to rear the larvae in the hatchery, postlarvae in the nursery and juveniles in the grow out systems with varying survival and growth performance depending on the qualities of diets,experimental design and water quality management.

One of the noteworthy advancements of nutritional research in the mid-seventies, has been the development and use of microencapsulated diet for the filter feeding crustacean larvae (Jones et al., 1974). Jones et al.(1976) and Moller et al. (1979)reared the larvae of P.merquiensis upto postlarvae II with micro-encapsulated diet. Subsequently, Jones et al. (1979a) employed the encapsulated diet consisting of chicken egg, short necked clam (Tapes philippinarum), Soybean cake and the purified diet-B of Kanazawa et al.(1977a), having particulate size ranging from 10 to 100 micron, to rear P.japonicus with encouraging results. Successful rearing of Penaeid Prawn larvae with micro-particulate and micro-encapsulated diets was demonstrated in P. japonicus (Kanazawa,1985), P.monodon, P.stylirostris and P.vannamei (Jones et al.,1987)and more recently by Galagani and AQUACOP (1988) in zoeal stages of some penaeid prawns.

The development of micro-encapsulated diets also helped to study the nutritional requirement of larvae. Thus, Jones et al. (1979b) studied the fatty acid requirement of the larvae of P. japonicus; Kanazawa (1982, 1983), Teshima and Kanazawa (1984) and Kanazawa et al. (1985) on protein, lipid, carbohydrate, phospholipid and vitamin requirements.

Studies on nutritional requirements of prawns and shrimps received considerable impetus in the recent years. Greater emphasis was given for understanding the protein requirement and determining optimum protein levels in the diet for different species (Kanazawa et al., 1970; Lee, 1970; Kitabhayashi et al., 1971 a, b, c; Deshimaru and Shigueno, 1972; Andrews et al., 1972; Balazs et al., 1973; Forster and Beard, 1973; Deshimaru and Kuroki, 1975a; Venkataramaiah, et al.,1975a; Colvin, 1976a; AQUACOP, 1977; Khannapa, 1979; Bages and Sloane, 1981; Kanazawa et al., 1981; Ahamad Ali, 1982 a; Charles John Bhasker and Ahamad Ali, 1984). In these investigations, a protein requirement ranging from 15% to 80% was reported for different species of penaeid prawns. The variations in the protein requirement among the different species were thought to be due to different factors such as the aminoacid profile of the protein source used, the carbohydrate level in the diet and factors such as differences in feeding habits and age of experimental animals. The amino acid requirements of penaeid prawns (Cowey and Forster, 1971; Kanazawa and Teshima, 1981) and also the Caridean prawns(Watanabe, 1975; Miyajima et al., 1976), were investigated and found that the same number of amino acids which were found to be essential for land animals were also found to be essential for these prawns.

Prawns have specific qualitative requirement of lipids rather than their quantity. Eventhough a lipid level of below 10% was found to be adequate in the prawn diet (Andrews et al., 1972; Forster and Beard, 1973), the fatty acid composition of the lipid source used is found to be more important for growth and survival. Employing radioisotope tracer technique, Kanazawa

et al. (1979b) and Kanazawa and Teshima (1977) have shown that prawns are not capable of synthesising polyunsaturated fatty acids (PUFA) such as linoleic acid (18:2 W6), linolenic acid (18: 3 W 3), eicosapentaenoic acid (20: 5 W 3) and docosahexaenoic acid(22: 6 W 3). These fatty acids are essential for prawns and should be supplied in their diet. Infact Kanazawa et al.(1977b, 1978, 1979d, 1979f) have demonstrated that the diets containing the fatty acids 18: 2 W 6, 18: 3 W 3, 20: 5 W 3 and 22: 6 W 3 produced faster growth in P. japonicus. Similar results have been obtained by Shewbart and Mies (1973) in P. aztecus. The optimum levels of the fatty acids, 20: 5 W 3 and 22: 6 W 3 were found to be 1.0% in the diet of P. japonicus (Kanazawa et al., 1979a).

Prawns are also found to require cholesterol at 0.5% in the diet (Teshima and Kanazawa, 1971; Teshima, 1982; Kanazawa et al., 1971a; Shudo et al., 1971). Further, Kanazawa et al. (1971b) demonstrated that P. japonicus could utilize ergosterol, sitosterol and stigmasterol to some extent as substitute for cholesterol. Based on the dietary value of different steroids, Teshima et al,(1982) suggested the metabolic pathway for the conversion of C 28 and C 29 sterols to cholesterol in prawns. Subsequently Teshima et al. (1983, 1986a,b,c,d) investigated the role of phospholipids in the diet of prawn.

The nutritive value of carbohydrates in the diet of prawns was investigated (Cowey and Forster, 1971; Forster and Gabbott, 1971; Sick and Andrews, 1973; Deshimaru and Yone,1978b; Abdel Rahman et al., 1979; Ahamad Ali, 1982b; Pascual et al., 1983; Alava and Pascual, 1987) and found that penaeid prawns

generally utilize disaccharides and polysaccharides better than monosaccharides. A carbohydrate level of 5 to 40% has been suggested in the diets of penaeid prawns. The role of amino sugar, N-acetylglucosamine in the diet of prawn has also been investigated, reporting conflicting results on the role of glucosamine in the diet. While Kitabhayashi et al, (1971a) have demonstrated that addition of 0.52% of glucosamine in the diet improved the growth of P. japonicus, Deshimaru and Kuroki (1974b) have pointed out that it is not necessary in the diet of the same prawn. However, Vaitheeswaran and Ahamad Ali (1986) observed positive growth promoting effect of glucosamine in the diet of P. indicus. Addition of cellulose to the diet is found to help better utilisation of nutrients by prawns (Venkataramaiah et al., 1975a; Fair et al., 1980).

Requirement of vitamins and minerals in the diet of prawn was investigated by several workers. Deshimaru and Kuroki (1976, 1979) have shown that the juveniles of P. japonicus require 300-1000 mg of ascorbic acid, 60 mg of choline, 200-400 mg of inositol, 6-12 mg of thiamine and 12 mg of pyridoxine per 100 g diet. Lightner et al. (1977, 1979) found that ascorbic acid deficiency could lead to abnormal symptoms (black death) in P. californiensis and P. stylirostris. Kitabhayashi et al. (1971b) found accelerated growth in P. japonicus fed with the diet having Vitamin C. Reviewing the metabolic functions of vitamins in crustaceans, Fisher (1960) reported that most of the B group vitamins were required in the diets of prawns. Although the vitamin D would be partly ingested, it could also be synthesised by the animals from ergosterol. The role of vitamin K was noted

to be antagonistic in some species of crustaceans. While the vitamin A might not be essential in prawn diets, its precursor β-carotene was required in the diet. The presence of β-carotene, astaxanthin, and canthaxanthin were demonstrated in P. japonicus (Kitayama et al., 1972). The importance of carotenoids in the prawn diets for the pigmentation had been demonstrated by Joseph and Williams (1975) and Sandifer and Joseph (1976).

In the case of minerals, the requirement of calcium and phosphorous in the diet of P. japonicus (Deshimaru and Yone, 1978a; Deshimaru et al., 1978; Kitabhayashi et al., 1971a) and P. aztecus (Hysmith et al., 1972; Shewbart et al., 1973; Huner and Colvin, 1977) was studied and varying results were obtained. It was demonstrated that prawns could absorb calcium from sea water. Recently Kanazawa et al. (1984) reported the requirement of calcium, phosphorous, magnesium, potassium, copper, iron and manganese in the diet of P. japonicus.

In India, the information on prawn nutrition is relatively less as compared to those available on finfish nutrition. Most of the observations on the food and feeding habits of prawns have been made during the course of biological investigation of the species (Rai, 1933; Panikkar, 1952; Gopalakrishnan, 1952; Panikkar and Menon, 1956; George, 1959; 1974; Subrahmanyam 1963; Bhimachar, 1965; Rao, 1967; Kuttyamma, 1973; Thomas, 1972, 1980). Although these studies have shown that the penaeids feed on a variety of plant and animal organisms and other detritus indicating their opportunistic omnivorous feeding behaviour, Rao (1967) discussed the relative importance of food in their diet. Selective feeding by different size groups in P. indicus

and Metapenaeus monoceros (George, 1974) and food species differences related to habitat preference of prawns (Kuttyamma, 1973) have been observed.

A series of investigations were carried out in recent years on the energy conversion, energy metabolism and food conversion in some of the penaeid prawns of India (Qasim and Easterson, 1974; Laxminarayana and Kutty, 1982; Sumitra Vijayaraghavan and Ramdhas, 1982; Thomas et al. 1984). Ravichandra Reddy and Katre Shakuntala (1982) investigated the use of Moina for the juveniles of M. affinis while Usha Goswami and Goswami (1979, 1982) formulated certain artificial diets and evaluated them for feeding the penaeid prawns.

As witnessed elsewhere in the world, nutritional studies on penaeid prawns received greater thrust in the country with the propagation and promotion of aquaculture of prawns. Hameed Ali (1980) and Hameed Ali et al. (1982)reported successful rearing of larvae of P. monodon, P. indicus, P. merguiensis, and Parapenaeopsis stylifera on crustacean tissue particles maintained in a suspension state. Microparticulate artificial diet prepared from mantis shrimp, prawn waste, groundnut cake, fishmeal and tapioca, fortified with vitamins and minerals was used by Mohamed et al. (1983) to rear P. indicus larvae. Silas et al. (1985) discussed the hatchery techniques of mass rearing of this species.

Studies on nutritional requirements of penaeid prawns particularly on P. indicus were carried out by several workers in recent years (Ahamad Ali, 1982a, 1982b, 1986; Udayaram

Jyothy 1983; Ahamad Ali and Sivadas, 1983; Charles John Bhaskar and Ahamad Ali, 1984; Ahamad Ali and Mohamed, 1985; Sally Anne Thomas, 1985; Vaitheeswaran, 1983). Gopal (1986) studied certain aspects of protein and vitamin requirements, while Chandge (1987) investigated the lipid requirements of larvae and juvenile P. indicus.

Some observations are also available on the culture of prawns in the grow out systems by feeding with compounded diets (Raman et al., 1982; Mohamed Sultan et al., 1982; Sambasivam et al., 1982). These diets were mainly used to supplement the natural food available in the pond and related to growth and production performance of prawns.

Although the above studies have considerably contributed to the information on the food and feeding habits and nutritional requirements of penaeid prawns of India, there are still very large gaps in our knowledge and much remains to be done. The studies available now are neither comprehensive nor exhaustive. Since nutrition is basic to aquaculture and since penaeid prawns offer great potential for large scale aquaculture in the vast coastal waters of the country, the present study to evaluate the feed sources of purified and natural proteins and carbohydrates and on the mineral requirements was taken up with a view to develop a balanced prepared diet and a practical feed for feeding penaeid prawns, particularly for P. indicus.

C H A P T E R - I

EVALUATION OF DIFFERENT SOURCES OF PROTEINS

Present status

Protein is the most important and the Principal nutrient in the diet of prawns. As in fishes, these crustaceans also show a preferential use of protein over the carbohydrates as dietary energy source. Because of this and the fact that Protein in a compounded feed forms the most expensive item, contributing to not only the cost of the feed but also to over all economics of culture operation, studies on protein nutrition, its requirement and evaluation of the sources in search of cheaper rawmaterials have received considerable attention from different quarters.

Feed stuffs can be classified into protein sources and energy supplements (Harris, 1978). Generally ingredients having more than 20% crude protein are considered as protein sources and those containing less than 20% crude protein and less than 18% crude fibre are considered as energy feeds. The efficiency of a protein source depends upon its quality and its digestibility by the recipient animal. Besides, the amino acid profile of the protein and the candidate species, feeding regime, developmental stages, proteins available in the aquatic food chain and its physical state, also influence significantly the protein requirement. Due to these reasons the determination of quantitative protein requirement not only vary from species to species but also within the same group. The observations made on the optimum protein requirement of juvenile penaeid prawns and protein sources used by different

workers are summarised below.

Species	Protein source	Optimum protein level(%) in diet	Authors
1	2	3	4
Penaeus japonicus	Silkworms, brine shrimp and fish meal	55.0	Kanazawa et al.(1970)
"	Squid meal, white fish meal, mysid meal, sludge and yeast	60.0	Shigueno et al.(1972)
"	Soybean, Hawaian fish and shrimp meals	40.0	Balazs et al. (1973)
"	Casein and egg albumen	52.0 to 57.0	Deshimaru and Yone (1978 c)
Penaeus aztecus	--	22.0 to 30.0	Shewbart et al.(1973)
"	Fish protein	40.0	Venkataramaiah et al. (1975a)
"	Soy flour	51.5	Zein-Eldin and Corliss, (1976)
P. setiferus	Menhaden meal	30.0	Andrews et al.(1972)
P. duorarum	Soybean meal	28.0 to 30.0	Sick and Andrews (1973)
P. merquiensis	Casein	43.0 to 55.0	AQUACOP (1978)
"	Mussel meat	34.0 to 42.1	Sedgwick (1979)
P. indicus	Prawn and fish meals	43.0	Colvin (1976 a)
"	Mantis shrimp and groundnut cake	42.9	Ahamad Ali (1982a)
Young post-larvae	Casein	40.0	Charles John Bhaskar and Ahamad Ali (1984)
Juveniles	"	30.0	"

1	2	3	4
Metapenaeus monoceros	Casein	55.0	Kanazawa et al.(1981)
P. monodon	"	40-30	Khannapa (1979)
Penaeus monodon	"	Growth proportional to the protein level	Bages and Sloane (1981)
"	Mixture of casein, squid meal, soybean meal, fish meal, shrimp meal, bread flour	40	Alava and Lim (1983)

The variations in optimum protein levels for same species, when two different sources are used, are mainly attributed to the difference in the quality between the two sources. Further, as the aminoacids are the building blocks of proteins, their profile in the protein source greatly determines the efficacy of its utilisation. Shewbart et al. (1972) investigated the amino acid requirement of the prawn Penaeus aztecus. By injecting radioactive isotope labelled acetate, Kanazawa and Teshima(1981) studied the aminoacid profile in the body of P. japonicus and reported that ten aminoacids were not synthesized by the animal and were required to be supplied in the diet. The aminoacids, identified as essential aminoacids for the penaeid prawns are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. The same ten aminoacids were also found to be essential for caridean prawns, Macrobrachium rosenbergii (Watanabe, 1975), M. ohione (Miyajima et al.,1976) and Palaemon serratus (Cowey and

Forster, 1971). Interestingly, Torres (1973), using the fluctuations between extreme values in the free aminoacid pool, indicated only eight aminoacids, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine as essential in Penaeus kerathurus. He also demonstrated the changes in the free amino acid pool of the abdominal muscle of P. kerathurus, fed on natural foods during moult cycle. Total free aminoacid content varied from a low, 0.85 mg/100 mg fresh weight at postmoult stage, B_1, to a high 7.27 mg/100 mg at intermolt stage, C_4. Dietary concentration of non-essential as well as essential aminoacids was found to effect the metabolic performance of the prawn and to play a significant role in the palatability of fresh diets (Takei and Ai, 1971).

The above studies, however, indicate only the qualitative essential aminoacid requirement. Information on the quantitative requirement is still scarce. Several workers have studied the effect of aminoacid supplementation to the diet. Cowey and Forster (1971) compared the weight gains in Palaemon serratus by feeding pure proteins deficient in essential aminoacids and mussel mantle tissue and found that the growth was 20% less in the animals fed with pure proteins. Supplementation of tyrosine and tryptophan to maize protein (zein) andtryptophan to gelatin did not improve their performance as proteins. Kitabhayashi et al. (1971 c) supplemented a basal diet of squid meal, squid meat extract and squid liver extract, with methionine and obtained superior growth in Penaeus japonicus. The authors found that the optimum level of methi-

onine was 0.53% above which growth was inhibited. Deshimaru and Kuroki (1974 c) prepared diets with pure crystalline aminoacid mixtures similar to the aminoacid composition of casein and albumen and fed to P. japonicus. Diets containing pure amino acids gave very poor growth rates, high mortality and reduced intake of food. Further studies by Deshimaru and Kuroki(1975a,b) confirmed that pure aminoacids and peptides were inferior to intact native proteins.

Deshimaru and Shigueno (1972) analysed the amino acid profiles of the Kuruma prawn Penaeus japonicus and that of the short necked clam, Tapes philippinarum, which is one of the best conventional food for this prawn, and found close resemblance between the two. They postulated that the protein sources whose amino acid profile is close to the amino acid profile of the prawn are the best high quality protein sources for preparing the diets. The authors observed that the diets prepared with fish meal were inferior to short necked clam. They also found that the aminoacid composition of fish meal is not similar to that of the prawn P. japonicus. (Colvin (1976a) and Ahamad Ali (1982a) found poor results with fish meal in P. indicus. Colvin suggested that the relative deficiency of the aminoacids, tyrosine and phenylalanine in the fish meal may be the reason for its relatively poor performance. Thus the amino acid profile of a protein source appears to greatly influence its performance.

Apart from the protein source and its aminoacid profile, the formulation and preparation of the diet, feeding regime and the abiotic factors can also influence the protein require-

ment of the animal. While formulating the diets for protein requirement study, only the protein is varied in graded levels, but the diet should have adequate levels of carbohydrate and lipid to meet the energy requirement and vitamins and minerals to make the diet otherwise balanced. It is also essential to ensure that all the diets are made isocaloric and should have same composition of the formula in respect of nutrients other than the protein source. Grinding the ingredients to a specific uniform particle size and homogenising the feed mixture are equally essential to eliminate the variability due to these factors. To minimise loss of nutrients and to make the diet sufficiently water stable, use of an appropriate binding material is as important as the diet formulation itself. The physical design of the diet to suit the feeding habits of the animal are equally important.

As the growth of animals and conversion efficiency depend upon the feeding regime and level of feeding, these also indirectly influence the protein requirement of the animal. The quantity of feed offered to the animals is generally based on the body weight and ranges from 100% to 5% dry diet of wet body weight, depending upon the size of the animal (Subrahmanyam and Oppenhiemer,1970; Venkataramaiah et al., 1972 a,b; 1975b). In nutritional studies, it is important and essential to ensure that the diet is available to the animals at all times by feeding 'ad libitum'. However, feeding level should be regulated according to the consumption and the diet left-over. Feeding the animals in devided doses twice or thrice a day was found to contribute towards better growth and conversion efficiency than

feeding the entire dose once in a day (Primavera et al., 1979; Metailler et al., 1980; Chua and Teng, 1982; Cuzon et al., 1982).

Abiotic factors such as age and size of the animal, environmental parameters such as salinity, oxygen, temperature and PH of the water are known to influence the food consumption, growth and conversion efficiency and hence the protein requirement of the animal. Charles John Bhaskar and Ahamad Ali (1984) demonstrated in P. indicus that the protein requirement in the diet varies according to the size of the animal and found that it decreases as the size of the animal increases. Similar observations were made by Khannapa (1979) in P. monodon and by Balazs et al. (1974) in the freshwater prawn, Macrobrachium rosenbergii. Sick et al. (1972) studied selected environmental and nutritional requirements of Penaeid shrimp and Venkataramaiah et al. (1975b) reviewed the influence of environmental and nutritional factors on brown shrimp P. aztecus. Lakshmikantham (1982) studied the salinity tolerance of post larvae of P. indicus and determined the optimum salinity required for different size groups of this prawn. While studying the effect of salinity on food intake, growth, conversion efficiency and proximate composition of the body in P. indicus, Kalyanaraman (1983) reported that the salinity had profound influence on food consumption, growth, food conversion efficiency and proximate composition. He also observed increased ammonia excretion in juvenile P. indicus at lower (5‰) and higher (35‰) salinity levels and related it to the increased catabolism of aminoacids at these salinity levels. Oxygen, temperature and

PH of water have marked effect on the metabolic activity of the animals and indirectly influence the protein requirement in the diet. It is therefore essential that while designing the feeding experiments, optimum levels of these environmental conditions should be provided for obtaining reliable results.

The techniques/approach employed in most of the studies on protein requirements of prawns followed the feeding of prawns on a balanced diet containing graded levels of protein over a period of time and to record the protein level which registers the optimum growth as the requirement. It was in 1970 Kanazawa et al. (1970) first prepared synthetic diets using purified ingredients and evaluated their performance against the shortnecked clam for P. japonicus. They found that the performance of the diet compounded with casein, glucose, sucrose, starch, glucosamine, sodium citrate, sodium succinate, cholesterol, pollack liver oil, minerals, vitamins and cellulose, was comparable to that of the control diet. Subsequently this diet was used as a basal diet for studying the nutritional requirements of P. japonicus. In recent years great strides have been made in protein evaluation methods especially in the introduction of newer methods and equipments to study nutritional biochemistry. Principally the efficiency of a protein is measured against growth of the animal. However, many expressions have been introduced for the evaluation of proteins which lead to very comprehensive testing of the quality of the proteins with greater insight. Thomas (1909) described first the quantitative method for evaluating protein quality and called it, Biological Value (BV). The biological value of a protein is defined as the percentage

of the absorbed protein which is utilized by the body. The method to determine the BV is laborious. However, the absorbed nitrogen is calculated from the intake of food nitrogen and the nitrogen in the faeces of food origin. When BV is multiplied by true digestibility of protein it gives an actual measure of the biologically complete protein in the food and is termed Net Protein Utilisation (NPU). Bender and Miller (1953) first determined NPU satisfactorily in rats. Subsequently it was employed for protein evaluation studies in fishes.

Another parameter employed to evaluate the protein is protein efficiency ratio (PER). It is defined as the increase in the weight of animal per unit protein consumed and in this, the protein used for body maintainance is not taken into account. Eventhough the procedures involved in determining various parameters to evaluate proteins are laborious, it is fairly easy in the case of homeothermic land animals to collect the metabolites like faeces and urine for analysis. On the other hand collection of metabolites in case of aquatic animals poses very complex problems since these are released into the water in which the animals are reared.

Reasonably good designs of equipments (Ogino et al., 1973 and Windell et al., 1978) are available for collection of faeces and urinary nitrogen for finfish. Protein evaluations through the measurement of BV, NPU, and PER have been made in finfish, especially fresh water fishes. However, little attempts have been made to measure these parameters in prawns. Nevertheless, digestibility studies in prawns using internal marker

like chromium oxide were made by Nose (1964) in crayfish and prawn fish, Forster and Gabbott (1971) in Palaemon serratus and Pandalus platyceros, Condrey et al. (1972) in Penaeus setiferus and P. aztecus and Colvin (1976a)in P. indicus. Recently Bordner et al. (1983) studied the digestibility of feed in lobster, Ashmore et al. (1985) in Macrobrachium rosenbergii, Lee and Lawrence (1985) in P. setiferus, Seidman and Lawrence (1985) in P. vannamei and P. monodon and Smith et al. (1985) in P. vannamei.

In the present study four purified proteins and nine practical protein sources were evaluated through the measurement of digestibility, protein efficiency ratio, Net Protein Utilisation, Biological Value, growth and food conversion ratio for the prawn, Penaeus indicus. For the first time, attempts were made to calculate the endogenous metabolic nitrogen in the prawn by feeding zero protein diets. The observations made and the results obtained are presented in the following sections.

MATERIAL AND METHODS

Four purified proteins and nine natural protein sources were evaluated for the juveniles of the prawn Penaeus indicus. The purified proteins were Albumen (egg), casein (milk), Fibrin (blood) and Gelatin. Out of the nine natural protein sources tested, five were of animal origin and four were of plant origin. The animal protein sources are clam meat, fishmeal, mantis shrimp, prawn waste meal, and silkworm pupa. Coconut cake, gingelly cake, ground nut cake and Spirulina were the plant protein sources. The details of the source of these materials are given below:

1. Albumen (egg) .. Albumen portion of the hen's egg and obtained from BDH.

2. Casein (fat free) .. Casein is the milk protein made free of fat; purchased from SISCO Research Laboratories Private Ltd. Bombay.

3. Fibrin (blood) .. This purified protein is prepared from the blood of animals; obtained from SIGMA Chemical Company, P.O. Box 14508, St.Louis MO 63178 USA.

4. Gelatin .. This is purified protein of animal origin, procured from BDH.

5. Cod Liver oil .. Cod liver oil is a commercial product under the brand name 'Seven Seas' prepared by Universal Generics Pvt. Ltd., Apollo Street, Bombay -23.

6. Clam meat powder .. Prepared from the meat of the clam Sunneta scripta; the extracted meat from the animal dried in the oven at 60°C for 12 hours.

7. Fish meal .. A commercial product obtained from the Tamil Nadu Fisheries Development Corporation, Mandapam.

8. Mantis shrimp .. Oratosquilla nepa, caught by trawl nets off Cochin and landed at the Cochin Fishing harbour, were collected. After cleaning the sample in fresh water and washing off the adhering salt, it was dried in the oven at 60°C for 12 hours.

9. Prawn waste meal .. Waste materials such as head, eyes, eye stalk, exoskeleton, hepatopancreas and residual meat of prawns were obtained from the processing units in fresh condition and dried in the oven at 60°C for 12 hours.

10. Silkworm pupa .. The residual material left after extraction of silk fibre from the cocoons, defated and sold as silkworm pupa, was obtained from Bangalore, Karnataka State.

11. Coconut cake .. Is the residue after extraction of oil from coconut; procured from local market.

12. Gingelly cake .. Is the residue after extraction of oil from gingelly seeds and purchased from local market.

13. Groundnut cake .. Is the residue after extraction of oil from groundnuts; purchased from local market.

14. Spirulina .. A blue green freshwater alga Spirulina platensis and popularly known as single cell protein, was obtained from the Central Food Technological Research Institute, Mysore.

For formulating diets with natural protein sources, tapioca (Mannihot utilissima) Powder was used as a source of carbohydrate and was purchased from the local market.

All the chemicals used in formulating diets, for chemical analysis, and as binders (agar agar, polyvinyl alcohol and sodium alginate) were guaranteed pure grade chemicals obtained from Standard Chemical Companies.

The purified and natural proteins selected for evaluation were analysed for their chemical composition, after drying them

Table 1(a). Proximate composition of purified proteins

Name of protein	% on dry basis (g/100 g)				
	Total nitrogen	Crude protein	Lipid	Carbo-hydrate	Ash
Albumen	12.49	78.1	1.68	0.73	7.56
Casein	13.42	83.86	0.92	0.77	3.28
Fibrin	13.56	84.72	2.40	1.46	3.23
Gelatin	15.47	96.71	0.40	0.69	1.14

Table 1 (b). Proximate composition of natural protein sources.

Name of material	% on dry basis (g/100 g)					
	Total nitrogen	Protein nitrogen	Crude protein	Lipid	Carbohydrate	Ash
Clam meat powder	7.69	6.35	48.10	13.55	16.69	7.62
Fish meal	10.30	6.93	64.40	4.70	0.97	19.26
Mantis shrimp meal	7.05	5.23	44.06	7.55	1.27	23.63
Prawn waste meal	5.63	4.34	35.20	6.60	0.97	23.95
Silkworm pupa	9.95	6.25	62.17	10.64	1.86	19.03
Coconut cake	3.80	3.80	25.96	11.20	22.19	8.88
Gingelly cake	4.90	4.27	34.03	10.80	24.76	12.52
Groundnut cake	7.74	6.16	48.42	7.56	28.18	6.03
Spirulina	9.74	8.05	60.89	9.00	6.63	13.00
Tapioca	0.32	--	2.00	0.54	68.50	1.45

in an electrical oven at 60°C for 12 hrs. Crude Protein was estimated by Kjeldahl method. Lipid was extracted by Chloroform-methanol (2:1) mixture (Bligh and Dyer, 1959). Carbohydrate was determined by spectrophotometric method using anthrone reagent. Ash was determined by incinerating the material at 550°C for 6 hrs in a muffle furnace. The chemical composition of the purified proteins is given in Table 1(a) and that of the natural protein sources and tapioca in Table 1(b).

Formulation of diets

(a) Purified proteins

Four groups of diets, group I with five types, Group II with six, group III with five and Group IV with seven types, were formulated with purified ingredients for evaluation of their relative superiority in four different experiments. The ingredients of the diets, the formulation characteristics and the evaluation objectives of each experiment are as follows.

Group of diets	Experiment No.	Type of diets	Diet formulation characteristics	Evaluation objectives
1	2	3	4	5
Group I	Experiment 1	PE_0, PE_1, PE_2, PE_3 and PE_4	While the diet PE_0 had no protein, each of the other diets contains one of the purified proteins of either albumen or casein or fibrin or gelatin, formulated on the basis of the purified diet 'B' prepared by Kanazawa et al. (1970, 1977a); each diet contained 40%	Evaluation of relative characteristics of the protein sources.

1	2	3	4	5
			protein, a mixture of sucrose and starch as source of carbohydrate and cod liver oil as lipid source:each of the diets contained vitamins and mineral mixture and additives such as glucosamine,cholesterol,sodium citrate and succinate and sodium carbonate; The diets also contained cellulose and chromium oxide to determine digestibility: Agar agar was used as the binder: Composition of diets given in Table 2.	
Group II	Experiment 2	PE_5, PE_6, PE_7, PE_8, PE_9 and PE_{10}.	The diets contained 0, 20, 30,40, 50 and 60% of albumen respectively as protein source; each diet contained fixed amount of lipid and all the diets were made isocaloric with carbohydrate. Each diet contained cellulose,Vitamins, minerals and other additives same as in diets of experiment 1. Agar agar was used as binder.Composition is presented in Table 3.	Evaluation of albumen
Group III	Experiment 3	PE_{11}, PE_{12}, PE_{13}, PE_{14} and PE_{15}	In this group,the diets contained 20, 30,40,50 and 60% casein respectively as protein source. The remaining components of the diets were same as in the diets of experiment 2	Evaluation of casein.

1	2	3	4	5
			Composition given in Table 4.	
Group IV	Experiment 4	PE_{16}, PE_{17}, PE_{18}, PE_{19}, PE_{20}, PE_{21} and PE_{22}.	Each diet contained a mixture of two purified proteins from among albumen, casein, gelatin and fibrin in equal proportion . In diet PE_{22} all the four proteins were present in equal proportions: The diets were iso-nitrogenous having same quantity of metabolisable energy: The additives were same as used in the diets of experiment 3. Sodium alginate was used as the binder; Composition of the diets given in Table 5.	Evaluation of mixed Protein Sources.

(b) Natural Protein sources.

Three groups of diets, group V, with nine types, five of animal protein sources and four of plant protein sources, group VI with nine types and group VII with seven types, were formulated with natural protein ingredients to evaluate their relative efficiencies in three different experiments. The ingredients of the diets, the formulation characteristics and evaluation objectives of each experiment are as follows:

Group of diets	Experiment No.	Type of diets	Diet formulation characteristics.	Evaluation objectives
1	2	3	4	5
Group V.	Experiment-5.	PE_{23}, PE_{24}, PE_{25}, PE_{26},	First five diets contained animal proteins clam meat powder	Evaluation of relative

1	2	3	4	5
		PE_{27}, PE_{28}, PE_{29}, PE_{30} and PE_{31}.	fishmeal, mantis shrimp, prawn waste and silkworm pupa respectively. The next four diets contained plant protein materials coconut cake, gingelly cake, groundnut cake and Spirulina respectively. All the diets contained 30% crude protein. The diet with coconut cake was formulated in combination with fishmeal to make up the protein content 30% in the diet. Tapioca powder was used as the source of carbohydrate. The diets were nutritionally balanced by adding lipid (fish oil), Vitamins and mineral mixture. Chromium oxide was included at 1% to determine the digestibility; polyvinyl alcohol was used as the binder. The composition of the diets is presented in Table 7.	efficiencies of natural Protein sources.
Group VI	Experiment 6.	PE_{32}, PE_{33}, PE_{34}, PE_{35}, PE_{36}, PE_{37}, PE_{38}, PE_{39} and PE_{40}.	To formulate this group of diets, a mixture of animal protein sources (APS) and another mixture of plant protein sources (PPS) were prepared first. The APS mixture contained clam meat powder(10%), fishmeal (30%), mantis shrimp(30%), and Prawn waste (30%). The PPS mixture contained coconut cake (30%), gingelly cake (30%) and groundnut cake (40%). The APS mixture and the PPS mixture were present in the diet, in the ratio 13:1 to 6:1, while the crude protein in the diets varied from 51.89% to 35.57% The diets were balanced as in the previous experiment. The composition of diets is given in Table 8.	Evaluation of animal and plant protein mixtures in different combinations.

1	2	3	4	5
Group VII	Experiment 7.	PE_{41}, PE_{42}, PE_{43}, PE_{44}, PE_{45}, PE_{46} and PE_{47}.	These diets were formulated taking APS and PPS mixtures in the ratio 70:30. All the diets were iso-caloric but the protein contents were 42, 38, 33, 28, 23, 19 and 14% respectively. The sources of carbohydrate and lipid and also the vitamins and mineral mixtures were same as used in the experiment 5. Polyvinyl alcohol was used as the binder; composition of the diets is given in Table 9.	Evaluation of protein levels in the diets formulated with natural protein sources.

Vitamin mixture

Vitamin mixture was prepared in the laboratory by mixing the individual vitamins. They were grouped into water soluble and fat soluble vitamins. Riboflavin, thiamine, cyanocobalamine, folic acid, choline chloride, ascorbic acid, para aminobenzoic acid, nicotinic acid, calcium pantothenate and prydidoxine were grouped as water soluble vitamins and biotin, β-carotene, menadione, α-tocopherol, inositol and calciferol as fat soluble vitamins. At the time of mixing with the diet, the first group of vitamins were dissolved in water and latter group in alcohol. The composition of the vitamins used is presented in Table 2 a.

Mineral mixture

The mineral mixture was also prepared in the laboratory. The individual salts were weighed, mixed together and homogenised in a varing blender. The mixture was stored in polythene containers until use. The composition of the mineral mixture is shown in Table 2 b.

Preparation of purified diets

Each ingredient used in formulating diets was seperately powdered and sieved through 250 micron sieve before using in the preparation of diets. The water soluble vitamins were dissolved in water and fat soluble vitamins in alcohol. The solid ingredients, including the mineral mixture and chromium oxide were first mixed together and blended thoroughly in an electrical varing blender. To this, cod liver oil was added and further homogenized the diet. The binder (agar agar or sodium alginate) was dissolved in approximately 40 ml of water (for 100 g diet) heated to 50-60°C. The diet mixture and vitamin solutions were added to the binder solution and the total diet was prepared into a dough. It was then steamed for ten minutes and extruded in a hand pelletizer using 3 mm diameter die. The pellets were collected in a suitable tray and dried in the oven at 60°C for 12 hours. The dry pellets were broken into small pieces (2-3 mm length) and packed in polythene covers. The diets were stored in descicator until use.

Preparation of Practical diets

The ingredients were individually powdered (approximately 250 micron) and weighed according to composition of the diet and mixed together. 40 ml. of water (for 100g of diet) was taken and

Table 2. Composition (g/100g) of the purified diets PE_0, PE_1, PE_2, PE_3 and PE_4.

Ingredients	Diet No.				
	PE_0	PE_1	PE_2	PE_3	PE_4
Albumen (egg)	0.00	40.0	--	--	--
Casein (fat free)	0.00	--	40.0	--	--
Fibrin (blood)	0.00	--	--	40.0	--
Gelatin	0.00	--	--	--	40.0
Sucrose	15.8	6.4	6.4	6.4	6.4
Starch	55.0	24.4	24.4	24.4	24.4
Cod liver oil	6.0	6.0	6.0	6.0	6.0
Glucosamine hydrochloride	0.8	0.8	0.8	0.8	0.8
Cholesterol	0.5	0.5	0.5	0.5	0.5
Sodium citrate	0.3	0.3	0.3	0.3	0.3
Sodium succinate	0.3	0.3	0.3	0.3	0.3
Vitamin mixture *	2.7	2.7	2.7	2.7	2.7
Mineral mixture *	8.6	8.6	8.6	8.6	8.6
Cellulose	6.5	6.5	6.5	6.5	6.5
Sodium carbonate	1.0	1.0	1.0	1.0	1.0
Chromium oxide	0.5	0.5	0.5	0.5	0.5
Agar agar	2.0	2.0	2.0	2.0	2.0

* The composition of vitamin and mineral mixture is given in table 2a and 2b respectively.

Table 2a. Composition of vitamin mixture

Water soluble vitamins: (g/100g diet)

Ascorbic acid	2.000
Choline chloride	0.120
Cyanocobalamine	0.00008
Folic acid	0.080
Nicotinic acid	0.040
Pantothenic acid (Calcium salt)	0.060
Para amino benzoic acid	0.010
Pyridoxine hydrochloride	0.0120
Riboflavin	0.0080
Thiamine hydrochloride	0.0040

Fat soluble vitamins: (g/100g diet)

Biotin	0.00004
β-carotene	0.0096
Calciferol	0.0012
Inositol	0.200
Menadione	0.004
α-Tocopherol	0.029

Table 2b. Composition of mineral Mixture: (g/100g diet)

Calcium lactate	2.72
Potassium dihydrogen orthophosphate	2.00
Sodium dihydrogen orthophosphate	0.79
Magnesium sulphate	3.02
Manganese chloride	0.004
Ferrous chloride	0.015

Table 3. Composition (g/100g) of the purified diets PE_5, PE_6, PE_7, PE_8, PE_9 & PE_{10}.

Ingredients	Diet No.					
	PE_5	PE_6	PE_7	PE_8	PE_9	PE_{10}
Albumen (egg)	0.00	20.0	30.0	40.0	50.0	60.0
Cod liver oil	6.0	6.0	6.0	6.0	6.0	6.0
Sucrose	16.3	11.0	8.6	5.8	3.0	0.4
Starch	50.0	44.4	33.2	22.3	11.4	0.4
Vitamin mix *	2.7	2.7	2.7	2.7	2.7	2.7
Mineral mix *	8.6	8.6	8.6	8.6	8.6	8.6
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5
Glucosamine HC1	0.8	0.8	0.8	0.8	0.8	0.8
Sodium citrate	0.3	0.3	0.3	0.3	0.3	0.3
Sodium succinate	0.3	0.3	0.3	0.3	0.3	0.3
Cellulose	--	0.9	4.5	8.2	11.9	15.5
Chromium oxide	0.5	0.5	0.5	0.5	0.5	0.5
Sodium hydrogen carbonate	1.0	1.0	1.0	1.0	1.0	1.0
Agar agar	3.0	3.0	3.0	3.0	3.0	3.0

* Vitamin and mineral mixtures were same as used in experiment 1 (Tables 2a and 2b)

Table 4. Composition (g/100g) of the purified diets PE_{11}, PE_{12}, PE_{13}, PE_{14} and PE_{15}.

Ingredients	Diet No.				
	PE_{11}	PE_{12}	PE_{13}	PE_{14}	PE_{15}
Casein (fat free)	20.0	30.0	40.0	50.0	60.0
Cod liver oil	6.0	6.0	6.0	6.0	6.0
Sucrose	11.0	8.6	5.8	3.0	0.4
Starch	44.4	33.2	22.3	11.4	0.4
Vitamin mix *	2.7	2.7	2.7	2.7	2.7
Mineral mix *	8.6	8.6	8.6	8.6	8.6
Cholesterol	0.5	0.5	0.5	0.5	0.5
Glucosamine HCl	0.8	0.8	0.8	0.8	0.8
Sodium citrate	0.3	0.3	0.3	0.3	0.3
Socium succinate	0.3	0.3	0.3	0.3	0.3
Cellulose	0.9	4.5	8.2	11.9	15.5
Sodium hydrogen carbonate	1.0	1.0	1.0	1.0	1.0
Chromium oxide	0.5	0.5	0.5	0.5	0.5
Agar agar	3.0	3.0	3.0	3.0	3.0

* Vitamin and mineral mixtures were same as used in experiment 1 (Tables 2a and 2b)

Table 5. Composition (g/100g) of the purified diets PE_{16}, PE_{17}, PE_{18}, PE_{19}, PE_{20}, PE_{21}, and PE_{22}.

Ingredients	Diet No.						
	PE_{16}	PE_{17}	PE_{18}	PE_{19}	PE_{20}	PE_{21}	PE_{22}
Albumen	20	20	20	----	----	----	10
Casein	20	--	--	20	20	----	10
Fibrin	--	--	20	--	20	20	10
Gelatin	--	20	--	20	--	20	10
Sucrose	6.4	6.4	6.4	6.4	6.4	6.4	6.4
Starch	24.4	24.4	24.4	24.4	24.4	24.4	24.4
Cod liver oil	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Vitamin mix *	2.7	2.7	2.7	2.7	2.7	2.7	2.7
Mineral mix *	8.6	8.6	8.6	8.6	8.6	8.6	8.6
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Glucosamine HCl	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Sodium citrate	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Sodium Succinate	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Cellulose	8.2	8.2	8.2	8.2	8.2	8.2	8.2
Sodium hydrogen carbonate	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Sodium alginate	3.0	3.0	3.0	3.0	3.0	3.0	3.0

* Mineral and vitamin mixtures were same as used in experiment 1 (Table 2a and 2b)

Table 6. Composition (g/100g) of the diets PE_{23}, PE_{24}, PE_{25}, PE_{26} & PE_{27} with natural protein (animal) sources.

Ingredients	Diet No.				
	PE_{23}	PE_{24}	PE_{25}	PE_{26}	PE_{27}
Clam meat powder	51.0	--	--	--	--
Fish meal	--	47.0	--	--	--
Mantis shrimp	--	--	68.5	--	--
Prawn waste	--	--	--	86.0	--
Silkworm pupa	--	--	--	--	50.0
Tapioca	40.5	44.5	23.5	5.5	41.5
Fish oil (Sardine)	3.0	3.0	3.0	3.0	3.0
Vitamin and mineral mixture *	1.5	1.5	1.5	1.5	1.5
Chromium oxide	1.0	1.0	1.0	1.0	1.0
Polyvinyl alcohol	3.0	3.0	3.0	3.0	3.0

* Vitamin and mineral mixture composition is shown in Table 6a

Table 6a. Vitamin and mineral mixture used in diets PE_{23} to PE_{27}.

Each 100g of the diet contained the following vitamins and minerals:

Vitamins	
Vitamin A (acetate)	5000 I.U.
Thiamine mononitrite	4.00 mg.
Riboflavin	6.00 mg.
Nicotinamide	50.00 mg.
Pyridoxine hydrochloride	3.00 mg.
Calcium pantothenate	10.00 mg.
Cynocobalamine	2.00 µg.
Ascorbic acid	100.00 mg.
Cholecalciferol	400 I.U.
Vitamin E (Alphatocopheryl acetate)	20.00 mg.
Biotin	0.10 mg.
Minerals	
Calcium phosphate	0.416 g.
Ferrous sulphate	21.24 mg.
Magnesium phosphate (dibasic)	96.00 mg.
Manganese hypophosphite	1.2 mg.

Table 7. Composition (g/100g) of the diets PE_{28}, PE_{29}, PE_{30} and PE_{31} with natural protein (plant) sources.

Ingredients	Diet No.			
	PE_{28}	PE_{29}	PE_{30}	PE_{31}
Coconut cake	72.5	--	--	--
Fish meal	16.0	--	--	--
Gingelly cake	--	88.5	--	--
Groundnut cake	--	--	62	--
Spirulina	--	--	--	50
Tapioca	3.0	3.0	29.5	41.5
Fish oil	3.0	3.0	3.0	3.0
Vitamin and Mineral mixture *	1.5	1.5	1.5	1.5
Chromium oxide	1.0	1.0	1.0	1.0
Polyvinyl alcohol	3.0	3.0	3.0	3.0

* Composition of vitamin and mineral mixture is same as given in Table 6a.

Table 8. Composition (g/100g) of the experimental diets PE_{32}, PE_{33}, PE_{34}, PE_{35}, PE_{36}, PE_{37}, PE_{38}, PE_{39} and PE_{40}.

Ingredients	Diet No.								
	PE_{32}	PE_{33}	PE_{34}	PE_{35}	PE_{36}	PE_{37}	PE_{38}	PE_{39}	PE_{40}
Animal Protein mix.1	90.0	80.0	70.0	60.0	50.0	40.0	30.0	20.0	10.0
Plant protein Mix. 2	10.0	20.0	30.0	40.0	50.0	60.0	70.0	80.0	90.0
Fish Oil	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Vitamin and mineral mixture*	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Chromium oxide	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Polyvinyl alcohol	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Ratio of animal and plant protein	12.1:1	5.7:1	3.3:1	2.2:1	1.4:1	1:1	0.6:1	0.4:1	0.2:1

* Vitamin and mineral mixture was same as used in experiment 5 (Table 6a)

1. Animal protein mixture (g/100g)

Clam meat powder	10
Fish meal	30
Mantis shrimp	30
Prawn waste	30
	100
Crude protein %	53.4

2. Plant protein mixture (g/100g)

Coconut cake	30
Gingelly cake	30
Groundnut cake	40
	100
Crude protein %	37.2

Table 9. Composition (g/100g) of the experimental diets PE_{41}, PE_{42}, PE_{43}, PE_{44}, PE_{45}, PE_{46} and PE_{47}.

Ingredients	Diet No.						
	PE_{41}	PE_{42}	PE_{43}	PE_{44}	PE_{45}	PE_{46}	PE_{47}
Protein mixture[1]	90.0	80.0	70.0	60.0	50.0	40.0	30.0
Tapioca	10.0	20.0	30.0	40.0	50.0	60.0	70.0
Fish oil(sardine)	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Vitamin and mineral mixture[2]	1.5	1.5	1.5	1.5	1.5	1.5	1.5
PVA	3.0	3.0	3.0	3.0	3.0	3.0	3.0

1 Protein mixture: A mixture of 70% of animal protein mix and 30% of plant protein mix (Table 8) used in Experiment 6.

2 Vitamin and mineral mixture was same as given in Table 6a.

the fish oil was emulsified in it using 1g of glycerol monolaurate with the help of a varing blender. The binder was seperately melted in a small quantity of hot water at 70°C. To this the dry ingredient mixture, the oil emulsion were added and thoroughly homogenized. The dough was steamed for ten minutes and extruded through a hand pelletizer using 3 mm diameter die. The resultant pellets were dried in the oven at 60°C for 12 hours. The dry pellets were broken into pieces of 2-3 mm size and stored in descicator until use.

Experimental animals: Early juveniles of the Indian white prawn, Penaeus indicus (H. Milne Edwards) (Plate 1) used in the experiments, were obtained from the hatchery of the Marine Prawn Hatchery Laboratory (MPHL) of the Central Marine Fisheries Research Institute (CMFRI) at Narakkal. For each experiment, the animals from a single brood were only used. In all the feeding experiments, the animals ranging from 20 to 30 mm in size (total length measured from tip of the rostrum to the tip of the telson) were used.

Rearing facility: Ten litre capacity, transparent circular containers, made of pursplux, were used for rearing the animals in feeding experiments (Plate 2). The background of the bottom of rearing tanks was specifically made milky white to facilitate easy recognition and collection of faeces for digestibility studies.

Water management: A mixture of filtered (through bolting cloth No.30) sea water and tap water, in the required proportion, to obtain the desired salinity, was used for rearing the animals in feeding experiments. Intermittant aeration (not less than 15 minutes per hour) was provided with the help of an air-.

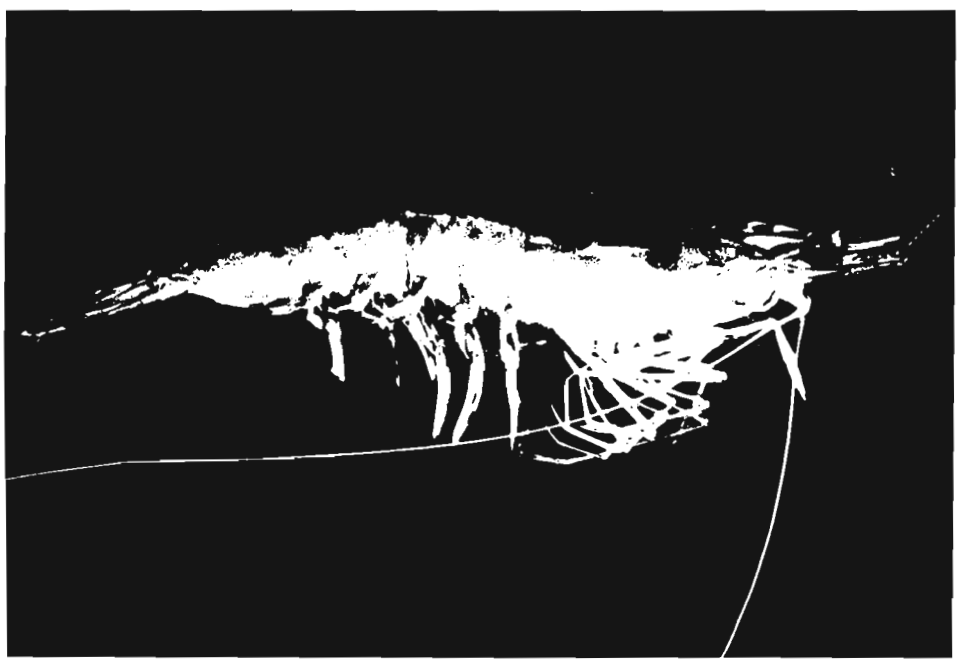

Plate 1. The Indian white prawn Penaeus indicus H. Milne Edwards.

blower using air stones. Sediment from each of the tanks was siphoned out everyday and three fourths of the water was replaced by freshly prepared water with the same salinity as mentioned above. Complete water was replaced in the tanks once in five days. Since the early juveniles of penaeid prawns were found to have (Venkataramaiah et al., 1975b) highest growth and survival at salinity between 15-20‰, the salinity of the water in the rearing tanks was selected within this range in all the feeding experiments. The hydrographical data in respect of each experiment are given separately in Table H_1.

Design of feeding experiment: In all the feeding experiments, the rearing tanks were arranged in randomized block design, in order to eliminate the influence of position and place of the tanks among different treatments. For each treatment there were three replicates. A group of eight animals were stocked in each tank. Thus there were twentyfour animals for each treatment. The duration of each feeding experiment was for a period of 30 days.

Feeding Procedure: In all the experiments group feeding procedure was adopted. The animals were fed at the rate of approximately 20% of body weight of dry diet in two divided doses in the morning and evening. Before feeding, the left-over feed was recovered, the adhering salt was washed gently and dried in the oven at 60°C to constant weight.

Faeces collection: For collection of faeces in digestibility studies, a pilot experiment was conducted first in which the animals were fed in the morning. It was observed that three

Plate 2. Experimental set up used for rearing animals for the biological evaluation of diets.

Table H_1. Hydrographical data of the feeding experiments 1, 2, 3, 4, 5, 6 and 7.

Experiment No.	Salinity ‰	Oxygen cc/L	PH	Temperature °C
1	16.7 ± 1	4.0	8.05 ± 0.1	28.5 ± 0.5
2	18.29 ± 1	3.9	8.29 ± 0.2	29.4 ± 0.5
3	18.14 ± 1	4.0	8.15 ± 0.1	29.1 ± 0.5
4	17.9 ± 1	4.1	8.20 ± 0.1	28.5 ± 0.5
5	18.13 ± 1	3.69	8.12 ± 0.1	29.0 ± 0.5
6	16.0 ± 1	3.69	8.1 ± 0.1	28.0 ± 0.5
7	17.19 ± 1	3.82	8.01 ± 0.1	29.1 + 0.5

hours after feeding, maximum sheding of faeces occurred. In all the experiments in which the digestibility was determined, collection of the faeces was therefore commenced three hours after the morning feeding. Generally the faeces used to come out as long strands. These were collected carefully with the help of a pipette on to a piece of bolting cloth. After gently washing the adhering salt with distilled water, the faeces were transfered to a petridish and dried in the oven at 60°C to constant weight. The dry faeces were homogenized in an agate mortar and used for analysis.

After commencement of the feeding experiment, the animals were allowed to fully acclimatize to the diet and no faeces were collected during the first week. In order to cover the inherent variabilities in the digestibility with time, faeces were collected regularily over a period of twenty days. The design and planning of the feeding experiments both for digestibility studies and growth evaluation simultaneously, helped to achieve faeces collection for long time, which had distinct advantages. Besides providing the opportunity to cover longer period for faeces collection, it helps to obtain sufficiently large sample of faeces adequate for chemical analysis.

Bacterial growth is known to occur in rearing tanks during the feeding experiments. Many a times it is practically difficult to quantify them and take into account for the purpose of feeding. As far as possible, to avoid such growth of bacteria, filtered water was used, the sediments were regularly removed and left-over food was taken out before fresh batch of food is given. However, no other precaution could be taken in addition to these measures.

measurements

Total length and weight: The total length of the animals, before, during and after the feeding experiment, was measured from the tip of the rostrum to the tip of the telson. For measuring the initial dry weight, three groups of eight animals each were sacrificed and after drying them at 60°C to constant weight, each group was separately weighed and the average dry weight of the animal was calculated. At the end of the experiment the dry weight was measured by sacrificing the total number of animals in each replicate. The initial and final live weight of the animals was determined by weighing the individual group of animals in each treatment after removing the external water with filter paper. Subsequently, the dried animals were finally powdered and used for estimation of nitrogen.

Growth: The growth of the animals in length, live-weight and dry weight was measured by using the following formula.

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

Food conversion ratio (FCR): Food conversion ratio of the diet is calculated using increase in live-weight and the food consumed, by the following formula.

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

Digestibility: Edin (1918) first introduced the use of chromium oxide as an inert digestion indicator in animals. This had obviated the need to collect the total faeces quantitatively. Phillips(1969) conducted extensive studies on digestibility of food components in fish. Later Smith and Lovell(1973) studied the digestibility of protein nitrogen using this method in catfish and found that the results obtained in catfish were similar to

the digestibility of protein in live stock. Austring (1978) confirmed the suitability of this procedure for measuring digestibility in fish. Forster and Gabbott (1971), Colvin (1976a), Bordner et al.(1983), Ashmore et al. (1985), Lee and Lawrence (1985), Seidman and Lawrence (1985), Smith et al. (1985) successfully used this method to study the digestibility of nutrients in prawns.

In the present study the digestibility of protein in the diet was determined using the same inert internal marker Chromium odixe (Cr_2O_3). The method consists of adding a known amount Cr_2O_3 in the diet. The chromium oxide is excreted out by the animal undigested. The faeces are collected for a period of time and the nutrient and Cr_2O_3 in the faeces and diet are determined. The apparent digestibility coefficient of the nutrient is calculated by the following formula.

$$\text{Apparent digestibility coefficient} = 100 - \frac{\%\ Cr_2O_3 \text{ in diet}}{\%\ Cr_2O_3 \text{ in faeces}} \times \frac{\%\ \text{nutrient in faeces}}{\%\ \text{nutrient in diet}} \times 100$$

Metabolic Faecal Nitrogen (MFN) and True digestibility

For the determination of Biological Value of a protein, the true digestibility of the protein by the animal is necessary. Calculation of digestibility of protein involves determination of faecal nitrogen which contains not only the undigested nitrogen from the diet but also the nitrogen excreted due to the metabolic activity in the body. Metabolic faecal nitrogen (MFN) comprises residues of the digestive juices, epithelial cells

derived from the walls of the alimentary canal, and bacterial residues (Maynard and Loosli, 1969). In prawns the secretion of a chitinous peritropic membrane around the faecal pellets (Forster, 1953) would also contribute to the MFN. To obtain the undigested dietary nitrogen in the faeces, the MFN should be subtracted from the total faecal nitrogen.

Metabolic faecal nitrogen is usually expressed in terms of nitrogen excreted per unit weight of dry diet consumed by the animal (milligrams of nitrogen per 100 grams of diet). It can be determined in two ways. One is by directly feeding animals a known quantity of a nitrogen free diet, and the nitrogen appearing in the faeces must therefore be MFN. The second method is by feeding diets containing different protein levels and determining the nitrogen in faeces. The level of faecal nitrogen, expressed as nitrogen per unit weight of food eaten, is then regressed against the level of dietary nitrogen, and the value when the level of dietary protein is zero, is taken as the estimate of MFN. These two methods have been critically evaluated by Mitchell and Bert (1954) for albino rats and have shown to give good agreement. Nose (1967b) also obtained reasonably good agreement of the two methods in rainbow trout. Both the methods were employed successfully by Forster and Gabbott (1971) for studying the assimilation of nitrogen in the prawns Palaemon serratus and Pandalus platyceros. In the present study MFN was determined by feeding zero protein diet to the prawns. A known quantity of chromium oxide was added as an inert marker. By determining the chromium oxide in diet as well as in faeces it is possible to calculate the MFN. It is

very advantageous to use the inert marker in the diet, because, by this method, accurate estimation of both the diet consumed and the faeces excreted need not be done. The ratio of the merker in the faeces and the diet could be directly used for calculating the amount of food consumed and MFN per 100 g diet. For this purpose, animals were individually held separately in six rearing containers and fed with zero protein diet PE_0, 'ad libitum' for 30 days. Faeces were collected every day as per the procedure described earlier. Nitrogen, chromium oxide in diet and faeces were determined. At the end of the experiment the carcass of the animals was analysed for nitrogen for determining NPU.

The metabolic faecal nitrogen was calculated using the following formula:

$$\text{MFN excreted when 100 g of diet consumed} = \frac{A \times B}{C}$$

Where A = % Nitrogen in faeces of animals fed zero protein diet.

B = % Chromium oxide in zero protein diet.

C = % Chromium oxide in faeces.

Correction of MFN in the faecal nitrogen of test diet for calculating true digestibility is obtained by using the formula.

$$\text{MFN due to the amount of test diet consumed} = \frac{E}{D} \times \frac{AB}{C}$$

Where D = % Chromium oxide in test diet.

E = % Chromium oxide in faeces of test diet fed animals.

The MFN thus obtained is subtracted from the total faecal nitrogen of the test group animals to obtain the corrected faecal nitrogen of the test group.

Using the corrected faecal nitrogen of the test group, the true digestibility of the protein was calculated using the formula of digestibility coefficient.

$$\text{True digestibility} = 100 - \frac{\%\ Cr_2O_3 \text{ in diet}}{\%\ Cr_2O_3 \text{ in faeces}} \times \frac{\%\ \text{corrected protein in faeces}}{\%\ \text{protein in diet}} \times 100$$

Protein Efficiency Ratio (PER):

The Protein efficiency ratio was calculated by the formula

$$PER = \frac{\text{Average live weight gain}}{\text{Average protein consumed}}$$

Net Protein Utilization (NPU):

Net protein utilization was determined using the modified formula (Castell and Tiews, 1980) for fish.

$$NPU = \frac{\text{Body nitrogen of test group animals} - \text{Body nitrogen of animals receiving zero protein diet}}{\text{Nitrogen consumed}}$$

Biological Value of Protein (BV):

The biological value of the protein is calculated by the following formula:

$$BV = \frac{\text{Net protein utilization}}{\text{True digestibility of protein}}$$

Analysis:

Total nitrogen and protein: Total nitrogen in dry diets, animals and faeces was determined by the standard Kjeldahl method. The percentage of protein was calculated by multiplying the percentage of total nitrogen by the factor 6.25.

Chromium oxide: The chromium oxide in the diets as well as in faeces was estimated by the method of McGinnis and Kasting(1964). The organic matter in the material was first digested with nitric

acid and the Cr_2O_3 was oxidised to $Cr_2O_7^{--}$. The dichromate ion thus obtained was determined by spectrophotometric method using diphenylcarbazide reagent. This is a very sensitive method in which the reagent gives intense pink colour even with traces of chromium which can be easily measured at 530 µm using potassium dichromate standard.

Statistical Analysis:

The data obtained in the feeding experiments on various parameters were subjected to the analysis of variance (ANOVA) and the least significant difference (LSD) was calculated in each case following Snedecor and Cochran (1973). The growth of the animals which was calculated as percentage was first changed to 'angular transfermations' and then subjected to ANOVA. The results of ANOVA in respect of each experiment were tabulated separately.

RESULTS

Purified Proteins

Evaluation of purified proteins

The results of evaluation of the four purified proteins for the early juveniles of the prawn Penaeus indicus, are presented in Table 10.

The feeding experiment carried out with the zero protein diet (PE_0) had given interesting results. There was a loss in weight in the body of the animals fed this diet. The animals lost 5.4% in live-weight and 4.8% in dry weight. Gradually the animals became less active and mortality occured. The survival was 60% at the end of the experiment. As a result of feeding zero protein diet the total body nitrogen came down to 9.91% from the initial value of 10.94%. The corresponding loss of body protein was 6.43%.

The excretion of nitrogen in the faeces and metabolic faecal nitrogen (MFN) of the animals fed with zero protein diet are given in Table 10 (A). There was variation in the nitrogen (N) excreted in the faeces by the animals. The lowest value of N excreted in the faeces was 1.04% and the highest value was 1.75%. The middle values of N excreted were 1.40%, 1.43% and 1.72%. The average value of N excreted was 1.46%. The MFN varied from 248.5 mg to 351.6 mg per 100 gram of diet, with an average of 326.4 mg per 100 gram of diet. Among the proteins tested albumen (egg) had produced the highest growth in length (52.1%), live-weight (359.4%) and dry weight (298.5%). The food conversion ratio (FCR) was 2.4 and the survival was 100%.

Table 10. Growth, food conversion ratio and survival of juvenile *P. indicus* fed with purified diets PE_0 to PE_4 for 30 days.

Particulars	Diet No.				
	PE_0	PE_1	PE_2	PE_3	PE_4
Initial average length (mm)	24.5	24.4	24.4	24.6	24.3
Initial average live weight (g)	0.0634	0.0631	0.0631	0.0636	0.0629
Initial average dry weight (g)	0.0168	0.0168	0.0168	0.0169	0.0167
Final average length (mm)	24.5	37.1	32.5	33.7	29.3
Final average live weight (g)	0.0600	0.2899	0.1840	0.1999	0.1376
Final average dry weight (g)	0.0160	0.0669	0.0396	0.0481	0.0274
Growth in length %	Nil	52.1^{a}	33.2^{b}	27.0^{c}	20.6^{d}
Growth in live weight %	-5.4	359.4^{a}	191.6^{b}	214.3^{b}	118.8^{bc}
Growth in dry weight %	-4.8	298.5^{a}	135.7^{b}	184.6^{c}	64.1^{d}
Food conversion ratio	--	2.40^{a}	4.17^{b}	5.08^{bc}	9.82^{d}
Survival %	60.0	100.0	53.3	100.0	53.0

Note: Values with same superscript are not significantly different among themselves. Growth in length, dry weight and food conversion ratio significant at 1% level ($P < 0.01$) and growth in live weight significant at 5% level ($P < 0.05$).

Table 10(A). Determination of metabolic Faecal nitrogen (MFN) in Juvenile P. indicus using zero protein diet.

Experiment No.	Chromium oxide %		Nitrogen in faeces %	Metabolic faecal nitrogen (MFN) (mg. of N per 100 g diet consumed)
	in diet	in faeces		
1	0.4342	1.8169	1.40	334.6
2	"	1.8169	1.04	248.5
3	"	1.8167	1.43	341.8
4	"	2.1500	1.72	347.4
5	"	2.1610	1.75	351.6
6	"	1.8167	1.40	334.6
	Average	1.9297	1.46	326.4

which were superior to the FCR and survival produced by the other proteins. Fibrin produced a growth of 27.0% in length, 214.3% in live-weight and 184.6% in dry weight, while casein obtained a growth of 33.2% in length, 191.62% in live-weight and 135.7% in dry weight. The FCR obtained by casein was 4.17 and that of fibrin was 5.08. However, the survival of the animals fed casein was low (53.3%) compared to that of the fibrin (100%). Gelatin produced low growth (20.6% in length, 118.8% in live-weight and 64.1% in dry weight), survival (53%) and poor food conversion ratio (9.82).

Interestingly gelatin had shown highest true digestibility (Table 10B) of 93.41 followed by fibrin, 82.42. The digestibility of albumen and casein was 72.48 and 76.44 respectively. While the diet with fibrin had shown the highest Net Protein Utilization (NPU), of 53, the NPU obtained by the diets with albumen, casein and gelatin were 43, 36 and 38 respectively. The Biological Value (BV) of albumen was 59 and that of casein, fibrin were 49 and 65 respectively. The BV obtained by gelatin was 39.

The Protein Efficiency Ratio (PER) produced by albumen was 1.34, whereas the PER obtained by casein, fibrin and gelatin respectively was 0.76, 0.67 and 0.27.

Analysis of variance (ANOVA) of the data (Table 10C) on growth, FCR, PER, true digestibility (TD), NPU and BV obtained by the four proteins had shown that the growth, FCR, PER and TD differ significantly among the treatments. The growth in length dry weight and PER were significant at 1% level ($P < 0.01$) and the growth in live weight, FCR and TD were significant at 5% level

Table 10(B). Digestibility, PER, NPU, and BV obtained by the diets PE_1, PE_2, PE_3 and PE_4 in juvenile P. indicus.

Diet No.	% Crude Protein			Chromium oxide %		True digestibility	PER	NPU	BV
	In the diet.	in animals after feeding with diets	In the faeces	in diet	in faeces				
PE_0	0.5	61.93	8.01	0.4342	1.8169	--	--	--	--
PE_1	31.86	68.07	32.62	0.4561	1.3394	72.48^a	1.34^a	43	59
PE_2	32.75	69.47	18.71	0.3772	0.8190	76.44^b	0.76^b	36	49
PE_3	30.60	71.20	29.05	0.4397	1.6327	82.42^c	0.67^b	53	65
PE_4	39.09	66.13	14.64	0.4238	1.8265	93.41^d	0.27^c	38	39

Note: Values with same superscript are not significantly different among themselves. Digestibility and PER significant at 1% ($P < 0.01$), NPU and BV not significant at 5% level ($P < 0.05$)

Table 10(C). Analysis of variance of the data obtained by diets PE_1, PE_2, PE_3 and PE_4.

Source	Degrees of freedom	Sum of squares	Mean sum of squares
1	2	3	4
1. Growth in length			
Treatment	3	602.26	200.75**
Error	8	13.22	1.65
Total	11	615.48	
2. Growth in live-weight			
Treatment	3	119321.38	39773.99*
Error	8	31666.96	3953.37
Total	11	150988.34	
3. Growth in dry-weight			
Treatment	3	84971.03	28323.67**
Error	8	2402.52	303.3
Total	11	87373.55	
4. Food conversion ratio			
Treatment	3	90.47	30.16*
Error	8	12.48	1.56
Total	11	102.95	
5. Protein Efficiency Ratio			
Treatment	3	1.7368	0.5789**
Error	8	0.1024	0.0128
Total	11	1.8392	
6. Digestibility			
Treatment	3	556.63	185.54*
Error	8	119.57	14.95
Total	11	686.20	

Table 10(C) (continued)

1	2	3	4
7. Net Protein Utilization			
Treatment	3	110.17	36.72^{N}
Error	8	35.00	4.42
Total	11	145.17	
8. Biological Value			
Treatment	3	11.49	3.83^{N}
Error	8	18.16	2.27
Total	11	29.65	6.10

** Significant at 1% level ($P < 0.01$)

* Significant at 5% level ($P < 0.05$)

N Not significant at 5% level.

($P < 0.05$). However, NPU and BV did not show any significant difference among the treatments ($P < 0.05$). The growth, FCR, and PER obtained by the diet with albumen were significantly higher than those with casein, fibrin and gelatin. There was no significant difference in the growth in live-weight and FCR obtained by the diets with casein and fibrin. The growth in length, dry weight and FCR of the diet with gelatin were significantly lower than those with casein and fibrin. However, no significant difference was observed in growth in live-weight produced by the three diets, with casein, fibrin and gelatin. The TD of gelatin is significantly higher followed by those of fibrin, casein and albumen. But the PER of albumen was significantly higher compared to the PER obtained by casein, fibrin and gelatin. No significant difference in the PER of casein and fibrin was observed, however, the PER of both casein and fibrin was significantly different to that of gelatin.

Comparison of the evaluation results (Fig. 1 & 2) of the four purified proteins albumen, casein, fibrin and gelatin had shown that albumen registered highest growth (Fig. 1a and 1b) and protein efficiency ratio (Fig. 1d) and the best food conversion ratio (Fig. 1c). While the growth, FCR and PER obtained by casein and fibrin were comparable, gelatin gave poor results. Gelatin showed highest true digestibility (Fig.2b) followed by fibrin, casein and albumen in decreasing order. Fibrin showed highest BV,(Fig.2c) followed by albumen. Gelatin had a very low BV. While fibrin showed comparitively higher NPU (Fig.2a) followed by albumen, there was no difference in NPU obtained by casein and gelatin.

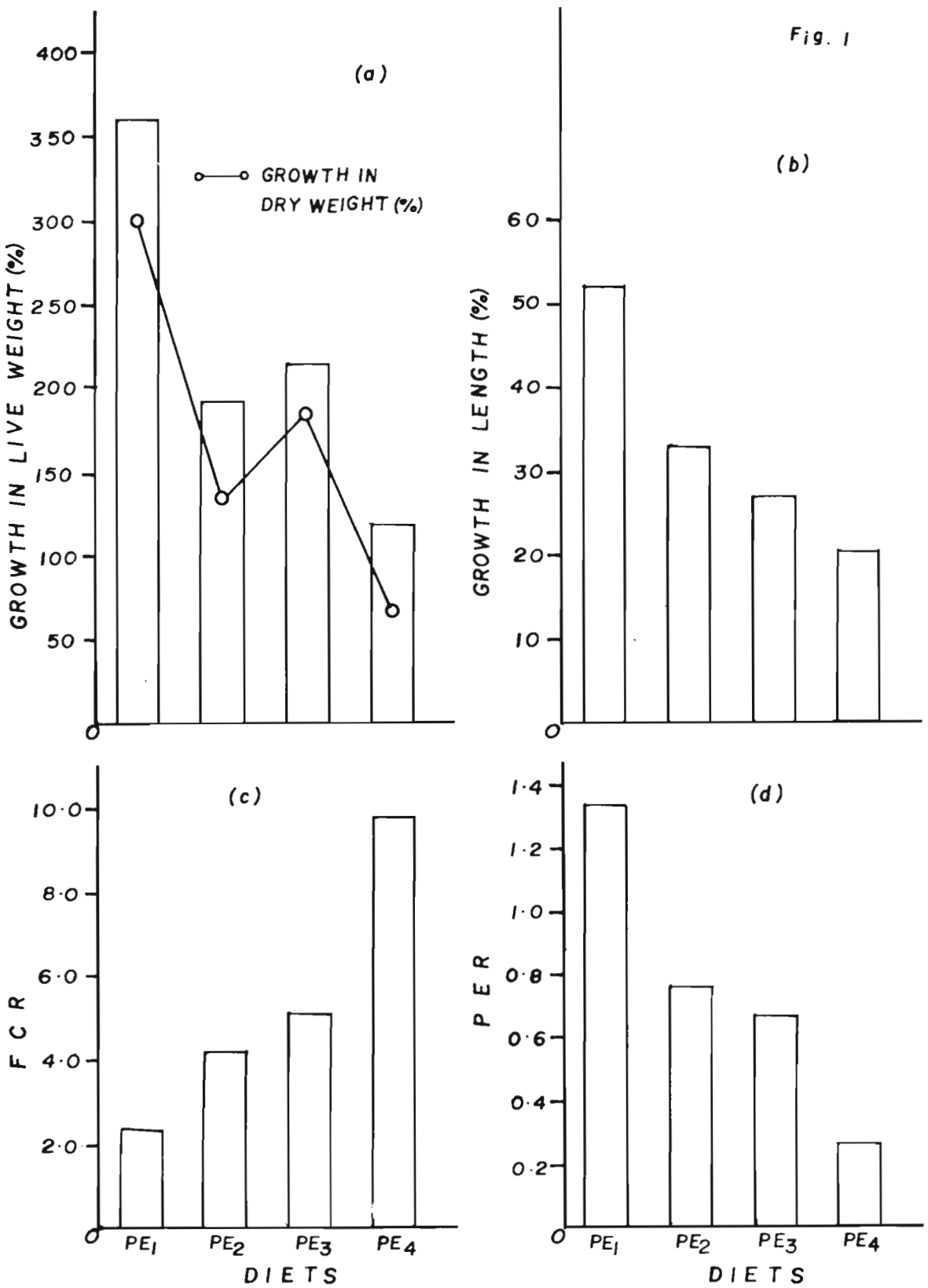

Figure 1. Evaluation of Purified Proteins for juvenile P. indicus. (a) growth in weight (b) growth in length (c) food conversion ratio (FCR) and (d) Protein efficiency ratio (PER)

Fig. 2

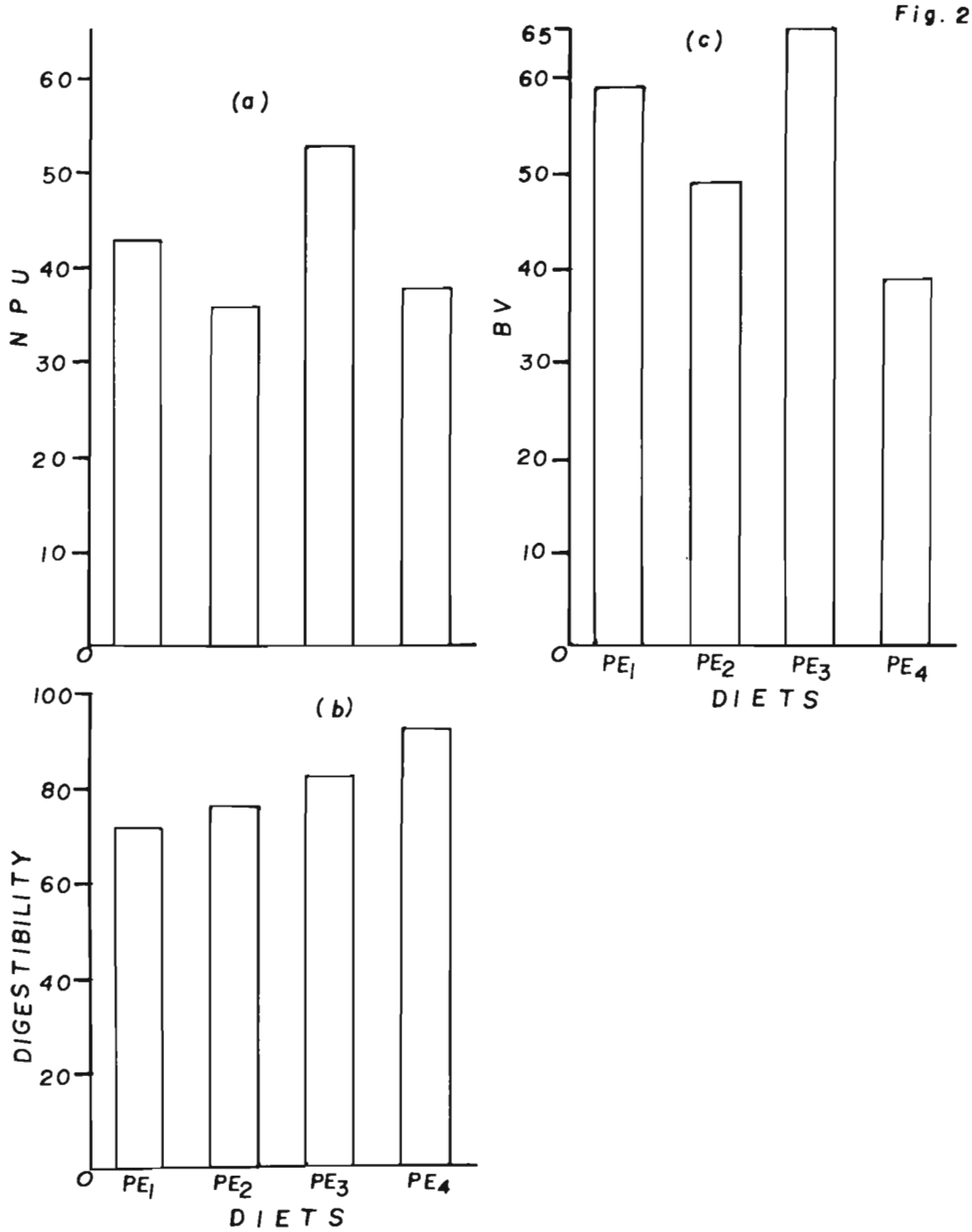

Figure 2. Evaluation of Purified Proteins for juvenile P. indicus (a) Net Protein Utilization (NPU) (b) digestibility and (c) biological value (BV).

Evaluation of albumen

The results of the feeding experiment conducted with diets PE_5 to PE_{10} are given in Table 11. As observed in the first experiment the animals fed with zero protein diet had shown negative growth. The loss in weight was 6.4% in live-weight and 5.8% in dry weight. There was a slight decline of 0.9% in length. The diets PE_6, PE_7, PE_8, PE_9 and PE_{10} produced a growth of 32.2%, 59.1%, 32.0%, 32.3% and 34.6% in length respectively. The corresponding growth in live-weight was respectively 146.7%, 256.6%, 138.7%, 148.8% and 126.3%, and the growth in dry weight being 154.5%, 262.7%, 154.2%, 155.8% and 167.1% respectively. The food conversion ratio obtained by the five diets, varied between 3.49 and 8.49, the maximum being for the diet PE_8. The survival of the animals in the case of zero protein diet (PE_5) was 30%. It was 45.8%, 75.0%, 75.0%, 50.0% and 58.3% in the case of the diets PE_6 to PE_{10} respectively.

The true digestibility (TD) (Table 11A)of protein in the diets PE_6 to PE_{10} was 87.34, 91.43, 79.35, 91.72 and 96.64 respectively, while the PER in respect of these diets was 0.6, 1.16, 0.43, 0.46 and 0.56. The NPU was varying from 33.90 to 47.39. The BV was highest in the diet PE_8 followed by PE_7, PE_9, PE_{10} and PE_6 in the decreasing order.

The ANOVA data is presented in Table 11B. The growth produced by the diet PE_7, in length, and dry weight was significantly higher ($P < 0.01$) compared to that of the other diets. No significant difference in the growth in length, live-weight and dry weight was found among the diets PE_6, PE_8, PE_9 and PE_{10}. The FCR obtained by different diets was not found significantly

Table 11. Growth, food conversion ratio and survival of juvenile P. indicus fed with albumen diets PE_5 to PE_{10}, for 30 days.

Particulars	Diet No.					
	PE_5	PE_6	PE_7	PE_8	PE_9	PE_{10}
Initial average length (mm)	22.7	22.7	22.5	22.5	22.6	22.8
Initial average live weight(g)	0.0658	0.0658	0.0652	0.0654	0.0658	0.0661
Initial average dry weight (g)	0.0154	0.0154	0.0153	0.0153	0.0154	0.0155
Final average length (mm)	22.5	30.0	35.8	29.7	29.9	30.7
Final average live weight(g)	0.0616	0.1623	0.2325	0.1561	0.1637	0.1496
Final average dry weight(g)	0.0145	0.0392	0.0555	0.0389	0.0394	0.0414
Growth in length %	-0.9	32.2^b	59.1^a	32.0^b	32.3^b	34.6^c
Growth in live weight %	-6.4	146.7^b	256.6^a	138.7^b	148.8^b	126.3^b
Growth in dry-weight %	-5.8	154.5^b	262.7^a	154.2^b	155.8^b	167.1^b
Food conversion ratio	--	6.15	3.49	8.49	8.47	3.63
Survival %	30.0	45.8	75.0	75.0	50.0	58.3

Note: Values with different superscripts are significantly different among themselves. Growth in length, live weight and dry weight significant at 1% level ($P < 0.01$). Food conversion ratio not significant at 5% level ($P < 0.05$).

Table 11 (A). Digestibility, PER, NPU and BV obtained by the albumen diets PE_6, PE_7, PE_8, PE_9 and PE_{10} in juvenile P. indicus.

Diet No.	Crude protein %			Chromium oxide %		True digestibility	PER	NPU	BV
	In diet	In animals after feeding with diets	In faeces	In diet	In faeces				
PE_5	0.5	61.19	8.97	0.4342	1.8167	--	--	--	--
PE_6	16.77	67.05	28.85	0.5734	2.3269	87.34	0.60^b	33.90	33.81
PE_7	24.75	70.90	47.22	0.6800	1.9690	91.43	1.16^a	45.86	50.16
PE_8	33.31	69.17	22.94	0.6340	1.2819	79.35	0.43^b	47.39	59.72
PE_9	41.28	68.10	12.14	0.6134	1.3698	91.72	0.46^b	44.70	48.74
PE_{10}	51.47	67.78	12.86	0.5260	1.5317	96.64	0.56^b	38.71	40.06

Note: Values with different superscripts differ significantly among themselves. PER significant at 5% level ($P < 0.05$). Digestibility, NPU, and BV not significant at 5% level ($P < 0.05$).

Table 11 (B). Analysis of variance of the data obtained by albumen diets PE_6, PE_7, PE_8, PE_9 and PE_{10}.

ANOVA			
Source	D.F.	Sum of squares	Mean sum of squares
1	2	3	4
1. Growth in length			
Treatment	4	1430.60	357.65**
Error	10	190.73	19.07
Total	14	1621.33	
2. Growth in Live-weight			
Treatment	4	29318.69	7329.67*
Error	10	10363.35	1036.33
Total	14	39682.04	
3. Growth in dry weight			
Treatment	4	124688.81	31172.20**
Error	10	6775.85	677.58
Total	14	131464.66	
4. Food conversion ratio			
Treatment	4	266.41	66.6^{N}
Error	10	113.54	11.35
Total	14	379.95	
5. Protein efficiency ratio			
Treatment	4	2.17	0.54*
Error	10	0.52	0.05
Total	14	2.69	

Table 11 (B) Continued.

1	2	3	4
6. Digestibility			
Treatment	4	685.67	171.42^{N}
Error	10	421.87	42.19
Total	14	1007.54	
7. Net Protein Utilisation			
Treatment	4	382.97	95.74^{N}
Error	10	547.80	16.48
Total	14	930.77	
8. Biological Value			
Treatment	4	33.85	8.46^{N}
Error	10	17.29	1.80
Total	14	51.14	

** Significant at 1% level ($P < 0.01$)

* Significant at 5% level ($P < 0.05$)

N Not significant at 5% level.

different at 5% level ($P < 0.05$), while only the PER of the diet PE_7 was significantly higher ($P < 0.05$) from that of all the other diets, the TD, NPU and BV had not shown any significant difference among the different diets at 5% level ($P < 0.05$).

The relationship between the dietary protein and the growth of P. indicus is shown in Fig. 3. The growth of Prawns in length (Fig. 3b) and weight (Fig.3a) increased as the protein in the diet increased from 0 to 25%. When the protein in diet further increased to 33% the growth, both in length and weight, declined. The growth curves leveled off as the protein in diet increased from 33.3 to 51.5%. The PER was the highest at 25% protein in the diet (Fig. 4a) and decreased at 33% protein and levelled off as the protein in the diet further increased to 51.5%. The diet with 25% protein recorded the lowest FCR (Fig. 4b), showing the high efficiency of the diet. Higher protein content (33.3% and 41.3%) in the diet only increased the FCR showing low efficiency of the diet. The diet with 51% protein had again shown low FCR, comparable to that of the diet with 25%.

True digestibility (Fig.5a) of the protein was seen increasing in the lower levels of protein, but decreased at 33.3% protein level and to increase again at higher levels. The NPU gradually increased (Fig.5b) with dietary protein level upto 33.3% and showed declining tendency as the dietary protein raised from 33.3% to 51.5%. Similarly the BV of the protein increased (Fig. 5c) with increase in the dietary protein and recorded a maximum value at 33.3% protein level. Thereafter it started declining with increase in the dietary protein.

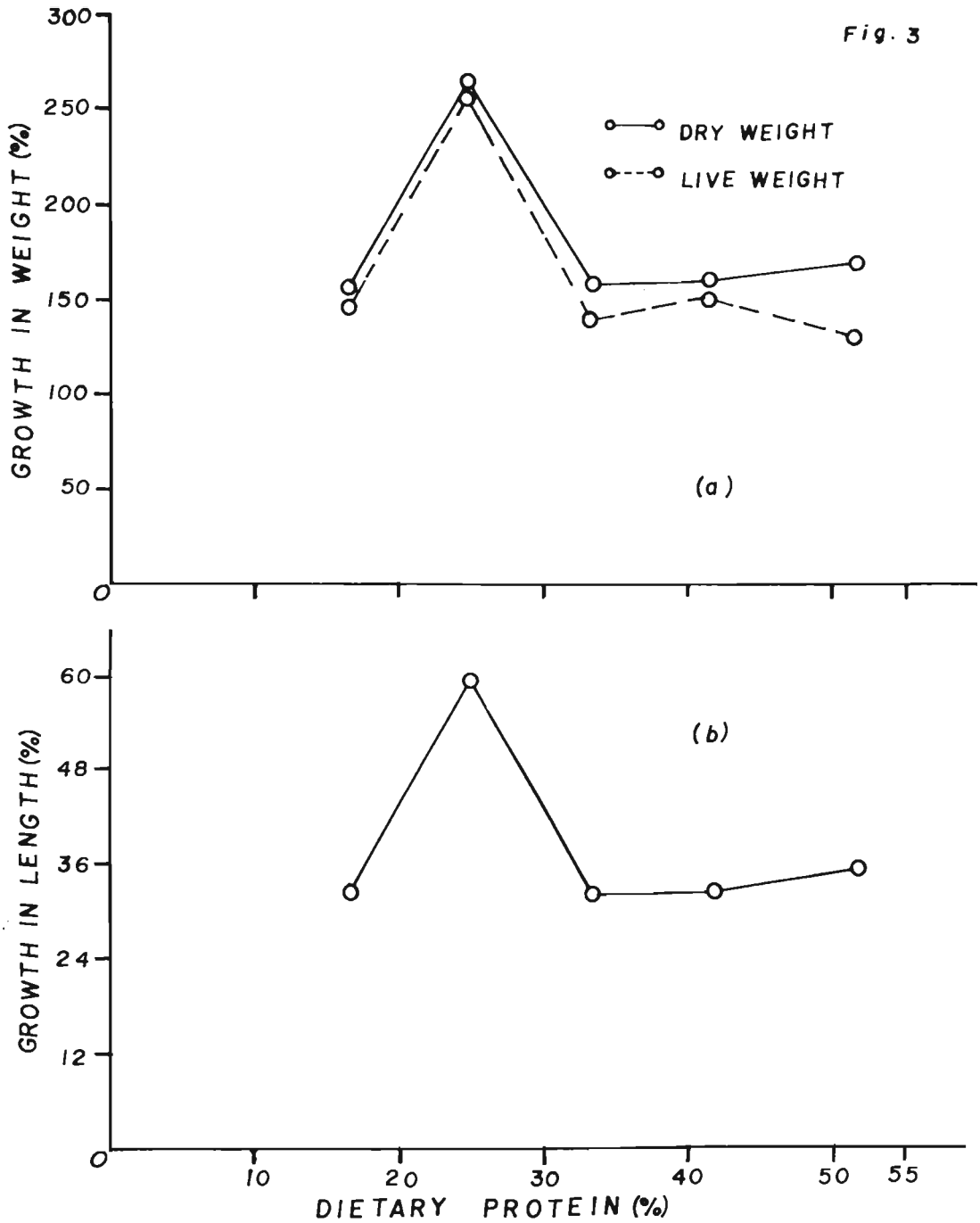

Figure 3. Evaluation of albumen diets for juvenile P. indicus. Relationship between dietary proteins and (a) growth in weight and (b) growth in length.

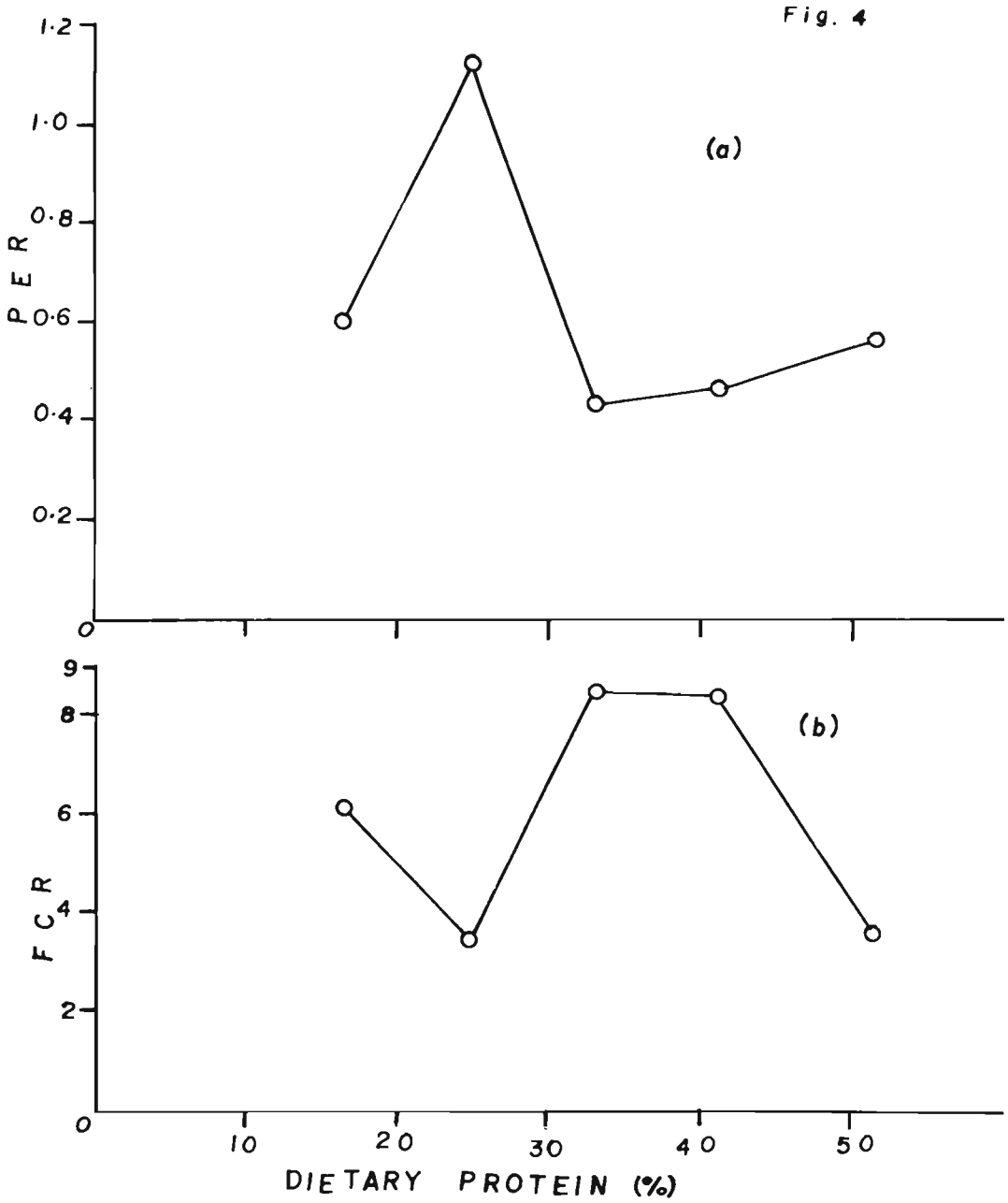

Figure 4. Evaluation of albumen diets for juvenile P. indicus. Relationship between dietary protein and (a) protein efficiency ratio (PER) and (b) food conversion ratio (FCR)

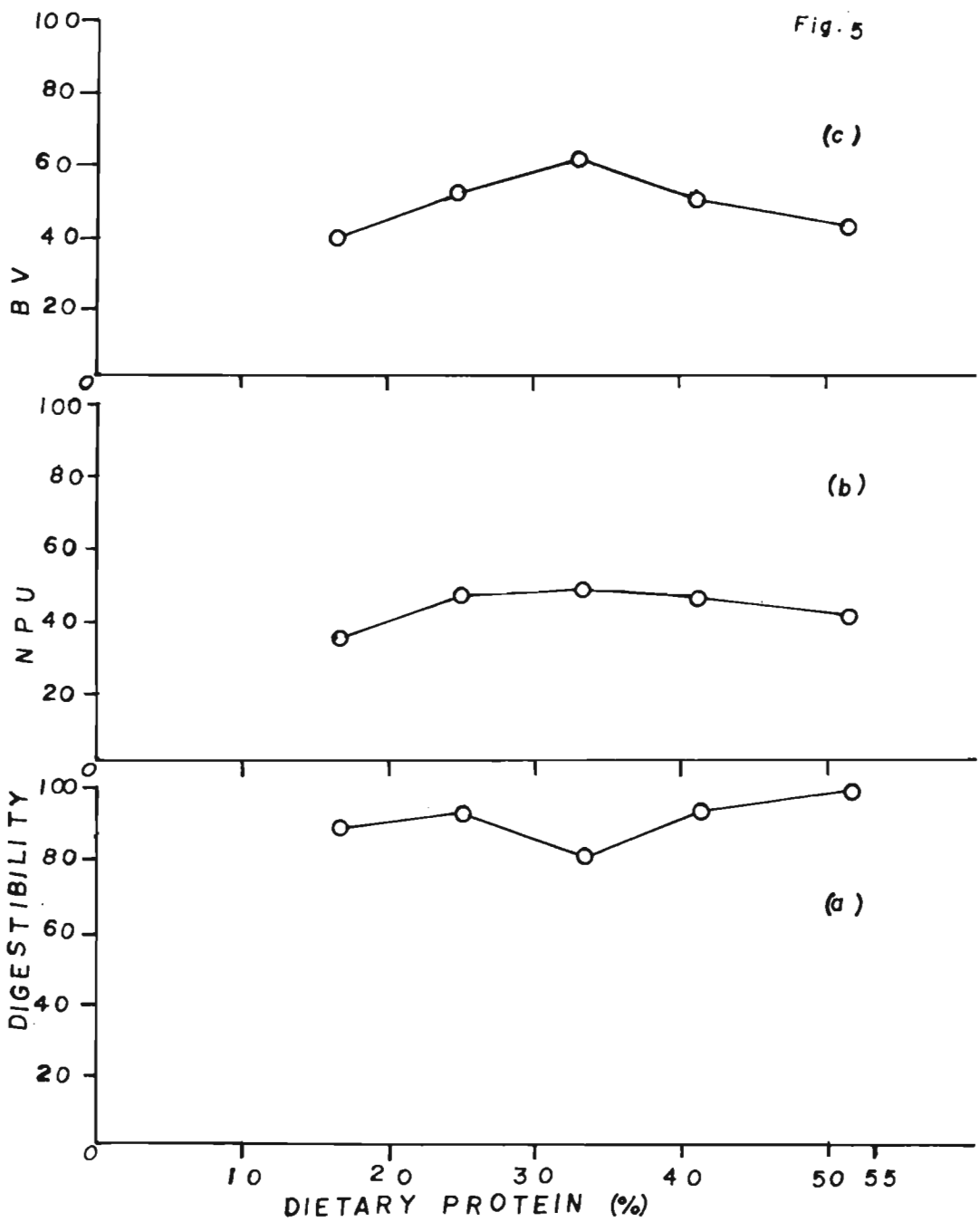

Figure 5. Evaluation of albumen diets for juvenile P. indicus. Relationship between dietary protein and (a) digestibility, (b) net protein utilisation (NPU) and (c) biological value (BV)

Evaluation of casein

From the results presented in Table 12, it can be seen that the diet PE_{12} realised the highest growth of 48.8% in length, 257.5% in live-weight and 238.8% in dry weight. The diets PE_{11}, PE_{13}, PE_{14} and PE_{15} had produced a growth of 45.2%, 36.1%, 24.1% and 28.0% in length, 179.5%, 137.6%, 90.1% and 111.4% in live weight and 122.3%, 106.9%, 82.2% and 93.7% in dry weight respectively. The FCR obtained by the diet PE_{14} was relatively higher (10.67), followed by PE_{13}, PE_{11}, and PE_{15} while higher survival rate was recorded with the diets PE_{11} and PE_{12}.

The protein in the diets PE_{11} to PE_{15} had shown a true digestibility (Table 12A) of 92.45, 95.05, 79.15, 98.52 and 97.37 and PER of 0.65, 0.92, 0.27, 0.19 and 0.34 respectively. As in the case of TD and PER, NPU and BV were greatest in PE_{12}. Analysis of variance of the data (Table 12 B) had shown that the treatments were highly significant among themselves. The growth in length and dry weight, TD, PER, NPU and BV were significant at 1% level ($P < 0.01$) while the growth in live-weight and FCR were significant at 5% level ($P < 0.05$).

The growth of the animals in length (Fig. 6b) and weight (Fig. 6a) increased with the dietary protein up to 29.3% and declined thereafter. The growth was significantly higher at this protein level in the diet (PE_{12}) than at the other protein levels. The same diet (PE_{12}) had shown highest PER (Fig.7b) and low FCR (Fig. 7a) showing higher efficiency of the diet. Higher protein levels in the diet lowered the PER and produced higher food conversion ratio decreasing the efficiency of the diet.

Table 12. Growth, food conversion ratio and survival of juvenile *P. indicus* fed with casein diets PE_{11} to PE_{15} for 30 days.

Particulars	Diet No.				
	PE_{11}	PE_{12}	PE_{13}	PE_{14}	PE_{15}
Initial average length(mm)	23.9	24.0	24.1	24.1	24.0
Initial average live-weight (g)	0.0605	0.0607	0.0609	0.0609	0.0623
Initial average dry weight (g)	0.0152	0.0151	0.0153	0.0153	0.0156
Final average length(mm)	34.7	35.7	32.8	29.9	31.5
Final average live-weight (g)	0.1691	0.2170	0.1446	0.1158	0.1317
Final average dry weight (g)	0.0337	0.0511	0.0316	0.0278	0.0302
Growth in length %	45.2^{a}	48.8^{a}	36.1^{b}	24.1^{c}	28.0^{bc}
Growth in live-weight %	179.5^{a}	257.5^{b}	137.4^{ac}	90.1^{cd}	111.4^{cd}
Growth in dry weight %	121.7^{a}	238.4^{b}	106.5^{ac}	81.7^{cd}	93.6^{cd}
Food conversion ratio	7.89^{a}	5.96^{ab}	9.92^{c}	10.67^{c}	5.50^{bd}
Survival %	66.0	62.5	50.0	45.8	58.0

NB: Values with different superscripts are significantly different among themselves. Growth in length, growth in dry weight are significant at 1% level ($P < 0.01$) and growth in live-weight and food conversion ratio significant at 5% level ($P < 0.05$).

Table 12(A). Digestibility, PER, NPU, and BV obtained by the casein diets PE_{11}, PE_{12}, PE_{13} PE_{14} and PE_{15} in juvenile P. indicus.

Diet No.	Crude protein %			Chromium oxide %		True digestibility	PER	NPU	BV
	in diet	in animals after feeding diets	in faeces	in diet	in faeces				
PE_{11}	17.7	63.85	25.59	0.5622	3.675	92.45^{a}	0.65^{a}	47.13^{a}	50.93^{a}
PE_{12}	29.3	71.14	16.41	0.5300	2.250	95.05^{a}	0.92^{a}	66.69^{b}	70.33^{b}
PE_{13}	37.4	68.00	31.31	0.5328	2.140	79.15^{b}	0.27^{b}	43.49^{a}	55.33^{ca}
PE_{14}	48.4	63.44	17.50	0.4527	2.520	98.52^{a}	0.19^{c}	28.68^{c}	29.67^{d}
PE_{15}	53.4	64.91	15.18	0.6089	2.410	97.37^{a}	0.34^{abc}	51.68^{d}	53.00^{ac}

N.B. Values with different superscripts are significantly different among themselves. True digestibility, PER, NPU and BV are significant at 1% level ($P < 0.01$)

Table 12(B). Analysis of variance of the data obtained by casein diets PE_{11}, PE_{12}, PE_{13}, PE_{14} and PE_{15}

ANOVA

Source	D.F.	Sum of squares	Mean sum of squares
1	2	3	4
1. Growth in length			
Treatment	4	1331.80	332.95**
Error	10	146.96	14.69
Total	14	1478.76	347.64
2. Growth in Live-weight			
Treatment	4	53186.76	13296.69*
Error	10	9354.86	935.49
Total	14	62541.62	14232.18
3. Growth in dry weight			
Treatment	4	48076.46	12019.11**
Error	10	1802.39	180.24
Total	14	49878.85	12199.35
4. Food conversion ratio			
Treatment	4	63.88	15.97*
Error	10	11.96	1.19
Total	14	75.84	17.16

Table 12(B) continued.

1	2	3	4
5. Protein efficiency ratio			
Treatment	4	1.12	0.28**
Error	10	0.06	0.006
Total	14	1.18	0.286
6. Digestibility			
Treatment	4	744.04	186.01**
Error	10	88.67	8.87
Total	14	832.71	194.88
7. Net Protein Utilisation			
Treatment	4	2270.69	567.67**
Error	10	50.06	5.01
Total	14	2320.75	572.68
8. Biological Value			
Treatment	4	2633.92	658.48**
Error	10	149.36	14.94
Total	14	2783.28	673.42

** Significant at 1% level ($P < 0.01$)

* Significant at 5% level ($P < 0.05$)

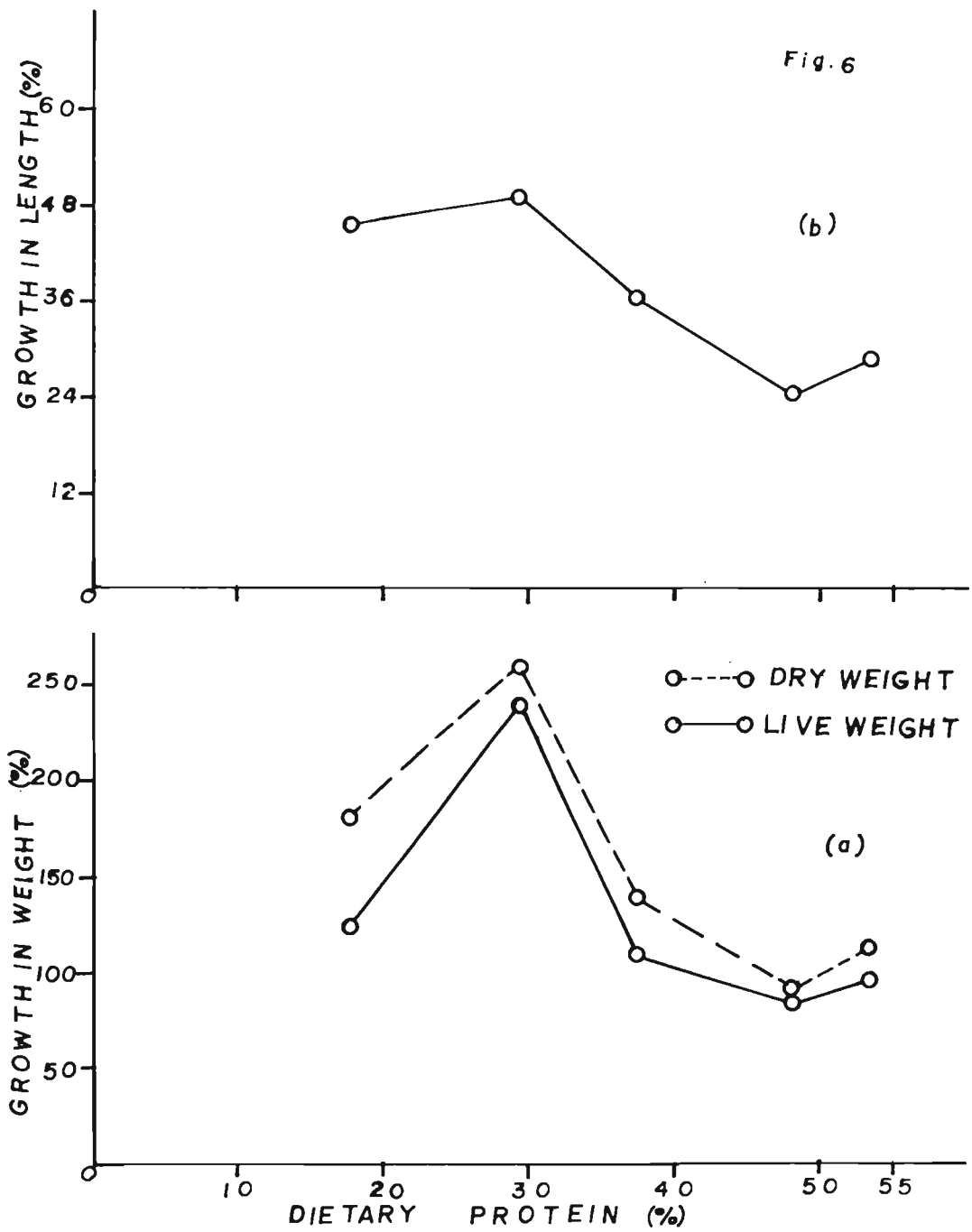

Figure 6. Evaluation of casein diets for Juvenile P. indicus. Effect of dietary protein on (a) growth in weight and (b) growth in length.

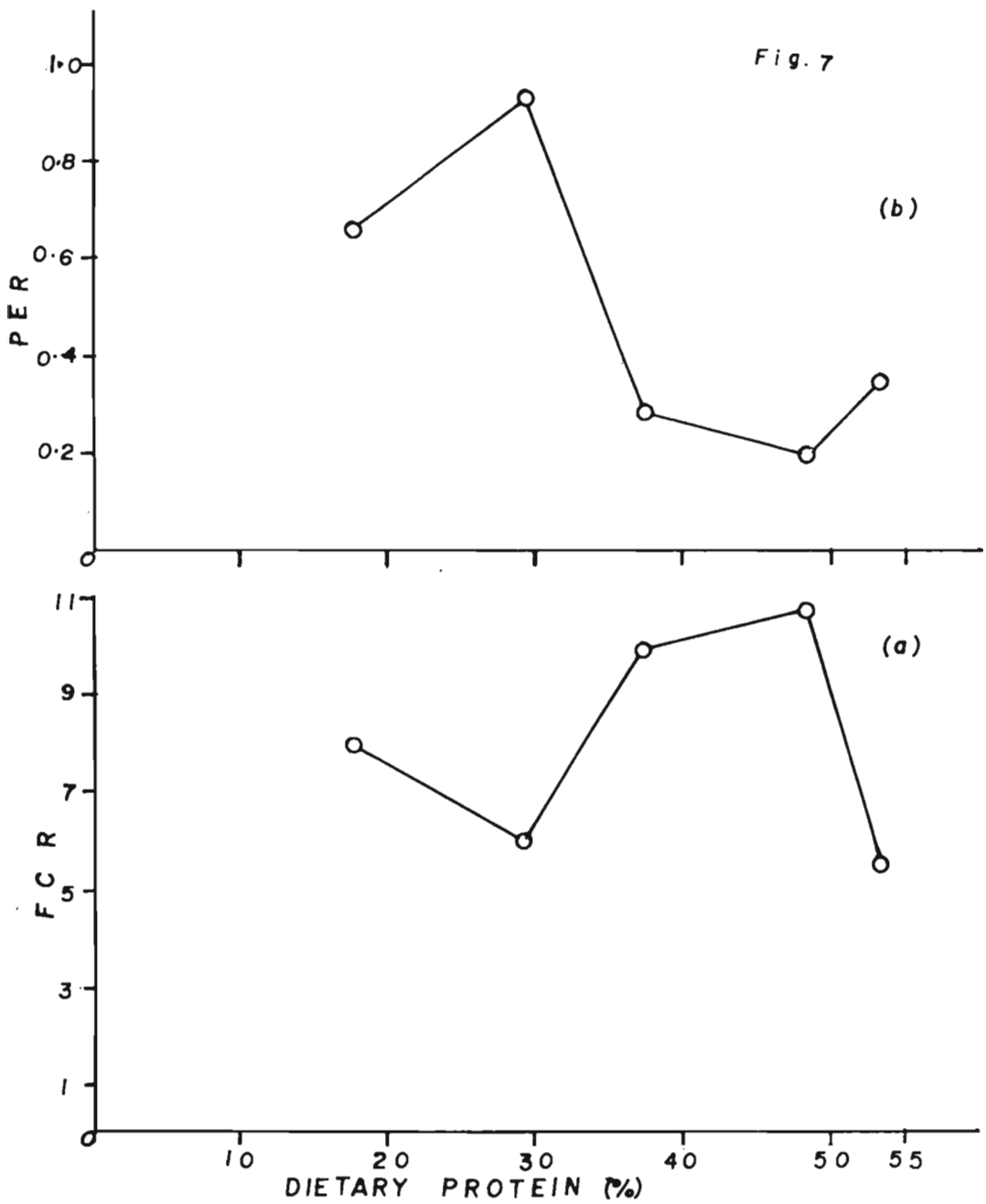

Figure 7. Evaluation of casein diets for Juvenile P. indicus. Effect of dietary protein on (a) food conversion ratio (FCR) and (b) Protein efficiency ratio (PER)

The TD (Fig. 8a) of protein tended to be higher at higher levels of protein in the diet, though the values were not significantly different except that of the diet with 37.4% protein (diet PE_{13}) which had shown low TD, significantly different from that of the other diets. The NPU (Fig. 8b) had shown a peak at 29.3% protein in the diet and decreased below and above this protein level. Similarly the BV (Fig. 8c) increased with dietary protein level with a peak at 29.3% protein and showed a downward trend with higher levels of dietary protein.

The results obtained with casein as protein source are practically similar to the results obtained with albumen as protein source in experiment 2. Both the protein sources apparently showed same effect of dietary protein level on growth, FCR, PER, digestibility, NPU and BV.

Dietary Protein level and excretion of faecal nitrogen

The excretion of faecal nitrogen (Fig. 9a) per 100 gm of the diet gradually increased with the increase in dietary protein level and registered a peak at a particular Protein level and decreased thereafter. The trend was similar with albumen and casein, when used separately as protein sources. In case of albumen, the faecal nitrogen per 100 g diet increased from 326.4 mg to 2667.5 mg as the protein in the diet increased from zero to 25%. From 2667.5 mg, it gradually came down to 706.6 mg as the protein in the diet further increased from 25% to 51.5%. In the case of casein the peak of maximum faecal nitrogen shifted to 37% protein in the diet. However, the faecal nitrogen decreased with further increase in the

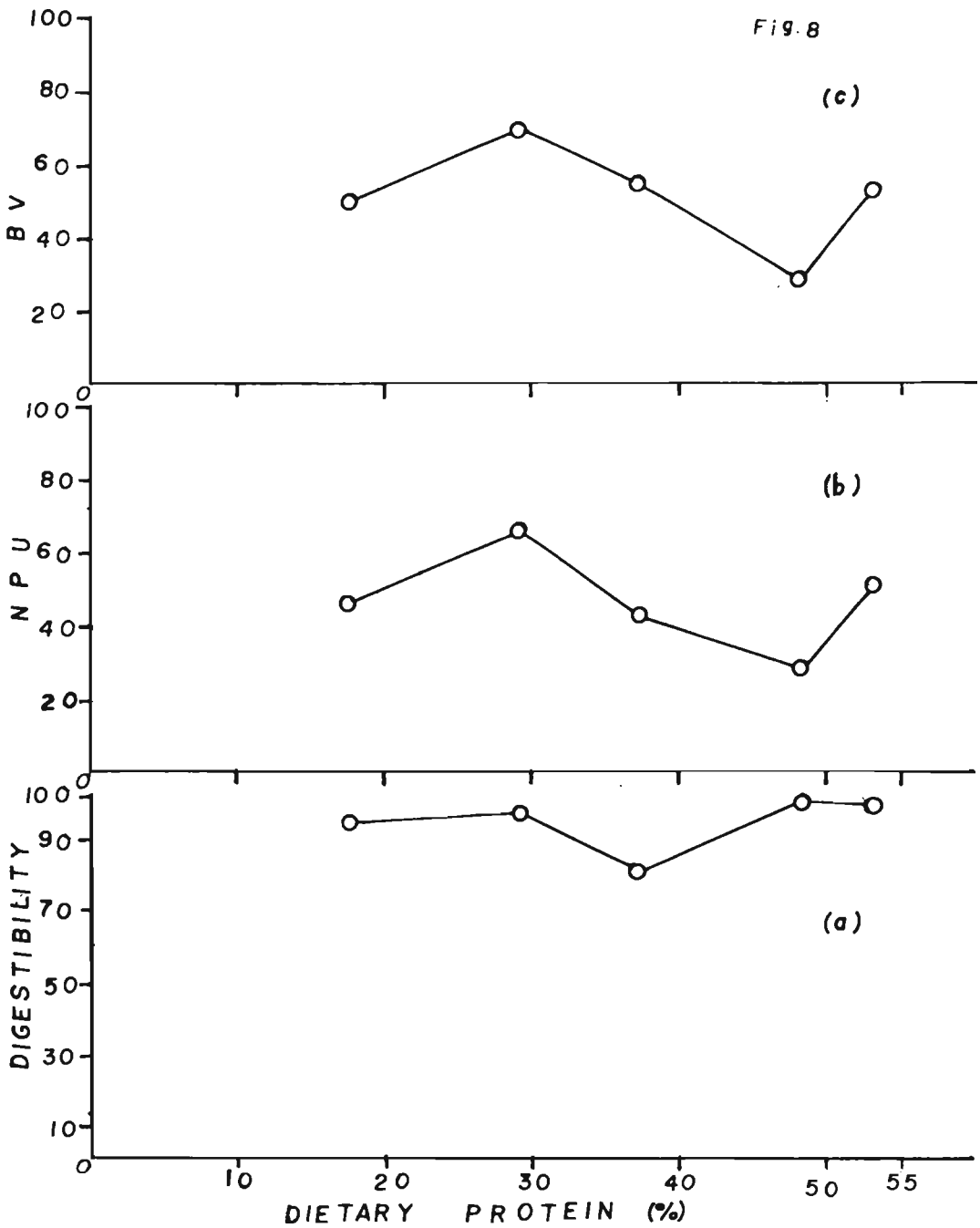

Figure 8. Evaluation of casein diets for juvenile P. indicus. Effect of dietary protein on (a) digestibility (b) net protein utilization (NPU) and (c) biological value.

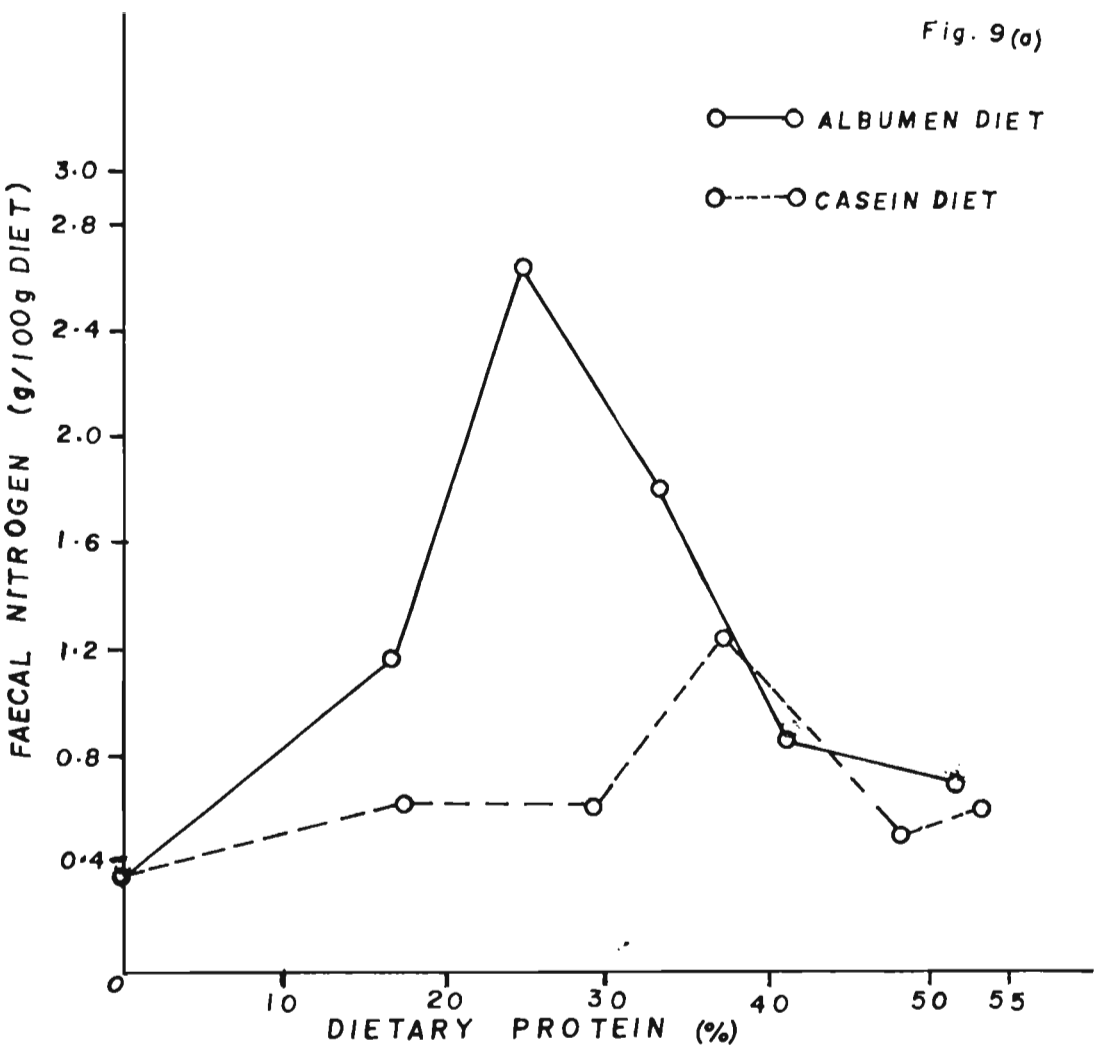

Figure 9(a). Relationship between dietary protein and faecal nitrogen in Juvenile P. indicus.

dietary protein level. The excretion of nitrogen was higher in the animals fed with albumen diet than those fed with casein diet.

Nitrogen balance

The nitrogen balance was calculated as the difference in the nitrogen of the diet and the nitrogen in the faeces of the animals fed that diet. The relationship between the dietary protein and the nitrogen balance is presented in Fig.9b. At lower dietary protein, the nitrogen balance was negative and as the protein in the diet increased, the nitrogen balance became positive and gradually increased. The results were practically similar between albumen and casein, when used as protein sources separately. In both the cases, the nitrogen balance curve intercepted the protein axis (Y-axis) at 22.5% protein. At this protein level, the nitrogen balance was zero, below which it was negative and above this level, it was positive.

Evaluation of mixed protein sources.

The results of the experiment with the diets PE_{16} to PE_{26}, in which the effect of mixed protein source in the diet on different parameters was tested, are given in Table 13. Among the different combination of purified proteins tested, the diet PE_{22} having the four proteins, albumen, casein, fibrin and gelatin in equal proportions had produced the highest growth in length (49.8%), live weight (321.4%) and dry weight (371.1%). The same diet had also shown lowest FCR (3.82) and high survival (87.5%). This was followed by the diets PE_{16}, PE_{17}, PE_{18} and PE_{19} having albumen-casein, albumen-gelatin,

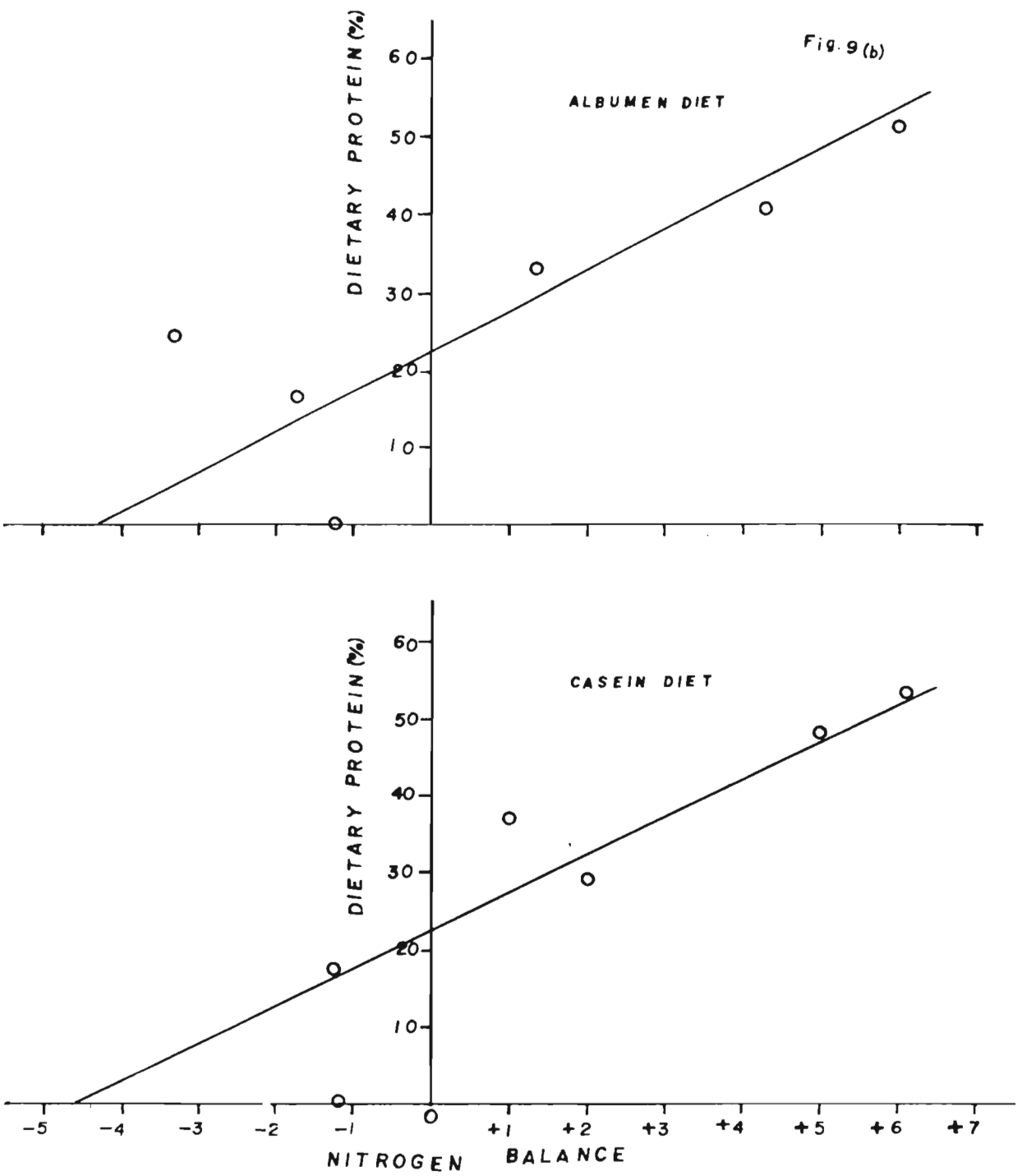

Figure 9(b). Relationship between dietary protein and nitrogen balance in juvenile P. indicus.

Table 13. Growth, food conversion ratio and survival of juvenile P. indicus fed the purified diets PE_{16} to PE_{22} for 30 days.

Particulars	Diet No.						
	PE_{16}	PE_{17}	PE_{18}	PE_{19}	PE_{20}	PE_{21}	PE_{22}
Initial average length (mm)	20.3	20.3	20.3	20.3	20.3	20.3	20.3
Initial average live weight(g)	0.0341	0.0341	0.0341	0.0341	0.0341	0.0341	0.0341
Initial average dry weight (g)	0.0083	0.0083	0.0083	0.0083	0.0083	0.0083	0.0083
Final average length (mm)	28.3	28.7	28.5	27.1	26.7	26.6	30.4
Final average live weight (g)	0.1121	0.1102	0.1178	0.1205	0.1010	0.0855	0.1437
Final average dry weight (g)	0.0291	0.0306	0.0276	0.0268	0.0251	0.0228	0.0391
Growth in length %	38.9^{b}	41.4^{b}	40.4^{b}	33.5^{c}	31.5^{c}	31.0^{c}	49.8^{a}
Growth in live-weight %	228.7^{bc}	223.2^{bc}	245.5^{b}	253.4^{b}	196.2^{cd}	150.7^{de}	321.4^{a}
Growth in dry weight %	250.6^{bc}	268.7^{b}	232.5^{cd}	222.9^{de}	202.4^{ef}	174.7^{g}	371.1^{a}
Food conversion ratio	7.05^{b}	7.45^{b}	9.30^{bc}	7.11^{b}	9.94^{cd}	10.32^{cd}	3.82^{a}
Survival %	62.5	75.0	37.5	75.0	50.0	37.5	87.5

Note: Values with different superscripts differ significantly among themselves. The growth in length, live weight, dry weight and FCR were significant at 1% level ($P < 0.01$)

Table 13(A). Analysis of variance of the data obtained by purified diets PE_{16}, PE_{17}, PE_{18}, PE_{19}, PE_{20}, PE_{21} and PE_{22}.

ANOVA

Source	D.F.	Sum of squares	Mean sum of squares
1	2	3	4
1. Growth in length			
Treatment	6	268.75	44.79**
Error	14	67.57	4.82
Total	20	336.32	49.61
2. Growth in live weight			
Treatment	6	49571.03	8261 .63**
Error	14	5601.73	400.12
Total	20	55172.76	8661.75
3. Growth in dry weight			
Treatment	6	71626.92	11937.82**
Error	14	1400.00	100.00
Total	20	73026.92	12037.82
4. Food conversion Ratio			
Treatment	6	90.48	15.08**
Error	14	9.87	0.705
Total	20	100.35	15.785

** Significant at 1% level ($P < 0.01$)

albumen-fibrin and casein-gelatin as protein combinations respectively. Analysis of variance of the data (Table 13A), had shown that there was significant difference ($P < 0.01$) in the growth and FCR obtained by these diets. The diets PE_{20} and PE_{21} having casein-fibrin and fibrin-gelatin as protein combinations, had produced significantly low growth and high FCR compared to all the other diets. The survival of the animals fed with the diets PE_{18}, PE_{20} and PE_{21} was very low compared to that of the other diets.

The performance of the diet having all the four proteins as a mixture was significantly superior (Fig. 10) over the performance of all the other combinations. Albumen-casein, albumen-gelatin and casein-gelatin had shown practically identical results. Though, the growth of prawns obtained by the albumen-fibrin combination (diet PE_{18}) was comparable to that of the diets PE_{16}, PE_{17}, and PE_{19}, the FCR was high and survival was very low. The combinations, casein-fibrin and fibrin-gelatin were distinctly inferior to all the other combination of proteins tested.

Natural protein sources.

Evaluation of relative efficiencies of natural protein sources.

The results of evaluation of the five animal protein sources, clam meat powder (CMP), fish meal (FM), mantis shrimp (MS), prawn waste (PW) and silkworm pupa (SWP) for *P. indicus* are given in Table 14. The diet PE_{26} prepared with PW registered the highest growth of 58.1% in length, 493.9% in liveweight and 548.9% in dry weight. Clam meat powder in diet PE_{23},.

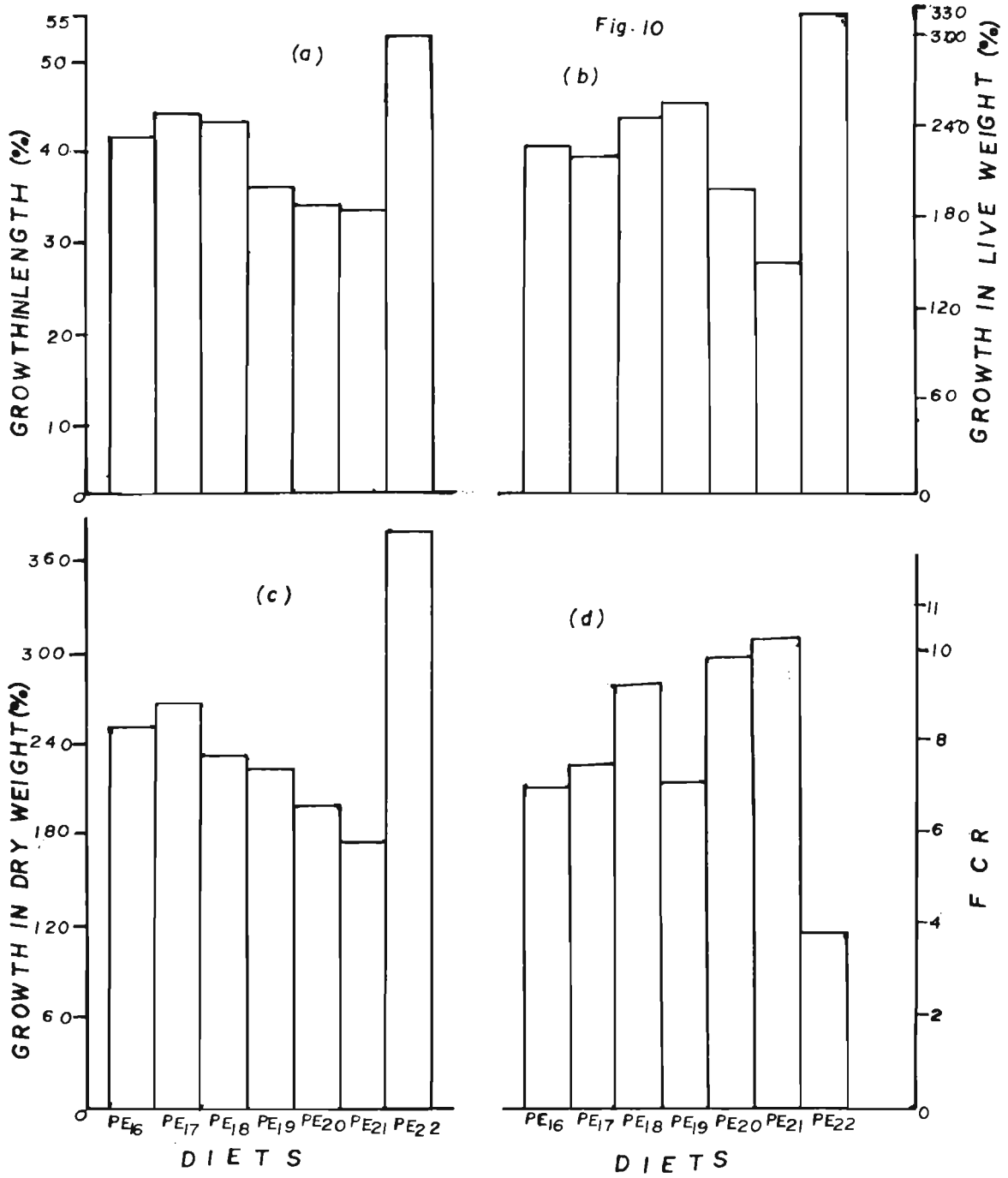

Figure 10. Evaluation of mixed proteins in different combinations for juvenile P. indicus. (a) growth in length (b) growth in live weight (c) growth in dry weight (d) food conversion ratio (FCR)

obtained a growth of 51.4%, 374.2%, 375.8% in length, live-weight and dry weight respectively. P. indicus fed by the diet prepared with FM had shown a growth of 48.5% in length, 317.4% in live-weight and 325.7% in dry weight. The diet PE_{25} containing MS had produced a growth of 40.2%, 234.5% and 263.8% respectively in length, live-weight and dry weight. Silkworm pupa in the diet PE_{27}, produced the lowest growth of 19.7% in length, 154.3% in live-weight and 170.8% in dry weight. The FCRs obtained by all the diets were good. However, the diets with CMP (1.83) and MS (1.95) were the best among the five protein sources tested. These were followed by FM (2.15), SWP (2.85) and PW (3.33). All diets had shown a survival of above 60%. CMP and MS had shown a survival of 66.6% and 62.5% respectively while FM, PW and SWP had shown 83.3%, 79.2% and 75.0% survival respectively. The growth in live-weight obtained by the five diets differ significantly (Table 14B) among themselves ($P < 0.01$). The difference was significant in the growth in length obtained by the diets PE_{24}, PE_{25}, PE_{26} and PE_{27}. However, there was no significant difference in respect of growth in length, between the diets PE_{26} and PE_{23} and between PE_{23} and PE_{24}. In the dry-weight, the growth obtained by the diets PE_{26}, PE_{23} and PE_{27} among them was only significantly different ($P < 0.01$). The growth in dry weight in case of diets PE_{23} and PE_{24} and also that of PE_{24} and PE_{25} was not significantly different between them, though it was significantly different between PE_{23} and PE_{25}. In respect of FCR, the diets PE_{23}, PE_{24} and PE_{25} did not show any significant difference ($P < 0.05$) among themselves though the three values were significantly different from that .

of the diets PE_{26} and PE_{27}. The FCR of the diets PE_{26} and PE_{27} had however shown significant difference between them.

The true digestibility (TD) (Table 14A) of clam meat protein was 81.19 and that of FM was 70.05. The digestibility of the protein of MS, PW and SWP was 66.91, 77.21 and 60.99 respectively. The diets containing CMP, FM, MS, PW and SWP produced a protein efficiency ratio of 1.77, 1.46, 1.52, 0.96 and 0.88 respectively. The net protein utilisation of clam meat powder, fish meal and mantis shrimp showed relatively higher values for the former two diets and lower (33.64) value for mantis shrimp. In the case of prawn waste and silkworm pupa it was 47.81 and 27.03. These proteins had shown a biological value of 74.6, 92.6, 53.1, 61.93 and 44.32 respectively.

The TD of protein of the diets with CMP, FM, PW and SWP was found to differ significantly (Table 14B) at 5% level ($P < 0.05$) among the diets . No statistical difference was found in the TD of protein of FM and MS, though the TD of protein of MS was significantly different from that of the other diets. In case of PER, there was no significant difference among the diets PE_{23}, PE_{24} and PE_{25} though the PER of these three diets was significantly different from the PER of other two diets. Similarly the PER obtained by the diets PE_{26} and PE_{27} did not differ significantly ($P < 0.05$). The NPU obtained for the diets PE_{23}, PE_{24} and PE_{26} did not differ significantly ($P < 0.05$), but it was significantly different from that of the diets PE_{25} and PE_{27}. The NPU of the diets PE_{25} and PE_{26} also did not differ significantly. The biological value shown by all the five diets (PE_{23} to PE_{27}) was significantly different ($P < 0.01$) among the diet

Table 14. Growth, food conversion ratio and survival of juvenile P. indicus fed with diets PE_{23} to PE_{27} for 30 days.

Particulars	Diet No.				
	PE_{23}	PE_{24}	PE_{25}	PE_{26}	PE_{27}
Initial average length (mm)	26.0	25.8	26.1	25.8	25.2
Initial average live weight (g)	0.0778	0.0771	0.0779	0.0771	0.0752
Initial average dry weight (g)	0.0199	0.0198	0.0199	0.0198	0.0192
Final average length (mm)	39.4	38.4	36.6	40.9	30.1
Final average live weight (g)	0.3689	0.3218	0.2605	0.4579	0.1912
Final average dry weight (g)	0.0947	0.0843	0.0724	0.01285	0.0520
Growth in length%	51.4^{ab}	48.5^{bc}	40.2^{d}	58.1^{a}	19.7^{e}
Growth ir live weight %	374.2^{a}	317.4^{b}	234.5^{c}	493.9^{d}	154.3^{e}
Growth in dry weight %	375.8^{b}	325.7^{bc}	263.8^{cd}	548.9^{a}	170.8^{e}
Food conversion ratio	1.83^{a}	2.15^{a}	1.95^{a}	3.33^{c}	2.85^{b}
Survival %	66.6	83.3	62.5	79.2	75.0

Note: Values with different superscripts differ significantly among themselves. Growth in length, live weight, dry weight significant at 1% level ($P < 0.01$) and food conversion ratio significant at 5% level ($P < 0.05$).

Table 14(A). Digestibility, PER, NPU and BV obtained by the diets PE_{23}, PE_{24}, PE_{25} PE_{26} and PE_{27} in juvenile P. indicus.

Diet No.	Crude Protein %			Chromium oxide %		True digestibility	PER	NPU	BV
	in diet	in animals after feeding with diets	in faeces	in diet	in faeces				
PE_{23}	31.62	70.07	26.75	0.9737	3.28	81.19^{a}	1.77^{a}	60.91^{a}	74.60^{b}
PE_{24}	32.02	71.21	30.09	1.0000	2.25	70.05^{b}	1.46^{a}	65.00^{a}	92.60^{a}
PE_{25}	32.16	67.02	27.18	0.9300	1.79	66.91^{b}	1.52^{a}	33.64^{bc}	53.10^{d}
PE_{26}	32.50	69.93	15.15	1.0499	1.77	77.21^{a}	0.96^{b}	47.81^{cd}	61.93^{c}
PE_{27}	31.73	68.54	30.51	0.8565	1.42	60.99^{d}	0.88^{b}	27.03^{d}	44.32^{e}

Note: Values with different superscripts differ significantly among themselves. BV significant at 1% level ($P < 0.01$) and Digestibility, PER and NPU significant at 5% level ($P < 0.05$).

Table 14 (B). Analysis of variance of the data obtained by the diets PE_{23}, PE_{24}, PE_{25}, PE_{26} and PE_{27}

ANOVA

Source	D.F.	Sum of squares	Mean sum of squares.
1	2	3	4
1. Growth in length			
Treatment	4	1172.03	293.01**
Error	10	44.83	4.48
Total	14	1216.86	297.49
2. Growth in live-weight			
Treatment	4	203042.63	50760 .65**
Error	10	1009.02	100 .90
Total	14	204051.65	50861 .55
3. Growth in dry weight			
Treatment	4	249053.41	62263.35**
Error	10	13858.97	1385.89
Total	14	262912.38	63649.24
4. Food Conversion Ratio			
Treatment	4	5.00	1.25*
Error	10	1.52	0.15
Total	14	6.52	1.40

Table 14(B) continued.

1	2	3	4
5. Protein efficiency ratio			
Treatment	4	1.74	0.44*
Error	10	0.49	0.05
Total	14	2.23	0.49
6. Digestibility			
Treatment	4	324.00	81.00*
Error	10	88.63	8.86
Total	14	412.63	89.86
7. Net Protein Utilisation			
Treatment	4	2565.06	641.26*
Error	10	574.25	57.42
Total	14	3139.31	698.68
8. Biological value			
Treatment	4	4421.91	1105.47**
Error	10	66.81	6.68
Total	14	4488.72	1112.15

** Significant at 1% level ($P < 0.01$)

* Significant at 5% level ($P < 0.05$)

The growth curves of the diets PE_{23} to PE_{27} are shown in Fig.11. The diet PE_{26} prepared with PW, showed the highest growth in length (Fig.11a), live-weight (Fig.11b) and dry weight (Fig.11c). This was followed by the diet PE_{23} with CMP, diet PE_{24} with FM, diet PE_{25} with MS and diet PE_{27} with SWP. Clam meat protein showed highest TD (Fig.12a) followed by the protein of PW. The TD of the proteins FM and MS were comparable and lower than that of CMP and PW. The protein of SWP showed the lowest digestibility of the five protein sources tested. Though the diet with PW gave higher growth, the PER (Fig.12d) was low and FCR (Fig.12c) was higher compared to that of the others. The PER and FCR of the diet with CMP were slightly superior, but were comparable to that of the diets with FM and MS. The three diets, with CMP, FM and MS, produced PER and FCR superior to the PER and FCR of the diet with PW. The diet with SWP recorded low PER and high FCR than that of the other diets, except that its FCR was slightly better than the FCR of the diet with PW. The NPU and BV (Fig.12b) produced by the diet with FM were superior to the NPU and BV obtained by all the other diets. This was followed by the diets with CMP, PW, MS and SWP in the decreasing order.

The results obtained with the diets PE_{28}, PE_{29}, PE_{30} and PE_{31}, prepared with the plant protein sources coconut cake (CNC), gingelly cake (GINC), groundnut cake (GNK) and Spirulina (SPL) respectively, are presented in Table 15. The growth of prawns fed with the diets PE_{28} (Growth in length 16.5%, growth in live-weight 127.3% and growth in dry weight 110.3%) and PE_{29} (Growth in length 16.2%, growth in live-weight 121.3% and growth in dry

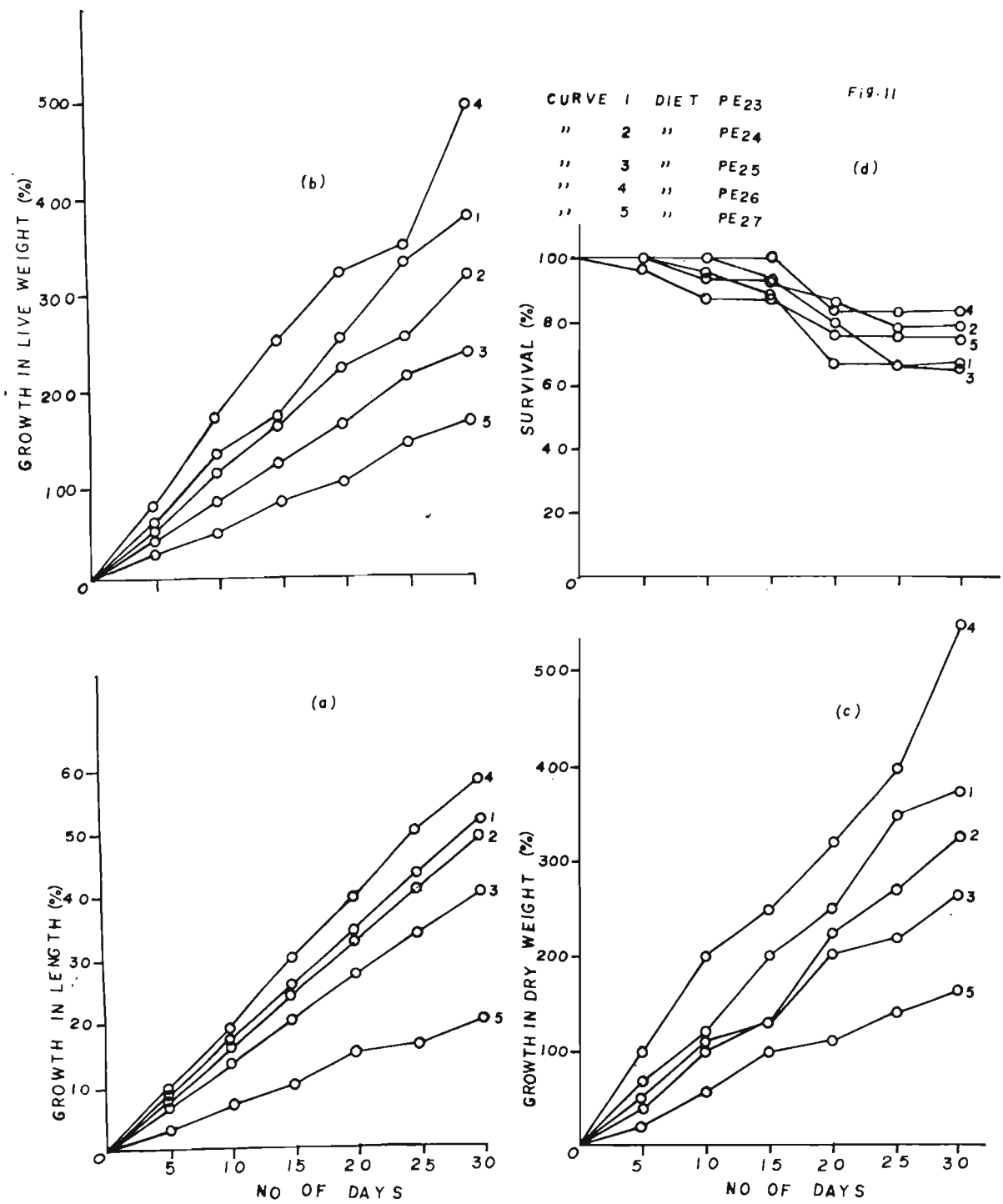

Figure 11. Evaluation of natural animal protein sources for juvenile P. indicus. Growth curves in (a) length (b) live weight (c) dry weight and (d) survival.

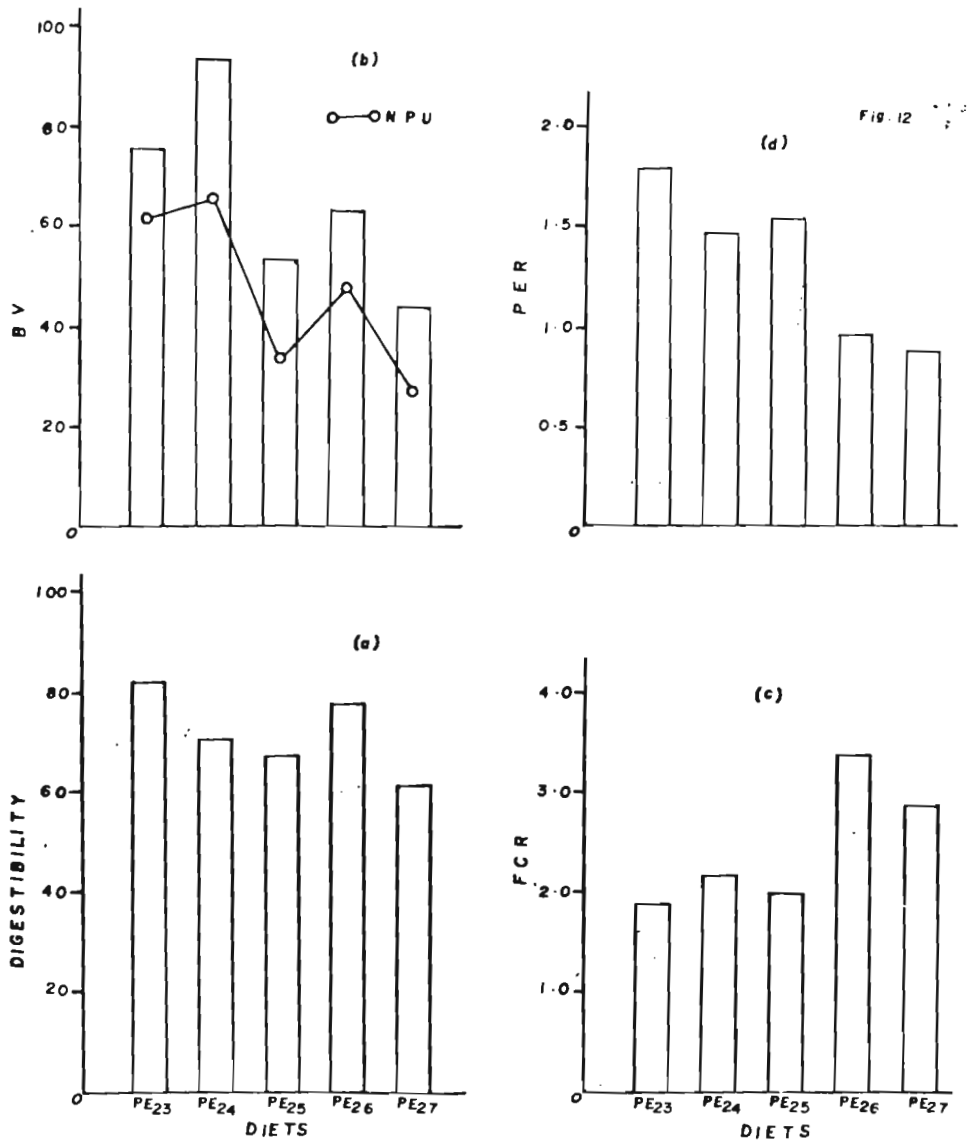

Figure 12. Evaluation of natural animal protein sources for juvenile P. indicus. (a) digestibility (b) biological value (BV) and net protein utilization (NPU) (c) food conversion ratio (FCR) and (d) protein efficiency ratio (PER).

weight 119.0%), was similar. The diet prepared with groundnut cake (PE_{30}) indicated a growth of 31.5% in length, 202.3% in live-weight and 227.1% in dry weight and the diet with Spirulina showed a growth of 23.8% in length, 240.4% in live-weight and 215.7% in dry weight. While the FCR obtained by CNC and GINC was 2.35 and 2.98 respectively, it was 4.99 and 4.02 in respect of the diets formulated with GNK and SPL. The survival of the animals fed CNC and GINC was 87.5% and 83.3% respectively, it was 66.6% in the prawns fed with GNK and 37.5% with that of SPL.

The four plant protein sources CNC, GINC, GNK and SPL, had shown a true digestibility (Table 15A) of 88.41, 85.02, 86.14 and 77.36 respectively. The PER of these protein sources laid between 0.63 and 1.29. The Net Protein Utilization of CNC and GINC was 23.27, and 31.82 respectively, while the NPU of GNK and SPL was 20.41 and 39.43. These plant proteins CNC, GINC, GNK and SPL had shown a BV of 27.02, 37.34, 23.36 and 50.96 in that order.

The ANOVA of the data of the diets PE_{28} to PE_{31} is given in Table 15(B). The TD of the different plant protein sources was significantly different ($P < 0.05$). The PER of CNC was significantly different from the PER obtained by GINC, GNK and SPL, while there was no significant difference in the PER of the latter three protein sources. The NPU of CNC and GNK and that of GINC and SPL was not found significantly different ($P < 0.01$) between them. But the NPU of CNC and GNK was significantly different from the NPU of GINC and SPL. The results were similar in the case of BV. The BV of CNC and GNK was significantly different

Table 15. Growth, food conversion ratio and survival of juvenile *P. indicus* fed with diets PE_{28} to PE_{31} for 30 days.

Particulars	Diet No.			
	PE_{28}	PE_{29}	PE_{30}	PE_{31}
Initial average length (mm)	25.4	25.3	26.1	25.2
Initial average live-weight(g)	0.0759	0.0754	0.0780	0.0751
Initial average dry weight (g)	0.0194	0.0193	0.0199	0.0192
Final average length (mm)	29.6	29.4	34.4	31.2
Final average live weight(g)	0.1725	0.1668	0.2358	0.2556
Final average dry weight(g)	0.0408	0.0422	0.0651	0.0606
Growth in length %	16.5^{b}	16.2^{b}	31.5^{a}	23.8^{b}
Growth in live weight %	127.3^{c}	121.3^{c}	202.3^{b}	240.4^{a}
Growth in dry weight %	110.3^{b}	119.0^{b}	227.1^{a}	215.7^{a}
Food conversion ratio	2.35^{a}	2.98^{a}	4.99^{b}	4.02^{c}
Survival %	87.5	83.3	66.6	37.5

Note: Values with different superscripts differ significantly among themselves. Growth in live weight, dry weight and food conversion ratio significant at 1% level ($P < 0.01$). Growth in length significant at 5% level ($P < 0.05$).

Table 15(A). Digestibility, PER, NPU and BV obtained by the diets PE_{28}, PE_{29}, PE_{30} and PE_{31} in Juvenile P. indicus.

Diet No.	Crude protein %			Chromium oxide %		True digestibility	PER	NPU	BV
	in diet	in animals after feeding diets	in faeces	in diet	in faeces				
PE_{28}	33.23	65.14	12.64	0.9960	2.1800	88.41	1.29^{a}	23.27^{b}	27.02^{c}
PE_{29}	33.22	66.39	15.36	0.9266	1.8351	85.02	0.78^{b}	31.82^{a}	37.34^{b}
PE_{30}	30.00	69.76	15.15	0.9960	2.9246	86.14	0.63^{b}	20.41^{b}	23.36^{c}
PE_{31}	31.46	67.66	31.74	0.8431	1.6409	77.36	0.74^{b}	39.43^{a}	50.96^{a}

Note: Values with same superscripts do not differ significantly among themselves. NPU and BV significant at 1% level ($P < 0.01$). PER significant at 5% level ($P < 0.05$). Digestibility not significant at 5% level ($P < 0.05$).

Table 15 (B). Analysis of variance of the data obtained by the diets PE_{28}, PE_{29}, PE_{30} and PE_{31}.

ANOVA

Source	D.F.	Sum of squares	Mean sum of squares
1	2	3	4
1. Growth in length			
Treatment	3	269.20	89.73*
Error	8	43.90	5.49
Total	11	313.10	95.22
2. Growth in live weight			
Treatment	3	30504.75	10168.25**
Error	8	2013.65	251.70
Total	11	32518.40	10419.95
3. Growth in dry weight			
Treatment	3	34452.99	11484.33**
Error	8	3047.61	380.95
Total	11	37500.60	11865.28
4. Food Conversion Ratio			
Treatment	3	12.14	4.04**
Error	8	0.09	0.12
Total	11	12.23	4.16

Table 15 (B) continued

1	2	3	4
5. Protein efficiency ratio			
Treatment	3	0.93	0.31*
Error	8	0.22	0.03
Total	11	1.15	0.34
6. Digestibility			
Treatment	3	125.09	41.69^{N}
Error	8	48.16	6.02
Total	11	173.25	47.71
7. Net Protein Utilisation			
Treatment	3	267.81	89.27**
Error	8	7.01	0.87
Total	11	274.82	90.14
8. Biological Value			
Treatment	3	493.51	164.50**
Error	8	43.11	5.39
Total	11	536.62	169.89

** Significant at 1% level ($P < 0.01$)
* Significant at 5% level ($P < 0.05$)

N Not significant at 5% level.

($P < 0.01$) from that of GINC and SPL, while it was not significantly different between the first two protein sources and between the second pair of protein sources.

The growth in length obtained by GNK was significantly higher than that obtained by CNC, GINC and SPL. No significant difference was observed in the growth in dry weight of CNC and GINC and that of GNK and SPL. However, the growth in live-weight of GNK and SPL was significantly different between them and was significantly higher than the growth obtained by CNC and GINC. Similar results were obtained in case of FCR. While there was no significant difference in FCR of CNC and GINC, it was significantly different from the FCR of GNK and SPL. The FCR of GNK and SPL was also found to be significantly different between them.

The growth curves of the diets PE_{28} to PE_{31} are shown in Fig.13. In general the diet prepared with GNK produced the highest growth (Fig. 13a, b and c) followed by the diet prepared with SPL. The growth obtained by the diets prepared with CNC and GINC was identical and was lower than the growth obtained by both GNK and SPL. The survival (Fig.13d) of the animals fed with the diet prepared with SPL was very poor. It was the highest in the case of the diets prepared with CNC and GINC.

The TD of all the four protein sources tested, was practically similar (Fig.14a). However, the NPU and BV of SPL were high and that of GNK were low. The NPU and BV (Fig.14b) of GINC were slightly higher than those of CNC, but the NPU and BV of both protein sources were higher than that of GNK and lower than that of SPL. Both GNK and SPL showed high FCR (Fig. 14c)

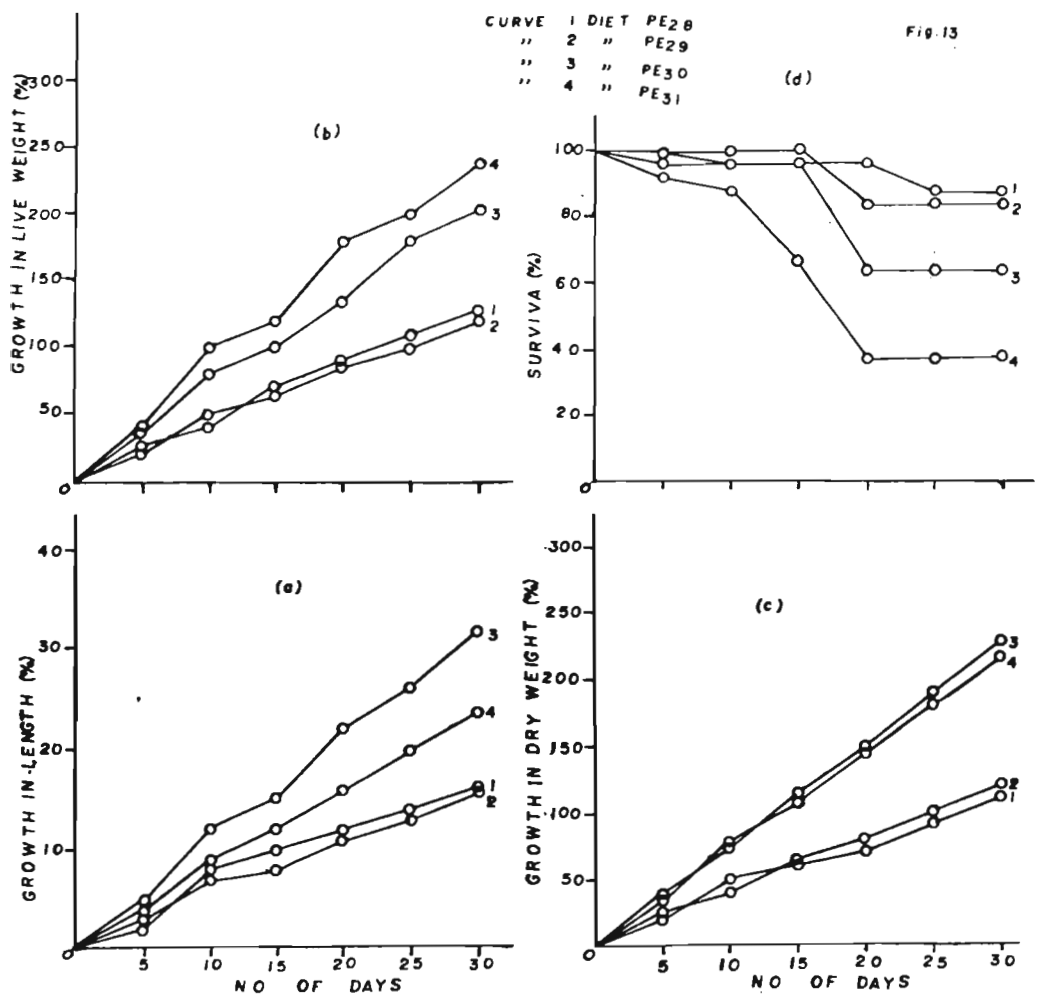

Figure 13. Evaluation of natural plant protein sources for juvenile P. indicus. Growth curves in (a) length, (b) live-weight, (c) dry weight and (d) survival.

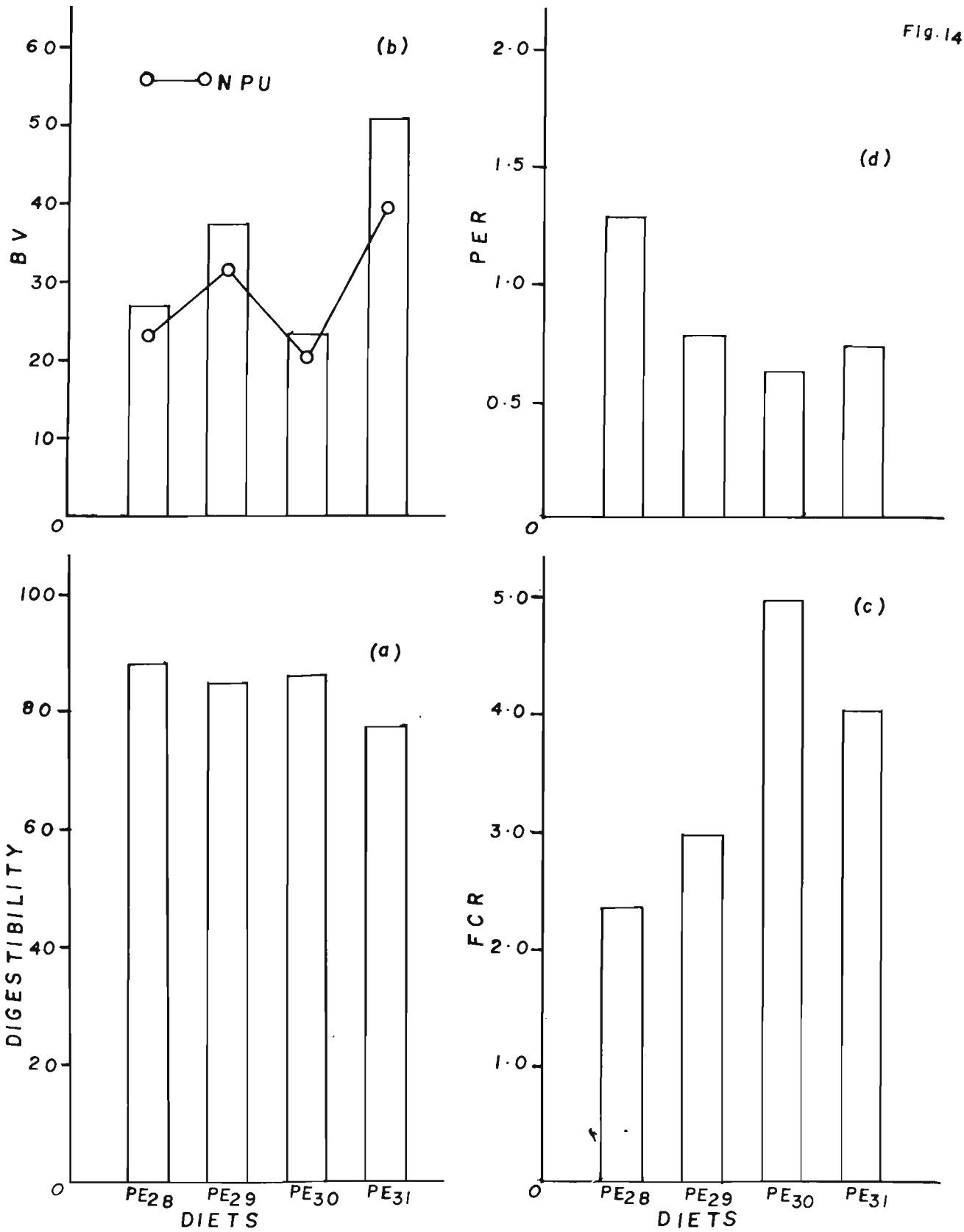

Figure 14. Evaluation of natural plant protein sources for juvenile P. indicus. (a) digestibility (b) biological value (BV) and net protein utilisation (NPU) (c) food conversion ratio (FCR) and (d) Protein efficiency ratio (PER).

and low PER (Fig. 14d) compared to CNC and GINC. CNC showed the lowest FCR and the highest PER compared to all the other three protein sources.

Evaluation of animal and plant protein mixtures in different combinations.

The results of the feeding experiment conducted with the diets PE_{32} to PE_{40} on P. indicus are given in Table 16. The growth of the animals (315.9% in live-weight and 346.5% in dry weight) fed with the diet PE_{34}, containing 70% animal protein source (APS) and 30% of plant protein source (PPS) was the highest among the different combinations of APS and PPS tested in diets. The growth of the animals fed the diets PE_{32} and PE_{33} (prepared with 90% APS and 10% PPS and 80% APS and 70% PPS respectively) was less than the growth obtained by the diet PE_{34}. The growth obtained by diets PE_{35} (244.3% in live-weight, 220.9% in dry weight) and PE_{36} (234.7% in live-weight and 247.9% in dry weight) was comparable. It was higher than the growth produced by the diets PE_{37} (156.2% in live weight, 170.5% in dry-weight), PE_{38} (197.4% in live weight, 229.2% in dry weight), PE_{39} (186.3% in live-weight, 226.2% in dry weight) and also PE_{40} which registered the lowest growth of 160.8% in live-weight and 155.4% in dry weight.

ANOVA of the data (Table 16B) had shown that the growth (in live weight and dry weight) obtained by the diet PE_{34} was significantly higher ($P < 0.01$) and that of the diet PE_{40} was significantly lower than the growth obtained by all the other diets. The difference in growth obtained by the diets PE_{32} and PE_{33} was not significant. And also no significant difference was observed

in the growth produced by the diets PE_{35}, PE_{36}, PE_{38} and PE_{39}. Similarly the growth obtained by the diets PE_{37} and PE_{40} was not significantly different. Though there were small variations in the growth in length and the FCR obtained by different diets, the difference was not found to be significant ($P < 0.05$). The survival of the animals fed with the diets PE_{32}, PE_{34}, PE_{35}, PE_{37}, PE_{38} was above 80%; that with the diets PE_{33}, PE_{36} and PE_{39} between 70 and 75% and PE_{40} showed the highest survival percentage of 95.8.

The data on true digestibility, PER, NPU and BV of the diets are presented in Table 16A. The TD of the protein in the diet PE_{32} (57.86) was low and that of the diet PE_{37} (81.24) was high, while the TD of the protein in the diets PE_{33} (62.87), PE_{34} (60.36), PE_{36} (65.48) was around 60. However, it was around 70 in the case of diets PE_{38} (69.99), PE_{39} (72.72) and PE_{40} (71.08). The TD of protein in the diet PE_{35}, was 58.58. The TD shown by the diet PE_{37} was significantly higher ($P < 0.01$) compared to that of all the other diets. The difference in the TD of protein in the diets PE_{38}, PE_{39} and PE_{40} was not significant. Similarly the diets PE_{35}, PE_{38} and PE_{40} did not show any significant difference in the TD among themselves. The true digestibility of protein in the diet PE_{32} was significantly lower than that of the diets PE_{36}, PE_{37}, PE_{38}, PE_{39} and PE_{40}. Only the PER obtained by the diets PE_{40} and PE_{34} was significantly higher compared to the PER obtained by all the other diets. The diets PE_{34}, PE_{35}, PE_{38} and PE_{39} produced a PER of around 0.7 and the difference in PER of these diets was not found to be significant. The PER of the diets PE_{32}, PE_{33}, PE_{36} and PE_{37} was

Table 16. Growth, food conversion ratio, and survival of juvenile P. indicus fed with diets PE_{32} to PE_{40} for 30 days.

Particulars	Diet No.								
	PE_{32}	PE_{33}	PE_{34}	PE_{35}	PE_{36}	PE_{37}	PE_{38}	PE_{39}	PE_{40}
Initial average length (mm)	24.1	24.2	24.7	24.7	24.6	24.8	24.5	24.6	24.3
Initial average live-weight (g)	0.0775	0.0779	0.0795	0.0796	0.0793	0.0797	0.0789	0.0793	0.0785
Initial average dry-weight (g)	0.0191	0.0192	0.0196	0.0196	0.0195	0.0196	0.0194	0.0195	0.0193
Final average length (mm)	37.5	37.6	37.3	37.5	36.3	34.7	35.9	36.5	34.8
Final average live-weight (g)	0.2909	0.2830	0.3306	0.2740	0.2654	0.2042	0.2346	0.2270	0.2047
Final average dry-weight (g)	0.0751	0.0785	0.0875	0.0629	0.0678	0.0530	0.0639	0.0636	0.0492
Growth in length %	55.6	55.2	50.9	51.8	47.4	39.9	46.4	48.2	42.9
Growth in live weight %	275.3^{b}	263.3^{bc}	315.9^{a}	244.3^{cd}	234.7^{d}	156.2^{e}	197.4^{f}	186.3^{f}	160.8^{g}
Growth in dry weight %	293.2^{b}	308.9^{b}	346.5^{a}	220.9^{c}	247.9^{c}	170.5^{d}	229.2^{c}	226.2^{c}	155.4^{d}
Food conversion ratio	3.21	3.73	3.32	3.14	3.68	4.02	3.82	2.66	3.22
Survival %	83.3	70.8	87.5	83.3	70.8	87.5	83.3	75.0	95.8

Note: Values with different superscripts differ significantly among themselves. Growth in live weight and dry weight significant at 1% level ($P < 0.01$). Growth in length and FCR were found not significant at 5% level ($P < 0.05$).

Table 16 (A). Digestibility, PER, NPU and BV obtained by the diets PE_{32} to PE_{40} in juvenile *P. indicus*.

Diet No.	Crude protein %			Chromium oxide %		True digestibility	PER	NPU	BV
	in diet	in animals after feeding the diets	in faeces	in diet	in faeces				
PE_{32}	51.89	64.03	35.46	1.0256	1.4235	57.86^{ef}	0.57^{d}	40.67^{a}	70.29^{a}
PE_{33}	47.32	63.39	25.62	0.9902	1.4783	62.87^{de}	0.56^{d}	28.75^{b}	45.72^{bc}
PE_{34}	44.92	64.23	30.06	1.0039	1.4080	60.36^{de}	0.77^{ab}	27.59^{bc}	45.70^{bc}
PE_{35}	42.81	66.72	27.68	0.9902	1.4651	58.58^{de}	0.71^{bc}	28.09^{bc}	47.95^{b}
PE_{36}	41.0	62.70	22.93	0.9604	1.4758	65.48^{cd}	0.55^{d}	28.18^{bc}	43.08^{cd}
PE_{37}	39.91	62.51	18.00	1.0337	1.5957	81.24^{a}	0.58^{d}	23.57^{bc}	29.01^{e}
PE_{38}	37.24	64.55	21.68	0.9941	1.4991	69.99^{bc}	0.71^{bc}	19.53^{bc}	27.90^{e}
PE_{39}	36.96	62.33	20.12	0.8039	1.3517	72.72^{b}	0.70^{bc}	18.76^{bc}	25.79^{ef}
PE_{40}	35.57	61.28	19.43	0.9941	1.5734	71.08^{bc}	0.85^{a}	16.66^{cd}	23.43^{fg}

Note: Values with different superscripts differ significantly among themselves. Digestibility, PER, NPU and BV are significant at 1% level ($P < 0.01$).

Table 16 (B). Analysis of variance of the data obtained by the diets PE_{32} to PE_{40}.

ANOVA			
Source	D.F.	Sum of squares	Mean sum of squares
1	2	3	4
1. Growth in length			
Treatment	8	769.88	99.61^{N}
Error	18	1493.40	82.96
Total	26	2290.28	182.57
2. Growth in live-weight			
Treatment	8	71531.32	8941.41**
Error	18	1391.51	77.31
Total	26	72922.83	9018.72
3. Growth in dry weight			
Treatment	8	94231.21	11778.90**
Error	18	2813.14	156.28
Total	26	97044.35	11935.18
4. Food Conversion Ratio			
Treatment	8	4.30	0.54^{N}
Error	18	4.59	0.26
Total	26	8.89	0.80

Table 16 (B) continued.

1	2	3	4
5. Protein efficiency ratio			
Treatment	8	0.28	0.03**
Error	18	0.03	0.0018
Total	26	0.31	0.0318
6. Digestibility			
Treatment	8	1434.23	179.27**
Error	18	1596.66	9.02
Total	26	3030.89	188.29
7. Net Protein Utilisation			
Treatment	8	1244.24	155.53**
Error	18	440.40	24.46
Total	26	1684.64	179.99
8. Biological Value			
Treatment	8	4494.48	561.81**
Error	18	40.39	2.24
Total	26	4534.87	564.05

** Significant at 1% level ($P < 0.01$)
* Significant at 5% level ($P < 0.05$)

N Not significant at 5% level.

not significantly different among themselves, but it was significantly low compared to the PER of the other diets. The NPU of the diet PE_{32} (40.67) was significantly higher ($P < 0.01$) and that of the diet PE_{40} (16.66) was significantly lower compared to the NPU of all the other diets. The NPU of the diets PE_{33} (28.75), PE_{34} (27.59), PE_{35}(28.09) and PE_{36}(28.18) was similar and not found to be significantly different among themselves. The NPU of the diets PE_{37}, PE_{38} and PE_{39} was 23.57, 19.53 and 18.76 respectively and the difference among them was not found to be significant. The BV of the protein in the diet PE_{32} (70.29) was significantly higher ($P < 0.05$) from the BV of the rest of the diets. The difference in the BV of the protein in the diets PE_{33} (45.72), PE_{34} (45.70), PE_{35}(47.95), PE_{36} (43.08) was not found to be significant. The BV of the protein in the diet PE_{40} (23.43) was significantly low as compared to the BV of the protein of all the other diets. However, the BV of the protein in the diets of PE_{37} (29.01), PE_{38}(27.9) and PE_{39}(25.79) were more or less similar and the difference among these values was not significant.

The growth of animals improved (Fig.15a & b) when the diet contained 10 to 30% PPS and 90 to 70% APS. The diet having 7:3 ratio of APS and PPS recorded the highest growth in weight. Higher levels of PPS above 30% in the diet resulted in gradual decrease in growth of animals. The FCR was low (Fig.16a) when the ratio of APS and PPS was 6:4 and also at 2:8. PER was high when the ratio was 7:3 and it decreased as APS and PPS ratio reached 5:5 in the diet (Fig. 16b). Though the rate of survival slightly varied (Fig.16c), it practically remained same in

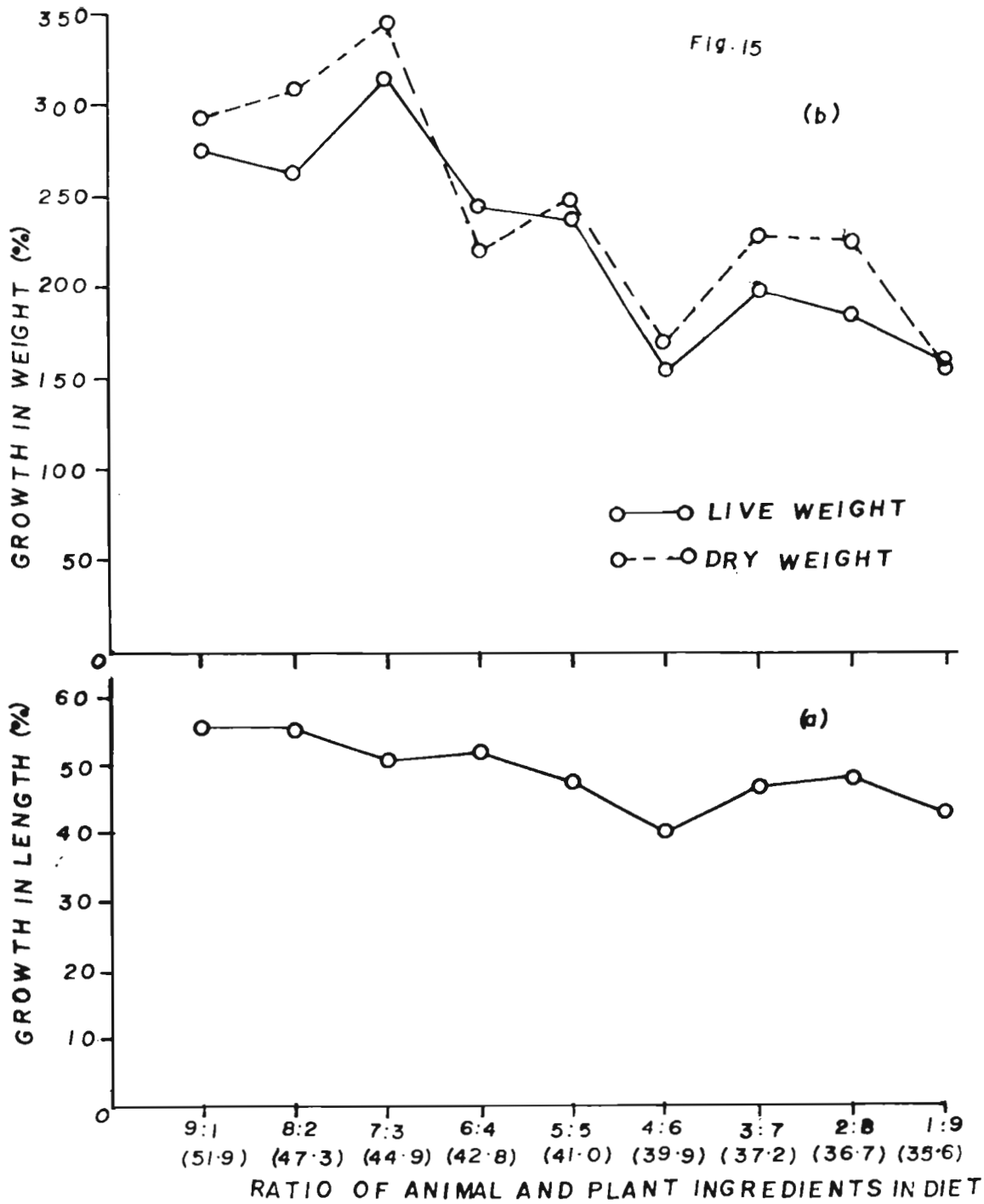

Figure 15. Effect of the ratio of animal and plant protein sources in the diet on (a) growth in length and (b) growth in weight in juvenile P. indicus. The values in parenthesis are percent crude protein level in the diet.

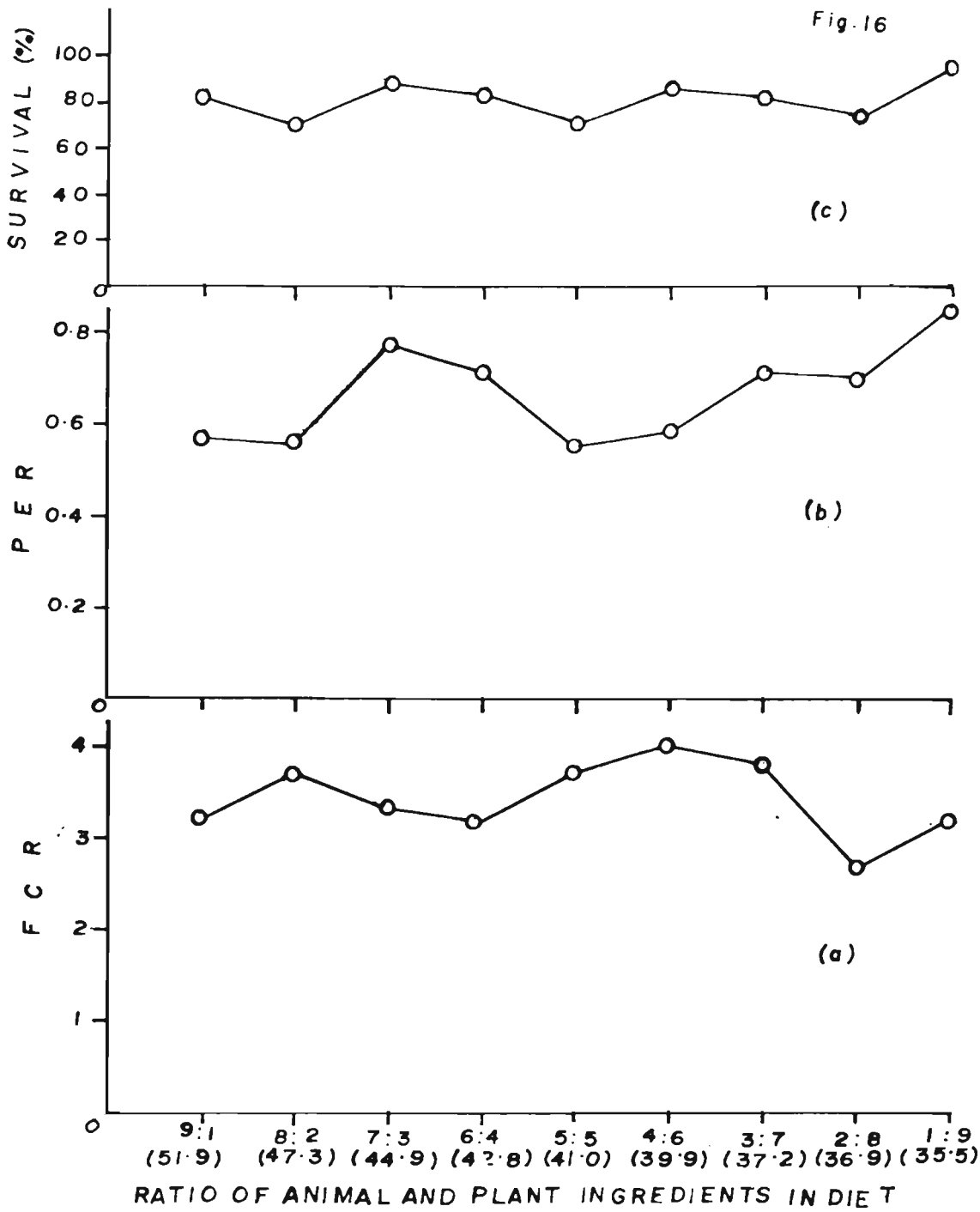

Figure 16. Effect of the ratio of animal and plant protein sources in the diet on (a) food conversion ratio, (b) Protein efficiency ratio and (c) survival in juvenile P. indicus. The values in parenthesis are percent crude protein level in the diet.

relation to APS and PPS ratio in the diet.

The true digestibility of the protein in the diet remained almost same (Fig.17a) until the APS and PPS ratio in the diet was 6:4; it increased to reach the maximum at 4:6 ratio. The TD decreased again at 3:7 ratio and levelled of thereafter. The NPU (Fig.17b) and also the BV (Fig.17c) gradually decreased as the PPS proportionately increased in the diet. However, the NPU remained almost constant between ratios 8:2 and 5:5 of APS and PPS in the diet. The BV from its peak value in the diet with 9:1 APS and PPS decreased sharply in the diet with 8:2 ratio, remained comparable between the diets PE_{33} and PE_{36} and decreased again in the diet PE_{37}, thereafter it remained constant.

Evaluation of protein levels in the diets formulated with natural protein sources:

From the results presented in Table 17, it can be seen that the feed PE_{44} having a crude protein content of 29.9% gave the highest growth in length (64.2%), in live weight (358.7%) and in dry weight (332.2%). The feed also gave a food conversion ratio of 1.99 and the survival rate of 83.3%. The feeds PE_{41}, PE_{42} and PE_{43}, which had higher protein contents of 41.81%, 37.89% and 33.27% respectively produced a growth of 56.3%, 62.9%, 63.8% in length, 291.0%, 348.7%, 319.1% in live weight and 264.3%, 323.6% and 311.3% in dry weight. The FCRs corresponding to these feeds were 2.19, 1.91 and 2.19 respectively. The animals fed with these feeds also had a high survival of 91.6%, 83.3% and 91.6% respectively. The feeds PE_{45}, PE_{46} and PE_{47}, which had lower protein than the feed PE_{44}, produced lower growth. The growth of the animals fed with the feeds PE_{45}, PE_{46} and PE_{47} was 57.8%, 53.7% and 48.6% in length, 279.6%, 264.5% and 229.5%

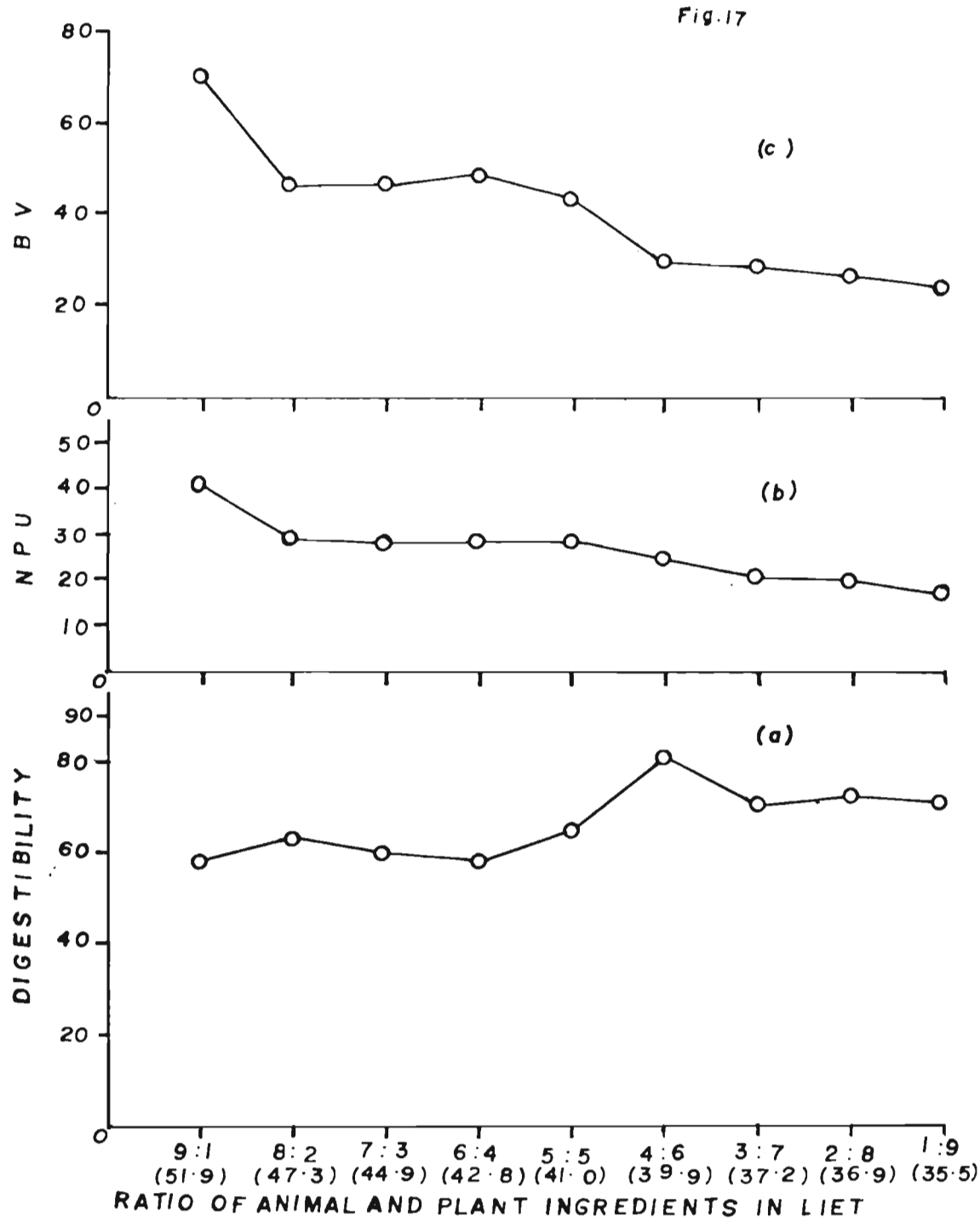

Figure 17. Effect of the ratio of animal and plant protein sources in the diet on (a) digestibility, (b) net protein utilisation (NPU) and (c) biological value (BV) in juvenile P. indicus. The values in parenthesis are percent crude protein level in the diet.

in live weight and 279.9%, 273.3% and 253.9% in dry weight respectively. The survival of the animals fed with the feeds PE_{45}, PE_{46} and PE_{47} was respectively 75.0%, 91.6% and 91.6% and their corresponding values of FCR being 2.37, 2.39 and 3.45.

ANOVA of the data (Table 17B) had shown that the growth obtained by the feed PE_{44} was significantly higher ($P < 0.01$) compared to the growth obtained by the feeds PE_{41}, PE_{45}, PE_{46} and PE_{47}. The difference in the growth obtained by the feeds PE_{42}, PE_{43} and PE_{44} was not found to be significant. Similarly the growth realised by the feeds PE_{41}, PE_{45}, PE_{46} and PE_{47} was not significantly different from each other. While there was no significant difference ($P < 0.01$) in the FCR produced by the feeds PE_{41} to PE_{46}, the difference was significant between the diet PE_{47} and the rest of the feeds.

The data obtained on the body protein of the animals fed with PE_{41} to PE_{47} and excretion of protein in the faeces is shown in Table 17A. The body protein of the animals experimented with different feeds remained almost the same. But the protein excreted in the faeces was high (35.02%) in the animals fed with the lower level protein feeds, and low (20.24%) in the animals reared with higher protein feed.

Protein balance:

The protein balance was calculated as the difference of the protein in the feed and the protein excreted in the faeces (Table 17A). The protein balance was negative (-19.93) when the protein level in the feed was 15%. It gradually increased with increase in the protein level of the feed to reach the positive value at 29.9% protein level. The highest positive value of

Table 17. Growth, food conversion ratio and survival of juvenile P. indicus fed with the diets PE_{41} to PE_{47} for 30 days.

Particulars	Diet No.						
	PE_{41}	PE_{42}	PE_{43}	PE_{44}	PE_{45}	PE_{46}	PE_{47}
Initial average length (mm)	25.0	25.0	24.7	25.2	24.8	24.9	24.8
Initial average live weight (g)	0.0768	0.0768	0.0759	0.0774	0.0762	0.0766	0.0762
Initial average dry weight (g)	0.0192	0.0192	0.0190	0.0194	0.0190	0.0192	0.0190
Final average length (mm)	39.1	40.7	40.5	41.4	39.1	38.3	36.9
Final average live weight (g)	0.3002	0.3446	0.3181	0.3550	0.2892	0.2792	0.2510
Final average dry weight (g)	0.0699	0.0813	0.0781	0.0838	0.0721	0.0716	0.0672
Growth in length %	56.3	62.9	63.8	64.2	57.8	53.7	48.6
Growth in live-weight %	291.0^{bc}	348.7^{ab}	319.1^{bc}	358.7^{a}	279.6^{cd}	264.5^{cd}	229.5^{e}
Growth in dry weight %	264.3^{cd}	323.6^{a}	311.3^{ab}	332.2^{a}	279.9^{bc}	273.3^{bc}	253.9^{cd}
Food conversion ratio	2.19^{a}	1.91^{a}	2.19^{a}	1.99^{a}	2.37^{a}	2.39^{a}	3.45^{b}
Survival %	91.6	83.3	91.6	83.3	75.0	91.6	91.6

Note: Values with different superscripts differ significantly. Growth in length, live-weight and dry weight are significant at 1% level ($P < 0.01$).

Table 17 (A). Protein in diet, body and faeces of juvenile *P. indicus* fed with diets PE_{41} to PE_{47}.

Diet No.	Protein %			Protein balance (Dietary protein-protein in faeces)
	in diet	in animals after feeding with diets	in faeces	
PE_{41}	41.81	67.50	25.59	+ 16.22
PE_{42}	37.89	67.27	20.24	+ 17.65
PE_{43}	33.27	68.49	22.64	+ 10.63
PE_{44}	29.91	66.88	22.69	+ 7.22
PE_{45}	27.07	67.11	28.76	- 1.69
PE_{46}	22.54	67.81	29.18	- 6.64
PE_{47}	15.09	65.29	35.02	- 19.93

Table 17 (B). Analysis of variance of the data obtained by the diets PE_{41} to PE_{47}.

Source	D.F.	Sum of squares	Mean sum of squares
	ANOVA		
1. Growth in length			
Treatment	6	209.40	34.90**
Error	14	61.24	4.37
Total	20	270.64	39.27
2. Growth in live weight			
Treatment	6	38654.40	6442.40**
Error	14	2390.67	170.76
Total	20	41045.07	6613.16
3. Growth in dry weight			
Treatment	6	17093.64	2848.94**
Error	14	4318.23	308.44
Total	20	21411.87	3159.38
4. Food Conversion Ratio			
Treatment	6	4.10	0.68**
Error	14	0.76	0.05
Total	20	4.86	0.73

** Significant at 1% level ($P < 0.01$)

17.65 was obtained with the feed PE_{42} having 37.89% protein.

The relationship between the dietary protein and the protein balance is shown in Fig.18. A straight line, inclined to X-axis, was obtained. The line intercepted the protein axis (Y-axis) at 26%, where the protein balance is zero. At this point the protein in the feed was same as the protein excreted in the faeces. Below this protein level in the feed, the protein balance was negative, above which it was positive. The results on protein balance obtained in this experiment were similar to the results shown in experiments 2 and 3 with regard to nitrogen balance. The results in all the three experiments had shown that the protein required in the diet for zero protein balance was between 20 and 25%.

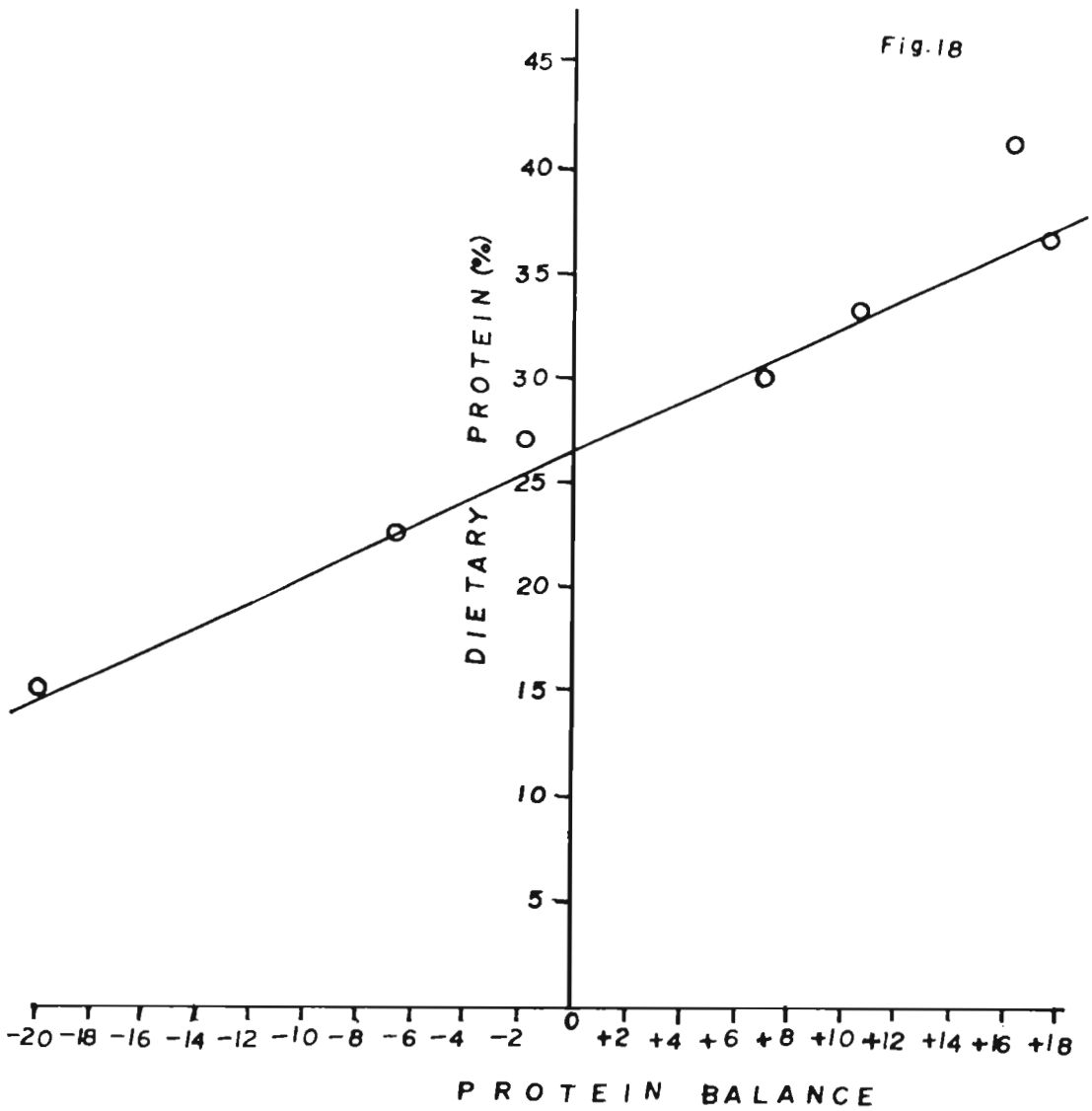

Figure 18. Relationship between dietary protein and protein balance in juvenile P. indicus.

DISCUSSION

Purified proteins

Among the four purified proteins evaluated for the early juveniles of Penaeus indicus, albumen (egg) gave the highest growth, protein efficiency ratio (PER) and low food conversion ratio (FCR). The growth, FCR and PER obtained by fibrin and casein were comparable. Gelatin showed poor performance among the proteins tested. The true digestibility (TD) of albumen was low and that of gelatin was high. Forster and Gabbott (1971), while studying the assimilation of nitrogen in the diets prepared with different protein ingredients, for Palaemon serratus and Pandalus platyceros, showed that the assimilation was 97.7 in casein based diet, 96.6 in albumen and 97.5 in the case of gelatin based diet. While the TD of protein in gelatin, obtained for P. indicus was comparable (93.4) to the results obtained for Palaemon serratus and Pandalus platyceros, the TD of protein in albumen and casein for P. indicus was low. The TD of fibrin was 82.42 and was also slightly lower than the assimilation of casein, gelatin and albumen by the two caridean prawns. This may be due to difference in the level of protein material used in the diet. Forster and Gabbott (1971) prepared the diets with 60% protein level whereas only 40% was used in the diets of P. indicus. However, in the diet which contained 60% albumen and 60% casein the TD of protein was 96 and 97 respectively. Nose (1964) determined the digestibility of albumen and a combination of albumen and gelatin and casein and gelatin in the crayfish Procambarus clarkii. The digestibility of albu-

men alone (at 55% in the diet) was 94.6. The protein from albumen-gelatin (54:15 in the diet) and casein-gelatin (54:5 in the diet) had shown a digestibility of 96.5 and 94.1 respectively. The TD of casein and albumen (at 40% in the diet) obtained in P. indicus in the present study was comparitively lower than the digestibility of these proteins obtained in the crayfish. But then it may be again due to the difference in level of the protein sources used in the diets in this study. In the subsequent experiments when the protein source was used at 60%, the digestibility was above 95 which was comparable to the results obtained both in the caridean prawns and the crayfish. Comparative studies of digestibility of albumen, fibrin and gelatin in prawns and fish are very limited. However, the digestibility of casein was determined in some finfishes. Atack et al.(1979) found a true digestibility (TD) of 93 protein at 30% protein level in mirror carp, Cyprinus carpio, using casein in the diet. Atack and Matty (1978) reported a TD of 98.7 in rainbow trout at the same dietary protein level. The digestibility of protein from casein in P. indicus, at 30% level, was lower than the values obtained in carp and rainbow trout.

The metabolic faecal nitrogen (MFN) determined for the first time, in the prawn Penaeus indicus, in the present study, varied from 248.5 mg to 351.6 mg N/100 g diet, with an average value of 326.4 mg N/100 g diet. The MFN was determined in some finfishes and caridean prawns. Using zero protein diet, Nose(1967b) determined MFN in young rainbow trout. The values obtained were 85.7 mg, 139.7 mg and 151.0 mg N per 100 g diet in three different experiments. Ogino and Chen(1973a), using the same method, obtained

170 mg N/100 g diet of MFN in carp (Cyprinus carpio). But Atack et al. (1979), while studying the utilisation of some single cell proteins, calculated the MFN in carp and found a value of 217 mg N/100g diet. Jauncey (1982) reported a MFN of 161 mg N/100 g diet in Tilapia (Sarotherodon mossambicus). In albino rats the MFN was found to be 132 mg N/100 g diet (Mitchell and Bert, 1954), and it was 100 mg N/100 g diet in humans (Maynard and Loosli, 1969). Maynard et al.(1981) summarised the results of various studies on MFN and reported that in general the MFN was about 200 mg N/100 g in non-ruminants and 545 to 576 mg N/100 g diet in ruminants. The high value of MFN in ruminants was thought to be due to the microbial residues and tissue desquammation. Forster and Gabbott (1971) determined MFN in Palaemon serratus and obtained a value of 185.2 ± 27.9 mg/100 g diet. From the above results it is obvious that the MFN varies from animal to animal. Differences were also found in the values of MFN determined by different workers within the same species as in the case of carp. It is quite interesting to note that the MFN is slightly higher in albino rats as compared to humans. The value of MFN obtained in finfish is higher than that is obtained in albino rats. In caridean prawn (P.serratus) the MFN is again higher than the value reported in finfish, though it is comparable to the value obtained in carp. On the other hand the MFN in ruminants is twice higher than the MFN in all the other animals. In the penaeid prawn, P. indicus the MFN was greater than the value shown by Palaemon serratus, and very much less than what was reported in ruminants. Prawns are known to secrete (Forster, 1953) a chitinous peritropic membrane round the faecal pellets.

In addition to this, digestive juices, epithelial cells abraded from the walls of the alimentary tract and bacterial residues contribute to MFN. The comparatively higher value of MFN in prawns in general among the aquatic animals, can be due to the secretion of chitinous peritropic membrane around the faecal pellets which is absent in the case of finfish. The difference in MFN between the prawns Palaemon serratus and Penaeus indicus was significant and might be due to the difference in the nature and quantity of faecal membrane in the two types of prawns. Determination of MFN in more species of penaeid prawns and the caridean prawns might throw more light on this aspect.

Forster and Gabbott (1971) observed that the influence of MFN on determining the true digestibility (TD) of protein was related to the dietary protein level. While the effect of MFN is more marked on the TD at low dietary protein level, its influence is negligibly small on TD at higher levels of protein in the diet. This is true due to the fact that at lower dietary protein levels the constant excretion of MFN makes up a greater proportion of faecal nitrogen output which becomes progressively less as the dietary protein levels increase (Maynard et al., 1981).

Eventhough, albumen had shown slightly low TD, compared to the other proteins, its protein efficiency ratio (PER) was higher. The PER of casein and fibrin were comparable while the PER of gelatin was very low. Fibrin had shown highest net protein utilisation (NPU), which was supported by high deposition of protein in the body of the animals fed this diet. The NPU of albumen was higher than the NPU of casein and gelatin.

Interestingly, gelatin which showed low PER, gave the NPU comparable to that of casein. But the biological value (BV) of gelatin came down because of low PER and high TD. By virtue of higher value of NPU, the BV of fibrin was superior to the BV of albumen and casein. As expected, the BV of albumen is higher than the BV of casein. While the data on NPU and BV of the purified proteins is not available for prawns, these were determined for some finfishes. Atack and Matty (1978) determined the NPU of casein in carp and rainbow trout and obtained the values of 49 and 40 respectively. The corresponding BVs were 52 and 41 respectively. Teshima *et al.* (1978) reported a NPU of 25.7 in *Tilapia zilli* using 35% casein in the diet. While the NPU and BV of casein obtained in *P. indicus* were comparable to those obtained in carp and rainbow trout, the NPU of casein in *P. indicus* was superior to the value reported in *Tilapia*. On the other hand, the NPU and BV of albumen and fibrin obtained in *P. indicus* were superior to the NPU and BV of casein obtained in finfishes. However, the values of NPU and BV of gelatin in *P. indicus* were inferior to those obtained in carp and rainbow trout, using casein.

While the digestibility of protein generally depends upon its method of processing, the digestive enzymes, and the age of the recipient animal, the NPU and BV are related to the quality of the protein in terms of amino acids. The animal body needs specific aminoacids which make up the protein or other nitrogenous tissues or compounds to be formed. The kind of aminoacids needed for protein synthesis,

are taken up from the available aminoacid pool in the required proportions, and the left overs are wasted in so far as protein nutrition is concerned. This loss is very substantial when the amino acid mixture absorbed is relatively deficient in any one essential aminoacid. If the consuming animal could utilize the aminoacids derived from the dietary protein, for the synthesis of protein in the tissue, that dietary protein will show high biological value. From the results obtained on the four purified proteins it can be seen that albumen and fibrin have high biological quality followed by casein for the prawn P. indicus. Gelatin alone is found to be an inferior protein source. Considering the growth of animals, FCR and PER, albumen appears to be the best protein among the four proteins tested for preparing research diets for prawns.

Protein requirement in the diet of penaeid prawns showed wide variations not only between the species but also within the same species when different protein sources were used by different workers for conducting the study. In general, the dietary protein requirement for penaeid prawns varies from a minimum of 20% in Penaeus aztecus (Shewbart et al., 1973) and maximum of 60% in P. japonicus (Shigueno et al., 1972), thus indicating large scale interspecies differences. Shigueno et al. (1972) used a mixture of squid meal, white fish meal, mysid meal, sludge and yeast as the protein source. But when Kanazawa et al. (1970) used silkworms, brine shrimp and fish meal, the dietary requirement of protein for this prawn was found to be 55%. Subsequently Balazs et al. (1973) showed that the protein requirement for P. japonicus was only 40%. These authors used

soybean, fish meal (Hawaiian)and shrimp meal mixture as protein source. Deshimaru and Yone (1978c), using casein and egg albumen had shown that the requirement was 52-57%. The results of protein requirement studies in P. aztecus were also quite interesting. First Shewbart et al.(1973) showed that the protein requirement was between 22 and 30%. Using fish protein, Venkataramaiah et al.(1975a) showed a protein requirement of 40%. In the subsequent year Zein-Eldin and Corliss (1976) found that the requirement was 51.5% in the same prawn, using soyflour as the protein source. Similar differences could also be seen in other species like P. merquiensis and P. indicus. IN P. merquiensis, AQUACOP (1978) showed a protein requirement of 43-55% using casein as source, while Sedgwick (1979) found the requirement between 34 and 42% using mussel meat as the protein source. While Colvin (1976a) using shrimp meal and Ahamed Ali (1982a) with mantis shrimp and ground nut cake mixture as protein source found a protein requirement of 43% for juvenile P. indicus, Charles John Bhaskar and Ahamad Ali (1984) using casein as protein source subsequently found that the post larvae (PL_1 to PL_{40}) had the requirement of 40% protein, and for the juveniles it was only 30%.

In all these studies, the protein requirement was determined by measuring the growth of animals and their food conversion ratio (FCR) and in some cases the protein efficiency ratio (PER). The differences in protein requirement shown within the same species were explained as due to the differences in quality of the protein sources in terms of aminoacids, especially the essential aminoacids (EAA). With a view to understand clearly,

the intricasies in the protein requirement, it had been decided to investigate the effect of dietary protein level on its digestibility, PER, NPU and BV in conjunction with the growth of the animals and the FCR in the present study. This approach yielded interesting results. For comparison, the effect of protein in the diet on the above mentioned parameters, was studied using two different protein sources albumen and casein. In the experiment in which albumen was used as the source, the diet with 24.75% protein gave the highest growth, best food conversion ratio and high value of protein efficiency ratio. In the experiment in which casein was used as the source, the diet with 29.3% protein gave the highest growth, best FCR and high PER. The difference in the protein requirement shown by albumen diets and casein diets was 4.55% and can be considered as an important difference. Though the true digestibility of protein did not show any clear trend with dietary protein, it tended to be high at higher dietary protein levels, and low at lower protein levels. Almost similar observations were made by Smith *et al.*(1985) in *P. vannamei*. As in the case of the growth, the PER, NPU and BV registered a peak at the optimum dietary protein level, and showed a decreasing tendency as the protein level in the diet increased. The excretion of nitrogen per 100 g diet also registered a peak value at a particular protein level and declined thereafter when the protein in diet further increased. The results were similar in both albumen and casein based diets. However, the peak of ^{N} excretion in the faeces was at a lower protein level in albumen diets (24.6%) than in casein diets (37.3%), indicating that the nitrogen required for basal meta-

bolism can be met at lower dietary protein level when albumen is used and when casein was used this requirement could be met only at higher protein level in the diet. These findings had clearly shown that the intraspecies differences in optimum dietary protein level were mainly due to the different protein sources used in preparing the diets. In the present case albumen had higher BV than casein. Albumen diets had shown lower protein requirement than the casein diets. This implies that when a protein with higher BV is used, the protein requirement will be less and vice versa. Since BV of a protein is related to its quality interms of essential aminoacids, it is emphasised that the protein having good essential aminoacid profile only should be used to determine the protein requirement in the animal. The protein requirement of P. indicus obtained by albumen (25%) and casein (30%) diets in the present study is lower than the protein requirement of P. japonicus, reported by Deshimaru and Yone (1978c), P. merguiensis, obtained by AQUACOP (1978), P. indicus shown by Charles John Bhasker and Ahamad Ali (1984), Metapenaeus monoceros reported by Kanazawa et al. (1981) and P. monodon, found by Alava and Lim (1983). In all these studies, casein was used as the protein source. However, such low protein requirement in the diet of penaeid prawns was also reported in literature. Andrews et al. (1972) concluded that the dietary protein requirement for Penaeus setiferus was around 30%. Shewbart et al. (1973) had shown a protein requirement of 22-30% for P. aztecus. Similarly for Penaeus duorarum, the requirement was found to be 28-30% by Sick and Andrews (1973).

New (1976), while reviewing the dietary studies of shrimp and prawn, concluded that the protein in the diet of penaeid prawns lies between 27 and 35%. In this context, the determination of NPU and BV and also the protein balance, in the protein requirement experiments in the present study, offered much more insight into the understanding of the dietary protein and the response of the animals to it. First of all, the results had indicated that all the purified proteins do not have the same rating of biological value. Eventhough fibrin has the highest BV, the growth of animals fed with the fibrin diet and its FCR were lower than those fed with the albumen diet, indicating that albumen should be preferred in protein requirement studies. When the suitable protein source is selected for preparing diet, the calorific value of the diet plays an important role in determining the protein required in the diet for the receipient animal. In the present study the diet with 25% protein (Albumen) gave the best performance. This diet had 31% of carbohydrate and 6% of lipid to provide the energy in the diet. Higher protein level lowered the performance of the diet, and showed enhanced assimilation of dietary protein. But the assimilated protein was not utilised for tissue growth. This is clearly reflected by low growth, low PER and low NPU. Consequently, the biological value of the protein came down. In the animals fed with high protein diet, which had proportionately less carbohydrate, the assimilated protein may be biologically oxidised for metabolic energy. The deamination of aminoacids and their subsequent oxidation need extra energy and thereby the growth of the animal

is reduced. This is evident from the fact that low protein deposition in the body of animals fed high protein diet and also low nitrogen output in the faeces. Decrease in PER, NPU and BV was experienced in finfish and also in higher animals as the protein in diet increased. Ogino and Saito (1970) found that the PER, NPU and BV decreased with increase in the dietary protein level in carp. Later Ogino and Chen (1973a,b) confirmed these findings. Similar results were obtained in Tilapia zilli by Mazid et al. (1979), Teshima et al. (1978) and also in Sarotherodon mossambicus by Jauncey (1982). In albino rats, the PER decreased as the protein in the diet increased (Albanese, 1972). The NPU and BV were higher at lower dietary protein levels and lower at higher levels of dietary protein. Explaining this interesting phenomenon Mitchell (1923-24) stated, that the decrease in the utilisation of protein in anabolism as the level of intake increases, is probably due to a lower utilisation for growth than for maintainance. He further stated that another possible factor for the reduction in the biological value of a given protein, as its concentration in the diet increases, is an inevitable wastage of aminoacids in the oxidative process of the cells. The aminoacids are not immune to biological oxidation even if enough energy is available for metabolic needs from other sources. The extent of oxidation of aminoacids will depend upon their concentration in the cellular fluids with respect to the concentration of non-nitrogenous nutrients. The amino acid concentration in the cells largely depends upon the protein content of the diet consumed by the animal. If the wastage of amino acids by oxidation increases

more rapidly than the protein intake, a greater percentage of loss of absorbed nitrogen would result with the diets containing higher percentage of protein. The oxidation of protein, referred to as Specific Dynamic Action (SDA), will be higher at higher protein levels than at the lower protein levels. This explanation therefore ascribes the lowering of biological value of protein as the level of intake of protein through diet increases.

Examination of nitrogenbalance (Fig.9b) shows that the curve intercepts the protein axis at 22% of dietary protein. At this point the intake and excretion in faeces of protein is same. The results are similar with albumen as well as with casein diets. A small increase in the protein (2.75% in case of albumen and 7.3% in case of casein) in the diet above this level had resulted in highest growth and Protein deposition in the body of the animals. Any further increase in the dietary protein, though resulted in positive nitrogenbalance, decreased the growth and body protein in the animals. From these findings it is clear that the dietary protein required to achieve highest growth and best food conversion is just above the dietary protein at which the nitrogenbalance is zero.

In the experiment in which the combination of different proteins were tested in the diet, the diet containing all the four proteins albumen, casein, fibrin and gelatin in equal proportion gave the highert growth and best food conversion ratio. Obviously, this diet having all the four proteins must be better balanced in the essential amino acids profile which resulted in significantly superior performance. Albumen-casein and albumen-gelatin were found to be the next best combination of proteins

without any significant difference between the two.Among the combinations tested,fibrin-gelatin showed significantly poor performance in P. indicus.Deshimaru and Yone(1978c) used a mixture of casein and egg albumen to study the protein requirement of P. japonicus.Deshimaru and Kuroki(1974a,1975a) used a mixture of vitamin free casein (54%)and egg albumen(6%) to study the nutritional requirements of the same prawn.But these results could not be directly compared with the results obtained in the present study due to the difference in proportions of proteins in the mixtures.However,the findings of the present study have indicated that it is advantageous to use a mixture of albumen,casein,fibrin,gelatin in equal proportions to study the protein requirement.In case this is not possible,albumen alone can be successfully employed for this purpose.Casein alone and other combinations may be used only as alternatives.

Natural protein sources.

In the animal protein sources,prawn waste meal(PW) gave the highest growth followed by clam meat powder(CMP)fish meal(FM),mantis shrimp(MS) and silkworm pupa(SWP) in the decreasing order. But clam meat powder gave the best food conversion ratio followed by mantis shrimp,fish meal,silkworm pupa and prawn waste meal. The true digestibility of protein in the clam meat powder was the highest and that of the protein of silkworm pupa was the lowest.The TD of prawn waste protein was also good and that of fish meal and mantis shrimp proteins were intermediate. The PER of clam powder,fish meal and mantis shrimp were high and that of prawn waste and silkworm pupa were low. In contrast to the PER,the NPU of clam meat powder and fish meal were only higher. The NPU of mantis shrimp meal was lower than

the NPU of prawn waste, and SWP showed a very poor NPU. In the case of biological value (BV) fish meal was on the top followed by clam meat powder, prawn waste, mantis shrimp and SWP.

Fresh clam meat was conventionally used as a feed for prawns. Kanazawa et al. (1970) reported that the fresh diet of short-necked clam, Tapes philippinarum gave superior growth compared to compounded diets in P. japonicus. But Villegas (1978) showed that the growth and survival of P. monodon larvae fed with Tapes was only next to the compounded diets. Superior results were obtained by Forster and Beard (1973, 1974) for Palaemon serratus using fresh mussel meat. Ahamad Ali (1982a) used fresh clam meat of Sunneta scripta as control diet while evaluating some protein materials for P. indicus and found inferior performance of fresh clam meat and heavy mortality. Colvin (1976a) used a diet of fresh mussel meat and prawn meat (50:50) as control diet for the same prawn and found remarkably inferior growth by this diet. But Venkataramaiah et al. (1975b)experienced that, although live foods, such as Artemia nauplii and shrimp meat gave superior growth in brown shrimp P. aztecus, a high rate of chitinoclastic bacterial infection leading to heavy mortality. The poor results of fresh clam meat observed by Ahamad Ali(1982a) might be due to similar reasons. In the same study, the author (Ahamad Ali, 1982a) using the dry meat powder of the clam, Villorita cyprinoidea, prepared the diet which gave the highest growth and best food conversion ratio in juvenile P. indicus. The results obtained with clam meat powder in the present study are comparable to these results.

Fish meals, prepared from different varities of fish, were extensively tested and used in feeds of both finfish and prawns. Nose (1964) found 89.0 assimilation of fish meal protein in the prawn P. japonicus, with the diet containing 96.4% of it. Deshimaru and Shigueno (1972) found comparitively poor results with fish meal in the same prawn. While Shigueno et al. (1972) used white fish meal, Balazs et al. (1973) used Hawaiianfish meal for studying protein requirement of P. japonicus. Andrews et al. (1972) prepared diets with menhaden meal to study the protein requirement of P. setiferus. Varying results were obtained by these authors. Forster and Gabbott (1971) found 90.5 assimilation of white fishmeal in Palaemon serratus and and 75.4 in Pandalus platyceros. Colvin (1976a) used fish meal along with prawn meal to prepare diets with constant protein level, and found an apparent protein assimilation of 69.4 to 72.8 in P. indicus. At 35% protein in the diet the assimilation of the protein from fish meal was 72.8. Ahamad Ali (1982a) compared fish meal (prepared from oil sardines) with other protein sources in P. indicus and found inferior results with it. In the present study, the fish meal, prepared from a mixed fish (Predominantly silver bellies) had shown a true digestibility of 70 in P. indicus. This is slightly lower than the assimilation of protein from fish meal found by Nose (1964) in P. japonicus, but comparable to the results obtained by Colvin (1976a) in P. indicus. The fish meal diet in the present study had shown high PER and NPU and excellent biological value. These findings are in agreement with the conclusions of Lee (1970) who obtained superior results with white fish meal in Penaeus monodon. But Pascual

and Destajo (1978) found inferior growth of P. monodon postlarvae fed fish meal diet, though their survival was higher on this diet. But the results were superior when fish meal was mixed with shrimp head meal. The assimilation of fish meal (Sardine) in gold fish was 92 (Nose, 1967a). Rainbow trout showed an assimilation of 90.8 of white fish meal at 50% level in the diet (Inaba et al., 1962). When flatfish meal was used in the diet (Nose and Mamiya, 1963), the assimilation in the same fish was 87.6. Using herring meal, Atack and Matty (1978) showed a true digestibility (TD) of 91.2 in trout and 80.3 in carp. The TD varied from 88.9 to 91.84 in Tilapia (Sarotherodon mossambicus when white fish meal was used in the diet at 8 to 56% level (Jauncey, 1982). The assimilation of fish meal in P. indicus obtained in the present study is slightly lower than the results obtained in different finfishes using various types of fish meals. However, the TD obtained in P. indicus is comparable to the assimilation of fish meal in poultry, which was 73.3 with white fish meal and 73.3 with brown fish meal (Ward, 1960). The same fish meals had shown an assimilation of 97 and 93.3 respectively, in pigs. From the above discussions it can be seen that the assimilation of fish meal protein varies from animal to animal and also depends upon the origin of fish meal and its method of preparation. Forster and Beard (1973), while studying the diets with different high protein materials for Palaemon serratus, found that Peruvian fish meal and Norwegian herring meal were inferior to white fish meal and confirmed the above observation. Deshimaru and Shigueno (1972) found that the aminoacid composition of fish meal was not similar to that of the prawn P. japonicus and thereby responsible for its

relatively poor performance. However, Colvin (1976a) observed that fish meal was relatively deficient in the essential amino acids tyrosine and phenylalanine and was of the opinion that these factors might be responsible for its inferior results. Nevertheless, the fishmeal tested in the present study scored the highest biological value, contrary to these observations.

Mantis shrimp protein showed good conversion efficiency next to clam meat. However, the TD of mantis shrimp protein was comparitively low. Eventhough the PER was higher than that of fishmeal, the NPU was low. The low TD was mainly due to the chitinous shell material of the mantis shrimp. The higher value of PER, good FCR and finally the BV of 53, show that the protein fraction of the material is of good quality. Garg et al.(1977) extracted protein from mantis shrimp. Using this extracted protein, excellent results were obtained (Ahamad Ali, 1982a) in the juveniles of P. indicus with the diet having 35% protein. The performance of mantis shrimp protein in that study was on par with that of the clam meat powder and superior to that of fish meal. Mantis shrimp has not been evaluated for any other penaeid prawns or finfish in literature. However, mantis shrimp was used for preparing feeds for rearing larvae in hatchery (Mohamed et al., 1983), post-larvae in nursery (Ahamad Ali and Sivadas, 1983) of the prawn P. indicus and also for its culture (Ahamad Ali and Mohamed, 1985) in grow-out-ponds. In all these experiments it was found that mantis shrimp is one of the potential animal protein sources for preparing prawn feed. Alikunhi et al.(1980) used ground tissue suspension of mantis shrimp (Oratosquilla

nepa) and Acetes prawn and successfully reared the larvae of P. indicus, P. monodon and other penaeid prawns. Banerjee(1978) listed Squilla as the new protein source for animal feeds.

Prawn waste meal produced the highest growth compared to the other animal protein sources tested. But the FCR was higher than those of the other protein sources. The TD of protein was slightly higher than that of silkworm pupa, mantis shrimp and fish meal but lower than the true digestibility of the protein from clam meat powder. Eventhough, the PER was low, prawn waste showed better NPU and BV than mantis shrimp and SWP. Forster and Gabbott (1971) determined the digestibility of Norwegian shrimp meal for the caridean prawns Palaemon serratus and Pandalus platyceros. The assimilation of protein (at 40% level) was 87 in the former prawn and 82 in the latter. As compared to these results, the true digestibility of prawn waste protein in P. indicus was low. Meyers and Rutledge (1971, 1973) identified shrimp meal as an important by-product from the prawn processing industry. Forster (1975) reported that Prawn waste protein had several essential aminoacids. The prawn head oil was found to contain polyunsaturated fatty acids (Joseph and Meyers, 1975) essential for crustaceans and it was a potential additive (Joseph and Williams, 1975) in the feeds of marine animals. Choubert and Luquet (1983) utilized shrimp meal in the diet of rainbow trout (Salmo gairdineri) for pigmentation. Sandifer and Joseph (1976) found waste shrimp heads of P. setiferus were a good source of fatty acids and pigments in the diets of the fresh water prawn Macrobrachium rosenbergii. Similar results were obtained by Forster and Beard (1973) for Palaemon serratus. While comparing

the nutritive value of prawn waste and high marsh grass, Venkataramaiah *et al.* (1978) found that the feed prepared with prawn waste gave very good growth in *Penaeus aztecus*. These observations are in agreement with the results obtained in *P. indicus*. But prawn waste was found to be inferior (Ahamad Ali, 1982a) to clam meat powder and mantis shrimp protein and superior to fishmeal for the prawn *P. indicus*. Pascual and Destajo (1978) found that shrimp head meal as one of the most promising protein sources in the diet of *Penaeus monodon*. Contrary to this finding, the authors observed slow growth and low survival of the postlarvae of *P. monodon* fed with the diet prepared exclusively with shrimp head meal. The authors concluded that shrimp head alone does not provide good growth and survival. But addition of shrimp head to fish meal diet improved the performance of the diet. This varying performance of prawn waste may be attributed to its varying quality, as prawn waste could be derived from different species and also the method of processing is varied. It was observed that the residual meat of prawn thrown along with the waste was different in different consignments of prawn waste obtained from the same processing unit. The utilization of prawn waste as potential feed ingredient for the culture of *P. indicus* was studied by Ahamad Ali and Mohamed (1985). Prawn waste was successfully used for preparing the feeds (Mohamed *et al.*, 1983, Ahamad Ali and Sivadas, 1983). Vaitheeswaran and Ahamad Ali (1986) demonstrated that prawn shell, prepared from prawn waste, when supplemented at 0.8 g per 100 g diet, was a positive growth promoting agent in *P. indicus*. Prawn head extract was an excellent lipid source (Ahamad Ali, unpublished) in the

diet of the prawn P. indicus. The higher value of FCR, though the growth of prawns was superior in the present experiments, shows that high ingestion of this diet by the animals. Does this mean that prawn waste meal is more palatable for prawns? It appears to be true when Pascual and Destajo(1978) observed improvement in the performance of fish meal diet when shrimp head meal was added to it for the post-larvae of P. monodon. Perhaps the flavour of prawn head meal is a good feed attractant in prawn feeds. And its high BV shows that it is a good protein source for compounding prawn feeds.

Silkworm pupa produced lowest growth among the five animal protein sources tested. The true digestibility of the protein was also low. But the FCR was not poor. Eventhough PER was not significantly lower than that of prawn waste meal, it gave very low value of NPU and poor BV. Less growth but not very high FCR shows that the diet prepared with SWP was not ingested very well. But whatever that was ingested efficiently converted into growth. This is further supported by moderate PER. The low TD may be due to comparatively higher percentage of non-protein nitrogen in the material, which also resulted in low NPU and biological value. Though silkworm pupa is extensively used in poultry feeds, the references on its use in fish and shellfish feeds are scarce. Kanazawa et al. (1970)used silkworm as one of the ingredients for preparing the diets for P. japonicus along with fish meal and brine shrimp. These results could not be compared with those obtained with SWP in the present study. Silkworm pupa is having very high lipid (about 36%). But this was extracted and only the defatted material is used for feed making. But it still had 10% lipid which can

produce high peroxide value and off flavour. The poor ingestion of the diet with SWP by the prawns in this study shows that the flavour of this material is not attractive to them. It is also not clear whether the poor growth is due to high peroxide value. However, the low BV of SWP and its non-palatability to prawns do not appear to make it a prospective protein source for compounding prawn feeds.

Among the plant protein sources, groundnut cake (GNK) gave comparitively better growth, followed by Spirulina, gingelly cake (GINC) and coconut cake (CNC). The growth obtained by groundnut cake and Spirulina is comparable between them while the growth obtained by coconut cake and gingelly cake was similar. Interestingly, the FCRs obtained by coconut cake and gingelly cake were similar and superior to the FCRs obtained by groundnut cake and Spirulina. The survival of the animals fed Spirulina diet was, however, very poor.

The true digestibility of protein in all the four protein sources was not significantly different. The PER of coconut cake was significantly higher and the PERs of all others were comparable. The NPU and BV of gingelly cake and Spirulina were higher than those obtained by coconut cake and groundnut cake. The BV of Spirulina was significantly higher among the four plant protein sources.

Both coconut cake and gingelly cake are commonly used for feeding cattle. In the present study, these plant protein materials have been systematically evaluated for penaeid prawns. Coconut meal was screened for the prawn P. monodon (SEAFDEC, 1978) and found that it was not a good protein source when used solely.

These results are similar to the results obtained with coconut cake in P. indicus.

Balazs and Ross (1976) used coconut cake along with tilapia meal in the diets of fresh water prawn Macrobrachium rosenbergii and obtained good growth in a 244 day feeding trial. Coconut cake was used at 7% level in fish feeds by Law et al. (1985) and the digestibility of the feed was evaluated for grass carp (Ctenopharyngodon idella). Pathmasothy (1985) included coconut cake from 7 to 14% in the brood stock diets of the fish Leptobarbus hoevenii. These observations indicate that coconut cake is gradually finding its use in aquatic diets. As one of the oil cakes, coconut cake is deficient in the essential amino acid lysine and may be because of which the BV is very low. It is therefore advisable that coconut cake should not be used as the major or sole protein source in prawn diet. It may however, be used to a limited extent in prawn feeds considering its superior PER and FCR.

Nearly 78% of the protein in gingelly cake was found to be digestible in higher animals (Banerjee, 1978). The true digestibility of GINC protein in P. indicus is higher than that was found in higher animals. Cho et al. (1985) observed that GINC is now being used in fish feeds in Asian countries. Just like the other oil cakes GINC is deficient in lysine. However, gingelly cake, with good digestibility of protein and moderate PER and BV can be used as one of the plant protein sources in prawn feeds. But it may not be used as a major or sole protein source in the feed, for it has very low protein content. It should be noted that the FCR of the diet prepared with GINC is low (2.98), which

shows that addition of this material in the feed would not affect the conversion efficiency of the feed. Groundnut cake is extensively used as the plant protein source in animal feeds. Forster and Gabbott (1971) determined the assimilation of groundnut cake in the caridean prawn Palaemon serratus, at 34% protein level in the diet. The mean assimilation of the protein was 89.5. The true digestibility of protein from GNK for P. indicus (86.14) is comparable to the assimilation obtained in the caridean prawn. The digestibility of groundnut protein for many animals including fish is about 89 (Harris, 1978), which is in agreement with the findings in P. indicus. However, the PER, NPU and BV of GNK are low in the present study. Though, GNK protein is one of the good plant proteins, the sulphur containing aminoacids, methionine and cystine are relatively deficient in it. The lysine is also found to be a limiting amino acid in GNK. The low score of BV in P. indicus might be due to these factors. Comparing the digestibility of GNK, yeast and white fish meal, Lee (1970) found that the diet with GNK having 47% protein was one of the best digested feeds for Penaeus monodon. Groundnut cake (also known as Peanut cake) has been used in various proportions (New, 1976) in both experimental diets and practical feeds of different species of prawns. It is used at 8 to 10% level in the commercial feeds (Lovell, 1978) of channel catfish (Ictalurus punctatus). Cho et al. (1995) reported that GNK is used as one of the protein sources in multi-ingredient feed formulations for finfish in Asia. But only in the present study, this plant protein has been individually evaluated for prawns through measuring the true digestibility of its protein, PER, NPU and the biological value. This

information would serve as the reference guide for the use of GNK in future research work as well as for the aquatic feed industry.

Spirulina, though gave growth and FCR comparable to that of GNK, the survival of the animals was very low. However, it emerged as the protein source with high NPU and BV among the four plant protein sources evaluated. This unicellular fresh water alga, is being cultured on large scale, at the Central Food Technological Research Institute (CSIR) at Mysore. Lim et al. (1978) prepared diets with Spirulina having 50% crude protein and fed to the postlarvae of P. monodon. The growth and survival (17%) of the post-larvae fed this diet were very poor and the diet had shown very low PER (0.6). The authors compared the results of Spirulina diet with other diets prepared with casein, shrimp meal and squid meal and found that the results of Spirulina diet were inferior to all other diets tested. While the growth of P. indicus fed Spirulina diet in the present study was not poor, the FCR, PER and survival were similar to what was found with P. monodon post-larvae by Lim et al. (1978). Cuzon et al.(1981) incorporated Spirulina in a standard diet of P. japonicus from 0 to 8%. The authors found good growth and FCR of the diet having 8% Spirulina. They also observed that Spirulina in the diet contributed to the pigmentation of the prawns. In another experiment in the same study, the authors extracted lipid from Spirulina and incorporated in the Standard diet at 4.57% level. The prawns fed this diet had very low survival of 16%. Investigations showed that the lipid extracted from Spirulina had a very high 'Peroxide value' due to the oxidation

of lipid. The low survival of prawns fed this diet was attributed to the toxic peroxide in the lipid added to the diet. The growth and FCR obtained in P. indicus with Spirulina diet in the present study are comparable to those obtained in P. japonicus. And the low survival of the prawn, P. indicus might be due to similar reasons put forward by these authors. It is reported that single cell proteins have high content of free nucleic acids (Personal discussions with Dr.Venkataraman, L.V.,Scientist, CFTRI, Mysore) and their influence on the survival of prawns need thorough investigation.

Atack et al. (1979) tested the digestibility of protein in Spirulina maxima (Mexico) for carp (Cyprinus carpio). At 30% protein level in the diet, the true digestibility was 87. The diet had shown a PER of 1.15 with a NPU of 36 and BV of 41. Similar results were obtained in some warm water fishes by Hepher et al. (1978). Eventhough the true digestibility and PER of Spirulina diet was low in P. indicus compared to that of carp, the NPU and BV were relatively higher in prawn than in carp. However, markedly low survival of prawn should be noted which was not observed in carp by the above authors.

The uni-cellular alga is emerging as a new source of plant protein and a potential ingredient in aquatic feeds. It may be used in prawn diet only at a limited level (upto 8%), as reported by Cuzon et al.(1981). It, however, is not advisable to use it as the sole protein source in the diet. As Spirulina is an expensive material at the moment, cautious approach should be adopted in using it in prawn feeds.

Comparing the animal protein sources (APS) to the plant protein sources (PPS), it can be seen that the digestibility of APS is low but they have higher NPU and BV. Whereas the PPS have high digestibility but low NPU and BV. These differences between APS and PPS are mainly due to the difference in their protein quality, especially of the essential aminoacids. Harris (1978) pointed out that the APS are generally rich in the essential amino acid lysine but deficient in sulphur containing amino acids, cystine and methionine. On the other hand plant protein sources are generally deficient in lysine but relatively rich in sulphur containing aminoacids. However, groundnut cake contains comparatively low levels of cystine and methionine. The essential aminoacid composition of some of the protein sources, collected from literature, is given in Table 18. It is established that the aminoacids arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine were essential both for caridean prawns such as Palaemon serratus, Macrobrachium ohione and M. rosenbergii (Cowey and Forster, 1971; Miyajima et al., 1976; Watanabe, 1975) and penaeid prawns, Penaeus aztecus, P. kerathurus and P. japonicus. (Shewbart et al., 1972; Torres, 1973; Kanazawa and Teshima, 1981). The quantitative requirements of these essential aminoacids in prawn diets are however, not yet known, though several workers have studied the effect of aminoacid supplementation in the diets (Cowey and Forster, 1971; Kitabhayashi et al., 1971c,d; Dehsimaru and Kuroki, 1974c, 1975a, b). Determination of quantitative requirement of essential aminoacids (EAA) for prawns will go a long in formulating practical feeds with exact EAA balance with optimum

Table 18. Essential aminoacid composition (percentage of total protein) of some protein materials.

Essential amino acid	Albumen[1] (egg)	Casein[1]	Fish[2] meal	Short[3] necked clam, *Tapes*.	Prawn[2] meal	Soybean[1] meal	Ground[1] nut cake	Muscle[2] of juvenile *P. indicus*.
Protein %	76.9	90.0	65.0	--	74.1	45.0	45.0	69.3
Arginine	4.18	4.30	5.20	6.30	5.30	2.61	4.46	5.80
Histidine	--	2.60	1.30	2.30	1.30	1.03	0.95	1.30
Isoleucine	4.31	8.50	5.5	3.75	2.80	1.80	1.58	3.30
Leucine	6.27	11.30	7.7	6.43	5.0	3.60	4.50	5.90
Lysine	4.43	7.5	8.0	7.52	5.0	2.43	1.35	5.90
Methionine	2.70	3.4	2.80	2.19	1.6	0.81	0.41	1.20
Phenylalanine	4.43	5.70	4.10	3.38	4.0	1.85	2.43	3.40
Threonine	3.69	4.5	4.0	4.07	2.6	1.80	0.68	2.90
Tryptophan	1.35	1.60	1.2	--	0.6	0.63	0.45	--
Valine	6.03	8.4	5.2	4.29	3.7	1.58	3.15	3.7

1. Harris (1978)
2. Colvin (1976a)
3. Deshimaru and Shigueno (1972)

protein level in the feed. This will greatly help in achieving better economics of feed and avoid wastage of valuable protein.

Studies on aminoacid requirements of rats and chicks showed that growth is a direct function of the amount of the limiting aminoacid; Mitchell and Block (1946) considered the amounts of aminoacids in whole egg protein as a standard reference since it was known to contain a good balance of aminoacids. Egg protein had a BV of approximately 100. Taking egg protein as standard, the authors suggested a chemical score for assessing the quality of a protein source depending upon the limiting aminoacid. Rao et al.(1964) found a high correlation between BV and chemical score in rats. Thus the Biological Value is a good indication of the quality of a protein for that animal for which it is tested. Campbell and Chapman (1959) developed a 'protein rating system' based on both the quality and quantity of the protein in the food. The protein rating was obtained by multiplying the PER of the food by the grams of protein in a reasonable daily intake. A food having a rating of 40 or more was designated as 'an excellent dietary source of protein' and the one with a rating of 20 to 39 as a good dietary source of protein. A similar rating system is followed in the "proposed Standard Identity of texture of Protein Products" in United States. But in this system, instead of daily reasonable intake, the quantity of food based on 100 calories was taken. The PER of the protein expressed as a fraction of the PER of casein, multiplied by the amount of protein in grams must not be less than 6.0 and the protein must also have a biological quality not less than 70% of that of casein. It was also pointed out that NPU would be more preferable to PER in the calculation

of ratings of proteins. Protein rating system is useful mostly in case of humans, because it is possible to estimate an average daily intake of a particular protein (such as milk, wheat bread, egg etc.) for calculating its rating. But it is not so in case of aquatic animals like prawns. The 'chemical score' may be more useful to undersand the quality of the protein, in general. Examining the BVs of the proteins evaluated for P. indicus, among the purified proteins albumen, casein and fibrin may be considered as good (BV above 40) and gelatin as not good (BV below 40). All the animal protein sources except SWP tested can be regarded as excellent (BV above 50) and SWP as good (BV below 50). In the plant protein sources, only Spirulina can be given a rating of good (BV, 50) and the others are only as satisfactory (BV between 20 and 40).

The experiment on the effect of the ratio of animal protein source (APS) and Plant Protein Source (PPS), in the diet on growth, digestibility, PER, FCR, NPU and BV gave interesting results. The APS and the PPS when present in the ratio 70:30 in the diet, gave the highest growth in the prawn P. indicus. Neither the diet having only APS nor the diet made exclusively with PPS gave better growth. Chen et al. (1985) observed improved performance of the feed when contained a mixture of APS and PPS in P. setiferus and P. vannamei. The FCR of the diet having 70% APS and 30% PPS in the present study was not inferior to FCRs obtained by the other diets. But the performance of the diet was reduced as the PPS in the diet increased. These results have shown that in compounded feeds with natural ingredients, a mixture of APS and PPS is essential. This is mainly because, that neither the APS nor the PPS

can provide all the essential amino acids (EAA) in adequate levels. While the animal protein sources are deficient in sulphur containing EEAs, the plant protein sources are deficient in lysine. To strike the balance of EEA, a mixture of APS and PPS in the diet is therefore imperative. It is evident from the fact that the diet prepared exclusively with APS had higher protein content than the diet which contained 70% APS and 30% PPS. Still, the growth and PER obtained by the former were low. However, the diet having only APS showed high NPU and BV. As the PPS content in the diet increased, the NPU and BV came down, showing the APS possess superior biological quality over the PPS. The diets having higher percentage of PPS did not show any inferiority in FCR and in fact gave higher values of PER. However, the NPU and BV of these diets were significantly low confirming the earlier statement regarding their protein quality. A close examination of the values of NPU and BV of the diets indicates that these are uneffected even if the diet contained 50% PPS. But the growth of the animals was significantly reduced if the diet had more than 30% PPS. The limiting of the aminoacid lysine might be becoming more pronounced beyond this PPS level in the diet. When Deshimaru and Shigueno (1972) first introduced the prawn, P. japonicus to the artificial diet, they used a diet made up of several ingredients such as squid meal, fish meal, whale meal, mysid shrimp meal, yeast, soybean protein, active-sludge, casein and gelatin. They found that the diets having the amino acid composition similar to the aminoacid composition of prawn gave good results. The authors prepared diets having aminoacid composition similar to that of P. japonicus by using a mixture of

animal and plant protein sources and established that the prawns could be successfully cultured on such artificial diets. Forster and Beard (1973) observed that the growth of the prawn Palaemon serratus, was high when the diet contained more than one ingredient. But when the animal protein white fish meal was completely substituted with soybean protein, the diet gave very poor results. However, Sick and Andrews (1973) found soybean to be a superior ingredient even when it was used as the sole protein source for P. duorarum. Soybean protein is more balanced in the essential aminoacids among the various plant proteins available. Venkataramaiah et al. (1975a) found better utilization of protein in P. aztecus, when the diet contained certain amount of vegetable matter. Zein-Eldin and Corliss (1976) reported similar results in the same prawn and found that inclusion of rice bran in shrimp meal based diet improved the growth.

Fenucci and Zein-Eldin (1976) postulated that synergistic effect between dietary components improves performance of the diet. But this synergism must be the result of improved balance of essential nutrients in the required level when more than one ingredient is used in the diet. In Macrobrachium rosenbergii, the results were much superior when the diet contained fishmeal-soybean-shrimp meal (Balazs et al., 1973) compared to the diet having only soybean meal. Subsequently Balazs et al. (1974) found that the diet with soybean meal-tuna meal gave significantly greater results than the diets containing only soybean, tuna meal and shrimp meal. These authors concluded that by using different ingredients (animal and plant) the possibility of approaching optimum aminoacid balance in the diet are greater and thus could

achieve superior results. The variations in the dietary protein requirement within the same species as obtained by different workers may largely be explained due to the different protein sources used in preparing the diet. In the diets where the optimum aminc acid balance is not reached, the animals may show higher protein requirement, and in the diets where the aminoacid balance is achieved, the dietary protein requirement may be much lower. The discussions presented above and also the results obtained in the prawn P. indicus clearly suggest that compounded feeds should be multiingredient based and no single material, either animal or plant origin, can be a complete feed by itself. It is only logical that animals like prawns feed on a variety of materials of plant and animal origin in nature. In doing so, the animals must be deriving the balanced nutrition and thus disposed to a healthy growth.

Formulation of compounded feeds has important bearing on the cost of resultant practical feed and influences the economics of production. Generally protein in the feed constitutes the most important and expensive nutrient in the feed. Further, animal protein sources are comparitively more expensive than the plant protein sources. It is in this context the results obtained in this particular experiment, in the present study, are very important. The results have clearly shown that the growth of prawns is higher when the feed is formulated using both animal and plant protein sources. In the present case, the plant protein sources can be used upto 30% with 70% animal protein source, without affecting the efficiency of the feed.

It was discussed earlier that combination of APS (70%) and PPS (30%) in diet might have helped in attaining more closer aminoacid balance required by the prawn, P. indicus. Using this protein combination as the protein source, diets were formulated with protein content varying from 15% to 40%. In this experiment, the feed with 30% protein gave the highest growth and good FCR. Low growth and high FCR were observed at protein levels lower than 30%. But at protein levels higher than 30%, though the FCR was not significantly different, the growth had shown a decreasing tendency. The protein requirement shown by P. indicus in this experiment is same as that obtained by casein diets and slightly higher than the value shown by the albumen diets. However, the protein requirement obtained in this study is lower than the protein requirement shown for this prawn by Colvin(1976a) and Ahamad Ali (1982a) which was found to be 40%. The former author used shrimp meal and the latter, a mixture of mantis shrimp protein and ground nut cake as protein source. But the results of the present study are in agreement with the findings of Charles John Bhaskar and Ahamad Ali (1984) in which it was shown that juveniles of P. indicus required 30% protein in the diet, using casein diets. The protein requirement shown by P. indicus in this study is comparable to the protein requirement shown by P. aztecus (Shewbart et al., 1973). P. setiferus (Andrews et al., 1972), P. duorarum (Sick and Andrews, 1973) and also the Juveniles of P. monodon (Khannapa, 1979). It is lower than the protein requirement of P. japonicus (Kanazawa, et al., 1970; Shigueno et al., 1972; Balazs et al., 1973; Deshimaru and Yone, 1978c; Deshimaru and Shigueno, 1972), P. aztecus (Venkataramaiah et al., 1975a;

Zein-Eldin and Corliss, 1976), P.merguiensis (AQUACOP, 1978; Sedgwick, 1979). P. monodon (Alava and Lim, 1983) and Metapenaeus monoceros (Kanazawa et al., 1981).

As seen under the purified proteins, the faecal nitrogen was comparitively higher in animals fed low protein diet and lower in the faeces of animals fed high protein diet. This has been found to be true even in land animals. At low levels of protein intake, the constant excretion of metabolic faecal nitrogen (MFN) makes up a greater proportion of the faecal nitrogen (Maynard et al., 1981). out put, which becomes progressively less as the feed protein levels increase. It was also found that apparent digestibility of protein increases in a curvilinear fashion as the level of dietary protein increases. Similar results are obtained in P. indicus both with purified proteins as well as the practical protein sources in the present study.

The protein balance (Fig.18), just like the nitrogen balance (Fig.9b) seen under the purified proteins, was negative at lower dietary protein level, then gradually increased with dietary protein level, and became positive at higher dietary protein levels. The protein balance curve (Fig.18), which is inclined to X-axis, intercepted the protein axis (Y-axis) at 26% protein level where the protein balance is zero. This is almost similar to the nitrogen balance curve (Fig. 9b) in which it intercepted the protein axis at 22.5%. The diet with 29.9% protein, which is just 3.9% higher than the level of zero protein balance, gave highest growth of the animals. Protein levels above and below this level tended to decrease the growth. The results once again emphasised that to obtain highest growth, the protein content in the diet

should be a little above (within 5% in the present case) the protein level at which the protein balance is zero. Even though the diet contains adequate levels of energy, the animals have a minimum essential protein catabolism for the maintainance of vital processes of the body. When the protein for this purpose is met fully from the diet, probably the protein balance is becoming zero. But even at this dietary protein, the growth in the animal is positive perhaps due to the available metabolisable energy in the diet consumed. Once the animal receives the protein a little in excess of this minimum requirement, the excess protein received must be fully utilised for growth registering highest growth at that protein level in the diet. But too much in excess of protein in the diet might be causing handling strain on the system, there by resulting in lower growth. Reviewing the dietary studies with shrimps and prawns, New (1976) suggested that it is reasonable to conclude that the optimum protein level for penaeid prawn diets, lies in 27-35% region. The results obtained in the present investigations are fully in agreement and are well within this limit. The possibility of achieving still low levels of dietary protein could be examined only after knowing the qualitative and quantitative essential aminoacid requirements of candidate species. Perhaps, the most urgent need is the determination of quantitative aminoacid requirement for each commercially importa t species of prawns to approach towards more economically efficient diet.

CONCLUSIONS

1. The purified proteins fibrin and albumen have high biological value (BV) for the prawn Penaeus indicus, followed by casein. Gelatin is a poor protein source for this prawn with low BV.
2. Considering growth, food conversion ratio (FCR) and protein efficiency ratio (PER) in conjunction with Net Protein Utilisation (NPU) and BV, albumen is the best protein source for formulating research diets for P. indicus.
3. The average metabolic faecal nitrogen (MFN) in juvenile P. indicus is found to be 324 mg N/100 g diet, when determined using zero protein diet.
4. The true digestibility of protein in the diet tended to be low at lower levels and high at higher dietary protein levels, though the difference was not statistically significant.
5. The PER, NPU, and BV were high at lower dietary protein levels and decreased as the protein level in the diet increased.
6. The dietary protein requirement of juvenile P.indicus is found to be 25% with albumen diets and 29% with casein diets.
7. The growth, FCR, PER, NPU and BV were the highest at the optimum protein level in both albumen and casein diets.
8. The nitrogen balance (calculated as the difference in nitrogen of the diet and the faeces) was zero at 22% dietary protein. When the dietary protein was raised a little above this level (3% in case of albumen diet and 7% in case of casein diet) the protein balance was positive at which the diet had shown the best performance.

9. In the different mixed protein combinations, the diet having, all the four proteins, albumen, casein, fibrin and gelatin in equal proportion gave the best results. This was followed by the combinations albumen-casein and albumen-gelatin. Fibrin-gelatin combination gave poor results.

10. To obtain balanced aminoacid profiles in the diet, it is suggested that a mixed portein source may be used for protein requirement studies. Albumen alone can be successfully employed for this purpose. Casein alone and other combinations mentioned above may be used only as alternatives. Proteins having high BV only should be used for protein requirement study to obtain realistic information.

11. Among the animal protein sources evaluated, fish meal, clam meat powder, prawn waste meal and mantis shrimp were found to be good protein sources with high PER, NPU and biological value in the decreasing order for P. indicus. Silkworm pupa was found to be a poor protein source for this prawn having low digestibility, PER, NPU and BV.

12. Among the plant protein sources, Spirulina and ground nut cake gave best growth, the former showed high biological value, but the BV of ground nut cake was very low. However, the animals fed Spirulina diet showed very low survival.

13. Coconut cake and gingelly cake, cannot be used as sole protein sources in the diet of P. indicus as they showed low growth and BV, though their protein had high digestibility and low FCR.

14. Animal protein sources in general had low digestibility but showed higher growth, PER, NPU and BV over the plant protein sources.

15. The diet having 70% animal protein source and 30% plant protein source gave the best growth and FCR, than the diets made exclusive either with animal protein source or plant protein source. The performance of the diet decreased as the plant protein source in the diet increased.

16. The juveniles of P. indicus showed a protein requirement of 30% in practical diet, when the diets were prepared with 70% animal protein source and 30% plant protein source. These results are similar to the protein requirement shown using casein diets.

17. The protein balance, which is the difference between the dietary protein and the protein in the faeces, was negative at lower dietary protein level and positive at higher dietary protein level.

18. The protein balance in practical diets was zero at a dietary protein level of 26% which was similar to the nitrogen balance obtained in casein and albumen diets.

19. The optimum protein level in the diet shown by the prawn was just 3.7% above the dietary protein level where the protein balance was zero.

20. Possibilities of achieving further lower levels of optimum protein in the diet could be examined only after the quantitative requirement of essential amino acids of candidate species of commercial prawns is known.

C H A P T E R - II

EVALUATION OF DIFFERENT SOURCES OF CARBOHYDRATES

Present status

Carbohydrate is an important source of energy component in the diet of animals including prawns. There are different types of carbohydrates available in nature. These are monosaccharides (eg. glucose, fructose, galactose), disaccharides (eg. sucrose, maltose, lactose) and polysaccharides (eg. starch, glycogen and cellulose). Most of the carbohydrates which contribute to the diet of the animals are of plant origin. The polysaccharide, glycogen is of animal origin and is generally known as animal starch. Carnivorous fish such as Atlantic salmon and Japanese yellow tail do not utilize appreciably, the dietary carbohydrate because their digestive system is not equipped to handle significant quantities of carbohydrate. On the other hand omnivorous fish such as common carp and channel catfish are able to digest fair amounts of carbohydrates in their diet. Digestion of carbohydrate in crustaceans has been demonstrated by Kooiman (1964). Dall (1964) studied the metabolism of carbohydrate in the Prawns (Metapenaeus sp.). Tyagi and Prakash (1967) investigated the nutritional importance of carbohydrates in Prawns. Subsequently Telford (1970) conducted comparitive study of carbohydrate activities of some crustacean tissues and Parvathy (1971) studied the carbohydrate metabolism in two crustaceans during starvation. The presence of enzymes involved in carbohydrate metabolism such as α-amylase, β-amylase, maltase, saccharase, chitinase, has been demonstrated. Amylase activity was found in the digestive tract of the prawn Penaeus indicu

(Karunakaran and Dhage, 1977). Carbohydrates are important for energy production in the Kreb's cycle, in glycogen storage, in chitin synthesis and in the formation of steroids and fatty acids. Prawns do not appear to utilise efficiently all types of carbohydrates available in nature. The ability to digest specific sources of carbohydrates varies from species to species. Andrews et al.(1972) found that addition of 20% glucose to a diet based on menhaden meal reduced the growth rate in Penaeus aztecus. When starch was included in the same diet at 30% level, increased growth rate was obtained. Andrews and Sick (1972) observed that when radio-active isotope (14_C) labelled starch and glucose were used separately in the diet, the incorporation of radioactivity in the tissue was at higher rate from starch than from glucose in Penaeus setiferus. It was postulated that the dietary glucose was very rapidly absorbed into the body but not utilized efficiently. On the other hand, glucose from digested polysaccharides was absorbed very slowly and thus utilized effectively. When glucose was used at lower levels (about 10%) the growth of the prawn Penaeus duorarum was not affected, but at higher levels (about 40%) the growth was very poor (Sick and Andrews, 1973). Deshimaru and Yone (1978b) tested different carbohydrates, glucose, sucrose, glycogen, starch and dextrin for P. japonicus. The authors found that higher growth was obtained with the group fed on the diet containing sucrose followed by glycogen. Glucose resulted in lowest gain in weight. The diets having glucose, starch and dextrin caused high

mortality. AQUACOP (1978) suggested that starch appears to be more suitable carbohydrate than glucose for P. merguiensis. Abdel Rahman et al. (1979) also evaluated different types of carbohydrates for P. japonicus and found that disaccharides and polysaccharides gave better growth. As reported earlier, these authors also found that glucose resulted in poor growth and high mortality. Pascual et al. (1983) demonstrated that sucrose and dextrin were better utilised than any other carbohydrate by P. monodon. When glucosamine was used in the diet in the place of glucose, the growth of Prawn P. japonicus was improved (Kitabhayashi et al., 1971b), and at 0.53% it was found to enhance the growth rate in P. japonicus (Kitabhayashi et al., 1971a). Similar results were obtained in P. indicus by Vaitheeswaran and Ahamad Ali (1986). Contrary to this, Deshimaru and Kuroki (1974b) found no beneficial effect of glucosamine in the diet of P. japonicus. A suitable source of carbohydrate in the diet seems to spare carbon chains from aminoacids for chitin synthesis (Cowey and Forster, 1971). Venkataramaiah et al. (1975a) reported that inclusion of vegetable fibre in the diet resulted in better utilisation of dietary protein in P. aztecus. Better growth was obtained in P. japonicus when 6% dextrin was included in the diet (Deshimaru and Kuroki, 1974a). Fair et al. (1980) studied the effect of dietary fibre on growth and assimilation and the presence of cellulase activity in the gaint prawn, Macrobrachium rosenbergii.

The quantitative requirement of carbohydrate in the

diet of prawns vary from species to species. Andrews et al. (1972) had reported that 30% starch in the diet gave better growth and spared protein in the diet in P. aztecus. Sick and Andrews (1973) found that 40% corn starch in the casein-based diets produced faster growth in P. duorarum. The growth of prawns P. indicus increased with increase in the dietary level of starch up to 40% (Ahamad Ali, 1982b). Recently Teshima and Kanazawa (1984), while studying the nutritional requirements of the larvae of P. japonicus, observed that the growth and survival of the larvae varied with protein and carbohydrate levels in the diet and not with the lipid levels. Alava and Pascual (1987) found that the diets containing trehalose and sucrose gave better weight gain in Juvenile P. monodon than the diets having glucose. They also observed that the best results were obtained with the diet having 20% carbohydrate.

Studies on the digestibility of dietary carbohydrates in prawns are very limited. Forster and Gabbott (1971) studied the assimilation of carbohydrate by the caridean prawns Palaemon serratus and Pandalus platycerog.

In the present study, three monosaccharides, two disaccharides and two polysaccharides were selected and evaluated for their suitability as carbohydrate source in the diet of P. indicus. The digestibility of each carbohydrate and the effect of dietary carbohydrate level on its digestibility, growth, and food conversion ratio were studied. The influence of cellulose in the diet was investigated. The protein sparing action of carbohydrate under different dietary conditions was studied and discussed in detail.

MATERIAL AND METHODS

Seven different carbohydrates were selected for evaluation in the diet of the prawn Penaeus indicus. Out of them, three were monosaccharides, glucose, fructose and galactose; two were disaccharides, maltose and sucrose, and two were polysaccharides, glycogen and starch. These materials were of analytical grade and were obtained from the companies described below:

1. Monosaccharides

a) Glucose	BDH Chemicals (Glaxo Laboratories, Bombay)
b) Fructose	E. Merck Chemicals, Bombay.
c) Galactose	Sarabhai M. Chemicals, Baroda.

2. Disaccharides

a) Maltose	LOBA Chemic Indoaustranal, Co., Bombay.
b) Sucrose	BDH Chemicals

3. Polysaccharides

a) Starch	BDH Chemicals
b) Glycogen	LOBA Chemicals

All the materials were solids in fine powder form, and were used in the diets as such.

Experiment 8: Evaluation of dietary characteristics of different sources of carbohydrates.

To evaluate the identified carbohydrates, seven separate diets were formulated using each carbohydrate in the diet at 30% level. The basal purified diet, having albumen(egg) and cod liver oil as protein and lipid sources

respectively and used for evaluation of purified proteins (vide Chapter-I), was adopted. A zero carbohydrate diet CE_0 was also formulated for comparison. For determining the digestibility of each of the carbohydrates, the inert marker chromium oxide was included at 0.5% level in all the diets. The composition of the experimental diets, designated as CE_0, CE_1,(glucose), CE_2(fructose) CE_3(galactose), CE_4(sucrose), CE_5(maltose), CE_6(starch) and CE_7(glycogen) is shown in Table 19.

Experiment 9. Evaluation of dietary characteristics of different combinations of carbohydrate sources.

Seven diets, comprising of carbohydrates, glucose-glycogen, glucose-starch, glucose-sucrose, glucose-maltose, glucose-sucrose-starch, glucose-maltose-starch and sucrose-maltose-starch were prepared and their effect on the growth, food conversion ratio (FCR) and survival of P. indicus was studied. In each combination the individual carbohydrates were used in equal proportions to obtain a mixed carbohydrate level of 30%. The detailed composition of these diets (CE_8 to CE_{14}) is given in Table 20.

Experiment 10. Evaluation of carbohydrate level in the diet at constant Protein and lipid.

In the earlier experiment it was found that the carbohydrate consisting of sucrose-maltose-starch in equal proportions was the best source of carbohydrate in the diet of P. indicus. Using this mixed carbohydrate source, the effect of carbohydrate level in the diet on its digestibility, growth, FCR and survival of P. indicus was studied

Table 19. Composition (g/100g) of the experimental diets CE_0 to CE_7.

Ingredients	Diet No.							
	CE_0	CE_1	CE_2	CE_3	CE_4	CE_5	CE_6	CE_7
Albumen (egg)	70.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Cod liver oil	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Glucose	--	30.0	--	--	--	--	--	--
Fructose	--	--	30.0	--	---	--	--	--
Galactose	--	--	--	30.0	--	--	--	--
Sucrose	--	--	--	--	30.00	--	--	--
Maltose	--	--	--	--	--	30.0	--	--
Starch	--	--	--	--	--	--	30.0	--
Glycogen	--	--	--	--	--	--	--	30.0
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Glucosamine HCl	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Vitamin mixture*	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7
Mineral mixture@	8.6	8.6	8.6	8.6	8.6	8.6	8.6	8.6
Cellulose	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9
Cr_2O_3	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sodium alginate	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Crude Protein %	55.0	31.5	31.5	31.5	31.5	31.5	31.5	31.5

* Vitamin mixture: Same as used for the diets PE_0 to PE_4 in chapter I (Table 2a)

@ Mineral mixture: Same as used for the diets PE_0 to PE_4 in chapter I (Table 2b)

Table 20. Composition (g/100g) of the experimental diets CE_8 to CE_{14}.

Ingredients	Diet No.						
	CE_8	CE_9	CE_{10}	CE_{11}	CE_{12}	CE_{13}	CE_{14}
Albumen (egg)	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Cod liver oil	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Glucose	15.0	15.0	15.0	15.0	10.0	10.0	--
Sucrose	--	--	15.0	--	10.0	--	10.0
Maltose	--	--	--	15.0	--	10.0	10.0
Starch	--	15.0	--	--	10.0	10.0	10.0
Glycogen	15.0	--	--	--	--	--	--
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Glucosamine HCl	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Vitamin mixture *	2.7	2.7	2.7	2.7	2.7	2.7	2.7
Mineral mixture @	8.6	8.6	8.6	8.6	8.6	8.6	8.6
Cellulose	8.4	8.4	8.4	8.4	8.4	8.4	8.4
Sodium alginate	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Crude protein %	31.5	31.5	31.5	31.5	31.5	31.5	31.5

* Vitamin mixture : Same as used for the diets PE_0 to PE_4 in Chapter I(Table 2a)

@ Mineral mixture : Same as used for the diets PE_0 to PE_4 in Chapter I(Table 2b)

in this experiment. Seven diets, numbering CE_{15} to CE_{21} were formulated varying the carbohydrate content from 5 to 50% in the diet but keeping the protein and lipid constant. Chromium oxide at 0.5% level was included in all the diets to determine the digestibility of carbohydrate. The composition of the diets is shown in Table 21.

Experiment 11. Determination of optimum level of carbohydrate in the diet at constant lipid.

To study the effect of different levels of carbohydrate at constant lipid on its digestibility, growth, FCR and survival of the animals and thereby determining the optimum level required in the diet, isocaloric (4.03 K cal/gram) diets were formulated with varying levels of carbohydrate, keeping the lipid constant. To make the diets isocaloric, protein content was adjusted. As a result, high protein-low carbohydrate to low protein-high carbohydrate diets were obtained. While the carbohydrate in the diet varied from 5 to 50%, the protein content varied from 21.9% to 50.7%. The composition of these diets, designated as CE_{22}, CE_{23}, CE_{24}, CE_{25}, CE_{26}, CE_{27}, and CE_{28}, is given in Table 22.

Experiment 12. Determination of optimum level of carbohydrate in the diet at constant protein.

Seven more iso-caloric diets were formulated to study the combined influence of varying levels of carbohydrate and lipid in diet at constant protein, on its digestibility, growth, FCR and survival of *P. indicus*. In these diets, keeping the protein constant, the carbo-

Table 21. Composition (g/100g) of the experimental diets CE_{15} to CE_{21}.

Ingredients	Diet No.						
	CE_{15}	CE_{16}	CE_{17}	CE_{18}	CE_{19}	CE_{20}	CE_{21}
Protein mix. *	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Cod liver oil	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Carbohydrate mix.+	5.0	10.0	15.0	20.0	30.0	40.0	50.0
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Glucosamine HCl	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Vitamin mixture @	2.7	2.7	2.7	2.7	2.7	2.7	2.7
Mineral mixture £	8.6	8.6	8.6	8.6	8.6	8.6	8.6
Cellulose	32.9	27.9	22.9	17.9	7.9	—	—
Cr_2O_3	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sodium alginate	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Crude protein %	34.8	34.8	34.8	34.8	34.8	34.8	34.8

* Protein mix: A mixture of albumen, casein, fibrin and gelatin in the ratio 1:1:1:1.

+ Carbohydrate mix: A mixture of sucrose, maltose and starch in the ratio 1:1:1.

@ Vitamin mixture : Same as used for diets PE_0 to PE_4 in Chapter 1 (Table 2a)

£ Mineral mixture : Same as used for diets PE_0 to PE_4 in Chapter 1(Table 2b)

Table 22. Composition (g/100g) of the experimental diets CE_{22} to CE_{28}.

Ingredients	Diet No.						
	CE_{22}	CE_{23}	CE_{24}	CE_{25}	CE_{26}	CE_{27}	CE_{28}
Protein mix. *	58.26	54.51	50.89	47.19	40.0	32.5	25.23
Cod liver oil	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Carbohydrate mix.+	5.0	10.0	15.0	20.0	30.0	40.0	50.0
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Glucosamine HCl	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Vitamin mixture @	2.7	2.7	2.7	2.7	2.7	2.7	2.7
Mineral mixture £	8.6	8.6	8.6	8.6	8.6	8.6	8.6
Cellulose	14.64	13.39	12.01	10.71	7.9	5.4	2.67
Cr_2O_3	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sodium alginate	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Crude protein %	50.7	47.8	44.3	41.1	34.8	28.3	21.9

* Protein mix: A mixture of albumen, casein, fibrin and gelatin in the ratio 1:1:1:1.

+ Carbohydrate mix: A mixture of sucrose, maltose and starch in the ratio 1:1:1.

@ Vitamin mixture : Same as used for diets PE_0 to PE_4 in Chapter 1 (Table 2a)

£ Mineral mixture : Same as used for diets PE_0 to PE_4 in Chapter 1(Table 2b)

hydrate was varied from 5 to 50%. At the same time the lipid in the diet was also varied to make the diets isocaloric, resulting in high lipid-low carbohydrate and low-lipid-high carbohydrate diets. The detailed composition of the diets, CE_{29} to CE_{35} is presented in Table 23.

Experiment 13. Effect of cellulose level in the diet on the growth and food conversion ratio of P. indicus.

In order to study the effect of cellulose level in the diet on the growth and food conversion ratio of P. indicus, eight diets, designated as CE_{36}, CE_{37}, CE_{38}, CE_{39}, CE_{40}, CE_{41}, CE_{42}, and CE_{43}, were formulated. The cellulose in the diets was varied from 0 to 20% replacing the corresponding amount of carbohydrate. The protein and lipid in the diets were kept constant. The composition of the diets is given in Table 24.

Experimental Procedures: The design of the feeding experiments, rearing facility, experimental animals, water management, feeding procedure, faeces collection, measurement of growth, food conversion ratio were same as described under Chapter I. The hydrographical data in respect of different feeding experiments are summarised in Table H_2. The apparent digestibility of carbohydrate was calculated using the following formula.

$$\text{Apparent digestibility of Carbohydrate} = 100 - \frac{\%\ Cr_2O_3 \text{ in diet}}{\%\ Cr_2O_3 \text{ in faeces}} \times \frac{\%\text{Carbohydrate in faeces}}{\%\text{Carbohydrate in diet.}} \times 100$$

Table 23. Composition (g/100g) of experimental diets CE_{29} to CE_{35}

Ingredients	Diet No.						
	CE_{29}	CE_{30}	CE_{31}	CE_{32}	CE_{33}	CE_{34}	CE_{35}
Protein mix. *	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Cod liver oil	16.8	14.7	12.59	10.42	6.0	1.58	--
Carbohydrate mix.+	5.0	10.0	15.0	20.0	30.0	40.0	50.0
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Glucosamine HCl	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Vitamin mix. @	2.7	2.7	2.7	2.7	2.7	2.7	2.7
Mineral mix. £	8.6	8.6	8.6	8.6	8.6	8.6	8.6
Cellulose	22.1	19.2	16.31	14.48	7.9	2.32	--
Cr_2O_3	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sodium alginate	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Crude protein %	34.8	34.8	34.8	34.8	34.8	34.8	32.8

* Protein mix: A mixture of albumen, casein, fibrin and gelatin in the ratio 1:1:1:1.

\+ Carbohydrate mix. A mixture of sucrose, maltose, and starch in the ratio 1:1:1.

@ Vitamin mixture: Same as used for diets PE_0 to PE_4 in Chapter 1 (Table 2a)

£ Mineral mixture: Same as used for diets PE_0 to PE_4 in Chapter 1 (Table 2b)

Table 24. Composition (g/100g) of the experimental diets CE_{36} to CE_{43}

Ingredients	Diet No.							
	CE_{36}	CE_{37}	CE_{38}	CE_{39}	CE_{40}	CE_{41}	CE_{42}	CE_{43}
Protein mix. *	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Cod liver oil	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Carbohydrate mix.+	40.0	39.0	37.0	35.0	32.0	30.0	25.0	20.0
Cellulose	0.0	1.0	3.0	5.0	8.0	10.0	15.0	20.0
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Glucosamine HCl	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Vitamin mix. @	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7
Mineral mix. £	8.6	8.6	8.6	8.6	8.6	8.6	8.6	8.6
Sodium alginate	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Crude protein %	34.8	34.8	34.8	34.8	34.8	34.8	34.8	34.3

* Protein mix: A mixture of albumen, casein, fibrin and gelatin in the ratio 1:1:1:1.

\+ Carbohydrate mix: A mixture of sucrose, maltose and starch in the ratio 1:1:1.

@ Vitamin mix: Same as used for diets PE_0 to PE_4 in chapter 1 (Table 2a)

£ Mineral mix: Same as used for diets PE_0 to PE_4 in Chapter 1 (Table 2b)

Table H_2. Hydrographical data of the feeding experiments 8 to 13.

Experiment No.	Salinity ‰	Oxygen CC/L	Temperature °C	PH
8	20.7 ± 1.0	4.2	29.0 ± 0.5	7.9 ± 0.2
9	16.0 ± 1.0	4.1	29.5 ± 0.5	8.0 ± 0.2
10	16.5 ± 1.0	3.78	28.0 ± 0.5	8.1 ± 0.2
11	18.1 ± 1.0	3.97	28.7 ± 0.5	8.1 ± 0.2
12	17.0 ± 1.0	3.95	29.0 ± 0.5	8.33± 0.2
13	18.5 ± 1.0	4.1	29.0 ± 0.5	8.1 ± 0.2

The methods followed for determining lipid, chromium oxide in diets, animals and faeces were same as those followed in Chapter I. While carbohydrate in diets, faeces and animals was determined by 'Anthrone' method using Junior Spectrophotometer, Protein in animals was determined by the biuret method.

The procedure followed for statistical analysis of the experimental data was also same as adopted under Chapter I.

RESULTS

Evaluation of dietary characteristics of different sources of carbohydrates

The results of the evaluation of three monosaccharides, two disaccharides and two polysaccharides in the diet of *P. indicus* are given in Table 25. The growth in length and dry weight (33.3%, 112.2%) obtained by the zero carbohydrate diet CE_0 was higher than the growth in length and dry weight obtained by the diets with glucose (16.3%, 44.5%), fructose (20.6%, 55.5%), galactose (19.2%, 42.3%), sucrose (28.25%, 47.65%) and glycogen (21.7%, 38.4%). The food conversion ratio (FCR) of the diets having glucose (8.06), fructose (10.9), galactose (8.19) and sucrose (5.14) was higher than the FCR obtained by CE_0 diet (3.53). Among the diets having the carbohydrates glucose, fructose, galactose, sucrose and glycogen, the diet with fructose showed slightly superior growth, but the FCR of the diet with sucrose was superior to the FCR of diets having glucose, fructose and galactose. However the FCR (2.4) of the diet with glycogen was more superior to not only to the FCR of these diets but also to that of the CE_0 diet.

In conctrast, the growth in length and dry weight obtained by the diets with maltose (40.6%, 123.9%) and starch (39.0%, 177.7%) was higher than the growth obtained by zero carbohydrate, CE_0 diet. The FCRs of the diets with maltose (2.19) and starch (2.18) were far superior to the FCR of the CE_0 diet. The survival of the animals fed the diet with glucose was comparitively low (54.1%) and that of glycogen was very

poor (29.2%); while the survival obtained by all the other diets was above 62% and comparable, it was 70.8% in case of CE_0 diet.

The protein, lipid and carbohydrate content of the animals fed with diets containing different sources of carbohydrates did not show any definite trend except that the carbohydrate in the animals fed with CE_0 diet was significantly low compared to that of the other group of animals.

The apparent digestibility of different sources of carbohydrates (Table 26) did not show much variation. The digestibility of glucose (89.19), fructose (96.45), galactose (92.37), sucrose (94.34) and maltose (91.55) was slightly higher than the digestibility of starch (88.04) and glycogen (88.96).

The results of the analysis of variance (ANOVA) of the data obtained by the diets CE_0 to CE_7 are given in Table 26A. The growth in dry weight obtained by the diet with starch was significantly higher ($P < 0.01$) than the growth recorded by all the other diets. The difference in the growth realised by the zero carbohydrate diet and the maltose diet was not significant while the growth obtained by both diets was significantly higher than that of the diets with glucose, fructose, galactose and sucrose. Similarly no significant difference was observed in the growth obtained by the diets with glucose, fructose, galactose, sucrose and glycogen. The FCRs of the diets having starch, maltose, glycogen, sucrose and of the zero carbohydrate diet, did not significantly differ ($P < 0.01$)

Table 25. Results of the feeding experiments with the diets CE_0 to CE_7 on juvenile *P. indicus* fed for 30 days.

Particulars	Diet No.							
	CE_0	CE_1	CE_2	CE_3	CE_4	CE_5	CE_6	CE_7
Initial average length (mm)	31.0	30.9	30.6	31.2	30.8	31.0	30.6	30.8
Initial average dry weight (g)	0.0404	0.0403	0.0408	0.0403	0.0402	0.0406	0.0399	0.0402
Final average length(mm)	41.4	34.6	36.9	37.2	39.6	43.8	42.7	40.5
Final average dry weight(g)	0.0859	0.0584	0.0621	0.0582	0.0590	0.0905	0.1110	0.0538
Growth in length %	33.3^{a}	16.3^{d}	20.6^{bcd}	19.2^{cd}	28.25^{abc}	40.6^{a}	39.0^{a}	21.7^{bcd}
Growth in dry weight %	112.2^{b}	44.5^{c}	55.5^{c}	42.3^{c}	47.65^{c}	123.9^{b}	177.7^{a}	38.4^{c}
Food Conversion ratio	3.53^{a}	8.06^{b}	10.9^{b}	8.19^{b}	5.14^{a}	2.19^{a}	2.18^{a}	2.4^{a}
Survival %	70.8	54.1	62.5	66.7	66.7	62.5	62.5	29.2
Protein content of *P. indicus* on termination of feeding experiment(%)	57.1	54.2	54.8	56.7	51.8	55.0	56.9	58.0
Carbohydrate content (%)	0.61	1.81	1.60	1.75	1.69	1.88	1.87	1.82
Lipid content (%)	13.41	14.86	16.40	11.54	15.71	14.31	11.72	17.19

Note:- The values with different superscripts differ significantly among themselves. Growth in length, dry weight and food conversion ratio at 1% level ($P < 0.01$).

Table 26. Digestibility of carbohydrates in the diets CE_0 to CE_7 at 30% level by P. indicus.

Diet No.	Carbohydrate %		Chromium oxide %		Apparent digestibility
	in diet	in faeces	in diet	in faeces	
CE_0	2.56	11.22	0.5125	1.8550	--
CE_1	32.07	15.83	0.5348	2.4400	89.19
CE_2	33.22	4.69	0.5183	2.0560	96.45
CE_3	34.75	9.62	0.5485	1.9900	92.37
CE_4	33.78	8.21	0.4524	1.9400	94.34
CE_5	33.91	15.60	0.5969	3.3200	91.55
CE_6	33.39	17.19	0.5115	2.2000	88.04
CE_7	33.36	15.04	0.5882	2.4000	88.96

Table 26(A). Analysis of variance of growth and food conversion ratio obtained by diets CE_0 to CE_7 in P. indicus.

Source	ANOVA		
	D.F.	S.S.	M.S.
1. Growth in length			
Treatment	7	785.76	112.25**
Error	16	256.61	16.04
Total	23	1042.37	128.29
2. Growth in dry weight			
Treatment	7	55719.33	7959.90**
Error	16	1198.97	74.93
Total	23	56918.30	8034.83
3. Food conversion ratio			
Treatment	7	236.27	33.75**
Error	16	28.70	1.79
Total	23	267.97	35.80

** Significant at 1% level ($P < 0.01$)

among themselves, but they were significantly different from the FCRs of the diets with glucose, fructose and galactose. However, the FCRs of the latter group were not found to differ significantly among themselves.

The growth curves of animals fed with the diets of different carbohydrate sources are shown in Fig.19. The diets formulated with three monosaccharides, glucose, fructose, and galactose, the disaccharide, sucrose, and the polysaccharide glycogen, showed poor growth. But the diets containing the disaccharide, maltose, and the polysaccharide, starch showed superior growth over the zero carbohydrate diet. The apparent digestibility of different carbohydrates was practically same (Fig.20a), but the diets with monosaccharides showed poor FCR (Fig.20b) than those with disaccharides and polysaccharides. While the FCR of diet with sucrose was slightly inferior, the diet with maltose gave FCR identical to that of starch and glycogen.

Evaluation of dietary characteristics of different combinations of carbohydrate sources.

The results obtained with the diets CE_8 to CE_{14} in which the effect of different combinations of various carbohydrates in the diet on the growth, FCR and survival of *P. indicus* was studied, are shown in Table 27. Among the diets tested, the diet CE_{14} produced the highest growth (64% in length, 339.5% in dry weight) followed by the diets CE_{13} (51.3% in length, 237.4% in dry weight), CE_{12} (41.5% in length, 172.8% in dry weight), CE_8 (33.6% in length, 126.7% in dry weight), CE_9

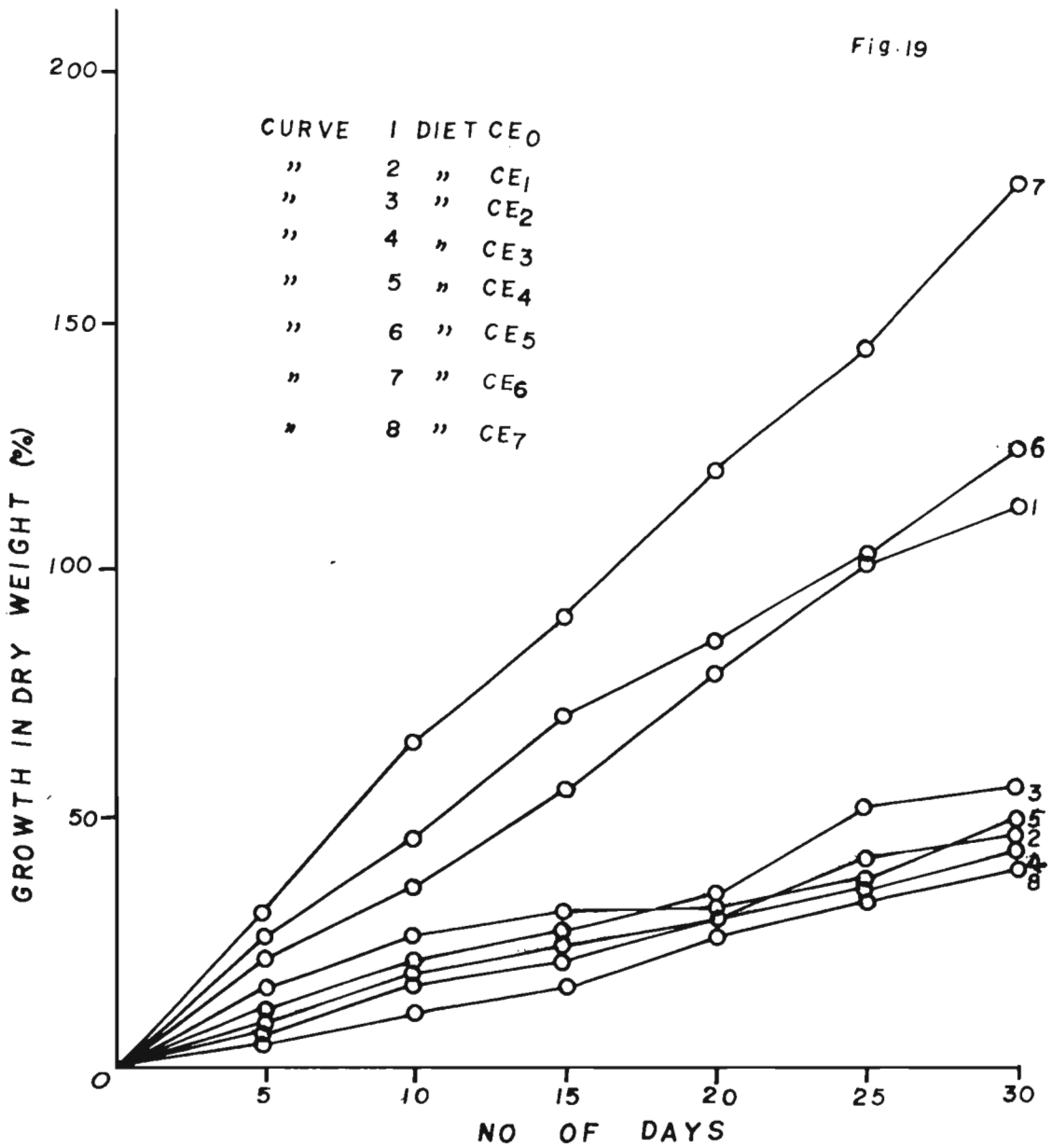

Figure 19. Growth curves of juvenile P. indicus fed with diets CE_0 to CE_7 having different carbohydrates. CE_0 zero carbohydrate, CE_1 glucose, CE_2 fructose CE_3 galactose, CE_4 sucrose, CE_5 maltose, CE_6 starch, CE_7 glycogen.

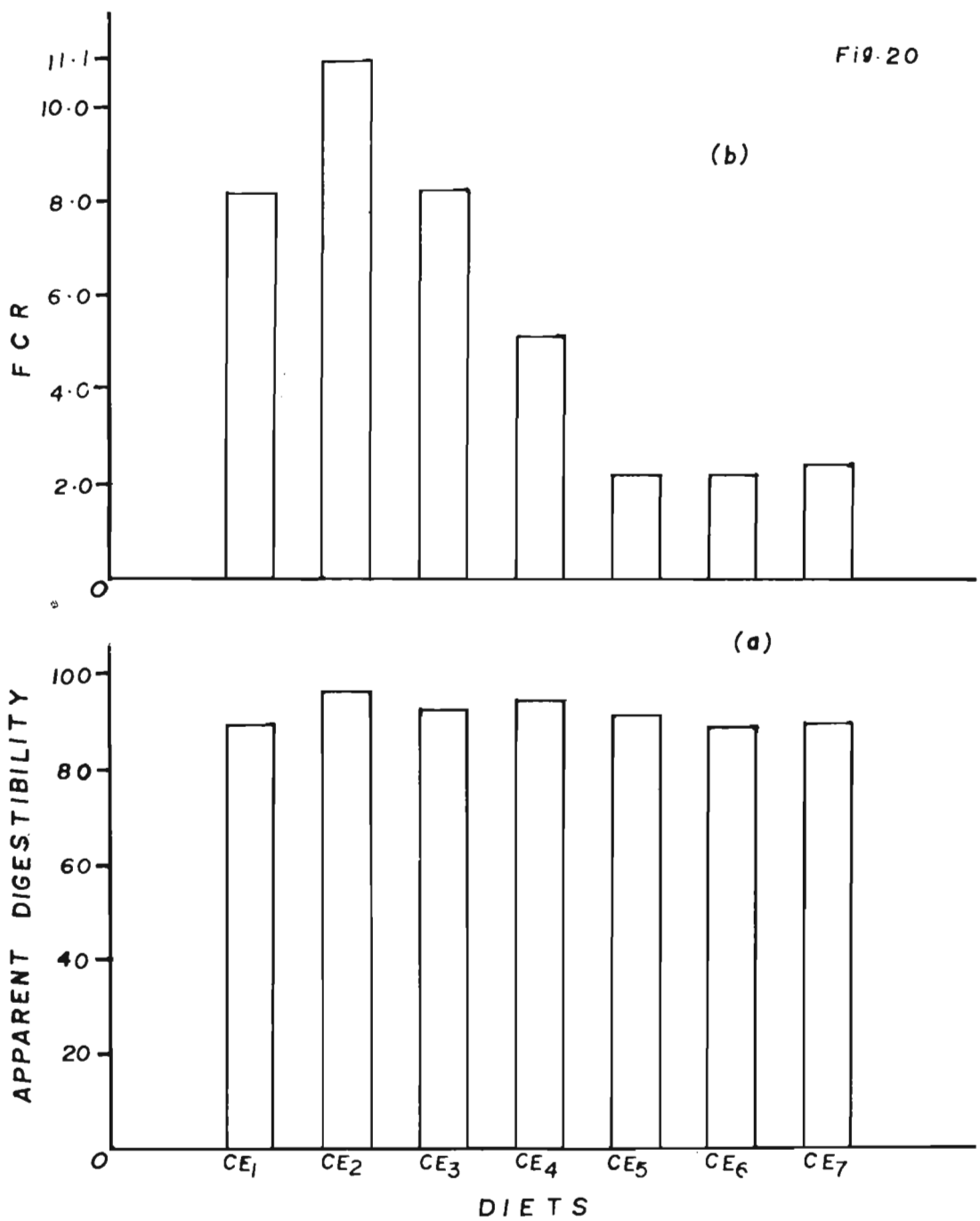

Figure 20. Evaluation of different carbohydrates in diets CE_0 to CE_7 for juvenile P. indicus. (a) Apparent digestibility and (b) Food conversion ratio (FCR)

(32.9% in length, 102.7% in dry weight) and CE_{10} (30.2% in length and 88.4% in dry weight). The diet CE_{11} recorded the lowest growth (30.4% in length, 82.2% in dry weight). Lowest FCR was obtained by the diet CE_{14} (3.45) followed by CE_{13}(4.37), CE_{12}(6.31), CE_{8}(8.11), CE_{11}(10.05), CE_{10}(10.29) and CE_{9}(11.89) in the increasing order. The survival of the animals fed with the diet CE_{8} was low (50%) whereas the survival of the animals fed all the other diets was above 65%.

The animals fed on the diets CE_{13} and CE_{14} had highest body protein (58.25 and 58.0% respectively) but low carbohydrate (1.06 and 1.29% respectively). P. indicus reared with the diet CE_{11} had the lowest body protein (51.53%) but the carbohydrate (1.65%) was not as low as those observed in the feeding experiments with diets CE_{13} and CE_{14}. The animals fed with the diet CE_{8} showed highest carbohydrate in the body (2.05%) when the body protein of the animals was 54.12%. With the diets CE_{9} and CE_{10} the body protein of P. indicus was 55.1 and 55.11% and the carbohydrate was 1.7 and 1.49% respectively. Only the animals fed with the diets CE_{9} (11.55%) and CE_{12} (13.74%) showed low lipid. The lipid in the body of the animals fed all the other diets was comparable. The growth (in length and dry weight) and FCR obtained by different diets were significant among the treatments (Table 27A). The growth obtained by the diets CE_{13} and CE_{14} was significantly higher ($P < 0.01$). The difference in the growth obtained by the diets CE_{12}, CE_{11} and CE_{8} was not found to be significant. Similarly no significant difference in the growth was observed among the diets CE_{9},CE_{10}

Table 27. Results of the feeding experiment with diets CE_8 to CE_{14} on juvenile *P. indicus* fed for 30 days.

Particulars	Diet No.						
	CE_8	CE_9	CE_{10}	CE_{11}	CE_{12}	CE_{13}	CE_{14}
Initial average length(mm)	22.6	22.8	22.5	22.7	22.9	22.8	22.8
Initial average dry weight(g)	0.0146	0.0147	0.0145	0.0146	0.0147	0.0147	0.0147
Final average length (mm)	30.2	30.3	29.3	29.6	32.4	34.5	37.4
Final average dry weight (g)	0.0331	0.0298	0.0275	0.0266	0.0401	0.0496	0.0646
Growth in length %	33.6^{cd}	32.9^{cd}	30.2^{cd}	30.4^{cd}	41.5^{bc}	51.3^{ab}	64.0^{a}
Growth in dry weight %	126.7^{bc}	102.7^{cd}	88.4^{cd}	82.2^{cd}	172.8^{ab}	237.4^{ab}	339.5^{a}
Food conversion ratio	8.11^{abc}	11.89^{cd}	10.29^{bc}	10.05^{bc}	6.31^{ab}	4.37^{a}	3.45^{a}
Survival %	50.0	87.5	87.5	70.8	66.7	75.0	75.0
Protein content of *P. indicus* on termination of feeding experiment (%)	54.12	55.10	55.11	51.53	54.18	58.25	58.0
Carbohydrate content (%)	2.05	1.70	1.49	1.65	1.59	1.06	1.29
Lipid content (%)	15.7	11.55	16.55	17.95	13.74	16.23	17.14

Note: The values with different superscripts differ significantly among themselves. Growth in dry weight and food conversion ratio significant at 1% level ($P < 0.01$). Growth in length significant at 5% level ($P < 0.05$)

Table 27(A). Analysis of variance of growth and food conversion ratio obtained by diets CE_8 to CE_{14} in P. indicus.

Source	ANOVA		
	D.F.	S.S.	M.S.
1. Growth in length			
Treatment	6	827.77	137.96*
Error	14	441.63	31.55
Total	20	1269.40	169.51
2. Growth in dry weight			
Treatment	6	160616.18	26769.36**
Error	14	35898.42	2564.17
Total	20	196514.60	29333.53
3. Food conversion ratio			
Treatment	6	199.36	33.22**
Error	14	59.51	4.25
Total	20	258.87	37.47

** Significant at 1% level ($P < 0.01$)

* Significant at 5% level ($P < 0.05$)

and CE_{11}. The FCRs obtained by the diets CE_{13} and CE_{14} were also significantly lower ($P < 0.01$). However, the difference in the FCRs obtained by the diets CE_{12} and CE_{8} was not found to be significant. The FCR obtained by the diet CE_{9} was significantly higher though it was not significantly different from the FCRs obtained by the diets CE_{8}, CE_{9}, CE_{10} and CE_{11}.

The performance of the diets CE_{8} to CE_{14} is depicted in Fig.21. The diets (CE_{8}, CE_{9}, CE_{10} and CE_{11}) having 15% glucose resulted in poor growth (Fig.21a). Only the combination of glucose-glycogen (diet CE_{8}) was slightly superior among them. The diets (CE_{8} to CE_{11}) having 15% glucose in combination with other carbohydrates, showed very poor food conversion ratio (Fig.21b). However, when the glucose content was reduced to 10%, the growth and FCR of the diets (CE_{12} and CE_{13}) improved. The glucose-starch-glycogen mixture (in the ratio 1:1:1) was superior to the glucose-sucrose-starch combination. But among the combination of different carbohydrates tested, the sucrose-maltose-starch combination (diet CE_{14}) gave the highest growth and the best FCR which were also significantly different from those obtained by all the other combinations.

Evaluation of carbohydrate level in the diet at constant Protein and lipid.

The results of the feeding experiment with diets CE_{15} to CE_{21} are given in Table 28. It could be seen that the diet CE_{18} produced the highest growth in dry weight of 560.7% (growth in length was 54.8%) and lowest FCR of 3.61, among the diets tested. Although the growth percentage of animals

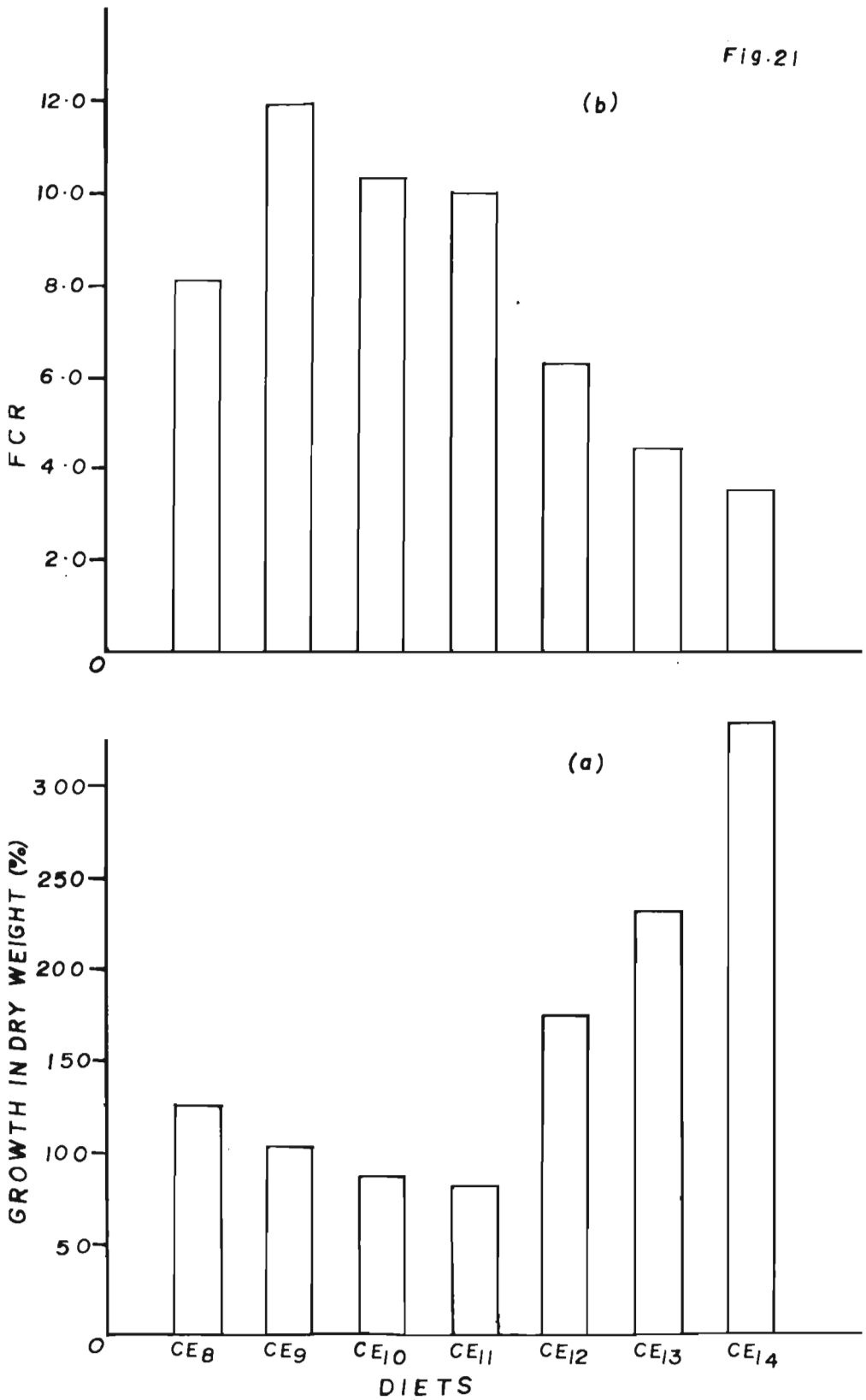

Figure 21. Evaluation of different combination of carbohydrate sources in diets CE_8 to CE_{14} for juvenile P. indicus. (a) Growth in dry weight and (b) Food conversion ratio (FCR).

fed different diets was fluctuating between 234.6% and 521.5%, the diets CE_{20}, CE_{21} and CE_{17} showed relatively better growth rates. The FCRs obtained by the diets CE_{15}, CE_{16}, CE_{17} (4.06, 4.34, 4.45 respectively) were comparable to those of the diets CE_{19}, CE_{20} and CE_{21} (4.54, 4.19 and 4.51 respectively). The survival of the animals fed on the diet CE_{15} was very low (33.3%). All the other diets showed a survival above 58%, the diet CE_{21} resulting in the highest survival (79.2%) followed by the diet CE_{16} (75%).

The body protein of animals fed with different diets did not show any appreciable difference. The carbohydrate content of the animals fed with the various diets was almost similar, except in those reared with diet CE_{18}. The lipid content of the animals fed with this diet (CE_{18}) was also comparatively higher.

The apparent digestibility of carbohydrate of the diet CE_{15} was 68.25 which was the lowest among the values (Table 29). The diet CE_{19} showed the highest apparent digestibility of 83.5, followed by the diets CE_{16} (81.63), CE_{20} (79.02), CE_{18} (77.60), CE_{21} (75.78) and CE_{17} (73.85) in the decreasing order. The ANOVA of the data on growth and FCR is given in Table 29A. The treatments were significant at 1% level ($P < 0.01$) with respect to growth in length and dry weight. The growth obtained by the diet CE_{18} was significantly higher, though it was not significantly different from those obtained by the diets CE_{17}, CE_{20} and CE_{21}. Similarly the growth obtained by the remaining

Table 28. Results of the feeding experiment with diets CE_{15} to CE_{21} on juvenile P. indicus fed for 30 days.

Particulars	Diet No.						
	CE_{15}	CE_{16}	CE_{17}	CE_{18}	CE_{19}	CE_{20}	CE_{21}
Initial average length(mm)	22.5	22.6	22.5	22.8	22.6	22.5	22.2
Initial average dry weight(g)	0.0108	0.0107	0.0107	0.0107	0.0106	0.0106	0.0106
Final average length (mm)	30.5	29.2	34.4	35.3	33.6	36.1	34.8
Final average dry weight (g)	0.0387	0.0358	0.0665	0.0707	0.0399	0.0579	0.0547
Growth in length %	35.6^{ab}	29.2^{ab}	58.9^{a}	54.8^{a}	48.7^{a}	60.4^{a}	56.8^{a}
Growth in dry weight %	258.6^{d}	234.6^{d}	521.5^{ab}	560.7^{a}	276.4^{d}	446.2^{ab}	416.0^{bc}
Food Conversion ratio	4.06	4.34	4.45	3.61	4.54	4.19	4.51
Survival %	33.3	75.0	58.3	58.3	62.5	66.7	79.2
Protein content of P. indicus on termination of feeding experiment (%)	54.06	52.66	55.01	52.77	53.51	54.82	54.55
Carbohydrate content (%)	1.28	1.06	1.03	1.45	1.11	1.26	1.19
Lipid content (%)	12.43	13.0	12.72	17.90	12.05	13.55	11.15

Note:- Values with different superscripts differ significantly among themselves. Growth in length and dry weight significant at 1% level ($P < 0.01$). Food conversion ratio not significant at 5% level ($P < 0.05$).

Table 29. Digestibility of carbohydrate in the diets CE_{15} to CE_{21} by P. indicus.

Diet No.	Carbohydrate %		Chromium oxide %		Apparent digestibility
	in diet	in faeces	in diet	in faeces	
CE_{15}	7.33	4.15	0.4301	0.7669	68.25
CE_{16}	12.65	4.75	0.4681	0.9565	81.63
CE_{17}	17.28	7.83	0.4709	0.8157	73.85
CE_{18}	22.53	14.05	0.4380	1.2392	77.60
CE_{19}	32.34	15.83	0.4380	1.2989	83.50
CE_{20}	42.07	27.34	0.4709	1.4583	79.02
CE_{21}	52.28	35.80	0.3980	1.1250	75.78

Table 29(A). Analysis of variance of growth and food conversion ratio obtained by the diets CE_{15} to CE_{21} in P. indicus.

Source	ANOVA		
	D.F.	S.S.	M.S.
1. Growth in length			
Treatment	6	11956.83	1992.80**
Error	14	514.36	36.74
Total	20	12471.19	2029.54
2. Growth in dry weight			
Treatment	6	271609.93	45268.32**
Error	14	27169.88	1940.70
Total	20	298779.81	47209.02
3. Food conversion ratio			
Treatment	6	1.95	0.64*
Error	14	9.02	0.32
Total	20	10.97	0.96

** Significant at 1% level ($P < 0.01$)

* Not significant at 5% level ($P < 0.05$)

diets was also not found to be significantly different. In the case of FCR, the treatments were not significant at 5% level ($P < 0.05$). However, diet CE_{18} produced distinctly low FCR though it was not found to be significantly different from the FCRs obtained by the other diets.

The influence of dietary carbohydrate on growth and FCR is depicted in Fig.22. The growth reached a peak (Fig. 22a) when the carbohydrate in the diet was 22.5%. Further increase in the dietary carbohydrate, brought down the growth of the animals. The diet with 22.5% carbohydrate registered the lowest FCR (Fig.22b), while the diets with carbohydrate levels below and above this showed higher values of FCR. The influence of dietary carbohydrate on its apparent digestibility (Fig.23a), though did not show a clear trend, the curve inclined towards improved digestibility at higher dietary carbohydrate levels. Similarly the survival (Fig.23b) was better at higher dietary carbohydrate levels. These results had indicated that dietary carbohydrate, at constant protein and lipid, influences the growth of prawns and the highest growth and the best FCR were obtained when the diet had 22.5% of carbohydrate, comprising sucrose, maltose and starch in the ratio 1:1:1.

Determination of optimum carbohydrate level in the diet at constant lipid.

From the results of feeding experiment with the diets CE_{22} to CE_{28} on juvenile *P. indicus*, shown in Table 30, it could be seen that the diet CE_{28} produced the highest growth

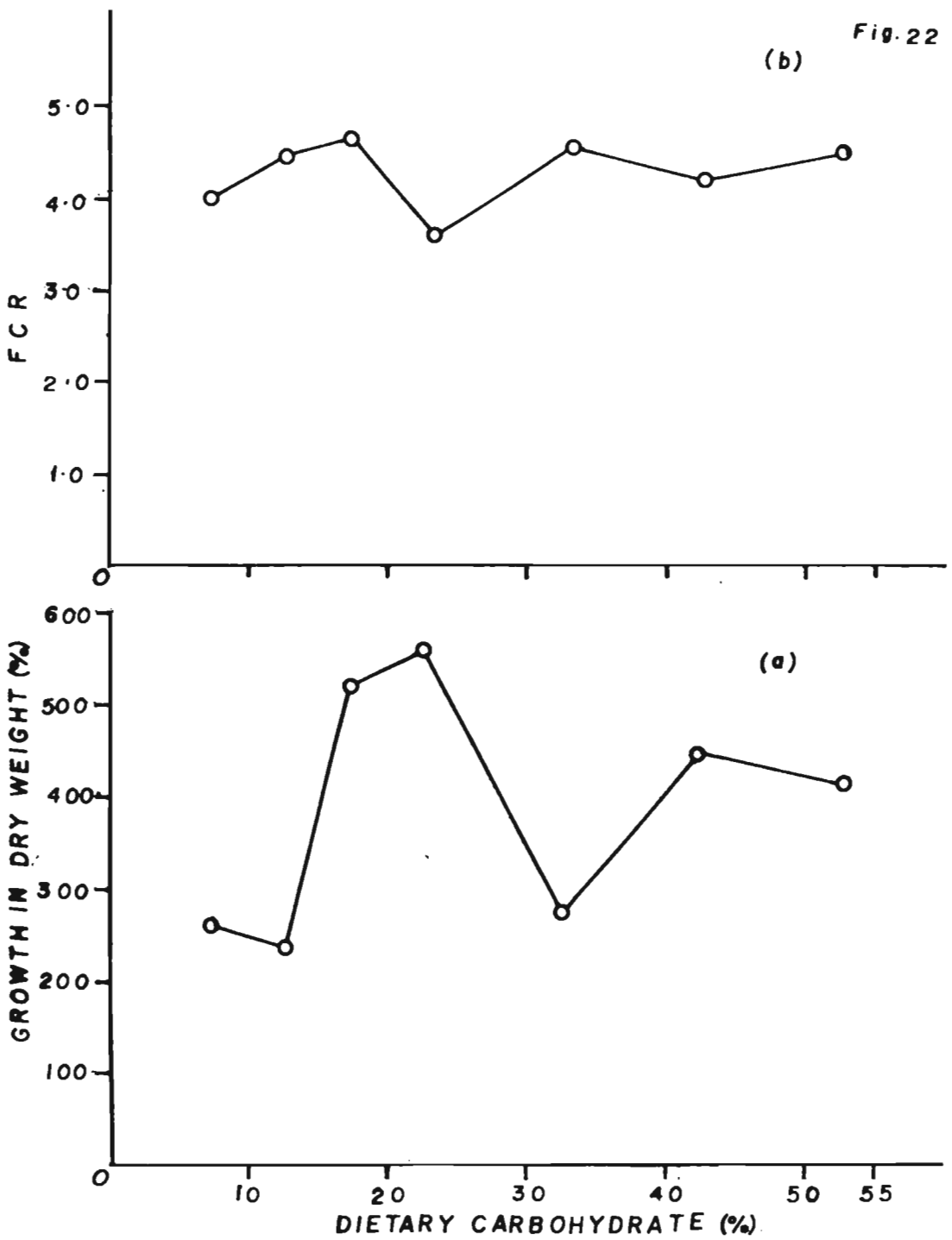

Figure 22. Effect of carbohydrate level in the diet at constant protein and lipid on (a) Growth in dry weight and (b) food conversion ratio (FCR) of juvenile P. indicus.

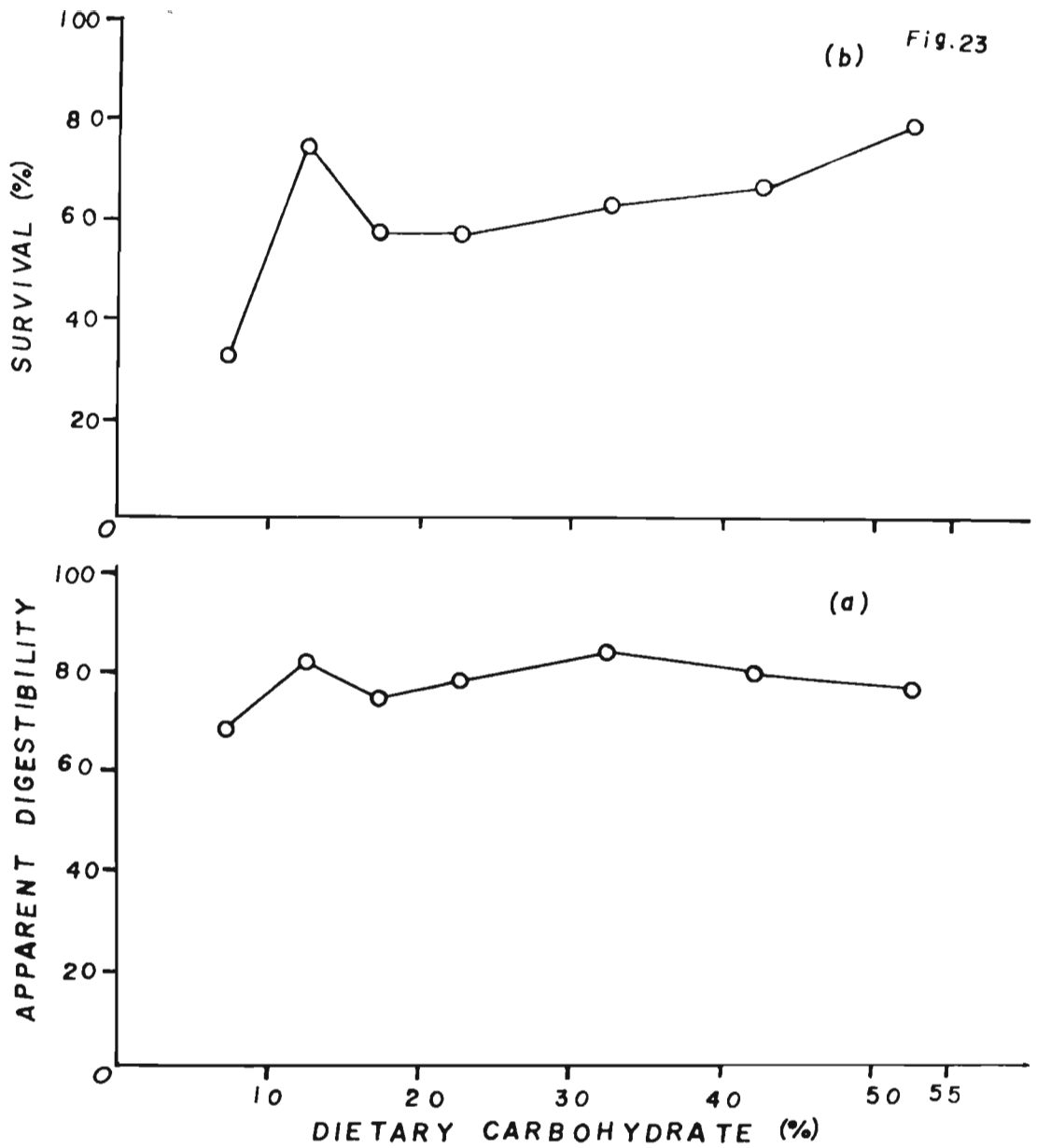

Figure 23. Effect of carbohydrate level in the diet at constant protein and lipid on (a) apparent digestibility and (b) survival in juvenile P. indicus.

in dry weight (684%) and length (89.6%). The same diet also realised the lowest FCR (3.45) among the diets tested. The diet CE_{22} showed the lowest growth (22.7% in length, 118.4% in dry weight) and high value of FCR (7.24). With the other diets (CE_{23}, CE_{24}, CE_{25}, CE_{26} and CE_{27}) the growth variation was between 147.4% and 614.7% in dry weight and 26.6% and 78.1% in length. The FCRs of these diets were 5.20, 5.16, 4.61, 4.57 and 3.46. The survival of the animals fed with the diets CE_{22} to CE_{28} was respectively, 66.7%, 50%, 62.5%, 62.5%, 79.2%, 75% and 58.3%.

The body protein of the animals fed with the diet CE_{28} recorded the highest value of 62.9%. While the body carbohydrate and lipid of the animals fed with the different diets were comparable, the lipid content of the animals fed the diet CE_{22} was particularly low (10.34%) compared to that of the other groups.

The apparent digestibility of carbohydrate (Table 31) in the first four diets, CE_{22}, CE_{23}, CE_{24} and CE_{25} was 71.5, 41.95, 54.78 and 50.83 respectively. In the remaining three diets, CE_{26}, CE_{27} and CE_{28} it was above 75.

ANOVA of the data (shown in Table 31A) indicated that the treatments were highly significant with respect to growth and the food conversion ratio . The growth (in length and dry weight) and FCR obtained by the diets CE_{27} and CE_{28} were significantly superior ($P < 0.01$) to the growth and FCR obtained by the other diets. Particularly, the growth and FCR obtained by the diet CE_{22} was significantly inferior($P < 0.01$).

Table 30. Results of the feeding experiment with diets CE_{22} to CE_{28} on juvenile *P. indicus* fed for 30 days.

Particulars	Diet No.						
	CE_{22}	CE_{23}	CE_{24}	CE_{25}	CE_{26}	CE_{27}	CE_{28}
Initial average length (mm)	20.3	20.3	20.3	20.3	20.1	20.1	20.1
Initial average dry weight(g)	0. 0076	0.0076	0.0076	0.0076	0.0075	0.0075	0.0075
Final average length(mm)	24.9	25.7	29.6	28.3	31.6	35.8	38.1
Final average dry weight (g)	0.0166	0.0188	0.0317	0.0272	0.0362	0.0536	0.0588
Growth in length %	22.7^{fg}	26.6^{ef}	45.8^{cd}	39.4^{de}	57.2^{bc}	78.1^{a}	89.6^{a}
Growth in dry weight %	118.4^{cd}	147.4^{bc}	$317,1^{bc}$	257.9^{bc}	$382,7^{ab}$	614.7^{a}	684.0^{a}
Food conversion ratio	7.24^{d}	5.20^{bc}	5.16^{bc}	4.61^{ab}	4.57^{ab}	3.46^{a}	3.45^{a}
Survival%	66.7	50.0	62.5	62.5	79.2	75.0	58.3
Protein content of *P. indicus* on termination of feeding experiment (%)	59.11	59.96	60.02	62.13	58.09	62.85	62.9
Carbohydrate content (%)	1.70	1.66	1.70	1.56	1.62	1.75	1.63
Lipid content (%)	10.34	12.98	13.51	14.10	12.69	14.96	11.44

Note: Values with different superscripts differ significantly among themselves. Growth in length and dry weight and food conversion ratio significant at 1% level ($P < 0.01$)

Table 31. Digestibility of carbohydrate in the diets CE_{22} to CE_{28} by *P. indicus*.

Diet No.	Carbohydrate %		Chromium oxide %		Apparent digestibility
	diet	faeces	diet	faeces	
CE_{22}	7.59	4.50	0.4903	1.0198	71.50
CE_{23}	12.89	10.63	0.6034	0.8571	41.95
CE_{24}	18.92	16.82	0.6474	1.2727	54.78
CE_{25}	23.72	16.65	0.6568	0.9375	50.83
CE_{26}	32.60	18.83	0.6568	1.6796	77.42
CE_{27}	44.50	17.83	0.7142	2.4347	88.25
CE_{28}	53.39	19.01	0.6609	2.2117	89.37

Table 31(A). Analysis of variance of growth and food conversion ratio obtained by the diets CE_{22} to CE_{28} in P. indicus.

Source	ANOVA		
	D.F.	S.S.	M.S.
1. Growth in length			
Treatment	6	4577.00	762.83**
Error	14	655.31	46.81
Total	20	5232.31	809.64
2. Growth in dry weight			
Treatment	6	849740.05	141623.34**
Error	14	146912.00	10493.71
Total	20	996652.05	152117.05
3. Food conversion ratio			
Treatment	6	29.95	4.99**
Error	14	7.00	0.50
Total	20	36.95	5.49

** Significant at 1% level ($P < 0.01$)

The effect of graded levels of dietary carbohydrate, on the growth and FCR is shown in Fig.24. The growth curve was seen increasing (Fig.24a) with the dietary carbohydrate level upto 19%, thereafter it showed a decrease only to increase again as the dietary carbohydrate level progressively increased. In a similar fashion, the FCR showed a decreasing trend (Fig.24b) with increase in dietary carbohydrate level.

The apparent digestibility of carbohydrate decreased (Fig.25a) as the dietary carbohydrate increased from 7.6 to 12.9%; it remained relatively low between the dietary carbohydrate levels of 12.9 and 23.7% and again showed an upward trend upto 44.5% and leveled off thereafter. The survival was higher (Fig.25b) at higher carbohydrate levels than at lower levels of carbohydrate in the diet.

The results of this experiment thus clearly indicated that the carbohydrate level in the diet would play very important role in promoting growth of animals and in improving the FCR, thereby sparing protein level in the diet.

Determination of optimum level of carbohydrate in the diet at constant Protein.

The results of the feeding experiment with the diets CE_{29} to CE_{35} are presented in Table 32. At constant protein, the diet CE_{33} having 6% lipid and 33.24% of carbohydrate produced the highest growth in length (48.4%) and dry weight (280.4%). The diet also showed the best food conversion ratio of 2.9. In contrast, the diet CE_{29} with 16.8% lipid and 8.44% carbohy-

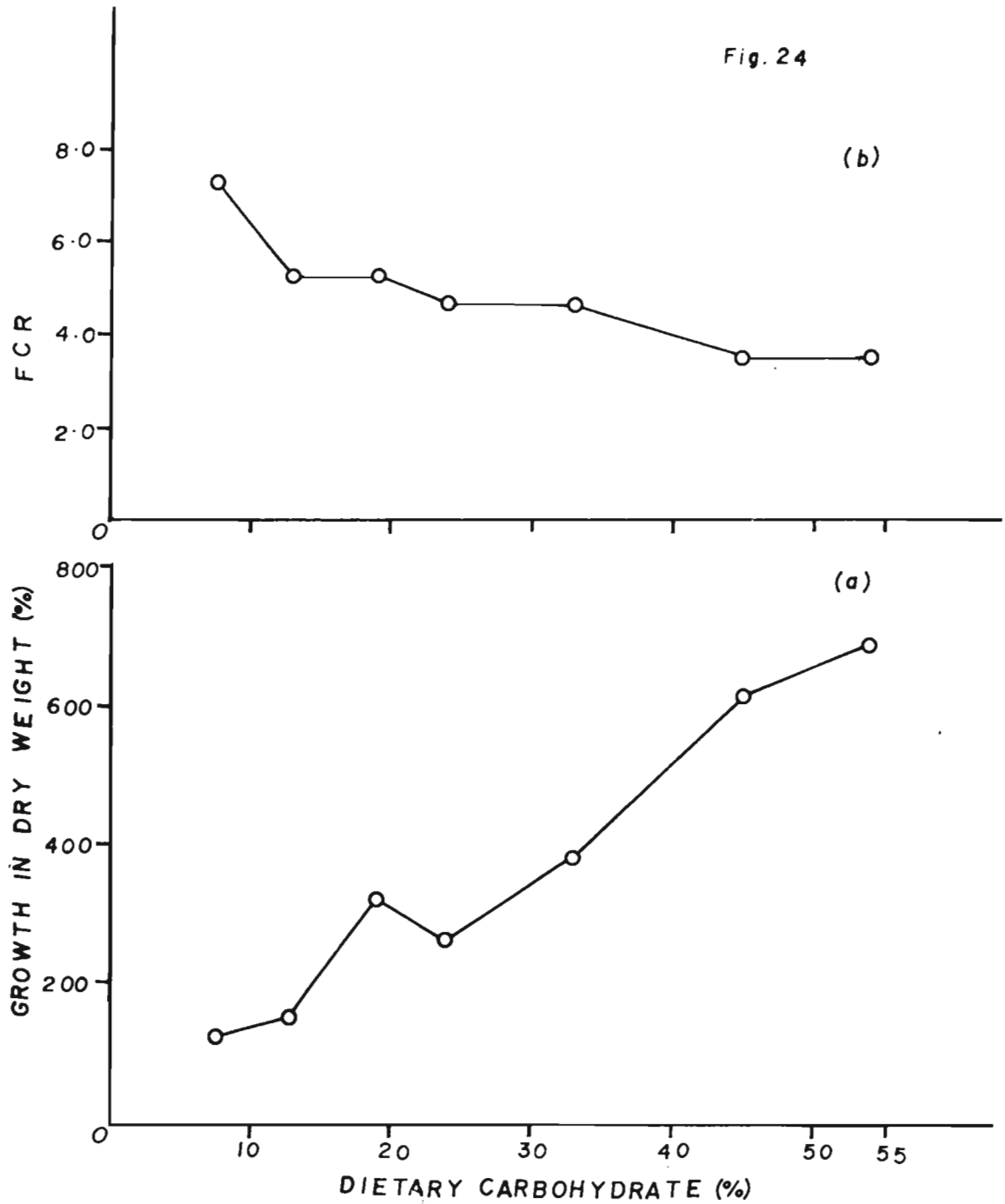

Figure 24. Influence of carbohydrate level in the diet at constant lipid on (a) growth in dry weight and (b) food conversion ratio (FCR) of juvenile *P. indicus*.

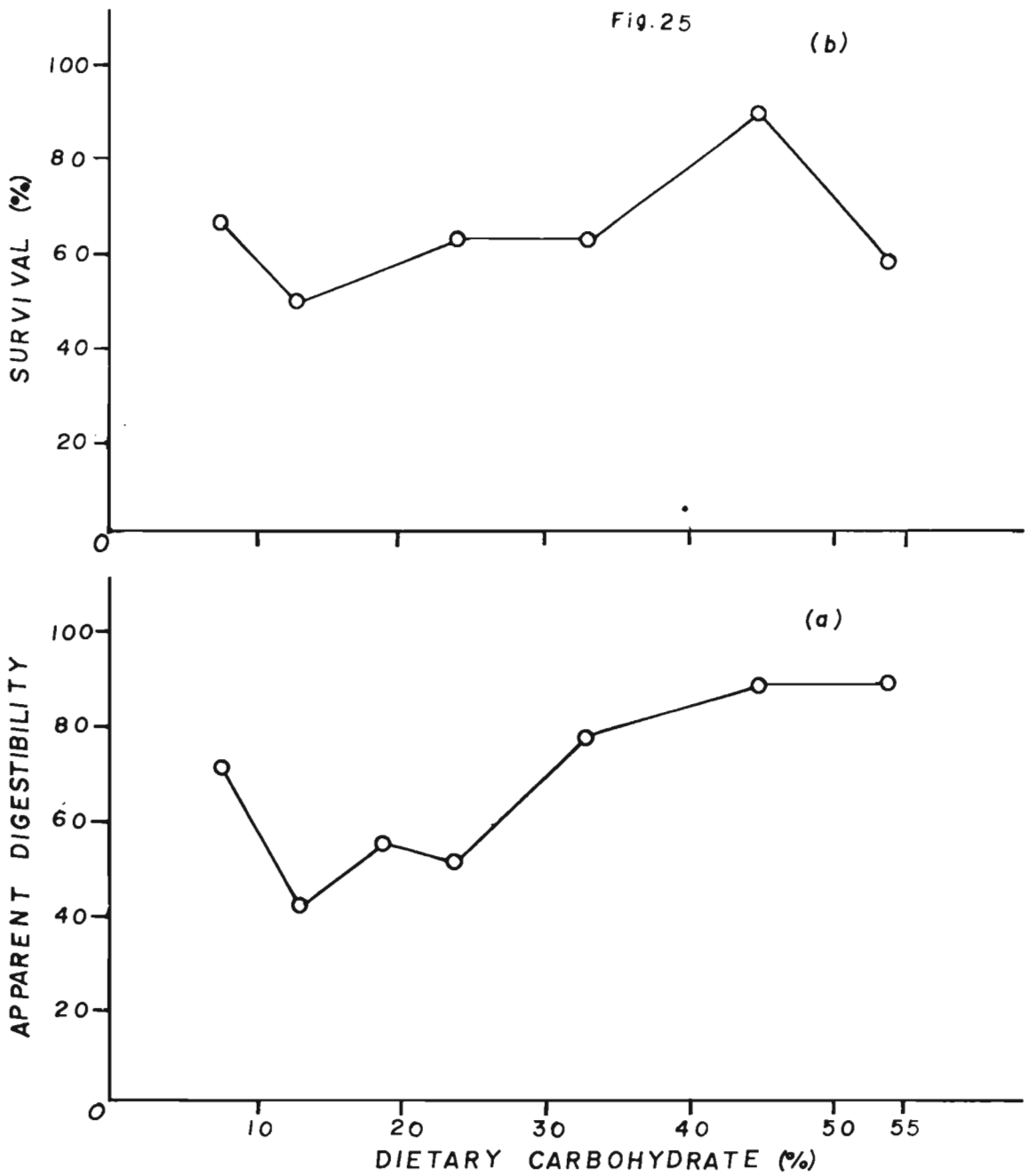

Figure 25. Influence of carbohydrate level in the diet at constant lipid on (a) apparent digestibility and (b) survival of juvenile P. indicus.

drate produced the lowest growth (10.2% in length and 45.5% in dry weight). Though the growth of animals fed with the diet CE_{35} having 53.36% carbohydrate and nil lipid, was comparable (108.3% in dry weight) to that of the diet CE32 with 23.31% carbohydrate and 10.42% lipid (109.7% in dry weight), the former resulted in high FCR (12.86). The growth obtained by the diets CE_{30}, CE_{31} and CE_{34} was 86.9%/19.8%, 71.7%/19.0% and 205%/44.4% in dry weight and length respectively. The FCRs of these diets were 3.75, 3.82 and 4.25. The survival of the animals fed with the diet CE_{33} was 91.7% while it varied from 54.2% to 70.8% in the other diets.

The body protein of the animals fed with the diets CE_{33} and CE_{35} was higher (57.12% and 57.3% respectively) than the groups fed with the other diets. The body carbohydrate of P. indicus fed with the different diets was almost similar. The body lipid of the prawns reared on the diets CE_{30} and CE_{32} was high (17.82%, 17.45%) while it was low in those fed with the diets CE_{29} and CE_{31} (10.11%, 10.77%). The lipid content of the animals fed the remaining diets was comparable among themselves.

The apparent digestibility of carbohydrate (Table 33) in the diet CE_{29} was very low (17.92) and it was as high as 95.82 in the diet CE_{35}. It was 54.63, 63.96, 67.73, 72.60 and 85.02 respectively in the diets CE_{30} to CE_{34}.

The treatments, with respect to growth (in length and dry weight) and FCR (Table 33A) were found to be significantly different. The growth realised by the diets CE_{33} and CE_{34} was

Table 32. Results of the feeding experiment with diets CE_{29} to CE_{35} on juvenile *P. indicus* fed for 30 days.

Particulars	Diet No.						
	CE_{29}	CE_{30}	CE_{31}	CE_{32}	CE_{33}	CE_{34}	CE_{35}
Initial average length (mm)	22.5	22.7	22.6	22.5	22.4	22.5	22.6
Initial average dry weight (g)	0.0145	0.0146	0.0145	0.0145	0.0145	0.0145	0.0145
Final average length (mm)	24.8	27.2	26.9	27.9	33.2	32.5	26.1
Final average dry weight (g)	0.0211	0.0273	0.0249	0.0304	0.0544	0.0443	0.0302
Growth in length %	10.2^{cd}	19.8^{bc}	19.0^{bc}	24.0^{b}	48.4^{a}	44.4^{a}	15.7^{bc}
Growth in dry weight %	45.5^{b}	86.9^{b}	71.7^{b}	109.7^{b}	280.4^{a}	205.5^{a}	108.3^{b}
Food conversion ratio	7.63^{ab}	3.75^{a}	3.82^{ab}	4.4^{ab}	2.90^{a}	4.25^{ab}	12.86^{c}
Survival%	54.2	54.2	66.7	54.2	91.7	70.8	50.0
Protein content of *P. indicus* on termination of feeding experiment (%)	53.83	52.98	53.94	55.75	57.12	55.13	57.30
Carbohydrate content (%)	1.69	1.52	1.52	1.48	1.52	1.29	1.47
Lipid content (%)	10.11	17.82	10.77	17.45	13.98	13.15	12.05

Note: Values with different superscripts differ significantly among themselves. Growth in length and dry weight and food conversion ratio significant at 5% level ($P < 0.05$)

Table 33. Digestibility of carbohydrate in the diets CE_{29} to CE_{35} by *P. indicus*.

Diet No.	Carbohydrate %		Chromium oxide %		Apparent digestibility
	in diet	in faeces	in diet	in faeces	
CE_{29}	8.44	12.50	0.5938	1.0714	17.92
CE_{30}	13.85	11.93	0.6041	1.1469	54.63
CE_{31}	18.35	13.27	0.6225	1.2488	63.96
CE_{32}	23.51	15.91	0.6423	1.3676	67.73
CE_{33}	33.24	23.96	0.5915	1.5560	72.60
CE_{34}	41.63	26.87	0.6113	2.6337	85.02
CE_{35}	53.36	18.74	0.5327	4.4700	95.82

Table 33 (A). Analysis of variance of growth and food conversion ratio obtained by the diets CE_{29} to CE_{35} in *P. indicus*.

Source	ANOVA		
	D.F.	S.S.	M.S.
1. Growth in length			
Treatment	6	1719.86	286.64*
Error	14	609.18	43.51
Total	20	2329.04	330.15
2. Growth in dry weight			
Treatment	6	117853.54	19642.26*
Error	14	36465.73	2604.69
Total	20	154319.27	22246.95
3. Food conversion ratio			
Treatment	6	221.79	36.97*
Error	14	106.76	7.63
Total	20	336.47	44.60

* Significant at 5% level ($P < 0.05$)

significantly higher ($P < 0.05$) than that registered by the other diets. The FCRs obtained by the diets CE_{29} to CE_{33} were significantly lower than the FCR of the diet CE_{35}. However, the difference in FCRs of all other diets was not found to be significant ($P < 0.05$).

The maximum growth in dry weight (Fig.26a) was obtained at 33.24% carbohydrate though it decreased between 13.85 and 18.35% carbohydrate in the diet. At higher levels the growth of the prawns decreased. The FCR gradually decreased (Fig.26b) and reached a minimum value at a dietary carbohydrate of 33.24%. Then again the FCR sharply increased with increase in the dietary carbohydrate level. The apparent digestibility showed gradual increase (Fig.27a) as the levels in the dietary carbohydrate enhanced. The survival (Fig.27b) was the highest when the diet had 33.24% carbohydrate. Above and below this dietary carbohydrate level, the survival was very low.

In summing up, the results of this experiment showed that the carbohydrate in the diet was utilised better in the diets having low lipid than in those having high lipid. The growth, FCR and survival were the best with low lipid (6%) and high carbohydrate (33.24%) diet.

Effect of cellulose level in the diet on the growth, FCR and survival of P. indicus.

The growth performance of P. indicus fed with the diets with 0, 1, 3, 5, 8, 10 and 15% cellulose (diets CE_{36} to CE_{42}) showed relatively better growth in length and weight by the diet CE_{36} and the lowest by the diet CE_{43} (Table 34). In the

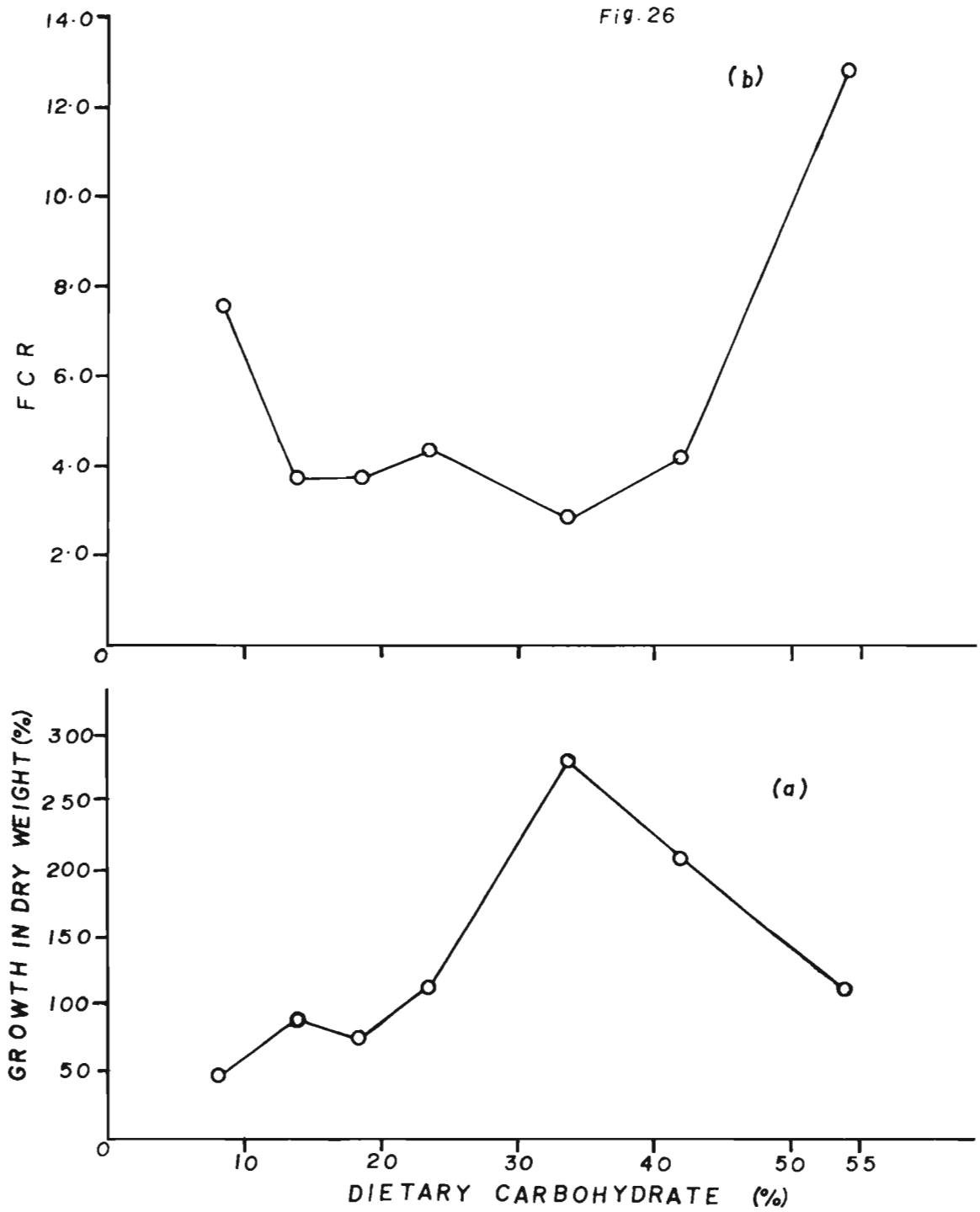

Figure 26. Effect of carbohydrate level in the diet at constant protein on (a) growth in dry weight and (b) food conversion ratio (FCR) of juvenile *P. indicus*.

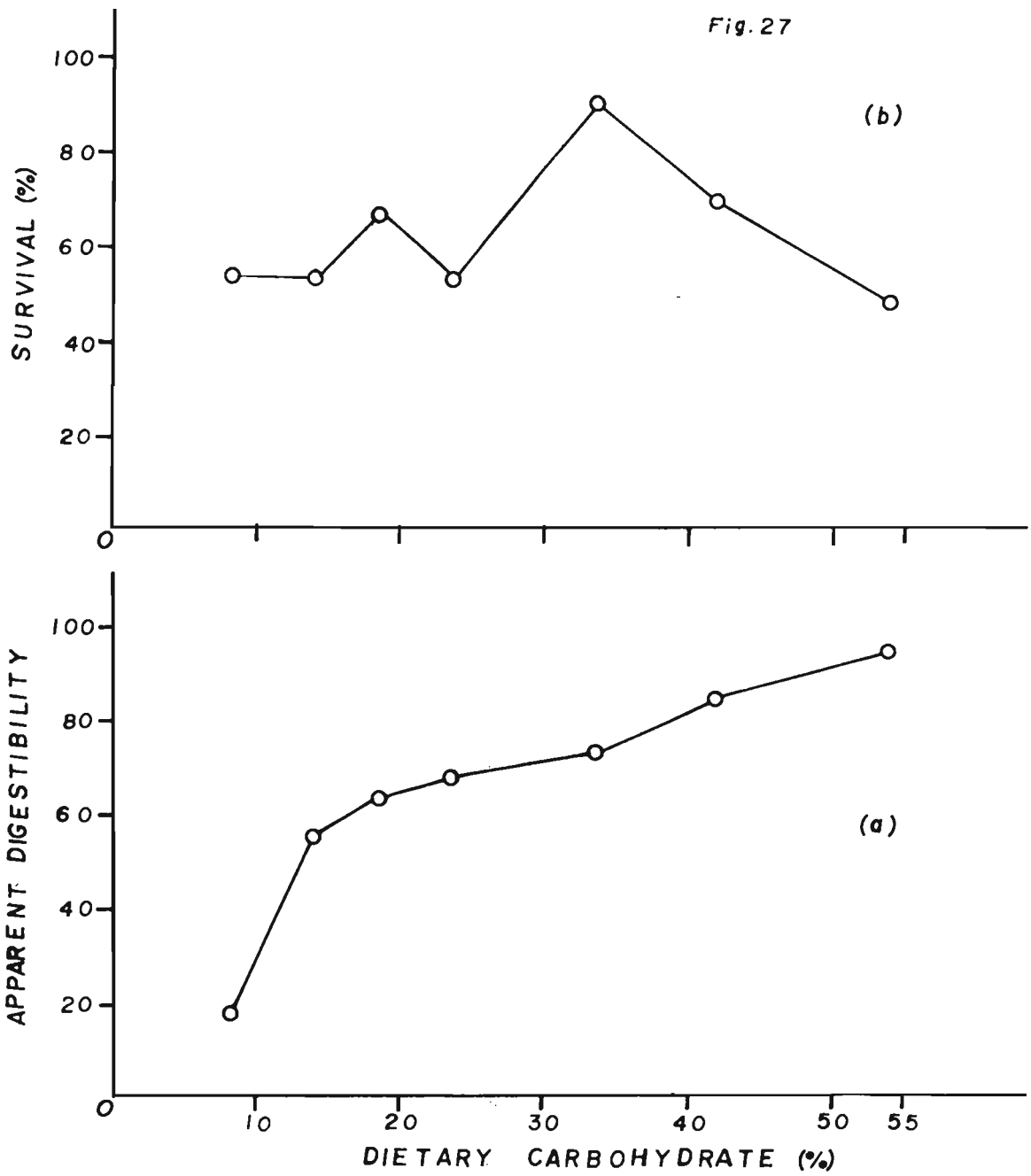

Figure 27. Effect of carbohydrate level in the diet at constant protein on (a) apparent digestibility and (b) survival of juvenile P. indicus.

diets CE_{38} to CE_{42} the growth in length was less than 60%. The FCRs of the diets having 1, 3, 5, 8, 10 and 15% cellulose were 3.61, 3.17, 3.32, 4.29, 3.04 and 3.66 respectively. The diet having 10% cellulose recorded the lowest FCR of 3.04. The ANOVA of the data (Table 34A) showed that the growth of the animals fed with them was not significantly different($P < 0.05$) among themselves. But the FCRs obtained by the diets having 1, 3, 5, 8, 10 and 15% cellulose were significantly superior ($P < 0.01$) to the FCR obtained (6.23) by the diet having no cellulose.

The body protein of the animals fed with the diets having cellulose was marginally higher than those fed the zero cellulose diet. The body lipid of the animals fed the zero cellulose diet was slightly higher, compared to those groups fed the diets having cellulose.

Figure 28 shows the growth, FCR and survival at different levels of cellulose in the diet. The growth of animals (Fig.28a) apparently showed slight decrease as the cellulose level in the diet increased. On the other hand, the FCR clearly improved (Fig.28b) with progressive increase in the dietary cellulose. The FCR registered a minimum value at 10% cellulose level in the diet. Further increase in the dietary cellulose, though resulted in decrease in growth, the FCR remained practically same. The survival of the animals fed the diets having different levels of cellulose varied widely (Fig.28C), however, the curve inclined towards improved survival with increase in dietary cellulose.

Table 34. Results of the feeding experiment with diets CE_{36} to CE_{43} on juvenile *P. indicus* fed for 30 days.

Particulars	Diet No.							
	CE_{36}	CE_{37}	CE_{38}	CE_{39}	CE_{40}	CE_{41}	CE_{42}	CE_{43}
Initial average length(mm)	22.7	22.6	23.0	22.8	23.0	23.1	23.2	22.9
Initial average dry-weight (g)	0 .0181	0.0181	0.0184	0.0183	0.0184	0.0185	0.0186	0.0183
Final average length(mm)	36.8	36.4	36.7	35.3	34.5	36.1	36.7	33.5
Final average dry weight(g)	0.0695	0.0665	0.0653	0.0627	0.0570	0.0673	0.0651	0.0530
Growth in length %	62.1	61.1	59.6	54.8	50.0	56.3	58.2	46.3
Growth in dry weight %	283.9^{a}	267.4^{a}	254.9^{ab}	242.6^{ab}	209.8^{bc}	263.8^{a}	250.0^{ab}	189.6^{cd}
Food conversion ratio	6.23^{d}	3.61^{ab}	3.17^{a}	3.32^{ab}	4.29^{bc}	3.04^{a}	3.66^{ab}	3.61^{ab}
Survival %	62.5	70.8	60.0	75.0	62.5	66.7	75.0	79.2
Protein content of *P. indicus* on termination of feeding experiment(%)	56.45	58.64	58.33	58.04	59.54	58.46	58.46	55.21
Carbohydrate content(%)	1.52	1.61	1.50	1.45	1.50	1.48	1.48	1.48
Lipid content (%)	17.04	17.94	14.39	16.44	15.40	16.42	15.40	15.67

Note: The values with different superscripts differ significantly among themselves. The growth in dry weight significant at 5% level ($P < 0.05$) and food conversion ratio significant at 1% level ($P < 0.01$). The growth in length was not found to be significant at 5% level ($P < 0.05$).

Table 34(A). Analysis of variance of growth and food conversion ratio obtained by the diets CE_{36} to CE_{43} in P. indicus.

Source	ANOVA		
	D.F.	S.S.	M.S.
1. Growth in length:			
Treatment	6	190.82	27.26^{N}
Error	14	141.76	8.86
Total	20	332.58	36.12
2. Growth in dry weight			
Treatment	6	19573.79	2796.25*
Error	14	10906.96	681.68
Total	20	30480.75	3477.93
3. Food conversion ratio			
Treatment	6	22.21	3.17**
Error	14	5.54	0.34
Total	20	27.75	3.51

** Significant at 1% level ($P < 0.01$)

* Significant at 5% level ($P < 0.05$)

N Not significant at 5% level.

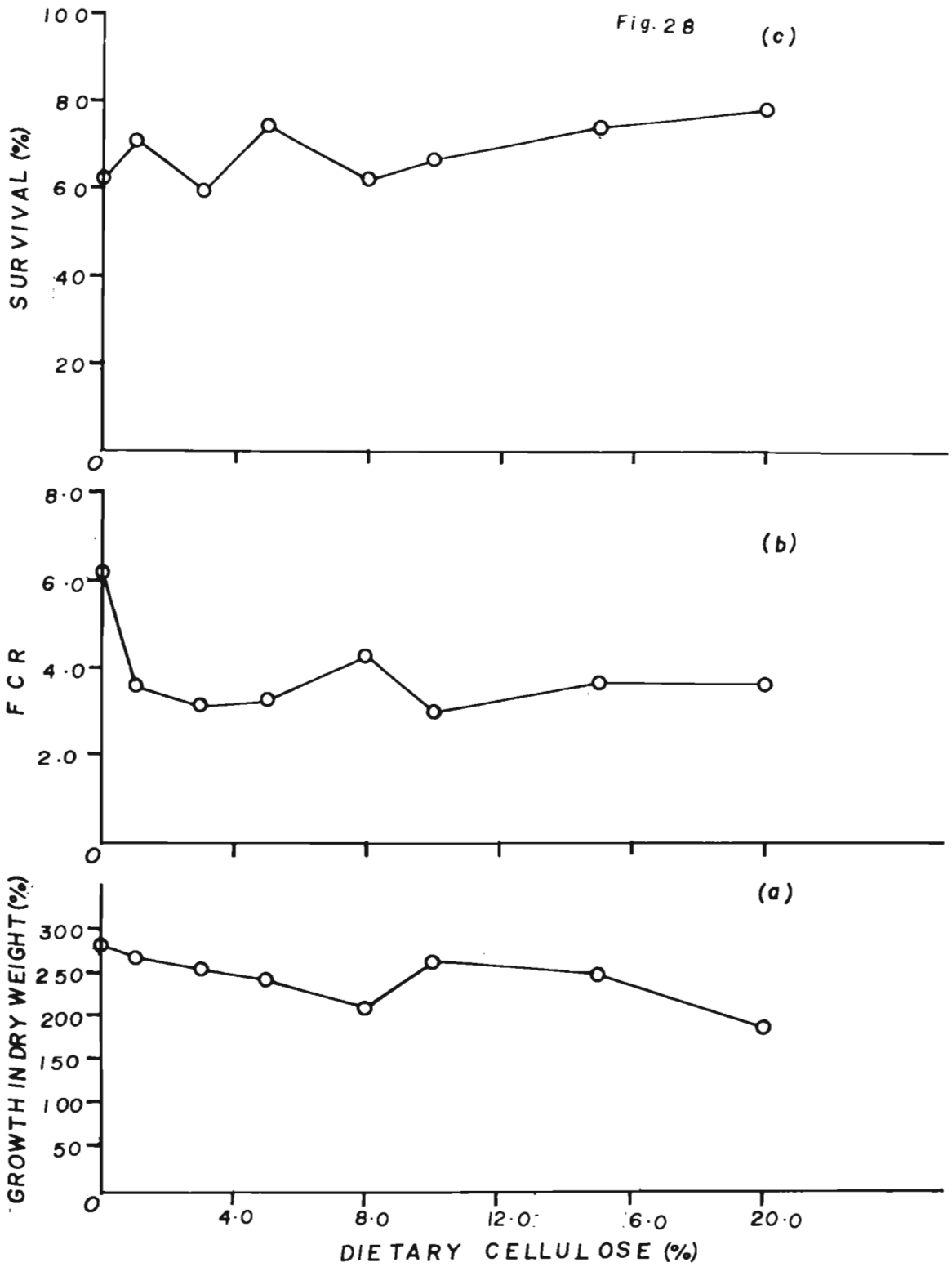

Figure 28. Influence of cellulose level in the diet on (a) growth in dry weight, (b) food conversion ratio and (c) survival of juvenile P. indicus.

To sum up the results, dietary cellulose appears to be required, even at the cost of dietary energy, for achieving efficient conversion of the feed and better survival of the cultured animals. The cellulose level between 1 to 10% in the diet does not seem to affect the growth of animals, but the best FCR was obtained with the diet having 10% cellulose. Higher levels of cellulose beyond 10% seem to affect the growth of animals.

DISCUSSION

Carbohydrate is an inexpensive source of energy in the diet. Though it is not considered as a physiologically essential nutrient, carbohydrate plays an important role in supplying the required energy to the animal through Kreb's cycle and also provides the carbon skeletons for synthesis of vital biochemicals such as chitin, steroids and fatty acids in the body. The importance of carbohydrate in the diet of the prawn, Penaeus indicus, could be clearly understood through the various experiments conducted in the present study.

Among the different types of carbohydrates tested, P. indicus has been found to utilize disaccharides and polysaccharides better than the monosaccharides. The three monosaccharides glucose, fructose and galactose tested, resulted in poor growth and food conversion ratio (FCR). The disaccharide maltose and the polysaccharide starch gave the best growth and FCR. Though glycogen (Polysaccharide) resulted in poor growth, the FCR was comparable to that of maltose and starch. However, the survival of the animals fed with the diet having glycogen was very poor. The disaccharide sucrose produced growth similar to that of the monosaccharides, but the FCR obtained by the former was much superior to that of the latter. It could be demonstrated that the carbohydrate in the diet is necessary and beneficial, since the diets having carbohydrate (maltose and starch at 30% level) produced better growth and FCR than the diet having no carbohydrate, though the latter had

a high protein (55%) in it.

Deshimaru and Yone (1978b) evaluated glucose, sucrose, dextrin, starch and glycogen in the diet of Penaeus japonicus. They found that the prawns fed with the diet having sucrose produced the highest growth, followed by the diet composed of glycogen. Whereas the prawns fed with the diet containing glucose resulted in very poor growth. They also found that the efficiency of the diets having starch was the highest followed by glycogen, sucrose and dextrin in the decreasing order. The efficiency of the diet with glucose was particularly poor and the survival of the animals was very low. Similar results were obtained by Kitabhayashi et al.(1971 b) in this prawn.

In the present study the growth of P. indicus was poor with the diets having glucose and other monosaccharides like fructose and galactose. The FCR of these diets was high, resulting in low survival. These findings in P. indicus are similar to those observed in P. japonicus with regard to monosaccharides by Deshimaru and Yone (1978b). Contrary to their findings, the diet with sucrose did not give highest growth in P. indicus as in the case of P. japonicus, but the FCR of sucrose diet was better than that recorded by the other monosaccharides used. On the other hand, the diets having maltose and starch gave the highest growth, best FCR, and the survival was also fairly superior. As in the case of P. japonicus, the growth of P. indicus fed the diets with glycogen was low with poor survival, but the FCR was comparable to that of the diets with maltose and starch.

Subsequently Abdel Rahman et al.(1979) evaluated different sources of carbohydrates for P. japonicus and found that the prawns fed with the diets containing sucrose, lactose, maltose, soluble starch, potato starch, dextrin and glycogen showed higher growth and those fed the diets having glucose, fructose and galactose showed very low growth with poor FCR and low survival. These findings are very much similar to those obtained in P. indicus in the present study. The poor performance of glucose as the source of carbohydrate in the diet was also observed in other penaeid prawns. Andrews et al.(1972) observed that when 20% glucose was used in a diet based on menhaden meal, the growth of P. aztecus was reduced. Sick and Andrews (1973)found that when 10% glucose was used in the diet, the growth of P. duorarum was similar to the diet having 10% starch. But when the glucose level was raised to 40%, the diet resulted in very poor growth. Alava and Pascual (1987) observed poor growth of P. monodon fed with the diet containing glucose, and reported further that the growth of P.monodon fed the diets having sucrose and trehalose (trisaccharide) was superior. Abdel Rahman et al.(1979) analysed the serum glucose levels of P. japonicus feeding orally, glucose, fructose, maltose and soluble starch. These authors found that the serum glucose of prawns which received glucose, rapidly rose to a very high value in one hour and maintained the same level for about 24 hours. But in the prawns which received fructose, the serum glucose did not raise as rapidly as it was in the case of glucose. This might be the reason that fructose gave better

growth than glucose in P. indicus. Abdel Rahman et al.(1979) further observed that in the prawns receiving maltose and starch, the serum glucose level gradually increased to a maximum level only in three hours and it came down to very low level after 24 hrs, in contrast to what observed in the case of glucose. It was postulated that dietary glucose is quickly absorbed from the alimentary canal and then released into the haemolymph, and it is not metabolised quickly causing physiological upsets leading to poor growth. On the other hand the glucose from digested disaccharides and polysaccharides is absorbed more slowly and metabolised effectively. Andrews and Sick (1972) using radio-active isotope (14_C) incorporated starch and glucose, found that 14_C from starch was incorporated into the tissues of P. setiferus at higher rate than from glucose, showing that starch is more effectively utilised than pure glucose as carbohydrate source. However, Kitabhayashi et al.(1971b) found that in the presence of adequate vitamin C, glucose was found to be utilised for energy production.

The apparent digestibility of monosaccharides at 30% in the diet was understandably high and that of the polysaccharides was slightly lower. Forster and Gabbott (1971), while studying the assimilation of different nutrients from the diet by caridean prawns, found that wheat starch and dextrin were completely digested by Palaemon serratus (at 47.5% level in the diet) and the apparent assimilation of wheat starch was 88.6, potato starch, 86.4, dextrin, 88.9 and that of glycogen, 88.9. The assimilation of potato starch in Pandalus platyceros was 86.1.

The results are similar to the apparent digestibility of starch and glycogen (88.04 and 88.96 respectively) obtained in P. indicus. But the poor growth and low survival of the animals fed by the diet with glycogen as carbohydrate could not be explained. Superior FCR of the glycogen diet, however shows that the animals did not ingest the diet very much but whatever that was ingested was digested well and effectively utilized. Whether glycogen in the diet influences the palatability is not known.

It was interesting to observe that the faeces of the animals fed with the CEO diet (2.56% carbohydrate) showed 11.22% carbohydrate. This experiment was repeated four times. It was found that the amount of faecal carbohydrate varied from 50 mg to 116 mg/g of dry diet consumed. Does this mean that the prawns constantly excrete carbohydrate just like nitrogen? Can this be called as the 'metabolic faecal carbohydrate' similar to metabolic faecal nitrogen? If a correction is applied to the faecal carbohydrate based on this, the true digestibility of all the carbohydrates tested will come to 100%. But this was not done, as the nature of compounds in the faeces was not known. Further studies are essential on this aspect to establish the existance of metabolic faecal carbohydrate, their quantity and then be calculated the true digestibility of the carbohydrate. Bergot and Breque (1983), while studying the digestibility of starch in the rainbow trout showed that the faecal carbohydrate of fish fed by gelatinised starch contained dextrin and other products. But no such observations are available in prawns.

The digestion of carbohydrate in crustacea has been demonstrated by Kooiman (1964). The presence of many carbohydrases, including α-and β-amylases, maltase, saccharase, chitinase and cellulase was shown. The activity of these enzymes was demonstrated not only in the carnivorous crab, Potamon martensi (Agrawal et al., 1967) but also in herbivorous prawns like Macrobrachium dayanum (Tyagi and Prakash, 1967). Karunakaran and Dhage (1977) already demonstrated the presence of amylase activity in the digestive tract of P. indicus and Metapenaeus monoceros. Penaeus indicus, which is an omnivorous species (Menon, 1954; Panikkar and Menon, 1955) might also possess most of the enzymes needed for carbohydrate digestion. The excellent digestibility of different varieties of carbohydrates tested in this study clearly demonstrates the presence of most of the carbohydrases in the prawn. The poor utilisation of monosaccharides by P. indicus might be due to similar reasons demonstrated in P. japonicus by Abdel Rahman et al. (1979). However, the disaccharide maltose and the polysaccharide starch are excellent individual sources of carbohydrate in the diet of P. indicus.

Experiments with the diets having mixed carbohydrate sources gave interesting results. When starch, glycogen, sucrose and maltose were used in combination with glucose in equal proportion (15% each), the growth and FCR were significantly low, though starch and maltose individually produced very superior growth and FCR. But when glucose was used in combination with sucrose and starch at 10% each, the growth of the animals and FCR considerably improved. And the combinations of glucose, maltose and starch at 10% ach produced

much higher growth and further improved the FCR of the diet. This indicates that prawns have a tolerable limit for glucose in the diet. Sick and Andrews (1973) observed in P. duorarum, that the diet having 10% glucose produced similar growth as to the diet having 10% starch, and when glucose was raised to 40% the growth was very much reduced. This shows that glucose can be used upto 10% in the diet. Kanazawa et al. (1970), when first designed artificial diets to study the nutritional requirements of P. japonicus, used a mixture of glucose (5.5%), sucrose (10%) and starch (4%) in the purified diets. With these diets the authors obtained growth comparable to the control diet, short necked clam (Tapes philippinarum) and evolved a purified diet designated as Diet-B. This diet was successfully employed by the authors in many subsequent nutritional studies. Abdel Rahman et al.(1979) prepared diets having 5, 10 and 20% glucose and found that the growth of P. japonicus was depressed as the glucose level in the diet increased. In the present study,the mixed carbohydrate having sucrose, maltose and starch in equal proportions produced the highest growth and the best FCR. It is, therefore, apparent that a mixed carbohydrate source, not having glucose (or any other monosaccharide) is more preferable and a mixture of di-and poly-saccharides is the best source of carbohydrate in prawn diets.

Increasing the level of carbohydrate in the diet, at constant protein and lipid, increased the growth of the prawn P. indicus and improved the FCR. The diet having 22.5% carbohydrate at 34.8% protein and 6% lipid, produced the highest growth and the best FCR. Further addition of carbohydrate to the diet resulted in marginal reduction in the per-

formance of the diet. The carbohydrate source used in this experiment was a mixture of sucrose, maltose and starch in equal proportions. These results indicate that carbohydrate can be used up to 22.5% in the diet, at constant protein and lipid and obtain higher growth and low FCR. Using starch as the source of carbohydrate, Ahamad Ali (1982b) found that the growth of juvenile P. indicus increased and FCR improved as the dietary starch level increased from 10 to 40%. But in the present study, higher growth and good FCR were obtained at a dietary carbohydrate level of 22.5%. Andrews et al. (1972) observed increased growth in P. aztecus when the diet contained 30% starch. Andrews and Sick (1972) obtained faster growth in P. duorarum using 40% starch in a casein based diet. The starch level in the diets at which higher growth was obtained in these species was higher than the carbohydrate level at which the higher growth was recorded in P. indicus in the present study. These differences could be due to the difference in source of the carbohydrate used. It is also attributed that starch contributes to the binding of the diet, improving its water stability resulting in better growth and FCR. On the other hand, Alava and Pascual (1987) found that the diet having 20% carbohydrate gave the best result in P. monodon and the diet having 30% carbohydrate, gave the lowest result. The authors used sucrose and trehalose as carbohydrate source. These findings are quite in agreement with findings in P. indicus in the present study.

Carbohydrate level above 22.5% in the diet, though did not bring any improvement in the growth, its apparent digestibility did not show any reduction. On the other hand it was

better than at lower carbohydrate levels. This is a clear indication that P. indicus is capable of digesting higher levels of dietary carbohydrate. The fact that the body carbohydrate of the animals fed with high carbohydrate diets did not show any difference with the other groups fed with low carbohydrate diets, indicates that there might be an optimum level of storage of glycogen in the body. Carbohydrate levels in the diet higher than 22.5% might exert handling strain on the system which might be resulting in marginal reduction in growth.

Subsequently, the role of carbohydrate level in the diet at constant lipid was studied. In this experiment the protein as well as the carbohydrate levels were varied, keeping the diets isocaloric. This has been done mainly to study the interaction of protein and carbohydrate in the diet and their combined role on the growth and FCR. The growth of the animals and FCR progressively improved as the carbohydrate level in the diet increased and protein level simultaneously decreased. Finally the diet with 21.9% protein and 53.4% carbohydrate recorded the highest growth and best food conversion ratio. These results have relevance to the protein requirement study in Chapter I. The dietary protein requirement of the animal depends not only on the quality of the protein source used but also on the amount of carbohydrate as the energy supplier. When the energy is balanced in the diet with carbohydrate, the optimum protein required in the diet could be as low as 22%. These results are very much in agreement with findings in Chapter I, in which the protein requirement in the diet was shown to be only 25% using albumen as the source, when the

diet had 42% carbohydrate. Animals need certain amount of metabolic energy for maintainance. The energy derived from the diet is used first to meet the maintainance energy requirement, and the balance only could be utilised for growth. Now in a high protein-low carbohydrate diet (at constant lipid), the animal has to derive the maintenance energy partly from the dietary protein in addition to the carbohydrate. Conversion of carbohydrate into energy through Kreb's cycle, is comparatively easy than conversion of protein to energy.

In the presence of adequate level of carbohydrate in the diet, the dietary protein can be effectively utilised for growth. Under this situation, the dietary carbohydrate can spare the dietary protein and the animals show low protein requirement in the diet for optimum growth. This protein sparing action of carbohydrate in the diet is also demonstrated in the present study in P. indicus from the fact that high carbohydrate-low protein diet produced higher growth, improved FCR, and the animals had increased protein deposition in their body. In the previous experiment in which the protein was kept constant, the diet with 22.5% carbohydrate gave the best result and higher levels of carbohydrate had no beneficial effect. It might be because that eventhough the carbohydrate level is increased beyond 22.5% in the diet, the protein level remained constant. This protein (34.8%) is at a higher level than required. That means the handling stress of excess protein (due to specific dynamic action) still persists in the animal, nullyfing the beneficial effect of increased carbohydrate in the diet. It is therefore logical to conclude that high carbohydrate diet could become efficient only when the protein is spared from

the diet. Increasing just carbohydrate level keeping the protein constant will not bring any beneficial effect beyond certain level. It is also appropriate to conclude that the combined effect of protein and carbohydrate in the right proportion in the diet is more relevant than considering each one of them in isolation.

Protein sparing action of carbohydrate was reported by Andrews et al. (1972)in Penaeus setiferus and Sick and Andrews (1973) in P. duorarum. These authors obtained best results with high carbohydrate level (up to 40%) in the diet. The diets had only 28% and 30% protein respectively. These results are comparable to those observed in P. indicus in the present study. The apparent digestibility was comparitively low at lower dietary carbohydrate than at higher dietary carbohydrate levels. In the first experiment it was pointed out that in the faeces of animals fed zero carbohydrate diet considerable amount of carbohydrate was measured. It was explained that this might be due to the excretion of so called metabolic faecal carbohydrate. This faecal carbohydrate might be responsible for the lower values of apparent digestibility at low dietary carbohydrate levels. But at higher dietary carbohydrate levels the apparent digestibility would show higher values even if the excretion of metabolic faecal carbohydrate remained same.

In the subsequent experiment, the diets were made isocaloric by varying lipid and carbohydrate, keeping the protein constant. As a result, high lipid-low carbohydrate and low lipid-high carbohydrate diets were obtained. This had been designed mainly to understand whether lipid could be used to enhance the calorific value of the diet. While the lipid-free and low-lipid diets reduced the growth and increased FCR. the high-lipid diet

depressed the growth and affected FCR. The negative effect is more pronounced in the high-lipid diets than in the low-lipid diet. These results indicate that lipid is essential in the diet but it may not be used to enhance the calorific value of the diet. Using a mixture of lipids, consisting of cod liver oil, sardine oil, soybean lecithin and prawn head oil (in equal proportions) the quantitative lipid requirement of juvenile P. indicus was studied (Ahamad Ali, unpublished). It was found that the prawn P. indicus required only 6% lipid in the diet. While the growth of prawns fed the diets with lower lipid levels (less than 6%) was poor, higher lipid levels depressed growth and caused mortality. Andrews et al. (1972) found adverse effect on the growth and survival of P. setiferus when the diet was added with 10% lipid. Similar adverse effect of high lipid (15%) on growth was also observed in Palaemon serratus by Forster and Beard (1973). Higher lipid levels in diet appear to increase the free fatty acid levels in the body of the animal which are toxic and retard growth. Lipid is an essential nutrient in the diet rather than an energy filler. On the other hand prawns have specific qualitative lipid requirement. It was well established that penaeid prawns such as Penaeus japonicus, P. monodon, P. indicus and P. merguiensis were found to have essential requirement for the polyunsaturated fatty acids (PUFA) like linolenic acid ($18:3W_3$), eicosapentaenoic acid ($20:5W_3$) and docosahexaenoic acid ($22:6W_3$) (Kanazawa and Teshima, 1977; Kanazawa et al.,1979a,b,c; Colvin, 1976b). Generally the lipids of plant origin are deficient in these fatty acids, where as the lipids of animal (marine) origin like fish oil and prawn head oil are very rich in PUFA.

The apparent digestibility of carbohydrate in the high lipid diet showed very low value. And these values are much lower than those obtained in low lipid (at 6%) diet. While this low digestibility might be partly due to the similar reasons of metabolic faecal carbohydrate explained earlier, the influence of high lipid in the diet on the digestibility of carbohydrate cannot be ruled out. As the lipid level in the diet is reduced, the apparent digestibility of carbohydrate increased and reached the highest value when diet had no lipid in it.

Hysmith et al.(1972) while investigating with P. aztecus, concluded that low protein-high energy diets gave better growth. With the possibility of using lipid to increase the dietary energy being ruled out, it is the carbohydrate which should be used for this purpose. From the results of the present investigations, it is clear that for achieving faster growth and best FCR, the diet should have low-protein and high-carbohydrate. These findings have very important economic implication in a package of prawn culture technology, because the cost of a practical feed, meant for commercial culture operations, has direct bearing on the cost of production of the cultured animals, as feed forms a major portion of the expenditure.

Addition of cellulose to diet, though did not improve the growth of animals, helps to realise improved food conversion ratio. The FCR progressively reduced with the addition of cellulose and reached a lowest value at a dietary cellulose of 10%. Since cellulose was added at the expense of carbohydrate in the diet, the results obtained with these diets had shown

that the cellulose was either acting as a nutrient by itself or aiding in better utilisation of the other nutrients. Venkataramaiah et al.(1975a) obtained improved food conversion efficiency without altering the original growth pattern in P. aztecus, by the addition of vegetable matter (fibre). These observations are similar to those found in P. indicus in the present study. Kooiman (1964) detected cellulase activity in the digestive juices of crayfish, Astacus fluviatilis and the lobster Homarus vulgaris. Yasumasu and Yokoe (1965) reported the biosynthesis of cellulose in the hepatopancreas of the crayfish, Procambarus clarkii. Forster and Gabbott (1971) found assimilation of small amounts of cellulose (20.8%) by the caridean prawn Palaemon serratus and indicated the presence of weak cellulase activity in the gut of this prawn. Fair et al.(1980) tested the effect of dietary cellulose at 0, 5, 15, 20 and 30% level and found that the growth of Macrobrachium rosenbergii was not affected at all the dietary cellulose levels. On the other hand, the growth was better with diets having 5 and 20% fibre than with the diet having zero cellulose. The authors detected high cellulase activity in the prawn and demonstrated that the enzyme activity was six times higher in adult prawn than in smaller ones. The findings in P. indicus in the present study with regard to dietary cellulose are in agreement with the observations made in different species of prawns discussed above. The fact that the growth of prawns is largely unaffected with the addition of cellulose in the diet up to 10% suggests that the cellulose is partly assimilated indicating limited cellulase activity. On the other hand, the dietary cellulose improved the FCR suggesting

that it is helping in better utilisation of diet by the animal. Perhaps in nature the detritus (Panikkar and Menon 1955; Williams, 1955) and algal filaments (George, 1959), on which the prawns feed, form the cellulose base. To sum up, cellulose (or roughage) is needed, if not essential, in the diet of P. indicus and the maximum limit of it may be 10%.

CONCLUSIONS

1. Among the different sources of carbohydrates tested, the disaccharides such as maltose and Polysaccharides like starch are found to be the best sources of carbohydrate in the diet of the prawn Penaeus indicus.
2. A mixed carbohydrate source consisting of sucrose, maltose and starch, in equal proportions was found to be relatively better combination for P. indicus. The combination of disaccharides and polysaccharides with 15% glucose was not found to be good carbohydrate sources.
3. At constant protein (34.8%) and lipid (6%) the diet with 22.5% carbohydrate produced the best growth and food conversion ratio (FCR).
4. At constant lipid (6%), carbohydrate has protein sparing action in the diet. Highest growth and best food conversion ratio were obtained with the diet having 22% protein and 53% carbohydrate.
5. At constant protein (34.8%) lipid is not suitable to increase the calorific value of the diet. High lipid (16.8%) and low carbohydrate (8.44) produced low growth and poor FCR. Only the diet with 6% lipid and 33% carbohydrate produced the highest growth and best FCR.
6. High energy-low protein feeds are more economical without compromising with the performance of the feed.
7. Cellulose is needed in the diet of the prawn P. indicus. Best FCR was obtained with the diet having 10% cellulose than the diet having zero cellulose, eventhough growth obtained by both diets was not significantly different. Cellulose

can be used up to 10% in the diet of *P. indicus*.

8. The faeces of animals fed zero carbohydrate diet had considerable amount of carbohydrate. The excretion of carbohydrate in the faeces varied from 50 to 116 mg/g of dry diet consumed. Whether this is a case of metabolic faecal carbohydrate similar to metabolic faecal nitrogen, needs further investigation.

C H A P T E R - III

DIETARY MINERAL REQUIREMENTS

Present status:

Compared to protein, lipid and carbohydrate, minerals are required in small quantities in the diet. Though minor in nature, the mineral nutrition in animals including prawns, is no less important. Minerals are essential because these cannot be synthesised by the animals and should be provided through external sources. Mineral ions are vital components of many biological chemicals such as enzymes, hormones and other organic compounds, involved in a number of biochemical life processes. Their non-availability for a prolonged period often leads to irrecoverable deficiency diseases. Mineral ions are particularly important to aquatic animals as these play very important role in osmotic regulation.

Mineral elements can be grouped into bulk elements such as calcium, phosphorous, chloride, potassium, magnesium and trace elements, copper, zinc, manganese, cobalt and aluminium. Crustaceans have high content of ash, 15.9%, in their body (Sze, 1973). Since sea water is rich in many mineral ions, they are capable of extracting most of the minerals required (Gilles and Pequeux, 1983) for the body from the surrounding environment. This often makes quite difficult to assess the dietary requirement of minerals. The fluctuation of different minerals and their seasonal variations in different ecosystems have been extensively studied (Sankaranarayanan and Qasim, 1969; Murty and Veerayya, 1972; Nair et al., 1975; Matkar et al., 1981; Venugopal et al., 1982; Nagarajaiah

and Gupta, 1983; Rajendran and Kurian, 1986; Subhash Chander, 1986). The variations in the body mineral composition of the crustaceans in relation to the ecosystem have also been investigated (Colvocorresses and Lynch, 1975; Zanders, 1980; White and Rainbow, 1982). Many a study indicate that these animals are capable of regulating the concentration of most of the body ions in tune with the ionic concentration of the surrounding medium. Among the minerals, calcium and phosphorous are studied in detail. Crustaceans in general were found to absorb Calcium from the surrounding water (Kleinholz and Bourquin, 1951; Guyselman, 1953; Robertson, 1960a, b; Lockwood, 1962; Smith and Linton, 1971; Hecht, 1975; Graf, 1978; Zanders, 1980). Calcium absorption from the water was specifically demonstrated in crayfish (Gibbs and Bryan, 1972; Greenway, 1972; Mills and Lake, 1976; Mills *et al.*, 1976; Chichibu, 1979) and in blue crab, *Callinectes sapidus* (Colvocorresses *et al.*, 1974). While Dall (1965) studied the metabolism of calcium in *Metapenaeus* sp. and Greenway (1985) reviewed the calcium balance and moulting in crustacea in general, Subhash Chander (1986) studied the relationship between the calcium content in different organs of the prawn *Penaeus indicus* and the surrounding medium under different ecosystems. Miyazaki and Jozuka (1964) and Deshimaru *et al.* (1978) confirmed the absorption of calcium by the crayfish and the prawn *Penaeus japonicus* respectively by using radio-active labelled calcium (45_{Ca}).

The body phosphorous of the aquatic animals appears to be related to the body calcium and magnesium and thus its variation is not directly linked to the fluctuations of the

environmental phosphorous. While working on phosphorous in the prawn Penaeus californiensis, Huner et al. (1979) opined that the variations of phosphorous levels are difficult to explain. Nevertheless, the dietary requirement of both calcium and phosphorous for prawns had been studied in greater detail. Kitabhayashi et al. (1971a) achieved the best growth rate in the prawn P. japonicus, by supplementing 1.24% calcium and 1.4% phosphorous in the diet. When the ratio of calcium and phosphorous was increased to 2:1, the growth was inhibited and the pigmentation decreased. This had lead to the suggestion that the ratio of calcium and phosphorous in the diet should be 1.2:1. Sick et al.(1972) provided a calcium phosphorous ratio of 1.3:1 (0.66% calcium and 0.51% phosphorous) in a casein based diet and obtained 18% increase in biomass in P. aztecus. At the same time Andrews and Sick (1972) used a mineral mixture containing only 0.26% calcium and 0.21% phosphorous in the diet of P. setiferus and Deshimaru and Kuroki (1974a) obtained the highest growth in P. japonicus with the diet having a calcium Phosphorous ratio of 0.76:1. On the other hand Parker and Holcomb (1973) reported the use of very high calcium phosphorous ratio (2:1 to 4:1) in the prawn diets due to addition of limestone to increase pellet density. While Shewbart et al. (1973) postulated that calcium, potassium, sodium and chloride requirements for P. aztecus might be satisfied through osmotic regulation, Deshimaru and Yone (1978a) did not find any improvement in the nutritive value of the diet when supplemented with 2% calcium along with other mineral elements in P. japonicus. But Kanazawa et al.(1984) reported that P. japonicus required 1% calcium and

1% phosphorous in the diet. It was emphasized that phosphorous might be essential in the diet of prawns, since sea water contains very little phosphorous in it.

White and Rainbow (1982) studied the regulation of trace metal concentration in the prawn Palaemon elegans in relation to the trace metal concentration in the surrounding water. Lewis and Cave (1982) reviewed the literature on copper in relation to the marine environment. While the importance of copper in crustaceans was highlighted by Lontie and Vanquickenborne, (1974) its variations in the different parts of the body was studied by Stickney et al. (1975) in P. aztecus and P. setiferus, Zindge et al. (1976) in P. monodon and Metapenaeus affinis, Horowitz and Presley (1977) in P. aztecus and Subhash Chander (1986) in P. indicus. Though it was suggested that copper is absorbed by crustaceans (Bryan, 1968; White and Rainbow, 1982; Subhaschander, 1986) from water, dietary studies have shown that copper is required in the diet of P. japonicus at 0.6% level (Kanazawa et al., 1984).

The investigations on the regulation of zinc in the body of lobster, Homarus vulgaris (Bryan, 1964), the crab, Callinectes sapidus (Colvocorresses and Lynch, 1975) and the prawns P. californiensis (Bryan, 1968), Palaemon elegans (White and Rainbow, 1984), P. indicus (Subhash Chander, 1986) had yielded varying results. These studies indicated that zinc was absorbed to some extent by these animals from the surrounding medium. However, Deshimaru and Yone (1978a) reported the requirement of 0.2% trace element mixture consisting of aluminium, Copper, Cobalt, Iodine, Manganese and zinc in the diet of P. japonicus.

Even though little seasonal variation in the magnesium content of the saline water (Naik, 1978; Gupta and Naik, 1981) was reported, the body magnesium of crustaceans appears to be related to the salinity of the water and the animals seem to regulate the body concentration of this element from the surrounding medium (Zanders, 1978). It was demonstrated that penaeid prawns were capable of absorbing magnesium from water (Graf, 1978; Subhashchander, 1986) and the food as its source is only secondary. However, Kanazawa *et al.* (1984) reported a requirement of 0.3% magnesium in the diet of *P. japonicus*.

The nutritive value of potassium in the diet of *P. japonicus* was studied by Deshimaru and Yone (1978a) and Kanazawa *et al.* (1984) and reported that the prawn required about 1% potassium in the diet for higher growth and feed efficiency. Addition of iron and manganese was found to inhibit the growth of prawns.

From the above discussions it can thus be seen that the mineral studies in prawns are directed mainly in relation to the environment and the investigations on their dietary requirement are very limited which are essential for preparing balanced feeds. With a view to obtaining information on these aspects, the dietary requirement of six minerals, calcium, phosphorous, magnesium, copper, zinc and manganese was individually investigated for the juvenile *P. indicus* and the results presented.

MATERIAL AND METHODS

Six sets of experiments were conducted to study the dietary requirement of six minerals namely calcium, phosphorous, magnesium, copper, zinc and manganese, for juvenile Penaeus indicus, by formulating purified diets with varying amounts of each mineral (Tables 36 to 41). The feeding experiments were carried out with these different diets and the level of requirement was determined by measuring the growth of the test animals and the food conversion ratio. The composition of animals for protein, lipid, carbohydrate, ash and for the concerned mineral, was analysed before and after the experiment to understand the influence of the mineral on these parameters. The proportion of diets used in this work was based on the purified diets formulated in Chapters I & II. But the protein source used was a mixture of fibrin and albumen (egg) in the ratio 80:20. For preparing mineral free diet, fibrin was found to be a better source of protein (Lovell, 1978) and it showed high biological value for P. indicus. Since albumen promoted better growth, it was used in combination with fibrin in the ratio indicated above. The mineral sources used were of analytical grade chemicals of standard brands. The mineral element studied in each experiment and the mineral source used and the supplier of the chemical are summarised below.

Experiment No.	Mineral element studied	Source Chemical	Supplier
14	Calcium	Calcium carbonate	Indian Drugs and Pharmaceuticals, Hyderabad.
15	Phosphorous	Potassium dihydrogen Orthophosphate	Glaxo Laboratories (BDH), Bombay.
16	Copper	Copper sulphate	- do -
17	Zinc	Zinc chloride	S.D. Fine Chemicals Pvt. Ltd., Boisar.
18	Magnesium	Magnesium sulphate	E. Merck Pvt. Ltd., Bombay.
19	Manganese	Manganese sulphate	Glaxo Laboratories (BDH), Bombay.

Formulation of diets.

The diets, to study the selected mineral requirements for juvenile *P. indicus*, were formulated with base diet (Table 35) having 35% protein mix, 30% carbohydrate mix and 6% cod liver oil. Keeping the major nutrients as well as the vitamin mix and other components at constant levels, the mineral to be studied was varied and seven diets in each of the groups were formulated. Thus to study the calcium requirement, the calcium carbonate was varied from 0 to 5%, for phosphorous requirement, the potassium dihydrogen orthophosphate was varied from 0 to 8.8%, for copper, copper sulphate was varied from 0 to 0.14%, for zinc, the zinc chloride was at levels varying from 0 to

0.12%, in the case of magnesium, the magnesium sulphate was varied from 0 to 7% and manganese sulphate from 0 to 0.008% for studying the manganese requirement. Sodium alginate was used as the binder. The diets in each of the groups were serially numbered to facilitate their identification. The composition of the diets formulated for calcium, phosphorous, copper, zinc, magnesium and manganese requirement studies are given in tables 36, 37, 38, 39, 40 and 41 respectively.

Preparation of diets:

The details of preparation of the diets are essentially same as described under the 'preparation of purified diets' in Chapter-I, except that in case of minerals copper, zinc and manganese, seperate solutions were prepared with the respective mineral salts in distilled water and appropriate amounts were included in the diets.

Feeding Experiments:

The design of the feeding experiments, rearing facility, experimental animals, stocking and number of replicates, water management, measurement of length and weight of animals, feeding shedule and procedure, measurement of growth and food conversion ratio were same as described in Chapter I. The hydrographical data of the different feeding experiments are shown in Table H_3. The duration of each feeding experiment was 45 days.

Chemical Analysis:

Preparation of samples for analysis and the methods of estimation of protein, lipid, carbohydrate and ash were same as described in Chapter I.

Phosphorous in the diets and in the animals was estimated by the Spectrophotometric method using Molybdovanadate reagent. The other mineral elements calcium, copper, zinc, magnesium and manganese both in diets and animals, were estimated by using Atomic Absorption Spectrophotometer (AAS). The samples for AAS analysis were prepared by 'Dry Ashing Method' described in the manual supplied along with the instrument. In this method, the ash obtained was dissolved in hydrochloric acid. The solution was gently boiled, cooled, filtered, and made upto a known volume using double distilled water. A blank was also prepared by same procedure. The sample thus prepared was analysed for the mineral element using Perkin Elmer Atomic Absorption Spectrophotometer of model 2380, in an air-acetylene flame (Plate 3).

Statistical analysis:

The data obtained on growth in length, dry weight and the food conversion ratio in all the feeding experiments were subjected to analysis of variance (ANOVA), following the same method described under Chapter I.

Table 35. Composition of the base diet used in mineral requirement studies.

Protein mix[1]	35.0 g.
Carbohydrate mix[2]	38.0 "
Cod liver oil	6.0 "
Glucosamine HCl	0.8 "
Cholesterol	0.5 "
Vitamin mix[3]	2.7 "
	83.0 g.

1. Protein mix : A mixture of fibrin and albumen in the ratio 80 : 20

2. Carbohydrate mix: A mixture of sucrose, maltose and starch in the ratio 1:1:1.

3. Vitamin mix : Same as used for the diets PE_0 to PE_4 in Chapter I (Table 2a)

Table 36. Composition (g/100g) of the experimental diets M_1 to M_7 to study calcium requirement.

Ingredients	Diet No.						
	M_1	M_2	M_3	M_4	M_5	M_6	M_7
Base diet	83.00	83.00	83.00	83.00	83.00	83.00	83.00
Calcium carbonate	0.00	1.30	2.0	2.7	3.25	3.8	5.0
Potassium dihydrogen ortho-phosphate	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Cellulose	10.00	8.7	8.0	7.3	6.75	6.2	5.0
Sodium alginate	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Ash %	5.14	6.86	7.56	8.26	8.81	9.36	10.20
Calcium %	0.024	0.525	0.774	1.025	1.275	1.525	2.024
Phosphorous %	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Table 37. Composition (g/100g) of the experimental diets M_8 to M_{14} to study Phosphorous requirement.

Ingredients	Diet No.						
	M_8	M_9	M_{10}	M_{11}	M_{12}	M_{13}	M_{14}
Base diet	83.00	83.00	83.00	83.00	83.00	83.00	83.00
Calcium carbonate	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Potassium dihydrogen orthophosphate	0.00	2.2	3.3	4.4	5.5	6.6	8.8
Cellulose	12.7	10.5	9.4	8.3	7.2	6.1	3.9
Sodium alginate	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Ash %	5.93	6.97	7.50	8.60	9.10	10.56	13.01
Calcium %	0.53	0.53	0.529	0.53	0.53	0.53	0.53
Phosphorous %	0.2155	0.6470	0.8482	1.0495	1.1810	1.3125	1.8303

Table 38. Composition (g/100g) of the experimental diets M_{15} to M_{21} to study Copper requirement.

Ingredients	Diet No.						
	M_{15}	M_{16}	M_{17}	M_{18}	M_{19}	M_{20}	M_{21}
Base diet	83.00	83.00	83.00	83.00	83.00	83.00	83.00
Calcium carbonate	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Potassium dihydrogen orthophosphate	4.4	4.4	4.4	4.4	4.4	4.4	4.4
Copper sulphate	0.0	0.007	0.0105	0.014	0.042	0.07	0.14
Cellulose	8.3	8.293	8.2895	8.286	8.258	8.23	8.16
Sodium alginate	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Ash %	7.5	7.96	7.6	7.0	6.94	7.0	6.94
Copper mg/100 g	2.4	4.2	5.5	7.1	10.2	13.6	22.7

Table 39. Composition (g/100g) of the experimental diets M_{22} to M_{28} to study Zinc requirement.

Ingredients	Diet No.						
	M_{22}	M_{23}	M_{24}	M_{25}	M_{26}	M_{27}	M_{28}
Base diet	83.00	83.00	83.00	83.00	83.00	83.00	83.00
Calcium carbonate	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Potassium dihydrogen orthophosphate	4.4	4.4	4.4	4.4	4.4	4.4	4.4
Copper sulphate	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Zinc chloride	0.00	0.02	0.04	0.06	0.08	0.10	0.12
Cellulose	8.23	8.21	8.19	8.17	8.15	8.13	8.11
Sodium alginate	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Ash %	6.9	7.54	7.50	7.54	7.45	7.4	7.5
Zinc (mg)/100g	6.0	14.10	18.90	23.60	30.4	38.6	50.2

Table 40. Composition (g/100g) of experimental diets M_{29} to M_{35} to study Magnesium requirement.

Ingredients	Diet No.						
	M_{29}	M_{30}	M_{31}	M_{32}	M_{33}	M_{34}	M_{35}
Base diet	83.00	83.00	83.00	83.00	83.00	83.00	83.00
Calcium carbonate	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Potassium dihydrogen orthophosphate	4.4	4.4	4.4	4.4	4.4	4.4	4.4
Copper sulphate	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Zinc chloride	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Magnesium sulphate	0.00	1.0	2.5	3.0	4.5	5.0	7.0
Cellulose	8.17	7.17	5.67	5.17	3.67	3.17	1.17
Sodium alginate	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Ash %	6.74	6.90	7.15	7.40	7.6	7.8	9.5
Magnesium (g)/100g	Trace	0.099	0.1980	0.2982	0.3976	0.4970	0.6958

Table 41. Composition (g/100g) of experimental diets M_{36} to M_{42} to study Manganese requirement.

Ingredients	Diet No.						
	M_{36}	M_{37}	M_{38}	M_{39}	M_{40}	M_{41}	M_{42}
Base diet	83.00	83.00	83.00	83.00	83.00	83.00	83.00
Calcium carbonate	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Potassium dihydrogen orthophosphate	4.4	4.4	4.4	4.4	4.4	4.4	4.4
Copper sulphate	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Zinc chloride	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Manganese sulphate	0.00	0.002	0.003	0.004	0.005	0.006	0.008
Cellulose	8.17	8.168	8.167	8.166	8.165	8.164	8.162
Sodium alginate	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Ash %	8.2	7.9	7.53	6.8	7.50	7.3	7.8
Manganese(mg)/100g	0.21	0.69	0.99	1.299	1.615	1.949	2.599

Table H_3. Hydrographical data of the feeding experiments conducted with different groups of diets in the mineral requirement study.

Experiment No. (diets)	Salinity ‰	Oxygen CC/L	Temperature °C	pH
(M_1 [14] to M_7)	18.6±1.0	5.4	27.0 ± 0.5	8.2 ± 1
(M_8 [15] to M_{14})	18.6±1.0	5.4	27.0 ± 0.5	8.2 ± 0.1
(M_{15} [16] to M_{21})	20.8±1.0	4.4	28.5 ± 0.5	7.98± 0.2
(M_{22} [17] to M_{28})	20.8±1.0	4.4	28.5 ± 0.2	8.0 ± 0.2
(M_{29} [18] to M_{35})	18.39±1.0	4.11	30.2 ± 0.5	8.1 ± 0.1
(M_{36} [19] to M_{42})	18.39±0.5	4.11	30.2 ± 0.5	8.1 ± 0.1

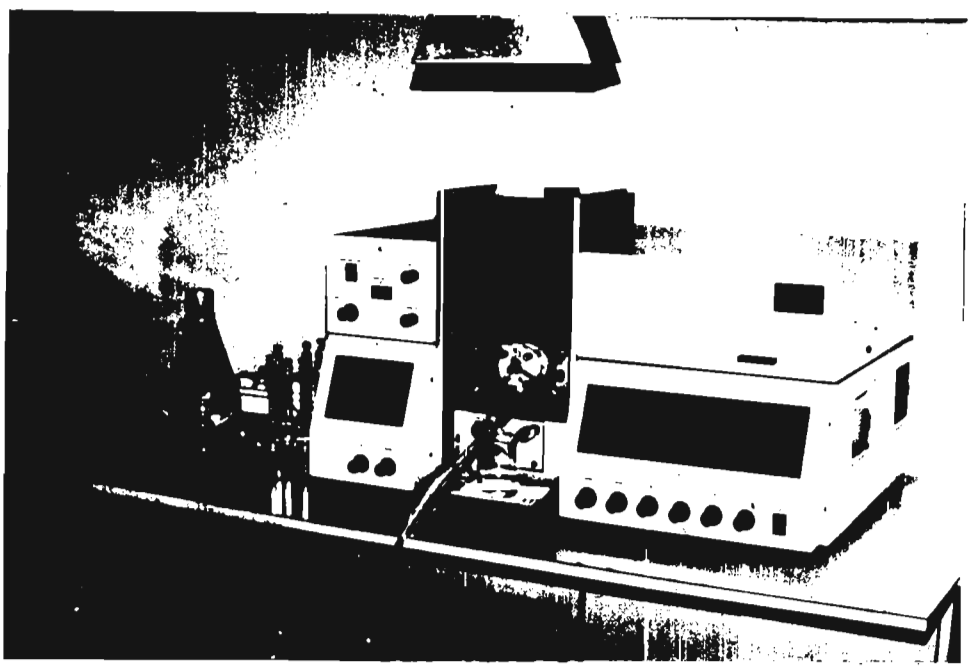

Plate 3. Perkin Elmer model 2380, Atomic Absorption Spectrophotometer used for analysing mineral elements.

RESULTS

Calcium requirement.

The results of the feeding experiment with diets M_1 to M_7 in which the dietary requirement of calcium for juvenile *P. indicus* was studied, are presented in Table 42. The growth in length obtained by the diets M_1, M_2, M_3, M_4, M_5, M_6 and M_7 was 60.0, 76.7, 76.1, 75.5, 66.1, 55.8 and 50.5% respectively and the corresponding growth in dry weight was 265.7, 383.0, 331.3, 275.8, 277.8, 213.1 and 291.9%. The diets in the same order produced, food conversion ratio (FCR) of 5.13, 3.89, 4.95, 5.69, 6.79, 6.88 and 4.85 respectively. The analysis of variance (ANOVA) of the data is given in Table 44. The treatments were significant among themselves in respect of growth in dry weight ($P < 0.01$) and FCR ($P < 0.05$). The growth in length was not significantly different ($P < 0.05$) among the treatments. The growth of animals fed with the diet (M_2), having 0.525% calcium, was significantly higher than the growth obtained by the diet (M_1) having 0.024% calcium and also those diets (M_3 to M_7) having higher levels of calcium above 0.525%. The FCR of the diet having the 0.325% calcium was significantly lower compared to the values obtained by all the other diets.

The body composition of the animals before and after feeding with diets M_1 to M_7 is presented in Table 43. The crude protein content of the animals before feeding (64.79%) and those of the prawns after feeding with diet M_2 (63.91%) were comparable. But there was a decreasing trend in crude protein of animals fed with the diets M_3 to M_7. On the other hand the body lipid of the animals fed with the diet M_1 was

Table 42. Results of the feeding experiment conducted with diets M_1 to M_7 on juvenile *P. indicus* for 45 days.

Particulars	Diet No.						
	M_1	M_2	M_3	M_4	M_5	M_6	M_7
Initial average length (mm)	21.0	21.0	20.9	20.8	20.8	20.8	20.8
Initial average dry weight(g)	0.0099	0.0100	0.0099	0.0099	0.0099	0.0099	0.0099
Final average length (mm)	33.6	37.1	36.8	36.5	34.5	32.4	31.3
Final average dry weight (g)	0.0362	0.0483	0.0427	0.0372	0.0374	0.0310	0.0388
Growth in length %	60.0	76.7	76.1	75.5	66.1	55.8	50.5
Growth in dry weight %	265.7^{bc}	383.0^{a}	331.3^{ab}	275.8^{bc}	277.8^{bc}	213.1^{cd}	291.9^{bc}
Food conversion ratio	5.13^{ab}	3.89^{a}	4.95^{ab}	5.69^{bc}	6.79^{cd}	6.88^{cd}	4.85^{ab}
Survival %	75.0	75.0	79.2	79.0	83.0	87.5	83.3

Note: Values with different superscripts differ significantly among themselves. Growth in dry weight significant at 1% level ($P < 0.01$) and Food conversion ratio significant at 5% level ($P < 0.05$). Gowth in length was not significant at 5% level.

Table 43. Body composition of P. indicus fed with the diets M_1 to M_7.

	% dry basis (g/100g)						Ca/P
	Crude protein	Lipid	Carbo-hydrate	Ash	Calcium	Phosphorous.	
Before feeding	64.79	16.36	1.66	16.15	2.62	1.76	2.63
After feeding with diets							
M_1	63.04	14.00	1.65	18.77	3.86	1.53	3.83
M_2	63.91	19.00	1.60	20.61	3.48	1.85	3.30
M_3	63.48	17.12	1.62	19.50	3.91	1.89	3.12
M_4	63.04	15.00	1.62	19.42	3.71	1.94	2.94
M_5	62.06	18.14	1.61	18.50	3.62	1.91	2.95
M_6	62.16	20.00	1.57	18.26	3.53	1.87	2.95
M_7	60.42	20.24	1.54	21.31	3.00	1.42	3.52

Table 44. Analysis of variance of the data obtained with the diets M_1 to M_7.

Source	Degrees of freedom	S.S.	M.S.
	ANOVA		
1. Growth in length			
Treatment	6	732.15	122.02^{N}
Error	14	1185.47	84.67
Total	20	1917.62	206.69
2. Growth in dry weight			
Treatment	6	50640.63	8440.10**
Error	14	15673.63	1119.54
Total	20	66314.26	9559.64
3. Food Conversion ratio			
Treatment	6	21.03	3.50*
Error	14	10.51	0.75
Total	20	31.54	4.25

** Significant at 1% level ($P < 0.01$)

* Significant at 5% level ($P < 0.05$)

N Not significant ($P < 0.05$)

lower (14%) than that of the animals before feeding (16.36%). The lipid showed increasing trend in the animals fed the diets M_2 to M_7. The body carbohydrate did not seem to be affected appreciably but the values were slightly less in the animals after feeding with different diets, than the initial value. The ash content in prawns fed different diets was higher than in those before feeding. Small variations could be noticed in the body phosphorous of the animals fed with the diets M_1 to M_7. Only the body phosphorous of prawns fed the diets M_1 and M_7 was less than that of the animals before feeding. The body phosphorous of all other groups was marginally higher than that of the initial value. The relationship between the dietary calcium and growth and FCR is shown in Fig.29. The growth curve showed a peak (Fig.29a) at a dietary calcium level of 0.5% and gradually declined with the increase in calcium level in the diet. The FCR showed a lowest value (Fig.29b) when the diet contained 0.5% calcium and further increase in the dietary calcium gradually enhanced the FCR. The body calcium slightly declined (Fig.30a) at the dietary calcium level of 0.5% and raised again at 0.774% dietary calcium, and beyond this point the body calcium gradually came down. Similarly the calcium-phosphorous (Ca/P) ratio decreased (Fig.30b) with progressive increase in the dietary calcium level. The survival of the animals did not show much variation (Fig.30c) with the dietary calcium level.

The results of this experiment showed that the juveniles of P. indicus require about 0.5% of calcium in their diet, for

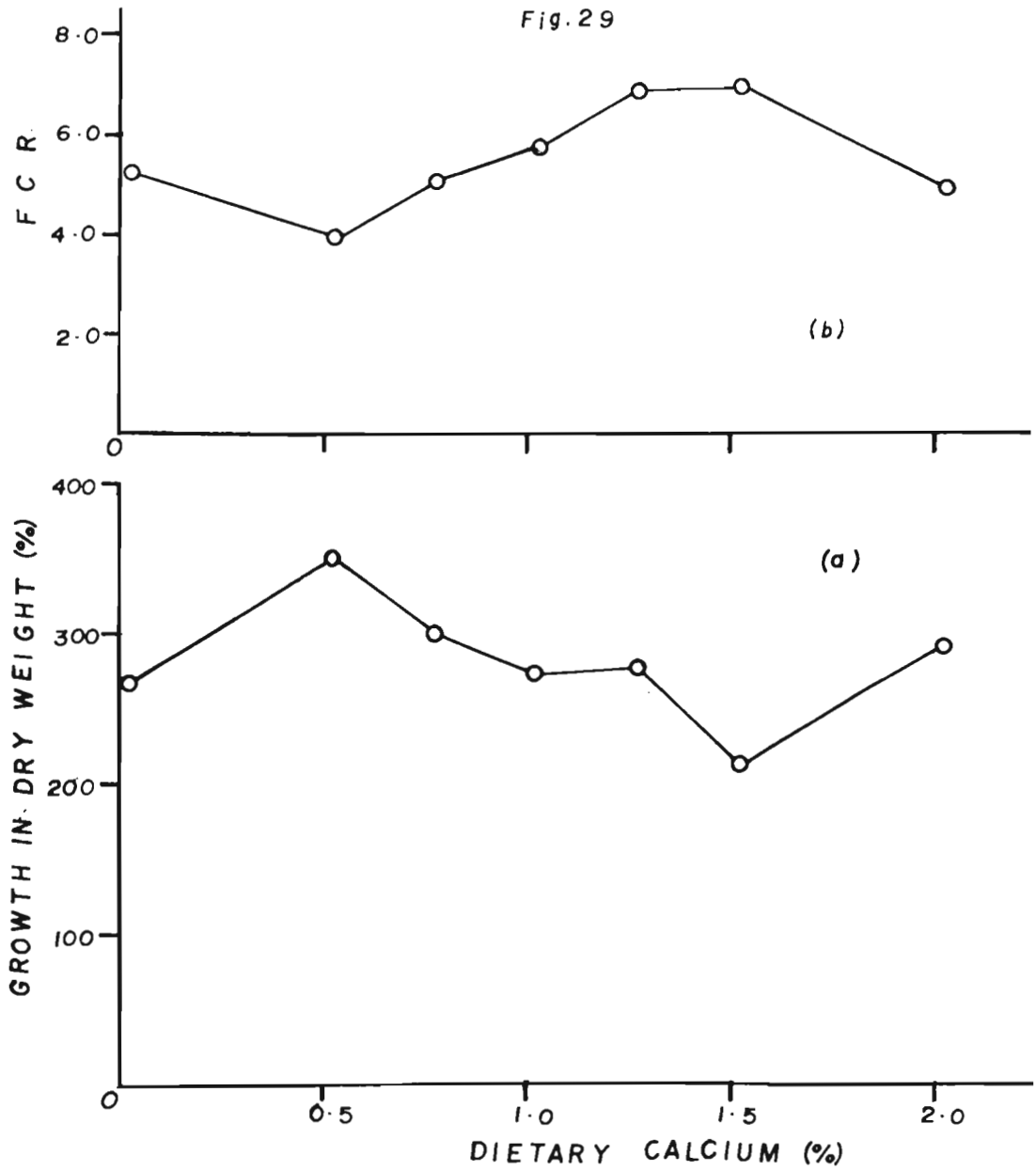

Figure 29. Growth (a) and food conversion ratio (b) of P. indicus fed with different levels of calcium in the diet.

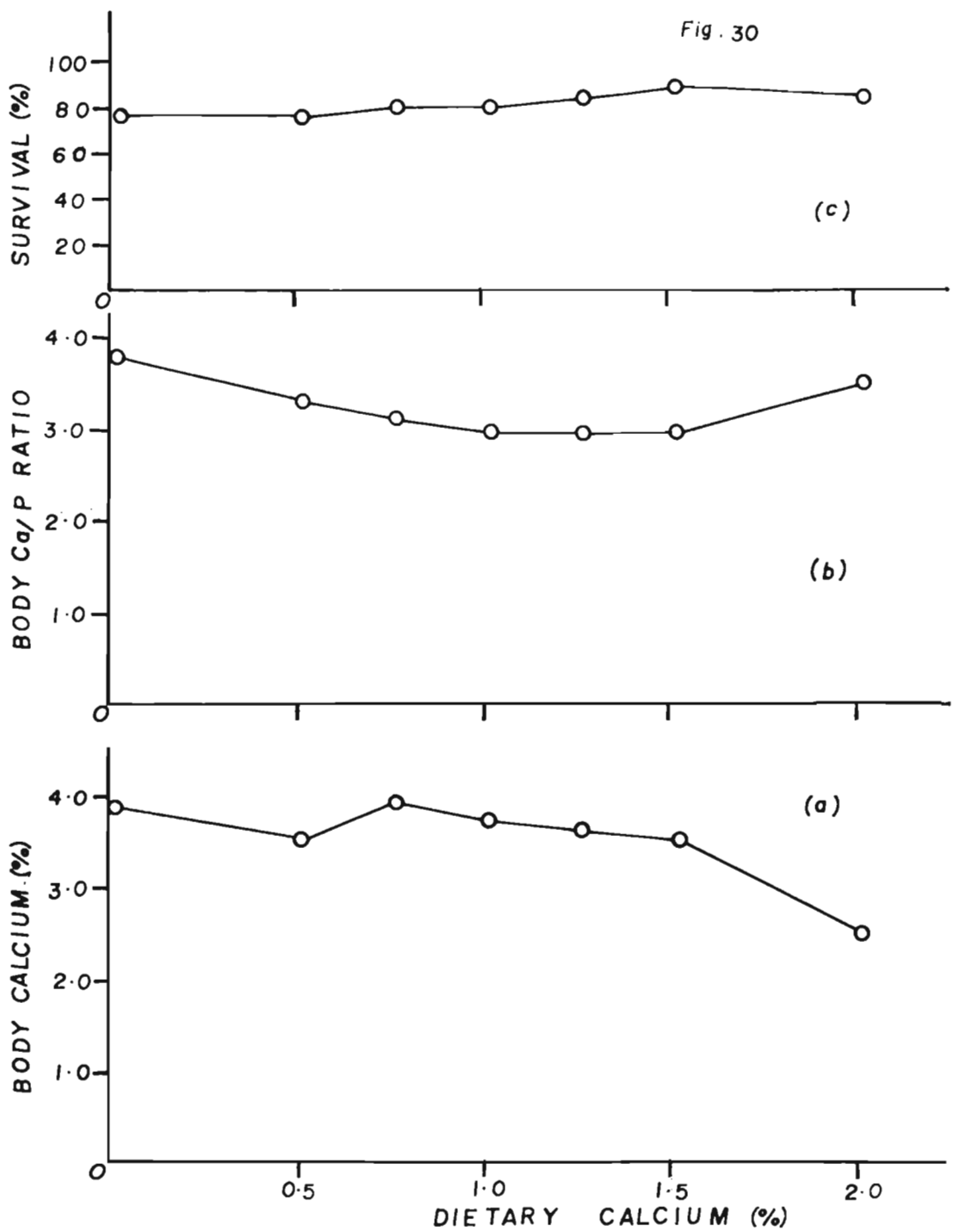

Figure 30. Effect of dietary calcium on (a) body calcium, (b) body calcium and phosphorous ratio (Ca/P) and (c) survival of P. indicus.

better growth and FCR. The body calcium of the animals fed with the diets having low and high calcium levels did not show significant variation.

Phosphorous requirement:

The results of the feeding experiment with the diets M_8 to M_{14} are given in Table 45 and the ANOVA of the data is presented in Table 47. The growth of the animals (13.1% in length and 12.2% in dry weight) fed with the diet M_8, having only 0.2155% of phosphorous, was very significantly low ($P < 0.05$). The animals became weak and sluggish. The growth was very much improved when the diets contained added amount of potassium dihydrogen orthophosphate as phosphorous source. The growth in dry weight of the animals fed the diets M_9, M_{10}, M_{11}, M_{12}, M_{13} and M_{14} was 287.6% (61.9% in length), 326.8% (63.4% in length), 365.9% (69.8% in length), 335.1% (61.0% in length), 301.0% (57.9% in length) and 326.8% (70.6% in length) respectively. The FCRs obtained by these diets were respectively 7.16, 7.08, 6.78, 7.52, 8.02 and 7.24. Due to very negligible growth, the FCR of the diet M_8 was not calculated but the consumption of diet by this group was in same order as in the case of other diets. The difference in the growth obtained by the diets M_9 to M_{14} was not found to be significant but the growth obtained by all these diets was significantly higher than the growth obtained by the diet M_8. However, the FCRs recorded by different diets were not found to differ significantly among themselves ($P < 0.05$).

The body composition of animals fed the diets M_8 to M_{14} is presented in Table 46. The crude protein content of the

Table 45. Results of feeding experiment conducted with diets M_8 to M_{14} on juvenile *P. indicus* for 45 days.

Particulars	Diet No.						
	M_8	M_9	M_{10}	M_{11}	M_{12}	M_{13}	M_{14}
Initial average length (mm)	20.6	20.5	20.5	20.5	20.6	20.7	20.4
Initial average dry weight(g)	0.0098	0.0097	0.0097	0.0097	0.0097	0.0098	0.0097
Final average length (mm)	23.3	33.2	33.5	34.8	33.17	32.7	34.8
Final average dry weight (g)	0.0110	0.0376	0.0414	0.0452	0.0422	0.0393	0.0414
Growth in length %	13.1^b	61.9^a	63.4^a	69.8^a	61.0^a	57.9^a	70.6^a
Growth in dry weight %	12.2^b	287.6^a	326.8^a	365.9^a	335.1^a	301.0^a	326.8^a
Food conversion ratio	--	7.16	7.08	6.78	7.52	8.02	7.24
Survival %	70.8	91.6	83.0	75.0	80.0	87.5	91.6

Note: Values with different superscripts differ significantly among themselves. Growth in length significant at 5% level ($P < 0.05$) and growth in dry weight significant at 1% level ($P < 0.01$). Food conversion was not significant 5% level.

Table 46. Body composition of *P. indicus* fed with the diets M_8 to M_{14}.

	% on dry basis(g/100g)						
	Crude protein	Lipid	Carbo-hydrate	Ash	Calcium	Phospho-rous	Ca/P
Before feeding	65.00	16.36	1.66	16.51	2.60	1.7600	1.47
After feeding with diets							
M_8	61.29	12.05	1.53	20.65	4.40	1.0762	4.08
M_9	66.67	15.62	1.52	19.60	4.08	1.3850	2.95
M_{10}	62.50	18.21	1.50	20.20	3.50	1.3500	2.59
M_{11}	60.40	21.81	1.52	22.20	3.84	1.4403	2.67
M_{12}	60.46	20.78	1.51	20.50	3.70	1.3700	2.70
M_{13}	62.16	21.87	1.56	19.00	3.60	1.4716	2.45
M_{14}	60.53	21.87	1.52	20.00	4.33	1.4990	2.88

Table 47. Analysis of variance of the data obtained with diets M_8 to M_{14}.

Source	Degrees of freedom	S.S.	M.S.
	ANOVA		
1. Growth in length			
Treatment	6	2230.20	371.70*
Error	14	936.02	66.85
Total	20	3166.22	438.55
2. Growth in dry weight			
Treatment	6	256086.58	42681.09**
Error	14	22685.96	1620.42
Total	20	278772.54	44301.51
3. Food conversion ratio			
Treatment	6	2.93	0.58^N
Error	14	13.12	0.93
Total	20	16.05	1.51

** Significant at 1% level ($P < 0.01$)
* Significant at 5% level ($P < 0.05$)
N Not significant ($P < 0.05$)

animals after feeding with the diets was slightly lower than the crude protein content of the animals before feeding, with the exception of the group fed with the diet M_9. The crude protein of prawns fed this diet was better (66.67%) than that of the animals before feeding (65.0%). The lipid content of the animals fed with the diet M_8 was reduced (12.05%) as compared to the lipid of the animals (16.36%) before feeding. But the lipid content gradually increased in the animals fed the diets M_9 to M_{14}. The body carbohydrate in animals fed different diets was slightly less than the value in the animals before feeding. The ash and calcium content of the prawns fed with different diets (M_8 to M_{14}) were higher than the ash and calcium content of the animals before feeding.

The influence of dietary phosphorous on growth and FCR is shown in Fig.31. The growth of the animals (Fig.31a) sharply increased as the dietary phosphorous increased from 0.2155% to 0.647%. It continued to raise with the increase in dietary phosphorous level up to 1.0495% registering a peak. The growth declined gradually with further increase in the dietary phosphorous, up to 1.3125% and levelled off with further increase in the dietary phosphorous. The FCR was gradually reduced (Fig.31b) with dietary phosphorous and reached a low value when the phosphorous in the diet was 0.8482%. It showed increasing tendency with further increase in the dietary phosphorous level.

The body phosphorous (Fig.32a) increased gradually with the dietary phosphorous between 0.2155% to 0.647% and remained almost constant with further increase in the dietary phosphorous level. The calcium phosphorous ratio in the body of the animals (Fig.32b) came down sharply with the addition of phosphorous

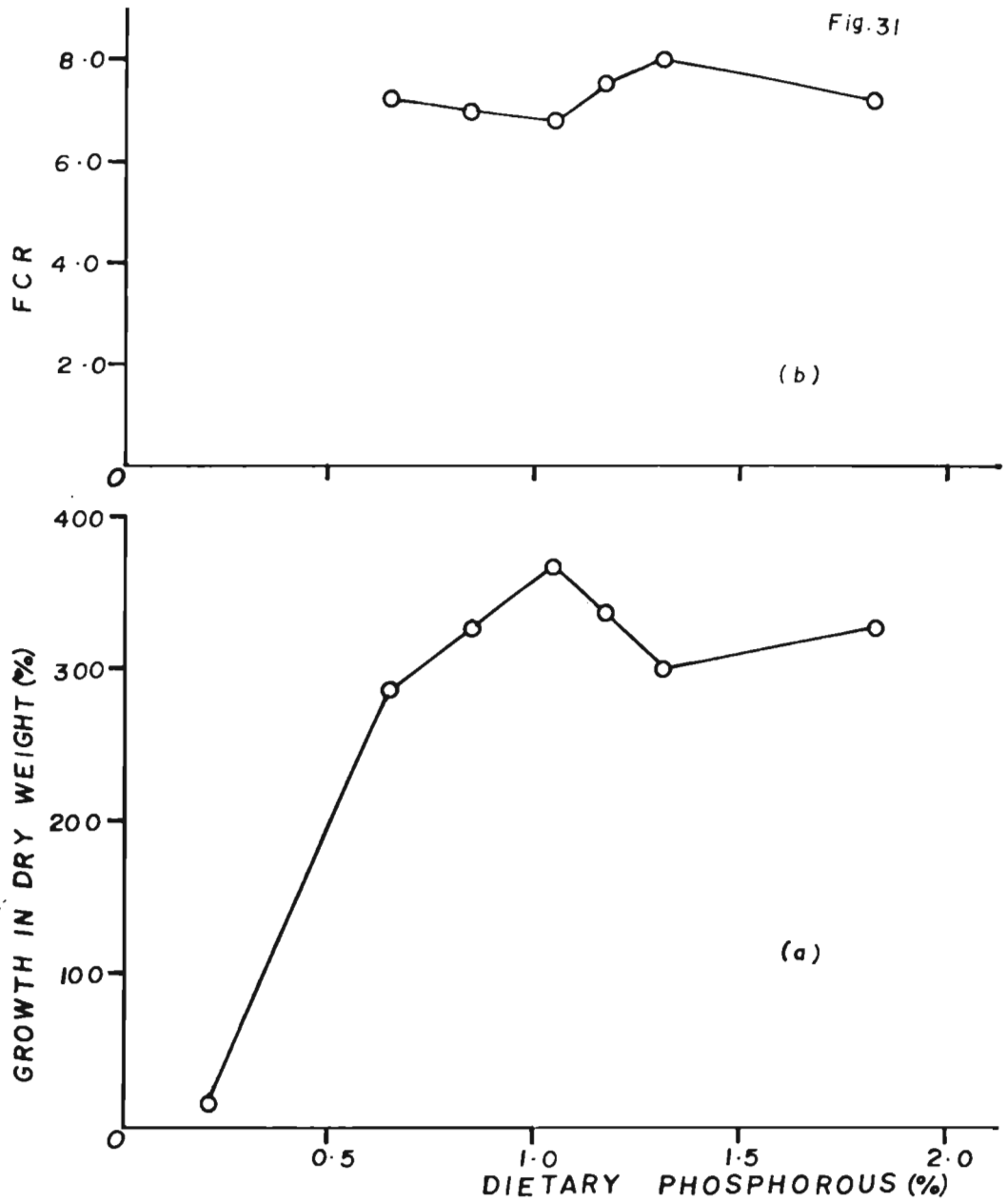

Figure 31. Growth (a) and food conversion ratio (b) of P. indicus fed with different levels of phosphorous in the diet.

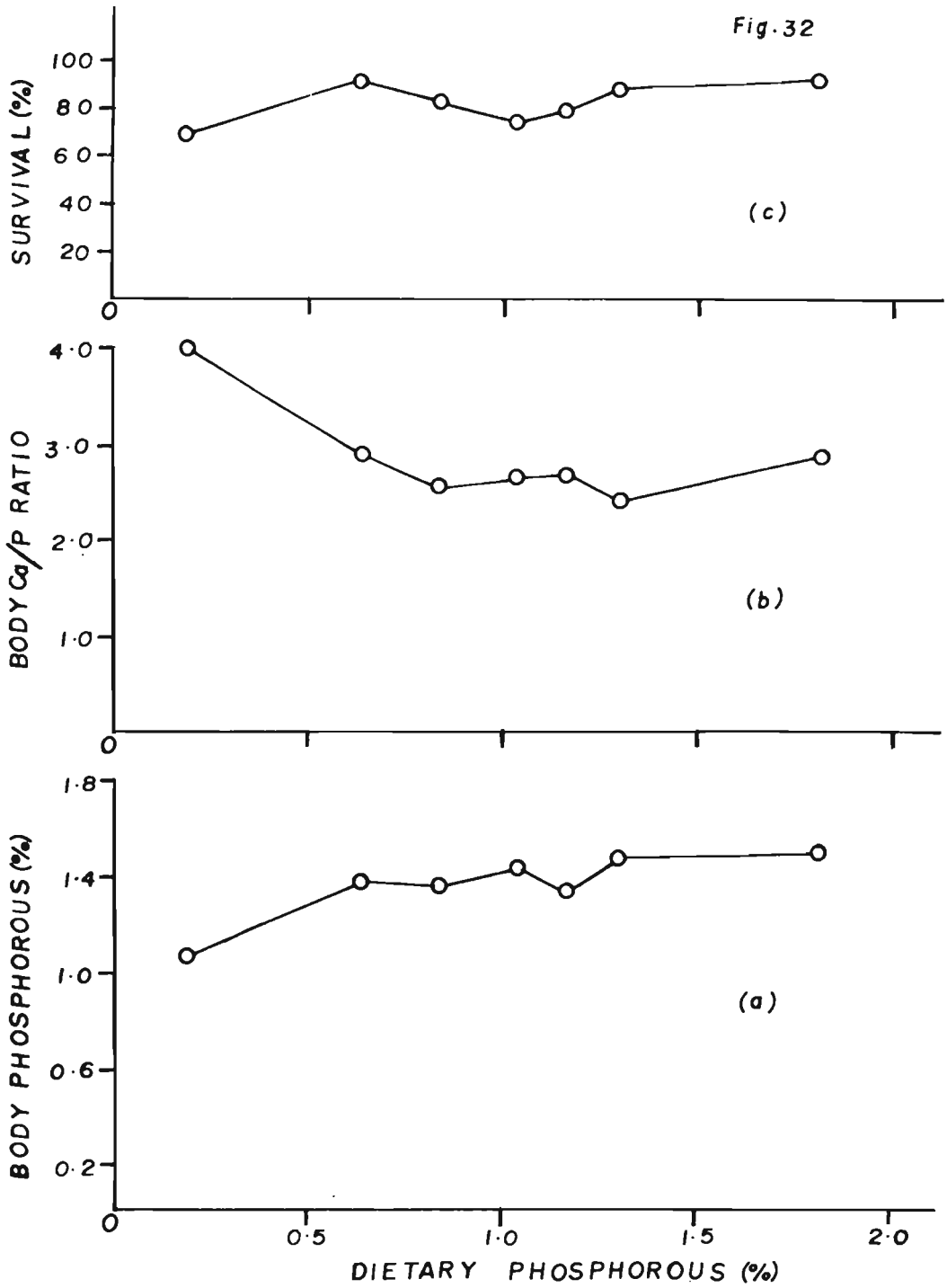

Figure 32. Effect of dietary phosphorous on (a) body phosphorous (b) ratio of body calcium and phosphorous and (c) survival in P. indicus.

in the diet (at 0.647%) and the decline continued until the phosphorous level in the diet reached 1.31%. The ratio started raising again when the diet had a phosphorous content of 1.83%. The survival of the animals (Fig.32c) marginally improved by the addition of phosphorous to the diet and largely remained same with increased levels of phosphorous in the diet. Thus the juveniles of P. indicus require about 1% phosphorous in the diet. Diets having phosphorous below 1% resulted in poor growth and FCR. Higher levels of phosphorous in the diet (above 1%) are not desirable as these diets lowered the performance.

Copper requirement:

The results of the experiment on the dietary requirement of copper (diets M_{15} to M_{21}) are presented in Table 48. The diet M_{15} in which no copper sulphate was added, had shown a copper content of 2.4 mg/100 g of diet. The diets M_{16}, M_{17}, M_{18}, M_{19}, M_{20} and M_{21} had copper contents 4.2, 5.5, 7.1, 10.2, 13.6 and 22.7 mg per 100 g diet respectively. The diet M_{15} produced the highest growth of 668.3% in dry weight and 100.9% in length. The diets M_{16} to M_{21}, on the other hand, showed a growth of 540.3% (85.3% in length), 600.8% (83.2% in length), 448.8% (80.8% in length), 460.2% (83.2% in length), 472.1% (86.2% in length) and 528.7% (85.4% in length) in dry weight respectively which were lower than the growth obtained by the diet M_{15}. Analysis of variance of the data (Table 50) had shown that the difference in growth in length and dry weight was however not significant ($P < 0.05$) among the treatments. The FCR of the diet M_{15} was 2.42 and that of the diets M_{16} to M_{21}

Table 48. Results of the feeding experiment conducted with diets M_{15} to M_{21} on juvenile *P. indicus* for 45 days.

Particulars	Diet No.						
	M_{15}	M_{16}	M_{17}	M_{18}	M_{19}	M_{20}	M_{21}
Initial average length (mm)	22.1	22.5	22.0	21.9	22.0	22.5	22.6
Initial average dry weight(g)	0.0126	0.0129	0.0128	0.0127	0.0128	0.0129	0.0129
Final average length (mm)	44.4	41.7	40.3	39.6	40.3	41.9	41.9
Final average dry weight(g)	0.0968	0.0826	0.0897	0.0697	0.0717	0.0738	0.0811
Growth in length %	100.9	85.3	83.2	80.8	83.2	86.2	85.4
Growth in dry weight %	668.3	540.3	600.8	448.8	460.2	472.1	528.7
Food conversion ratio	2.42	2.48	2.83	2.53	2.82	2.27	2.14
Survival %	68.8	75.0	75.0	75.0	68.0	66.7	81.3

Note: Growth in length, in dry weight and food conversion ratio were not found to be significantly different among the treatments at 5% level ($P < 0.05$)

Table 49. Body composition of P. indicus fed with the diets M_{15} to M_{21}.

	% on dry basis(g/100g)				
	Crude protein	Lipid	Carbo-hydrate	Ash	Copper mg/100g
Before feeding	61.1%	11.76	1.64	13.40	33.0
After feeding with diets					
M_{15}	65.66	19.00	1.45	16.66	36.0
M_{16}	67.41	20.0	1.44	15.46	36.1
M_{17}	67.14	18.16	1.46	14.91	37.2
M_{18}	67.41	20.00	1.50	14.00	39.6
M_{19}	65.93	19.62	1.51	16.76	38.9
M_{20}	66.53	21.00	1.58	17.66	38.8
M_{21}	65.66	20.20	1.43	17.00	43.0

Table 50. Analysis of variance of the data obtained with the diets M_{15} to M_{21}.

ANOVA			
Source	Degrees of freedom	S.S.	M.S.
1. Growth in length			
Treatment	6	799.90	133.31[N]
Error	14	940.97	67.21
Total	20	1740.87	200.52
2. Growth in dry weight			
Treatment	6	102256.76	17042.79[N]
Error	14	163779.58	11698.54
Total	20	206036.34	28741.33
3. Food conversion ratio			
Treatment	6	1.19	0.199[N]
Error	14	1.16	0.083
Total	20	2.35	0.232

N Not significant at 5% level ($P < 0.05$)

were 2.48, 2.83, 2.53, 2.82, 2.27 and 2.14 respectively. As in the case of growth, the FCRs obtained by these diets were not significantly different ($P < 0.05$) among themselves.

The body composition of P. indicus before and after feeding with the diets M_{15} to M_{21} is presented in Table 49. The crude protein content and lipid in the prawns after feeding with the diets were higher than in those before feeding. But the values remained largely the same in the groups of animals fed with different diets. The body carbohydrate showed reduction in all the groups of prawns after feeding the diets as compared to the value in the animals before feeding.

The ash content in the body of P. indicus after feeding with the diets was higher than in the animals before feeding. But the values varied among the groups fed different diets.

The influence of copper in the diet on growth and FCR is shown in Fig.33. The growth decreased (Fig.33a) as the copper content in the diet increased from 2.4 to 7.1 mg/100 g diet and thereafter the growth remained parallel to X-axis with further increase of copper in the diet. The FCR fluctuated (Fig.33b) as the dietary copper raised from 2.4 mg to 10.2 mg/100 g diet. At 13.6 mg/100 g diet, the FCR came down and the decline continued up to 22.7 mg copper/100 g diet.

The copper content in the body of animals gradually increased (Fig.34a) upto a dietary copper level of 7.1 mg/100 g, it remained more or less at the same level between 7.1 mg and 13.6 mg copper/100 g diet, but showed increase as the copper level in the diet went up to 22.7 mg/100 g. The survival of the animals showed gradual improvement (Fig.34b) with the progressive increase in the dietary copper level.

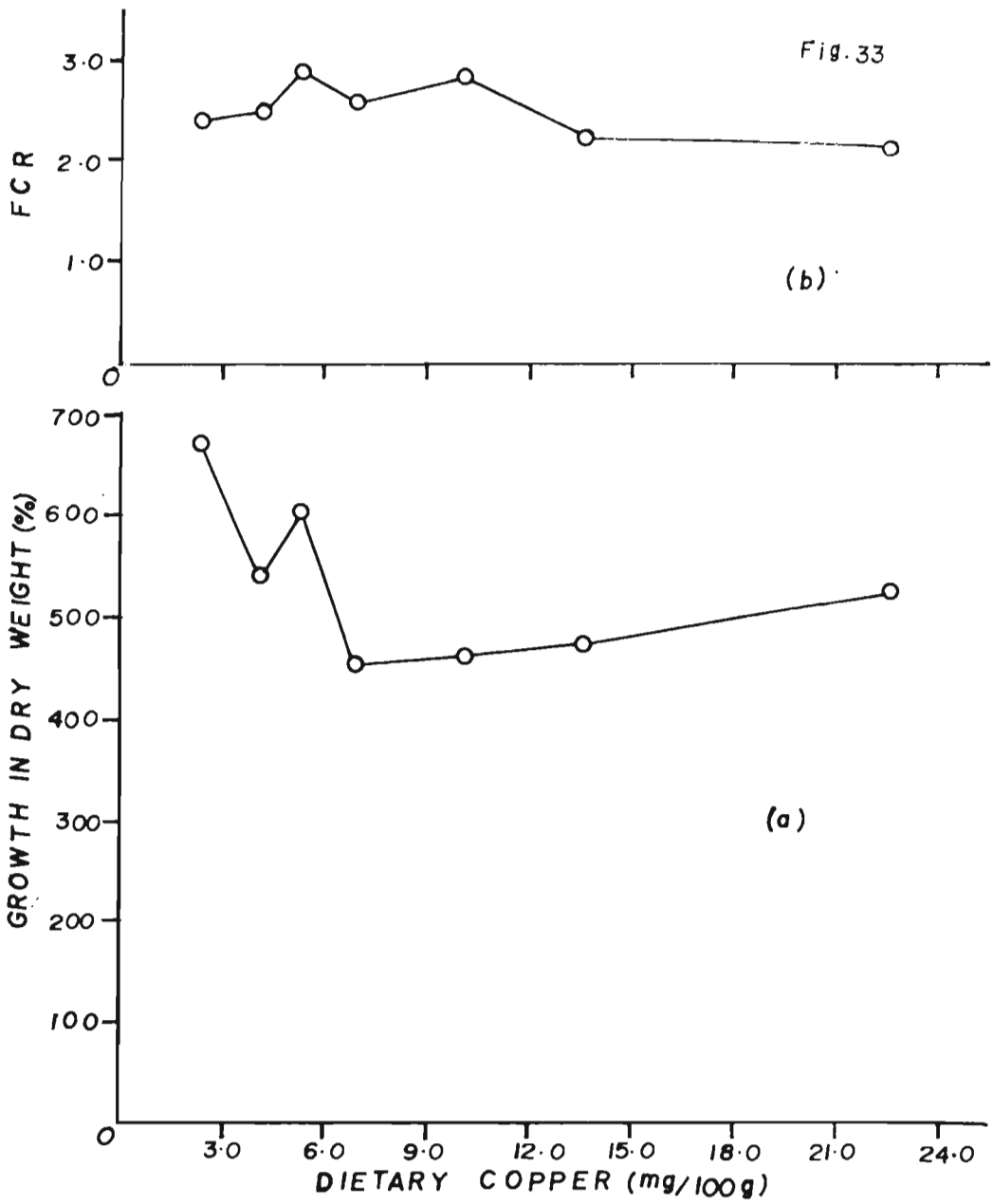

Figure 33. Growth (a) and food conversion ratio (b) of P. indicus fed with diets having different levels of copper.

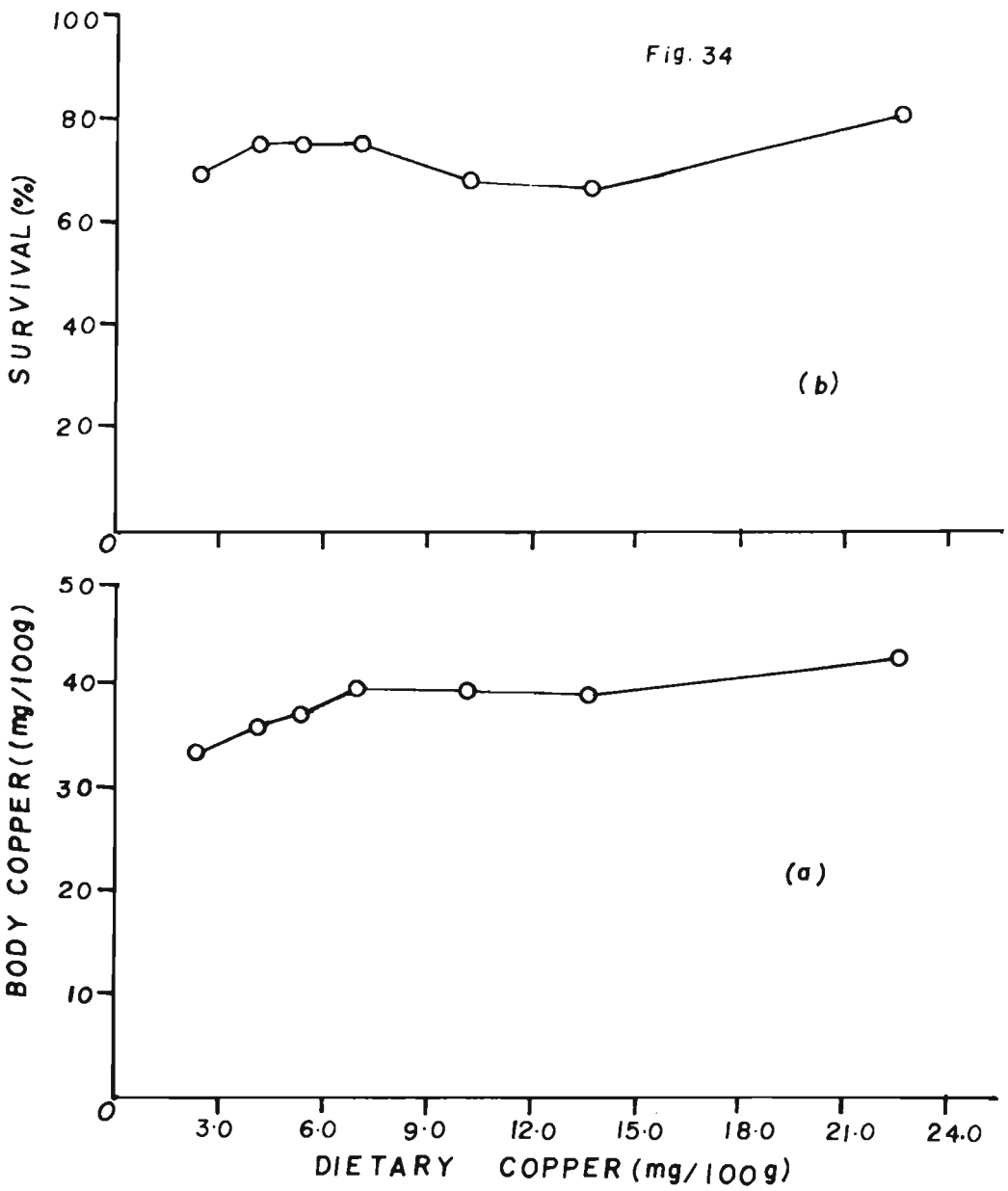

Figure 34. Effect of dietary copper on (a) body copper and (b) survival in P. indicus

The results showed that addition of copper, through copper sulphate, in the diet had no beneficial effect on the growth of the animals. But the FCR improved when the diet contained 13.6 mg of copper per 100 g. The animals fed this diet had higher lipid and ash in their body.

Zinc requirement:

From the results presented in the Table 51, obtained by the diets M_{22} to M_{28}, it could be seen that the diet M_{25} recorded the highest growth of 83.4% in length and 482.8% in dry weight. The FCR obtained by this diet was 2.81. The growth of the prawns fed with the diets M_{22}, M_{23} and M_{24} was 366.2% (64.8% in length), 445.0% (76.3% in length) and 465.9% (78.2% in length) respectively. On the other hand the growth obtained by feeding with the diets M_{26}, M_{27} and M_{28} respectively was 476.4% (74.1% in length), 448.1%(69.9% in length) and 310.9% (65.5% in length). The ANOVA of the data presented in Table 53, showed that the growth obtained by the different diets was not significantly different ($P < 0.05$). Eventhough the FCRs recorded by the diets M_{22} to M_{28} were 2.70, 2.66, 2.76, 2.81, 2.94, 2.30 and 3.03 respectively, the difference among themselves was not found to be significant ($P < 0.05$).

The body protein of the animals (Table 52) fed with the diet M_{22} was slightly higher (68.29%) than the body protein of the prawns fed all the other diets. But lipid was significantly lower in the group fed by the diet M_{22} and higher in the animals fed with the diet M_{25}. Both crude protein and lipid were higher in the animals after feeding with the diets than in the animals before feeding. Only the animals fed with the diet M_{27} had

Table 51. Results of feeding experiment conducted with diets M_{22} to M_{28} on juvenile *P. indicus* for 45 days.

Particulars	Diet No.						
	M_{22}	M_{23}	M_{24}	M_{25}	M_{26}	M_{27}	M_{28}
Initial average length (mm)	22.7	22.8	22.5	22.3	22.4	22.9	22.3
Initial average dry weight(g)	0.0130	0.0131	0.0129	0.0128	0.0127	0.0131	0.0128
Final average length (mm)	37.4	40.2	40.1	40.9	39.0	38.9	36.9
Final average dry weight(g)	0.0606	0.0714	0.0730	0.0746	0.0732	0.0718	0.0526
Growth in length %	64.8	76.3	78.2	83.4	74.1	69.9	65.5
Growth in dry weight %	366.2	445.0	465.9	482.8	476.4	448.1	310.9
Food conversion ratio	2.70	2.66	2.76	2.81	2.94	2.30	3.03
Survival %	79.2	79.2	70.8	70.8	66.3	56.3	81.3

Note: The growth of animals in length and dry weight and the food conversion ratio were not found to be significantly different among themselves at 5% ($P < 0.05$)

Table 52. Body composition of P. indicus fed with the diets M_{22} to M_{28}.

	% on dry basis(g/100g)				Zinc mg/100g.
	Crude Protein	Lipid	Carbo-hydrate	Ash	
Before feeding	61.11	11.76	1.64	13.40	38.50
After feeding with diets					
M_{22}	68.29	12.60	1.55	15.00	23.25
M_{23}	66.53	17.00	1.38	16.80	26.25
M_{24}	66.53	18.62	1.58	14.90	28.52
M_{25}	66.53	20.00	1.52	12.50	32.00
M_{26}	66.53	19.14	1.57	14.50	33.52
M_{27}	66.53	18.65	1.72	13.00	33.25
M_{28}	64.79	19.00	1.52	14.30	37.75

Table 53. Analysis of variance of the data obtained with the diets M_{22} to M_{28}.

Source	Degrees of freedom	S.S.	M.S.
	ANOVA		
1. Growth in length			
Treatment	6	948.80	158.13^{N}
Error	14	1453.48	103.80
Total	20	2402.28	261.21
2. Growth in dry weight			
Treatment	6	61556.56	10259.42^{N}
Error	14	235985.90	16856.13
Total	20	297542.46	27115.55
3. Food conversion ratio			
Treatment	6	0.99	0.16^{N}
Error	14	2.96	0.21
Total	20	3.95	0.37

N Not significant at 5% level ($P < 0.05$)

higher carbohydrate than the initial value. All the other groups showed lower body carbohydrate after feeding as compared to the animals before feeding.

The ash content of *P. indicus* fed with the diet M_{25} was lower than the ash content of the animals before feeding (13.4%). The highest ash content (16.80%) was seen in the prawns fed the diet M_{23} among the different groups.

The relationship between the dietary zinc and growth and FCR is depicted in Fig.35. The growth curve was a smooth (Fig.35a) parabolic shaped curve with a transitory apex. The growth increased with dietary zinc up to 23.6 mg/100g diet. It remained constant between 23.6 and 30.4 mg/100 g level and again declined thereafter with further increase in the zinc level in the diet. The FCR (Fig.35b) showed a slight decline at 14.10 mg/100 g level and gradually increased as the zinc level in diet raised up to 30.4 mg/100 g. The FCR showed the lowest value at 38.6 mg/100 g diet and again raised sharply as the zinc in diet increased to 50.2 mg/100 g. The zinc in the body of the animals showed gradual increase (Fig.36a) with progressive increase in the dietary zinc. The survival of animals (Fig.36b) decreased with the increase in the dietary zinc, but at 50.2 mg zinc/100 g diet the survival was significantly higher than that fed with the diet having only 6 mg zinc/100 g.

Thus the diet having 23.6 mg zinc/100 g produced better growth than the diets having lesser amounts of zinc. Higher amounts of zinc in the diet above 23.6 mg/100 g again depressed the growth. However, the FCR of the diet having 23.6 mg zinc/100 g was not significantly different from the FCR of the diets having less or more amounts of zinc.

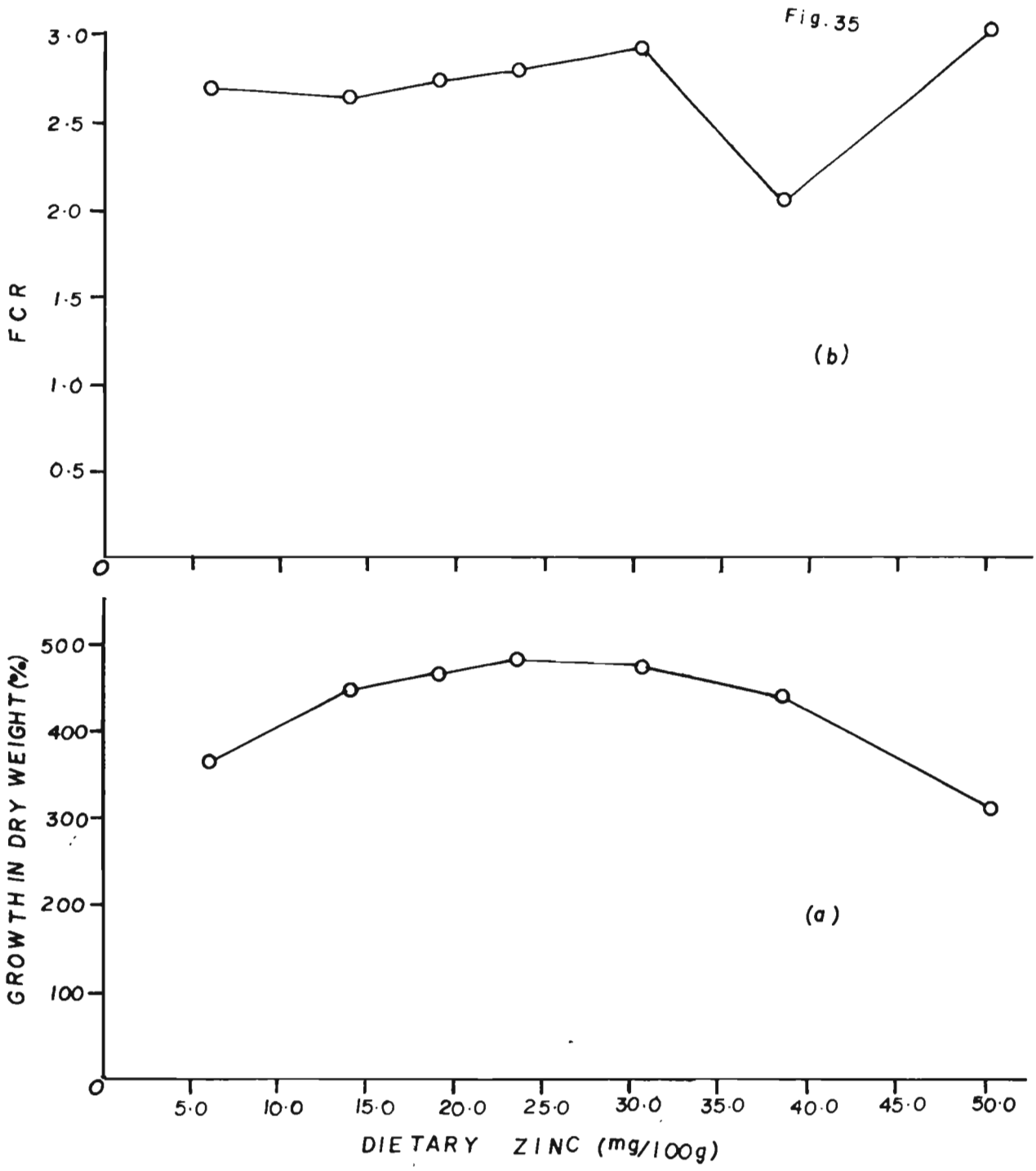

Figure 35. Growth (a) and food conversion ratio (b) of P. indicus fed with different levels of zinc in the diet.

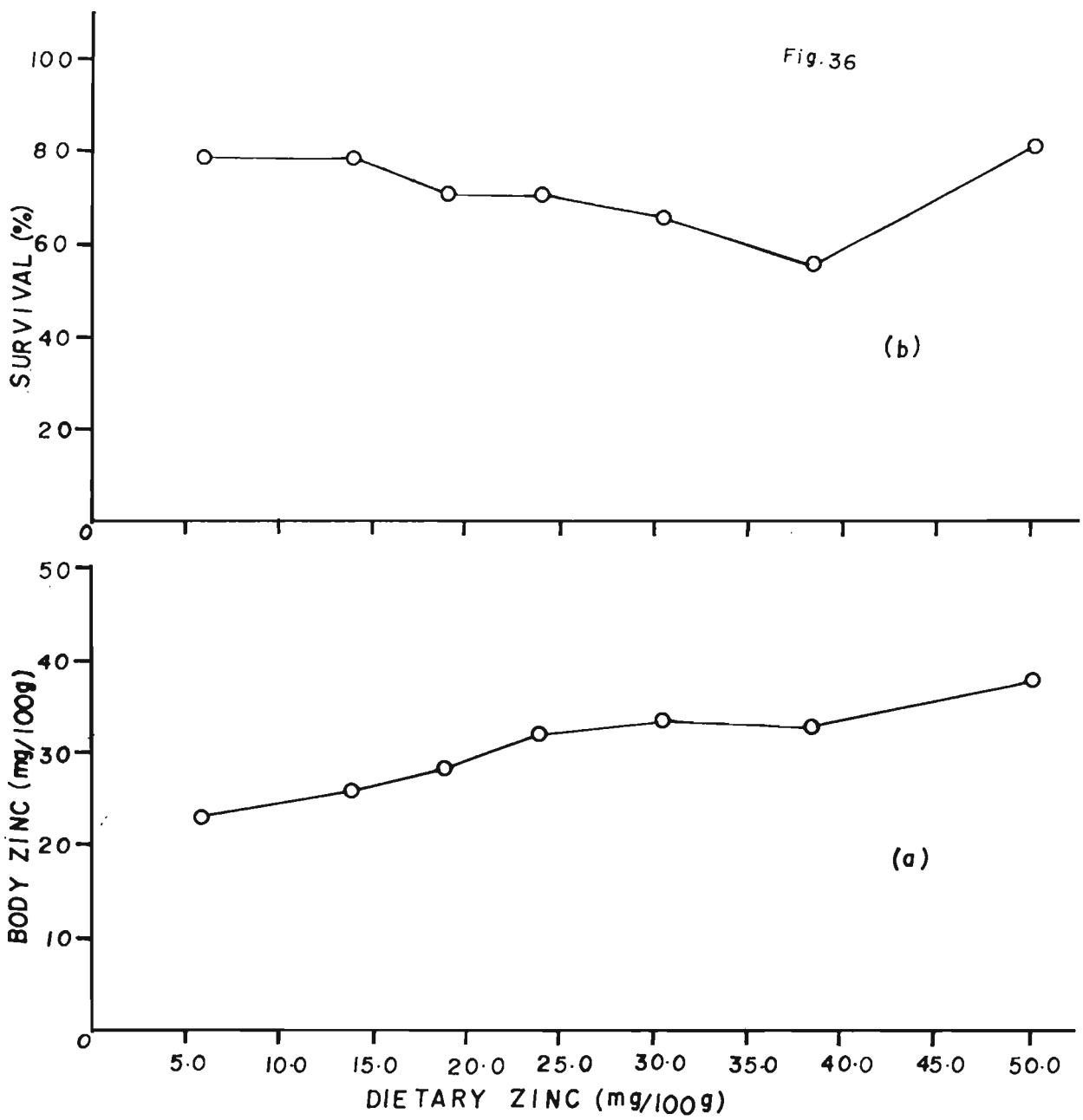

Figure 36. Effect of dietary zinc on (a) body zinc and (b) survival in P. indicus.

Magnesium requirement.

The results of the experiment with diet M_{29} to M_{35}, in which the magnesium was varied from trace to 0.6958%, are shown in Table 54. It could be seen that the growth of the animals was depressed by addition of magnesium to the diet. The diet M_{29} in which no magnesium was added, produced a growth of 44.5% in length and 155.2% in dry weight. The FCR of this diet was 5.29. Whereas the diets M_{30}, M_{31}, M_{32}, M_{33}, M_{34} and M_{35} in which graded amounts of magnesium were added, could produce a growth of only 83.4% (32.9% in length), 85.5% (32.2% in length), 87.4% (24.6% in length), 98.1% (30.7% in length), 102.5% (32.8% in length) and 117.1% (34.4% in length) respectively. The corresponding FCRs were respectively 9.56, 10.5, 12.5, 10.88, 10.33, and 7.82. ANOVA of the data (given in Table 56) indicated that the growth (in length and dry weight) and FCR obtained by the different diets were not significantly different ($P < 0.05$) among the treatments. The prawns fed with the diet M_{29} without any magnesium only had high values of crude protein, lipid, and ash (Table 55). Whereas the values of protein, lipid, carbohydrate and ash in the body of the animals fed all other diets were lower. However, the carbohydrate was higher in the animals after the feeding experiment than the initial value.

The effect of dietary magnesium on growth and FCR is depicted in Fig.37. The growth sharply declined when the diet contained 0.1% of magnesium (Fig.37a). This depression in the growth curve remained practically constant with the addition of higher amounts of magnesium to the diet. The FCR

Table 54. Results of feeding experiment conducted with diets M_{29} to M_{35} on juvenile *P. indicus* for 45 days.

Particulars	Diet No.						
	M_{29}	M_{30}	M_{31}	M_{32}	M_{33}	M_{34}	M_{35}
Initial average length (mm)	24.5	24.3	24.5	25.2	24.1	24.1	24.4
Initial average dry weight(g)	0.0163	0.0163	0.0165	0.0167	0.0161	0.0161	0.0164
Final average length (mm)	35.4	32.3	32.4	31.4	31.5	32.0	32.8
Final average dry weight(g)	0.0416	0.0299	0.0306	0.0313	0.0319	0.0326	0.0356
Growth in length %	44.5	32.9	32.2	24.6	30.7	32.8	34.4
Growth in dry weight %	155.2	83.4	85.5	87.4	98.1	102.5	117.1
Food conversion ratio	5.29	9.56	10.50	12.50	10.88	10.33	7.82
Survival %	62.5	56.3	58.7	56.3	60.1	56.3	68.8

Note: The growth in length and dry weight and food conversion ratio obtained by different diets were not found to be significantly different among themselves at 5% level ($P < 0.05$)

Table 55. Body composition of P. indicus fed with the diets M_{29} to M_{35}.

	% dry basis (g/100g)				Magnesium mg/100g
	Crude Protein	Lipid	Carbo hydrate	Ash	
Before feeding	70.04	18.50	1.44	16.67	206.12
After feeding with diets					
M_{29}	68.65	22.00	1.63	19.50	382.17
M_{30}	60.03	19.62	1.61	19.00	386.25
M_{31}	63.76	17.21	1.65	17.40	393.52
M_{32}	65.67	18.34	1.68	15.94	400.90
M_{33}	62.27	17.68	1.70	15.00	385.90
M_{34}	63.12	18.28	1.69	15.00	379.17
M_{35}	66.15	19.52	1.71	19.00	402.90

Table 56. Analysis of variance of the data obtained by the diets M_{29} to M_{35} in P. indicus.

Source	Degrees of freedom	S.S.	M.S.
	ANOVA		
1. Growth in length			
Treatment	6	740.21	123.36[N]
Error	14	1310.41	93.60
Total	20	2050.62	216.96
2. Growth in dry weight			
Treatment	6	7975.05	1329.17[N]
Error	14	27750.85	1982.20
Total	20	35725.90	3311.37
3. Food conversion ratio			
Treatment	6	99.38	16.56[N]
Error	14	63.62	4.54
Total	20	163.00	21.00

N Not significant at 5% level ($P < 0.05$)

reached a highest value at 0.3% magnesium (Fig.37b) in the diet and further increase in the dietary magnesium brought down the FCR, but the decline did not reach the value of the diet having no magnesium.

Body magnesium of animals remained same (Fig.38a) after feeding with the diets having 0 to 0.7% of magnesium. Similarly, the survival of the animals was not affected significantly (Fig.38b) by the dietary magnesium.

The results of the experiment showed that addition of magnesium to the diet, depressed the growth of the animals and adversely affected the food conversion ratio.

Manganese requirement.

The manganese requirement for P. indicus was studied by using the diets M_{36} to M_{42} and the results obtained are given in Table 57. The growth (in length and dry weight) and FCR were subjected to ANOVA (Table 59). The results showed that the treatments were not significant ($P < 0.05$) among themselves in respect of growth and FCR. However, the diet M_{36}, to which manganese was not added, produced 159.8% growth in dry weight (35.9% in length) and its FCR was 7.40. In contrast, the growth obtained by the diets M_{37}, M_{38}, M_{39}, M_{40}, M_{41} and M_{42} was 143.6% (34.4% in length), 54.6% (31.3% in length), 96.9% (27.2% in length), 121.5% (27.5% in length), 146.6% (34.8% in length) and 126.9% (38.7% in length) respectively, which was less than the growth obtained by the diet M_{36}. The FCRs obtained by the diets M_{37} to M_{42} were respectively 8.02, 9.00, 9.79, 8.51, 7.08 and 8.75. These values were higher than the value obtained by the diet M_{36}, except that of the diet M_{41}.

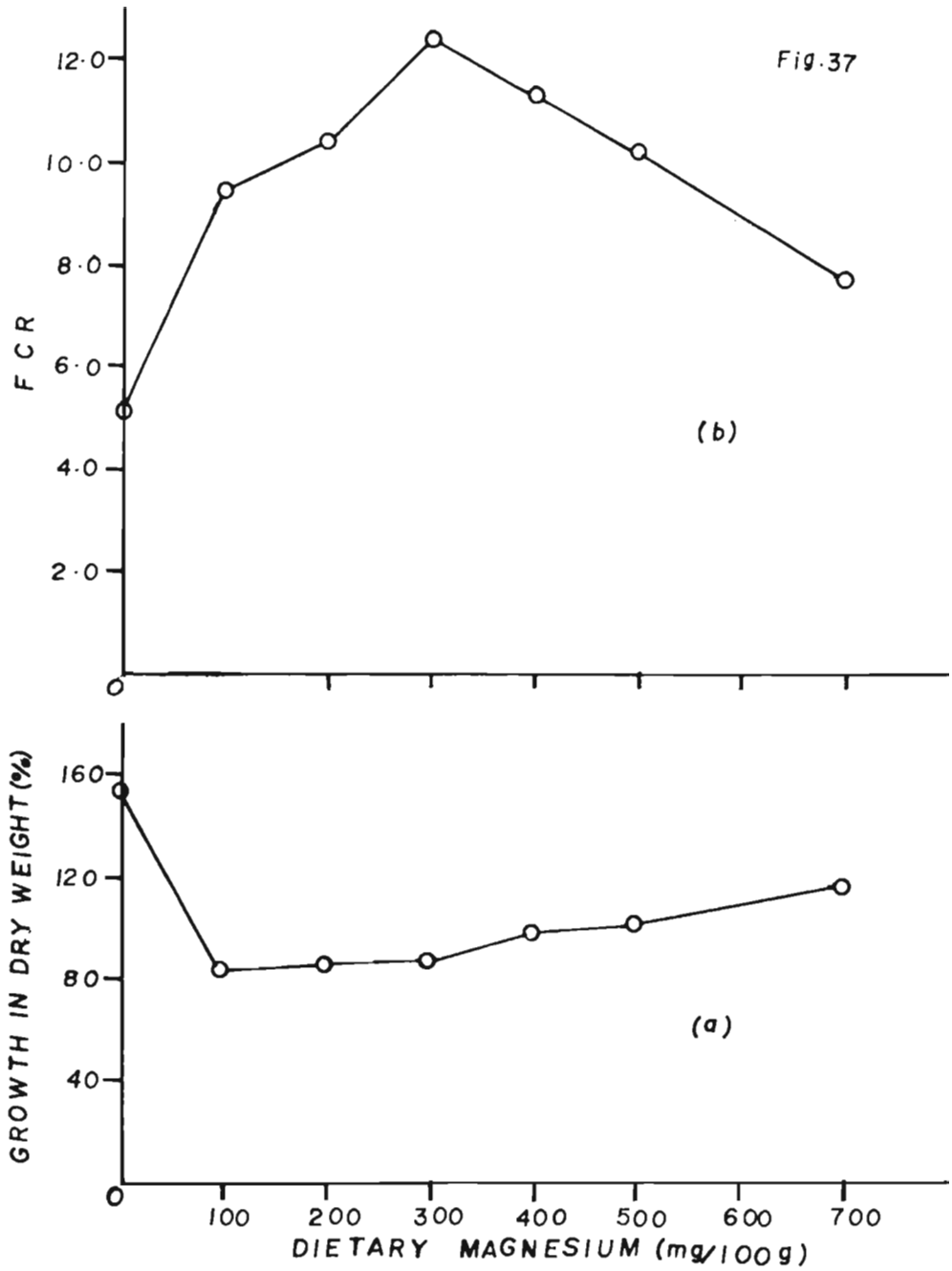

Figure 37. Growth (a) and food conversion ratio (b) of P. indicus fed with diets having different levels of magnesium.

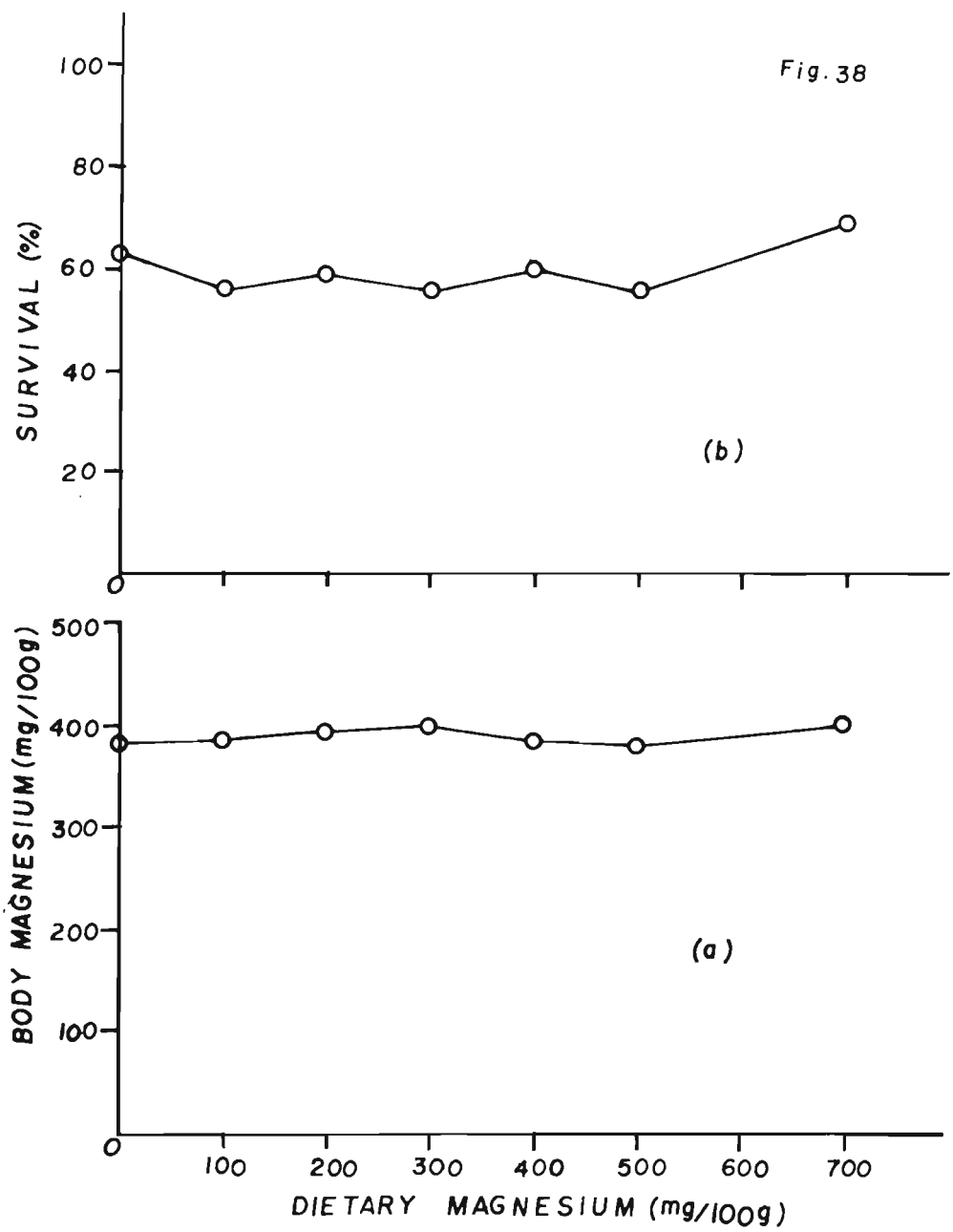

Figure 38. Effect of dietary magnesium on (a) body magnesium and (b) survival in P. indicus.

Table 57. Results of feeding experiment conducted with diets M_{36} to M_{42} on juvenile *P. indicus* for 45 days.

Particulars	Diet No.						
	M_{36}	M_{37}	M_{38}	M_{39}	M_{40}	M_{41}	M_{42}
Initial average length (mm)	24.5	24.4	24.3	24.3	24.4	24.4	24.3
Initial average dry weight(g)	0.0164	0.0163	0.0163	0.0163	0.0163	0.0163	0.0163
Final average length (mm)	33.3	32.8	31.9	30.9	31.1	32.9	33.7
Final average dry weight (g)	0.0426	0.0397	0.0359	0.0321	0.0361	0.0402	0.0370
Growth in length %	35.9	34.4	31.3	27.2	27.5	34.8	38.7
Growth in dry weight %	159.8	143.6	54.6	96.9	121.5	146.6	126.9
Food conversion ratio	7.40	8.02	9.00	9.79	8.51	7.08	8.75
Survival %	50.0	43.8	48.5	54.2	58.3	62.5	79.2

Note: The growth in length and dry weight and also the food conversion ratio obtained by different treatments were not found to be significantly different at 5% level ($P < 0.05$).

Table 58. Body composition of P. indicus fed with the diets M_{36} to M_{42}.

	% on dry basis (g/100g)				
	Crude Protein	Lipid	Carbo-hydrate	Ash	Manganese mg/100 g
Before feeding	70.04	18.50	1.44	16.67	0.31
After feeding with diets					
M_{36}	60.40	12.12	1.99	17.50	0.55
M_{37}	67.37	18.57	1.80	17.20	0.90
M_{38}	64.23	14.37	1.78	17.25	1.11
M_{39}	61.28	11.53	1.74	18.00	1.33
M_{40}	60.88	13.35	1.73	19.80	1.35
M_{41}	61.28	15.00	1.83	21.00	1.40
M_{42}	62.16	14.70	1.61	19.40	1.75

Table 59. Analysis of variance of the data obtained by the diets M_{36} to M_{42}.

Source	Degrees of freedom	S.S.	M.S.
	ANOVA		
1. Growth in length			
Treatment	6	7201.93	1206.32[N]
Error	14	9806.99	700.49
Total	20	17008.92	1906.81
2. Growth in dry weight			
Treatment	6	21813.60	3635.60[N]
Error	14	58921.01	4208.64
Total	20	80734.61	7844.24
3. Food conversion ratio			
Treatment	6	17.14	2.85[N]
Error	14	13.43	0.95
Total	20	30.57	3.80

N. Not significant at 5% level ($P < 0.05$)

The body composition of the animals before and after feeding with the diets M_{36} to M_{42} is given in Table 58. It could be seen that the crude protein and lipid decreased after feeding with different diets from the initial value. However, the body carbohydrate and ash were higher in the prawns after feeding with different diets than the initial values.

The effect of manganese in the diet on the growth and FCR is show in Fig. 39. The growth curve declined with in increase in dietary manganese (Fig.39a) and reached the lowest point at dietary manganese level of 100µg/100 g diet. It started raising again and reached a peak at the dietary manganese level of 196 µg/100 g diet. The growth again declined between dietary manganese levels of 196 and 260µg/100 g diet. The FCR increased (Fig.39b) upto a dietary manganese level of 130µg/100 g, then declined and reached lowest point at 196µg of manganese/100 g diet. It again started raising as the dietary manganese was increased further.

The manganese content in the body of prawns gradually increased (Fig.40a) with the increase in the dietary manganese level. The survival of the animals first slightly declined (Fig.40b) at about 70µg of manganese/100 g diet, after that it showed continuous improvement as the manganese level in the diet increased.

From these results it could be concluded that addition of manganese to the diet did not have any appreciable effect either on accelerating the growth or on improving the FCR. Higher levels of manganese in the diet resulted in increased accumulation of the metal ion in the body of prawns, but improved the survival of the animals.

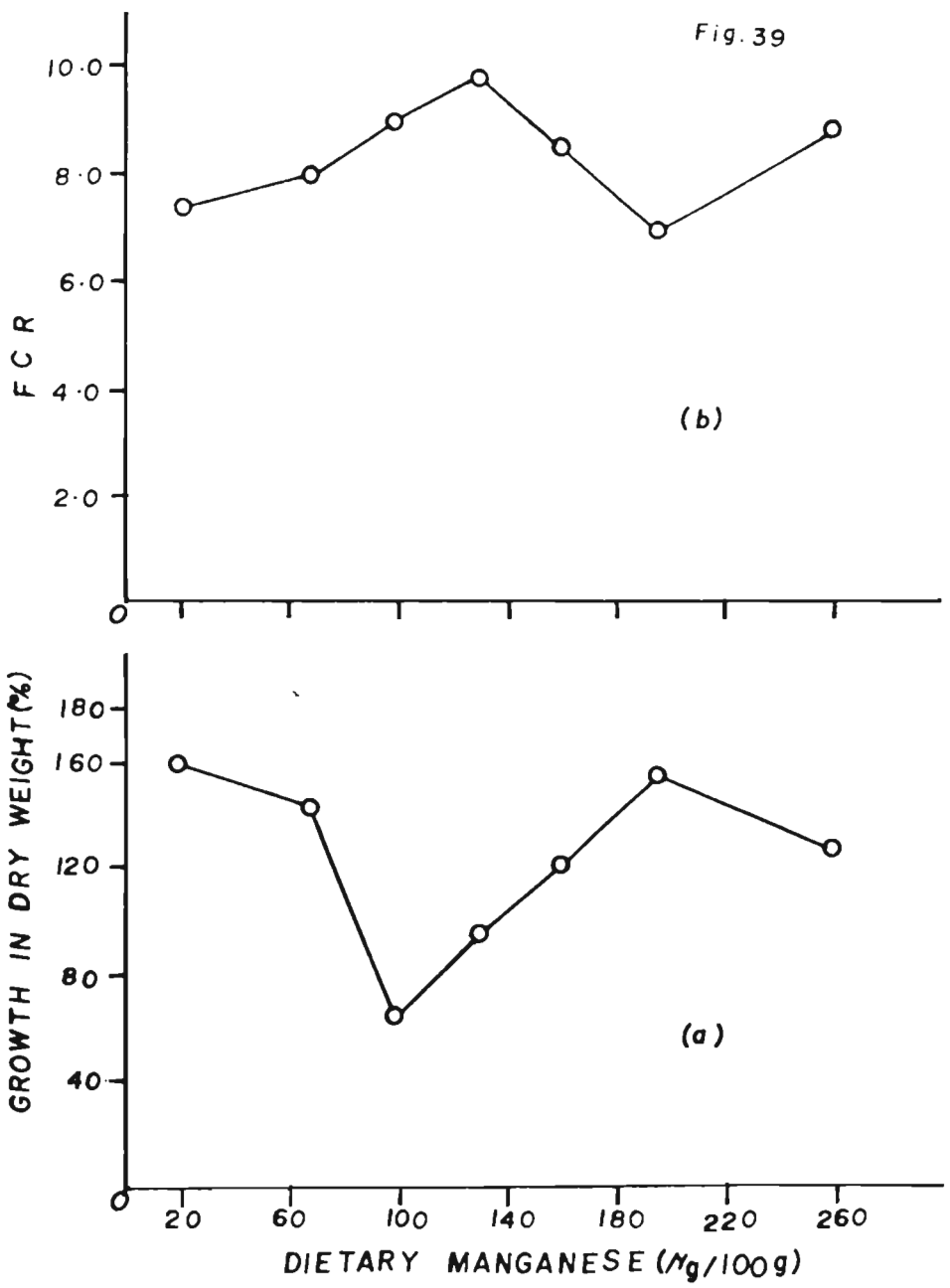

Figure 39. Growth (a) and food conversion ratio (b) of P. indicus fed with different levels of manganese in the diet.

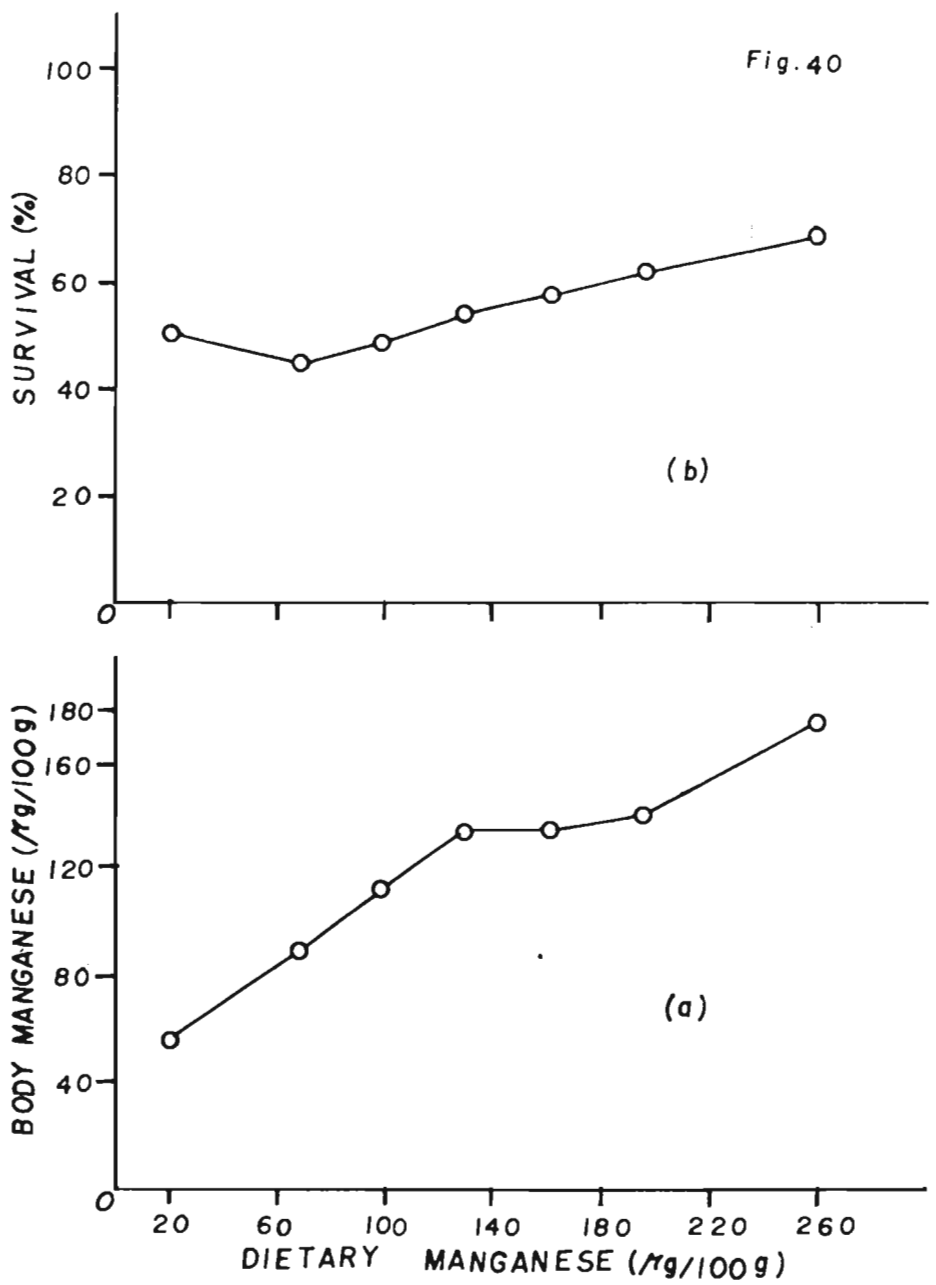

Figure 40. Effect of dietary manganese on (a) body manganese and (b) survival in P. indicus.

DISCUSSION

Due to lesser quantitative disposition in the diet as well as in the body, minerals are considered as micro nutrients. Nevertheless, they are involved in body structure and in many vital life processes and should be considered more as essential nutrients rather than as just required nutrients. Further, their deficiency causes abnormalities/disease and at the same time excess causes toxicity. The mineral nutrition of finfishes was studied to some extent, but very little information is available on the dietary mineral nutrition of prawns. In the present study, therefore, the important minerals such as calcium, phosphorous, copper, zinc, magnesium and manganese are studied with a view to formulate a suitable nutritionally balanced diet including the minerals.

The growth of prawn P. indicus was higher and food conversion ratio was low when the diet contained 0.525% of calcium. If the calcium content in the diet was less or more than 0.5%, the growth was reduced and the FCR increased. Thus the prawns showed a requirement of 0.5% calcium in the diet. Regarding the calcium requirement in the diet of penaeid prawns, varying results were obtained in different species and also within the same species. Kitabhayashi et al. (1971a) obtained faster growth in P. japonicus with diets having 1.24% calcium, in combination with 1.04% phosphorous. When the calcium-phosphorous ratio was increased to 2:1 by these authors, the growth of prawns was inhibited and pigmentation decreased. The authors also observed that increasing calcium and phosphorous in the

diet (keeping the ratio of Ca/P 1.2:1), inhibited the growth. Kanazawa et al. (1984) reported a requirement of 1.0% calcium in the diet of P. japonicus. However, Deshimaru and Yone (1978a) found that supplementing the diet with 2% calcium did not have any beneficial effect on the growth of P. japonicus. The results found in P. indicus in the present study are in agreement with the findings of Kitabhayashi et al. (1971a) and Kanazawa et al.(1984). But the calcium requirement shown in the diet of P. japonicus by these authors was higher than that was found for P. indicus. The observations made by Deshimaru and Yone (1978a) were also true, because the authors used 2% calcium in the diet which was higher than the required level.

The body calcium of P. indicus fed with the diet having lowest calcium was not less than the body calcium of the animals fed the diet having higher levels of calcium; on the contrary, there was decreasing tendency (Fig. 30a) in the body calcium at higher levels of dietary calcium. Similarly, the Ca/P in the body of the animals also decreased (Fig.30b) as the calcium content in the diet increased. Thus higher levels of calcium in the diet are not beneficial and produce negative effect on growth.

While investigating the mineral requirements of P. aztecus, Shewbart et al. (1973) postulated that along with other mineral elements, calcium requirement of prawns might be met through osmotic regulation. The exoskeleton (shell) of crustaceans contains fair amount of calcium carbonate (Richards, 1951; Venkataramaiah et al., 1978). During the premolt period calcium concentration in the blood increases to a very high value.

The large amount of calcium resorbed from the old exoskeleton accounts for most of the excess calcium in the blood. Heavy deposition of calcium takes place in the new exoskeleton during the postmoult period. But the calcium level in the blood is rapidly restored to its intermoult period by the regulatory mechanism which includes, absorption of sea-water calcium, reduced calcium excretion, and expenditure of calcium reserves in tissue. These observations suggest that calcium is an important mineral element essential for crustaceans including prawns. It should be available to the animals in adequate levels for carrying out the physiological functions for healthy growth. Deshimaru et al. (1978) demonstrated that the prawn P. japonicus absorbed radioactive isotope labelled calcium from sea water. It was calculated that approximately 0.83 mg of calcium was absorbed by the prawn per day per gram of body weight, when the sea water contained 0.44 mg of calcium per ml. The authors concluded that the requirement of calcium could be met from the surrounding water when lacking in the diet. Positive correlation between the haemolymph calcium and that of surrounding water was found in penaeid prawns by Dall (1981). While studying the fluctuation in calcium levels in the exoskeleton, muscle and haemolymph of P. indicus cultivated in a brackish water pond, Rao et al. (1982) observed that the body calcium level was linked to the calcium level in the water. Similar observations were made by Subhash Chander (1986) in the same prawn. The findings discussed above clearly suggest that prawns are capable of absorbing calcium from the environment.

Nevertheless, the results of the present study showed that the dietary calcium at 0.5% level enhances the growth and improves the FCR, proving that P. indicus can successfully utilise dietary calcium readily available to them, and at the same time they are capable of absorbing calcium from the environment when it is not available in the diet. The differences in dietary calcium requirement in different penaeid prawns might be due to that the requirement is different for different species. However, the differences within the same species could be due to the differences in the availability of the element in surrounding water and in the diet.

The prawns fed with the diet without phosphorous had stunted growth, became sluggish and weak. Addition of phosphorous to the diet restored normal growth indicating that phosphorous is very essential in the diet of P. indicus. The growth of the animals reached the highest value when the diet contained 1.05% of phosphorous. At this level the FCR of the diet also showed improvement. However, higher amounts of phosphorous did not have any beneficial effect. Kitabhayashi et al. (1971a) achieved best growth in P. japonicus by supplementing 1.05% of phosphorous in the diet. But Deshimaru and Yone (1978a) found that 2% of phosphorous in the diet was very effective only in the absence of calcium, in the same prawn. On the other hand Kanazawa et al. (1984) found that the requirement of phosphorous was 1.0% in the diet of P. japonicus. The phosphorous requirement found in the diet of P. indicus was same as that was found in P. japonicus by Kitabhayashi et al. (1971 a) and Kanazawa et al. (1984) but it was lower than the phosphorous requirement as

shown by Deshimaru and Yone (1978a). Sick et al.(1972) achieved increased growth in P. aztecus by adding 0.51% phosphorous in the diet which was lower than the value shown by P. indicus in the present study.

Phosphorous is not only found associated with calcium in the body structure, but also forms an important element in the high energy compounds such as AMP, ADP and ATP, nucleic acids and in phospholipids which are involved in the vital biochemical life processes. The deficiency of phosphorous in the diet results in reduced growth, poor feed efficiency, low haematocrit levels as observed in channel catfish and red sea bream (Cho et al., 1985). Prolonged feeding of phosphorous deficient diet results in deformities and accumulation of fat in the liver (lardosis) in common carp. Feeding phosphorous deficient diet to P. indicus had resulted in stunted growth and poor FCR. The body phosphorous declined disturbing the Ca/P ratio. The possibility of prawns absorbing phosphorous from the environment being practically nill, it is essential to provide it in the diet.

Apart from the individual levels of calcium and phosphorous, their ratio in the diet seems to play an important role (Huner and Colvin, 1977) in the nutrition. Kitabhayashi et al. (1971a) reported that the calcium-phosphorous ratio (Ca/P) of 1.2:1 in the diet produced the best results in P. japonicus. When this ratio was increased to 2:1, the growth was inhibited. On the other hand, Deshimaru and Kuroki (1974a) reported the highest growth in P. japonicus fed with the diet having Ca/P ratio of 0.76:1. In the present study, Ca/P ratio of 0.5:1 (or 1:2) gave the best result in P. indicus and changing the ratio to 1.3:1

depressed the growth and reduced the food conversion efficiency. Sick et al. (1972) obtained increased growth in P. aztecus with the diet having 0.66% of calcium and 0.51% of phosphorous in the diet with Ca/P ratio of 1.3:1. Hysmith et al.(1972) observed that when the Ca/P ratio was 1:2, the growth of P. aztecus was lower than when it was 2:2. The Ca/P ratio found to be required in the diet of P. indicus in the present study was lower than the Ca/P ratio reported to be required for P. aztecus both by Sick et al.(1972) and Hysmith et al. (1972). High Ca/P ratios, ranging from 2:1 to 4:1, were also reported by Parker and Holcomb (1973) in the diets of shrimp due to the addition of lime stone to increase the pellet density. The calcium-Phosphorous ratio in the body of vertebrates like finfishes was found to be generally in the order of 2:1 (Chow, 1978). But in P. indicus it was slightly higher (Tables 43 and 46) and inconsistant. Similar observations were made in the prawn Penaeus californiensis (Huner et al., 1979). Gallagher et al. (1978) fed the lobsters (Homarus americanus) with diets having different Ca/P ratios and obtained best results with the diet having 0.56% calcium and 1.10% phosphorous (Ca/P ratio 1:2). The findings are similar to those found in P. indicus in the present study.

Copper is found to be required in the diets of finfishes. Diets having 3 mg copper/kg showed higher weight gains in common carp than the diets having lower levels. But rainbow trout did not show any response to the dietary copper levels while copper concentrations of 20-30 mg/kg diet resulted in reduced growth in channel catfish (Cho et al., 1985). In crustaceans copper is

an important element found in the haemolymph and is associated with protein in the formation of haemocyanins which participate in oxygen transport. The copper content found in the haemocyanins of decopod crustaceans such as Homarus and Linpulus is about 0.25%. This is remarkably higher concentration (Goodwin, 1960) as compared to the copper content generally found in sea water (0.01µg/100ml). Whether copper is required in the diet of prawns or they are capable of extracting this element from the surrounding water to meet the requirement has been the subject matter of discussions, eventhough there are evidences that crustaceans do absorb copper from water (Bryan,1968; White and Rainbow,1982; Subhash Chander,1986). But Deshimaru and Yone (1978a) opined that copper may not be required in the diet of prawn.

However,some of the natural foods used for rearing prawns contain appreciable amounts of copper. For example, copper content in rotifiers (Brachionus plicatilis) varies from 4 to 23µg/g on dry basis. In Artemia nauplii, it is found from 11 to 159µg/g and in Tigriopus 18 to 82µg/g. Whereas in Daphina,it varies from 11 to 13µg and in Moina spp. from 28 to 58µg/g depending upon the season and strain(Watanabe et al.,1978). Therefore,it may be considered that the diet is a source of copper for prawns. In the present study addition of copper to the diet did not help in increasing the growth of the prawns,on the other hand the growth slightly declined with the increase in the dietary copper. However, the growth obtained by the low copper diet(2.4mg/100g) and high copper diet (22.7mg/100g) was not statistically different ($P < 0.05$). But the food conversion ratio showed constant improvement with increase in the dietary copper. From these results it cannot be

said that copper is not essential in the diet of P. indicus. May be that 2.4 mg copper/100 g diet is meeting requirement since this diet had shown highest growth. But when the diet contained 13.6 mg copper/100g, the FCR was low indicating that the dietary copper is involved in the effective utilisation of the diet. Deshimaru and Yone (1978a) found that 0.2% of trace element mixture was required in the diet of P. japonicus, which contained 0.0066% copper chloride. The effect of copper as an individual element on the growth and FCR of the prawns was, however, not ascertained by these authors. Recently Kanazawa et al. (1984) recommended 0.6% copper requirement in the diet of P. japonicus. It is very much higher than the copper content (0.013%) in the diet of P. indicus, which showed improved FCR. The dietary level of copper (13.6 mg/100g) found to improve the FCR in P. indicus is comparable to the copper content recorded in Artemia nauplii, which forms famous live food in the culture of larvae of penaeid prawns.

Experiments on the requirement of zinc revealed interesting results. The growth of prawns increased (Fig.35a) with the increase in the dietary zinc upto 23.6 mg/100g diet. Higher amounts of zinc in the diet only reduced the growth. The FCR, however, did not show any significant difference ($P < 0.05$) with the different levels of dietary zinc (Fig.35b). These results indicate that zinc is required in the diet of P. indicus, and 23.6 mg/100 g diet (or 0.06% of zinc chloride) would meet the requirement. Deshimaru and Yone (1978a) indicated that 0.2% of trace elements were required in the diet of the prawn P. japonicus. The trace element mixture used, contained zinc sulphate which was equivalent to about 34.5 mg of zinc per 100 g

diet. Compared to this, the zinc requirement shown by P. indicus was lower. Zinc is an important component of mettallo-enzymes such as super oxide dismutase and carboxy pepsidase. Its deficiency may cause disfunction of many metabolic processes. In rainbow trout, zinc requirements are normally met by dietary levels of 15-30 mg/kg diet (Cho et al., 1985). Its deficiency caused mortality, cataract in the eyes and erosion of skin and the fins (Chow and Schell, 1978). But dietary zinc levels up to several hundred milligrams per kilogram diet do not seem to be injurious to rainbow trout.

Requirement of zinc in the diet of prawns is not studied very much though its regulation in the body of some crustaceans was studied (Bryan, 1964, 1968; Colvocorresses and Lynch, 1975; White and Rainbow, 1984; Subhash Chander, 1986) in relation to the environment. Perhaps the present study is the first attempt in this direction in which the requirement of zinc as an individual element for P. indicus has been investigated. The zinc level in the body of the animals, fed diets with low levels of zinc, showed reduction as compared with the value found before the start of the feeding experiment (Table 52). As the amount of zinc in the diet increased, the zinc level in the body also increased (Fig. 36a). These results clearly show that prawns effectively absorb the dietary source of zinc, while the water in which the feeding experiments were conducted showed 5.4 ppm of zinc. Watanabe et al. (1978) reported that zinc content in the rotifers varied from 43 to 99 µg/g (on dry basis). In Artemia nauplii, it varied from 160 to 960 µg/g, depending upon the season and the strain. On the other hand, Tigriopus showed a zinc content of 81 to 406 µg/g; whereas Moina, 94 to 172 µg/g; Acartia 390 to

1300µg/g and *Daphnia*, 128µg/g. From these findings it could be understood that some of the conventional live foods used for the culture of prawns are good suppliers of zinc to the recipient animals. It is therefore logical that the prawn *P.indicus* showed a dietary requirement of 23.6 mg zinc per 100 g in the diet.

Magnesium constitutes one of the most important prosthetic (non-protein) groups in many metallow-enzymes. Magnesium ions act as co-factors in the enzymes involved in the metabolism of fats, carbohydrates and proteins and also as an important structural component of cell membranes. Magnesium is found to be essential in homeothermic land animals. Its deficiency causes loss of appetite, poor growth, sluggishness and convulsions followed by tetani, often leading to high mortality (Maynard *et al.*, 1981; Georgieveskii *et al.*, 1981; NRC, 1977). Magnesium is also found to be required in aquatic animals like finfish. But fish are capable of extracting magnesium from the environment (Chow and Schell, 1978). Due to comparatively low concentrations of this mineral element in fresh water, non-marine species appear to depend upon dietary source to meet their requirement of magnesium. Magnesium requirement of common carp was found to be 0.04-0.05% and that of rainbow trout 0.06-0.07% (Cho *et al.*, 1985). Deficiency of magnesium in fish was found to cause symptoms similar to those found in land animals. Its deficiency in rainbow trout leads to renal Calcinosis and flaccidity (accumulation of water) of the muscle. Retarded growth and behavioural abnormalities are also observed in common carp.

Kanazawa et al.(1984) reported that 0.3% of magnesium is required in the diet of the prawn P. japonicus. But the experiments conducted on P. indicus in the present study did not show any dietary magnesium requirement for this prawn. On the other hand depressed growth and poor food conversion ratio were observed (Fig. 37a and 37b) with the addition of magnesium in the diet. These findings are in agreement with the findings of Deshimaru and Yone (1978a) who found no improvement in the nutritive value of a purified diet when supplemented with 0.3% magnesium in P. japonicus. Graf (1978) and Subhash Chander (1986) observed that penaeid prawns are capable of absorbing magnesium from water and the source of magnesium through food is only secondary. Perhaps because of these reasons the prawn P. indicus did not show dietary requirement for magnesium.

Discussing osmotic and ionic regulations in crustacea, Robertson (1960a) indicated that almost all the crustaceans are capable of exchanging many inorganic ions in which Mg^{++} ion is one among them. The plasma concentration of Mg^{++} varies between individual species. The Decapods such as the anonurans and brachyurans, including crabs, have the highest levels of magnesium ions in the blood, while the stomatopods (Squilla) have the lowest Mg^{++} concentration in their blood. It was experimentally demonstrated that in the uptake of ions from water, the gills are primarily involved. Chemical proof of the absorption of Mg^{++} and Na^{+} ions was established by the help of intermoult specimens. It was found that the animals attained equilibrium between ions present in the body and in the surrounding water by absorbing and excreting equal amounts of these ions.

Examination of the body magnesium of P. indicus fed with the diets having different levels of magnesium, showed little variation (Fig.38a). It remained constant irrespective of the dietary magnesium. Further, the magnesium content in the prawns fed with the diet having no additional magnesium, was appreciably high as compared to the value present before the start of the feeding experiment (Table 55). The water in which the feeding experiment was conducted, contained magnesium equivalent to 10.5 ppm. Viewing these results vis-a-vis the capacity of crustaceans to regulate the absorption of inorganic ions from the environment, it is amply clear that prawns are extracting magnesium from the surrounding water. Since magnesium ions are involved in the osmotic regulation, the absorption of this element from the environment may be more primary than from the diet. In the light of these discussions it may be concluded that magnesium is required for P. indicus, but meet their requirement by absorbing it from water.

Feeding the diets with manganese varying from 0.21 to 2.599 mg/100g to juvenile P. indicus did not improve the growth and food conversion ratio. The growth and FCR of the control diet were slightly better than that of the other diets, though, the difference was not statistically significant ($P < 0.05$). Deshimaru and Yone (1978a) included manganese sulphate (20 mg in a mixture of 150 mg trace metal salts) in the diet of P. japonicus. But the authors did not evaluate the requirement of manganese individually. On the contrary, Kanazawa et al. (1984) reported that inclusion of 0.003% manganese in the diet depressed growth in P. japonicus. These results are similar to those obtained in P. indicus in the present study.

Manganese is found as co-factor in some of the metabolic enzymes such as arginase, involved in bone formation and erythrocyte regeneration in the animals. Manganese had been found to be essential in finfishes such as rainbow trout and Tilapia mossambica (Chow and Schell, 1978). Higher growth was obtained in carp and rainbow trout when fed on the diet having 12-13 mg/kg of manganese (Cho et al., 1985). P. indicus have shown progressive increase in the manganese content in their body (Fig.40a) as the dietary manganese increased, indicating accumulation of this element in the body from the diet. But the survival of the animals improved with the dietary manganese. The water in which the feeding experiment was conducted had shown 0.024 ppm manganese in it.

Some of the zooplankton cultured and used as food for postlarvae of prawns, such as rotifers (4-22µg manganese/g on dry basis), Artemia nauplii (2-980µg/g), Tigriopus (9 - 21µg/g), Acartia (2 - 10µg/g), Daphnia (132µg/g) and Moina (5 - 35µg/g), had shown fairly good amounts of manganese in them. This means that diet might be the source of manganese for prawns. The control diet in present study had 210µg manganese/100 g which had shown better growth and FCR. May be the amount of manganese present (210µg/100 g) in this diet is meeting the requirement of the prawns. And higher amounts of manganese in the diet might be exerting undesirable affect on growth as the animals had shown accumulation of this element in their body. In conclusion it can be said that the manganese requirement of P. indicus is very low and the amount of this element present in the various ingredients of the diet meets the requirement of the animals. Addition of

manganese to the diet had no beneficial effect and higher amounts might prove even toxic to the prawns.

CONCLUSIONS

1. 0.53% of calcium is required in the diet of the prawn Penaeus indicus for best growth and food conversion ratio.
2. Phosphorous is essential in the diet of P. indicus. Feeding phosphorous deficient diet resulted in very poor growth and FCR. 1.05% of phosphorous is required in the diet.
3. The food conversion ratio of the diet was improved when the diet contained 13.6 mg of copper per 100 gram diet.
4. Best growth and FCR were obtained by the diet having 23.6 mg of zinc. Zinc levels in the diet lower or higher than this level depressed growth.
5. Magnesium was not found to be required in the diet of P. indicus. Addition of magnesium to the diet decreased growth and increased FCR.
6. The control diet had 0.21 mg of manganese/100 g which gave the best results. Addition of manganese to the diet depressed growth and increased FCR.

C H A P T E R - IV

A PURIFIED DIET AND A PRACTICAL FEED FOR PRAWNS

INTRODUCTION

Compounded diets can be classified as purified diets and practical feeds. The former diets are formulated using purified ingredients and are generally used for studying the nutritional requirements of the animals, whereas the practical feeds are formulated based on the nutritional requirements of the recipient animal, using naturally available feed materials and are used for different phases of culturing the animals. Different purified diets, for standard reference, have been published in literature for finfish (Halver, 1972), Prawns (Kanazawa et al., 1977a) and lobsters (Conklin et al., 1980; Boghen et al., 1982; Kean et al., 1985). Any one of these diets could be used for nutritional studies in finfish and shellfish. But very often, it happens that these diets could not be formulated in totality in a given region as some of the diet components enlisted may not be available in that particular region. This situation necessitates the development of a purified diet formulated using the ingredients locally available and suitable for the species that are cultured in the prevailing conditions of the region.

In the traditional prawn and fish culture practices in the brackishwater region followed in India and some of the south-east Asian countries, feeding of the population in the pond is generally not practised. The prawn and fish population grow by feeding on the natural food available in these ponds. Supplementary feeding is resorted to only when the productivity or

the growth of the natural food in the pond is found depleted. In such cases and in the early years of development of semi-intensive culture systems, several kinds of supplementary feeds have been used with varying degrees of success. The most important supplementary feeds used are clams, mussels, trash (cheaper varieties) fish, animal flesh, oil cakes such as groundnut, coconut, gingelly, mustard and soybean; rice bran, wheat bran, and other grains either singly or in combinations.

With the rapid progress of semi-intensive and intensive systems of culture, the formulation and development of practical feeds, meeting the nutritional requirements of candidate species for obtaining optimum growth and survival during the different phases of its growth and in consideration of the cost of manufacture of feed, have been assigned high priority. Accordingly, during the past two decades, considerable advancements have been made in the various aspects of feed development and feed technology. As a result of the Research and Development Programmes, several types of compounded feeds are developed and being used for the culture of prawns in different regions. (Asia: Hirasava, 1984; Kungvankij, 1984;. Japan: Shigueno, 1984; Philippines: SEAFDEC, 1981, 1983; Liu and Mancebo, 1983; Apud, 1984; Pascual, 1984; Tibbu et al., 1984; Taiwan: Liao, 1981; Chiang and Liao, 1985; Tahiti: AQUACOP, 1984a,b; United States: Caillouet et al., 1974; Parker and Fred, 1974; Elam and Green, 1974; Huang et al., 1984; Lee and Shlesser, 1984; Latin America: Escobar, 1984; Scura, 1985).

In India attempts were made to develop the practical feeds with the locally available materials by Alikunhi et al. (1980),

Ahamad Ali and Sivadas (1983) and Mohamed et al.(1983) to rear the larvae in the hatchery and post larvae in the nursery. With a view to developing suitable artificial feed for feeding the prawns in the grow-out systems, different types of feeds were formulated and feeding experiments were conducted by many workers (AICRIP, 1978; Ahamad Ali, 1982a; Mohammed Sultan et al., 1982; Raman et al., 1982; Ahamad Ali and Mohamed, 1985.) Recently a pelletized feed under the brand name 'Tamco feed' has been introduced by M/S.TATA, Madras and it was supplied to the farmers at a subsidised price by Marine Products Export Development Authority.

Although the above endeavours have provided certain information on the use of the compounded feeds, the results and the Production rate obtained have been inconsistant and it is found imperative that an appropriate formulated feed has to be further developed and perfected for large scale use in the commercial operations. In this context, on the basis of results obtained in the present study, a purified diet is formulated with the intention that this formula diet can be used as a basal purified diet for studying the nutrition of penaeid prawns in this region, as all the diet components listed in the diet are easily available. A practical feed is formulated using feed ingredients evaluated in the present study and attempts are made to balance it according to the requirements of the prawn P. indicus. Long term feeding experiments are conducted in the laboratory with the purified diet and the practical feed formulated on P.indicus. The results are compared with those of the conventionally used feed fresh clam meat. The prospects of using the purified diet for nutritional studies in this region and the practicalfeed for the culture of penaeid prawns are discussed.

MATERIAL AND METHODS

The ingredients, albumen (egg), sucrose, maltose, starch, cod liver oil, used for preparing purified diet, and prawn waste, mantis shrimp, fish meal, groundnut cake and tapioca, used for preparing practical feed are already described in Chapter I.

Formulation of diet and feed

Purified diet: The purified diet, designated as PDP, was formulated using albumen (egg) as the protein source, a mixture of sucrose, maltose, starch (in equal proportion), as carbohydrate source and cod liver oil, as the source of lipid. Cholesterol, glucosamine and the vitamin mixture were added based on the purified diet formulated by Kanazawa et al. (1970) for P. japonicus The mineral mixture consisted of calcium carbonate, potassium dihydrogen orthophosphate, copper sulphate and zinc chloride, prepared according to the requirement of these minerals shown by P. indicus in the present study. The diet had 28.9% crude protein, 40% mixed carbohydrate and 6.17% of cellulose which were found to be adequate for this prawn. Sodium alginate was used as the binder. The composition of the diet is given in Table 60.

Practical Feed: The practical feed, designated as PFP, was formulated using prawn waste, mantis shrimp, fish meal, groundnut cake and tapioca. The protein base in the feed was made up of 65% of animal protein source and 35% plant protein source. Tapioca was used as the source of carbohydrate. A commercial vitamin mixture (Becadex) manufactured by Glaxo Laboratories, Bombay and meant for human consumption, was included in the feed. Since the feed had adequate levels of calcium and phosphorous,

Table 60. Composition (g/100g) of purified diet -PDP

Ingredients	g/100g
Albumen (egg)	35
Carbohydrate mix	40
Cod liver oil	6
Cholesterol	0.5
Glucosamine HC1	0.8
Vitamin mixture 1	2.7
Mineral mixture 2	5.83
Cellulose	6.17
Sodium alginate	3.00
Crude protein (%)	28.90

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1. Vitamin mixture: Same as used for the diets PE_0 to PE_4 in Chapter I (Table 2a)

2. Mineral Mixture:

Calcium carbonate	=	1.3
Potassium dihydrogen orthophosphate	=	4.4
Copper Sulphate	=	0.06
Zinc chloride	=	0.07
		5.83

these were not added, but copper and zinc were supplemented at the required level in the feed. Since tapioca acts as the binder, no additional binder was used. The composition of the feed is given in Table 61.

Control feed: The fresh meat of the clam, Sunneta scripta was used as the control feed. The composition of the clam meat was same as given in Chapter I (Table 2).

Preparation of purified diet and practical feed: The method of preparation of purified diet and the practical feed is same as described under the respective sections in Chapter I.

Feeding experiments: Hatchery reared, early juveniles of the prawn Penaeus indicus with an average length of 20.7 mm and average live-weight of 0.0249g were used in the feeding experiments. The animals were stocked in 3' x 2' circular plastic pools (Plate 4), containing 200 L of a mixture of filtered sea water and tap water to give a salinity of about 16‰. Each pool had ten animals and there were three replicates for each treatment. The water in the pools was completely replaced once in five days and aeration was provided with the help of an air compressor. The hydrographical data is presented in Table H_4.

The animals were fed at 20% of the body weight approximately in two devided doses in the morning and evening. Feed was placed in petridishes kept in the pools. Left over feed was removed, washed gently with water and dried in the oven at 60° for 12 hrs. The feeding experiment was continued for a period of 100 days.

The total length and live-weight were measured before and after feeding experiment individually for each treatment.

Table 61. Composition of practical feed - PFP

Ingredients	(g)
Prawn waste	14.0
Mantis shrimp	14.0
Fish meal	11.0
Groundnut cake	21.0
Tapioca	40.0
Vitamin mixture[1]	1.0
$CuSO_4$	0.06
$Zncl_2$	0.07

Proximate composition	(g/100g)
Moisture	5.60
Crude protein	28.02
Lipid	10.00
Carbohydrate	34.40
Ash	16.80
Crude fibre	5.18
Calcium	2.54
Phosphorous	1.23

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1. Vitamin mixture (1 g consists of)

Vitamin A	5000 I.U.
Vitamin D_3	400 I.U.
Vitamin B_1	4 mg
Vitamin B_2	4 mg
Nicotinamide	50 mg
Vitamin C	60 mg
Calcium phosphate	500 mg.

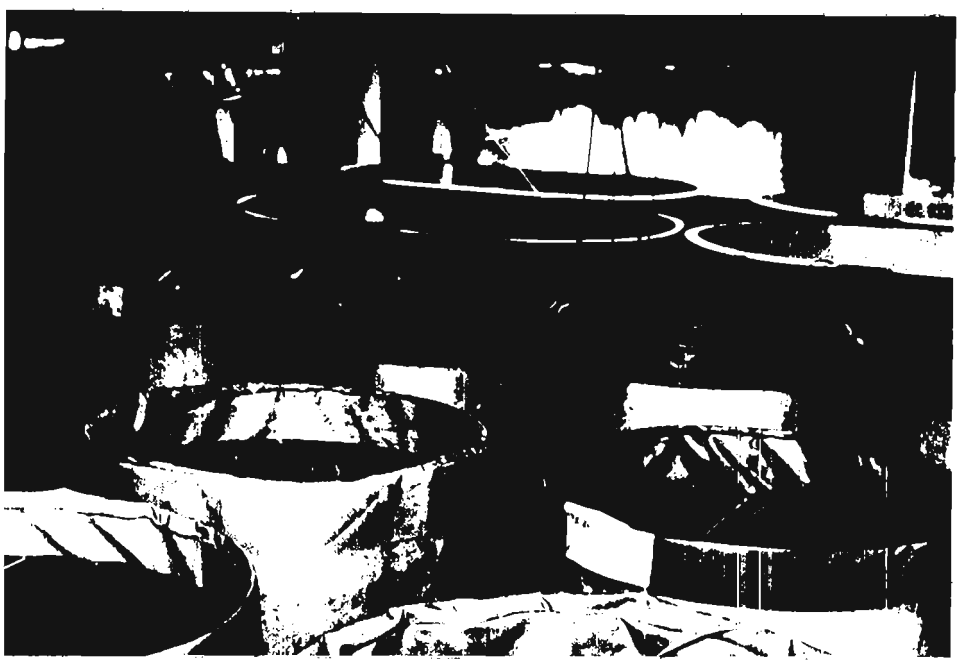

Plate 4. Plastic liner pools used for long term feeding experiments with purified diet (PDP), practical feed (PFP) and fresh clam meat.

Table H_4. Hydrographical data of the feeding experiment with PDP, PFP and clam meat.

Salinity ‰	Oxygen cc/L	Temperature °C	pH
21.2 - 25.9	3.81 - 4.21	28.4 - 30.2	8.1 - 8.29

Biochemical Analysis: The preparation of samples of animals and the diets for analysis and the methods used for analysing them for protein, lipid, carbohydrate and ash are the same as described in Chapter I. Moisture was estimated by drying the sample at 60°C to constant weight. While calcium and phosphorous were estimated by the same methods described in Chapter III, crude fibre was determined by the AOAC method.

Statistical Analysis: The data obtained by the purified diet, practical feed and the control feed on growth and food conversion ratio were subjected to analysis of variance and the results are shown in Table 62 A.

RESULTS

The results of the feeding experiment conducted with FDP, PFP and fresh clam meat are presented in Table 62. The practical feed PFP produced highest increase in length (72.2mm) and live weight (4.69g), followed by the fresh clam meat(63.7mm in length and 4.42g in live-weight) and the purified diet(54.7mm in length and 3.83g in live-weight). The increase in length and live-weight obtained by the practical feed and clam meat were significantly higher ($P < 0.05$) than the increase in length and weight obtained by the purified diet. However, the difference in length and weight obtained by the practical feed and fresh clam meat was not significant. The food conversion ratio (FCR) obtained by the practical feed was the lowest (1.80) followed by the clam meat (2.09) and purified diet (2.37), though the values were not statistically different ($P < 0.05$). The survival of the animals was the highest in the case of purified diet (90%), followed by the practical feed (63.3%) and clam meat (53.0%).

The crude protein in the animals fed the practical feed, PFP was the highest (66.53%) while the animals fed the clam meat had low protein (60.4%). However, the lipid of the animals fed clam meat had shown high value (21.0%) and that of the animals fed practical feed was low (13.6%). The carbohydrate and ash did not differ significantly among the animals fed the different feeds.

The growth curves of the animals fed the three different feeds are shown in Fig. 41. For the first ten days, the increase in live weight (Fig.41a) and length (Fig.41b) was similar

Table 62. Results of the feeding experiment conducted with PDP, PFP and fresh clam meat on P. indicus for 100 days.

Particulars	Feed		
	PDP	PFP	Fresh clam meat
Initial average length(mm)	20.9	20.7	20.8
Initial average live-weight (g)	0.0249	0.0249	0.0249
Final average length(mm)	75.5	92.9	88.1
Final average live-weight (g)	3.86	4.72	4.45
Increase in length (mm)	54.7^{c}	72.2^{a}	63.7^{b}
Increase in live-weight(g)	3.83^{b}	4.69^{a}	4.42^{a}
Food conversion ratio	2.37	1.80	2.09
Survival %	90.0	63.3	53.0
Composition of animals (% on dry basis) after completion of feeding experiment.			
Crude protein	63.90	66.53	60.40
Lipid	18.60	13.60	21.00
Carbohydrate	1.41	1.44	1.52
Ash	17.23	16.93	16.81

Note: Values with different superscripts differ significantly among themselves. Increase in length and weight significant at 5% level ($P < 0.05$). Food conversion ratio not significant at 5% level ($P < 0.05$).

Table 62 A. Analysis of variance of the data obtained by PDP, PFP and fresh clam meat

Source	D.F.	S.S.	M.S.
		ANOVA	
1. Increase in length			
Treatment	2	588.15	294.07*
Error	6	52.93	8.82
Total	8	441.08	302.89
2. Increase in live-weight			
Treatment	2	1.18	0.59*
Error	6	0.19	0.03
Total	8	1.37	0.62
3. Food conversion ratio			
Treatment	2	0.495	0.2475^{N}
Error	6	1.50	0.2500
Total	8	1.995	0.4975

* Significant at 5% level ($P < 0.05$)

N Not significant ($P < 0.05$)

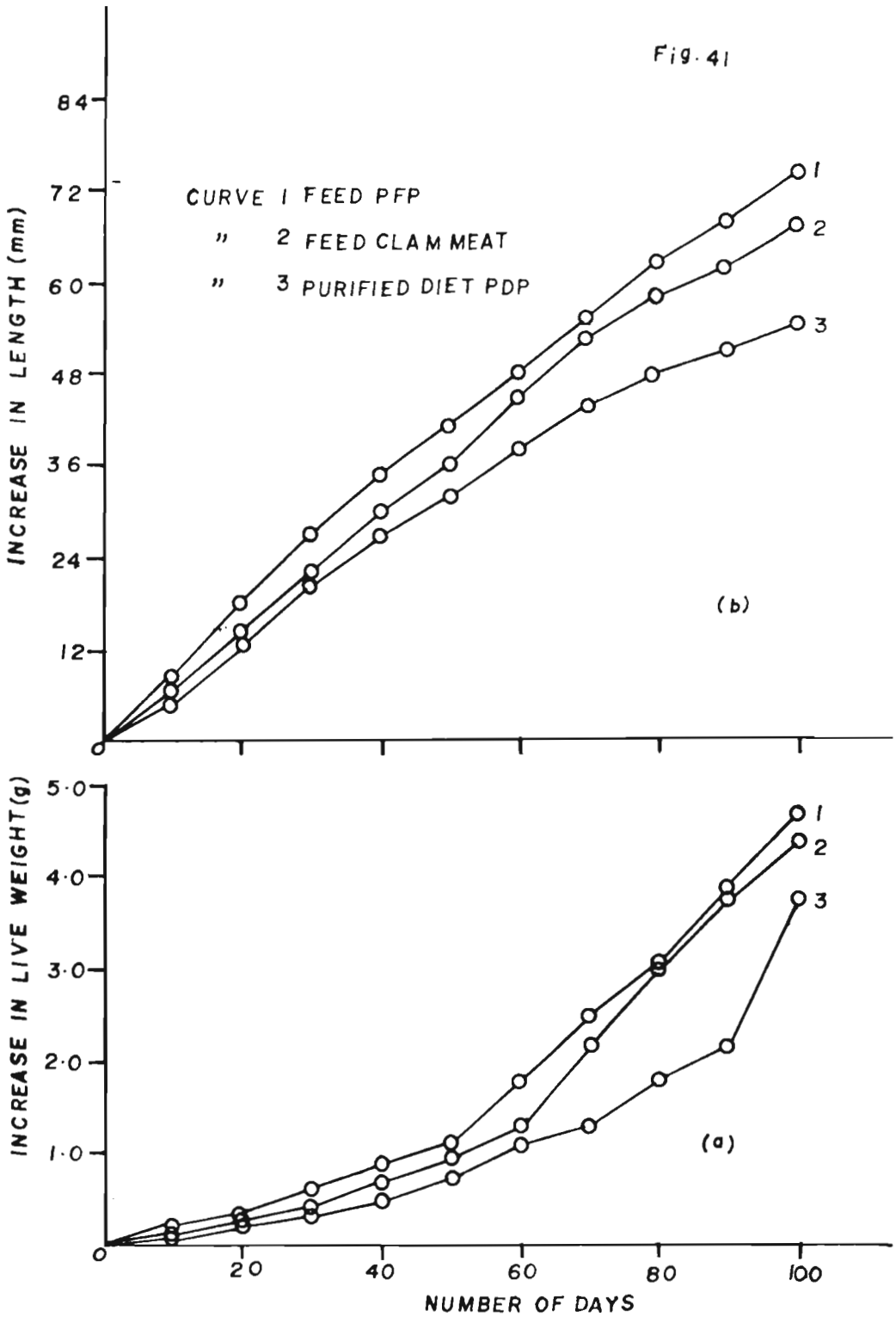

Figure 41. Growth curves (a) weight and (b) length of P. indicus fed with purified diet (PDP), Practical feed (PFP) and fresh clam meat.

in the case of all the three feeds. With time, the growth curves separated out, with the practical feed PFP occupying the top position and the purified diet PDP, occupying the lower position. The control feed clam meat remained intermediate between the practical feed and purified diet.

DISCUSSION

The purified diet PDP, formulated with albumen (egg), mixed carbohydrate, cod liver oil, vitamins, minerals and other additives resulted in the highest survival (90%) of the prawn Penaeus indicus at the end of 100 days rearing. The average increase in length of the animals fed this diet was 54.7mm and the average increase in weight was 3.83g. The food conversion ratio of the diet was 2.37. Purified diets were first formulated by Kanazawa et al. (1970) for the prawn P. japonicus and subsequently by Kitabhayashi et al. (1971a, b, c, d) and Deshimaru and Kuroki (1974a,b,c, 1975a, b) for studying different aspects of nutrition, AQUACOP (1978) and Kanazawa et al. (1981) formulated purified diets for studying the nutritional requirements of P. merguiensis. Lim et al. (1978) prepared casein diets and compared with those of shrimp meal, squid meal and Spirulina for the post larvae of P. monodon. Ahamad Ali (1982b), Charles John Bhasker and Ahamad Ali (1984) and Gopal (1986) prepared purified diets for the prawn P. indicus to study carbohydrate and protein requirements in the diet. Alava and Pascual (1987) formulated and prepared purified diets for P. monodon. While Conklin et al. (1980) evolved a purified diet for the culture of juveniles of American lobster Homarus americanus, D' Abramo et al. (1981, 1982, 1984) studied the requirements of different nutrients for the same lobster using purified diets. In all the above mentioned works, casein was used as the protein source whereas albumen (egg) was used in the purified diet PDP in the present study. Most of the diets formulated in these studies were test diets and the protein content varied from 15 to 80% where as the protein content of the

purified diet PDP was only 28.9%.

The growth obtained by the purified diet PDP in juvenile P. indicus compares well with growth of P. japonicus obtained by Kanazawa et al.(1970), P. merguiensis by AQUACOP (1978) and Kanazawa et al. (1981) and of P. monodon reported by Lim et al. (1978) and Alava and Pascual (1987). The growth of the prawns obtained in the present study is higher than the growth recorded in P. indicus fed with casein diets by Ahamad Ali (1982b) and Charles John Bhasker and Ahamad Ali (1984). Besides, the food conversion ratio and the survival rate obtained by the present diet in P. indicus are superior to those obtained in P. japonicus P. merguiensis and P. monodon in the studies mentioned earlier. These indicate that the purified diet PDP, prepared with albumen is superior to the casein diets.

However, the growth and food conversion ratio shown by the diet PDP are lower than the growth and food conversion ratio recorded by the Practical feed PFP and also the control feed clam meat in this study. Only the survival of the animals fed with the purified diet was higher as compared to those fed with the control feed and the practical feed. But such inferior growth of prawns fed with the synthetic purified diets is not uncommon in nutritional studies. Poor growth of prawns, fed with purified diets prepared with casein, gelatin, albumen and amino acid mixtures, compared to the diets prepared with natural ingredients, was reported by Sick et al. (1972), Deshimaru and Kuroki (1974c, 1975a, 1975b), Ahamad Ali (1982b) and Teshima et al. (1986e). The difference in the growth of the prawns fed with purified diet and natural feed might be due to the non-palatability of the purified diet as it

is prepared with purified materials and chemicals. Nevertheless, the use of purified diets is essential as the effect of a particular nutrient could be clearly understood, without the interference of extraneous factors, only through purified ingredients.

It is well known that purified diets are primarily meant for studying the nutritional requirements of candidate species. However, these are also used for practical purposes, Villegas and Kanazawa (1980) and Jones et al. (1979a) used the purified diet for rearing the larvae of P. japonicus, and Conklin et al. (1980) for culturing the juveniles of the lobster, Homarus americanus. Some of the purified proteins are also mixed with natural ingredients in formulating diets (Lee, 1971; Andrews and Sick, 1972; Alava and Lim, 1983) either to enhance the protein level of the diet or to balance the aminoacid profile. A balanced purified diet can be used for maintaining animals in laboratory where practical feeds are not available. Feed attractants such as Squid extract, mussel mantle, shrimp extract and aminoacids like glutamic acid are often used in purified diets to increase their acceptability.

Feeding constitutes a major cost of aquaculture production. It is thought that the triumph of future aquaculture depends largely on the development of a nutritionally balanced and economical feed and its sustained supply. In this context, the results of the experiments conducted on the practical feeds in this study are compared with those similar observations and discussed. The control feed fresh clam meat produced low growth and survival compared to the practical feed in the present study. Similar results were reported in P. indicus with fresh clam meat by

Colvin (1976a) and Ahamad Ali (1982a), especially it resulted in high mortality in both the studies. Ahamad Ali (1982a) observed frequent moulting and high incidence of cannibalism in the prawns fed with fresh clam meat. Contrary to the results obtained in P. indicus, Kanazawa et al. (1970) reported superior growth in P. japonicus fed with the meat of short-necked clam (Tapes philippinarum). Similar observations were made by Forster and Beard (1973) in the Prawn Palaemon serratus. However, clam meat by itself may not be considered as a nutritiously balanced feed for prawns. Besides, it is relatively expensive (5 to 8 rupees per kilogram of fresh meat with 80% moisture) and is used for human consumption. Further, the availability of clam meat in adequate quantity for feeding large scale culture of prawns may not be assured. It may, however, be used as supplementary feed for prawns wherever it is available at a competitive price.

The practical feed PFP prepared with prawn waste, mantis shrimp, fish meal, groundnut cake and tapioca, fortified with vitamins and minerals gave highest growth (72.2mm in length and 4.69 g in weight) and best food conversion ratio (1.80) in P. indicus among the three diets tested. The survival of the prawns fed with the practical feed was 63.3%.

Several different feeds were formulated in India for feeding the prawns. At the Kakdwip Research Centre (AICRP I 1978), supplementary feeds were formulated, consisting of soybean flour, brewer's yeast, maize powder, wheat flour, calcium phosphate, vitamins and algin, for feeding the post larvae of P. monodon, and another feed consisting of goat offal, yeast, algal powder, wheat flour and terramycin for feeding the post larvae of P.

indicus. It was reported that the survival of the post larvae fed with the above feeds, ranged between 57% and 90% depending upon the temperature of the medium. In another experiment, four feeds were prepared with prawn meal alone, prawn meal + maize (1:1), prawn meal + Wheat powder (1:1) and wheat powder and maize (1:1) and fed to the post larvae of P. monodon at different feeding rates. It was found that the feed with prawn meal + wheat powder (1:1) gave the highest survival of 90% at 10% (of body weight) feeding level at the end of 8 weeks. The survival of P. indicus in the present study is 63.3% which is slightly less than that recorded in P. monodon post larvae in the above study.

At the Kakinada Brackishwater Research Centre(AICRIP, 1978), feeding trials were also conducted on P. monodon in cement cysterns with three feeds prepared with a mixture of fish meal, groundnut cake and rice bran, fish meal alone, and flesh of trashfish (Oryzias melastigme). It was reported that the feed prepared with flesh of trashfish gave the highest growth and best food conversion ratio (FCR). The post larvae of P.monodon fed with the feed mixture had grown from 0.015 g to 0.332 g on an average in 30 days, where as those fed with fish meal alone had grown from 0.015g to 0.2415g and those fed with trashfish, from 0.015g to 0.368g. The FCR recorded by the feed mixture was 3.5,while those recorded by the fish meal and trashfish were 4.37 and 1.26 respectively. In the present study,P.indicus fed with the practical feed had grown from 0.0249g to 4.72 g in 100 days. While the FCR (1.80) of the practical feed prepared in present study is slightly inferior compared with the FCR of flesh of

trashfish obtained in P. monodon, it is superior to the FCRs recorded by the feed mixture and fish meal.

Raman et al. (1982)conducted feeding trials on P.indicus using different combinations of feed ingredients consisting of fish meal, prawn factory waste, groundnut oil cake, gingelly cake, black gram husk, Bengal gram husk, bajra, wheat flower, wheat bran, rice bran and tapioca. Among the feed combinations tested, fish meal, rice bran and tapioca in the ratio 1:1:1 and 2:2:1 gave satisfactory results. The FCR obtained by the first combination feed was 1.69, while it was 3.21 and 3.32 by the second combination feed in two different experiments. The FCR (1.80) of the practical feed PFP for P. indicus compares well with the FCR obtained by the first combination but superior to the FCRs of the second combination feed reported by Raman et al. (1982). Mohammed Sultan et al. (1982) formulated feeds with frog flesh waste and reported a food conversion ratio between 3.01 and 4.96 for P. indicus and 5.87 and 8.21 for P. monodon. The food conversion ratio obtained by the feed in the present study is superior to FCRs obtained by the feeds with frog flesh both in P. indicus and P. monodon in the above study. Compounded feeds were formulated using prawn waste, mantis shrimp, groundnut cake and tapioca for feeding juveniles of P. indicus by Ahamad Ali and Mohamed (1985) and the best feed among the tested, recorded a food conversion ratio of 3.22. Compared to this FCR, the FCR obtained (1.80) in P. indicus is superior in the present study.

Out side India, Colvin (1976a) studied the growth, digestibility and FCR of some diets formulated with fish meal and shrimp meal in P. indicus and reported a growth of about 44 mg/

day and the lowest FCR of 2.72. While the growth of P. indicus in present study is 46.9 mg/day, the FCR is 1.80 which are superior to those reported by Colvin (1976a). Recently Apud et al. (1984) studied the growth, survival and production of the prawn P. indicus in brackishwater ponds in the Philippines. Supplementary feeding of the prawns was done with a compounded feed. The production was 343.2 Kg/ha in the test pond (where feeding was done) and 180 Kg/ha in the control pond. The average survival was 70.36%. The FCR of the feed was not reported for comparison with that of the present practical feed.

The performance of the present practical feed compares well with some of the diets formulated for other species. Feeding trials conducted on P. monodon cultured in cages using a practical feed in the Philippines (SEAFDEC, 1981) had shown a food conversion ratio of 4.8. In a semi-intensive culture experiment with the same prawn (SEAFDEC, 1983), a commercial prawn feed with 45% protein and an experimental feed with 35% Protein produced FCRs 3.4 to 4.6 and 6.1 respectively. Similarly in another intensive culture experiment (SEAFDEC, 1983), a crustacean pelleted feed resulted in a FCR of 3.01 in the same prawn. Certainly, the FCR (1.80) shown by the practical feed in P. indicus in the present study is much lower than the FCRs obtained by the feeds tested in P. monodon in the above experiments. Further, in pond culture experiments with P. monodon in the Philippines, Liu and Mancebo (1983) used a commercial formula feed developed by the 'President Enterprises Corporation', Taiwan, and obtained food conversion ratios of 1.69 and 1.78. The FCR of the present practical feed in P. indicus is in agreement with these values.

Venkataramaiah et al. (1972a,b, 1975a,b), while studying the effect of feeding level and salinity on growth and FCR in P. aztecus, reported that the FCR of a standard prawn feed varied from 3.73 to 1.25, and the FCR of the present practical feed (1.80) in P. indicus falls within this range. But the FCR of the diets formulated with fish meal, having different protein levels, in P. aztecus (Venkataramaiah et al., 1975a) ranged from 2.8 to 19.0 which are inferior to the FCR obtained by the feed in the present study.

Elam and Green (1974) conducted feeding experiments with different formula feeds on P. setiferus and obtained food conversion ratios ranging from 1.8 to 2.3 which are comparable to the value recorded by the feed in the present study. However, the pink shrimp P. duorarum fed with wheat bran and a catfish ration attained 6.0 g and 8.0 g weight in 78 and 92 days respectively, in pond experiments (Caillout et al., 1974). Parker and Fred (1974), while studying the shrimp production module for P. aztecus observed that the Prawns fed standard commercial rations attained a weight of 10.77 g. in 139 days, while P. stylirostris attained a weight of 6.54 g in 136 days. Compared to the size of the prawns attained in above studies, the weight attained by P. indicus in present study is low. But these differences are obvious because of the species difference and also the difference in experimental conditions in these investigations.

From the above discussions it is clear that the performance of the present practical feed PFP is comparable to that of many of the standard formula feeds used for feeding penaeid prawns and it is superior to the performance of some of the formulated feeds.

Moreover, the material cost of the present practical feed is found to be Rs. 3/- per kilogram, based on the existing retail prices of the ingredients (prawn waste Rs. 2/-per kg; mantis shrimp Rs. 2/- per kg; Fish meal Rs. 6/- per kg; tapioca Rs. 2/- per kg) including the cost of vitamin and mineral mixture (which is equivalent to Rs. 0.35 per kg of the feed). With a food conversion ratio of 1.8, the cost of the feed to produce one kilogram of prawns is only Rs. 5.40 which can be considered as very economical.

Prawn waste and mantis shrimp are available in sufficiently large quantities in most of the maritime states. A major portion of these two materials is not utilised at present and are practically thrown away. Only a small quantity of prawn waste and mantis shrimp are collected, sundried and used as manure or mixed with some plant fertilizer mixtures and in poultry feeds. Nevertheless, these two materials are potential feed ingredients to reckon with for formulating prawn feeds. Both prawn waste and mantis shrimp are good sources of animal protein rich in essential aminoacids (Forster, 1975; Sandifer and Joseph, 1976) and have shown good digestibility and biological value (Chapter I) Besides, prawn waste is a good source of lipid rich in poly - unsaturated fatty acids and carotenoids (Joseph and Meyers, 1975; Joseph and Williams 1975; Ahamad Ali, unpublished) essential for prawns. It also contains calcium and phosphorous and the Chitin present in it has growth promoting effect (Vaitheeswaran and Ahamad Ali, 1986) when mixed with the diet of P. indicus. The composition of prawn waste sometimes varies according to the size of the prawns from which it is derived. Often it was found that the residual meat was left more in the peelings of smaller

size prawns such as Metapenaeus dobsoni, M. monoceros and Parapenaeopsis stylifera, compared to the larger prawns such as P. indicus and P. monodon. In the case of mantis shrimp large size animals (contain higher amount of meat) are landed generally from December to May. In other months, the landings of mantis shrimp are very less and also the size of the animals is very small. The composition of mantis shrimp meal is very much influenced by the presence of trashfish, molluscs, crabs, starfish and other biomass, which are generally discorded along with this material.

Fish meal, though available in fairly large quantities at present, it is in great demand as a feed ingredient of other animals, especially poultry. Of late the production of fish meal is on the decline in the country. As a potential raw material for prawn feeds, fish meal production should be encouraged for the development of large scale prawn farming in the years to come.

Fish meal is a good source of protein with essential amino acids and has high biological value. The composition and quality of fish meal varies according to the rawmaterial used for preparing it. Generally fish meal with a protein content of above 60% is considered as good quality fish meal. White fish meal prepared using predominantly silver belleis and anchovies are of high quality compared to the brown fish meal prepared using sardines and other trashfish.

Groundnut cake, which is a versatile feed ingredient of many animals, is available in fairly large quantities. It is a good source of plant protein. The composition of groundnut cake

is known to vary according to the process used for expelling the oil. The cake obtained from mechanical expellers contains higher amount of lipid than that obtained from the solvent extraction process. Seasonal and regional variations are known to cause minor differences in the composition of groundnut cake.

Tapioca is a double action material in prawn feeds. It is a very good source of carbohydrate and at the same time acts as the natural binding agent in aquatic feeds. The water stability of feed pellets prepared with tapioca as binder was tested (Ahamad Ali, 1986) and found that the Pellets prepared with 20% of it were stable for more than 6 hours in the water. Its binding capacity was comparable to chemical binders such as agar agar, sodium alginate and polyvinyl alcohol. Tapioca is available in large quantities in Kerala, Tamil Nadu and Andhra Pradesh.

Taking into account, the good performance of the feed, its low cost, the adequate availability of ingredients, the detailed information available on the techniques of preparation of water stable pellets, the practical feed PFP can be recommended for the large scale culture of the prawn Penaeus indicus.

GENERAL REMARKS

On a perusal of the available information at present, the live feeds, especially, the diatoms seem to meet adequately the requirements for rearing the larvae of penaeid prawns. Mixed culture of phytoplankton are successfully used for rearing protozoea to post larvae. But after post larvae, diatoms alone are not adequate. Introduction of micro-particulate compounded feeds of different types, for rearing the post larvae both in hatchery and nursery until they become stockable size, has helped in largely replacing the use of live feeds such as Artemia, rotifers and other organisms. However, the success of using artificial feeds for rearing the early stages of larvae (Protozoea to Post larvae) is yet to take off on large scale. Eventhough it is possible to prepare a nutritionally balanced feed, the greatest impediment has been the physical design of the diet. The diet should have a particle size below 50 microns, having all the nutrient package in it and these particles should be kept in suspension in the water column without appreciable loss by leaching. Tissue suspensions (Alikunhi et al., 1980, 1982; Hameed Ali, and Dwivedi, 1982) and powdered formula feeds (Mohamed et al., 1983) were used for culture of larvae of penaeid prawns in out door tanks. In these systems, the excess feed helps in the growth of diatoms which are also consumed by the larvae. Nevertheless, it is reported that the technique works successfull resulting in good survival of post larvae comparable to the

survival rates obtained in the conventional systems. It is emphasised that this mixed diet (artificial diet + diatoms grown in the culture tank) technique obviates seperate production of diatoms for this purpose. Further, the same artificial feed could be used for subsequent rearing of the post larvae in hatchery and also in the nursery. It may therefore, be worthwhile that investigations may be carriedout on the comparitive economics of this technique with that of the standard techniques.

In the recent past, considerable technological advancements have been made in the preperation of larval feeds through encapsulation and micro-binding. Reports of rearing the larvae using these diets in the hatchery are available (Kanazawa, 1985; Jones et al., 1987). However, further information on their large scale production and economic use are required before they could substitute the live feeds being used at present for rearing the larvae of prawns.

As juveniles and adult prawns are capable of holding the food and nibble on, pelleted feeds appear to be more appropriate than the soft moist feeds which appear to be more acceptable. Preparation and storage of dry pellets are more practicable. The performance of an aquatic feed depends upon many factors such as its nutritional balance, the ingredients used, the method of preparation and the abiotic properties. It is also important to adopt proper storage procedures of the feed to ensure its shelf life. Based on the requirements of receipient animal, the feed can be nutritionally balanced by the

judicious selection of the ingredients. This could be more effectively achieved by formulating multi-ingredient formula feed rather than single ingredient feeds. If necessary, the feed can be fortified with vitamins and mineral mixtures to meet the requirement.

The range of feed ingredients used for formulating feeds are compiled in Table I. Certain feed stuffs, particularly of plant origin, are known to contain antinutritional factors which may inhibit growth of animals if not removed. Principal among these are soybean, which contains a digestive enzyme inhibiter (Urease), groundnut cake, containing phytates and cotton seed cake which contains gossypol. In addition, isothiocyanates and cyanogenic glycosides are found in linseed and hydrocyanic acid in tapioca. All these anti-nutritional factors are easily destroyed just by heat treatment of the material except the gossypol present in cottonseed cake which should be removed by a special process (Chow, 1978).

In a culture operation, since the cost of the feed constitutes the major expenditure, it should be kept at its minimum possible to make economically viable. But at the same time the nutritional quality of the feed cannot be compromised with. Selection of raw materials plays an important role in containing the cost of the feed. Materials which are used for human consumption should generally be avoided as it creates competition for them and escalates the cost. One of the important criterea for selection of feed ingredients is that they should be available in considerably large quantities with consistant quality.

Employing appropriate technique for preparing feed is essential to ensure its desired quality. Grinding feed ingredients to uniform particle size is imperative to prepare a homogeneous feed mixture. Non-uniformity of particles of ingredients not only results in poor quality of feed pellets but also leads to selective feeding by the animals disturbing the nutrition package of the feed. Grinding the materials into fine powder also helps in improving the digestibility and preparing compact and water stable feed pellets. It was reported that grinding the rawmaterials of feed to about 200 microns had resulted in highest water stability of the pellets, enhanced digestibility and best food conversion ratio (Rani, 1984) in the prawn Penaeus indicus.

As the feed has to be provided under the water column to the aquatic animals, its water stability plays a major role in its performance. Faster disintegration of the feed not only pollutes the culture medium but also leads to wastage of feed resulting in poor conversion ratio. Water stability of crustacean diets using different binding materials have been studied (Meyers et al., 1972; Meyers and Zein-Eldin, 1972; Balazs et al., 1973; Meyers and Brand, 1975; Meyers, 1980). Prominant binding materials used in the prawn feeds are agar agar, alginates (Sodium), gelatin, gums, carboxy methyl cellulose (CMC), Polyvinyl alcohol (PVA), Carrageanan (Polysaccheride), Zein (Corn protein) and starch. Selection of the binding agent has to be done carefully as some of the binders are very expensive and can considerably enhance the cost of the final feed. Natural feed materials such as wheat flour, wheat gluten,

rice flour and tapioca are also used as binders besides being feed components. While tapioca, which contains more than 70% starch, is a good source of carbohydrate in the feed, it is also found to be a potential binding agent (Ahamad Ali, 1986) in prawn feeds. It was found that feed pellets prepared having 20% tapioca were stable under water for more than six hours. Its binding quality compared well with the binding quality of agar agar, sodium alginate and polyvinyl alcohol and was superior to that of pure starch.

Steaming the feed does many a good to it. It not only kills the unwanted micro-organisms and makes the feed inoccuous but also gelatinises the starch present in the feed and improves the digestibility and pellet stability. Steaming also removes some of the antinutritional factors present in certain feed materials. However, steaming the feed at higher temperature, especially under preseure, may bring in undesirable alterations in the feed ingredients and destroy some of the heat lebile components particularly, the vitamins. It is advisable to incorporate vitamins only after the steaming process is completed.

Proper storage of feed is as important as the development of a good feed. The lipid, particularly, the polyunsaturated fatty acids are very much prone to atmospheric oxidation leading to rancidity. The end products, epoxides and hydroperoxides are not only toxic to animals but also render some of the nutrients in the feed unavailable to the recipient animal. Eventhough the lipids contain the natural antioxidants, such as tocopherols, chemical antioxidants like Butylated Hydroxy Anisole (BHA), Butylated Hydroxy Toluene (BHT) and ethoxyquin are used to prevent the lipid oxidation.

Improper storage of feed may lead to insect attack, destruction by rodents and mould growth. This will make the feed unsafe and causes economic loss. Moisture plays a critical role in the storage of feed. The moisture content in the feed should be below 10%. Higher moisture content invites fungal growth which makes the feed stale and musty. Some of the species of fungi (Aspergellus flavus) contaminate the feed with aflatoxin which is poisonous to the animals. Absorption of moisture by the feed, in places where the relative humidity is very high, can be prevented by storing in air-tight containers. Eventhough, potassium metabisulphite (KMS), Sorbic acid, benzoic acid and propionic acid are used in many food stuffs to prevent mould growth, their use in the prawn feed needs to be investigated.

The use of certain growth promoting agents, such as anabolic steroids, hormones, and some antibiotics in the feeds of land animals, especially poultry, is well known. As such there are 'starter' and 'booster' feeds available for poultry farming. Investigations on the possible use of growth promoting agents in the feeds of prawns are very few. Encouraging results were not observed on the use of antibiotics (oxytetracycline) in prawn diets (Corliss et al., 1977; Vaitheeswaran and Ahamad Ali, 1986). It is reported that testosterone, glucosamine, prawn shell, and alfalfa extract had growth promoting effect (Rao et al., 1983; Vaitheeswaran and Ahamad Ali, 1986) in P. indicus. On the other hand thyroid and ethylostrenol (Oestrogen derivative) have no such effect. This is an areawhere work needs to be carried out. Invention of legally acceptable growth

promoting substances may go a long way in achieving faster growth of prawns and increasing the efficiency of the feed.

The concept of Linear Programming (LP), in which the nutritionist first lays down a set of constraints and then lists all available rawmaterials which he wishes to be considered for selection by the computer to achieve the objective of least cost feed formula that will satisfy all the constraints, had been introduced in the animal feed industry some time ago. Its application in finding least cost feed formula for live-stock and poultry has gained widespread acceptance in most countries of the world. Recently LP in the least cost feed formulation for finfish (fresh water) has been attempted. This is a promising area in which the Linear programming techniques have to be introduced in the formulation of prawn feeds. For this purpose, the existing gaps in the knowledge of prawn nutrition need to be completely filled up. Data on the digestibility of common feed stuffs by the cultivable species of prawns, as in the case of present study, need to be obtained and documented, which will help in formulating cost-effective formula feeds for the large scale culture of prawns to augment their production.

TABLE - I. Some rawmaterials and their composition, used for compounding feeds.

Name of rawmaterial	Percent on dry basis				
	Crude protein	Fat/ lipid	Carbohydrate	Crude fibre	Ash
1	2	3	4	5	6
I. Energy feeds:					
Barley	9.9-20.29	2.17	16.34	5.81	2.36
Corn (Maize)	6.2- 9.6	4.3-5.5	69.6-70.7	1.4	1.4
Oats	12.0	2.4	63.7	5.0	--
Rice (whole)	8.4	2.1	76.7	0.7	0.8
Rice (broken)	7.5	0.5	79.9	0.3	0.5
Rice bran	13.24-15.80	18.2	47.43	9.0	14.8
Rye	11.6	1.7	69.8	1.9	2.0
Sorghum (Milo)	11.0	2.8	71.6	2.0	--
Tapioca	2.0	0.54	68.50	--	1.45
Wheat grain	13.07	1.96	63.61	3.91	3.85
Wheat flour	10.80	1.10	74.60	0.20	0.5
Wheat bran	13.90	4.20	55.60	10.50	5.3

TABLE - I (Continued)

1	2	3	4	5	6
II. Protein Supplements:					
(a) Plant materials.					
Brewer's grains	26.0	6.0	41.8	15.0	--
Coconut cake	25.96	11.2	22.19	--	8.88
Cottonseed cake	42.0	2.0	30.0	11.0	--
Distiller's grains	27.0	--	41.0	12.0	--
Gingelly cake	34.03	10.8	24.76	--	12.52
Gluten (Wheat)	25.0	2.0	48.0	8.0	--
Groundnut cake	48.42	7.56	28.18	--	6.03
Linseed	35.0	2.0	39.0	9.0	--
Malt sprouts	26.0	1.0	44.0	14.0	--
Rapeseed cake	46.0	1.0	28.0	14.0	--
Soybean cake	46.0	1.0	31.0	5.0	--
Sunflower cake	47.0	3.0	24.0	11.0	--

TABLE - I (Continued)

1	2	3	4	5	6
(b) Animal materials:					
Blood meal	80.0	2.0	--	--	0.52
Clam meat (Sunneta scripta)	48.10	13.55	16.69	--	7.62
Crab meal	30.0	1.7	--	--	--
Fish meal (Brownfish meal, low protein; white fish meal, high protein)	52-74	1.0-10.0	--	--	14.0-31
Mantis shrimp (Squilla)	44.06	7.55	1.27	--	23.63
Meat meal	53.0	10.0	--	--	12.03
Meat and bone meal	51.0	10.0	--	--	16.07
Mysid meal	76.05	2.72	5.57	--	15.66
Prawn waste meal	35.20	6.60	0.97	--	23.95
Silkworm pupa (i) whole	55.91-57.5	24.5-29.7	5.58	--	2.98
(ii) defated	75.36	1.75	8.40	--	5.59
Shrimp meal	36.0-48.0	3.0	--	--	--
Squid meal	81.38	9.63	5.33	--	3.66

TABLE - I (Continued)

1	2	3	4	5	6
III Non-conventional feed ingredeints					
House fly larvae	45.0	15.0	--	--	8.0
Poultry feather meal (Hydrolysed)	80-85	2.5	--	1.5	3.0
Single cell protein					
(i) Krill	55.0	10-15	--	--	15.2
(ii) Marine yeast	25.63	2.69	63.50	4.27	3.91
(iii) Petrolium yeast	61.22	2.10	26.24	3.9	6.54
(iv) Sludge	43.0	0.43	15.0	28.0	3.0
(v) Spirulina	60.89	9.0	6.63	--	13.0
Snail (Vivipara)	84.93	2.40	--	--	--
Worms:					
(i) Limnodrilus	47.21	24.15	--	--	--
(ii) Tubifex	64.48	16.0	15.40	--	0.9

SUMMARY

Penaeid prawn farming is being practiced in the coastal water impoundments in India to supplement the production of prawns from the capture fishery resources. Adequate seed and appropriate feed are the two important requisites for the culture of prawns. For preparing balanced and low cost feeds, knowledge on the nutritional requirements of the candidate species and the evaluation of locally available raw-materials are essential. In this context, evaluation of different sources of proteins and carbohydrates and the requirement of minerals in the diet of the prawn Penaeus indicus, an important species for culture along the entire Indian coast, has been undertaken in the present study. The data obtained are presented in four different chapters.

1. In the first chapter four purified proteins and nine natural protein sources were evaluated for the juveniles of P. indicus.

2. The purified proteins were albumen (egg), casein, fibrin (blood) and gelatin.

3. Among the natural protein sources, five were animal materials - Clam meat, fish meal, mantis shrimp (Squilla), Prawn waste, Silkworm pupa and four were plant materials - Coconut cake, gingelly cake, groundnut cake and Spirulina (single cell protein).

4. The evaluation of these proteins was carried out through standard methods of nutritional biochemistry by measuring digestibility, biological value (BV), net protein utilisation (NPU), Protein efficiency ratio (PEP) and growth, in

statistically designed feeding experiments.

5. For the first time, endogenous nitrogen excretion (metabolic faecal nitrogen) was determined for penaeid prawns, using zero protein diet. For P. indicus the metabolic faecal nitrogen was found to be on an average 324 mg N per 100 gram diet.

6. With purified proteins, four sets of experiments were conducted in which relative efficiencies of individual proteins and their different combinations were studied.

7. The influence of dietary protein level on its digestibility, BV, NPU, PER and growth was investigated.

8. Comparative studies of protein requirement in the diet were carried out using different protein sources.

9. The significance of dietary protein level in relation to faecal nitrogen, biological value and nitrogen balance was elucidated.

10. Among the purified proteins tested, fibrin and albumen had high biological value for P. indicus, followed by casein. Gelatin was found to be a poor protein source with low BV, for this prawn.

11. Biological value, NPU, PER were high at low dietary protein level and showed decreasing tendency with the increase in the dietary protein level, while the digestibility of protein in the diet tended to be low at lower levels and high at higher levels of dietary protein, though the difference was not statistically significant.

12. The requirement of protein in the diet of P. indicus was found to be 25% with albumen diets and 29% with casein diets.

13. The nitrogen balance (calculated as the difference in nitroge of the diet and the faeces) was zero at 22% dietary protein and whe it was raised a little above this level (3% in case of albumen diet and 7% in case of casein diet), the nitrogen balance was positive, at which the diet had shown the best performance.

14. In the different combination of proteins tested, the diet having all the four proteins, albumen, casein, fibrin and gelatin in equal proportions, gave the best results.

15. From the results, it was concluded that proteins having high BV only should be used for protein requirement study to obtain realistic information. A mixed protein source can be employed for protein requirement study. Albumen alone can be employed for protein requirement study. Casein alone and other combinations may be used only as alternatives.

16. Among the natural protein sources, the animal proteins were significantly superior to the plant proteins.

17. Fish meal, clam meat, prawn waste and mantis shrimp gave good results with high BV, NPU and PER in the decreasing order respectively of the ingredients. Whereas silkworm pupa was found to be a poor protein source.

18. In the plant protein sources, Spirulina and groundnut cake gave the best growth while the BV of the former was higher than that of the latter. However Spirulina diet resulted in very low survival of the prawns.

19. Coconut cake and gingelly cake showed low growth and BV, eventhough their protein had high digestibility.

20. Animal protein sources in general had low digestibility and showed higher growth, PER, NPU and BV over the plant protein sources.

21. The diet having 70% animal protein source and 30% plant protein source gave the best growth and FCR than the diets made exclusively either with animal protein or plant protein source. Under these conditions the diet with 30% protein showed best performance which is in agreement with the results obtained with casein diets.

22. The protein balance, which is the difference between the dietary protein and the protein in the faeces, was negative at lower dietary protein levels and positive at higher dietary protein level.

23. The protein balance in the practical diets was zero at 26% protein and the optimum protein requirement shown by the prawn was just 3.7% above the dietary protein level, where the protein balance was zero.

24. In the second chapter, seven different carbohydrates - three monosaccharides, glucose, fructose, galactose; two disaccharides, maltose, sucrose; two polysaccharides, glycogen and starch, were evaluated by measuring the digestibility, growth and food conversion ratio (FCR).

25. Only disaccharide, maltose and polysaccharide starch were efficiently utilised by P. indicus. Glucose, fructose and galactose gave poor results while the results of the diet with sucrose were slightly superior to that of the monosaccherides.

26. Using a mixed carbohydrate - sucrose, maltose, starch (in equal proportions), which gave the best results, the effect of dietary carbohydrate level on digestibility, growth survival and FCR was studied.

27. The role of carbohydrate level in the diet at constant protein, at constant lipid and at constant protein and lipid was investigated and discussed.

28. The diet with 22.5% carbohydrate produced the best performance at constant protein (34.8%), while at constant lipid (6%), the dietary carbohydrate showed protein sparing action and the diet having 53% carbohydrate and 22% protein gave the highest growth and best FCR. High lipid (16.8%) and low carbohydrate (8.44%) produced low growth and high FCR. Only the diet with 6% lipid and 33% carbohydrate gave the best performance.

29. The results had shown that, the carbohydrate is preferable over lipid for increasing the calorific value of the diet. High energy - Low Protein feeds are more economical without compromising with the performance of the feed.

30. Further, cellulose was found necessary for the efficient utilisation of the diet. Best FCR was obtained with diet having 10% cellulose than the diet having zero percent cellulose. It is concluded that cellulose can be used upto 10% in the diet.

31. The faeces of animals fed with zero carbohydrate diet had considerable amount of carbohydrate. The excretion of carbohydrate in the faeces varied from 50 to 116 mg/g of dry diet consumed. Further investigations are needed to establish whether this is metabolic faecal carbohydrate similar to the metabolic faecal nitrogen.

32. The third chapter deals with the studies on the requirement of six minerals - Calcium, phosphorous copper, zinc, magnesium and manganese, in the diet of P. indicus.

33. The Prawns were fed with diets prepared with different levels of selected minerals and their growth, FCR and survival were measured. The effect of each mineral level in the diet on the body mineral level in animal was investigated.

34. 0.53% of calcium was required in the diet of P. indicus for best growth and FCR.

35. Phosphorous was essential in the diet of P. indicus and it was required at 1.05% level in the diet for the best growth and FCR.

36. The food conversion ratio of the diet was the best when the diet contained 13.6 mg copper per 100 gram diet.

37. The best growth and FCR were obtained by the diet having 23.6 mg of zinc per 100 g. Higher level of zinc in the diet depressed the growth.

38. Magnesium was not found to be required in the diet. Addition of magnesium in the diet decreased growth and increased FCR.

39. The control diet had 0.21 mg of manganese per 100 gram diet which gave the best results. Addition of manganese to the diet depressed growth and increased FCR.

40. Using the information obtained on the nutritional requirements of P. indicus and also the ingredients evaluated in the present study, a purified diet and a practical feed were formulated.

41. While the purified diet was made up of albumen (egg), mixed carbohydrate (sucrose, maltose, starch, in 1:1:1 ratio), cod liver oil, vitamins and minerals, and other additives, the practical feed consisted of prawn waste, mantis shrimp, fish meal, groundnut cake and tapioca, fortified with vitamins. These were fed to P. indicus in long term feeding experiments

in the laboratory and the results were compared to those of the conventional feed, clam meat.

42. The practical feed produced the highest growth and the best FCR, followed by the control feed and the purified diet. However, the purified diet resulted in the highest survival of animals among the three diets tested.

43. The possibilities of using the purified diet as standard basal diet for nutritional studies on prawns in this region, and the practical feed for the culture of penaeid prawns in the nursery and grow-out systems were discussed.

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* Papers not referred in original.

A P P E N D I X - I

Reprints of some scientific papers on Nutrition and compounded feeds for the Prawn Penaeus indicus Published by the author.

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RELATIVE EFFICIENCIES OF PELLETIZED FEEDS COMPOUNDED WITH DIFFERENT ANIMAL PROTEINS AND THE EFFECT OF PROTEIN LEVEL ON THE GROWTH OF THE PRAWN *PENAEUS INDICUS*

SYED AHAMAD ALI

Central Marine Fisheries Research Institute, Cochin-682 018

ABSTRACT

Laboratory experiments with four pelletized feeds, compounded separately with the animal proteins from prawn waste meal, mantis shrimp protein, clam meat powder and fish meal in combination with the plant protein from groundnut cake, were conducted to study the relative efficiency by feeding juvenile *Penaeus indicus*. The animal and the vegetable proteins in each feed were approximately adjusted in the ratio 1 : 1. Tapioca powder was used as the source of carbohydrate as well as the binding agent. The control experiment was carried out with the feed prepared solely from fresh clam meat. Feeds with mantis shrimp protein and clam meat powder gave high increase in live weight and good food conversion values followed by the feeds with fresh clam meat, prawn waste meal and fish meal.

Feeding experiments with pelletized feeds, consisting of mantis shrimp protein, groundnut cake and tapioca powder with crude protein content ranging from 20.5% to 46.5% were conducted on the juvenile *P. indicus*. Progressive increase in the live weight gain was recorded with the increase in the crude protein level upto 42.9% and declined thereafter, while the protein efficiency ratio was the highest at 20.5% crude protein level.

INTRODUCTION

THE white prawn *Penaeus indicus* has been identified as one of the most suitable species for intensive culture in coastal aquaculture practices. The development of a suitable feed is an important pre-requisite for the successful culture operations. For that the basic knowledge on the nutritional requirements of the prawn is essential. Commendable work has been done in this direction (New, 1978) and a number of feeds have been patented in various countries of the world. Very often these feed formulations cannot be directly utilized due to either the non-availability of the raw materials or their prohibitive cost.

Protein forms the most important constituent in prawn nutrition. Several workers have conducted studies on the protein requirement of different species (Deshimaru and Shigeno, 1972 : Sick *et al.*, 1972; Venkataramaiah *et al.*, 1975 ; Balazs and Ross, 1975 ; Colvin, 1976).

In the present study, an attempt has been made to study the relative efficiencies of some of the locally available animal protein materials to be included in prawn diets and the protein requirement in the feeds in terms of the raw materials for the culture of *P. indicus*.

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Materials and Methods

Raw materials

The prawn waste includes head and exoskeleton obtained from the peeling sheds. Mantis shrimp protein was prepared from *Oratosquilla nepa*. The wet material was boiled in water and the coagulated protein was separated and dried (Garg *et al.*, 1977). This material was obtained from the Central Institute of Fisheries Technology, Cochin. The clam (*Vellorita cyprenoidea*) meat was separated from the shells by heating and the whole meat was dried. Fish meal was obtained from the Kerala Fisheries Corporation, Azhikode. Groundnut cake and Tapioca (*Manihot utilissima*) were procured from the local market. All the raw materials were dried in an oven at 70-80°C and ground in an electrical grinder and sieved through 60 mesh sieve. Fresh clam meat (*Sunneta scripta*, 82% moisture), stored in the deep freeze, was given as the control diet.

The crude protein contents of the raw materials, determined by the micro Kjeldahl method, are given in Table 1.

Preparation of the experimental feeds

In each feed the animal and the plant proteins were approximately adjusted in the ratio 1:1. Groundnut cake was the common plant protein source in all the feeds. Tapioca powder was used as the main source of carbohydrate as well as the binding agent. Calcium lactate and potassium dihydrogen phosphate and multivitamins were included in all feeds.

Tapioca powder was first cooked with 40-50% of water for 10-15 minutes until the starch gelatinised. The other premixed ingredients were added to the paste and thoroughly mixed into a dough. The dough was passed through a 3 mm diameter die in a screw press. The pellets were dried at 70-80°C and found to be quite water stable.

Rearing

The juveniles of *Penaeus indicus*, used in the feeding experiments were collected from the backwater canals around the Vypeen Island. The animals were acclimatized for 5-7 days and starved for one day before the start of the experiment.

Feeding experiments were conducted in 50 litre capacity circular plastic troughs. Ten animals were stocked in each trough. The troughs were covered with velon screens to prevent the animals from jumping out. The sediments were removed regularly and the water was changed once in three days. Aeration was provided with an air compressor.

Feeding was done at the rate of 10-15% of the live body weight once in a day in the evening hours in petri dishes kept at the bottom in the middle of the trough. The food left

Table 1. *Crude protein values of the raw materials*

Ingredients	Prawn waste meal	Mantis shrimp protein	Clam meat powder	Fish meal	Ground-nut cake	Tapioca
Crude protein content, % on dry basis	35.2	59.2	48.1	60.7	48.5	2.0

over was recovered every day before feeding. Weight and measurements were taken every ten days. No attempts were made to control the environmental conditions.

Feeding experiment I

Four experimental feeds 1, 2, 3 and 4 were compounded separately with the animal proteins from prawn waste meal, mantis shrimp protein, clam meat powder and fish meal in combination with the common plant protein from groundnut cake. The ingredients in each feed were adjusted to give the same crude protein content of 35%. The percentage composition of the feeds is given in Table 2.

Prawns with an average body weight of 100 mg were separately fed with feeds 1-4 and fresh clam meat for 30 days. For each feed two sets of experiments were run concurrently.

The salinity of the water increased from 1.8‰ to 7.5‰. The temperature ranged between 28.5°C to 32.5°C and the pH between 8.0 and 8.7.

The results of the feeding experiments are given in Table 3.

TABLE 2. *Percentage composition and crude protein values of the experimental feeds* 1-4

Ingredients %	Experimental feeds			
	1	2	3	4
Groundnut cake	35.0	38.0	40.0	35.0
Tapioca powder	17.0	31.0	19.0	37.0
Prawn waste meal	45.0	—	—	—
Mantis shrimp protein	—	28.0	—	—
Clam meat powder	—	—	38.0	—
Fish meal	—	—	—	25.0
Mineral mix*	2.0	2.0	2.0	2.0
Vitamin mix**	1.0	1.0	1.0	1.0
Total	100.0	100.0	100.0	100.0
Crude protein %	34.4	35.0	33.3	33.9

* Mineral mix : Each kg of the feed contains Calcium lactate 14 g ; Potassium dihyrogen phosphate 8g ; Ferrous sulphate 106 mg, Magnesium phosphate 480 mg.

** Vitamin mix Each kg of the feed contains vitamin A 40000 I.U., Thiamin mononitrate 100 mg, Riboflavin 20 mg, Nicotinamide 100 mg, Cynocobalamine 10 mg, Ascorbic acid 50 mg, Calciferol 4000 I.U., vitamin E 15 mg, Biotin 0.5 mg.

TABLE 3. *Results of the feeding experiment* 1 *fed with the feeds* 1-4 *and fresh clam meat for* 30 *days*

Description	Experimental feeds				Fresh clam meat
	1	2	3	4	
Initial average body weight (mg)	100	100	100	100	100
Final average body weight (mg)	380	513	497	250	400
Increase in average body weight (mg)	280	413	397	150	300
Percentage increase in average body weight ..	280	413	397	150	300
Average growth (mg/day)	9.30	13.80	13.20	5.00	10.00
Total food consumed (g)	6.38	5.16	5.79	5.62	—
Average food ingestion (mg/animal/day) ..	21.30	23.60	19.30	21.00	—
Food conversion* ..	2.27	1.71	1.46	4.20	—
Survival %	100	80	100	70	30

* Food conversion : $\frac{\textit{Average rate of food ingestion}}{\text{Average growth rate.}}$

Feeding experiment 2

Experimental feeds 5, 6, 7, 8 and 9 were prepared consisting of mantis shrimp protein, groundnut cake and tapioca powder with the crude protein contents of 20.6, 28.5, 35.0, 42.9 and 46.5% respectively. The percentage composition of the feeds 5-9 is presented in Table 4.

TABLE 4. *Percentage composition and crude protein values of the experimental feeds* 5-9

Ingredients	Experimental feeds				
	5	6	7	8	9
Mantis shrimp protein	14.0	21.0	28.0	35.0	42.0
Ground nut cake	19.0	28.5	38.0	47.5	57.0
Tapioca powder	64.0	47.5	31.0	14.5	10.0
Mineral mix	2.0	2.0	2.0	2.0	2.0
Vitamin mix	1.0	1.0	1.0	1.0	1.0
Total	100.0	100.0	100.0	100.0	112.0
Crude protein %	20.6	28.5	35.0	42.9	46.5

The animals with an average body weight of 200-230 mg were fed separately with feeds 5-9 for 30 days. For each feed three sets of experiments were run concurrently.

The salinity of the water used in the experiment ranged from 17.3‰ to 20.3‰, temperature varied between 30.8 to 32.5°C and pH between 7.9 and 8.0. The results are presented in Table 5.

RESULTS

In the first experiment, feeds 2 and 3 with mantis shrimp protein and clam powder topped among the protein materials tested by producing an average weight increase of 313% and 297% and an average growth of 13.8 and 13.2 mg/day respectively (Table 3). These were followed by fresh clam meat (200% and 10 mg/day), feed 1 with prawn waste (181% and 9.3 mg/day) and feed 4 with fish meal (50% and 5 mg/day). Clam powder gave the best food conversion (1.46) followed by mantis shrimp protein (1.71), prawn waste (2.27) and fish meal (4.2). The food conversion in the case of fresh clam meat was not determined, as there was heavy mortality.

The average rate of food ingestion was the highest in the case of mantis shrimp protein (23.6 mg/day/animal) and the lowest in the

TABLE 5. *Results of the feeding experiment* 2 *fed with the feeds* 5-9 *for* 30 *days*

Description	Experimental feeds				
	5	6	7	8	9
Initial average body weight (mg)	210	200	230	200	200
Final average body weight (mg)	590	590	700	890	800
Increase in average body weight (mg)	380	390	470	690	600
Percentage increase in average body Weight	181	195	204	345	300
Average growth (mg/day)	12.70	13.00	15.70	23.00	20.00
Total food consumed (g)	8.66	7.05	8.97	8.88	8.77
Average rate of food ingestion (mg/animal/day)	28.90	23.50	29.90	29.60	29.20
Food conversion	2.28	1.80	1.90	1.29	1.46
Protein efficiency ratio*	2.10	1.96	1.50	1.50	1.18
Survival %	100	100	100	100	100

* Protein efficiency ratio : Live weight gain per gram of protein consumed.

case of claim powder (19.3 mg/day/animal), while for prawn waste and fish meal the food intake was 21.3 and 21.0 mg/day/animal respectively.

Weight gain increased with time (Fig. 1) in general. The increase in weight was the highest in the last ten days and lowest in the

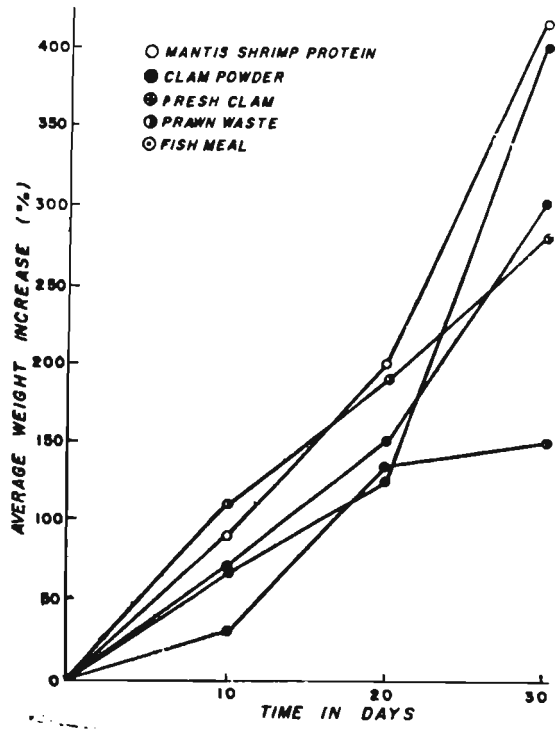

Fig. 1. Growth curves of the prawn *P. indicus* fed on the feeds compounded separately with prawn waste, mantis shrimp protein, clam meat powder, fish meal and fresh clam meat.

first ten days in the case of feeds 2, 3 and fresh clam meat, whereas in the case of feeds 1 to 4 it was vice versa. In the case of fresh clam meat frequent moulting, heavy mortality and cannibalism were observed.

In general the animals were immediately attracted to all the foods. However, the preference was observed for prawn waste, mantis shrimp protein, fresh clam meat, clam powder and fish meal in the decreasing order.

The gain in live-weight increased with the increase in crude protein level in the diet upto 42.9% (Table 5) and declined thereafter in the second experiment. The feed with 42.9% crude protein gave the highest increase in live weight gain (345% and 23 mg/day) and best food conversion (1.29). The protein efficiency ratio declined gradually with successive increase in the dietary protein level.

There was a sharp increase in weight between 35.0 and 42.9% crude protein levels, while the increase in weight from 20.6 to 35.0% protein levels was gradual (Fig. 2, curve A) and again there was a gradual decline between 42.9 and 46.5% protein. The protein utilisation gradually declined from 20.6 to 35.0 crude protein level (Fig. 2, curve B); it was steady between 35.0 and 42.9% and it sharply declined between 42.9 and 46.5%.

Discussion

Mantis shrimp protein and clam powder gave almost identical growth rates, while clam powder gave better food conversion. But both are superior to fresh clam meat. The growth obtained by prawn waste is comparable to that of the fresh clam meat. Venkataramaiah *et al.* (1978) observed that *Penaeus aztecus* fed with shrimp waste pellets gave good results. Sandifer and Joseph (1976) found waste shrimp heads (*P. setiferus*) were a good source of fatty acids and pigments in the diets for *Macrobrachium rosenbergii*. Similar results were obtained by Forster and Beard (1973) for *Palemon serratus*. Prawn waste protein is reported to be having several essential amino acids (Foster, 1975). Fresh clam meat failed to give superior growth results compared to that of the compounded diets with mantis shrimp protein and clam powder. Kanazawa

et al. (1970) reported that the fresh diet of short-necked clam (*Tapes philippinarum*) gave superior growth compared to the compounded diets for *Penaeus japonicus*. Similar results were obtained by Forster and Beard (1973) for *Palaemon serratus*. But Venkataramaiah *et al.* (1975) found that although the live foods such as *Artemia* nauplii and shrimp meat gave superior growth in brown shrimp, a high rate of chitinoclastic bacterial infection, leading to heavy mortality was observed in these experiments. The relatively poor performance obtained by the fresh clam meat in the present study may be due to similar reasons.

Frequent moulting was observed during feeding experiments with fresh clam meat suggesting that this contains a factor which induces moulting. The probable factor may be the iodine rich thyro-proteins in the fresh clam (Personal discussion with Kai W. Chow, Aquaculture Department and Co-ordination Prog., FAO).

Fish meal gave comparatively poor results as reported earlier by Deshimaru and Shigeno (1972) and Colvin (1976). The former workers found that the amino acid composition of fish meal was not similar to that of the prawn *P. japonicus*. The latter suggested that the relative deficiency of the amino acids, tyrosine and phenylalanine in the fish meal may be the reason for its relatively poor performance.

Prawns were observed to prefer prawn waste. The odour it possesses may be attracting the animals and making the feed more palatable.

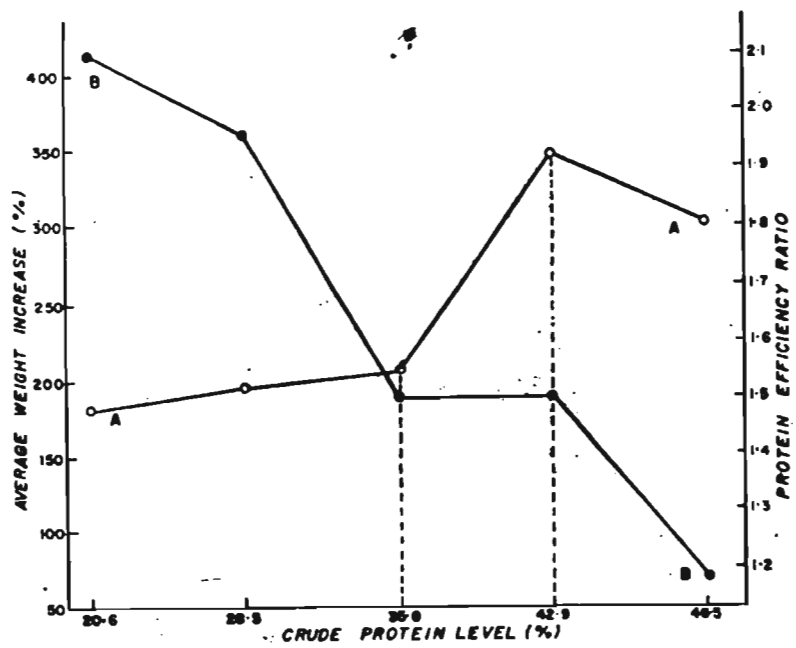

Fig. 2. Growth, dietary protein level and protein efficiency ratio curves of the prawn *P. indicus*: A. Relationship between the dietary protein level and live-weight increase and B. Relationship between dietary protein level and protein efficiency ratio.

The protein quality of the prawn waste may be further improved by blending it with other high quality protein materials such as mantis shrimp, in suitable proportions.

Deshimaru and Shigeno (1972) observed that the growth of *P. japonicus* was found to correlate with the amount of crude protein in the diet. A similar trend was observed by Balazs and Ross (1976) that high protein content produced larger prawns (*M. rosenbergii*). Venkataramaiah *et al.*, (1975) found best growth with the food containining 40% protein (*P. aztecus*) and Colvin (1976) recorded highest live-weight gain with a 43% protein diet (*P. indicus*). The findings of experiments carried out in the present study conform to the above results. The growth values recorded in the present study (23 mg/day with initial weight 0.2 g) are comparable to that of Colvin (1976, 44 mg/day with intial weight 0.95) taking into consideration the initial mean weights of the prawns. The food conversion values obtained are comparatively superior.

The optimum protein level in the diets for penaeid prawns appears to be between 35 and 40%. This is indicated by the steep rise in weight increase (Fig. 2, curve A) between 35.0 and 42 9%, while the increase in weight was gradual in the lower protein levels. This is further supported by the fact that the protein utilisation is steady (Fig. 2, curve B) between the two protein levels. Results from other workers confirm that protein levels compatible with maximum or near-maximum growth were between 30 and 40% for penaeid prawns (Sick *et al.*, 1978 ; Forster and Beard, 1973 ; Venkataramaiah *et al.*, 1975). In general it is observed that the high protein levels in the diet beyond the optimum level do not produce significant increase in growth or the growth is not proportional to the increase in protein level. The probable explanation for this may be that the increase in protein decreases the quantity of other energy giving nutrients such as fat and carbohydrate in the diet, from which the animals normally derive most of the energy required for their metabolic activities. In that case the protein in excess of the optimum level is mostly utilised for the metabolic energy required and not for tissue growth. This is indirectly supported by the fact that the protein utilisation, in terms of live-weight gain, declines with the increase in the dietary protein level. However, this needs further study to establish the fact.

The results of the present study indicate that the quality of the protein in the diets depends upon its source and influences the growth. This is in accordance with the findings of Forster (1975). Therefore for the practical utilization, it will be more realistic to select the suitable raw materials available in the region concerned and find the nutritional requirements in terms of the raw materials, instead of purified protein materials, such as casein. For the growth and food conversion obtained by the diets containing casein at a particular protein level may be entirely different from that of the diets containing the selected raw materials depending upon their protein quality.

The results of the present study show that the mantis shrimp protein is one of the high quality animal protein sources for diets. Little Information is available in the literature about the use of mantis shrimp in prawn diets. Considerable quantities of mantis shrimp are landed along with the prawns. But most of it is not properly utilised. The utilisation of mantis shrimp as one of the prawn feed ingredients may be a promising proposition.

Conclusion

Mantis shrimp protein and dry clam powder may be considered high quality animal protein sources to be used in the prawn diets. Prawn waste provides desirable flavour and palatability in the diets for prawns. Fresh clam meat contains a factor which induces moulting in the

juvenile prawns. Fish meal alone may be a relatively poor animal protein source for prawns. Maximum growth rate is obtained with diets containing 9% crude protein and the optimum protein level in the diets for penaeid prawns lies between 35-40%.

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EFFECT OF CARBOHYDRATE (STARCH) LEVEL IN PURIFIED DIETS ON THE GROWTH OF *PENAEUS INDICUS*

SYED AHAMAD ALI
Central Marine Fisheries Research Institute, Cochin.

ABSTRACT

Four purified diets were formulated using casein, gelatin, starch, fish oil, groundnut oil, vitamins, minerals and cellulose. The carbohydrate in the diets was increased from 10 to 40% by varying the starch content to study the effect of dietary carbohydrate level on the growth, survival and food conversion of the prawn *Penaeus indicus* The protein and the lipid contents were kept constant in all the diets. Feeding experiments conducted in the laboratory on the juvenile *P. indicus* indicated that the growth of prawn increased with increase in the dietary carbohydrate level from 10 to 40% ($P < 0.01$). The food-conversion efficiency and the rate of survival improved with the increase of carbohydrate level in the diet. The growth also increased with the increase of carbohydrate calorie ratio, while it was better with lower protein carbohydrate ratio of the diets.

INTRODUCTION

Carbohydrate is a cheap source of energy in the diet of animals, including fish and prawns. If a large percentage of the metabolic energy requirements of the animal can be met from the carbohydrate, the more expensive protein can be spared for growth. The protein sparing action of the carbohydrate in the diet at 30% level was reported by Andrews et al (1972) in *Penaeus aztecus*. Sick and Andrews (1973) found that 40% corn starch in casein-based diets produced faster growth in *P. duorarum*. The carbohydrate requirements of the Indian white prawn *P. indicus* are not known. With a view to finding out the effect of dietary carbohydrate level on the growth and food conversion of *P. indicus*, the present study had been carried out. Since Abdel Rahiman et al (1979) had reported that the polysaccharides like starch, dextrin and glycogen gave better growth, along with disaccharides, in *P. japonicus*, starch was selected as the source of carbohydrate in the present study.

MATERIAL AND METHODS

Fat-free casein was obtained from Centron Laboratories, Bombay. Gelatin used in the diets was obtained from B.D.H., and starch from Merck. Sardine oil and refined groundnut oil were locally purchased. The vitamin mixture was obtained from Roche Pharmaceuticals; the mineral mixture was prepared in the laboratory. Cellulose powder was procured from Johnson Chemicals, Bombay.

Preparation of the diets

Four purified diets (C1, C2, C3 and C4) were formulated using casein, gelatin, starch, a mixture of sardine oil and refined groundnut oil (in ratio 1 : 1).The starch content was varied from 10 to 40% in the diets C1 to C4. Cellulose powder was used as the non-nutrient filler. Vitamins and minerals were included in all the feeds. No extra binding material was used since all the diets contained gelatin and starch. The protein content in all the diets was kept approximately at 35%. By virtue of variation in the carbohydrate content, the protein carbohydrate ratio varied from 3.5 to 0.92 in the diets from C1 to C4 while the carbohydrate calorie ratio changed from 2.079 to 8.831. The percentage composition of the diets is given in table 1.

The starch and the gelatin were dissolved in 80 ml (for 100 g of diet) of water and cooked into a paste, while the other ingredients were separately

TABLE 1. *Percentage composition of the diets.*

Ingredients %	Diets			
	C_1	C_2	C_3	C_4
Casein	30	30	30	30
Gelatin	06	06	06	06
Oil *	06	06	06	06
Starch	10	20	30	40
Vitamin and mineral (mix **)	03	03	03	03
Cellulose powder	45	35	25	15
Total	100	100	100	100
Proximate composition % on dry basis				
Crude protein	35.75	33.25	37.62	36.75
Lipid	6.00	6.00	6.00	6.00
Carbohydrate (Starch)	10.0	20.0	30.0	40.0
Ash	4.0	4.25	4.03	6.25
Calorific value (K cal/g)	4.81	4.79	4.66	4.53
Protein carbohydrate ratio	3.50	1.75	1.25	0.92
Carbohydrate calorie ratio	2.079	4.166	6.437	8.831

* The oil used consists of a mixture of fish oil (Sardine) and groundnut oil in the ratio 1 : 1.

** The mineral and vitamin mixture : Eevery 500 g consists of Vitamin A 6,25,000 I.U; Vitamin D^3, 62,500 I.U; Vitamin E, 250 I.U; Vitamin B2, 200 mg, Calcium, 126.0 g; Phosphorous, 90.0 g, Copper, 1.25 g, Iodin as iodate, 0.25 g. Manganese 1.00 g, Cobalt 0.10 g and Zinc 0.50 g.

powdered and passed through a 30-mesh sieve and added to the paste. The mixture was then thoroughly homogenised and passed through a 3mm-diameter extruder. The extruded part was dried in an electric oven at 65 ± 2° for 12 h, and stored in air tight plastic containers until use as diet.

The crude protein of the diet was determined by Kjeldahl method and the calorific value was determined using bomb calorimeter.

Feeding experiments

Juveniles of *P. indicus* from the same brood, reared in the prawn culture laboratory of CMFRI, Narakal, were randomly selected and used in the experiments. The average initial length was about 27 mm and weight 0.1 g.

The animals were stocked in 3'-diameter collapsable plastic pools with 300 l sea water, filtered through boltingsilk. In each pool, 20 animals were kept, with two replicates for each treatment. Aeration was provided intermittently in all the pools. The water in the pools was changed every six days.

Feeding was done at the rate of 25% of the body weight per day, to start with. But subsequently, feeding was adjusted, to 15 to 10% of the body weight per day, according to the left over food. Feeding was done once a day, in the evening. The feeding experiment was carried out for a period of 30 days.

The salinity of the water was maintained at 16 ± 1‰ throughout the experiment. The temperature ranged between 26°C to 27°C and the pH of the water varied from 7.9 to 8.0 during the experimental period.

Results

The results of the feeding experiment are presented in table 2. It can be seen that the growth of the prawns, both in length and weight, gradually increased (from 62.3% to 147.6%) with the successive increase in the carbohydrate level of the diets. The diet with 40% starch recorded the highest growth (fig. 1 curves A and B). The food conversion efficiency improved with the increase in the dietary carbohydrate level up to 30%. There is a slight decline theafter. The percentage of survival is maximum (95%) at 40% carbohydrate level.

The experimental data were subjected to statistical analysis and were analysed by the method of analysis of non-orthogonal data by fitting constants. For this, the model used was:

$$Y_{ijk} = \mu + a_i + b_j + (ab)_{ij} + e_{ijk}$$

Where Y_{ijk} is the length of the K^{th} individual in the j^{th} replicate of the i^{th} diet level.

a_i the effect of i^{th} diet level.

b_j = the effect of j^{th} replicate.

$(ab)_{ij}$ the interaction effect.

e_{ijk} = random error component.

The analysis is summarised in table 3. It can be seen that the interaction is highly significant along the treatments and replications, indicating that treatments and replicates are not independent. That is, there is a differential response to treatments over the replications and vice versa. From individual mean composition, it is observed that treatments significantly differ among themselves, diets C3 and C4 scoring over C1 and C2. Thus there is increase in growth with the increase in carbohydrate level in the diet ($P < 0.01$) from C1 to C4.

The results also indicate that the low protein carbohydrate ratio and high carbohydrate calorie ratio produce higher growth rates (Fig. 2. curves C, D, E, and F) and survival and improve the food conversion efficiency of the diet.

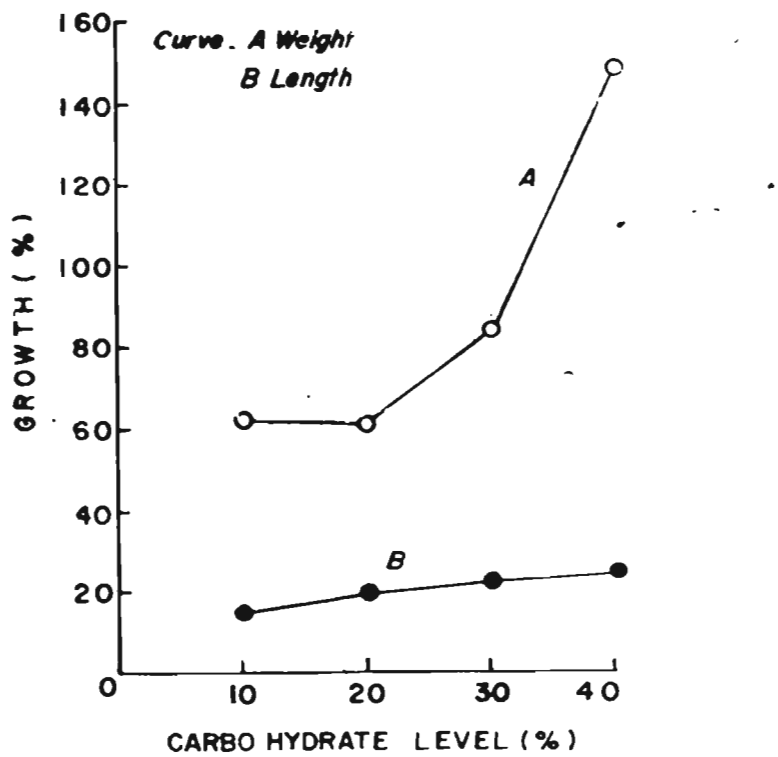

FIG. 1. Relationship between the dietary carbohydrate level and growth

TABLE 2. *Data on the feeding experiment with feeds C1 to C4 on P. indcus for 30 days.*

	Diets			
	C_1	C_2	C_3	C_4
Initial mean length (mm)	27.05	27.37	27.40	27.87
Initial average weight (g)	0.105	0.105	0.105	0.105
Final mean length (mm)	31.01	32.68	33.43	34.62
Final average weight (g)	0.170	0.169	0.193	0.260
Percentage increase in length	14.70	19.30	21.90	24.30
Percentage increase in weight	62.30	61.0	84.20	147.60
Percentage survival	72.5	87.5	82.5	95.0
Food conversion*	11.15	5.55	4.07	4.25

* Food conversion = $\frac{\text{Weight of food consumed}}{\text{live weight gain}}$

TABLE 3. *Statistical analysis : ANOVA*

Source	Degrees of freedom	Sum of squares S.S.	Mean square
Feed levels	3	233.5612	77.8537 X X
Replicate	1	14.9718	14.9718 X X
Treatments	3	187.8687	62.6226 X X
Errors	129	811.3187	6.2893

X X = Highly significant ($P < 0.01$)

DISCUSSION

The increase in the carbohydrate level in the diets enhanced the growth in prawns. This may be due to the protein sparing action of carbohydrate in which the increased levels of carbohydrate in the diet might have provided larger quantums of energy required for the metabolic activities of the animals, while more and more protein had been spared for growth. The enhanced growth at higher levels of carbohydrate might be responsible for better food conversion values. At the lower levels of carbohydrate in the diet, the aminals might be deriving the metabolic energy partly from protein, thus accounting for lesser growth even though the protein and lipid levels in the diets at lower and higher carbohydrate levels were the same.

The diet with 40% carbohydrate produced the highest growth which is maximum level tested. Andrews et al (1972) have reported that 30% starch in the diet gave better growth and spared protein in the diet in *P. aztecus*. Sick and

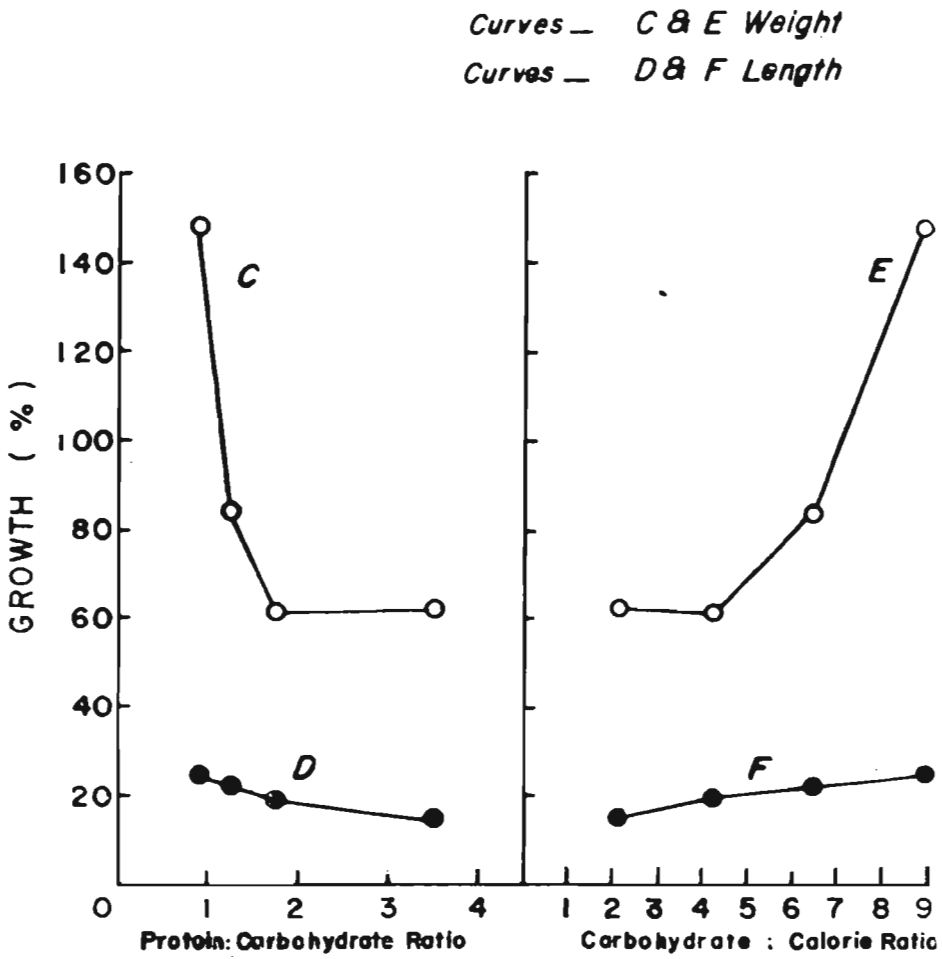

FIG. 2. Relationship between growth, protein-carbohydrate ratio and carbohydrate- calorie ratio

Andrews (1973) found that 40% corn starch in the casein-based diets produced faster growth in *P. duorarum*. The results obtained in the present study on *P. indicus* are in agreement with these findings.

The performance of the diet can be improved by reducing the protein carbohydrate ratio. As indicated in the present study (Fig. 2 curves C and D), it can be less than unity. That is, the carbohydrate level in the diet can be as much as that of the protein or even more. The growth of prawns also gradually

increased with the increase in carbohydrate calorie ratio (Fig. 2 curves E and F). These relationships indicate that by increasing the carbohydrate level (up to 40%) in the diet, the protein can be spared and the calorific value of the diet can be maintained and thus improve the overall performance of the diet.

The growth of prawns obtained with purified diets in the present study (147.6%) is low compared to the growth obtained in the laboratory experiments in *P. indicus* (413% to 690%), with feeds compounded with natural ingredients reported earlier by the author (Ahamad Ali 1980). Similar inferior growth was reported in prawns with purified diets prepared using casein, amino acid, mixtures and peptides by Kanazawa, et al (1970), Sick et al (1972) and Deshimaru and Kuroki (1974 c, 1975 a and b).

The use of cellulose powder as a non-nutrient filler in purified diets appears to affect the palatability of the diets for prawns in the present study. The maximum levels of cellulose that can be used in the purified diets without affecting the palatability needs further investigation.

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POSTER PAPER

COMPOUNDED FEEDS FOR POSTLARVAL REARING OF MARINE PRAWNS

SYED AHAMAD ALI AND M. G. SIVADAS
Narakkal Prawn Cultute Laboratory of CMFRI, Cochin-682 505

Availability of appropriate feed has been the main constraint for rearing the various stages of prawn larvae. This is largely overcome by the use of live feed cultures. But the maintenance of these live feed cultures require specialised technical inputs, labour and time. Compounded feeds have been tried for rearing prawn larvae with varied success in different countries of the world. Attempts are being made to develop compounded feeds for rearing different stages of prawn at the NPCL. One of the successful feeds developed for the post-larval rearing has been described here.

Several feeds were prepared and pelletized with protein contents ranging from 30 to 60%, using clam meat, groundnut oil cake, fish meal, mantis shrimp *(squilla)*, trash fish, yeast and cassava (tapioca). The feeds were prepared by mixing dry' powdered ingredients with 50% water and steaming for 15 minutes. The homogenised wet dough was extruded in 1 mm diameter pellets and dried in oven at 70 $\pm$ 2° C for 12 hours. The dry pellets are stored in polythene bags. By keeping the moisture content below 10%, the feeds could be stored for a period of six months.

Using these feeds several experiments were conducted in which over 1,50,000 postlarvae of *Penaeus indicus* were stocked and reared in 24' dia. pools. Among the feeds tested, the feed PLF - 3 consisting of mantis shrimp 20%, prawn waste 20%, groundnut oil cake 30%, fish meal 10% and cassava 20% with a protein content of 36 8% gave the best performance in terms of growth and survival. The postlarvae were reared using this feed from PL_5 to PL_{20} with a survival rate of 90.3% and the larvae had grown from an initial average length of 6 0 mm to a final average length of 18.0 mm. Feeding was done at the rate of 100% of the body weight for the first two days and it was gradually brought down to 10% of the body weight finally. The cost of preparation of the feed was approximately Rs. 2/- per kilogram. The details of the experiments conducted and the results obtained using this feed are illustrated in the posters. O

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Edited by:

K. Ravindran
N. Unnikrishnan Nair
P. A. Perigreen
P. Madhavan
A. G. Gopalakrishna Pillai
P. A. Panicker
Mary Thomas

Society of Fisheries Technologists (India)
Matsyapuri P. O., Cochin-682 029, India

1985

Utilisation of Prawn Waste and Mantis Shrimp for Compounding Feeds for the Culture of Penaeid Prawns

SYED AHAMAD ALI and K. H. MOHAMED

Central Marine Fisheries Research Institute, Cochin - 682 018

Waste materials like prawn waste (Protein 38%, energy 3.69 Kcal/g) and mantis shrimp (Protein 45%, energy 3.72 Kcal/g) were identified for providing animal protein in compounded feeds for culture of penaeid prawns. Four different feeds were formulated and pelletized using prawn waste, mantis shrimp, groundnut cake and cassava to evaluate their acceptability as feeds. These feeds were tried on the Indian white prawn *Penaeus indicus* and its growth and food conversion efficiencies were found out. The efficiency of these feeds were compared with the results obtained by the use of conventional feed of fresh clam meat taken as control. Short term feeding experiments conducted on juvenile *P. indicus* at the Narakal Prawn Culture Laboratory showed that the feed No. $NPCL_{21}$ having a composition of prawn waste 25%, mantis shrimp 35%, groundnut cake 20% and cassava 20% gave the highest growth rate (length increase 75.7%, weight increase 400%). Among the feeds tested the highest food conversion efficiency of 3.22 was also obtained by the use of this feed. The feed was readily accepted by the prawn and that their growth was far superior in comparison with the growth parameters obtained in the control.

The nutritional values and characteristics of these two low cost animal protein sources as feed ingredients have been discussed and detailed methods of preparation described.

Appropriate feed is the most important pre-requisite for the development of scientific prawn culture through its various stages. Earlier workers in Japan used clams and mussels for feeding prawns (Fujinaga, 1935; Hudinaga, 1969) in culture systems. Later, through the development of balanced compounded feeds (Deshimaru & Shigano, 1972) it had been possible to develop intensive culture of Kuruma shrimp *Penaeus aponicus* (Shigeno, 1975). Subsequently several studies were conducted in different parts of the world, on penaeid prawns to develop feeds (Cook & Murphy, 1969; Cook, 1973 and New, 1976) using various feed stuffs available in the concerned region. Several compounded feeds have also been patented by various workers in different countries for culturing of aquatic organism on commercial scale.

In recent years high priority is given for the development of marine prawn culture in India, utilising the coastal swampy areas, to augment the production. Among the several candidate species suitable for culture (Forster & Beard, 1974) the Indian white prawn *Penaeus indicus* has been identified as the most suitable species for culture in coastal waters. Suitable hatchery techniques have been developed for mass production of the seeds of this species at the Prawn Culture Laboratory of Central Marine Fisheries Research Institute, Narakal.

With a view to develop low cost nutritionally balanced feeds for the culture of *P. indicus*, locally available low cost materials like prawn waste, mantis shrimp, groundnut cake and cassava (tapioca) were identified as the feed ingredients. The acceptability and the relative efficiencies of these feed materials for the prawn were already reported by Ahamad Ali (1980). The present study is taken up for exploring diversified utilisation of these products for compounding low cost feeds for the culture of penaeid prawns in the grow out systems.

Materials and Methods

Raw materials used were prawn waste, mantis shrimp, groundnut cake and cassava (tapioca). Prawn waste consists of the entire exhoskeleton, the head, eyes and eyestalks, hepato pancreas, gills, residual meat in head and the intestine of prawns. For feed compounding this conglamorate material was collected and brought to the laboratory and was sun dried on cement platform without washing. When fully dried it was made into a fine powder using micro pulverizer.

The mantis shrimp is a stomatopod caught in abundant in the early fishing season for prawns. Large quantities of *Orato squilla nepa* occur in the trawl catches along with prawns during November to January. During the season tonnes of this species are sorted out and discarded in the fishing harbour and other trawler landing centres. The meat content of the animal is very low and major portion of its body consists of

chitin. These were collected, dried and powdered in the same way as prawn waste. The cassava powder as well as the groundnut cake were obtained from the local market.

Preparation of feeds

Four feeds nos $NPCL_{20}$, $NPCL_{21}$, $NPCL_{22}$ and $NPCL_{23}$ were formulated using the above ingredients in different proportions keeping the protein level at 34%. The percentage of prawn waste and mantis shrimp were varied from 15 to 45% while groundnut cake and cassava were kept constant in all the four experimental feeds (Table 2). The powdered ingredients were weighed separately and then mixed and homogenized in an electrical blender. Water (900 ml for 1 kg of feed) was added and cooked at 1 psig for 15 minutes. The feeds were then kneaded into a dough and extruded through 3 mm diameter extruder and dried in the oven at 70 ± 2°C for 12 h. The control feed was prepared from clam *(Sunneta scripta)* meat.

The crude protein was estimated by Kjeldahl method, lipid was estimated by using methanol chloroform mixture (1:2 ratio) and moisture and ash were determined by the A.O.A.C. methods. The calorific value was determined by the bomb calorimeter. While calcium was estimated by the titrimetric method, phosphorus was determined by the colourimetric method using molybdo vanadate reagent.

The composition of prawn waste, mantis shrinp, groundnut cake and cassava used for the preparation of the feeds is given in Table 1 and the composition of different feeds and fresh clam meat in Table 2.

Table 1. *Biochemical composition of prawn waste, mantis shrimp, groundnut cake and cassava*

Description	Prawn waste	Mantis shrimp	Groundnut cake	Cassava
Crude protein % (TN x 6.25) dry wt. basis	38.20	45.71	48.54	2.00
Carbohydrate (% on dry basis)	—	—	14.50	68.50
Lipid ,,	7.52	7.55	10.40	0.54
Ash ,,	23.95	23.63	6.30	1.45
Acid insoluble ash ,,	3.93	1.38	—	—
Calcium ,,	7.18	8.02	—	—
Phosphorus	1.22	1.26	—	—
Calorific value (k cal/g)	3.69	3.72	—	—

Table 2. *Composition of the feeds and fresh clam meat*

	Feeds				
	$NPCL_{20}$	$NPCL_{21}$	$NPCL_{22}$	$NPCL_{23}$	Fresh clam meat
(a) Ingredients					
Prawn waste	15	25	35	45	—
Mantis shrimp	45	35	25	15	—
Groundnut cake	20	20	20	20	—
Cassava (tapioca)	20	20	20	20	—
Total	100	100	100	100	—
(b) Proximate composition					
Crude protein (% on dry basis)	33.71	32.83	33.29	33.71	45.10
Lipid ,,	6.50	6.72	8.20	6.43	13.00
N-free extract (by difference)	40.41	43.15	41.62	43.03	39.71
Ash ,,	19.38	17.30	16.89	16.83	2.19
Acid insoluble ash	0.39	0.67	0.88	2.31	Trace

Feeding experiments

Juveniles of *P. indicus* (average 30 mm length, 0.2 g weight), reared in the hatchery were selected at random for feeding experiments. They were stocked in 90 cm diameter plastic pools containing 300 litres of sea water (filtered through No.20 bloting silk cloth). The specimens were individually measured for length, but the initial average weight was taken from a sample of 50 animals sacrificed for this purpose. Ten animals were stocked in each pool and two replicates were kept for each treatment. Aeration was provided intermittantly in all the pools and the entire water was changed once in three days.

Feeding was done at the rate of 12% of the body weight once a day in the evening hours. The feed was kept in petridishes at the bottom of the pool. The left over food was carefully collected, dried and weighed and the sediments were removed regularly before feeding. The feeding experiments were continued for a period of 30 days.

The salinity, oxygen and pH of the water were monitored whenever water was changed and special care was taken to see that these parameters had least variation. No temperature control was exercised since the experiments was conducted at the given ambient temperature. The range of these parameters during the experiments were, salinity 14 ± 2‰, pH 8 to 8.05 and temperature 26.6°C to 28.2°C.

Results and Discussion

The results obtained from the four experiments using the formulated feeds together with those in the control are given in Table 3. In all these cases, the offered food had been generally acceptable to the prawns even from the first day. Within the 30 days of duration of the experiment there was no significant variation in the acceptability of these feeds and it was also noticed that the quantity of feed offered (12% of body weight) was more than what was taken, indicating that the feeding was more or less '*ad libitum*'. In all the treatments the animals were active and healthy and no signs of defficiency were noticed throughout the experimental period.

The growth achieved in the control experiment with fresh clam meat as feed was 9.5 mm with in 30 days and that was only an increase of 30.9%. In the matter of weight the increase was to the extent of 130%. This growth of prawns obtained by the use of control feed was inferior to the results obtained by use of all the compounded feeds. Among the four compounded feeds tested, the use of feed $NPCL_{21}$ produced the highest growth rate of 75.7% in length and 400% in weight and the best food conversion efficiency of 3.22. This is followed by the results obtained by the use of feed $NPCL_{20}$ in which the growth rate was 52.5% in length and 280% in weight and food conversion efficiency was 5.97. The growth and food conversion obtained in prawns by the use of feeds $NPCL_{22}$ and $NPCL_{23}$ were almost similar, the results obtained by these feeds being 63.5%, 242% and 7.78 and 57.6%, 242% and 7.648, the growth in length, weight and food conversion efficiency respectively.

The components groundnut cake and cassava being the same in all the feeds, the combination of prawn waste (25%) and mantis shrimp (35%) in the ratio 5:7 gave

Table 3. *Results of the feeding experiments conducted with the feeds $NPCL_{20}$ to $NPCL_{23}$ and clam meat on the juveniles of P. indicus*

No. of animals stocked: 10 nos/pool; Duration of experiment: 30 days

Description	Feed No. NPCL 20	NPCL 21	NPCL 22	NPCL 23	Fresh clam meat
Initial mean length mm	28.40	28.00	27.40	27.60	30.70
Initial average weight g	0.15	0.15	0.15	0.15	0.20
Final average length mm	43.30	49.10	44.10	43.50	40.20
Final average weight g	0.57	0.757	0.513	0.513	0.46
Increase in average weight g	0.42	0.60	0.363	0.363	0.26
Increase in mean length mm	14.90	21.20	17.40	15.90	9.50
Growth in length %	52.50	75.70	63.50	57.60	30.90
Growth in weight %	280.00	400.00	242.00	242.00	130.00
Total food given g	20.90	20.90	20.90	20.90	—
Total left over food g	1.3163	nil	1.4397	1.7788	—
Total food consumed g	19.5837	20.90	19.4603	18.1212	—
Food conversion value (+)	5.59	3.22	7.78	7.648	—
Survival %	75	85	90	90	50

(+) Food conversion value = Total food consumed (g)/total weight increase (g)

the best performance in feed $NPCL_{21}$. Higher contents of prawns waste above 25% and mantis shrimp above 35% in the feed lowered the performance. Therefore, the best combination of these two materials in the feed appears to be in the ratio 5:7 under the present experimental conditions.

The use of prawn waste and mantis shrimps for preparation of feeds for the culture of prawns appears to be very promising. Although, the four compounded feeds prepared were readily acceptable to the prawns, there appears to be a relative preference to flavour of prawn waste which possibly is enhancing the palatability of the feeds. The feeds produced very superior growth in prawns in comparison with the results obtained by using the control feed of fresh clam meat. While clam has been conventionally used as control feed in prawn rearing experiments, Ahamad Ali (1980) observed in such cases heavy mortality and cannibalism leading to poor performance.

The growth of 400% in body weight of prawns *P. indicus* obtained in the experiment using the feed $NPCL_{21}$ is far superior to the growth of 196.88% obtained by Colvin (1976) using prepared feed with 33.4% of protein consisting of yeast, molasses, prawn meal (whole prawn) and fish meal under more or less same conditions. By using pelleted feeds consisting of prawn shell, fish meal and high marsh grass, Venkataramaiah *et al.* (1978) obtained a growth of 31.4% in length and 137.9% in weight in *P. aztecus*. The growth obtained by the feed $NPCL_{21}$ in *P. indicus* in present study is far superior. New (1976) has reviewed the various works on preparation, use and effect of different compounded feeds in different species of prawns. The comparison of the results obtained in these studies is rendered difficult due to variations in experimental conditions and procedures. However the growth of 75.7% in length and 400% in weight and food conversion efficiency of 3.22 obtained by using feed $NPCL_{21}$ in *P. indicus* can be considered as very good under laboratory conditions and are superior to that of the control feed fresh clam meat.

While no reference is available on the use of mantis shrimp in prawn feeds, prawn waste had been used for preparing prawn feeds by Forster (1975), Sandifer & Joseph (1976) and Venkataramaiah *et al.* (1978). The relative efficiencies of feeds compounded separately with prawn waste and mantis shrimp protein for rearing *P. indicus* were reported earlier (Ahamad Ali, 1980), in which the feed prepared using mantis shrimp protein gave the highest growth and best food conversion value. Both prawn waste and mantis shrimp contain fairly high percentage of protein, calcium, phosphorus and chitin (Madhavan & Ramachandran Nair, 1975) which are very important factors in prawn nutrition. Kitabhayashi *et al.* (1971) showed glucosamine the breakdown product of chitin, is a growth promoting factor for the Japanese prawn *P. japonicus*. That the essential amino acids, fatty acids and pigments contained in the prawn waste material have growth promoting role when fed to prawns in compounded feeds, has been reported by Forster (1975) and Sandifer & Joseph (1976). It is also seen that additic of these materials in the compounded feeds enhances their flavour and palatability in respect of prawns.

These two materials are presently wasted in large quantities in the fishing harbour and peeling sheds and the cost of these materials is only nominal. It is important that these materials are put to rational use for enhancing the production by preparing low cost feed materials for the fast developing prawn culture industry in the country.

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