DIETARY PHOSPHORUS REQUIREMENT AND DEFICIENCY SYNDROMES IN *MACROBRACHIUM ROSENBERGII* JUVENILES

By

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THESIS

Submitted in partial fullfilment of the requirement for the degree

MASTER OF FISHERIES SCIENCE

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DEPARTMENT OF AQUACULTURE

COLLEGE OF FISHERIES

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1996

TO
MY PARENTS
AND BROTHERS

DECLARATION

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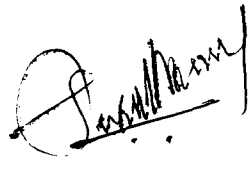

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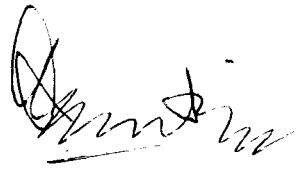

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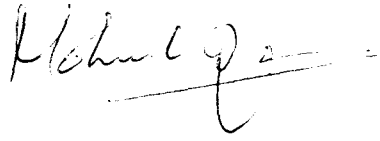

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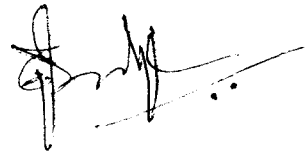

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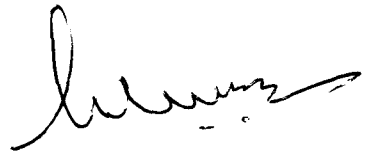

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I. INTRODUCTION

Aquaculture is gaining considerable importance over the years, as it is the fastest growing sector in food production (Csavas, 1994). Production of aquatic food items through culture is ever rising. These increases are guided by market demands,production limitations in the capture fisheries,and by improved techniques for increased production through culture (Fast, 1992).

The giant freshwater prawn *Macrobrachium rosenbergii* (De Man) is cultured in the warm waters world wide,and constitutes 3.2% of the total cultured crustaceans in 1992 (New, 1995). The species is an omnivore, requires only low level of dietary protein and is cultured both in fresh and brackish waters. The prawn is known for its consumer preference, growth potential, adaptability, combatibility and as a commodity earning foreign exchange. Growth rate of the prawn is upto 100-120 g in 7-9 months (New, 1995) and the production ranges from 500 kg to 10,000 kg\ha\crop (New, 1994). In recent years, this species has become very popular in sport fishing (Lio, 1990).

The prawn in natural environment is able to satisfy its particular nutrient requirements from a variety of sources, which include aquatic worms, insects, small molluses, crustaceans, plankton and organic detritus (Ling, 1969). However, under the controlled conditions of culture, it will become necessary to provide the prawn with appropriate levels of nutritionally balanced feeds,which include proteins, amino acids, lipids, fatty acids, carbohydrates, minerals and vitamins. Proper nutrition is essential for survival, growth and reproduction of any animal species.The role of nutrition in controlling susceptibility of animals to diseases has also been recognised.

Initial attempt to use non living natural feeds or formula feeds for the culture of *M.rosenbergii* originated in Thailand (Singholka *et al.*,1980). The prawns eat prepared formula feed as well as the pond biota produced as a result of the fertilization due to the feed(Biddle *et al.*,1977). The commercial culture of the prawn most often uses farm made feeds with locally available raw material. About 41,000 tonnes of freshwater prawn feeds were manufactured in Asia in 1990 (New and Csavas, 1993). This figure is

expected to rise to over 1,12,000 tonnes by the year 2000 A.D.

The maximum growth in terms of increase of weight and size is determined by nutrition and on optimum nutritional regime. Growth is characterized primarily by an increase in protein, minerals and water(Shepherd and Bromage, 1985).

For the cultured crustaceans, only a few studies have been reported on the subject of mineral nutrition. Works on this aspect have been deferred, based on the assumption that most mineral needs can be satisfied by direct absorption from the environment. Of all the minerals, phosphorus is one of the most important, mainly because of its essential requirement in growth, formation of exoskeleton and also in lipid and carbodydrate metabolism (Halver,1989). The ability of phosphate anion to form polymers is vital to many life processes. The living organisms cannot synthesize phosphate anions and it is limiting in the natural environment. The element, therefore, is essential in the diet of all living organisms.

The aquaculture production of *M.rosenbergii* can be supported by defining the optimum physiological requirements of the prawn. Such data will be valuable in detecting and correcting the environmental,nutritional and pathological stresses. Also, application in excess without taking water and soil quality into account affects the environment and also the economics of cultivation.In the present investigation,attempt has been made to find out the optimum requirement of phosphorus in the diet and to characterise the symptoms of disease if any, due to deficiency and excess of phosphorus in the diet of *M.rosenbergii* juveniles.

II. REVIEW OF LITERATURE

2.1 Mineral nutrition in fishes and crustaceans.

Fishes and crustaceans require minerals as essential factors in their metabolism and growth. However, in contrast to terrestial animals, which are entirely dependent on a dietary supply of minerals, fishes and crustaceans can absorb part of their mineral requirements directly from the water through their gills and body surface(Halver,1989;New,1976)

Dietary requirements of shrimps for some minerals will depend on the amounts present in the aquatic environment (Cowey and Sargent, 1979). The calcium requirement of the fish may be affected by its concentration in water (Andrew *et al.*, 1979). On working with red seabream, Yone (1975) observed that the fish does not demonstrate the dietary need for major mineral elements such as Ca,Mg,S and other elements such as Al, Zn, Mn, Cu, I and Co. It seems that sufficient amount of various elements required by the fish can be obtained from the water environment.

Marine shrimps live in a hypertonic environment and continuously drink small amounts of water thus their mineral requirements like that for calcium are partially met (Tacon, 1987). On the contrary, freshwater shrimp drinks little or no water (Piedad-Pascual, 1989) and so dietary source is essential.

Studies on fishes show that the rate of mineral absorption varies among species and with variations in certain environmental factors such as the mineral concentration, water temperature, pH, etc. (Hepher, 1988). Fishes derive inorganic elements from the surrounding water as well as from diets and a dual source of mineral is therefore available to the fishes. The contribution of dissolved minerals as nutritional elements for fish depends largely on the capability of the fish to take up dissolved mineral from the water (Phillip *et al.*, 1959). Strategies for trace metal sequestration on aquatic organ-

isms was reviewed by Phillips and Robinson (1989).

Although minerals are very important, as constituents of skeletal systems, in organic compounds such as proteins and lipids, as cofactors for enzymes, as soluble salts in the blood and other body fluids,for maintenance of acidbase equilibria and for proper functioning of muscle and nerves (Halver, 1989), the mineral nutrition in aquaculture is one of the least advanced area. The study on the mineral requirements of shrimps has been quite neglected (Piedad-Pascual, 1989) and that with regard to the prawn was absent upto 1990 (New, 1995). Metabolism of minerals differs from that of most of the nutrients since they are neither produced nor consumed by the organisms.

Dietary requirements of minerals have virtually been ignored in research investigations with *Macrobrachium spp.* (Biddle, 1977). Many micronutrient additions to formula feeds used for *Macrobrachium spp.* by virtue of *ad libitum* supplementation may actually be deleterious for maximum rates of growth (Sick and Milliken, 1983).

Dietary requirements for approximately 9 minerals (calcium, phosphorus, magnesium, copper, iron, zinc, manganese, selenium and iodine) have been identified for fish (Lall, 1978) and 7 minerals (calcium, phosphorus, copper, potassium, magnesium, selenium and zinc) have been recommended for shrimp and lobster (Davis and Gatlin, 1991).

Shewbart *et al.* (1973)while working with *Penaeus aztecus* postulated that calcium, potassium, sodium and chloride requirements might be satisfied through osmotic regulations, while phosphorus may be essential in the diet because it is present in large quantities in shrimps but not in sea water.

There is little information regarding trace element requirements except for the studies of Deshimaru and Kuroki, (1974), Deshimaru and Yone (1978) and Kanazawa *et al.* (1970, 1984). The suitable level of trace metal supplemented in the diet was found to be 0.2% in *Penaeus japonicus*, while levels over 0.2% resulted in a lower nutritive value than the diet without the supplement (Deshimaru *et al.*, 1978).

Mineral deficiency in the diet resulted in marked depression of growth and feed efficiency in rainbow trout but carps were relatively insensitive to mineral level in the diet (Halver,1976).Andrew *et al.* (1973) observed that the inclusion of high levels of fishmeal in rations for cultivated fish obviated the need for supplements of calcium and phosphorus. It is pointed out that, the substitution of other proteinacious meterial necessitated more precise information on calcium and phosphorus requirements, which is later demonstrated by Ketola(1975). Soybean meal is deficient in these minerals and supplementation with either the ash or fish protein concentrate or dicalcium phosphate alleviates this deficiency. Yeast grown on hydrocarbon is considered to be an effecitive substitute for fishmeal in formulated fish feeds. This yeast has particular mineral components, being high in phosphorus and low in calcium. Addition of calcium to the yeast diet at approximately 250 mg/100g stimulated the growth of rainbow trout (Arai *et al.*,1975).

A complication with many of the minerals is that they are not available for absorption because of binding by other feed ingredients. Gallagher *et al.* (1982) reported that calcium and phosphorus in the formulated diet for lobsters were more available for absorption than these elements held in the protein matrix of animals consumed in the wild.

One of the major considerations with regard to aquaculture nutrition has been on the interaction between dietary nutrients. A number of interactions between minerals (Ca-P; Cu-Zn; Se-Cu) and between vitamins and minerals (vit D-Ca; vit E-Se; vit C-Cu; vit E-Fe) exists (Kaushik, 1981). Sedgwick (1980) observed that when a mineral supplement was incorporated at 7% by weight in addition to the vitamins in diet on freeze-dried *Mytilus edulis* meal, improvement of growth response was achieved in *Penaeus merguiensis*. This indicates that in *P. merguiensis*,a relationship exists between the levels of dietary vitamin and minerals which act to permit normal metabolic function and therefore growth. The inclusion of minerals alone in the diet at either 7.0% or 14.0% by weight not only fails to increase growth but also resulted in severe mortalities.

It is indicated that a physiological interrelationship exists between minerals and the nitrogen components of a diet. It is possible that the high turn over of minerals in the marine environment may affect the amino acid metabolism and increases the requirements of certain amino acids in fish, which would explain the high protein intake of salt water fish (Lall and Bishop, 1979). The influence of minerals on availability of dietary lipids is also demonstrated. A higher level of dietary ash may reduce dietary lipid availability leading to reduced body lipid stores when isoenergetic diets containing differing levels of ash are fed (Shearer *et al.*, 1992).

Studies on mineral composition of fish carcasses (Lall and Bishop, 1979) indicate that calcium and phosphorus levels were lower in salt water fish than in fresh water fish, whereas magnesium and potassium levels were higher in fish grown in salt water. The levels of dietary protein had no significant effect on the mineral composition of fish.

Artificial diets for crustaceans usually include a salt mixture (Kanazawa *et al.*, 1970; New, 1976).Penaeid shrimp received diets supplemented with different levels (2 to 19.5% of the dry diet) of mineral mixtures containing a wide range of Ca:P ratio of 0.76:1 to 4:1. Ash levels of commercial shrimp feeds should not exceed 15%. (Akiyama *et al.*, 1992). This limitation on ash is specified to ensure a minimum amount of organic nutrients in the feed.

Studies of Davis *et al.* (1992) in *Penaeus vannamei* for 4 week experimental period indicate that the individual deletion of magnesium, manganese, iron, zinc and copper from the mineral supplement resulted in reduced tissue mineralisation whereas the deletion of calcium and phosphorus from the mineral mix did not produce significant responses. Deletion of selenium from the mineral premix resulted in a significant increase in the ash content of the carapace. Deshimaru and Yone (1975) found that there was a dietary requirement for phosphorus, potassium and trace metals but not for calcium,magnesium and iron in *Penaeus japonicus*.

Many investigations on fishes show correlation between the dietary level of minerals and body content (Brown *et al.,* 1993; Yone and Toshima, 1977; Liu *et al.,* 1993). On the other hand, in lobster *Homarus americanus,* mineral determinations were quite variable and statistical analysis revealed no trend in ash calcium and phosphorus content of carapace or body tissue with respect to diet (Gallagher *et al.,* 1977). However, Davis *et al.* (1993) observed that although the calcium and phosphorus content of hepatopancreas and the carapace responded to dietary supplementation, there was no clear correlation of tissue mineralization to shrimp growth.

Of all the minerals, phosphorus is one of the most important, mainly because of its high requirement in growth and mineralization of skeletal system and also in lipid and carbohydrate metabalism (Shepherd and Bromage,1988). The levels of phosphorus required are the highest of all the inorganic ions, with the dietary inclusion of 0.4 - 0.9% of available phosphorus being required for most fish species.

The main portion of hepatopancreas mineral resources consists of Ca and Mg phosphates. The cations are utilized in post ecdysial cuticular mineralization and phosphates are necessary for chitin synthesis. During the intermoult, the mineral (or ash) content of hepatopancreas doubles (Passano, 1960). Exokeletal calcium, magnesium and phosphorus in *Penaeus californiensis* showed maximum concentration in late premoult and postmoult stage. (Huner and Colvin,1979). The three minerals in haemolymph gave the maximum values in late premoult stages, followed by a decline through the postmoult to reach the minimum values in intermoult and early premoult stages in *P.indicus*(Vijayan, 1988).

About 99% of the total inorganic composition of the exoskeleton widely varies among species,and according to location on the body and stage of the moult cycle (Conklin, 1981).Crustaceans may need dietary sources of minerals for growth because of repeated moulting wherein minerals are lost.in the form of exuviae.

Studies in fishes show that whole body ash levels can be lower than normal if certain essential elements are deficient in the diet (shearer and Hardy, 1987). But the

level of dietary ash appears to have no effect on the ash content of the fish provided sufficient levels of essential elements are present (Kirchyessnes and Schwarz, 1986; Shearer *et al., 1992*).

Similar ash content (1.03 - 1.26%) is observed in peeled and deveined bull males, batchelor males, berried females,non-berried females and in stunted indeterminates of *Macrobrachium rosenbergii*.However its components of Ca and P showed significant variation, while iron showed no significant variation (Sherief *et al.*,1992).

Difference between shrimp and prawn feed formulations are apparent with the *Macrobrachium* premix having no calcium, phosphorus and potassium components (Corbin *et al.,* 1983). These dietary minerals can probably be supplied by the other feed ingredients and from natural productivity. Palaemonids are known to assimilate inorganic fractions from the compounded diet. Forster and Gabbott (1971) observed that the assimilation efficiency of inorganic or ash fraction of a compounded diet by *Palaemon serratus* was 32.2 ± 2.6%.

An increase in mineral ash and water content during the development of eggs of *Macrobrachium rosenbergii* was reported by clark *et al.,* (1990). Sze(1973) and Iwai(1976) determined the ash content of *Macrobrachium rosenbergii* as high as 15.9% and 21.3% of the dry weight respectively, suggesting that mineral nutrition may be important to overall animal health and well-being.

2.2 Ionic regulation

Studies on composition of haemolymph and tissue of animals exposed to varying salinities were reviewed by Prosser (1973), Shaw (1964), Lockwood(1964,1967), Potts and Parry(1964) and Robertson(1960). The mechanism of ionic regulation was described by Spaargaren(1978), Kirchner(1979) and Lockwood(1977).

In freshwater fish, the urinary loss of sodium chloride to the hypotonic external medium is compensated by an active uptake of sodium chloride across the gill into the

plasma (Cowey and Sargent, 1979). To avoid desication through the waterloss, marine teleost fish constantly drinks small amounts of water, and pump the incoming sodium chloride across gill epithelim in to the external water. Calcium decreases the permeability of the fish's membrane to actively oppose loss of ions to the environment following abrupt ionic changes of the environment (Podoliak and Holden, 1965).

In freshwater, the crustaceans are hyperosmotic to their normal medium and are faced with diffusive loss of ions and gain of water. These movements may be minimised by reduction of permeability to water or to ions or to both. Studies of Mc Namara *et al.* (1991) in freshwater prawn *Macrobrachium olfersii* suggest that neurofactors apparently located within the ganglia of the central nervous system may alter the apparent ionic permeabilities of this prawn depending on the salinity characteristics of the external medium. The data support the notion that invasion of specific physiological mechanisms capable of compensating for the osmotic dilution and ion loss is typically encountered in the organism.

Decapod crustaceans adapted to freshwater can tolerate relatively high salinities as well, and have osmotic concentration of haemolymph greater than 350 mosm when in freshwater (Passano,1960). Some decapods notably crayfishes and certain shrimps are able to produce a hyposmotic urine. Shrimps that are known to have very dilute urine when in freshwater are the palaemonids, *Macrobrachium australiensis*, (Denne 1968)and *M. rosenbergii*(Kamemoto and Tallis,1972).At higher salinities, the concentration of haemolymph remains at a relatively constant level. However, atleast in *M. australiensis* the concentration of urine rapidly becomes isosmotic. The palaemonid prawns are well known for their ability to penetrate into freshwater and the importance of production of dilute urine as a factor in this penetration is unknown (Mantal and Farmer, 1983).

Osmoregulation can satisfy a part of the mineral requirements of aquatic organisms (Shewbart *et al.*,1973). However, phosphorus is deficient in seawater and it has to be delivered through the diet(New,1976).

The level of phosphorus in the haemolymph of crustaceans show considerable variation with respect to the moult cycle. Inorganic phosphorus in the blood is about 0.45 mg per litre in *Panulirus argus* (Passano,1960). A rise in phosphorus concentration in haemolymph occurs at the time of moulting but is always followed by a significant fall following the moult. This is probably due to the cuticular hardening and phosphorus store depletion in the hepatopancreas (Passano,1960).In *Homarus vulgaris* a significant rise in the organic phosphate concentration before moulting has been reported(Passano,1960).

2.3 Nutritional requirements of minerals

The food contains certain amounts of minerals, which form the 'ash' part of the food. However, the amounts are not always sufficient to meet the requirements. There are factors such as leaching loss and availability of minerals, which also need due consideration. The adequate supply of minerals is ensured by mineral supplementation in the diet.

The requirements of minerals for cultivable fishes and shrimps are given in Table 1 and Table 2, respectively. Table 3 provides a summary of information on mineral supplementation of fish (Cho and Schell, 1980) and shrimp (Akiyama *et al.*, 1992).

Very recently, Shearer (1995) proposed factorial model for determining the relationship between the net requirement for an essential element in fish and the dietary concentration necessary to meet this requirement.

2.3.01 Calcium.

The mineral is sequestered from water through the gill tissue in trout. It is also absorbed through the gut when adequate amounts of vitamin D_3 are present in the ration (Halver, 1976).

The calcified nature of exoskeleton in many crustaceans suggests a possible func-

Table 1 Dietary mineral requirements of cultivable fishes.

Element	Species	Requirement mg/100 g	source
Calcium			
	Ictalurus punctatus	1500	Andrew *et al.*. , 1973.
	Salmo gairdneri	240	Arai *et al.*, 1975
	" "	340	Ogino and Takeda, 1978
	Cyprinus carpio	300	Ogino and Takeda, 1976
	Anguilla japonica	270	Arai *et al.* , 1974
	Pagrus major	340	Sakamoto and yone , 1973
	Tilapia aurea	170-650 *	Robinson *et al.* ,1984.
	Oreochromis aureus	700 *	Robinson *et al.*,1987.
Phosphorus			
	Salmo gairdneri	780-800	Ogino and Takeda, 1978
	Oncorhychus keta	500-600	Watanabe *et al.*, 1980
	Salmo salar	600	Ketola ,1975b
	lctalurus punctatus	450	Lovell,1978
	" "	400	Wilson *et al.*. ,1982
	" "	800	Andrew *et al.* ,1973
	Sciaenops ocellatus	860	Davis and Robinson,1987
	Poecila reticulata	530-1230	Shim and Ho , 1989
	Pagrus majar	680	Sakamoto and yone, 1973
	Tilapia nilotica	900	Watanabe *et al.* ,1980
	Oreochromis aureus	500 *	Robinson *et al*,. 1987
	Sparus macrocephalus	680	Liu *et al.* ,1993
	Cyprinus carpio	600-700	Ogino and Takeda ,1976
	Morone chrysops x M.saxatilis (sunshine bass)	540	Brown *et al.* , 1993
Magnesium			
	Salmo gairdneri	50	Knox *et al.*, 1981
	Salmo gairdneri	60-70	Ogino *et al.*, 1978
	" "	140	Shearer, 1988b
	lctalurus punctatus	40	Gatlin *et al.*, 1982
	Cyprinus carpio	60	Dabrowska and Dabrowski, 1990
	" "	40-50	Ogino and chiou, 1976
	Tilapia sp.	60	Dabrawska *et al.*, 1989
	Tilapia sp.	50	Reigh *et al.*, 1991
	Poecilia reticulata	54	Shim and Ng, 1988
	Anguilla japonica	40	Arai *et al.*, 1975
Potassium			
	Oncorhychus tshawytscha	800	Shearer,1988a
	Ictalurus punctatus	260	Wilson and El Naggar, 1992
Iron	*Pangrus major*	15	Sakamoto and yone, 1978

Table 1. (cont.)

Element	Species	Requirement mg/100 g	source
	Ictalurus punctatus	3.00	Gatlin and Wilson,1986a
	Anguilla japonica	1.67	Nose and Arai ,1979
	Salmo gairdneri	0.30	Ogino and yang, 1980
Copper			
	Ictalurus punctatus	0.15	Murai *et al.*, 1981
	" "	0.50	Gratlin and wilson, 1986b
Zinc			
	Salmogairdneri	1.5-3.0	Ogino and yang, 1978
	Ictalurus punctatus	2.0	Gatlin and Wilson, 1983
	Sciaenops ocellatus	2.0-2.5	Gatlin *et al.*, 1991
	Tilapia sp.	2.0	Mc clain and Gatlin, 1988
	Cyprinus carpio	1.5-30	Ogino and young, 1979b
Manganeese			
	Salmo gairdneri	1.2-1.3	Ogino and yang, 1980
	Cyprinus carpio	0.24	Gatlin and Wilson, 1984b

* Fish were raised in calciumfree water.

Table 2. Dietary mineral requirements of crustaceans.

Element/species	Dietary requirement	Source
Calcium		
Penaeus japonicus	1-2 %	Kanazawa *et al.*, 1984
" "	1.24 %	Kitabayashi *et al.*, 1971
" "	1.00 %	Kanazawa, 1985
" "	< 0.50 %	Deshimaru *et al.*, 1978
Penaeus orientalis	!.5 %	Xu Xinzhang and Li Aijie, 1988
Phosphorus		
Penaeus japonicus	1.04 %	Kitabayashi *et al.*, 1971
" "	2.00 %	Deshimaru and yone, 1978
" "	1.20 %	Kanazawa *et al.*, 1984
Penaeus orientalis	3.00 %	Xu , Xinzhang and Li Aijie, 1988
Penaeus vannamei	0.50-2.00 %	Davis *et al.*, 1993a
Ca : p ratio		
Penaeus mondon	1:1	Bautista and Baticados, 1990
Penaeus californiensis	2.42 : 1	Huner and Colvin, 1977
Penaeus japonicus	2.8 :1	Deshimaru and Shigueno, 1972
" "	1 : 1	Kanazawa *et al.*, 1984
" "	1 : 1	Kitabayashi *et al.*, 1971
Penaeus merguiensis	1.3 : 1	Sick *et al.*, 1972
Penaeus aztecus	1.3 : 1	Sick *et al.*, 1972
Penaeus orientalis	1 : 2	Xu Xinzhang and Li Aijie, 1988

Table 2. (contd.)

Element/species	Dietary requirement	Source
Penaeus orientalis	1 : 1.7	Lie *et al.*, 1986
Homarus americanus	1 : 2	Conklin *et al.*, 1983
" "	0.51 : 1	Gallagher *et al.*, 1977
Homarus americanus	1 :1	Gallagher *et al.*, 1982
Magnesium		
Penaeus japonicus	0.30 %	Aquacop,1978
" "	ND	Deshimaru and yone, 1978
Penaeus merguiensis	0.30 %	Kanazawa *et al.*, 1984
Iron		
Penaeus japonicus	ND	Kanazawa *et al.*, 1984
" "	ND	Deshimaru *et al.*, 1978
Manganese		
Penaeus japonicus	ND	Kanazawa *et al.*, 1984
Copper		
Penaeus japonicus	ND	Kanazawa *et al.*, 1984
Penaeus vannamei	0.0034 %	Davis *et al.*, 1993b
Potassium		
Penaeus japonicus	0.9 %	Kanazawa *et al.*, 1984
" "	1.0 %	Deshimaru *et al.*, 1978
Penaeus aztecus	ND	Shewbart *et al.*, 1973
Sodium chloride		
Penaeus aztecus	ND	Shewbart *et al.*, 1973
Trace metals		
Penaeus japonicus	0.2 %	Deshimaru *et al.*, 1978

ND - No dietary requirement demonstrated.

Table 3. Recommended level of mineral supplementation in fish and shrimp feeds.

Element	Requirement /Kg For fish	dry diet for shrimp
Calcium	5g	maximum 23g
phosphorus	7g	available 8g Total 15g
Magnesium	500 mg	2g
Sodium	1-3g	6g
Potassium	1-3g	9g
Sulphur	3-5g	--
Chlorine	1-5g	--
Iron	50-100mg	300mg
Copper	1-4g	35mg
Manganese	20-50 mg	20mg
Cobalt	5-10 mg	10mg
Zinc	30-100mg	110mg
Iodine	100-300mg	--
Molybdenum	Trace	--
Chromium	Trace	--
Flourine	Trace	--
Selenium	----	1mg

tion for vitamin D in the class but little is known about these aspects of crustacean metabolism. Crustaceans may obtain part of their vitamin D from phytoplankton. Studies show that requirement of crustaceans for vitamin D is of a negative or inconclusive character (Fisher, 1960).

The absorption of radioactive calcium (^{45}Ca) from surrounding sea water by *Penaeus japonicus* fed on a diet without supplemental calcium, was compared with that of the shrimp fed on calcium supplemented diet (Deshimaru *et al.*, 1978). The group fed on the diet without supplemental calcium exhibited faster absorption and higher activity of ^{45}Ca, than the group fed on diet with calcium.

The haemolymph calcium in *P.indicus* cultured in the brackish water environment was found between 28 and 80 mg/100 ml in males and between 22 and 68 mg/100 ml in females. The calcium content of exoskeleton varies between 4 and 15%, which is reported to be low compared to other crustaceans (Rao, *et al.*, 1982). The calcium content of the pond water showed a gradual increase from December to May when haemolymph calcium was also relatively high. In June, when there was an appreciable fall in salinity, temperature, pH and calcium of pond water, due to ecological changes consequent on the onset of monsoon, the calcium level in haemolymph and exoskeleton of prawn also decreased , but not in the pond soil and in the muscle of the prawn. The difference in the calcium level in the haemolymph, muscle and exoskeleton indicates some imbalance in the absorption and transportation of calcium from the external medium to the exoskeleton,particularly from haemolymph to the integument via muscle, during the period of monsoon. Baticados *et al.* (1987) reported that hepatopancreas of soft-shelled *Penaeus monodon* stained more intensely for calcium than that of hard-shelled one. Soft-shell syndrome in prawn reduces the return from prawn farming industry and the condition may be further deteriorated by secondary invasion by bacteria and other commensal organisms(Sarac and Rose, 1995; Baticados *et al.*, 1986).

The prawn shell contains about 63% of ash (Sarac and Rose, 1995). Ash component of prawn shell consists primarily of calcium (up to 99%) and some magnesium,phosphorus and sulfur (Welinder,1975). The calcium is present in the form

of calcium carbonate, usually as calcite or in amorphous form (Passano,1960).

The amount of calcium salts in the shell of a newly moulted crustacean is about 15% of the weight of the shell. This level rapidly increases to 60% within four days after moulting(Passano,1960). It has been reported that sea water alone can provide the total requirement of calcium for prawns (Deshimaru *et al.*, 1978). However, pond conditions may affect calcium availability in sea water. In ponds, where pH of water is below 6.0,the ability of prawns to take up calcium would be limited. When the pH of water is above 8.0, calcium precipitates as calcium phosphate and becomes unavailable to prawns. These situations either prolong hardening of prawn's shell or result in soft-shell syndrome. Calcium concentration of pond water and haemolymph showed a direct relation with salinity (Deshimaru *et al.*, 1978).

The effect of salinity on haemolymph osmotic Ca^{2+} regulation in *Macrobrachium carcinus* was investigated by Moreira *et al.* (1988). Ca^{2+} haemolymph concentration was hypertonic to those of the media in the tested salinities.Large fluctuations in haemolymph calcium were observed 0-6 hours after moult. In low salinities, haemolymph calcium peaked at 3 hours postmoult to a value 30% higher than those during moult. These values subsequently decreased rapidly after moulting. At 44 ppt, calcium concentration was highest during molt, then gradually declined by about 15% to inter molt value. Total haemolymph calcium was largely affected by moult stage and less so by salinity in *P. monodon* (Parado-Estepa *et al.*, 1989). A sharp transient increase in haemolymph calcium occured 3-6 hours postmoult, followed by an intermoult peak. Concentrations of total calcium was greater in low salinities (8 and 20 ppt) than in high salinities.

In crustaceans, the metabolism of calcium is very much active. The highest concentrations of calcium in exoskeleton are generally found in areas where rigidity and strength are required. Most of the calcium stored in the midgut gland is in the form of calcium phosphate and is probably an important store of calcium as well as phosphorus. The mitochondria appears to play a role in the formation of calcium phosphate

crystals (Chen *et al.*, 1974). Sherief and Xavier (1994) reported that the content of calcium in hepatopancreas of *Macrobrachium rosenbergii* and *Penaeus indicus* are 82.20 mg% and 148.70 mg%, respectively. The marine crabs store calcium as spherules of calcium phosphate and this may reflect the paucity of phosphorus in sea water (Greenaway,1985). Muscle is suggested as a calcium reservoir to be utilized in post-moult stage. The calcium stored in the muscle is not sufficient for complete hardening of new shell, and so an external source of calcium is required (Romero, 1990).

Palaemonetes pagio exposed to barite medium accumulated higher levels of Barium in their exoskeleton and soft tissues than control shrimp in sea water. The relative concentration of the minerals in the exuvia of barite-exposed shrimp were Ca>Ba>Sr, while those of control shrimp were Ca>Sr>Ba(Brannon,1979)

Total body calcium increased from low post-moult levels to peak during intermoult and declined rapidly during pre moult in post larvae and juveniles of *P.californiensis* (Huner and Colvin,1979). Calcium reached in relatively constant levels within 24 hours postmoult and total body calcium of intermoult declined with age. A thin layer of cristalline calcium deposit was reported between the exocuticle and endocuticle of *Penaeus chinensis* (Chen-Kuanzhi *et al*, 1992). Wang Fangguo and Liu-Jincan (1992) reported the relation between diseases of the chinese shrimp and the environmental factors such as temperature, salinity, pH, dissolved O_2, phosphate and calcium in the water.

Arlot-Bonnemains *et al.* (1986) detected a molecule, immunologically related to salmon calcitonine in the haemolymph of the shrimp *Palaemon serratus,* the concentration of which varies inversely with the calcium level during the moult cycle, a maximum (14 ng/ml) is found in the postmoult stage and the minimum (0.5 ng/ml) during the premoult stage. Later, presence of both salmon calcitonine and human calcitonine gene related peptide was investigated in the tissue of *Palaemon serratus* by Lamharzi *et al.*(1992). In the haemolymph, calcium fall observed was correlated with salmon calcitonine increase.However,no correlation appeared between the circulating calcium and human calcitonine gene related peptide concentration.

Maximum growth of *M.rosenbergii* juveniles was reported at a water hardness of between 40 and 60 mg/l of $CaCO_3$ and the growth rate was found declining at higher levels (Brown *et al.*, 1991). Survival was impaired at the high hardness levels. Greater deposition of calcium in the carapace occured in low hardness water and the lower levels of calcium found in the cast carapace from those prawns indicate greater withdrawal of calcium from the carapace before moulting.

Carapace weight loss but with constant calcium content was reported in *Penaeus monodon* as a result of low pH. The withdrawal of more calcium salts in preference to magnesium from old carapace occured prior to ecdysis under low pH conditions, compared to more normal conditions (Wickins,1984b). Studies made on the effect of hypercarbonic sea water on mineralization of penaceid prawn revealed that calcium levels in prawn cuticle increased with increasing exposure while no increase was noted in strontium and magnesium levels (Wickins, 1984a).When exposed to hypoxia and low saline conditions, circulating levels of calcium progressively increased in *Crangon crangon* (Hagerman and Uglow, 1982).

Calcium is essential for blood clotting, activation of enzymes, muscle contraction and cell permeability and is believed to be essential for the absorption of vitamin B_{12} (Akiyama *et al.*, 1992). Eventhough calcium is not considered a dietary essential, calcium level in the feed needs to be monitored to maintain a calcium phosphate ratio of 1:1 to 1.5:1 and calcium should not exceed 2.3% in the feed (Akiyama *et al.*, 1992). The addition of calcium to the artificial diets improved the mineralization of exoskeleton but survival or growth was not enhanced in shrimps (Conklin *et al.*, 1983). In the presence of replete phosphorus, supplementation of 1.0 and 2.0 % calcium to the diet depressed survival and did not appear to increase the nutritive value of the diet, indicating that a dietary calcium supplement is not required under these conditions in *Penaeus vannamei* (Davis *et al.*, 1993).

Sarac *et al.* (1993) observed substantial variation in the ash content (9.0-15.5%) and calcium content (1.3 - 3.3%) in the commercial prawn feeds. Zimmerman *et al.*

(1993) reported the highest survival of 69.32% for *M.rosenbergii* at intermediate alkalinity and higher calcium content in the diet. The lower calcium produced better performance in the higher alkalinity. There is narrow relation between calcium from the diet and dissolved calcium from rearing water, showing the need for dosing the calcium in diet as a function of the alkalinity of culture water. Serum calcium in *M.rosenbergii* was found greater than in panaeid shrimp eventhough the level in sea water was more than 18 times greater than in pond water (Balazs *et al.* 1974).

2.3.02 Phosphorus.

Phosphorus is a major component of structural tissues and is an important constituents of nuclic acids and cell membrane. Phosphorus plays an important role in carbohydrate, lipid and aminoacid metabolism and in muscle and nervous tissue metabolism as well as various metabolic processes involving buffers in body fluids. It is directly involved in all energy producing reactions of the cell (Halver, 1989).

The uptake of ^{32}P from water has been demonstrated by Lall (1979). However food is the main source of phosphorus since phosphate concentration is low in both fresh water and sea water (Boyd, 1981; Halver, 1989).

The phosphocompounds are vital exchange currencies in life process and are distributed throughout the organs and tissues of the fish. The skin like the skeleton also appears to be an important repository of phosphorus in some species (Chow and Schell, 1978). The regulation of phosphorus is considered to be more critical than that of calcium. Lui *et al.*(1974) observed that labelled phosphorus is readily incorporated by the ovary into phosphotidyl choline, a mojor yolk lipid.

Distinct relatioship of *P. monodon* production with available phosphorus in the soil phase was reported (Chakrabarti *et al.*1985). However its availability in pond water depends on the O_2 level and temperature of water (Baticados *et al.* 1986). Inorganic phosphates are released at higher temperature and taken up at lower temperature by

algae. In fishes, the phosphorus absorption through the gut is enhanced by increasing water temperatures and by the presence of glucose in the diet (Chow and Schell,1978). In semi-intensive and intensive shrimp culture, phosphorus supplement in feeds is very important. High dietary level of calcium inhibit the bioavailability of the dietary phosphorus (Davis and Gatlin, 1991).

Dietary phosphorus level affects greatly the growth, the body composition, and the mineral composition of the body and vertebra of rainbow trout (Ogino and Takeda, 1978). The supplement of phosphorus to commercial carp diet was found to be effective not only for prevention of cranial deformity but also for promotion of growth (Murakami, 1970). The lipid content of muscle and viscera were remarkably reduced by the addition of phosphate to the diet and protein content of fish tissue consequently increased. This suggests the important role of phosphorus in the catabolic metabolism of lipids resulting in high retention of protein in the fish body. This is also demonstrated by Sakamoto and Yone (1973). In Atlantic salmon *Salmo salar* supplementation of inorganic phosphorus significantly improved growth, food utilization and bone ash content (Ketola, 1976). Feeding with low phosphorus diet in carp *Cyprinus carpio* resulted in reduced growth, low feed efficiency and deformity of head (Ogino and Takeda, 1976).

There are contradictory observations on the interaction of phosphorus and calcium. Shepherd and Bromage (1988) observed that, generally phosphorus requirements are not affected by dietary calcium levels. However, growth of *Penaeus japonicus* was improved by the supplement of 1-2% phosphorus to the diet, but such an effect of phosphorus was reported to be affected by dietary calcium levels by Kanazawa *et al.* (1984).

Huner and Colvin (1979) reported that wholebody phosphorus of *Penaeus californiensis* remained fairly constant in postlarvae but rose constantly from a post moult low to a late premoult peak in young juveniles. Phosphorus values of rostrum , carapace and abdominal region vary from 0.5 to 1.2%. Sherief *et al.* (1992) observed significant variation in body phosphorus in bull males, bachelor males, berried fe-

males, non berried females and stunted indeterminates of *M. rosenbergii*. A slow but steady increase of hepatopancreas phosphorus from early post moult to early premoult and its fall immediately before and after moulting in *Penaeus indicus* was reported by (Vijayan,1988).This probably indicates the build up of phosphorus during tissue growth and its subsequent use at the time of moult as an energy source as reported by Huner *et al.*.(1979). Sherief and Xavier(1994) observed appreciable amount of phosphorus in hepatopancreas of *M.rosenbergii* (216.10 mg%) and *p.indicus*(168.70 mg%). Serum inorganic phosphorus of *M.rosenbergii*(2.0 mg/100ml) was found lower than that of *P.marginatus*4.6 mg/100 ml) (Balazs *et al.*, 1974) but higher than that of *Panulirus*(0.7 mg/100ml) (Passano,1960). Serum levels of phosphorus was found slightly higher in females than in males of *M.rosenbergii* (Balazs *et al.*, 1974).

Rise in inorganic phosphate concentration in the blood of *Panulirus argus* occurs at the time of moulting but is always followed by a significant fall following the moult (Passano,1960). In *Homarus vulgaris*, a significant rise (up to 64 mg P per liter of blood) in the organic phosphate concentration before moulting was reported (Passano,1960).

Much of the phosphorus in the exoskeleton of crustaceans may be in organic form such as phospholipids (Passano,1960). Huner and Colvin,(1979) while working on *P.californiensis* did not observe much variation in the distribution of exoskeletal phosphorus. A similar result was observed in *P.indicus* (Vijayan, 1988). More or less uniform phosphorus level in the exoskeleton probably indicates its insignificant role in hardening of the exoskeleton.

However, in soft-shelled *P.monodon* calcium and phosphorus levels were significantly higher in the hepatopancreas and phosphorus was significantly lower in the exoskeleton, than in hardshelled prawn. Though chitinoclastic bacteria were isolated from soft-shelled prawns, experimental infection with these bacteria to induce soft-shelling gave largely negative results.A 96 hour exposure in atleast 0.0154 ppm of organostannous pesticide could result in soft shelling in 47-60% of prawns (Baticados

et al.,1986). Inclusion of either calcium or phosphorus alone in diets of prawns increases the recovery from soft-shell syndrome. The recovery improves further,reaching 89%, when calcium and phosphorus are added to diets at a ratio of 1:1 (Batista and Baticados, 1990). Pond water conditions and presence of pesticides in the rearing water might probably reduce the availability of these minerals, which can result in soft shell syndromes in shrimps.

Calcium deposits formation and moult death syndrome in the American lobster *Homarus americanus* was related to the amount of phospholipid-soylecithin-in purified ration (Bowser and Rosemark, 1981). Increasing the soylecithin to 7.5% of the dry weight of the diet significantly decreased mortality.

Phosphorus is considered the most limiting mineral in feeds. Sources of phosphorus include dried distillers solubles, cotton seed meal, crab meal, fish soluble, fish meal, krill meal, rice bran and byproducts, shrimp meal,squid meal, wheat bran and byproducts and yeast (Akiyama *et al.*,1992). Substantial variation in phosphorus content (1.2-2.0%) in commercial prawn feed was noticed. Phosphorus was observed as one of the limited nutrient constituents in the process of compounding artifical feed for *Penaeus chinensis,* based on 18 raw materials that were found most frequently on the east China coast (Sarac *et al.*, 1993). Sodium phosphate ($NaH_2Po_4.H_2O$) or non calcium phosphate supplements are recommended as phosphate suppliments.Total phosphorus need to be monitored to maintain acceptable Ca:P ratio of the feed for the shrimp (Akiyama *et al.*, 1992).

2.3.2.1 Availability of phosphorus.

Among inorganic compounds, primary calcium phosphate and primary sodium or potassium phosphate were utilized effectively by fishes as a dietary phosphorus source; whereas secondary and tertiary calcium phosphate were found to be low in availability.

Availability of phosphorus contained in fish meal was fairly low in carp com-

pared with that in rainbow trout. Thus supplementation of primary sodium phosphate to the diet resulted in acceleration of the growth responses of carp. On the other hand both carp and rainbow trout effectively utilized phosphorus contained in casein and yeast, but phosphorus in vegetable materials was relatively low in availability (Ogino *et al.*, 1979) since it is present as phytate (Halver, 1976).

Availability of phosphorus for channel catfish as determined by net absorption from the digestive tract was 90-94% for monosodium or monocalcium phosphate, 65% for dicalcium phosphate, 40% for fishmeal, 50-54% for soybean meal and 25-28% for cereal products (Lovell, 1978). Phosphorus combined with either protein as in casein or nucleic acid as in yeast was highly accessible to fishes (Nose and Arai, 1979).

The bioavailability of phosphorus is found to be low in stomachless fish like Common carp and high in fish with a stomach such as rainbow trout (Watanabe *et al.*,1988).Phosphorus availability values for various feed ingredients are given by Akiyama *et al.* (1992).

Studies on the availability of phosphorus supplement to shrimps and prawns are limited. However, phosphorus availability values of shrimps and prawns should be more similar to those of common carp than other fishes since the digestive system of crustaceans, where the pH is approximately 5-7 is not very acidic as in the case of carp. The availability of minerals to shrimp is dependent on the dietary source and form of the mineral that is ingested, amount stored in the body, interaction of other elements present in the gastrointestinal tract and body tissues and mineral interactions with other dietary ingredients or metabolites (Tacon, 1987). Soluble monobasic inorganic salts or bioavailable organic salts must be provided in the diet of stomachless shrimps. Large intake of some minerals such as aluminium, magnesium and iron by prawns resulted in the formation of insoluble phosphates. This reduces the absorption of phosphorus. Dietary levels of magnesium and vitamin D may also affect the bioavailability of calcium and phosphorus by limiting their absorption in the gut of the prawns (Davis and Gatlin, 1991).

Phytate phosphorus fed *Penaeus japonicus* grew faster than those fed with mono-,di- or tri-sodium phosphate.The reason for this high nutritive value of phytate phosphorus is unknown (Lovell, 1989).

Very recent studies by Davis and Arnold (1994) indicated that leaching losses of phosphorus is highest in diet supplementation with highly water soluble phosphorus such as potassium phosphate and sodium phosphate. However it is also noted that bioavailability of phosphorus sources also appears to be positively correlated with the solubility of phosphorus source in water. The apparent phosphorus availability (APA) of various sources such as calcium phosphate monobasic, calcium phosphate dibasic,calcium phosphate tribasic,potassium phosphate monobasic and sodium phosphate monobasic for *P.vannamei* are observed to be 46.3%,19.1%,9.9%,68.1% and 68.2% respectively. APA value of diet utilizing sodium phosphate monobasic as the source of phosphorus in *P.vannamei* was significantly depressed by the presence of calcium lactate (APA value 50.0%) but not by calcium carbonate (APA value 65.5%) or calcium chloride (APA value 68.2%). The study also indicated that APA values are not influenced by leaching in *P.vannamei.*

2.3.02.2 Phosphorus as pollutant.

Although phosphorus occurs in natural water in very small quantities, the element is recognised to be the most critical single factor in the maintenance of fertility. In fresh water environment, dissolved inorganic phosphorus is considered the most important growth limiting factor, substantial increase of which leads to hypernutrification and eutrophication.Phosphorus is one of the major factors contributing to environmental pollution from aquaculture (Rijin and Shilo, 1989;Akiyama, 1992;Boyd and Musig, 1992;Persson, 1991). For the sustainability of aquaculture, nutritional strategies for minimizing waste output are to be developed (Cho *et al.*,1994).

5-15 g of phosphate was produced in the effluent for each kg of dry pelleted feed

with which the trout was fed.Use of deflourinated rock phosphate and low phytin phosphorus diets reduced the discharge of phosphorus into the effluent (Ketola, 1985). On the other hand leaching did not influence the apparent phosphorus availability of shrimp. Decreased bioavailability of dietary phosphorus to shrimp and reduced leaching losses from the shrimp feed indicate pollution loads would follow a pattern different from that of fish production system (Davis and Arnold, 1994).

The commercially produced feeds provide the largest source of environmental pollution in intensive aquaculture systems (Seymour and Benjamin, 1991). The aquaculture feeds must therefore be designed to maximize nutrient retention and minimize nutrient losses to the environment. The low pollution diets can be prepared only by knowing the bioavailability of nutrients from feed stuff and the nutrient requirements of the cultured species. Recentely it is proved that, use of better quality diet and improvewd field management reduced waste phosphorus loadings from cage sites of trout (Gavin *et al.*,1995).

2.3.02.3 Ca:P ratio of the diet.

The metabolism of calcium and phosphorus are closely interrelated. In *Cyprinus carpio* and *Salmo gairdneri*, the level of phosphorus in the diet sets the rate at which calcium is retained in the body. An increasing level of dietary phosphorus will be accompanied by increasing retension of both mineral elements in the body tissue, thus maintaining the ratio of calcium and phosphorus within narrow limits. In common carp, the wholebody Ca:P ratio is about 1.4, except when phosphorus is severely lacking in the diet. Fish appears to have an ability to balance Ca:P ratio by controlling the absorption and excretion of calcium for optimal utilization of both mineral elements (Chow and Schell, 1978).

In mineral nutrition studies in Crustacea, considerable attention has been given to the importance of Ca:P ratio in the mineral supplementals. But the overall ratio in the final diet is often neglected. A high mortality rate for very soft prawn may indicate

a possible imbalance in Ca:P ratio in the diet (Conklin, 1980). Soft shelling may therefore be related to the Ca:P balance in the prawn body (Kitabayashi *et al.*, 1971; Deshimaru and shigueno, 1972; Parker and Holecomb, 1973; Shewbart *et al.*, 1973; Conklin , 1982). Lobsters fed with pellet diets with Ca:P ratio which varied from 3.0 to 6.0 often had soft shells and died just after ecdysis (Gallagher *et al.*, 1977). The Ca:P ratio of the prepared diet is found to have significant influence on the growth (weight gain)of adult lobsters (Gallagher *et al.*,1982).

The survival and growth rate of postlarvae of *Penaeus orientalis* are found to be the best when total content of calcium and phosphorus of the diet was about 1% and the Ca:P ratio was 1:7.3. When the total content of calcium and phosphorus was lower,despite the Ca:P ratio also low, the survival and the growth rate of the postlarvae decreased. The growth and food conversion of juveniles of *P.orientalis* were best when the total content of the calcium and phosphorus were 2% and the Ca:P ratio was 1:1.7 (Lie *et al.*,1986).

Eventhough the ratio of phosphorus to calcium in the diet for *P.japonicus* was found to be 1:1, large amounts of these minerals resulted in inhibition of prawn growth, making the prawn shell greyish white (Kanazawa *et al.*, 1984). A similar result was obtained when the ratio of phosphorus to calcium was about 1:2 (Kitabayashi *et al.*, 1971). Andrew *et al.* (1973) observed that hypercalcemic effect could not be prevented by adjusting the Ca:P ratio by the addition of phosphorus to the diet in cat fish *Ictalurus punctatus*. These results suggest the need for specification of both phosphorus level and Ca:P ratio of the diet.

2.3.03 Magnesium.

Magnesium is an essential constituent of bone in fish and its metabolic activity is interrelated with calcium and phosphorus metabolism. Magnesium is an essential element in some enzymatic process in the metabolism of carbohydrate(Hepher, 1988).

In rainbow trout magnesium deficiency may give rise to renal calcinosis (Cowey,

1970). No renal calcinosis occured at dietary calcium levels of 2.6% when suplemental magnesium (0.1%) was provided in the diet. Recently, it is observed that cobalt chloride at a concentration of 7.5 ppm and magnesium chloride at a concentration of 250ppm were found to be effective in the growth promotion of common carp fry(Xavier *et al.*,1994).

Magnesium is abundant in seawater and is excreted by most Crustacea, resulting in blood level lower than that of the external medium. In shrimp the majority of the magnesium is found in the exoskeleton. Magnesium is essential for several enzymatic processes including protein, lipid and carbohydrate metabolism, muscle and nerve function and osmoregulation. (Akiyama *et al.*, 1992).

Effect of exposure of *Penaeus monodon* to low pH included carapace weight loss, increased magnesium content and slightly decreased strontium content (Wickins, 1984b). Huner and Colvin (1979) reported that total body magnesium increased from low postmoult levels to peaks during intermoult and declined rapidly during premoult in the postlarvae and young juveniles of *P.californiensis*. The Magnesium level reached a relatively constant level within 24 hour postmoults.The rostrum and carapace magnesium maximum values were higher (0.9-1.2%) than that of abdomen (0.6%) and the total body magnesium level is found declining with age (Greenaway,1985). The proportion of magnesium in the exoskeleton is higher in marine Crustacea than in freshwater species (Greenaway, 1985).

The sources of magnesium include crabmeal,cotton seed meal, krill meal,ricebran, shrimp meal and wheat bran (Akiyama *et al.*,1992). Magnesium may be supplemented as magnesium sulphate (Akiyama *et al.*, 1992).The bioavailability of magnesium in white fish meal is very low(Watanabe *et al.*,1988).

2.3.04 Sodium, Potassium and Chloride.

Sodium,potassium and chloride are found in the fluids and soft tissues of the

body. They serve in regulating osmotic pressure and acid-base balance and also play important role in water metabolism (Akiyama *et al.,* 1992).

Dietary pretreatment with high sodium diets stimulates early elevated ATP-ase activity, when trout has been environmentally conditioned to smolt and then migrate to the sea (Halver, 1976). *Macrobrachium carcinus* is a strong hyperosmotic regulator in fresh water or low salinities while at high salinities, it is a hypoconformer. Na^+ haemolymph concentrations stayed fairly constant in salinities from 0-14 ppt and increased slightly with the increase in the external sodium ion concentration. K^+ haemolymph concentration increased slightly from 0-21 ppt and sharply in salinities beyond this range (Moreira *et al.,* 1988). Chloride level in serum (160 meq/l) from *M.rosenbergii* (Balazs *et al.,* 1974) is found higher than that of freshwater *Cambarus* (117 meq/l) (Schlatter, 1941).

The chloride neutralizes 80 - 95% of the total inorganic cations in crustaceans. Regardless of salinity of the medium, the chloride cencetrations in the haemolymph of *Penaeus monodon* become stable within one day after moulting (Ferraris *et al.,* 1986). When exposed to hypoxia, and low salinity conditions, the shrimp *Crangon crangon* showed a progressive loss of blood chloride untill a new stable value still higher that of the medium is reached (Hagerman and Uglow, 1982).

Sources of sodium include crab meal, fish solubles, fish meal, krill meal and shrimp meal. Sources of potassium include dried distillers solubles, cottonseed meal, fish solubles, rice bran, soybean meal,wheat bran and yeast. Crab meal, fish soluble, fish meal and shrimp meal also form the sources of chloride. Supplemental potassium sources include potassium chloride and potassium iodate (Akiyama *et al.*,1992). Sodium chloride may be supplemented at 0.2% as a flavour enhancer especially in high plant product formulations.

2.3.05 Sulphur.

Sulphur is an important element in the intracellular fluid. It constitutes an essential part of aminoacids such as methionine, cystine and cysteine. The elements can be

absorbed directly from the water but the absorption of sulphur from water is appreciably low and most of the sulphur required by the fish must therefore be supplied through the diet (Hepher, 1988).

Inorganic sulphur supplementation may spare some of the sulphur containing aminoacid requirements. Sources of suphur include ingredients which are high in sulphur containing aminoacids such as fishmeal, cotton seed meal, rapeseed meal and yeast (Akiyama *et al.*, 1992).

2.3.06 Iron.

Iron is an essential element in the formation of haemoglobin in blood erythrocytes. Diet deficient in iron resulted in hypochromic microcytic anaemia when fed to eel *Anguilla japonica* (Arai *et al.*, 1975a),yellow tail *Seriola quinqueradiata* (Ikeda *et al.*, 1973) red seabream, *Pagrus major* (Sakamoto and Yone, 1978) and catfish,*Heteropneustes fossilis* (Firdus *et al.*, 1994). Iron absorption may be depressed by high level of phosphates, calcium, phytates, copper and zinc (Akiyama *et al.*, 1992).

It was shown that iron is accumulated in the mid gut region of crustaceans (Icely and Nott, 1980). High content of iron in hepatopancreas of *M.rosenbergii* (13-30 mg%) and *P.indicus* (8-10 mg%) was reported (Sherief and Xavier, 1994). However its role in metabolism is unknown. Sherief *et al.* (1992) observed that iron content of the body showed no significant variation in bull males, bachelor males, berried females, non-berried females and stunted indeterminates of *M.rosenbergii*.

Sources of iron include blood meal, dried distillers solubles, crab meals, fish solubles and fish meal. Supplemented iron sources include ferrous gluconate and ferric sulphate, but there may not be a need to supplement iron (Akiyama *et al.*, 1992).

2.3.07 Copper.

Copper is absolutely essential for normal growth and development. It is utilized in various oxidation reduction enzyme systems and is a component of haemocyanine for oxygen transport in shrimps and prawns. Higher levels of copper is stored as free ions in proteinacious granules in shrimp *Crangon crangon* (Djangmab and Grove, 1970). Shrimp fed with copper deficient diet showed depressed growth, enlarged heart and depressed copper level in the haemolymph, carapace and hepatopancreas (Davis *et al.*,1993).

Sources of copper include dried distillers solubles, fish solubles, krill meal and yeast. Copper may be supplemented by cupric sulphate and cupric chloride (Akiyama *et al.*, 1992).

2.3.08 Zinc.

Zinc is a component of metalloenzymes and co-factors in enzymes systems involved in protein, nucleic acid, lipids, carbohydrate and micropolysaccharide metabolism(Hepher, 1988). It stimulates the appetite and promotes absorption of nutrients. It stabilizes the membrane of the cells and ribosomes (Anon, 1996).

The bioavailability of zinc contained in various fish meal depends on the tricalcium phosphate content of the meal. In the case of rainbow trout, zinc availability is the lowest in white fish meal, which contains the highest level of tricalcium phosphate and slightly better in brown fish meal, which contains less tricalcium phosphate (Watanabe *et al.*,1988). The supplementary source of zinc, zinc methionine has approximately 3 times the potency of zinc sulphide in a purified diet and 4.5 times the potency of Zinc sulphide in a practical diet containing phytic acid for meeting the dietary zinc requirements of channel catfish *Ictalurus punctatus* (Paripatananont and Lovell, 1994).

Postlarvae of *M.rosenbergii* fed with diet containing 90 mgZn/ kg diet showed the best growth, survival, FCR and SGR (Rath and Dube, 1994)

Sources of zinc include dried distillers soluble, corngluten meal, fish solubles,

fishmeal, krill meal, rice mill byproducts, wheat bran and byproducts and yeast. (Akiyama *et al.*, 1992)

2.3.09 Manganese.

Manganese serve as a co-factor for a number of enzymes. Knox *et al.* (1981) indicate that the requirement of manganese may be affected by the age in rainbow trout. No improvement in growth of *P.japonicus* was obtained with dietary supplement of manganese at a level of 0.01 - 0.1% (Kanazawa *et al.*, 1984).

Sources of manganese include dried distillers solubles, crab meal, fish soluble, rice bran and byproducts. Manganese may be supplemented as manganese sulphate (Akiyama *et al.*, 1992).

2.3.10 Selenium.

Selenium is a component of the enzyme glutathione peroxidase which serves to protect cellular tissues and membranes against oxidation. Selenium and vitamin-E function synergistically (Hepher, 1988).

Dietary selenium deficiency in Atlantic salmon fry and fingerlings supressed glutathione peroxidase activity, while supplementation of both Vitamin E (500 IU D.L 2-Tocopherol acetate/kg dry diet) and selenium (0.1 mg/kg dry diet) prevented muscular dystrophy (Poston *et al.*, 1976). Rainbow trout can readily take up selenium from water at ambient concentrations as low as 0.4 mg/l. Dietary selenium concentration as low as 0.07mg/kg and 400 IU vitamin E/kg dry diet prevented deficiency signs such as degeneration of the liver and muscle in rainbow trout fingerlings (Hilton *et al.*, 1980).

Davis *et al.* (1992) observed that deletion of selenium from the mineral premix of the diet resulted in a significant increase in the ash content of the carapace of

Penaeus vannamei. Sources of selenium include blood meal, corn gluten meal, fish solubles, fish meal, rapeseed meal and yeast. Selenium may be supplemented as sodium selenite (Akiyama *et al.*, 1992).

2.3.11 Cobalt.

Cobalt is an essential component of vitamin B_{12} or cyanocobalamin. This vitamin is produced in some fishes and shrimps by the intestinal bacterial flora. Cobalt can be absorbed by fish directly from solution in water. The calcium concentration in water affect the metabolism and absorption of cobalt (Hepher, 1988).

Addition of cobalt to the rearing water increased the survival rate of fry of Indian carp (Das, 1967) and mullet *Mugil parsia* (Ghosh,1975). The best result was obtained when 0.1 ppm Cobalt was added. Dietary supplementation of cobalt in the form of cobaltous chloride at a rate of 0.01 mg/fry/day affected the survival and growth of young fry of Indian major carp after their hatching (Sen and Chatterjee., 1979; Chatterjee, 1979). Khanna and Bhatt (1974) observed that administration of cobaltous chloride to Catfish *Clarias batrachus* caused hyperglycemia and decreased liver glycogen within 5 hours, which was accompanied by histological changes in the islets of Langerhans. Addition of cobalt chloride in the conventional feed influences the growth and maturity of cultivable carps (Rajagopalaswami *et al.*, 1985).

Cobalt chloride at a concentration of 7.5 ppm and manganese chloride at a concentration of 250 ppm were found to be effective in the growth promotion of common carp fry (Xavier *et al.*, 1994). Singhal (1995) observed a weight gain of 422mg/g/day in carp with diet supplemented cobaltous chloride at 200mg concentration as against weight gain of 42mg/g/day without supplementation.

Sources of cobalt include cotton seed meal, soybean meal, fishmeal and yeast. Cobalt may be supplemented as Cobalt sulphate and cobalt chloride (Akiyama *et al.*, 1992).

2.4 Mineral deficiencies

Deficiency symptoms of phosphorus, magnesium, iodine, iron, manganese and zinc in fishes are given in Table 4.

Although minerals may be present in adequate quantities in food stuffs for shrimps and prawn diets, mineral deficiency can occur under intensive culture conditions. The lack of certain specific minerals may be due to the presence of certain compounds that bind the elementary form of mineral that is used in the feed and antagonistic or synergistic reactions in the gastro-intestinal tract are factors that sometimes cause dietary mineral imbalances or deficiency (Tacon, 1987). Reduced growth in *P. japonicus* was reported when there was a deficiency in phosphorus and magnesium. Magnesium deficiency also resulted in decreased survival and feed efficiency (Kanazawa *et al.*, 1984).

2.5 Mineral toxicity

Some minerals become toxic at a certain level of intake and may result in retarded growth and mortality.

Studies done under laboratory conditions in *P.japonicus* showed that levels of iron above 0.006 to 0.012% and manganese 0.01 to 0.1% retarded growth (Kanazawa *et al.*, 1984). In *M.rosenbergii*, the toxicity of heavy metals like copper, cadmium and zinc have been demonstrated (Ismail *et al.*, 1990 ; Chan *et al.*, 1992;Liao and Itsich, 1990). Eggs of *M.rosenbergii* is reported to have high tolerance for heavy metals but

Table4. Mineral deficiency symptoms in fishes.

Element/species	Deficiency symptoms	source
Phosphorus		
Salmo gairdneri	Poor growth and bone development, Low bone Phosphorus.	Lovel, 1989
Oncorhynchus keta	Reduced growth , Low feed conversion, insufficient development of bones.	Watanabe *et al.*, 1980
Anguilla japonica	Anarexia and reduced growth	Arai *et al.*, 1974
Ictalurus punctatus	Reduced growth, poor food efficiency, low bone ash and hematocrit levels	Andrew *et al.*, 1973
Cyprinus carpio	Reduced growth, low feed efficiency, deformity of head.	Ogino and Takeda , 1976.
Pagrus major	Reduced growth, poor food coversion efficiency, bone demineralization, increased muscle, liver and vertibrae lipid content ,curved ,enlarged and spongy vertibrae , decreased liver glycogen.	Sakamoto and yone , 1979.
Sparus macrocephalus	Slow growth rate, poor food convertion ratio, high level of body lipid .	Lie *et al.*, 1993
Magnesium		
Salmo gairdneri	Poor appetite, reduced growth rate, reduced ash content deformations,convulsions, cataracts, mortality	Ogino *et al.*, 1978
Cyprinus carpio	Growth retardation, lordosis, convulsions, mortality	Satoh *et al.*, 1983
Anguilla japonica	Poor appetite and growth retardation	Nose and Arai ,1979
Iodine		
Salmo gairdneri	Proliferation of thyroid tissue	Lall , 1979
Iron		
Anguilla japonica	Hypochromic microcytic anaemia.	Arai and Hashimoto, 1975
Seriola quinqueradita	" "	Ideka *et al.*, 1973
Pagrus major	" "	Yone , 1975.
Cyprinus carpio	" "	Sakomoto and Yone, 1978 b
Hetropneustes fossilis	" "	Firdaus *et al.*, 1994

Table 4. (contd..)

Element/species	Deficiency symptoms	source
Manganese		
Salmo gairdneri	Cataracts , growth inhibition, malformation of backbone and tail	Satoh *et al.*, 1986 b
Zinc		
Salmo gairdneri	Lower growth rate ,high mortality, high incidence of cataracts, high incidence of finerosion and reduced protein and carbohydrate digestibility.	Ogino and Yang ,1978

Table 5 . Composition of basal diets.

Inediants /Diets	S1	S2	S3	S4	S5	S6	S7	S8
Casein	36.0	36.0	36.0	36.0	36.0	36.0	36.0	36.0
Egg albumin	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Glucose	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Sucrose	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
α – corn starch.	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4
glucose amine Hcl	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Sodium succinate	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Mineral Mix	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Vitamine Mix	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
cod liver oil	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Corn oil	4..0	4..0	4..0	4..0	4..0	4..0	4..0	4..0
Carboxye methyl cellulose	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Cholestrol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Phosphate ($Na H_2 Po_4 .2H_2O$)	0	2.5	5.0	7.5	10.0	12.5	15.0	20.0
Cellulose	20.0	17.5	15.0	12.5	10.0	7.5	5.0	0
Total	100	100	100	100	100	100	100	100

the levels of copper, cadmium and zinc in larval rearing tank should not exceed 0.1, 0.057 and 2.97 mg/l, respectively. Among metals, copper is recognised as the most toxic to *Macrobrachium* larvae.

III.MATERIAL AND METHODS

The experiment was conducted at the *Macrobrachium* hatchery of the College of Fisheries, Panangad, Kochi. The study was designed to find out the optimum requirement of phosphorus in the diet and to characterise the symptoms of deficiency and excess of phosphorus in the giant freshwater prawn *Macrobrachium rosenbergii* juveniles. Since phosphorus is a micronutrient which is required only in small quantities, the experiment was conducted for a longer duration of 84 days in order to obtain objective results.

3.1 Experimental animals

M.rosenbergii juveniles produced at the hatchery of the College of Fisheries were used for the experiment.

The postlarvae were produced from berried female obtained from the wild. The larval rearing was done in recirculating clear water system with biological filter and at water salinity of 12 - 14 ppt (Nair & Thampy, 1988; Sebastian, 1990). The larvae were fed with prepared feed composed of 'thelly' meat (*Metapenaeus dobsoni*) and hen's egg on three hourly intervals during day time and Artemia nauplii during night. Full siblings settled on the 25th day were separated into a flat bottomed oval tank of 1.2 tonne capacity and reared in freshwater. The postlarvae were fed with clam meat based commercial feed at a rate of 20% of their body weight twice daily to reach the juvenile stage. Dried twigs were kept in the tank to provide substratum for attachment and to reduce cannibalism during moulting(Smith and Sandifer,1975).Cleaning of the tank and water exchange were done once in five days.

3.2 Experimental rearing facilities

The experiment was conducted in a shed with good ventilation, roofed partially

with translucent fibreglass reinforced plastic (FRP) sheets for moderate light conditions. The experimental tanks were placed on concrete floor with gentle slope towards one side to facilitate easy drainage of water. Freshwater taps and aeration facilities from oil-free Roots airblower were provided at the site as shown in Plate I.

Round bottomed fibreglass tank with the following specifications was used for the experiment.

Capacity	-	83 litres
Diameter	-	0.55 m
Bottom Area	-	0.2376 m^2
Height	-	0.35 m
Thickness	-	4 mm
Rim Width	-	3 cm
Colour	-	Aquamarine

Flat tiles (20 x 20 cm) kept in an inclined position by means of a piece of stone inside the tank provided substrate and shelter for the prawns. A controlled aeration was provided in each tank and the tank was arranged as shown in Plate II.

Freshwater from the pond was used for rearing the prawn during the experiment. The water was collected in a sump and the suspended matter was allowed to settle at the bottom. The clear water was pumped to an overhead tank from where it was made available at the site by gravity. The water was filtered through fine meshed filter cloth to remove algae and other debris before using in the experimental tank.

3.3 Experimental diets

Eight casein based semipurified isoproteinacious diets with supplementary phosphorus concentrations varying from 0 to 4%, made in one batch were used for the study. The basal diet as used by Sherief *et al.*, (1992) was modified as shown in the table 5. The source of w_6 fatty acid, sunflower oil was replaced by corn oil and binder carragenan by carboxymethyl cellulose. The vitamin mix used is given in

Plate I A view of experiment site

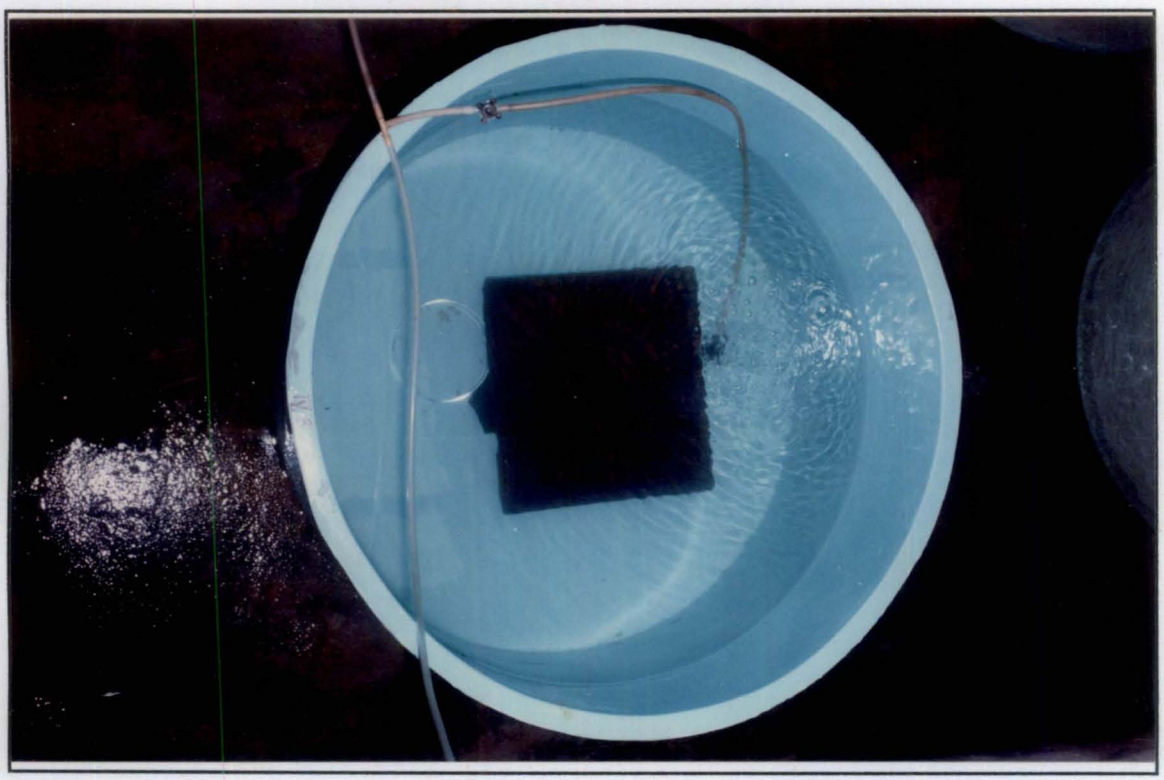


Plate II Experimental tank

Table6. Composition of Vitamin mix.

Vitamins	Quantity (mg)
Thiamin Hcl	5.0
Riboflavin	8.0
Paraaminobenzoic acid	10.0
Biotin	0.4
Inocitol	400.0
Niacin	40.0
Calcium Pantothenate	60.0
Pyridoxin Hcl	12.0
Menadione	4.0
B carotene	9.6
X - tocopherol	20.0
Calciferol	100.0
Vitamin B_{12}	0.1
Folic acid	0.9
Choline chloride	600.0
Vitamin -C	2730.0
Total	4000.0

Table 7. Composition of mineral mix.

Minerals	Quantity (mg)
Ca CO_3	2500.0
Mg SO_4	520.0
Kcl	311.0
Sodium citrate	298.8
Nacl	240.0
$FeSO_4$	100..0
Zn SO_4	20.0
KI	6.0
$MnSOS_4$	3.0
$AlCl_3$	0.4
CuCl	0.4
Co Cl	0.4
Total	4000.0

Table8. Proximate composition of experimental diets.

Components/Diets (g/100g)	S1	S2	S3	S4	S5	S6	S7	S8
Crude protein	38.05	37.47	37.90	36.98	38.95	38.66	37.98	36.98
Moisture	8.92	9.08	8.82	10.94	8.48	8.79	8.66	8.10
Fat	7.42	6.89	6.21	5.98	6.26	6.51	7.12	6.61
Crude Fibre	8.25	7.89	7.13	6.93	5.14	3.38	2.95	2.80
Ash	5.85	6.60	7.40	8.90	10.20	12.80	14.30	18.10
Carbohydrate	31.51	32.07	32.54	30.27	30.97	29.86	28.99	27.41
Calcium	1.04	1.00	1.05	1.10	1.03	1.10	0.99	1.08
Phosphurus	0.26	0.79	1.22	1.76	2.26	2.77	3.30	4.29
ca : p ratio	1 : 0.25	1 : 0.79	1 : 1.16	1 : 1.68	1 : 2.19	1 : 2.52	1 : 3.33	1:3.9 7

Table 6 which is the same as described by Kanazawa *et al.*(1982). Mineral mix described by Stahl and Ahearn (1978) and Deshimaru and Yone (1978) was modified as given in the table 7 and is used to formulate the diet.

Sodium dihydrogen phosphate (NaH_2PO_4. $2H_2O$) was used as the source of phosphorus and cellulose as the filler in the feed. NaH_2PO_4. $2H_2O$ and cellulose was added in such a way that the feeds contain supplementary phosphorus at 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 4.0% levels, in the diet s1, s2, s3, s4, s5, s6, s7 and s8, respectively. Calcium carbonate($CaCO_3$) served as the source of clacium which was maintained in constant level of about 1% in all the feeds.

The ingredients were finely powdered and sieved through 250 micron sieve. It was then separately weighed in the electronic balance (SHIMADZU-LAROR-AEV 130V,with an accuracy of 0.0001 g) as per the composition. Except vitamins and oils, all other ingredients were mixed thoroughly in a food mixer. The mixture was then blended with boiling water and hand kneaded until a consistency appropriate for pelleting was obtained. Vitamins and oils were added and the dough was pelleted with a hand pellet mill using 3mm die. The pellets were collected in an enamel tray and dried in an oven at 50°C for 12 hours to a moisture content of < 10%. The pellets were then broken into pieces and sieved to get particles of 1-2 mm size. The feeds were stored in refrigerated condition in plastic containers covered with black plastic paper, in order to prevent loss of nutrients(Paulraj, 1993).

3.4 Experimental procedure

Healthy well pigmented juveniles with weight 0.0684 ± 0.0068g and length 2.12 ± 0.09 cm were used for the experiment. The prawns were acclimatized in the experimental tanks to their corresponding semi-purified diets for five days. Feeding was done *ad libitum* twice daily during this period.

The experiment was planned using completely randomised design with twenty

four tanks were randomly alloted to 8 treatments in 3 replications each. About 50 litres of freshwater was maintained in the experimental tanks. 10 numbers of prawns were maintained in each tank after being acclimatized with their corresponding feeds. The prawn were then starved for 24 hours in order to remove their gut contents, before weighing and the weighing was done using the electronic balance. Standardised blotting technique was used to weigh the prawn. The total lengths of the prawn were also noted in each tank.

The prawns were fed with the experimental diets at the rate of about 20% of their body weight for the first 3 fortnights and it was reduced to 10% in the last 3 fortnights. Feeding was done twice a day, on morning and evening hours.Feed for each tank for a period of one week was weighed and kept in separate small plastic containers. The feed with no supplementary phosphorus (Diet s1) served as control. The feed was supplied in petri-dishes kept in the tank. The feed remnants were collected daily and dried to constant weight to compute the actual feed consumption of the animal.

A water exchange of 75% by volume was done in every alternate day to maintain water quality. The sides of the tank were cleaned daily to prevent the devolpment of algae and possible alteration in phosphorus assimilation, and to maintain hygienic condition in the tank.

The experimental tanks were checked daily for dead specimens, if any, and their weight was recorded for computation of total weight gain. Growth assessments were carried out at fortnightly intervals. The tanks,tiles, stone and the diffusion stones were cleaned and dried at the time of the assessments.

At the end of the 6th fortnight the prawns were captured and the final assessment was made. The prawn fed on different diets were thoroughly examined and the external characteristics were studied. Prawn under each treatment were pooled and used to estimate the whole body phosphorus and calcium contents.

3.5 Physico-chemical parameters

The physico-chemical parameters of rearing water such as temperature, pH, dissolved oxygen, total alkalinity, total hardness, phosphorus and calcium levels were measured during the experiment.

Temperature : Using mercury bulb thermometer with accuracy of 0.1°C

pH : Using Universal pH indicator solution

Dissolved Oxygen: Using standard Winkler method (Strickland and Parson, 1972)

Total Alkalinity: Using chemical methods (Boyd and Pillai, 1984)

Total Hardness : Using chemical methods (Boyd and Pillai, 1984)

Phosphorus : Using photometric methods (Fiske and Subbarow, 1925)

Calcium : Using Permanganometry (Clark and Collip, 1925)

3.6 Evaluation methods

3.6.1 Proximate composition of feed.

Proximate analysis of the prepared feed was conducted to quantify the level of

nutrients and to estimate the calcium and phosphorus in each case ; analysis was carried out in triplicate and the mean values were determined. The methods used for analysis were the following.

Moisture : By drying the sample at 105 °C for 12 hours (Boyd's Method, 1979)

Crude Protein : By Microkjeldhal's method(AOAC,1975)

Crude Fat : By solvent extraction method using petroleum ether (Bp 40-60°C) in a Soxhlet extraction apparatus for 6 hours (Flachlees and Solonestanley, 1957)

Ash : By combustion at 450 degree C for 12 hours

Crude Fibre : By method of Pearson (1976).

Carbohydrate : By difference in dry weight (Hasting, 1976)

3.6.2 Water quality parameters.

Temperature and pH were measured in the morning and evening hours and the mean values for every fortnight were obtained. Other parameters like dissolved oxygen, total alkalinity, total hardness, phosphorus and calcium levels were estimated fortnightly and the mean values during the period of experiment were obtained.

3.6.3 Percentage weight gain (PWG).

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

3.6.4 Percentage gain in length (PGL).

$$PWL = \frac{\text{Final average length - Initial average length}}{\text{Initial average length}} \times 100$$

3.6.5 Specific growth rate (SGR).

$$SGR = \frac{\text{In Wf - In Wi}}{\text{Tf - Ti}} \times 100$$

Where, SGR is the specific growth rate in percentage (assuming exponential growth); Wi is the initial weight of the experimental animal at the day Ti ; Wf is the final weight at the day Tf ; In, denotes logarithm (base e)

3.6.6 Percentage survival rate.(PSR)

$$PSR = \frac{\text{Final number of prawns}}{\text{Initial number of prawns}} \times 100$$

3.6.7 Food conversion ratio and food conversion efficiency (FCR & FCE).

$$FCR = \frac{\text{Feed intake on a dry matter basis}}{\text{Weight gain on wet matter basis}}$$

$$FCE = \frac{\text{Weight gain on wet matter basis}}{\text{Feed intake on a dry matter basis}}$$

3.6. 8 Protein efficiency ratio (PER).

$$PER = \frac{\text{Total weight gain on wet matter basis}}{\text{Crude protein intake on a dry matter basis}}$$

3.6.9 Wholebody phosphorus and Ca:P ratio

The prawn samples (Intermoult stage) were oven dried and dry ashed according to procedures described in the Association Of Analytical Chemists(1984). Pooled sampes were used for estimation of phosphorus and calcium. Phosphorus was determined photometrically (Fiske and Subbarow, 1925) and calcium by Permanganometry (Clark and Collip, 1925).

3.7 Statistical Methods

Data were analysed using analysis of varience as the experiment was planned using CRD. Indices such as PWG, PGL, SGR, PSR, FCE and PER were analysed for significant variation using ANOVA (at 5 % level). Pairwise comparison was performed using 't' test technique (Snedecor and Cochran, 1968). In order to guarantee the normality of distribution, Arc sine transformation was carried out where ever necessary, as follows:

$$Y = \sin^{-1} (x/100)^{0.5}$$

where Y is the transformed value and x is the observation in percentages.

To obtain the optimum dietary phosphorus level from the wholebody phosphorus content, second order polynomial regression analysis was employed. It describes the relationship between dietary phosphorus level and wholebody phosphorus content in a curvilinear fashion ($y = a+bx+cx^2$).

IV. RESULTS

4.1 Proximate composition of diet

The proximate composition , the calcium and phosphorus content and the Ca:P ratio of the experimental diets are given in Table 8. The average protein content was 37.87 ± 0.67 g/100g, fat content 6.625 ± 0.46 g/100g, carbohydrate content 30.45 ± 1.58 g/100g and calcium content 1.05 ± 0.04 g/100g . The Control diet without phosphorus supplementation contained 0.26 ± 0.04 g/100g phosphorus.

4.2 Water quality maintenance

The water temperatures of the experimental tanks is given in Table 9 . The temperature varied between 27 and 31°C. The recorded values of pH are shown in Table 10 which ranged from 7.0 to 9.5. Table 11 gives the values of other parameters such as dissolved oxygen, total alkalinity, total hardness, phosphorus and calcium levels of the rearing water.

4.3 Determination of optimum dietary phosphorus

Determination of optimum phosphorus requirement is based on the following criteria.

1. Percentage Survival Rate (PSR)
2. Percentage Weight Gain (PWG)
3. Percentage Gain in Length (PGL)
4. Specific Growth Rate (SGR)
5. Food Conversion Ratio and
   Food Conversion Efficiency (FCR & FCE)
6. Protein Efficiency Ratio (PER)

Table 9. Temperature of rearing water.

Temp / Fortnights	1	2	3	4	5	6
Mean	29.19	29.95	29.08	28.46	28.12	28.81
± SE	1.31	0.84	1.17	1.20	1.13	1.36
Range	27.0 - 31.0	28.5 - 31.0	27.5 -30.5	27.0 - 30.0	27.0 - 29.5	27.0 - 30.5

Table 10. pH of rearing water.

pH/ Fortnights	1	2	3	4	5	6
Mean	7.79	7.82	7.91	7.81	7.65	7.82
± SE	0.59	0.48	0.61	0.58	0.81	0.57
Range	7.0 - 8.5	7.0 - 8.5	7.0 - 8.5	7.0 - 9.0	7.5 -9.5	7.0 - 8.5

Table 11. Chemical Parameters of rearing water.

Parameter (mg/l)	Dissolved oxeygen	Total alkalinity	Total hardness	Phosphorus	Calcium
Mean	5.5407	94.0033	112.4	0.0476	49.833
± SE	0.57 88	2.4846	3.0523	0.0137	4.2979
Range	4.6929 - 6.2153	90.96 - 95.81	1.098-116.8	0.0282-0.0570	42.0-56.0

Table 12. Percentage survival rate of prawn fed with different levels of phosphorus

Treatment	Replication	Survival (%)	Mean	± SE
S1	1	90	86.67	4.71
	2	90		
	3	80		
S2	1	70	83.33	9.43
	2	90		
	3	90		
S3	1	90	90.00	8.16
	2	100		
	3	80		
S4	1	80	80.00	8.16
	2	90		
	3	70		
S5	1	90	83.33	9.43
	2	70		
	3	90		
S6	1	80	76.67	4.71
	2	70		
	3	80		
S7	1	90	93.33	4.71
	2	90		
	3	100		
S8	1	100	93.33	
	2	100		

Table 13. ANOVA table for percentages survival rate of prawn.

(Data subjected to arc sine transformation)

Source	Sum of Square	Degrees of freedom	Mean sum of square	F ratio
Treatment	1039.0156	7	148.4308	1.5399
Error	1542.1462	16	96.3841	
Total	2581.1618	23		

(Calculated F value < table value at 5% level of significance)

Table 14 Percentage weight gain of prawn fed with different levels of phosphorus

Treatment	Replicatoion	Av. initial wt . (mg)	Av. final wt. (mg)	Av. wt. gain (mg)	% wt. gain	Mean ± SE
S1	1	73.8	323.2	249.4	337.94	339.94
	2	69.9	269.2	199.3	285.12	45.35
	3	73.1	362.7	289.6	396.17	
S2	1	58.8	313.4	254.6	432.99	329.32
	2	78.6	284.6	206.0	262.09	77.72
	3	64.1	240.3	176.2	274.88	
S3	1	60.8	233.1	172.3	283.39	296.19
	2	60.5	243.1	182.6	301.82	9.07
	3	68.7	277.1	208.4	303.35	
S4	1	58.4	336.4	278.0	476.03	395.95
	2	79.6	347.8	268.2	336.93	58.71
	3	73.8	350.5	276.7	374.90	
S5	1	74.6	264.2	189.6	254.16	329.44
	2	66.0	358.6	292.6	443.33	82.02
	3	64.0	249.7	185.7	290.96	
S6	1	53.0	240.8	187.8	354.34	340.73
	2	74.8	278.8	204.0	272.73	.59.88
	3	65.6	324.8	259.2	359.12	
S7	1	73.0	342.8	269.8	369.59	390..75
	2	72.2	392.9	320.7	444.18	38.05
	3	65.5	300.3	234.8	358.47	
S8	1	69.7	257.8	188.1	269.87	320.35
	2	65.5	292.8	227.3	347.02	35.72
	3	77.2	342.9	265.7	344.17	

Table 15 ANOVA table for percentage weightgain of prawn (Data subjected to arcsine transformation).

Source	Sum of square	Degrees of freedom	Mean sum of square	F ratio
Teatment	0.5992	7	0.0856	0.8235
Error	1.6631	16	0.1039	
Total	2.2623	23		

(Calculated F value < table value at 5 % level of significance).

Table 16. Average fortnightly net increment in weight of prawn fed with different levels of phosphorus.

Dietary level% /Fortnights	1	2	3	4	5	6
0.26	0.0281	0.0331	0.0370	0.0359	0.0584	0.0536
0.79	0.0297	0.0328	0.0248	0.0320	0.0459	0.0471
1.22	0.0203	0.0238	0.0239	0.0337	0.0356	0.0504
1.76	0.0310	0.0332	0.0336	0.0505	0.0596	0.0406
2.26	0.0336	0.0246	0.0372	0.0403	0.0440	0.0428
2.77	0.0255	0.0264	0.0299	0.0368	0.0500	0.0485
3.30	0.0334	0.0342	0.0295	0.0543	0.0571	0.0668
4.29	0.0294	0.0311	0.0343	0.0348	0.0490	0.0484

Table 17. Percentage gain in length of prawn fed with different levels of phosphorus.

Treatment	Replication	Av. in. length (cm)	Av. fin. length in (cm)	Av. gain in length (cm)	% gain in length	Mean ± SE
S1	1	2.12	3.42	1.30	61.32	65.43
	2	2.12	3.36	1.24	58.49	7.89
	3	2.04	3.60	1.56	76.47	
S2	1	1.94	3.38	1.44	74.23	63.65
	2	2.08	3.24	1.16	55.77	7.77
	3	2.10	3.38	1.28	60.95	
S3	1	2.10	3.28	1.18	56.19	58.67
	2	2.12	3.34	1.22	57.55	2.60
	3	2.12	3.44	1.32	62.26	
S4	1	1.94	3.92	1.98	102.06	74.95
	2	2.28	3.26	0.98	42.98	24.36
	3	2.08	3.74	1.66	79.81	
S5	1	2.18	3.60	1.42	65.14	67.25
	2	2.14	3.58	1.44	67.29	1.70
	3	2.02	3.42	1.40	69.31	
S6	1	2.18	3.20	1.20	46.79	53.06
	2	2.12	3.26	1.14	53.77	4.86
	3	2.32	3.68	1.36	58.62	
S7	1	2.02	3.70	1.68	83.17	77.26
	2	2.12	3.70	1.58	74.53	4.19
	3	2.16	3.76	1.60	74.07	
S8	1	2.22	3.32	1.10	49.55	61.90
	2	2.16	3.62	1.46	67.59	8.74
	3	2.10	3.54	1.44	68.57	

Table 18. ANOVA table for percentage gain in length of prawn.

Source	Sum of square	Degrees of freedom	Mean sum of Square	F ratio
Treatment	1343.8839	7	191.9834	1.2138
Error	2530.5760	16	158.1610	
Total	3874.4599	23		

(Calculated F Value < table value at 5% leval of significance.)

Table 19. Specific growth rate of prawn fed with different levels of phosphorus.

Treatement	Replicatim	Av. In Wt (mg)	Av. fin wt. (mg)	SGR	Mean ± SE
S1	1	73.8	323.2	1.76	1.76
	2	69.9	269.2	1.61	0.12
	3	73.1	362.7	1.91	
S2	1	58.8	313.4	1.99	1.70
	2	78.6	284.6	1.53	0.21
	3	64.1	240.3	1.57	
S3	1	60.8	233.1	1.60	1.64
	2	60.5	243.1	1.66	0.03
	3	68.7	277.1	1.66	
S4	1	58.4	336.4	2.08	1.90
	2	79.6	347.8	1.76	0.13
	3	73.8	350.5	1.85	
S5	1	74.6	264.2	1.51	1.71
	2	66.0	358.6	2.01	0.21
	3	64.0	249.7	1.62	
S6	1	53.0	240.8	1.80	1.76
	2	74.8	278.8	1.57	0.14
	3	65.6	324.8	1.90	
S7	1	73.0	342.8	1.84	1.89
	2	72.2	392.9	2.02	0.09
	3	65.5	300.3	1.81	
S8	1	69.7	257.8	1.56	1.71
	2	65.5	292.8	1.78	0.10
	3	77.2	342.9	1.78	

Table20. ANOVA table for the specific growth rate of prawn (Data subjected to arc sine transformation)

Source	sum of square	Degrees of freedom	Mean sum of square	F ratio
Treatment	0.8372	7	0.1196	0.8408
Error	2.2759	16	0.1422	
Total	3.1131	23		

(Calculated F value < Table value at 5 % level of significance)

7. Wholebody Phosphorus Content
8. Wholebody Calcium Content
9. Wholebody Ca:P Ratio

4.3.1 Effect of varying dietary phosphorus levels on percentage survival rates.

The percentage survival rates of prawn fed with varying phosphorus levels are given in Table 12.

The results of analysis of variance (Table 13) show the influence of varying dietary levels of phosphorus on percentage survival rate as not significant. Fig. 1 demonstrates the effect of phosphorus level of the diet on survival rate. Among the levels of phosphorus used in the present study the highest survival of 93.33% is obtained at higher levels of phosphorus such as 3.3% (Diet s7) and 4.29 % (Diet s8).

4.3.2 Effect of varying dietary phosphorus levels on percentage weight gain.

The percentage weight gain of prawns obtained by feeding varying levels of supplementary phosphorus are given in the Table 14 and Fig. 2.

The analysis of variance (Table 15) indicates that there is no significant variation among different phosphorus levels used in the diets on their influence on percentage weight gain of the animal . Among the different levels of phosphorus used in the diet the highest percentage weight gain of 395.95% is recorded for 1.76% (Diet s4).

Fortnightly increment in the weight of prawn by feeding different levels of phosphorus over the experimental period is presented in Table 16. The highest

Figure 1. Effect of dietary phosphorus levels on percentage survival of prawn.

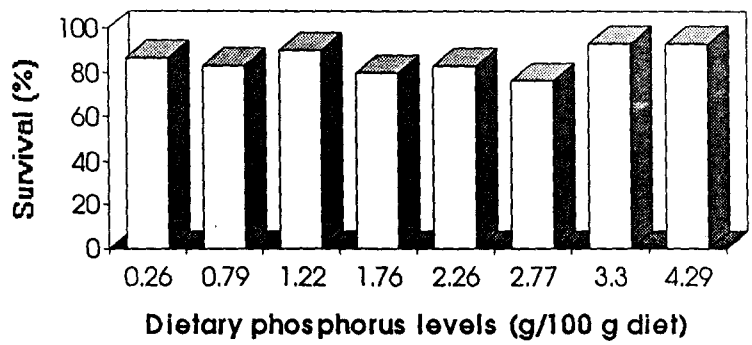
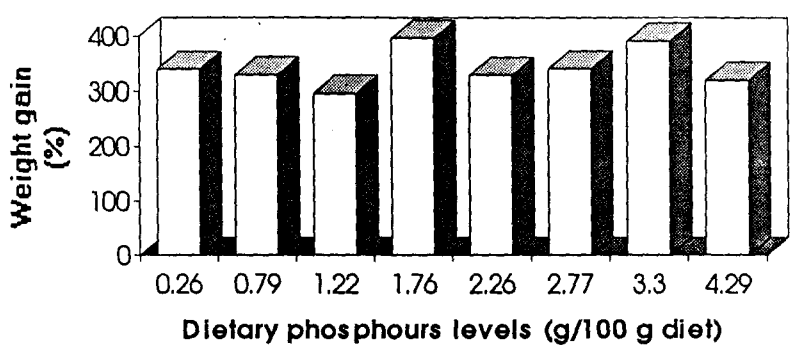


Figure 2. Effect of dietary phosphorus levels on percentage weight gain of prawn



increment in weight of 0.0668g is obtained in prawn fed with 3.3% phosphorus (Diet s7) during the 6th fortnight, which is followed by 0.0596g fed with 1.76% phosphorus (Diet s4) during the 5th fortnight. The prawn fed with control diet (Diet s1) having 0.26% phosphorus gives maximum growth increment of 0.0854g during the 5th fortnight and those fed with 4.29 % phosphorus yielded the highest growth increment of 0.0490g during the 5th fortnight. Fig.3 gives the variations of gain in weight by prawn fed with varying levels of phosphorus on fortnightly intervals during the course of the experiment.

4.3.3 Effect of varying phosphorus levels on percentage gain in length

The percentage gain in length of prawn with respect to varying dietary phosphorus levels is given in Table 17 and also depicted in Fig.4.

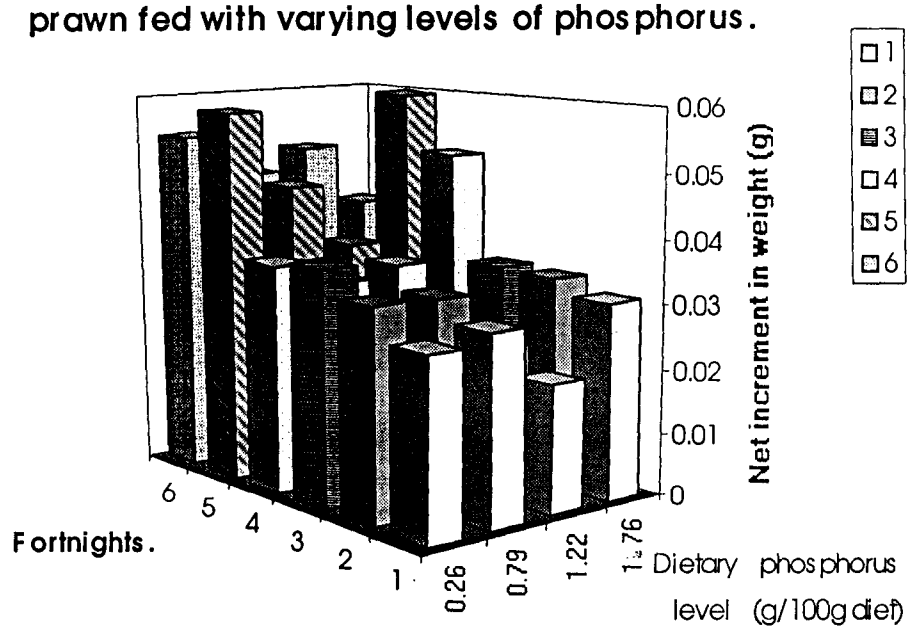
The analysis of variance indicates that the influence of different phosphorus levels exhibit no significant variation in percentage gain in length of the animal (Table 18). The highest percentage gain in length of 77.26% is obtained with dietary phosphorus level of 3.3% (Diet s7) followed by 74.95% gain in length with dietary phosphorus level of 1.76% (Diet s4).

4.3.4 Effect of varying phosphorus levels on specific growth rate

The specific growth rate of prawns fed with different levels of phosphorus are given in the Table 19 and graphically represented in Fig.5.

Results of analysis of variance as given in Table 20 indicates that the variations in specific growth rate exhibited by prawns fed with different levels of phosphorus are not significant.

Figure 3. Fortnightly net increment in weight of prawn fed with varying levels of phosphorus.



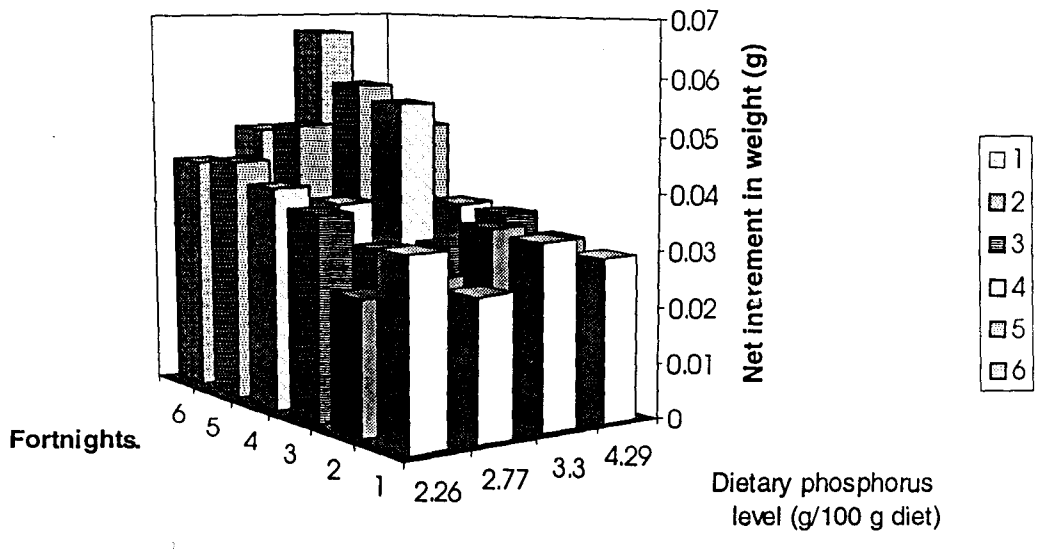


Figure 4. Effect of dietary phosphorus levels on percentage gain in length of prawn.

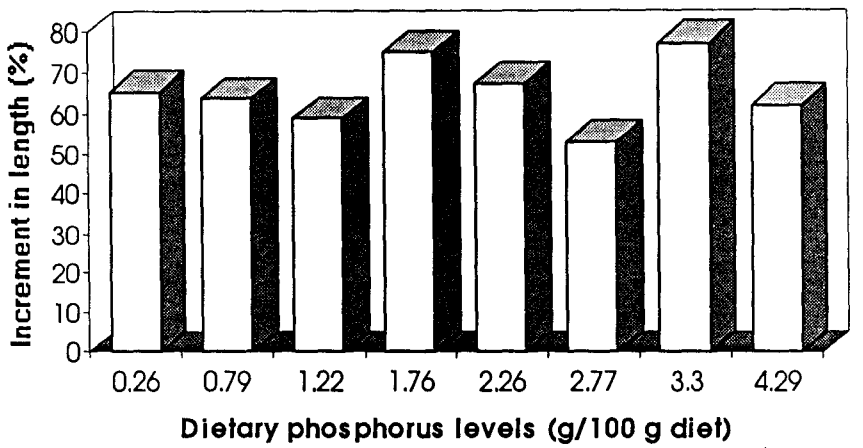
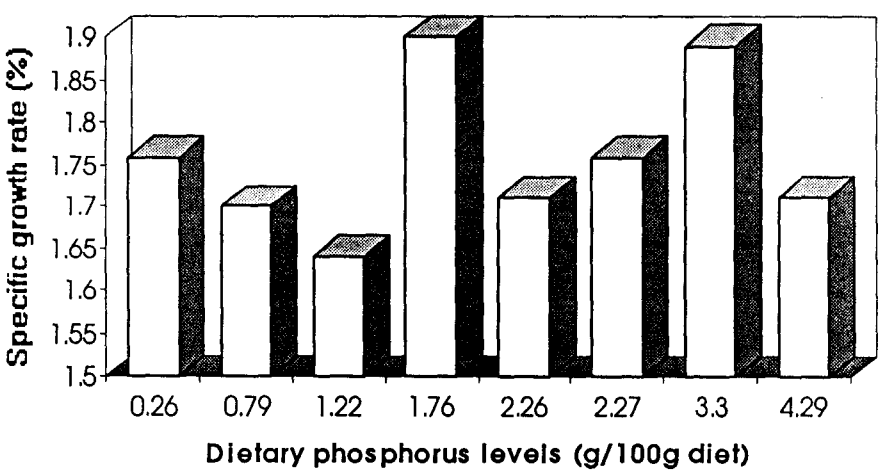


Figure 5. Effect of dietary phosphorus levels on specific growth rate of prawn.



The highest SGR of 1.9% is observed in prawns fed with 1.76% phosphorus (Diet s4) followed by 1.89% in prawns fed with 3.3% (Diet s7). In the figure, it can be noted that as the dietary level of phosphorus increases from 0.26 to 4.29%, there exist two peaks in SGR values the highest being at a level of 1.76% (Diet s4). Table 8 indicates that the Ca:P ratio of the diet s4 is 1:1.68.

4.3.5 Effect of varying phosphorus levels on FCR and FCE

The FCR and FCE of the diet with varying levels of phosphorus obtained with the experimental animal is shown in Table 21. FCE (Fig.6) with respect to the different phosphorus levels are also represented graphically.

Analysis of variance indicates that there exists significant variation in the influence of varying dietary phosphorus levels on food conversion efficiency(Table 22). Pairwise comparison of the mean values of FCE shows that the feeds used in the experiment are involved in three groups based on their relative significance on conversion efficiency. It can be noted that there is no significant variation between the feeds containing 1.76%(Diet s4), 3.3% (Diet s7) and 0.26% (Diet s1) of phosphorus with respect to the food conversion efficiency. The food conversion efficiency is maximum (0.1902) for feed (Diet s4) containing 1.76 % phosphorus (Table 21), the influence of which is significantly different from other feeds except the feed containing 3.3 % (Diet s7) and 0.26 % (Diet s1) phosphorus. The lowest food conversion value (5.2745) is obtained for feed containing 1.76 % phosphorus (Diet s4). The Fig.6 demonstrates that as the dietary level of phosphorus increases from 0.26 to 4.29 %, the FCE values show 3 peaks, the maximum being at dietary phosphorus level of 1.76 % (Diet s4). The reverse is the case with FCR values (Table 21), where the lowest value is observed at 1.76 % of dietary phosphorus (Diet s4).

Table 21. Food conversion ratio and food conversion efficiency of the diets with different levels of phosphorus.

Treatment	Replication	wt. gain (g)	Feed intake (g)	FCE	Mean FCE ± SE	FCR	Mean FCR ±SE
S1	1	2.4284	15.5704	0.1560	0.1498	6.4118	6.7791
	2	1.8913	15.0674	0.1255	0.018	7.9667	0.860
	3	2.6040	15.5168	0.1678		5.9588	
S2	1	2.2329	14.3146	0.1560	0.1362	6.4108	7.4182
	2	1.9211	14.9986	0.1281	0.014	7.8073	0.718
	3	1.6644	13.3761	0.1244		8.0366	
S3	1	1.6893	14.1470	0.1194	0.1254	8.3745	7.985
	2	1.8260	13.9711	0.1307	0.005	7.6512	0.298
	3	1.8917	15.0040	0.1261		7.9315	
S4	1	2.3770	13.4890	0.1762	0.1902	5.6748	5.2745`
	2	2.5437	13.2053	0.1926	0.011	5.1914	0.299
	3	2.5077	12.4313	0.2017		4.9573	
S5	1	1.8328	13.4464	0.1363	0.1378	7.3365	7.4227
	2	2.1843	13.3404	0.1637	0.021	6.1074	1.111
	3	1..6935	14.9440	0.1133		8.8243	
S6	1	1.8174	13.7422	0.1322	0.1434	7.5615	7.1515
	2	1.7386	14.3026	0.1215	0.024	8.2286	1.086
	3	2.4661	13.9691	0.1765		5.6644	
S7	1	2.4450	15.5294	0.1574	0.1795	6.3515	5.7061
	2	3.1137	14.1022	0.2208	0.029	4.5291	0.834
	3	2.3495	14.6557	0.1603		6.2378	
S8	1	1.8815	15.6210	0.1204	0.1377	8.3024	7.3264
	2	2.2730	15.0686	0.1508	0.013	6.6294	0.711
	3	2.2652	15.9637	0.1419		7.0474	

Table 22. ANOVA table for the food conversation efficiency of the diets.

Source	Sum of Square	Degrees of freedom	mean sum of square	F Ratio
Treatement	0.0109	7	0.0016	3.1537*
Error	0.0079	16	0.0005	
Total	0.0188	23		

Critical difference for the comparison of treatement mean values (at 5 % level of significant) = 0.0387
Comparison of mean value of FCE with critical difference value :

Treatment	Mean value of FCE
S4	0.1902
S7	0.1795
S1	0.1498
S6	0.1434
S5	0.1378
S8	0.1377
S2	0.1362
S3	0.1254

Side scored mean FCE values are not significantly different at 5 % level

* Significantly different at 5 %level

Table 23. Protein efficiency ratio of the diet with different levels of phosphorus.

Treatment	Replication	Total wt. gain (g)	Total protein consumption (g)	PER	Mean ± SE
S1	1	2.4284	5.9245	0.4099	0.3936
	2	1.8913	5.7331	0.3299	0.05
	3	2.6040	5.9041	0.4410	
S2	1	2.2329	5.3637	0.4163	0.3634
	2	1.9211	5.6200	0.3418	0.04
	3	1.6644	5.0120	0.3321	
S3	1	1.6893	5.3617	0.3151	0.3309
	2	1.8260	5.2950	0.3449	0.01
	3	1.8917	5.6865	0.3327	
S4	1	2.3770	4.9882	0.4765	0.5143
	2	2.5437	4.8833	0.5209	0.03
	3	2.5077	4.5971	0.5455	
S5	1	1.8328	5.2374	0.3499	0.3537
	2	2.1843	5.1961	0.4204	0.05
	3	1.6935	5.8207	0.2909	
S6	1	1.8174	5.3127	0.3421	0.3710
	2	1.7386	5.5308	0.3143	0.06
	3	2.4661	5.4005	0.4566	
S7	1	2.4450	5.8981	0.4145	0.4726
	2	3.1137	5.3560	0.5813	0.08
	3	2.3495	5.5662	0.4221	
S8	1	1.8815	5.7766	0.3257	0.3724
	2	2.2730	5.5724	0.4079	0.03
	3	2.2652	5.9034	0.3837	

Table 24. ANOVA table for protein efficiency ratio of the diet.

Source	sum of square	Degrees of freedom	Mean sum of square	F ratio
Treatment	0.0844	7	0.0121	3.5203*
Error	0.0548	16	0.0034	
Total	0.1392	23		

Critical difference for comparison of treatment mean values. (at 5 % level of significtance) = 0.1009

Comparison of mean value of PER with critical difference value :

Treatment	Mean values of PER
S4	0.5143
S7	0.4726
S1	0.3936
S8	0.3724
S6	0.3710
S2	0.3634
S5	0.3537
S3	0.3309

Sidescored mean PER values are not significantly different at 5 % level.

* Significantly different at 5 % level.

Figure 6. Effect of dietary phosphorus levels on food convertion efficiency.

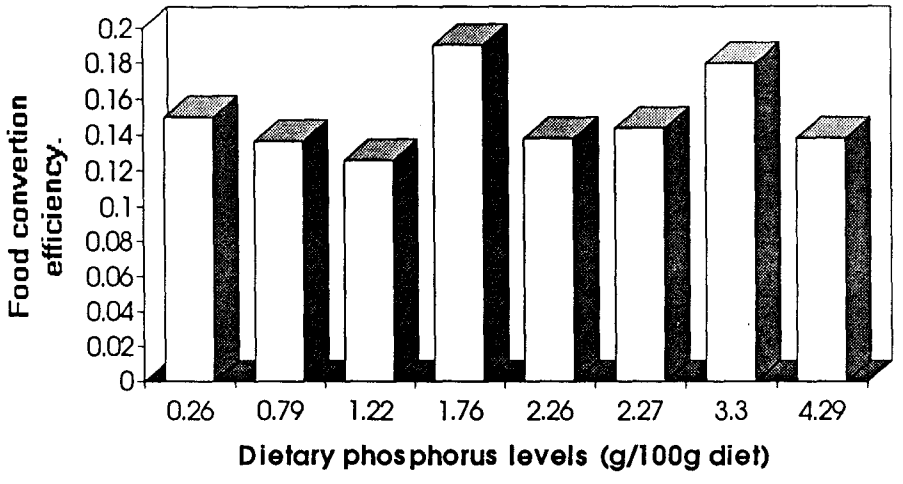
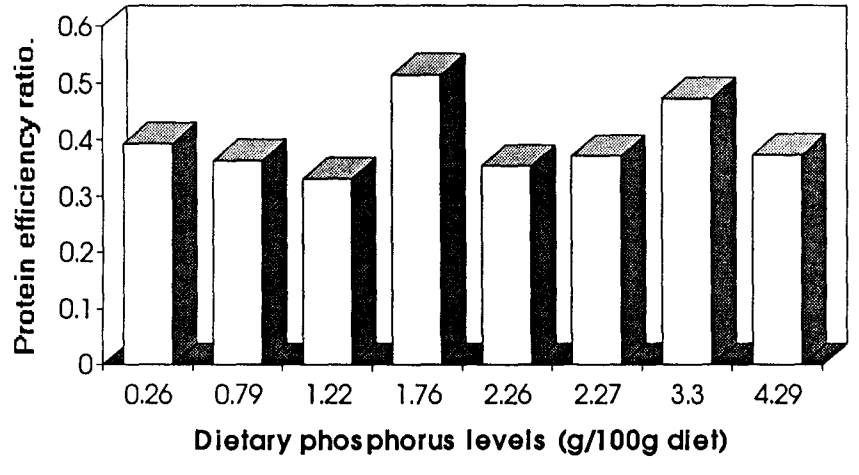


Figure 7. Effect of dietary phosphorus levels on protein efficiency ratio.



4.3.6 Effect of varying phosphorus levels on protein efficiency ratio.

The protein efficiency ratio obtained for feeds having varying levels of phosphorus are shown in Table 23, which is presented graphically in Fig.7.

Analysis of variance (Table 24) shows that there exist significant variations among the different levels of phosphorus tested in the experiment based on their influence on PER. Pairwise comparison of mean PER values indicates the presence of 3 groups of feeds. The highest PER of 0.5143±0.03 (Table 23) is obtained for feed containing 1.76 % phosphorus (Diet s4), which is followed by PER of 0.476±0.08 for feed containing 3.3% (Diet s7) phosphorus, and it is noted that as per Table 24, there is no significant variation between these two feeds with respect to the protein efficiency ratio. Fig.7 demonstrates the presence of 2 peaks of PER values when the dietary phosphorus level varies from 0.26% to 4.29%, the maximum being at 1.76% phosphorus (Diet s4).

4.3.7 Variation of wholebody phosphorus content with dietary phosphorus levels.

The wholebody phosphorus level of the prawn obtained with respect to the varying dietary phosphorus levels are given in Table 25. The Fig.8 shows that the wholebody phosphorus level increases as dietary phosphorus level increases from 0.26 to 1.76% and then decreases up to a dietary phosphorus level of 3.3%. A sharp increase in wholebody phosphorus level is observed, when the dietary phosphorus level increases to 4.29%. The first peak value of 525.8545 mg/100g wholebody phosphorus content (Table 25) is observed when feeding diet with 1.76% phosphorus (Diet s4), and the second peak of 527.05 mg/100g wholebody phosphorus, when feeding 4.29% phosphorus (Diet s8).

Optimum requirement of dietary phosphorus was estimated by fitting second order polynimial regression line ($Y = 432.4102 + 83.0932x - 23.2612x^2$). Table 26 indicates that, there is no significant variation between the observed values and the

Table 25. Wholebody phosphorus, calcium and Ca :P ratio of the prawn fed with different levels of phosphorus.

Dietary P level (%)	Wholebody P (mg/100g)	Wholebody Ca(mg/100 g)	Wholebody Ca :P ratio
0.26	457.665 ± 22.15	289.4054 ±7.09	0.6323
0.79	472.9448 ± 18.19	375.5738 ± 15.53	07941
1.22	494.8745 ± 20.50	472.9448 ± 13.28	0.9642
1.76	525.8545 ± 15.37	517.0903 ± 15.13	0.9833
2.26	495.7965 ± 27.06	528.1311 ± 22.76	1.0652
2.77	477.0234 ± 32.89	569.2168 ± 39.82	1.1932
3.30	457.0747 ± 28.13	616.0572 ± 36.91	1.3478
4.29	527.0500 ± 35.69	620.0589 ± 42.33	1.1765

Comparison of mean values of wholebody phosphorus and calcium :

Dietary P (%)	Mean wholebody P (mg/100g)	Dietary P (%)	Mean wholebody Ca (mg/100g)
3.30	457.0747	0.26	289.4064
0.26	457.6650	0.79	375.5738
0.79	472.9448	1.22	472.9448
2.77	477.0234	1.76	517.0903
1.22	494.8745	2.26	528.1311
2.26	495.7965	2.77	569.2168
1.76	525.8545	3.30	616.0572
4.29	527.0500	4.29	620.0589

Sidescored mean value are not significantly different at 5 % level.

Table 26 Comparison of observed and expected values of wholebody phosphorus with respect to different dietary phosphorus levels

$Y = 432.4102 + 83.0932 x - 23.2612 x^2$

Dietary p level (x)	Observed wholebody P (o)	Expected wholebody P (y)	$\frac{(o-y)^2}{y}$
0.26	457.6650	452.4419	0.0603
0.79	472.9448	483.5365	0.2320
1.22	494.8745	499.1619	0.0368
1.76	525.8545	506.6003	0.7318
2.26	495.7965	501.3919	0.0624
2.77	477.0234	484.0975	0.1034
3.30	457.0747	453.3033	0.0314

($\Sigma \frac{(o-y)^2}{y}$ = 1.2581 < Chi-square at degrees of freedom, 6 and 5 % level of significance)

Optimum level of dietary phosphorus = -b/2c =1.7861

Figure 8. Variation of wholebody phosphorus content with dietary phosphorus levels.

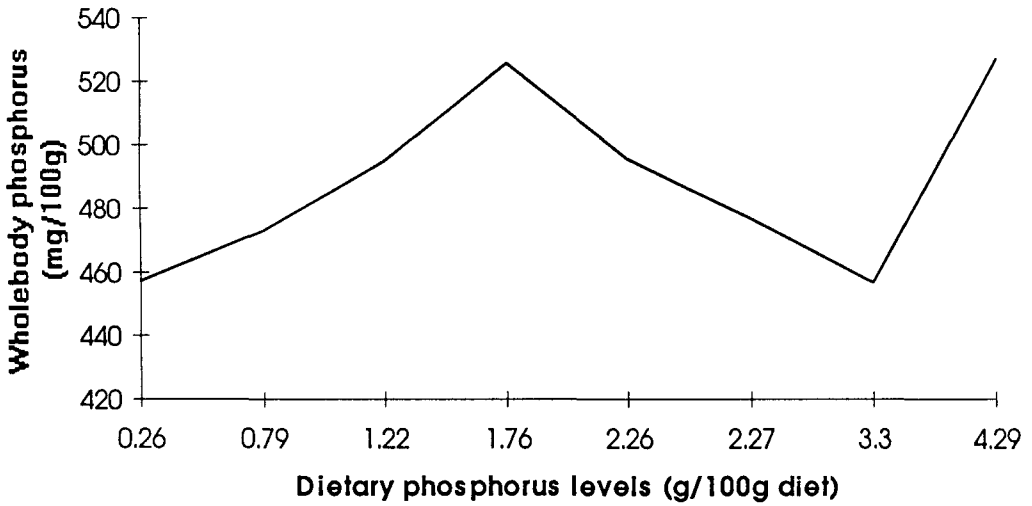


Figure 9. Variation of wholebody calcium content with dietary phosphorus levels.

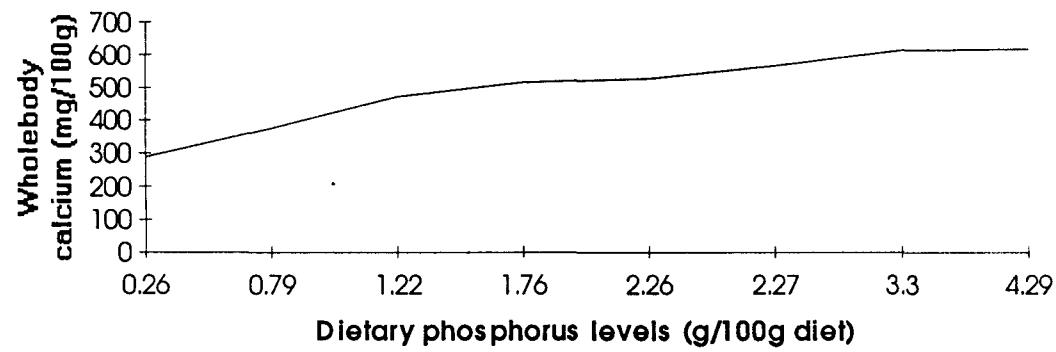
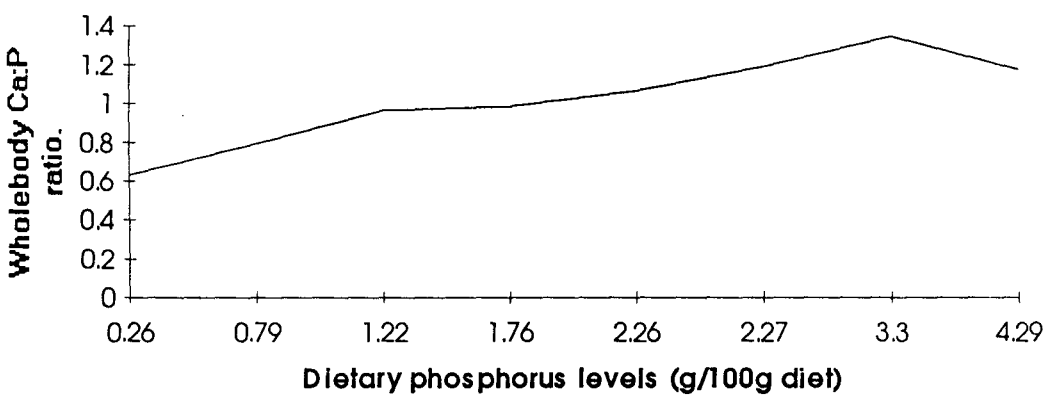


Figure 10. Variation of wholebody Ca:P ratio with dietary phosphorus levels.



values estimated by the second degree equation, of wholebody phosphorus level with respect to dietary levels. The estimated optimum value of dietary phosphorus based on the wholebody phosphorus content of the prawn was 1.7861%.

Since the dietary phosphorus level varies uniformly only from 0.26% to 3.3% and, also 4.29% phosphorus is an exorbitantly high level for crustaceans, the latter value was not considered for fitting the curve.

4.3.8 Variation of wholebody calcium content with dietary phosphorus level.

The variation of wholebody calcium content with respect to varying dietary phosphorus level is given in Table 25. The values show a more or less uniform increase in the wholebody calcium in response to increase in dietary phosphorus.

Fig.9 depicts the accumulation of body calcium in reponse to an increase in phosphorus level in the feed. The highest wholebody calcium of 620.0589 mg/100 g is observed in prawn fed with 4.29% phosphorus (Diet s8). It can be noted that on increasing the dietary phosphorus from 0.26% to 1.22%, the body calcium increases rapidly and then the slope decreases upto a dietary phosphorus level of 2.26%. On further increase in dietary phosphorus level the wholebody calcium content sharply increases. The curve shows a plateau between the dietary phosphorus levels of 1.76% and 2.26%.

4.3.9 Variation of wholebody Ca:P ratio with dietary phosphorus levels.

The variation of wholebody Ca:P ratio in response to varying levels of dietary phosphorus is given in Table 25. It indicates that, as the dietary phosphorus level increases from 0.26 to 3.3% (Fig.10), wholebody Ca:P ratio increases, and further increase in dietary phosphorus level to 4.29% results in a sharp decline in wholebody Ca:P ratio. The highest wholebody calcium phosphorus ratio of 1.3478 is observed in

response to phosphorus level of 3.3% in the feed (Diet s7). The slope of the curve Fig.10) is found to be declining and a plateau is observed as the dietary phosphorus level varies between 1.22% and 2.77%. No objective estimate of optimum dietary phosphorus level is available based on the influence of varying dietary phosphorus level on wholebody Ca:P ratio of the animal.

4.4 Characterisation of dietary deficiency and excess symptoms of phosphorus

4.4.1 Deficiency symptoms

The prawn fed with control diet containing 0.26% phosphorus (Diet s1) developed deficiency symptoms. The symptoms may be categorised broadly as external symptoms , variation in growth, feed effiency and wholebody phosphorus and calcium contents.

The external symptoms include lack of pigmentation and body opaqueness in contrast to prawn fed with medium level of phosphorus , where the body is with moderate to good pigmentation and trasparency.

The prawns fed with control diet yield a percentage weight gain of 339.74 ± 45.35% (Table 14) and those fed with 1.76% phosphorus(Diet s4) yield a weight gain of 395.95 ± 58.71%. Table 17 shows that percentage gain in length of prawns fed with control diet is 65.43 ± 7.89% and that of prawns fed with 3.3% phosphorus (Diet s7) is 77.26 ± 4.19%. Table 19 shows that SGR of prawns fed with control diet is 1.76 ± 0.12% whereas that of prawns fed with 1.76% phosphorus (Diet s4) is 1.90 ± 0.13%.

The diet with 0.26% phosphorus produced a feed conversion efficiency of 0.1498 (Table 21) and that of diet with 1.76% phosphorus (Diet s4) is 0.1902.Table 23 shows that the protein efficiency ratio of feed containing 0.26% phosphorus (Diet

s1) is 0.3936 ± 0.05, which is significantly lower than the protein efficiency ratio of feed containing 1.76% phosphorus (Diet s4), which is 0.5143 ± 0.03 (Table 24).

The prawns fed with control diet contain 457.665mg/100g whole body phosphorus (Table 25), which is significantly lower than that of prawns fed with 1.76% phosphorus (Diet s4) that is 525.4064 mg/100g. The Table also indicates that wholebody Ca:P ratio is at its minimum in prawns fed with control diet.

4.4.2 Excess symptoms

Prawns fed with 4.29% dietary phosphorus (Diet s8) developed excess symptoms of phosphorus. It can be described as external characters, variation in growth, variation in feed efficiency and variation in wholebody phosphorus and calcium contents.

As a result of excess phosphorus in the diet , the prawns developed general body opaqueness and stunted growth. The colour remained dull yellow , with whitish patches on body and white spots on legs especially on chelate legs. 7% of the prawns fed with 4.29% phosphorus (Diet s8) developed black melanised lesions on the dorsal aspect of the abdomen at the end of the experimental period which is clearly shown in Plate III and Plate IV.

The prawns fed with 4.29% phosphorus (Diet s8) exhibited the lowest percentage weight gain of 320.35 ± 35.72% (Table 14), whereas the control diet produced a weight gain of 339.74 ± 45.35% . Table 17 indicates that the percentage gain in length of prawns fed with control diet is 65.43 ± 7.89% which is reduced to 61.90 ± 8.74% when fed with diet containing 4.29% phosphorus (Diet s8). The specific growth rate value obtained for prawns fed with 4.29% phosphorus (Diet s8) is 1.71 ± 0.10% which is lower than that of prawns fed with control diet for which the value is 1.76 ± 0.12% (Table 19).

The food conversion efficiency obtained for feed with 4.29 % phosphorus

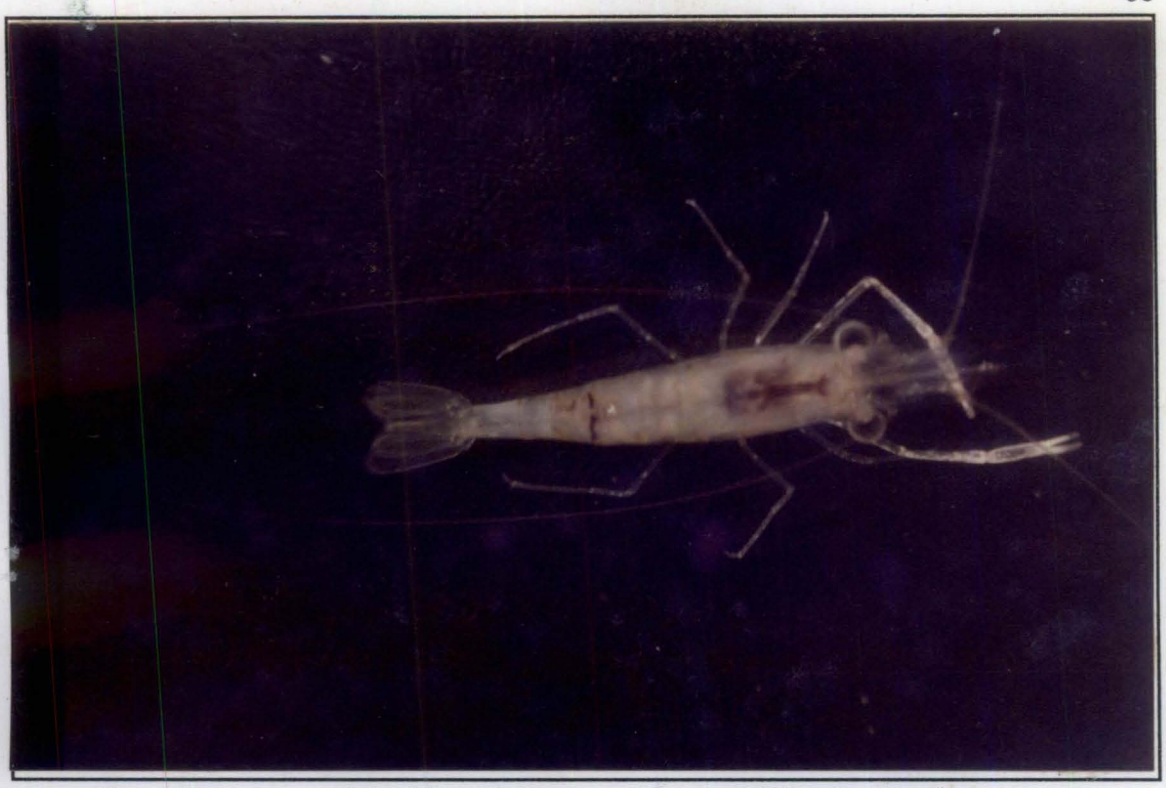


Plate III Specimen fed with 4.29 % phosphorus, with its abnormal symptoms.

Plate IV Specimen fed with 4.29 % phosphorus, with its abnormal symptoms.

(Diet s8) is 0.1377, which is lower than that of feed with 0.26% phosphorus (Diet s1). The food conversion ratio obtained for control diet is 6.7791 and it is increased to 7.3264 as the dietary phosphorus level increases to 4.29% in diet s8 (Table 21). The Table 23 shows that the protein efficiency ratio obtained with feed having 4.29% phosphorus(Diet s8) is 0.3724 ± 0.03, which is significantly lower than the protein efficiency ratio obtained for feed which contained 1.76% phosphorus in diet s4 (Table 24).

Table 25 and Fig.8 show that the highest level of wholebody phosphorus content of 527.05mg/100g is observed in prawns fed with 4.29% phosphorus (Diet s8), when the dietary phosphorus level varies from 0.26 to 4.29%. The wholebody calcium level shows the maximum value of 620.0589mg/100 (Table 25) in prawns fed with 4.29% phosphorus (Diet s8). The wholebody Ca:P ratio (Table 25) shows a decreasing trend (Fig.11) when dietary phosphorus level increases from 3.3% to 4.29% as a result of sharp increase in wholebody phosphorus level (Fig.9).

V. DISCUSSION

Although minerals are very important in the diet of crustaceans, the study of mineral requirements of shrimps and prawns has been somewhat neglected. However some studies have been conducted in *Penaeus japonicus* (Kanazanwa *et al.*, 1984; Kitabayashi *et al.*, 1971; Kanazawa, 1985; Deshimaru and Yone, 1978; Deshimaru and Shigueno, 1972), *P.monodon* (Batista and Baticados, 1990), *P. merguiensis* (Sick *et al.*, 1972; Aquacop, 1978), *P. aztecus* (Sick *et al.*, 1972; Shewbart *et al.*, 1986) and in *P.vannamei*(Davis *et al.*, 1993). Dietary mineral studies with freshwater prawns were lacking before 1990, the tendency being to treat their mineral requirements as similar to those of the marine shrimps (New, 1995).

Phosphorus is one of the most important mineral nutrients required by the prawn. It is found in low concentrations in natural waters (Boyd, 1981; Halver, 1989). Consequently, absorption of significant amounts of phosphorus from water is unlikely. On the other hand, most of the practical diets, especially for penaeid shrimps, are commonly supplemented with high levels of phosphorus and it is one of the major factors contributing to environmental pollution from aquaculture (Rijin and Shilo, 1989; Akiyama,1992; Boyd and Musig,1992; Persson, 1991). Reduction in outputs of phosphorus from the dietary source can greatly reduce aquaculture wastes as well as potential eutrophication effects in the receiving water bodies. It was observed that aquaculture can be done in a sustainable way through nutritional strategies for management of aquaculture wastes by minimizing waste outputs from the dietary source. This stresses the need for obtaining the optimum dietary level of phosphorus for the cultured organism with respect to maintenance of general health, growth, feed efficiency, culture economics and pollution free environment.

5.1 Composition of formulated feed

In the present investigation, semipurified diet with casein and egg albumin as the source of protein was used. These sources have been previously used for

P.japonicus (Deshimaru and Yone, 1978), *P.vannamei* (Davis and Arnold, 1994), *P. aztecus* and *P.setiferus* (Sick *et al.*, 1970) in studies on mineral nutrition. Gomez *et al.*(1988) observed that casein based semipurified diet with a protein level of 13-25% is enough to produce maximum growth in *M.rosenbergii*. Diet containing 39% crude protein with casein and egg albumin as protein sources was found very effective in promoting weight gain and survial in postlarval *M.rosenbergii*(Read and D'Abramo, 1989). In the present study,diet with protein level of 37.87 ± 0.67% was used.

Varying levels of lipid are reported in commercial feed of *M.rosenbergii*. Sheen D'Abramo(1989) found that using 2:1 cod liver oil corn oil mixture a 6% inclusion rate was optimal. Lacroix and Gondowin(1992) recommeneded 3-15% lipid levels in experimeantal feeds for prawn. Teshima *et al.*(1992) observed that both 18:2w_6 and 18:3 w_3 fatty acids are required by fresh water prawns. In the present study cod liver oil and corn oil were used as the source of w_3 and w_6 fatty acids which give a lipid level of 6.25± 0.46%.

Cho and Schell(1978) reported that phosphorus from the diet is enhanced by presence of glucose in the diet.The level of carbobydrate used in the present study was 30.45 ± 1.59%. Fair *et al.* (1980) reported that dietary fibre level up to 30% does not appear to suppress growth in *M.rosenbergii*.

Soluble monobasic inorganic salts or bioavailable organic salts are to be provided as source of phosphorus in the diet of stomachless prawns. Phosphorus availability among the inorganic phosphorus source is high for sodium phosphate monobasic and its apparent phosphorus availability is not significantly depressed in the presence of calcium carbonate (Davis and Arnold ,1994). In the present study , sodium dihydrogen phosphate ($NaH_2PO_4.2H_20$) was used as the source of phosphorus and calcium carbonate ($CaCO_3$) as the source of calcium. The calcium content of the diet was 1.05±0.04%.

Calcium and phosphorus requirements of shrimps are within the range of 1 to

2%(Piedad-Pascal,1989). Andrew et al. (1972) used calcium carbonate ($CaCO_3$) and sodium phosphate monobasic (NaH_2PO_4) or calcium phytate substituted for cellulose in basal diet, in dietary calcium and phosphorus studies with catfish. In *Penaeus vannamei* sodium phosphate as source of phosphorus , clacium carbonate as source of calcium and diatomaceaous earth as filler were used by Davis *et al.*1992. Davis *et al.* (1993) used sodium phosphate monobasic,calcium chloride dihydrate and clacium phosphate dibasic in studies with *P.vannamei.* The filler used was a 1:1 ratio of acid washed diatomaceaous earth and sucrose. The apparent phosphorus availability of sodium phosphate monobasic is 68.2% for *P.vannamei* and the leaching did not influence the aparent phosphorus availability (Davis and Arnold, 1994).

In the present study , the control diet contained 0.26±0.04 g/100g phosphorus derived from endogenous origin. To estimate the total available phosphorus for the various dietary treatments, it is assumed that the availability of $NaH_2PO_4.2H_2O$ for *M.rosenbergii* is 100%.

5.2 Water quality parameters

Phosphorus available in water can be absorbed by the aquatic organisms (Lall,1979). However, the availability of phosphorus in the rearing water depends on the oxygen level, temperature and other water conditions (Baticados *et al.*,1986;Batista and Baticados, 1990). Studies on dietary requirement of calcium in *M.rosenbergii* revealed the possible inter relationship between dietary requirement of minerals and water quality parameters (Zimmermann *et al.*,1993).

M.rosenbergii adults tolerate a wide temperature range of 18-34°C(New,1990). For postlarvae 18-22°C markedly stunts growth, the optimum being 29-31°C (New, 1995). Chow and Schell(1978) report that phosphorus absorption from the diet is enhanced by water temperature. In the present study , the water temperature varied between 27 and 31°C.

The optimum range of pH reported for adult was 7.0-8.0. While for larval rearing the desirable pH is 7.0-8.5 (New, 1995). High pH and high level of unionized

ammonia produce synergistic toxic effect on prawns (Strauss *et al.*,1991) . In the present investigation , the pH level varied between 7.0 and 9.0.

The optimum level of dissolved oxygen for *Macrobrachium* culture pond is 6-8 ppm(Vasquez *et al,*1989). Avault (1986) noted that when dissolved oxygen level falls to below 2 ppm, prawns were stressed and 0.5 ppm was normally lethal. In the present study , the dissolved oxygen level varied between 4.69 ppm and 6.22 ppm.

High dietary calcium combined with high alkalinity was detrimental to prawns and the diet with 1.8% calcium performed better at the highest alkanility level (Zimmermann *et al.*, 1994). In the present investigation , a dietary calcium level of 1.05±0.04% was used and the total alkalinity varied between 90.96 and 95.81 mg/l as $CaCO_3$.

There exist discrepancy in the optimum water hardness for culture of *M.rosenbergii* (Brown ,1991). This may be due to the interaction of different water quality parameters.The total hardness of water in the present study varied between 109.8 and 116.8 mg/l as $CaCO_3$.

The growth in *M.rosenbergii* in relation to varying phosphorus and calcium levels in water is illdefined. In the present study , the phosphorus and calcium levels of water varied between 0.0282 and 0.0570 mg/l, and 42.0 and 56.0 mg/l, respectively.

5.3 Effect of varying levels of dietary phosphorus on survival.

The prawns fed with varying levels of phosphorus exhibit no significant differnence in percentage survival rate. The result indicates that the dietary phosphorus level has no significant influence on the percentage survival of the animal. However, the highest survival rate of 93.33% is obtained on feeding the prawn with

3.3% phosphorus and 4.29% phosphorus . The Ca:P ratio of the diets were 1:3.33 and 1:3.97, respectively.

A lower survival rate was observed in red drum *Sciaenops ocellatus* for fish fed with inorganic phosphorus deficient diet(Davis and Robinson, 1987). A depression in survival was noticed in *P.vannamei* when calcium supplementation was 1.0 and 2.0 in the presence of replete phosphorus (Davis *et al.*, 1993). Depression in survival in response to and/or Ca:P ratio have been reported for lobster *Homarus americanus*(Gallagher *et al.*,1978) and for *P.japonicus*(Deshimaru and yone, 1978; Kanazawa *et al.*, 1984) and is reported as characteristic of the deficiency. Best survival of *H.americanus* were shown by animals fed on experimental diets containing 0.51 Ca:P ratio (0.56% calcium and 1.10% phosphorus)as reported by Gallagher *et al.* (1978).

However, *P.vannamie* fed with casein based diet and with complete mineral premix or mineral premixes from which calcium and phosphorus were individually deleted, produced no significant difference among treatments for survival data (Davis *et al.*, 1992).

In the present study it is conclusively proved that the dietary phosphorus levels have no significant effect on percentage survival rate of *M.rosenbergii* juveniles. However, higher percentage survival was observed in prawns fed with higher levels of phosphorus.

5.4 Effect of varying levels of dietary phosphorus on growth.

In the present investigation, the effect of varying levels of dietary phosphorus on growth was studied based on four parameters, such as percentage weight gain, percentage gain in length , specific growth rate and fortnightly net increment in weight.

The highest percentage weight gain of 395 ± 58.71% was obtained in prawn fed with diet containing 1.76% phosphorus. The diet contained 1.10% calcium and the Ca:P ratio was 1:1.68. It was followed by 390.75 ± 38.05% by the prawn fed with diet containing 3.3% phosphorus, 0.99% calcium and calcium phosphorus ratio 1:3.33.

Data on percentage gain in length show that the highest value of 77.26 ± 4.19% was observed for prawn fed with diet containing 3.3% phosphorus and with calcium content and Ca:P ratio 0.99% and 1:3.33 respectively. This is followed by 74.95 ± 24.36% observed for prawn fed with diet containing 1.76% phosphorus and with calcium content and Ca:P ratio, 1.10% and 1:1.68, respectively. Eventhough the variations of percentage gain in length in response to varying dietary phosphorus level are not significant, a difference in dietary phosphorus level which results in maximum growth in terms of length and weight is noticed.

The diet with 1.76 % phosphorus with the calcium content and Ca:P ratio 1.10 % and 1:1.68 respectively yielded the highest specific growth rate of 1.9 ± 0.13%. This is followed by the diet with 3.3% phosphorus with calcium content and Ca:P ratio 0.99% and 1:3.33, respectively which showed a specific growth rate of 1.89 ± 0.09%. These results are similar to the effect of dietary phosphorus level on percentage weight gain of the prawn.

The fortnightly net increment in weight of the prawn shows that the highest fortnightly net increment of 0.0668g was obtained in prawns fed with 3.3% phosphorus during the period of the 6th fortnight. This is followed by the prawns fed with 1.76% phosphorus which show the highest fortnightly increment in weight of 0.0596g during the 5th fortnight.

The result of the present study confirms that the variation in dietary phosphorus level is not having any significant influence on the growth of *M.rosenbergii* juveniles. The growth data indicate that 1.76% phosphorus in the diet shows the best performance, however, optimum requirement estimate cannot be the same (Baker,

1986). So it can be stated that based on the growth rate, the recommended level of dietary phosphorus and Ca:P ratio in *M. rosenbergii* is 1.76% and 1:1.68, respectively.

The phosphorus requirement studies in finfishes revealed the essentiality of the mineral for the growth. Ketola (1975b) reported that supplements of inorganic phosphorus up to 0.6% of the diet containing 0.7% phosphorus from the plant source significantly improved growth in Atlantic salmon, *Salmo salar*. Weight gain is significantly affected by dietary available phosphorus in juvenile sunshine bass (*Marone chrysops x M. saxatilis*). The non linear regression analysis of weight gain indicated minimum available phosphorus requirement of 0.41% of dry diet (Brown *et al.*, 1993). Weight gain data in *Oreochromis aureus* indicated a 0.30% dietary phosphorus was adequate for good growth (Robinson *et al.*, 1987). The available phosphorus requirement of catfish is approximately 0.8% (Andrew *et al.*, 1973).

Calcium and phosphorus requirement studies in carp (Ogino and Takeda, 1976), catfish (Lovell,1978) and chum salmon (Watanabe *et al.*, 1980) indicated that the growth rate correlates positively with dietary phosphorus level but not with calcium levels. Available phosphorus level in a diet producing the maximal growth in carp was found to be 0.6 to 0.7% and that of chum salmon was 0.5 to 0.6%.

Growth of black seabream is greatly affected by the phosphorus content and Ca:P ratio of the diet (Liu *et al.*, 1993). The optimum value of phosphorus and Ca:P ratio in the diet are 0.68% and 1:2, respectively. However, in juvenile red drum *Sciaenops ocellatus* no significant difference in weight gain was noticed on feeding graded levels of inorganic phosphorus (Davis and Robinson, 1987). Studies in fishes show that based on the growth, the requirement of phosphorus varies between 0.3 and 0.8%.

Penaeids may need dietary source of minerals for growth because of repeated molting wherein minerals are lost (Kanazawa, 1985). Shewbart *et al.* (1973) postulated that phosphorus may be essential in the diet of shrimp because it is present in large quantities in prawn but not in sea water. *P.indicus* fed without phosphorus had

stunted growth and became sluggish and weak (Ali,1988). In *P.japonicus* improved effect of phosphorus on growth was reported at 2% level in the diet and this was more pronounced in the absence of calcium (Deshimaru and Yone, 1978). Davis *et al.* (1992) observed that *P.vannamei,* fed with casein based diet and with complete mineral premix or mineral premixes from which calcium and phosphorus were individually deleted for a period of 4 weeks produced no significant difference among treatments for growth data.

With replete phosphorus supplementation, 1.0 and 2.0% calcium to the diet did not appear to increase the nutritive value of the diet for *P.vannamei* indicating that a dietary calcium supplement is not required under these conditions (Davis *et al.*, 1993). In the presence of 1.0 and 2.0% supplemental calcium, supplementation of 0.5-1.0% phosphorus and 1.0-2.0% phosphorus, respectively were required to maintain normal growth of shrimp. The studies show that although the calcium and phosphorus content of hepatopancreas and carapace responded to dietary supplementation, there was no clear correlation of tissue mineralisation to shrimp growth. The minimum level of dietary phosphorus supplementation required for maximum growth of shrimp is dependent on the calcium content of the diet. It is suggested that the increased dietary calcium may affect the bioavailability of phosphorus. Best growth of *H.americanus* was shown by animals fed with experimental diet containing 0.56% calcium and 1.10% phosphorus (Ca:P ratio 0.51).Gallagher *et al.*(1982) reported that the Ca:P ratio can significantly affect weight gain by adult lobsters and it is suggested that calcium and phosphorus in the formulated diets were more available for absorption than these elements held in the protein matrix of animals consumed in the wild. The total amount of mineral required by lobster fed with formulated diets may therefore be less than that required by lobster in the wild.

Kanazawa *et al.* (1984) observed that the best growth of *P.japonicus* was found with the diet supplemented with 1.2% levels of calcium and phosphorus at the Ca:P ratio of 1:1. A Ca:P ratio of 1:1 was necessary to prevent soft shell disease in *P.monodon* (Piedad-Pascual, 1989) while in juvenile *P.californiensis* a Ca:P ratio of 2.1:1 was optimum for growth (Hunner and Colvin, 1977).

Best growth rate of *P.japonicus* was achieved with a test diet containing 1.04% phosphorus and 1.24% calcium and adequate ratio of phosphorus to calcium was about 1:1 (Kitabayashi *et al.*, 1971). Optimum content of calcium and phosphorus together and Ca:P ratio in compounded diet for *P.orientalis* are 4.5 % and 1:2, respectively. Andrew and Sick (1972) used 0.26% calcium and 0.21% phosphorus in shrimp diets. In shrimp Sick *et al.* (1972) used Ca:P ratio of approximately 1.3:1 and inhibition of growth rate has been reported when the ratio was raised to 2:1

Influence of dietary phosphorus level on the growth of *M.rosenbergii* is not well defined. The recommended level of dietary phosphorus in the present study (1.76%) conforms to the optimum range of dietary phosphorus levels (0.21 - 3.0%) demonstrated in other crustaceans. The phosphorus content of the water in the present study ranges between 0.0282 and 0.057 mg/l. The very recent studies in prawn (Zimmermann *et al.*,1994) demonstrated the dependance of water quality parameters on the influence of dietary nutrient level on growth and survival.Animals fed with diets containing low levels of minerals can often sacrifice mineral stores to compensate for dietary deficiencies and thus growth is often a less sensitive indicator of mineral status than is the mineral content of specific tissue (Baker,1986). This may be the reason for the lack of significant influence of dietary phosphorus on growth of *M.rosenbergii* juveniles in the present investigation.

5.5 Effect of varying levels of dietary phosphorus on feed efficiency.

Feed efficiency is commonly measured in terms of food conversion ratio (FCR). However, food conversion efficiency (FCE) is widely preferred over food conversion ratio. The FCR and FCE values of prawn fed with varying levels of phosphorus indicate that there exists significant influence of dietary phosphorus on these values. The lowest FCR value and the highest FCE value are shown by the prawn fed with 1.76% phosphorus. It is also demonstrated that there is no significant difference between diet S1,S4 and S7 having the dietary phosphorus content

0.26%, 1.76% and 3.3%,respectively. The levels of calcium in the diets are approximately equal and the Ca:P ratio of the diets are 1:0.25, 1:1.68 and 1:1.33, respectively.The result thus confirms that in *M.rosenbergii* juveniles, the dietary phosphorus level and the Ca:P ratio have significant influence on the FCR and FCE of the feed. Based on the observation the optimum dietary phosphorus level and Ca:P ratio is 1.76% and 1:1.68, respectively.

The highest protein efficiency ratio of 0.5143±0.03 is obtained on feeding with the diet having 1.76% phosphorus. Statistical analysis shows that there exists significant influence of dietary phosphorus level on protein efficiency ratio of the feed. The diets with 1.76% phosphorus and 3.3% phosphorus have no significant difference on their influence on protein efficiency ratio. As the calcium level of the diets are approximately equal,the variation of Ca:P ratio is the other factor which influence the protein efficiency ratio. The Ca:P ratio of the diets are 1:1.68 and 1:3.33 respectively, the latter being almost double the former value. Since the highest protein efficiency ratio is obtained with Ca:P ratio 1:1.68, it can be stated that the optimum dietary phosphorus level is 1.76%.

In the Atlantic salmon(*Salmo salar*) inorganic phosphorus up to 0.6% of the diet containing 0.7% phosphorus from the plant source improved the feed utilization (Ketola,1975b). Nonlinear regression analysis of food efficiency data in juvenile sunshine bass (*Morone chrysops x M.saxatilis*) indicated a minimum available requirement of 0.46% phosphorus in the dry diet(Brown *et al.,* 1993).

In juvenile red drum *Sciaenops ocellatus* no significant difference in feed conversion was noticed on feeding with graded levels of inorganic phosphorus (Davis and Robinson, 1987). Feeding with low phosphorus diet resulted in low feed efficiency in carp (Ogino and Takeda, 1976).

Phosphorus level in the food significantly influences the feed efficiency in *P.japonicus* (Kanazawa *et al.*, 1984). Deshimaru and Yone (1978) reported improved effect of phosphorus supplementation on feed efficiency at 2% level in the diet,

which was more pronounced in the absence of calcium.

Nutrient conversion of prawn feeds, as in aquaculture generally, is inefficient. It is stated that prior to 1990, less than 7% of the inorganic carbon in prawn feeds added to Hawaiian ponds was harvested as prawn biomass (New, 1995). The optimum phosphorus and Ca:P ratio in the diet of prawn is therefore considered critical to reduce the wastage of valuable ingredients of the formulated diet and the resultant pollution of the environment. In the present study, the highest FCE and PER values of 0.1902 and 0.5143 respectively were recorded with dietary phosphorus of 1.76% and Ca:P ratio of 1:1.68.

5.6 Effect of varying levels of dietary phosphorus on wholebody phosphorus, calcium and Ca:P ratio

The wholebody phosphorus content shows an increasing trend when the dietary phosphorus level varies from 0.26% to 1.76%, and the maximum value observed in prawn is 525.8545 ± 15.37mg/100g. As the dietary phosphorus level varied from 1.76% to 3.3%, the wholebody phosphorus level shows a decreasing trend. When the dietary phosphorus level rises to 4.29%, wholebody phosphorus level becomes 527.05 ±35.69mg/100g. The nonlinear regression line fitted is found to have no significance deference from the observed line of wholebody phosphorus content of prawn and the optimum dietary phosphorus level estimated is 1.7861%, which is approximately equal to the recommended level of dietary phosphorus based on the growth and the optimum level observed based on the FCE and PER values (1.76%). So the present study concludes that the optimum phosphorus requirement in the diet of *M.rosenbergii* juvenile is 1.8%. At exorbitant high levels of dietary phosphorus (4.29%), abnormal deposition of phosphorus in the body is observed, as shown by the second peak in Fig.8.

The body calcium level showed a steady increase as the dietary phosphorus level increased from 0.26% to 4.29%. The body Ca:P ratio also showed an increase from 0.26 to 3.3%. On increasing the dietary phosphorus level to 4.29%, a sharp decline in

the curve is noticed. In Fig.9, a plateau is observed between the dietary phosphorus level 1.22% and 2.26% and in Fig.10 it is between 1.22 and 2.52% . This indicates that the regulatory mechanism of the prawn is within a narrow range above which the animal shows abnormal symptoms. This is confirmed by the facts that the optimum dietary phosphorus level of 1.8% lies within the plateaus of the curves and the observation of abnormal symptoms on very high levels of diearty phosphorus (Plate III and Plate IV). It is important to note that under the present conditions the calcium absorption of the animal from the water is in direct relation to the dietary phosphorus level which is demonstrated in the Fig.9.

The prawn exhibits regulation of body phosphorus to a considerable extent while the regulation of body calcium is comparatively less and is entirely dependent upon the dietary phosphorus level. However, at exorbitant levels of dietary phosphorus an abnormal increase in the wholebody phosphorus and calcium are noticed with a lowering of wholebody Ca:P ratio. This may be because of the deposition of calcium as calcium phosphorus in the body of the prawn. The appearance of white patches and spots in the prawn (Plate III and Plate IV) points to this fact. However, further study is required to understand whether white patches and spots are entirely due to deposition of calcium phosphate and how it affects the growth, survival and moulting of the prawn. The high wholebody calcium and phosphorus content may have its impact on the normal physiology and metabalism of the prawn. The black melanised lesions appearing on the prawn (Plate III and Plate IV), fed with exorbitant high levels of phosphorus point to this aspect which needs to be studied further.

In juvenile sunshine bass the mineral composition of bone and scale tissue were significantly affected by dietery available phosphorus.The non-linear requirement estimate based on the phosphorus mineralization of wholebody tissue was 0.49 % of the diet (Brown *et al.*,1993).

Studies on common carp and rainbow trout indicate that an increasing level of dietary phosphorus will be accompanied by increasing retension of both calcium and phosphorus in body tissue, thus maintaining the ratio of calcium and phosphorus within

a narrow limit. Fish appears to have an ability to balance Ca:P ratio controlling the absorption and excretion of calcium for optimum utilization of both the mineral elements (Cho and Schell ,1978).

In *P.vannamei* , deletion of calcium and phosphorus from the mineral mix did not produce significant response in hepatopancreas and carapace with respect to ash, Ca,P Na,K, Mg, Mn, Fe, Zn and Cu content (Davis *et al.*,1992).Studies of Gallagher *et al.*(1978) and Kanazawa *et al.*(1984) show that tissue levels of calcium and phosphorus by themselves may be poor indicaters of dietary intake for marine crustaceans.Gallagher *et al.* (1978) found that ash, calcium and phosphorus contents of the carapace and body tissue of *H.americanus* were variable, presumably due to the moult cycle, and not related to dietary levels of calcium and phosphorus. Davis *et al.*(1993) also arrived at the same conclusion in *P.vannamei.*

However, in the present investigation , strong correlation between the dietary phosphorus level and wholebody phosphorus and calcium levels were observed in *M.rosenbergii.* This may be due to the longer duration of the experiment and selection of intermoult prawn for wholebody analysis. Mineral content of specific tissue is often a more sensitive indicator than the growth when animals are fed with diets containing low levels of minerals (Baker, 1986). Feeding phosphorus deficient diet to *P.indicus* leads to declined body phosphorus level disturbing the Ca:P ratio (Ali,1988).

As reported by Cho and Schell(1978) in fishes, prawns show reduced accretion of calcium by the body tissue when they are fed with diet limited in phosphorus. However , on higher dietary phosphorus levels, the prawns show an increase in Ca:P ratio . This is presumably due to excretion of phosphorus and accretion of calcium in the tissue. It appears that, the process of excretion of calcium from the body in order to retain the Ca:P ratio in *M.rosenbergii* juveniles, under higher levels of wholebody phosphorus is poor. Fig.11 demonstrates that the prawns retain the body Ca:P ratio arround 1:1 when the dietary phosphorus level varies from 1.22% to 2.77%.

5.7 Abnormal symptoms on feeding low and excess levels of phosphorus

In the present study the diet s4 containing 1.76% phosphorus produced the maximum growth, feed efficiency and protein efficiency ratio in *M.rosenbergii* juveniles.

Eventhough the control diet is without phosphorus supplementation it contains 0.26±0.04g/100g endogenous phosphorus. The prawns fed with control diet developed lack of pigmentation and general body opaqueness. They exhibit stunted growth and showed low values of percentage weight gain, percentage gain in length, specific growth rate and food conversion efficency than the prawns fed with 1.76 % phosphorus. The diet with no phosphorus supplementation produced a significantly lower protein efficiency ratio in the animal.

The wholebody analysis of the prawn fed with the diet with no phosphorus supplementation shows that the body phosphorus content is significantly lower than that of the prawns fed with 1.76% dietary phosphorus(Table 25). The wholebody calcium content of the prawn fed with diet with no phosphorus supplement is also found significantly lower than that of the prawn fed with the diet containing 1.76% phosphorus.

Excess phosphorus in the diet produced abnormal symptoms in prawn . It involves general body opaqueness, stunted growth, whitish patches on the body and white spots on the legs. Black melanised lesions were developed on the dorsal side of the abdomen of the prawn towards the end of the experimental period.The feeding with excess phosphorus resulted in low values of percentage weight gain, percentage gain in length, specific growth rate and food conversion efficency in prawn. There is a significant reduction in protein efficency ratio of prawns fed with excess phosphorus than with the prawns fed with diet containing 1.76% phosphorus.

At exorbitant high level of dietary phosphorus (4.29%) the whole body phospho-

rus level increases to the maximum value of 527.05 mg/100g .The whole body calcium level also increases to the maximum value of 620.0589 mg/100g.However, the Ca:P ratio shows a decline at the dietary phosphorus level of 4.29%.

The prawns fed with diet having excess phosphorus, when compared with prawn fed with diet having no phosphorus supplementation on the basis of percentage weight gain (Table 14), percentage gain in length (Table 17), specific growth rate (Table 19), food conversion efficency (Table 21), and protein efficency ratio(Table 23) , it can be noted that there exsist a reduction in the performance of prawn fed with excess phosphorus. This is presumably due to the inhibitory effect of excess phosphorus supplementation .The fortnightly net increment in weight (Table 16)also indicates the inhibitory effect of excess phosphorus, where the growth of the prawns fed with excess phosphorus showed a decreased fortnightly net increment in weight than that of the prawns fed with diet with no phosphorus supplementation, in all the fortnights except in the first.

Black melanised lesions were observed in 7% of the prawns fed with excess phosphorus.The crustaceans fed with diets deficient in vitamin C develop melanized lesions distributed throughout the collagenous tissue underlying the exoskeleton, decolourization and abnormal colouration and mortality(Deshimaru and Kuroki,1979;Lightner *et al.*,1979;Shigueno and Itoh,1988).The lesions observed in the present study are similar to the black lesions reported for the defeciency of vitamin C.The black lesion are reported to be due to the inhibition of alkaline phosphatase activity(Paulraj,1993). So the excessive deposition of phosphorus and Calcium in the body of the prawn may impose disturbances in the alkaline phosphatase activity which warrants further study.

The phosphorus deficiency symptoms reported in fishes involve poor growth and backbone development , low bone phosphorus, anorexia, poor food efficiency, low bone ash and hematocrit levels, deformity of head , increased muscle , liver and vertebrae lipid content, curved enlarged and spongy vertebrae and decreased liver glycogen (Lovell,1989;Arai *et al.*,1974; Andrew *et al.*,1973;Ogino and Takeda,1976; Watanabe

et al.,1980; Sakamoto and Yone ,1979;Lie *et al*,1993).

Histological studies in *H.americanus* revealed that animals received diets with Ca:P ratio of 1.55 or above produced subtle deleterious effects in the endocuticle (Gallagher *et al.*,1978). Kitabayashi *et al.* (1971) stated that in *P.japonicus*, the adequate ratio of phosphorus to calcium is about 1:1 . However , large amount of these minerals resulted in inhibition of prawn growth , making the prawn shell greyish white. A similar result was produced when the ratio of phosphorus to calcium was about 1:2. Reduced growth in *P.japonicus* was reported due to deficiency of phosphorus in the diet (Kanazawa *et al.*,1984). In prawn , several workers have used Ca:P ratio of approximately 1.3:1 and growth rate and pigmentation inhibition has been reported when the ratio was raised to 2:1 (New,1976).

Studies on mineral requirement, its deficiencies and excess in prawn and shrimp diets are very much limited. In this respect , the present study may be of great importance as it is the first of its kind depicting the interaction of dietary phosphorus level and aquacultural performance and also characterising the deficiency and excess symptoms of phosphorus in the giant freshwater prawn *M.rosenbergii*.

VI. SUMMARY

1. The present study in the juveniles of the prawn *Macrobrachium rosenbergii* was conducted to find out the optimum requirement of phosphorus in their diet and to characterise the symptoms of deficiency and excess of phosphorus in their diet.

2. The experiments were conducted for a period of 84 days by feeding the prawn using casein based semipurified diet containing 37.87 ± 0.67% protein, 6.625 ± 0.46% lipid, 30.45 ± 1.59% carbohydrate, 1.05 ± 0.04% calcium and with graded levels of supplemental phosphorus.Sodium dihydrogen phosphate($NaH_2PO_4.2H_2O$) was used as the source of phosphorus.

3. The experiments were carried out under defined water quality conditions; at temperature, 27 - 31°c ; pH, 7.0-9.0 ; dissolved oxygen, 4.69 - 6.22 ppm ; total alkalinity, 90.96-95.81 mg/l as $CaCo_3$; total hardness, 109.8 - 116.8 mg/l as $CaCo_3$; phosphorus, 0.0282 - 0.0570 mg/l and calcium, 42.0 - 56.0 mg/l.

4. No statistically significant influence of dietary phosphorus on the survival of the prawn was observed during the study.

5. At the end of 84 days, the highest percentage survival rate of 93.33% was obtained by feeding the prawn with diets containing 3.33% and 4.29% phosphorus.

6. The highest percentage weight gain of 395 ± 58.71% was obtained in prawns fed with 1.76% phosphorus.

7. The percentage gain in length of the prawns was at the maximum of 77.26 ± 4.19% when fed with 3.3% phosphorus.

8. The diet with 1.76% phosphorus and Ca:P ratio of 1:1.68 produced the maximum specific growth rate of 1.9 ± 0.13%.

9. The percentage weight gain, percentage gain in length and specific growth rate of the prawn with respect to the varying levels of dietary phosphorus demonstrate that there is no significant influence of dietary phosphorus on growth.

10. The diet with 3.3% phosphorus produced the maximum fortnightly net increment in the weight of 0.0668 g during the sixth fortnight. The diet with 1.76% phosphorus produced the maximum fortnightly net increment in weight of 0.0596 g during the fifth fortnight.

11. The lowest FCR value of 5.2745 ± 0.299 and the highest FCE value of the 0.1902 ± 0.011 were shown by the prawns fed with the diet containing 1.76% phosphorus and with a Ca:P ratio of 1:1.68 .

12. The dietary phosphorus level and Ca:P ratio show significant influence on the food conversion efficiency. It is observed that, there is no significant difference on the dietary phosphorus level of 0.26%, 1.76% and 3.3% on their effect on the food conversion efficiency of the feed.

13. The diet containing 1.76% phosphorus and with a Ca:P ratio of 1:1.68 produced the maximum protein efficeincy ratio of 0.5143 ± 0.03.

14. The protein efficiency ratio of the feed is significantly influenced by the phosphorus content and Ca:P ratio of the diet. The diet with 1.76% phosphorus and 3.3 % phosphorus have no significant difference on their influence on protein efficiency ratio.

15. The phosphorus level in the diet shows significant influence on the wholebody phosphorus content of the prawn exhibiting two peaks as the dietary level varies from 0.26% to 4.29%. The maximum values of wholebody phosphorus content, 525.8545 ± 15.37 mg/100g and 527.05 ± 35.69 mg/100 g were observed in prawn fed with diet

containing 1.76% and 4.29% phosphorus respectively.

16. The nonlinear regression estimate of dietary phosphorus based on the phosphorus minerlization of wholebody tissue is 1.7861%.

17. The dietary phosphorus level show significant influence on the wholebody calcium content of the prawn, the latter varying in direct relation with the former.

18. The wholebody Ca:P ratio of prawn varies in direct relation with phosphorus level in the diet, as the dietary level varies from 0.26% to 3.3%. However at 4.29%, sharp decline in the ratio is noticed. The prawns maintain the body Ca:P ratio at 1:1 when the dietary phosphorus level ranges from 1.22% to 2.77%.

19. Excess phosphorus in the diet (above 3.3 %) developed inhibitory effect on growth and feed efficiency in the prawn.

20. Symptoms of very low level of dietary phosphorus in prawn include lack of pigmentation, general body opaqueness, stunted growth, reduced protein efficiency and low wholebody phosphorus, calcium and Ca:P ratio.

21. Symptoms of excess level of dietary phosphorus include generel body opaqueness, stunted growth, whitish patches on the body , white spots on the legs, black melanised lesions on the abdomen, reduced protein efficiency and abnormal increase in wholebody phosphorus and calcium with a lowering of Ca:P ratio.

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DIETARY PHOSPHORUS REQUIREMENT AND DEFICIENCY SYNDROMES IN *MACROBRACHIUM ROSENBERGII* JUVENILES

By

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ABSTRACT OF THE THESIS

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ABSTRACT

The objective of the present study is to find out the optimum dietary requirement and to characterise the symptoms of the dietary deficiency and excess of phosphorus in *Macrobrachium rosenbergii* juveniles.

The prawns were fed with casein-based semipurified diet containing graded levels of supplemental phosphorus in the form of sodium dihydrogen phosphate($NaH_2PO_4.2H_2O$) under defined environmental conditions for a period of 84 days.

The survival and growth of the prawn was not significantly influenced by the varying dietary phosphorus supplementation. The maximum specific growth rate of 1.9 ± 0.13% was obtained in prawns fed with diet containing 1.76% phosphorus and with a Ca:P ratio of 1:1.68. Phosphorus level in the diet and the Ca:P ratio showed significant influence on the food conversion efficiency, protein efficiency ratio, wholebody phosphorus and wholebody calcium content of the prawn. The wholebody calcium increases with phosphorus level of the diet. Non-linear regression estimate based on the phosphorus mineralization of wholebody tissue of prawn indicates an optimum requirement of 1.7861% of phosphorus in the diet. The prawns maintain the body Ca:P ratio of 1:1 at or near the optimum dietary phosphorus level. Very low levels of phosphorus in the diet produced dificiency symptoms such as lack of pigmentation general body opaqueness, stunted growth, reduced protein efficiency and low wholebody phosphorus, calcium and Ca:P ratio. Symptoms of excess level of dietary phosphorus were general body opaqueness, stunted growth, whitish patches on the body, white spots on the walking legs, black melanised lesions on the abdomen, reduced protein efficiency and abnormal increase in wholebody phosphorus and calcium with a lowering of Ca:P ratio.

This study shows the optimum requirement of 1.8% dietary phosphorus for the giant fresh water prawn, the deficiency and excess of which leads to adverse effects.