EVALUATION OF PHOSPHORUS SOURCES IN THE COMPOUNDED DIETS OF *Penaeus indicus*

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BY

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CERTIFICATE

Certified that the dissertation entitled **"EVALUATION OF PHOSPHORUS SOURCES IN THE COMPOUNDED DIETS OF *Penaeus indicus*"** is a bonafide research work done by **Mr.Biswamitra Patro** under our guidance at Central Marine Fisheries Research Institute, Kochi during the tenure of his M.F.Sc. (Mariculture) programme (1998-2000) and that it has not previously formed the basis for the award of any other degree, diploma or other similar titles or for any publication.

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DECLARATION

I hereby declare that this dissertation entitled **"EVALUATION OF PHOSPHORUS SOURCES IN THE COMPOUNDED DIETS OF *Penaeus indicus.*"** is based on my own research work and has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.

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सारांश

किशोर *पेनिअस इंडिकस* के लिए संयोजित आहार में अकार्बनिक फॉस्फोरस की प्रभावोत्पादकता के चुने गए श्रोतों का मूल्यांकन करने के लिए 30 दिवसीय परीक्षण चलाया गया. परीक्षण चलाए गए फॉस्फोरस् श्रोत ये हैं: कैल्शियम फोस्फेट द्विबेसिक, सोडियम फोस्फेट एकबेसिक, पोटासियम फोस्फेट एकबेसिक और 1:1अनुपात में कैल्शियम फोस्फेट द्विबेसिक और पोटासियम फोस्फेट एकबेसिक का मिश्रण. भार लब्धि, खाद्य रूपांतरण अनुपात (एफ सी आर) , दृष्ट खाद्य पाच्यता गुणांक और दृष्ट फॉस्फोरस पाच्यता के वक्त आहारों की प्रभावोत्पादकता का मूल्यांकन किया गया. रिकार्ड किए गए प्रतिक्रिया प्राचलों के परीक्षणों (पी > 0.05) के बीच विशेष. प्रकार की विभिन्नताएं नहीं देखी गई. सोडियम फोस्फेट एकबेसिक से संपूरक आहार खिलाए गए झींगों में अच्छी बढ़ती दर (4.05±0.32) आहार का दृष्ट पाच्यता गुणांक (ए डी सी) (93.58±0.39) और फोस्फरस की दृष्ट पाच्यता (ए डी पी) (55.08±2.05) दृष्टमान थे. लेकिन 1:1 अनुपात में कैल्शियम फोस्फेट द्विबेसिक और पोटासियम फोस्फेट एकबेसिक से संपूरक आहार खिलाए गए झींगों का खाद्य रूपांतरण अनुपात (एफ सी आर) अच्छा पाया गया. अतः यह अध्ययन यह साबित करता है कि निश्चित लवणता (17-19 पी पी टी) में *पेनिअस इंडिकस* के संयोजित आहार में अकार्बनिक फॉस्फोरस के परीक्षित श्रोतों में सोडियम फोस्फेट एकबेसिक सबसे उत्कृष्ट है.

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ABSTRACT

A 30- day feeding experiment was conducted to evaluate the efficacy of selected sources of inorganic phosphorus in a diet compounded for juvenile *Penaeus indicus*. The phosphorus sources tested were calcium phosphate dibasic, sodium phosphate monobasic, potassium phosphate monobasic and a mixture of calcium phosphate dibasic and potassium phosphate monobasic in the ratio 1:1. The efficacy of the diets was evaluated in terms of weight gain, food conversion ratio (FCR), apparent feed digestibility coefficient and apparent phosphorus digestibility. No significant differences were observed among the treatments ($P>0.05$) in the response parameters recorded. However the best specific growth rate (4.05± 0.32), apparent digestibility coefficient (ADC) of the diet (93.58± 2.05) and apparent digestibility of phosphorus (ADP) (55.08±2.05) were recorded for shrimps fed the diet supplemented with sodium phosphate monobasic, but the best FCR (2.00±0.33) was observed with the diet supplemented with a mixture of calcium phosphate dibasic and potassium phosphate monobasic in the ratio 1:1. The present study suggests that a diet containing good quality ingredients with sufficient available P (0.81 %) as in the control diet (diet-1) is adequate to promote survival, growth and phosphorus retention in juvenile *P. indicus*. However if a supplement source of P is required sodium phosphate monobasic is recommended in the diet as it provided the best response when incorporated in the diet in the tested salinity (17-19 ppt).

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CONTENTS

INTRODUCTION

Aquaculture dates back to the fifth century B.C. in China (Fan Li, 1983). It has now become an important avenue for animal protein production to meet the nutritional demands of the burgeoning global population. Worldwide attention on the role of aquaculture in augmenting fisheries production came into focus in the year 1966, when the FAO held a World Symposium on Warm Water Fish Culture in Rome, Italy (Rabanal, 1996). Since then the attention of participant countries was awakened on the need to accelerate and sustain production through aquaculture. As a result of intensive research and development efforts the global aquaculture production showed a steady growth from 6.1 million mt. in 1975 (Pillay, 1976) to 28.8 million mt. in 1997 (FAO, 1999). Aquaculture remained by and large as a household traditional farming activity in freshwater ponds and tanks for centuries. However, in recent years aquafarming has witnessed spectacular growth and spread to brackishwater and marine waters. Technologies have been developed for breeding, seed production and grow-out for a wide variety of aquatic organisms. Significant progress has also been achieved in the development of practical feeds, feed, disease and environment management strategies. However there are several unresolved technical problems, which need concerted research investigations to make aquaculture an eco-friendly activity.

Shrimp is called the "Pinkish Gold" of the sea because of its universal appeal, unique taste, high unit value and ever increasing demand in the world market (Sakthivel, 1987). However, due to the declining catches commercial scale production of shrimps through culture assumed importance. At present shrimp production through culture (9,41,814 mt.) accounts for only 3.27 % of world aquaculture production (28.8 million Mt.) in quantity but 13.36 % in terms of value (FAO, 1999).

The pioneering work of Hudinaga in 1942 on the successful larval rearing of *Penaeus japonicus* was a major breakthrough for shrimp seed production and culture (Hudinaga, 1942). The subsequent technological advancements made in hatchery technology of shrimp seed production, in the physiological and biochemical studies and the realization of the role of feeds in sustaining shrimp culture stimulated intensive interest in penaeid shrimp nutrition.

Nutritional studies in shrimp were initiated in the early 1970's (Akiyama *et al.*, 1992). Researches carried out during the last three decades have substantially enriched our knowledge of nutrition of marine shrimp and brought to focus the need to continually develop, test and apply new nutritional concepts. In view of the diversity of research methodologies, research diets, variables such as species, size, source and physiological status of the shrimp, environmental conditions, experimental design and facilities and diet form, composition and processing, employed by various authors a meaningful comparison of results reported by various authors is a difficult exercise. Nevertheless, nutritional studies have been the major tools for the development of commercial shrimp feed industry.

A good deal of contribution to nutrition, feeds and feeding of shrimp has come forth during the past three decades from several laboratories in the world. Compilations and reviews of these studies have been made by New (1976, 1987), Kanazawa (1984), Akiyama *et al.* (1992), Paulraj (1993), Cuzon *et al.* (1994) and D'Abramo *et al.* (1997). Feed holds the key not only for success of shrimp culture, but also for its sustainability. Intensive shrimp farming practices involve a huge amount of feed and consequently results in substantial waste generation. Nitrogen, phosphorus and organic wastes principally derived from feeds are major contributors to pollution and the consequent biological degradation of the culture environment (Briggs and Funge-Smith, 1994). In order to sustain the growing shrimp farming sector, and make it eco-friendly greater attention in nutrition research has to be focussed on developing nutrients balanced, quality-assured feeds and feed management strategies.

REVIEW OF LITERATURE

Nutrition research has brought to light the need for more than 40 essential dietary nutrients by shrimp. These nutrients are often grouped as macro-nutrients and micro-nutrients based on their quantitative requirement. Protein, lipids and carbohydrates are grouped under macro-nutrients. The micro-nutrients group includes the fat-soluble and water-soluble vitamins and minerals. Minerals are further grouped as macro-minerals and micro-minerals or trace elements. Macro-minerals include calcium, phosphorus, potassium, magnesium, sodium, chlorine and sulphur. Micro-minerals include iron, copper, zinc, manganese, cobalt, selenium, iodine, nickel, fluorine, vanadium, chromium, molybdenum, tin, silicon and silver.

Protein and amino acid requirement of many cultivable species of shrimp have been well defined including that of *Penaeus indicus* (Colvin, 1976; Kanazawa *et al.*, 1981; Gopal and Paulraj, 1990). The importance of lipids, especially the essentiality of *polyunsaturated fatty acids*, cholesterol and phosphoplipids in the diets has been well established and recommendations on sources and optimal levels of these nutrients in diets have been made (Kanazawa *et al.*, 1971, 1985, 1993; Teshima *et al.*, 1986; Piedad-Pascual 1986; Chandge and Paulraj, 1990, 1997a, b; Chen and Jenn, 1991; Chen, 1993; Kanazawa, 1993).

Requirement of carbohydrate and utilization of various carbohydrate sources by shrimp have been reported by Andrews *et al.* (1972), Deshimaru and Yone (1978a), Abdel-Rahman *et al.* (1979), Alava and Pascual (1987), Ali (1988), Shiau *et al.* (1991) and Hemambika and Paulraj(1999).

Despite a good deal of research on vitamin requirements of shrimp the *recommendations* emanated from these studies are under reinvestigation in view of the complexity of vitamin research, interaction among nutrients, gut microbial contributions and bioavailability, processing and storage losses especially of the heat labile and water soluble vitamins (Kanazawa *et al.*, 1976; Deshimaru and Kuroki, 1974 b,1976; Shigueno and Itoh 1988; Chen *et al.*, 1991; Chen and Hwang, 1992; Chen and Chang, 1994; Shiau and Lung, 1993; Shiau and Liu, 1994; Catacutan and Lavilla-Pitogo, 1994).

Mineral nutrition:

Mineral ions are essential components of many biological chemicals such as enzymes, hormones and other organic compounds involved in a number of biochemical and physiological life processes and form structural components. Their non-availability for a prolonged period often leads to irrecoverable deficiency diseases. With the exception of osmoregulation, biochemical functions of minerals in aquatic animals appear to be similar to those of terrestrial animals (Lovell, 1989). The more soluble minerals, viz. calcium, phosphorus, sodium, potassium and chlorine, function in osmoregulation, in the maintenance of acid-base balance and as membrane components.

Since seawater is rich in many mineral ions, shrimp are capable of extracting most of the minerals required (Gilles and Pequeux, 1983) from the water. Consequently, the determination of quantitative requirements is difficult (Lall, 1989). But dietary sources of minerals for growth may be necessary especially to recoup the losses incurred during moulting (Piedad-Pascual, 1990). Several studies have been made to establish the essentiality and dietary levels of minerals and trace elements for shrimps (Kitabayashi *et al.*, 1971; Deshimaru and Kuroki, 1974 a; Deshimaru and Yone, 1978 a & b; Kanazawa *et al.*, 1984; Castille and Lawrence, 1989 and Davis *et al.*, 1992).

Calcium, phosphorus and potassium constitute the major chunk of the ash contents in shrimp. The concentration of calcium varies between 2% and 3%, phosphorus 1.2 % and 1.3 % and potassium 0.8% and 1.2 % of the body weight (Boyd and Teichert-Coddington, 1995). Deshimaru and Kuroki (1974 a) using a semi-purified diet found that mineral rich diets (as high as 19.5 % ash) produced the best growth in *Penaeus japonicus*. Castille and Lawrence (1989) showed that growth rates of juvenile *Penaeus vannamei* were significantly reduced when fed a practical feed without mineral supplementation.

Role of phosphorus:

Phosphorus(P), a macro-mineral, is an essential nutrient for shrimp. In association with calcium, phosphorus forms a major component of the exoskeleton. It has functional roles in many-metabolic processes. As an essential component of phospholipids (eg. lecithin and cephalin), nucleic acids, phosphoproteins, high-energy compounds

(Adenosine triphosphate), many metabolic intermediates and co-enzymes (Akiyama *et al.*, 1992). Inorganic phosphates also serve as important buffers to maintain normal pH of intracellular and extracellular fluids.

Phosphorus in water:

Phosphorus occurs in nature almost exclusively as phosphate. Phosphate is found in the dissolved form in natural waters as a result of the natural weathering and solution of the phosphate minerals, soil erosion and transport, soil fertilization and resultant transport, biological transfer (assimilation and dissimilation processes involving phosphorus in agriculture etc.) and use of soluble phosphate compounds in detergent manufacture, water treatment and industry. Phosphorus is generally found at low concentration in natural waters (Boyd, 1981). Consequently absorption of significant amounts of phosphorus from water is unlikely, making a dietary source essential for most aquatic animals (Akiyama *et al.*, 1992).

Sources of phosphorus in shrimp diets:

From an economic point of view, phosphorus accounts for the major cost of mineral supplement in feeds. Sources of phosphorus and their bioavailability to the shrimp are very critical as excess of phosphorus in the feed not only results in an unnecessary investment in a nutrient that will not be efficiently utilised by the cultured species, but also adds to the nutrient loading of the culture systems and effluent waters possibly increasing the pollution load of receiving waters.

The various sources of phosphorus used in diets are organic compounds such as plant products, animal products, microbial products and inorganic compounds such as sodium phosphate (monobasic and dibasic), calcium phosphate (monobasic, dibasic and tribasic) and potassium phosphate (monobasic and dibasic). The phosphorus content in plant products varies from 0.25 % (wheat), to 1.04 % (rapeseed meal) while in animal products it varies from 0.7 % (feather meal) to 5.90 % (bone meal) (Cho *et al.*, 1994).

Several workers have conducted experiments using diets with different sources of phosphorus. Phosphates of potassium and sodium (monobasic and dibasic), calcium

phosphate (monobasic, dibasic and tribasic) have been used as source of phosphorus in the purified diets for *P. aztecus* (Sick *et al.*, 1972), *P. japonicus* (Kanazawa *et al.*, 1984; Deshimaru and Shigueno, 1972; Deshimaru and Yone, 1978b;Civera and Guillaume, 1989), *P. vannamei* (Civera, 1994; Civera and Guillaume, 1989; Davis *et al.*, 1992; Davis and Arnold, 1994, 1998; Velasco *et al.*, 1998), *P. monodon* (Penaflorida, 1999); *P. indicus* (Ali, 1988) and in the compounded diets of juvenile *P. californiensis* (Huner and Colvin, 1977).

Cheng and Guillaume (1984) and Civera and Guillaume (1989) reported sodium phosphate dibasic to be the best inorganic source of phosphorus in a casein-gelatin based purified diet for *P.japonicus*. Cuzon *et al.* (1994) reported that shrimps utilize phosphorus more efficiently if phosphates, which dissociate at basic pH such as sodium phosphate, are provided in the diet rather than calcium phosphate dibasic.

When calcium phosphate dibasic, sodium phosphate dibasic and sodium phosphate monobasic were tested in diets sodium phosphate monobasic produced the best response in terms of growth, but the response was not significantly different among the treatments when supplemented at 0.8 % level in the semi-purified diet for *P.vannamei* (Velasco *et al.*, 1998).

Requirement of phosphorus for shrimp:

Several studies have focussed on the dietary phosphorus requirements of shrimp (Kitabayashi *et al.*, 1971; Sick *et al.*, 1972; Deshimaru and Kuroki, 1974a ; Deshimaru and Yone, 1978 b); Kanazawa *et al.*, 1984; Cheng, 1984; Civera and Guillaume, 1989; Davis *et al.*, 1993a; Davis and Arnold, 1994, 1998; Velasco *et al.*, 1998; Penaflorida, 1999).

Review of the results in terms of growth performance achieved by the above workers indicates wide variations caused by diet composition. Kitabayashi *et al.* (1971) reported the best growth rates in *P. japonicus* by feeding diets supplemented with 1.24% calcium and 1.04% phosphorus.They also showed that when the calcium/phosphorus ratio was increased to 2:1 growth was inhibited with a decrease in pigmentation whereas Deshimaru and Kuroki (1974a)reported the best growth increment for *P. japonicus* when

Ca: P ratio of 0.76:1. Subsequently, Deshimaru and Yone (1978 b) reported the best growth in *P.japonicus* when phosphorus was supplemented at 2 % level as sodium phosphate monobasic in the purified diet.

Kanazawa *et al.* (1984) concluded that supplements of 1 to 2 % of Ca and P at the Ca: P ratio of 1:1, to the purified diets was indispensable for the growth of P.japonicus juveniles. Further they assumed that a supplemental Ca might play some role in the effective utilization of dietary P by the shrimp.

Civera and Guillaume (1989) found that for *P. japonicus* and *P. vannamei* juveniles, a casein-gelatin-based diet without phosphorus supplements, but containing 0.56 % and 0.41% phosphorus in the basal diet was adequate for sustaining good growth and survival. Civera (1989) recommended a supplementation of 1 % Ca and 0.78 % P in the diet for *P. japonicus*.

A casein-based diet supplemented with a mineral premix providing 0.66 % Ca and 0.51 % P in the ratio 1.3:1 for *P. aztecus* gave an 18 % increase in biomass over the control (Sick *et al.*, 1972).

Huner and Colvin (1977) recommended a calcium-phosphorus ratio of 2.06:1 in the diet for juvenile *P. californiensis*. They also recommended that Ca: P ratios higher than 2.42:1 should be avoided in dietary formulations for this shrimp.

An experiment on juvenile *P. vannamei* fed with a casein/gelatin based semi-purified diet indicated that the deletion of Ca and P from the mineral premix produced no significant decrease in growth rate (Davis *et al.*, 1992). Davis *et al.*, (1993a) reported that in the absence of calcium, the casein-gelatin based semi-purified diet containing 0.35 % P was adequate to maintain good growth and survival of *P. vannamei* post-larvae indicating that a dietary calcium supplementation was not required. It was also demonstrated that the minimum level of dietary phosphorus supplementation required for maximum growth of the shrimp was dependent on the calcium content of the diet. However, Davis and Arnold (1998) reported that anchovy and soybean meal based practical diets containing 0.22% available P (0.98% total phosphorus) was not adequate to meet the physiological requirement of juvenile *Penaeus vannamei* for sustaining

maximum growth and survival. Velasco *et al.*, (1998) reported the phosphorus requirement of *P. vannamei* post-larvae to be 0.4 % (Ca: P ratio 1:2) in semi-purified diet for good survival and growth.

Experiment with juveniles of *P.orientalis* showed that the growth and food conversion rate were the best when the total contents of Ca and P were 2 % and Ca/P ratio was 1:1.7 (Li *et al.*, 1986).

Bioavailability of phosphorus to the shrimp:

Excess of phosphorus in the feed not only results in increased feed cost but also leads to wastage of phosphorus into the culture system and the effluent water culminating in eutrophication. The possible effect of excess phosphorus in discharged waters can be understood from the estimate that if phosphorus is the limiting factor, 1 mg of P is able to synthesize approximately 0.1 g of algal biomass by dry weight in one single cycle of limnological transformation (Kramer, 1967). After settling to the deeper layers, this biomass exerts a biochemical oxygen demand of approximately 140 mg/l for its mineralization.

The lack of comprehensive information on the dietary requirement of phosphorus and its bioavailability from various sources for shrimp leads to over supplementation of P in formulated feeds. Usually, commercial shrimp diets contain 1.5-2.5 % P mostly derived from fish meal.

Owing to the difficulty in prediction of feed intake and optimum level of feeding, feed waste contributes to a relatively large proportion of total waste output in most intensive culture operations (Bergheim *et al.*, 1984; Person,1988; Cho *et al.*, 1991; Seymour and Bergheim, 1991). Nitrogen, phosphorus and organic wastes from feeds are the major factors contributing to the environmental pollution from aquaculture (Rijn and Shilo, 1989; Akiyama, 1992; Boyd and Musig, 1992). Dissolved reactive phosphorus constituted 50-60 % of the total phosphorus losses from feeds and 30-40% from faeces (Philips *et al.*, 1993).

Thus the determination of phosphorus availability from various sources for shrimp is imperative to facilitate reduction in feed costs and phosphorus loading to the culture environment. New (1987) gave tentative estimates of phosphorus availability to the shrimp from various sources as follows: plant and plant products (30%), animal products (70%), microbial products (90 %), monobasic sodium, potassium or calcium phosphates (95%), dibasic calcium phosphate (70%), tribasic calcium phosphate (65%). Akiyama *et al.* (1992), assuming the pH of the digestive system of shrimps to be similar to that of common carp, which lacks a HCl acid secreting stomach suggested the phosphorus availability to shrimp to be similar to the values for common carp. They estimated the availability of phosphorus for various ingredients as follows: plant products (30%), animal products (30 %), microbial products (90 %), calcium phosphate monobasic (94%), calcium phosphate dibasic (45 %) and calcium phosphate tribasic (15 %). Apparent phosphorus availability for *P.vannamei* had been reported for a casein/gelatin based semipurified diet and calcium phosphate dibasic as 86.3 % and 33.5 % respectively (Davis, 1990). Utilizing chromic oxide as an inert marker, Davis and Arnold (1994) determined the apparent phosphorus availability (APA) from inorganic sources for *P.vannamei*. The APA values for calcium phosphate monobasic, calcium phosphate dibasic, calcium phosphate tribasic, potassium phosphate monobasic, sodium phosphate monobasic were 46.3 %, 19.1 %, 9.9 %, 68.1 % and 68.2% respectively. The same study also indicated that APA values for diets containing sodium phosphate monobasic were significantly depressed by the presence of calcium lactate (50.0 % APA) but not by calcium carbonate (65.5 % APA) or calcium chloride (68.2 % APA). The APA value for phytate phosphorus was determined to be 8.4 % for *P.vannamei* and 47.3 % for *P.japonicus* (Civera *et al.*, 1990).

Apparent phosphorus digestibility of shrimp meal, fish meal, squid meal, soybean meal, rice bran was determined to be 29.8%, 46.5%, 76.8%, 39.9% and 26.1% respectively for *P. vannamei* (Akiyama *et al.*, 1992). Davis *et al.* (1993b) reported reduced availability of dietary phosphorus and zinc to *P. vannamei* when fed a diet containing 1.5% phytate. Davis and Arnold (1998) reported that a basal diet containing anchovy and soybean meal and without phosphorus supplementation had an APA value of 23.1 % for *P. vannamei* juveniles. The same study revealed Dynafos (primarily dibasic calcium phosphate) had a relative biological value of 63.8 % of Cefkaphos (primarily monobasic calcium phosphate) based on the final weights of the shrimp offered diet

containing 1.25 % of supplemental P/kg of diet. Penaflorida (1999) reported that in the absence of supplemented Ca, 0.5 % supplemental P (0.74 % total P) in a casein-gelatin based diet provided maximum growth of *P. monodon* post-larvae.

Ali (1988) reported phosphorus requirement of 1 % in the presence of 0.5 % Ca in a purified diet for optimal growth of *P. indicus*. The study appears incomplete as the mineral mixture in the diet lacked other essential minerals and trace elements.

The above findings suggest that for the development of eco-friendly practical feeds for shrimp, optimization of dietary phosphorus levels and identification of suitable inorganic P sources are pre-requisites. Optimization of phosphorus level will facilitate reduction in feed costs as well as reduction in phosphorus loading in the culture environment and in the effluent discharge. Recognising these aspects the present work was carried out on juvenile *Penaeus indicus* to evaluate the efficacy of supplements of selected inorganic phosphorus sources in a compounded diet. Among the 15 species of shrimps available in Indian waters suitable for aquaculture, the Indian white prawn *Penaeus indicus* is identified as one of the important commercial species. India contributes about 164 mt. of *P. indicus* to the global *P. indicus* aquaculture production of 4655 mt. (FAO, 1999).

The outline of the work is as follows:

(1) Formulation of a basal diet using selected natural ingredients

(2) Supplementation of selected inorganic phosphorus sources: potassium phosphate monobasic, sodium phosphate monobasic and calcium phosphate dibasic and determining their efficacy.

(3) Determination of digestibility of these phosphorus sources.

(4) Evaluation of waste-output in terms of phosphorus.

MATERIALS AND METHODS

A 30-day culture experiment was conducted to evaluate the efficacy of a few inorganic phosphorus sources in the compounded diets of juvenile *Penaeus indicus*. The phosphorus sources tested were calcium phosphate dibasic, sodium phosphate monobasic, potassium phosphate monobasic and a mixture of calcium phosphate dibasic and potassium phosphate monobasic in the ratio1:1. Data on growth in terms of weight gain , survival,food conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER) and body composition of the experimental animals, digestibility of the feed and phosphorus sources were obtained from the experiment.

Collection of feed ingredients

Fish (anchovies), squilla(mantis shrimp), clam and squid were procured fresh from local markets and fish landing centers in and around Kochi. These were cleared off extraneous materials, washed properly to remove the adhering salt and dirt, oven-dried at 60 ±2 ^{o}C, pulverized and sieved through 250 μ mesh sieve. Defatted soybean cake was procured from local market, dried, pulverized and sieved as earlier. Tapioca flour and wheat flour in powdered form was procured from local market. These ingredients were stored in plastic bottles until the feed preparation. The ingredients were analysed to obtain information on nutrient composition.

Biochemical Analysis

Feed ingredient samples were dried to constant weight in a hot-air oven at 105 ±5 ^{o}C. After cooling in a desiccator, samples were weighed to the nearest 0.001 g and the differences in weight were used to calculate the moisture percentage in the samples.

Total nitrogen in the samples was determined by Kjeldahl method. Crude protein was calculated from the total nitrogen by multiplying by a conversion factor of 6.25 (A.O.A.C. 1990).

Crude fat in the sample was determined by soxhlet extraction method (A.O.A.C., 1990) using petroleum ether (boiling point 40^{o}-60^{o} C) as solvent.

Crude ash of the ingredients was determined by incinerating samples in a muffle furnace at 550 ^{o}C for 4 hrs (A.O.A.C., 1990).

Phosphorus content in the ingredients was analysed by Molybdovanadate method (A.O.A.C., 1990) from a standard curve.

Diet Preparation

The composition of the diets formulated and used in this study is presented in Table-1(a, b). The experimental diets except the control were supplemented with one of the sources of phosphorus: potassium phosphate monobasic, sodium phosphate monobasic, calcium phosphate dibasic and a mixture of potassium phosphate monobasic and calcium phosphate dibasic at 1:1 ratio by adjusting with cellulose to obtain 0.5 % of phosphorus supplement. Chromic oxide was incorporated in the diets at 0.5 % level for digestibility studies.

The dry ingredients except vitamins, cholesterol and lecithin were mixed in a mixer and steam cooked for 10 minutes to obtain gelatinization of the wheat flour and tapioca flour and to enhance the binding effect. After cooling the mixture, vitamin premix, cod liver oil, sunflower oil, cholesterol and lecithin were added to it and thoroughly blended with little hot distilled water to a dough consistency and pelleted through a 3 mm die by using a hand pelletizer. The pellets were oven- dried at 60 ^{o}C to a moisture content less than 10 %, crumbled to 1-2 cm length and stored in air-tight plastic bottles. The diets were analysed following the methods as described for feed ingredients.

Crude fibre of the sample was determined by digesting it with 1.25 % HCl, then with 1.25 % NaOH followed by acetone washing, drying and ashing in the muffle furnace at 550 ^{o}C (A.O.A.C., 1990).

Calcium in the diet was determined using the residue from ash by titration method (A.O.A.C., 1990) and calculated as follows:

$$\text{Calcium (\%)} = \frac{\text{ml. permanganate solution}}{\text{Weight of the sample (g)}} \times \frac{\text{aliquot used (ml)}}{250} \times 0.1$$

Table –1a: Ingredient composition of the control diet.

Ingredients	Inclusion level(%)
Fish meal	20
Squid meal	5
Squilla meal	5
Clam meal	5
Soybean meal	20
Wheat flour	19
Tapioca flour	15
Lecithin	1
Cholesterol	0.5
Cod liver oil	3
Sunflower oil	1
[a]Vitamin mix	0.8
[b]Mineral mix(Ca and P free)	0.7
BHT	0.05
α-Cellulose	3.45
Chromic oxide	0.5
	100.00

[a]Vitamin mix (g/100g diet): Retinol acetate-0.02, Cholecalciferol-0.007, Tocopherol-0.05, Menadione-0.05, Thiamin hydrochloride-0.02, Ca-Pantothenate-0.02, Folic acid-0.008, p-Amino benzoic acid-0.01, Choline chloride-0.15, Inositol-0.1, Biotin-0.002, Cyanocobalamin-0.1mg, Pyridoxine hydrochloride-0.008, Riboflavin-0.008, Niacin-0.02, Stay C(35% active)-0.2, α-cellulose-0.127.

[b]Mineral mix(g/100g diet):$MgSO_4.7H_2O$-0.5,$MnSO_4.H_2O$-0.06, $FeSO_4.7H_2O$-0.06, $ZnSO_4.7H_2O$-0.06, $CoCl_2$-0.01, $CuSO_4.5H_2O$-0.01, KI-0.03mg, $NaHSeO_3$-0.07mg.

Table-1b: Phosphorus sources and their inclusion level (g/100 g diet) to provide 0.5 % phosphorus supplement

Phosphorus source	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Calcium phosphate dibasic. ($CaHPO_4$)	0.000	2.197	0.000	0.000	1.098
Sodium phosphate monobasic. ($NaH_2PO_4.2H_2O$)	0.000	0.000	2.518	0.000	0.000
Potassium phosphate monobasic (KH_2PO_4)	0.000	0.000	0.000	2.196	1.098
α-Cellulose[c]	3.450	1.253	0.932	1.254	1.254

[C] Inert Filler

Hydrostability test of thediets

The water stability of the diets was determined over a period of 3 hours by employing the method described by Jayaram and Shetty (1981) with minor modifications. Five gram samples of each diet was taken in a 4" × 4" No. 30 bolting silk pouch (prestitched) and immersed separately in plastic tubs containing 15 *l* of 18 ppt seawater provided with light aeration. At set intervals of 30 minutes, one hour, two hours and three hours 3 pouches for each diet were removed and after rinsing with double distilled water, to remove adhering salts, the excess water was drained and the residue dried in a hot air-oven at 105 ± 5 ^{o}C for 30 minutes, followed by further drying at 65 ±2 ^{o}C to a constant weight and cooled in a desiccator. The mean difference in weights of pouches containing the diets before immersion and after drying were used to calculate the percentage dry matter loss, which is a measure of the water stability of the pellets for the corresponding time intervals.

Experimental set-up

Experiment was conducted using plastic tubs of 50 *l* capacity. The tubs were arranged on vertical racks and provided with aeration from air pumps (Plate-2). Aeration was maintained uniformly by using regulators through out the experimental period. Each tub was covered with nylon screen and clipped to prevent the escape of animals.

Seawater for the experiment was collected from the sea off Kochi and transported by tanker. The water was chlorinated at 30 ppm to disinfect. The sediments were removed and after dechlorination for over a period of one week the water was filtered through bolting silk (40 μ). The water was diluted with tap water to the required salinity of 18 ppt since juvenile *P. indicus* prefer lower salinities (Paulraj and Sanjeeva raj, 1990) and stored in 1000 *l* FRP tanks.

Experimental Animals

Juveniles of *Penaeus indicus* (Plate 1) were procured from the Fisheries Station, Puduvyppu near Kochi and transported in polyethylene bags with oxygen packing.

Plate 1 : Juvenile *Penaeus indicus*

Plate 2: Experimental set-up

The shrimps were acclimated to experimental condition for a week after which the animals were hand-graded and selected for the experiment. The initial average weight of the animals was 0.416 ± 0.127g. The weight was taken after blot drying the animal. They were randomly distributed into the experimental tubs. Ten juvenile shrimps were stocked in each tub. Three replicates were maintained for each diet. Feeding was suspended and animals were starved for 48 hrs before the commencement of the experimental feeding.

Three groups of shrimps (15 per group) were removed from the initial stock, weighed individually and dried in hot-air oven at 60 ±2 ^{o} C for 48 hrs. The dried samples were stored in desiccator and used for analysis of initial body composition.

Feeding Rates and Methods

Initially feeding was done thrice a day at 8 a.m., 2 p.m. and 7 p.m. The shrimps were fed approximately 20 % of their body weight. The daily ration was split into three doses of 30 %, 20 % and 50 % for each daily feeding regime. The feeding rate and frequency were decreased to 10 % of the body weight and twice a day respectively consistent with the feed intake to minimize the feed wastage. The diet was offered in a petridish to the animals to facilitate easy recovery of the left-over feed. Changes in the daily feed allowance were made so as to meet the increased feed demand of the animals and to minimize the feed waste. The left-over feed in the experimental tubs was collected daily before the first feeding, after siphoning out the waste faecal matter. The collection of faeces was started seven days after the commencement of the experiment for 2 days in every week. The faecal matter was collected by siphoning into a collection sieve (48 μ), followed by a rinse with distilled water to remove adhering salts. The faeces were dried in oven at 60^{o} C for 24 hrs and stored in plastic vials. The entire faecal collection over the experimental period was pooled by tub and used for phosphorus digestibility analysis. The phosphorus level in the whole body and faeces was measured following the method described for feed ingredients. Apparent digestibility of phosphorus was determined for the diets based on relative change in chromic oxide percentage in feed and faeces. Chromic oxide in the faeces as well as in the diet was determined by the method of Furukawa and Tsukahara (1966)

Water exchange

Daily one-third of the water from the tubs was siphoned out and replenished with an equal amount of the fresh water of required salinity. Complete water replacement was done once in 15 days.

On 15th day of the experiment, the animals were weighed to adjust the feed amount to meet their food demand. The experiment was terminated on the 30th day and the final weight of the animals was recorded. The animals were dried in an oven at 60 ±2 ^{o} C for 48 hours. Dried samples were then powdered using a porcelain mortar and pestle and stored for biochemical analysis.

Physico-chemical parameters like temperature, pH, salinity, and dissolved oxygen were recorded daily. Salinity was estimated by Mohr-Knudsen method and dissolved oxygen using the modified Winkler's method (Strickland and Parsons, 1968). The pH of water was measured using a digital pH meter. Ammonia, nitrate and dissolved orthophosphate were measured once a week. Ammonia was measured by phenol-hypochlorite method (Solorzano, 1969). Dissolved orthophosphate was measured by ascorbic acid method and nitrite in the water was measured following the method by APHA (1976).

Nutritional parameters evaluated

$$\text{1. Survival Rate (\%)} = \frac{\text{Final number of shrimps}}{\text{Initial number of shrimps}} \times 100$$

$$\text{2. Food Conversion Ratio (FCR)} = \frac{\text{Food consumed (g)}}{\text{Average live weight gain (g)}}$$

$$\text{3. Protein Efficiency Ratio (PER)} = \frac{\text{Average live weight gain (g)}}{\text{Total protein consumed (g)}}$$

Table-2: Water quality parameters recorded during the experiment.

Parameter	Range
Salinity (ppt)	17- 19
Dissolved oxygen (ml/l)	4.2-5.3
pH	7.52-8.2
Temperature (°C)	27-31
Ammonia-nitrogen (μg at /l)	0.063-0.069
Nitrite-nitrogen (μg at /l)	0.170-0.292
Dissolved orthophosphate (μg at /l)	1.202-1.326

4. Specific Growth Rate was calculated using the formula:

$$W_t = W_o(1 + a/100)^t$$

where W_o = Average initial weight (g)

W_t = Average final weight (g)

t = duration of experiment in days

a = specific growth rate

5. Apparent Digestibility of Phosphorus:

$$ADP = 100 - \left\{ \frac{\%\ \text{nutrient in the faeces}}{\%\ \text{nutrient in the feed}} \times \frac{\%\ Cr_2O_3 \text{ in the feed}}{\%\ Cr_2O_3 \text{ in the faeces}} \right\} \times 100$$

6. Apparent Digestibility Co-efficient of the feed :

$$ADC = \frac{\text{Feed intake(g) - Faecal output(g)}}{\text{Feed intake (g)}} \times 100$$

7. Gross Conversion Efficiency: $= \frac{\text{Average weight gain (g)}}{\text{Consumption (g)}} \times 100$

8. Phosphorus Retention (%) $= \frac{\text{Gain in phosphorus (g)}}{\text{Total phosphorus consumed(g)}} \times 100$

9. Estimated Faecal Phosphorus Production (kg P/ton of shrimp production):

= FCR X Total P (Kg / ton of feed) X Faecal P (% of the phosphorus in the feed)

Statistical Analysis

The data obtained on the nutritional parameters from this experiment were subjected to statistical analysis. One-way analysis of variance (ANOVA) was performed to test whether any significant differences existed among the treatment means. The ANOVA was performed using Microsoft – Excel package.

RESULTS

The results of the various parameters obtained from the 30-day experiment are presented below.

Biochemical Composition of the feed ingredients:

The results of the biochemical analysis of the ingredients used for the diet preparation are presented in Table-3.

The moisture content of the ingredients varied from 5.48% for soybean meal to 9.97 for wheat flour. Squid meal had the highest crude protein (75.86%) on dry matter basis. Fish meal prepared from cleaned dry anchovies fish had 72.22% crude protein. Tapioca flour had the lowest crude protein content (1.75%). Crude fat was the highest in squid meal (8.33%) and the lowest in tapioca flour (0.35%) . The highest and lowest crude ash levels were recorded for squilla meal (24.61%) and tapioca flour (1.56%) respectively.

Phosphorus was high in fish meal (2.49%) and squilla meal (1.7%). Tapioca flour had the lowest phosphorus content (0.078%). Tapioca flour had the highest P/N ratio of 0.28 and soybean meal had the lowest P/N ratio of 0.075 .

Biochemical composition of the Diets

Biochemical composition of the five diets is given in Table-4. The levels of moisture, crude protein, crude fat, crude ash, crude fibre, nitrogen free extract did not differ markedly among the diets. The control diet (diet-1) had 0.81% phosphorus. Calcium content of the diets varied from 1.2% to 1.87%.

Diet-3 supplemented with sodium phosphate monobasic had the lowest Ca/P ratio (0.88:1) while the control diet had the highest Ca/P ratio (1.5:1).

Gross energy for the diets varied from 1832.98 KJ/100g for diet-4 to 1887.2 KJ/100g for diet-1. P/N ratio for the diets varied from 0.136 (control diet) to 0.237 (diet 3).

Table-3: Proximate composition of the ingredients used for the experimental diet.

Ingredients	Moisture (%)	Dry matter (%)	Crude protein * (%)	Crude fat (%)	Crude ash (%)	Phosphorus (%)	N (%)	P/N
Fish meal	4.48	95.52	72.22	6.22	15.54	2.490	11.550	0.215
Squid meal	9.57	90.43	75.86	8.33	4.19	0.937	12.130	0.077
Clam meal	6.22	93.78	54.35	7.35	4.53	0.694	8.696	0.079
Squilla meal	6.91	93.09	56.40	2.11	24.61	1.700	9.024	0.188
Soybean meal	5.48	94.52	50.60	0.86	8.81	0.611	8.096	0.075
Wheat flour	9.97	90.03	14.63	2.80	1.62	0.394	2.342	0.168
Tapioca flour	9.40	90.60	1.74	0.35	1.56	0.078	0.278	0.280

* Crude protein = Total Nitrogen × 6.25

Table-4: Proximate composition of the diets.

Constituents	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Moisture (%)	4.26	3.82	5.14	5.68	5.69
Dry matter (%)	95.74	96.18	94.86	94.32	94.31
Crude protein (%)	37.01	37.23	36.82	37.54	37.81
Crude fat (%)	7.52	7.55	7.6	7.32	7.58
Crude ash (%)	8.08	9.66	9.19	9.5	9.22
Crude fibre (%)	1.46	1.56	1.4	1.71	1.66
* Nitrogen free extract (%)	41.67	40.18	39.85	38.25	38.04
Phosphorus (%)	0.81	1.25	1.4	1.42	1.32
Calcium (%)	1.22	1.87	1.2	1.25	1.56
Calcium : Phosphorus	1.5:1	1.49:1	0.85:1	0.88:1	1.18:1
P/N Ratio	0.136	0.21	0.237	0.236	0.218
** Gross Energy (kJ/100 g)	1887.2	1867.94	1854.57	1832.98	1846.01

* Nitrogen free extract (NFE %) = 100-(Moisture + Crude protein + Crude fat + Crude fibre + Crude ash)

** Gross Energy value calculated as protein 23.6 kJ/g, fat 39.5 kJ/g, and carbohydrate 17.2 kJ/g (Brafield and Llewellyn, 1982); fibre was assumed to have zero energetic value.

Hydrostability of the diets

The hydrostability i.e. the dry matter retained in the diets after the set period of half-an-hour, one hour, two hours and three hours was estimated and presented in Figure-1. The hydrostability of the diets varied from 93.26% to 97.24% after half -an-hour, 88.98% to 93.89% after one hour, 84.75% to 90.08% after two hours and 82.0% to 86.62% after three hours. So the highest hydrostability of 86.62% was observed for the diet-4 after three hours.

Response parameters

The survival rate, food conversion ratio, specific growth rate, gross conversion efficiency, protein efficiency ratio, apparent digestibility coefficient of the diets and apparent phosphorus digestibility are presented (Table 5-11) and statistical analysis revealed that these response parameters did not vary significantly among the treatments ($P > 0.05$).

Survival Rate

Survival rate of the animals (Table-5 and Fig: 2) ranged from 93.33 ± 5.77% (mean ± standard deviation) (diet 1 and diet 3) to 100% (diet 5).

Food Conversion Ratio (FCR)

The FCR (Table-6) did not vary significantly between the treatments. However the diet-5 provided relatively low FCR (2.00).

Specific Growth Rate (SGR)

The specific growth rate (Table-7) of the shrimps ranged from 3.56 ±0.15 to 4.05±0.32 in the various treatments.

Gross Conversion Efficiency

The gross conversion efficiency (Table-8) varied from 46.905 ±15.123% (diet 1) to 50.68 ± 8.486% (diet 5) in the various treatments.

FIGURE 1:
HYDROSTABILITY TEST OF THE DIETS

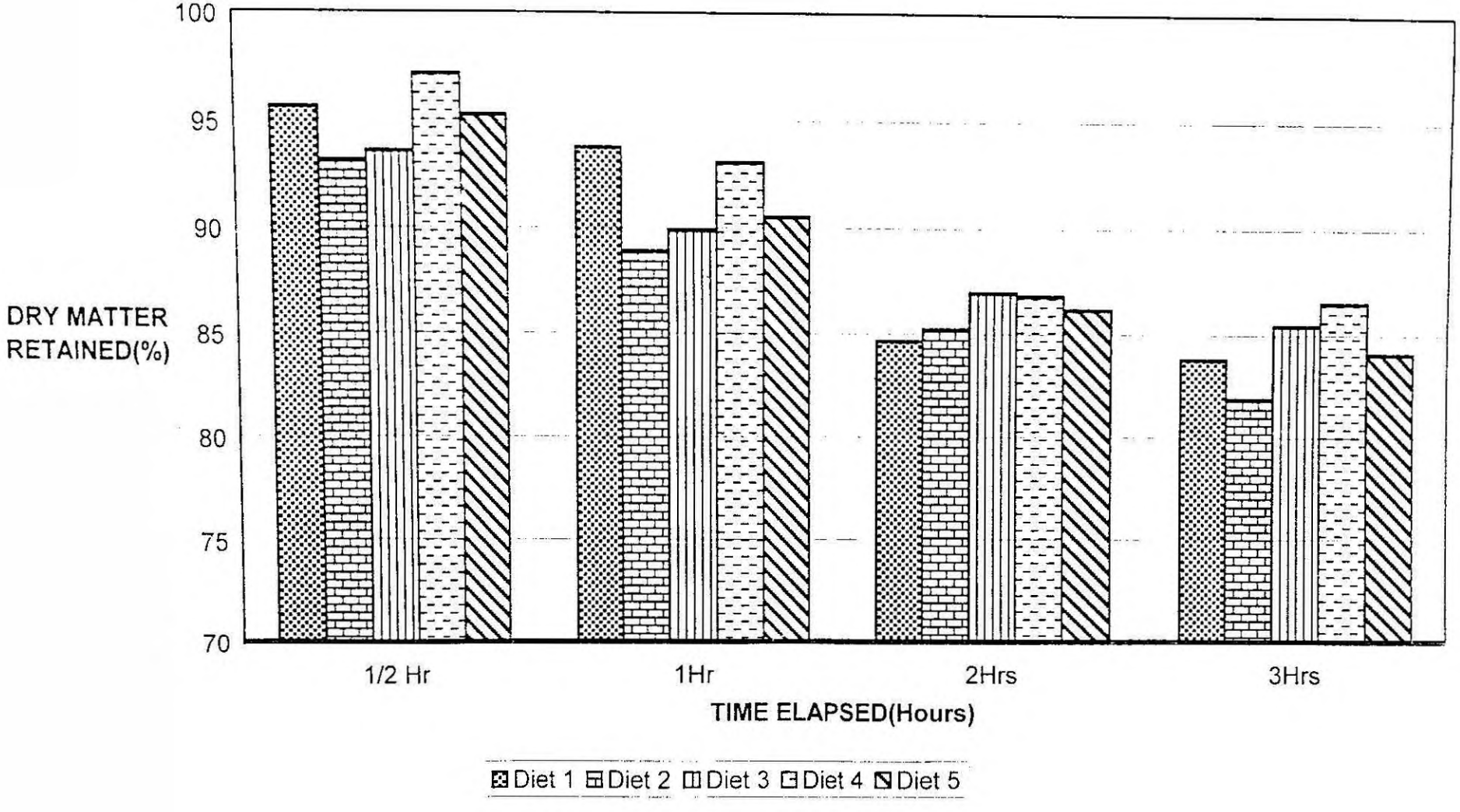

FIGURE-2 : SURVIVAL RATE (%) OF *P.indicus*

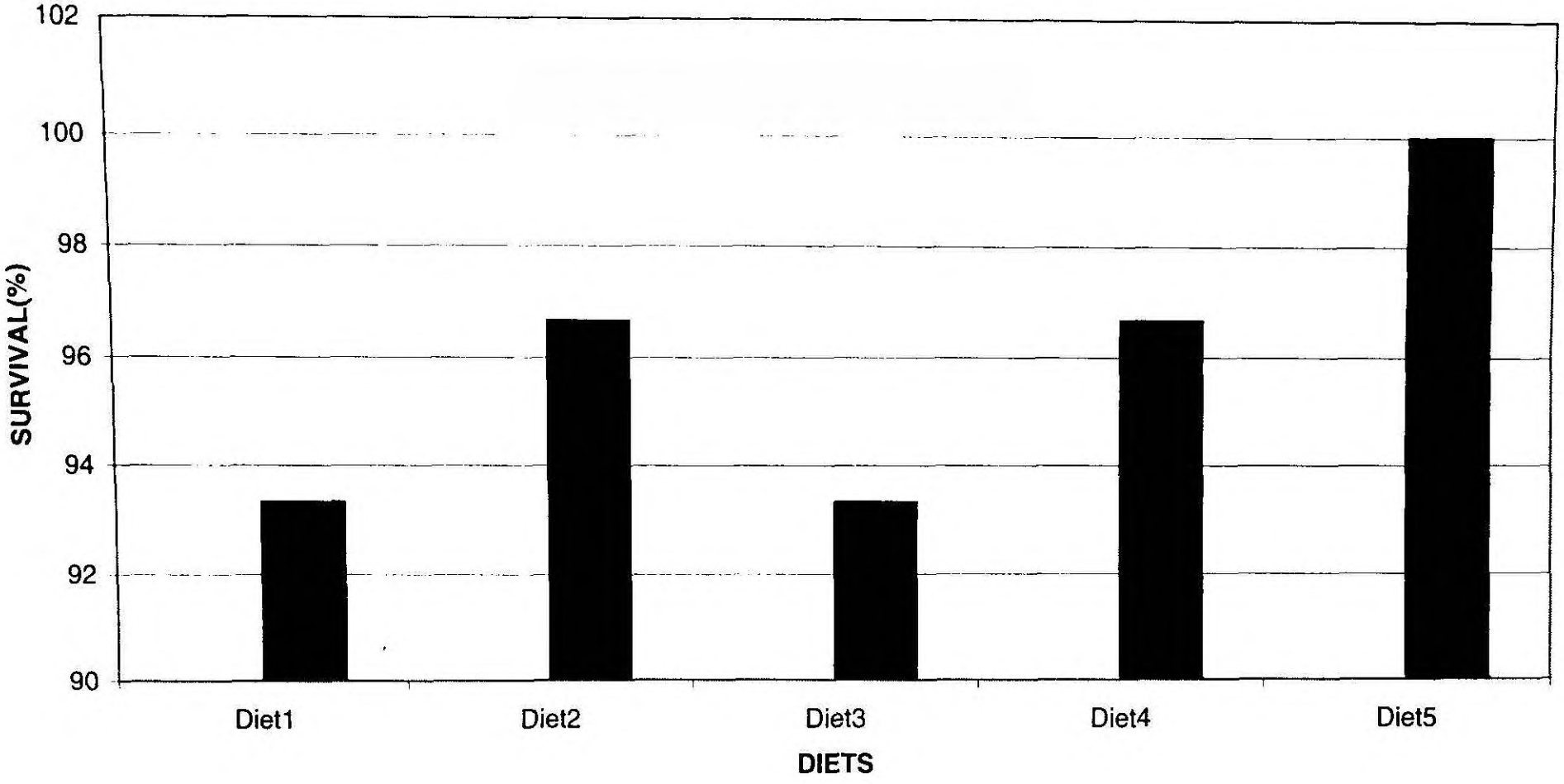

TABLE 5: SURVIVAL RATE OF *P.indicus*

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Diet1	3	280	93.3333	133.333
Diet2	3	290	96.6667	33.3333
Diet3	3	280	93.3333	33.3333
Diet4	3	290	96.6667	33.3333
Diet5	3	300	100	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	93.3333	4	23.3333	0.5	0.7368	3.4780
Within Groups	466.6667	10	46.6667			
Total	560	14				

RESULT: NOT SIGNIFICANT

TABLE 6 : FOOD CONVERSION RATIO OF THE DIETS

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
DIET 1	3	6.95	2.317	0.7845
DIET 2	3	6.39	2.1300	0.0327
DIET 3	3	6.09	2.0300	0.0307
DIET 4	3	6.37	2.1233	0.0044
DIET 5	3	6.02	2.0067	0.1124

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.179	4	0.045	0.2321	0.9140	3.4780
Within Groups	1.930	10	0.1930			
Total	2.109	14				

RESULT: NOT SIGNIFICANT

TABLE 7: SPECIFIC GROWTH RATE

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
DIET 1	3	11.16	3.7200	0.5439
DIET 2	3	10.76	3.5867	0.0505
DIET 3	3	12.17	4.0567	0.1062
DIET 4	3	10.7	3.5667	0.0226
DIET 5	3	11.6	3.8667	0.1670

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.5052	4	0.1263	0.7093	0.6038	3.4780
Within Groups	1.7807	10	0.1781			
Total	2.2859	14				

RESULT: NOT SIGNIFICANT

TABLE 8: GROSS CONVERSION EFFICIENCY(%)

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
DIET 1	3	140.72	46.907	228.717
DIET 2	3	141.32	47.107	16.1361
DIET 3	3	148.38	49.46	18.5364
DIET 4	3	141.01	47.003	2.42093
DIET 5	3	152.04	50.68	72.0207

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	36.0996	4	9.0249	0.13357	0.96634	3.4780
Within Groups	675.6628	10	67.566			
Total	711.7624	14				

RESULT: NOT SIGNIFICANT

Protein Efficiency Ratio (PER)

The PER (Table-9) varied from 1.263 (diet 1 and 2) to 1.36 ±0.183 (diet 5) in various treatments.

Apparent Digestibility Coefficient (ADC) of Diet

The ADC (Table 10) ranged from 91.12 ±3.274 (diet 4) to 93.587 ±0.398 (diet 3) in various treatments.

Apparent Digestibility of Phosphorus (ADP)

The ADP (Table 11) of the diets varied from 45.6 ±3.88 (diet6 2) to 55.083 ±0.053 (diet 3). Statistical analysis showed that there were no significant differences among the treatments (P=0.0649).

Carcass Composition

The result of the initial carcass analysis is presented in Table-12. The moisture content was 77.44 ±0.15 %, crude protein, crude fat, crude ash and phosphorus content on dry matter basis were 53.43 ±1.01%, 5.34 ±0.8%, 17.6 ±0.31% and 0.85 ± 0.07%respectively.

The carcass of the shrimps fed on the test diets was analysed after termination of the experiment. The phosphorus content (Table 13) varied from 1.18% (diet 2) to 1.25% (diet 4).

Phosphorus Retention (%)

Phosphorus retention (Table 14) was found to be superior with the diet-1 (control) that had no phosphorus supplementation.

Estimated Faecal Phosphorus Production

The estimation of faecal phosphorus per tonne of shrimp production is presented in Table-15. The control diet produced the least faecal phosphorus, as waste, while the diet with potassium phosphate monobasic produced the highest faecal phosphorus.

TABLE 9:Protein Efficiency Ratio

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Diet1	3	3.789	1.263	0.1646
Diet2	3	3.79	1.2633	0.0121
Diet3	3	4.02	1.34	0.0133
Diet4	3	3.74	1.2467	0.0016
Diet5	3	4.08	1.36	0.0507

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.0318	4	0.0080	0.1642	0.9518	3.4780
Within Groups	0.4847	10	0.0485			
Total	0.5165	14				

RESULT: NOT SIGNIFICANT

TABLE 10: APPARENT DIGESTIBILITY COEFFICIENT OF THE DIETS

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Diet1	3	273.87	91.29	4.5036
Diet2	3	275.31	91.77	9.0649
Diet3	3	280.76	93.587	0.2377
Diet4	3	273.36	91.12	16.087
Diet5	3	277.12	92.3733	1.3049

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	11.953	4	2.9883	0.4789	0.7509	3.4780
Within Groups	62.396	10	6.2396			
Total	74.349	14				

RESULT: NOT SIGNIFICANT

TABLE 11: Apparent Digestibility of Phosphorus(ADP)

SUMMARY

Groups	Count	Sum	Average	Variance
Diet 1	3	140.67	46.89	42.2397
Diet 2	3	136.81	45.6033	22.5860
Diet 3	3	165.25	55.0833	6.3250
Diet 4	3	139.77	46.59	9.8161
Diet 5	3	159.3	53.1	8.1687

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	223.7608	4	55.9402	3.1379	0.0649	3.4780
Within Groups	178.2711	10	17.8271			
Total	402.0319	14				

RESULT: NOT SIGNIFICANT

TABLE 12: Initial carcass composition of the juvenile *P. indicus.*

Constituents	Level in the body(% dry weight basis)
Moisture	77.44 ±0.15
Crude Protein	53.43 ±1.03
Crude Fat	5.34 ±0.8
C rude Ash	17.6 ±0.31
Phosphorus	0.85 ± 0.07

TABLE 13: Moisture,crude ash and phosphorus content of the final carcass(% dry weight basis)

DIETS	MOISTURE	CRUDE ASH	PHOSPHORUS
DIET 1	75.896	14.88	1.19
DIET 2	75.655	14.38	1.18
DIET 3	75.313	15.58	1.22
DIET4	76.195	14.50	1.25
DIET5	75.888	15.23	1.22

TABLE 14: Phosphorus retention (%) by *Penaeus indicus*.

Diets	Phosphorus retention (%)
Diet 1	25.54
Diet 2	16.91
Diet 3	20.16
Diet4	18.18
Diet5	21.56

TABLE 15: Estimated faecal phosphorus production (Kg P /t of shrimp production).

Diet	Phosphorus
Diet 1	9.96
Diet 2	14.48
Diet 3	12.76
Diet 4	16.1
Diet 5	12.41

DISCUSSION

Growth of shrimps and feed efficiency by and large depends on the quality of raw materials and additives, used in feeds. In the present study, ingredients of high quality were incorporated in the experimental diets to provide the essential nutrients to juveniles of *P. indicus*. Fish meal, soybean meal, squid meal are very good sources of digestible protein (Ali, 1982; Akiyama *et al.*, 1988: Paulraj, 1993). Squid meal also contains a small peptide, which increases the digestive efficiency of shrimp as well as enhances the growth rate. Squid meal is also an excellent chemo-attractant (Akiyama *et al.*, 1992) for shrimp inducing a good feeding response (Cruz-Ricque *et al.*, 1987). Squilla meal and clam meals are also very good sources of protein and have been used in practical feed formulations (Paulraj, 1993; Sridhar *et al.*, 1999). High crude protein content of squilla meal can be attributed to its high chitin (N- acetyl glucosamine) content, which contains predominantly non-protein nitrogen. The high crude protein content of the fish meal used in this study can be attributed to adequate processing to remove sand and silica particles. Soy lecithin was incorporated at 1% in the diets to satisfy the phospholipid requirement of the shrimp as its inclusion in the diet improves protein retention (Chandge, 1987). The diets were supplemented with 0.5% cholesterol to satisfy the shrimp's requirement for the synthesis of various physiologically important compounds such as steroid hormones, brain and moulting hormones and vitamin D (Kanazawa *et al.*, 1971; New, 1976; Chandge and Paulraj, 1997b). Cod liver oil and sunflower oil were incorporated in the diets as sources of n3 and n6 polyunsaturated fatty acids which are found to be essential for *P. indicus* (Read, 1981; Chandge and Paulraj, 1990, 1997a). The diets were formulated to contain a crude protein level of about 37% which satisfies the protein requirement of juvenile *Penaeus indicus* (Gopal and Paulraj, 1990). The diets were supplemented with vitamins at adequate level keeping in view the leaching loss in water. Mineral mixture was included in the diets at level suggested by Paulraj (1993) with slight modification. The experimental diets did not differ significantly in proximate composition (Table – 4).

Leaching of nutrients from feed is a problem inherent in working with an aquatic environment. Potential nutrient losses are emphasized when dealing with shrimp, which eat relatively slowly and externally masticate their feed. The observed hydrostability of

the diets up to 3 hours fall within a period when most of the pellets are consumed by the shrimps implying minimal loss of nutrients from the diets.

Excellent survival and growth of shrimps were obtained regardless of the phosphorus source in the diets. Besides, the control diet which had 0.81 % phosphorus itself was found to be adequate to sustain high survival rate, which implies that the diets provided the essential nutrients for their normal survival and growth. Phosphorus supplementation did not effect any significant improvement in survival and growth. Civera and Guillaume (1989) found that for *P. japonicus* and *P. vannamei*, a casein – gelatin based purified diet without phosphorus supplements but containing 0.56% phosphorus was adequate for good growth and survival. Davis et al. (1992) reported that 0.35 % phosphorus in the casein – gelatin based purified diet was adequate for normal growth and survival. Velasco *et al.* (1998) observed that survival of *P. vannamei* postlarvae did not differ significantly irrespective of the phosphorus source supplemented in a wheat-starch / casein based semi-purified diet. In contrast to the above observations Davis and Arnold (1998) observed that phosphorus supplementation was necessary in the practical diet containing 0.22 % available phosphorus (0.98 % total P) to improve the growth rate of *P. vannamei* juveniles. However Penaflorida (1999) reported that phosphorus supplement at various levels to casein – gelatin based purified diet (0.35% P) produced significant difference in growth but not in survival rate of *P. monodon* postlarvae.

The relatively higher digestibility of phosphorus recorded for the feed supplemented with sodium phosphate monobasic was not significantly reflected in the retention of phosphorus in the body. Though the ADP value was relatively lower for control diet (Diet-1), it produced the best response in terms of phosphorus retention (25.5%) in the shrimp implying better phosphorus assimilation and utilization by the shrimps receiving the control diet. The lowest P retention was observed with the diet supplemented with calcium phosphate dibasic. The addition of Ca in the form of $CaHPO_4$ to provide phosphorus in the diet might have reduced the bioavailability of phosphorus as Davis *et al.* (1992) observed that calcium supplementation inhibits phosphorus bioavailability in *P. vannamei*. The diet containing sodium phosphate monobasic enabled better phosphorus retention than the diet supplemented with calcium phosphate dibasic, because bioavailability of phosphorus has been shown to be positively

correlated with the solubility of the mineral in the water (Davis and Arnold, 1994). Moreover, shrimps utilize more efficiently phosphates, which dissociate at basic pH such as sodium phosphate rather than dicalcium phosphate (Cuzon *et al.*, 1994)

The carcass analysis of shrimps revealed that the phosphorus level in the body remained similar irrespective of the phosphorus supplementation and source of phosphorus. The phosphorus levels (1.18% to 1.25%) are quite comparable to the values obtained *for P. vannamei* and *P. stylirostris* (Boyd and Teichert–Coddington, 1995) and for *P. monodon* (1.07 – 1.16 %). Kanazawa *et al.* (1984) found that Ca and phosphorus levels in the tissue do not relate to dietary Ca and P. Davis *et al.* (1993) observed no clear relationship between tissue mineralization, shrimp growth and dietary phosphorus levels. The lack of correlation was assumed to be associated with variations due to moult cycle.

The present finding suggests that a diet containing good quality ingredients with sufficient available phosphorus (0.81% P) as in the control diet was adequate to promote survival, growth and phosphorus retention in the shrimp. The control diet appears to have met the phosphorus requirement of the shrimp. The calcium / phosphorus ratio in the control diet (1.5: 1) was higher than that in other diets. Ali (1988) obtained best response in *P. indicus* juveniles fed 1% total phosphorus in a fibrin-albumin based purified diet with a Ca: P ratio of 1: 2. The level of calcium in the diet has been found to influence the phosphorus requirement of shrimps. Kanazawa *et al.* (1984) opined that juvenile *P. japonicus* requires a Ca: P ratio of 1:1 in the purified diet whereas Huner and Colvin (1977) reported this to be 2.06: 1 in the diet for juvenile *P. californiensis*.

Faecal phosphorus is considered as a major contributor to pollution in shrimp culture systems. Estimates of faecal P output shows that the control diet produced the least faecal phosphorus (9.96 Kg P/t of shrimp production) among diets tested. Faecal phosphorus production was relatively high (16.1Kg P/t of shrimp production) for the diet supplemented with potassium phosphate monobasic. The difference in the faecal phosphorus production for different diets is presumably due to the variations in FCR, total P content in the diet and phosphorus retention by the shrimps. A feed with 1.34 % P and with FCR (1.9 –2.1) contributes about 38-60 % of the total phosphorus input to the system and every 0.1 % reduction in wet weight FCR has been estimated to decrease P

waste level by 2.3 % (Briggs and Funge-Smith, 1994). The ingredients used in the present study except fish meal and tapioça flour had lower P/N ratios which are desirable characteristics for formulating a low pollution diet (Cho *et al.*, 1994). The study also suggests that the diet resulting in maximum phosphorus retention in the body generates the lowest faecal phosphorus output.

Fish meal and squid meal are ingredients reported to have very high apparent phosphorus availability (Akiyama *et al.*, 1992) and it appears inclusion of these ingredients in the experimental diets has facilitated good growth in *P.indicus* juvenile when reared at 17-19 ppt salinity. It is suggested that supplementation of phosphorus in the compounded diets based on animal products may be dispensed with or done cautiously with the objective to reduce the feed cost as well as faecal phosphorus loading to the culture environment. Besides, supplementation if at all is required, sodium phosphate monobasic is recommended in the compounded diet at relatively lower level to sustain good growth in *P.indicus*.

Owing to limitations in the study such as a constant level (0.5%) of phosphorus supplementation and a single combination of phosphorus sources tested in the diets, the effect of different sources of phosphorus is not pronounced .So further research to define optimum level of phosphorus in the diets and combinations of P sources to identify correct phosphorus sources, appears warranted to formulate low- pollution diets for *P. indicus*.

SUMMARY

The efficacy of supplementation of the inorganic phosphorus sources, *viz.*, sodium phosphate monobasic, calcium phosphate dibasic, potassium phosphate monobasic and a mixture of calcium phosphate dibasic and potassium phosphate monobasic in the ratio of 1:1 was evaluated in a compounded diet of juvenile *P. indicus*. These phosphorus sources were incorporated in the compounded diet to obtain a 0.5 % P supplementation. The salient features of this experiment of 30-days period are:

1. The test diets (diet1 to diet 5) had similar moisture, crude protein, crude fat, crude fibre, nitrogen-free extract and gross energy as all the diets were prepared using the same ingredients in the same proportion.

2. The control diet without any P supplement had 0.81 % total P and Ca /P ratio of 1.5:1. The P content of the other diets varied from 1.25 % (diet-2) to 1.42% (diet – 4). The Ca: P ratio in the other diets varied from 0.85 to 1.49.

3. The P/N ratio of the diets varied from 0.136 (control diet) to 0.237(diet –3).

4. The hydrostability of the diets varied from 93.26 % to 97.24% after half-an –hour, 88.98% to 93.89% after one hour, 84.75% to 90.08% after two hours and 82.0% to 86.62% after three hours. The highest hydrostability of 86.62 % was observed for the diet supplemented with potassium phosphate monobasic.

5. The observed response parameters such as survival rate, FCR, specific growth rate (SGR), gross conversion efficiency (GCE), apparent feed digestibility and apparent phosphorus digestibility did not show any significant differences among the diets ($P>0.05$).

6. The survival rate varied from 93.33% (diet 1and 3) to 100% (diet 5). The SGR varied from 3.56 (diet 4) to 4.05 (diet 3). The FCR of the diets varied from 2.3 (diet 1) to 2.0(diet 5) .The gross conversion efficiency varied from 46.9 % to (diet1) to 50.68% (diet 5). The protein efficiency ratio varied from 1.26 (diet 1 and 2) to 1.36 (diet5).

7. The apparent digestibility coefficient of the diets varied from 91.12 (diet 4) to 93.58 (diet 3). The apparent digestibility of phosphorus varied from 45.6 % (diet 2) to 55.08%(diet3).

8. The P retention (%) was the highest (25.54 %) in the shrimp receiving the control diet and the lowest value (16.91%) was observed for the diet supplemented with calcium phosphate dibasic.

9. The faecal P production (Kg P / t of shrimp production) varied from 9.96 (control diet) to 16.1 (diet 4).

10. The present study suggests that when quality ingredients, especially of animal origin are used P supplementation is unnecessary. However, if supplementation is considered essential sodium phosphate monobasic is recommended as a source for *P. indicus*.

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