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# Calcium, Phosphorus and Magnesium Requirements in the Diet of Shrimp *Penaeus indicus*

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## Abstract

Calcium, phosphorus, and magnesium requirements in the diet of Indian white shrimp *Penaeus indicus* were studied using semi-purified diets. During a 45 day feeding trial, shrimps with an initial average length of 25 mm and weight of 0. 15 g, showed sig-nificantly higher growth ($p< 0.01$) and low FCR ($p< 0.05$) when fed with diet containing 0.53% of calcium and 1.05% phosphorus. Higher levels of dietary calcium supressed growth and increased FCR, though survival was unaffected. Shrimps fed with a phosphorus defi-cient diet were sluggish and weak and levels of phosphorus exceeding 1.05% showed no beneficial effect. The body calcium of shrimps fed with diets containing different levels of calcium was lower than the initial value and decreased as dietary levels of calcium in-creased. The body phosphorus of shrimps on the other hand, increased as levels of dietary phosphorus was increased. A dietary Ca-P ratio of 1: 1.98 gave the best growth and FCR for *P. indicus*, while the Ca-P ratio in shrimps showed a decreasing trend with dietary cal-cium.

Addition of magnesium to the diet from 0.1 to 0.7% also supressed growth and in-creased FCR. The total ash content of shrimps decreased as levels of magnesium was in-creased; however, the magnesium level of shrimps remained practically constant. Results suggest that the magnesium requirement may be satisfied through absorption from the water.

## Introduction

Minerals are essential in shrimp nutrition. Aside from playing important role in osmotic regulation and moulting (Vijayan and Diwan 1996), mineral ions are also components of many biological compounds such as enzymes, hormones and high energy compounds. The evaluation of dietary requirements of minerals for marine animals including shrimp is particularly difficult because sea water is rich in mineral ions which can be absorbed (Gilles and Piquex 1983). Nevertheless, dietary requirements of important mineral elements are known for selected species of shrimp such as *Penaeus japonicus* (Deshimaru and Yone 1978 ; Kanazawa 1985) *P. aztecus* (Hysmith et al. 1972 ; Sick et al.

1972) and *P. vannamei* (Davis and Lawrence 1993). In the present study the dietary requirements of calcium, phosphorus and magnesium were investigated for the Indian white shrimp *Penaeus indicus* which is widely cultured in India.

## Materials and Methods

### *Formulation and preparation of diets*

Semi-purified diets were formulated using a mixture of fibrin (blood) and albumen (egg) in a 4:1 ratio, cod liver oil and a mixture of maltose, sucrose and starch (1:1:1) as a basal diet (Table 1). Calcium carbonate, potassium dihydrogen orthophosphate, and magnesium sulphate were used as the sources of the respective dietary minerals. Purified (chemical grade-LOBA) cellulose was used as a no-nutrient filler. In diets C1 to C7, different levels of calcium were evaluated, while keeping the level of phosphorus constant. After the calcium requirement was determined, diets P1 to P7 containing different levels of phosphorus were also evaluated. Response to dietary magnesium was evaluated using seven dietary levels (diets M1 to M7) keeping the calcium and phosphorus levels constant. The composition of the diets is presented in Table 2.

The solid ingredients were first finely ground and passed through a 300 micron sieve. The binder, sodium alginate, was dissolved in hot water ( 40 ml for 100 g of dry mixture) and added to the dry diet mixture. After homogenization, the diet was steamed for five minutes at atmospheric pressure and made into pellets of 3 mm in diameter using a hand pelletizer. The pellets were dried at 70 °C and manually ground into granules, approximately 1 mm.

### *Biological evaluation of diets*

The experiments were conducted using hatchery reared (from a single brood) *P. indicus* shrimps with an average length of 25 mm and live weight of 0.15 g. The shrimps were stocked in circular tanks containing 10 L of saline water. Eight shrimps were stocked in each tank with three replicates for each treatment. The shrimps were initially fed at a rate of 20% of their body weight and later adjusted based on the left over diet. The diet was offered twice daily. The tanks were cleaned by removing sediments and totally replacing the water daily. Aeration was provided in all experimental tanks. The duration of the entire feeding trial was 45 days. Salinity, temperature, pH, and dissolved oxygen of water used in the experiments were 21.2 ± 2.0 ppt, 28.4 ± 1 °C, 8.3 ± 0.2 and 4.2 ± 0.5 mg $L^{-1}$ respectively.

### *Analysis of samples*

At the end of the experiment, shrimps from each tank were pooled, dried in oven at 55 °C, and powdered for analysis. Calcium and magnesium in diets and animals were estimated using a Perkin Elmer model 2380 Atomic Absorp-

Table 1: Ingredient composition (%) of basal diet mix.

| Ingredient | % |
|---|---|
| Protein mixture* | 38.0 |
| Cod liver oil | 8.0 |
| Carbohydrate mixture** | 36.0 |
| Glucosamine HCl | 0.8 |
| Cholesterol | 0.5 |
| Vitamin mixture[1] | 2.7 |
| Binder (sodium alginate) | 3.0 |
| | 89.0 g |
| Crude protein (on analysis) of basal diet % | 39.55 |
| Calcium % | 0.027 |
| Phosphorus % | 0.242 |
| Magnesium % | Trace |

*Protein mixture : Fibrin (blood) (Sigma) 30.5 g and albumin (egg) (BDH) 7.5g.
**Carbohydrate mixture : Maltose, sucrose and starch in the ratio 1:1:1.
[1]Vitamin mixture : Ascorbic acid 2.0 g, choline chloride 0.12g Cyanocobalamine 0.08 mcg, folic acid 0.08g, nicotinic acid 0.04g, calcium pantothenate 0.06g, para-aminobenzoic acid 0.01g, pyridoxine 0.012g, riboflavin 0.008g, thiamine hydrochloride 0.004g, biotin 0.04mcg B-carotene 0.0096g, calciferol 0.0012g, inositol 0.2g, menadione 0.004g and α - tocopherol 0.02g.

Table 2. Composition of test diets.

| Diet | Basal diet mix | Calcium carbonate | Potassium dihydro. orthophosphate | Magnesium sulphate | Cellulose | g/100g diet | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Ca | P | Mg |
| C1 | 89.0 | 0.0 | 4.0 | - | 7.0 | 0.024 | 1.0 | - |
| C2 | 89.0 | 1.3 | 4.0 | - | 5.7 | 0.525 | 1.0 | - |
| C3 | 89.0 | 2.0 | 4.0 | - | 5.0 | 0.774 | 1.0 | - |
| C4 | 89.0 | 2.7 | 4.0 | - | 4.3 | 1.025 | 1.0 | - |
| C5 | 89.0 | 3.3 | 4.0 | - | 3.7 | 1.275 | 1.0 | - |
| C6 | 89.0 | 3.8 | 4.0 | - | 3.2 | 1.525 | 1.0 | - |
| C7 | 89.0 | 5.0 | 4.0 | - | 2.0 | 2.024 | 1.0 | |
| P1 | 89.0 | 1.3 | 0.0 | - | 9.7 | 0.53 | 0.215 | - |
| P2 | 89.0 | 1.3 | 2.2 | - | 7.5 | 0.53 | 0.647 | - |
| P3 | 89.0 | 1.3 | 3.3 | - | 6.4 | 0.53 | 0.848 | - |
| P4 | 89.0 | 1.3 | 4.4 | - | 5.3 | 0.53 | 1.049 | - |
| P5 | 89.0 | 1.3 | 5.5 | - | 4.2 | 0.53 | 1.181 | - |
| P6 | 89.0 | 1.3 | 6.6 | - | 3.1 | 0.53 | 1.313 | - |
| P7 | 89.0 | 1.3 | 8.8 | - | 0.9 | 0.53 | 1.830 | - |
| M1 | 89.0 | 1.3 | 4.4 | 0.0 | 8.3 | 0.53 | 1.05 | trace |
| M2 | 89.0 | 1.3 | 4.4 | 1.0 | 7.3 | 0.53 | 1.05 | 0.099 |
| M3 | 89.0 | 1.3 | 4.4 | 2.5 | 4.8 | 0.53 | 1.05 | 0.198 |
| M4 | 89.0 | 1.3 | 4.4 | 3.0 | 4.3 | 0.53 | 1.05 | 0.298 |
| M5 | 89.0 | 1.3 | 4.4 | 4.5 | 2.8 | 0.53 | 1.05 | 0.398 |
| M6 | 89.0 | 1.3 | 4.4 | 5.0 | 2.3 | 0.53 | 1.05 | 0.497 |
| M7 | 89.0 | 1.3 | 4.4 | 7.0 | 0.3 | 0.53 | 1.05 | 0.696 |

tion Spectrophotometer. Phosphorus was analysed through the (AOAC 1965) spectrophotometric method using molybdo-vandate reagent. Crude protein and ash were also estimated using AOAC (1965) methods.

Growth and FCR were subjected to analysis of variance and the means were compared using the Least Significant Difference (LSD) method.

## Results

The results of the feeding trials are summarized in Table 3. Diet C2 having 0.525% of calcium produced significantly ($p < 0.01$) higher growth and best FCR ($p < 0.05$). Higher levels of dietary calcium resulted in a decrease in growth and increase in FCR. Survival rate of shrimps however, did not show any change. Body calcium levels and calcium-phosphorus (Ca-P) ratio also showed decreasing trend (Table 4) with an increase in dietary calcium. The body ash content at the end of the feeding trial was higher (18.26 - 21.31%) than the initial value (16.15%) and showed variation (Table 4).

The growth of shrimps fed with diet P1, devoid of added phosphorus (P) was significantly ($p < 0.05$) less than that of shrimps fed with all other diets. Shrimps in this treatment became sluggish and weak. Addition of phosphorus to diet greatly improved their growth. Shrimps fed with diet 1.05% P registered higher growth (Table 3) and low FCR. Further increase in dietary P had no beneficial effect, rather it supressed growth. The phosphorus content in shrimps showed an increasing trend (Table 4) with dietary levels of P. However, the Ca-P ratio declined with an increase in dietary P. The total body ash content (20.52%) was nearly 4% more on the average after feeding shrimps with phosphorus diets than the initial value (16.5%).

Shrimps fed with the control diet with no added magnesium (Mg) recorded higher growth (Table 3) and low FCR. Addition of Mg in the diet supressed growth and increased FCR. The survival of shrimp was also largely unaffected. The total body ash content decreased (Table 4). However, the body Mg levels practically remained the same regardless of dietary Mg levels.

## Discussion

### *Calcium*

Calcium is an essential mineral element for shrimp not only because it constitutes a major proportion of shell (exoskeleton) in the form of calcium carbonate (Venkataramaiah et al. 1978), but it is also involved in osmoregulation (Shewbart et al. 1973). Shrimps fed with calcium deficient diet in the present study, showed no reduction in body calcium. Obviously this could be because the shrimp might be extracting calcium from water (rearing medium). Using radioisotope labelled calcium, Deshimaru et al. (1978) demonstrated that *P. japonicus* extracted Ca from water. Similar observations were made in *P. indicus* by Rao et al. (1982) and in other penaeid shrimps by Dall (1981). It is

Table 3. Results of feeding trial with test diets on *P. indicus* for 45 days.

| Diet | Increase in length (mm) | Increase in weight (g) | FCR | Survival (%) |
|---|---|---|---|---|
| C1 | 12.6 | $0.1315^{bc}$ | $5.13^{ab}$ | 75.0 |
| C2 | 16.1 | $0.1915^{a}$ | $3.89^{a}$ | 75.0 |
| C3 | 15.9 | $0.1640^{ab}$ | $4.95^{ab}$ | 79.2 |
| C4 | 15.6 | $0.1365^{bc}$ | $5.69^{bc}$ | 79.0 |
| C5 | 13.7 | $0.1286^{bc}$ | $6.79^{cd}$ | 83.0 |
| C6 | 11.6 | $0.1055^{cd}$ | $6.88^{cd}$ | 87.5 |
| C7 | 10.5 | $0.1145^{bc}$ | $4.85^{ab}$ | 83.3 |
| | $(p>0.05)$ | $(p<0.01)$ | $(p<0.05)$ | |
| P1 | $2.70^{b}$ | $0.0055^{b}$ | - | 70.8 |
| P2 | $12.17^{a}$ | $0.1355^{a}$ | 5.16 | 91.6 |
| P3 | $13.00^{a}$ | $0.1585^{a}$ | 5.08 | 83.0 |
| P4 | $14.30^{a}$ | $0.1775^{a}$ | 4.78 | 75.0 |
| P5 | $12.50^{a}$ | $0.1626^{a}$ | 5.52 | 80.0 |
| P6 | $12.00^{a}$ | $0.1475^{a}$ | 6.02 | 87.5 |
| P7 | $14.40^{a}$ | $0.1565^{a}$ | 5.24 | 91.6 |
| | $(p<0.05)$ | $(p<0.05)$ | $(p>0.05)$ | |
| M1 | 10.90 | 0.1465 | 5.29 | 62.5 |
| M2 | 8.00 | 0.1100 | 7.56 | 56.3 |
| M3 | 7.90 | 0.1205 | 6.50 | 58.3 |
| M4 | 6.20 | 0.1230 | 8.50 | 56.3 |
| M5 | 7.40 | 0.1250 | 7.88 | 60.1 |
| M6 | 7.90 | 0.1130 | 7.33 | 56.3 |
| M7 | 8.40 | 0.1460 | 5.82 | 68.8 |
| | NS | NS | NS | |
| | $(p>0.05)$ | $(p>0.05)$ | $(p>0.05)$ | |

Note:
Values with same superscript do not differ significantly; NS = Not significant.

Table 4. Ash and mineral composition of *P. indicus* fed with test diets.

| Diet No. | Total ash % | Calcium % | Phosphorus % | Ca/P | Magnesium % |
|---|---|---|---|---|---|
| C1 | 18.77 | 3.66 | 1.53 | 2.39 | - |
| C2 | 20.61 | 3.48 | 1.85 | 1.88 | - |
| C3 | 19.50 | 3.91 | 1.89 | 2.07 | - |
| C4 | 19.42 | 3.71 | 1.94 | 1.91 | - |
| C5 | 18.50 | 3.62 | 1.91 | 1.90 | - |
| C6 | 18.26 | 3.53 | 1.87 | 1.89 | - |
| C7 | 21.31 | 3.00 | 1.42 | 2.11 | - |
| P1 | 20.65 | 4.40 | 1.08 | 4.07 | - |
| P2 | 19.60 | 4.08 | 1.39 | 2.94 | - |
| P3 | 20.20 | 3.50 | 1.35 | 2.59 | - |
| P4 | 22.20 | 3.84 | 1.44 | 2.67 | - |
| P5 | 20.50 | 3.70 | 1.37 | 2.70 | - |
| P6 | 19.00 | 3.60 | 1.47 | 2.45 | - |
| P7 | 20.00 | 4.33 | 1.49 | 2.91 | - |
| M1 | 19.50 | - | - | - | 0.38 |
| M2 | 19.50 | - | - | - | 0.39 |
| M3 | 19.00 | - | - | - | 0.39 |
| M4 | 17.40 | - | - | - | 0.40 |
| M5 | 15.94 | - | - | - | 0.39 |
| M6 | 15.00 | - | - | - | 0.38 |
| M7 | 15.00 | - | - | - | 0.40 |

well known that during premoulting the Ca concentration in the blood increases to a relatively high level (Vijayan and Diwan 1996). The large amount of Ca resorbed from old exoskeleton accounts for most of this "excess" Ca in the blood. Heavy deposition of Ca takes place in the new exoskeleton during the postmoulting period (Passano 1960 ; Robertson 1960). The Ca level in the blood is rapidly restored to its intermoulting level using a regulatory mechanism which includes absorption of Ca from sea water.

The shrimp *Penaeus indicus* has shown positive response in growth and FCR at a dietary level of 0.53% of calcium in the presence of 1.05% of phosphorus, which is significantly higher than the growth and FCR shown by the diet not supplemented with Ca. This Ca level in the diet of *P. indicus* is lower than the requirement shown by *P. japonicus* for dietary calcium (Kitabhayashi, et al. 1971; Kanazawa 1984). Davis and Lawrence (1993) reported that *Penaeus vannamei* grew well on a diet without Ca supplementation at 0.35% phosphorus and higher levels of Ca in diet supressed growth. However, the authors concluded that Ca-P ratio has a bearing on the growth and nutritive value of these minerals. In spite of the fact that shrimps are capable of absorbing Ca from water, shrimps seem to respond to dietary Ca for improving growth and FCR as shown by the results of the present study as well as the similar observations made in other penaeid species. Depending upon the availability of Ca in water, shrimps may show varying levels of dietary requirement for this element. The saline water in which the feeding experiment was conducted had a Ca level of 278 to 300 mg per litre. The ratio of Ca and P in the diet also has a bearing on the body Ca-P ratio. It decreased with dietary Ca levels and reached a minimum level at 1.5:1 Ca-P ratio (Table 4) and increased thereafter. Kitabhayashi et al. (1971) reported better growth and FCR in *P. japonicus* when the diet contained a Ca-P ratio of 1.2:1 while growth was supressed when the Ca-P ratio in the diet was increased to 2:1.

### *Phosphorus*

The results of the present study showed that dietary phosphorus is essential for the growth of *P. indicus*. The saline water in which the experiment was conducted had a P content of 0.05 ppm. While shrimps fed with phosphorus deficient diet showed poor growth and sluggishness, addition of phosphorus to diet improved growth and FCR. The shrimps have shown higher growth and better FCR at a dietary phosphorus level of 1.05%. These results are similar to those shown by *P. japonicus* (Kitabhayashi et al. 1971; Kanazawa et al. 1984). However a lower P requirement of 0.51 was reported for *P. aztecus* by Sick et al., (1972). In the present study, since potassium dihydrogen orthophosphate was used as the source of P, the potassium requirement of the diet might have also been met.

Apart from the individual requirement, phosphorus appears to be linked with Ca, and their dietary ratio plays an important role in shrimp nutrition (Huner and Colvin 1979). Davis and Lawrence (1993) had shown that phosphorus is essential in the diet of *P. vannamei*. The requirement varied from 0.5 to 2.0% according to the levels of Ca in the diet. The authors also emphasized that Ca-P ratio influences the response of shrimps to dietary levels of these mineral elements. As discussed ealier, a dietary Ca-P ratio of 1.2:1 was found to produce the best results in *P. japonicus* (Kitabhayashi et al 1971).

However, Deshimaru and Kuroki (1974) observed that 1:1.3 was the best Ca-P ratio in the diet of *P. japonicus*. They also observed that further change of the ratio to 2:1 resulted in poor growth and FCR. For *P. indicus* the best Ca-P ratio in the diet is 1:1.98, which is different from what is reported for *P. japonicus* and *P. aztecus* (Hysmith et al. 1972). Gallaghar et al. (1978) while studying Ca and P needs for lobster, *Homarus americanus* with different Ca-P ratios in diet obtained best results in diet having 0.56% of Ca and 1.10% of P. The Ca-P ratio in this case was 1:2 which is similar to what is found for *P. indicus* in the present study. The Ca-P ratio in the body of *P. indicus* varied from 1.47:1 to 4.08:1 (Table 4). Similar observations were made in the shrimp *Penaeus californiensis* by Huner and Colvin (1979).

***Magnesium***

Addition of dietary magnesium through magnesium sulphate did not improve growth and FCR in *P. indicus*. Similar observations were made by Deshimaru and Yone (1978) in *P. japonicus*. In contrast, Kanazawa et al. (1984) reported that 0.3% of magnesium is required in the diet of the same shrimp.

Discussing osmotic and ionic regulations in crustacea, Robertson (1960) indicated that almost all the crustaceans are capable of exchanging magnesium ions from saline water. Chemical proof of such absorption was established with the help of intermoulting specimens. Subhash Chander (1986) observed that penaeid shrimps are capable of absorbing $Mg^{++}$ from water. Body Mg of *P. indicus* in the present study fed with different levels of Mg showed little variation (Table 4). In addition, the Mg content (382.17 mg%) of shrimps fed with no additional Mg was appreciably high compared to the value (206.12 mg%) present before the start of the feeding trial. Recently Vijayan and Diwan (1996) observed a rapid increase in Mg ion concentration in the hemolymph of *P. indicus* during moulting. The saltwater used for rearing the experimental animals in the present study contained 10.5 ppm of Mg. Shrimps have the ability to absorb magnesium ions from the surrounding water and it appears that *P. indicus* can meet the requirement by absorbing it from the rearing medium.

## Conclusions

The shrimp, *Penaeus indicus* showed a positive response in growth and FCR when fed with diet having 0.53% calcium. Phosphorus is essential in the diet and its dietary requirement is 1.05%. A dietary Ca-P ratio of 1:1.98 gave the best growth and FCR. Results also showed that magnesium requirement may be satisfied through absorption from water. Addition of magnesium sulphate to diet supressed growth.

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