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Effect of Dietary Phosphorus on Growth and Its Excretion in Tiger Shrimp, *Penaeus monodon*

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Abstract

Six semi-purified diets were formulated with supplemented phosphorus levels of 0, 0.5, 1.0, 1.5, 2.0 and 2.5% at a fixed calcium level of 1.25% and the effect of dietary phosphorus on growth, FCR, body phosphorus and its excretion in shrimp *Penaeus monodon* was studied. The results of a 45 day feeding trial, in triplicate, on the juveniles of *P. monodon* (initial average live –weight 2.54 ±0.28g) had shown that phosphorus is indispensable in the diet and the best performance (147.3 and 154.5% increase in live-weight and 1.44 and 1.57 FCR) was recorded by the diet supplemented with 1.0 and 1.5% phosphorus which was significantly ($P<0.05$) higher than the performance of the other diets tested. Higher phosphorus supplementation suppressed growth and increased FCR. The shrimp maintained body phosphorus and calcium levels in a ratio of 1:3 irrespective of dietary levels of phosphorus. There was no significant change in the proximate composition of post feeding trial shrimp. Phosphorus excretion in faeces of *P. monodon* increased proportionately with dietary phosphorus while the excretion level far exceeded the body phosphorus level in the shrimp fed with diets having supplemented phosphorus level above 1.5%. Even the shrimp fed with 0% phosphorus diet had 0.10% phosphorus in their faeces. The phosphorus levels in shrimp feeds vis-a-vis its excretion into aquaculture environment have been discussed.

Introduction

Crustaceans including shrimp have relatively high content of ash (15.9%) in their body (Sze 1973). Minerals have significant importance in shrimp nutrition. It is however complicated by the fact that some of the mineral ions are involved in osmoregulation and since sea water is rich in many mineral ions, they are capable of extracting most of the minerals (Gilles and Pequeux 1983) from the surrounding environment. Calcium and phosphorus are the major minerals found in shrimp. Unlike calcium, phosphorus is generally found at low concentration in natural water (Boyd 1981). Diet is the main source of phosphorus, which necessitates to determine the dietary needs of the element for shrimp (Ambasankar and Ahamad Ali 2002). While inadequate levels of phosphorus in shrimp diet may cause its deficiency, excess phosphorus in feeds may lead to loading of the element in the aquaculture environment. Therefore an attempt has been made in the present study to determine the optimal dietary requirement of phosphorus for *Penaeus monodon* and its effect on excretion in the faeces, with the objective of fixing a precise level of dietary phosphorus and making the shrimp diets more environment friendly.

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Materials and Methods

Preparation of test diets

Six semi-purified diets consisting of casein (SIGMA), albumen (egg) (BDH), fish oil from sardines, cereal flour and other additives were formulated containing 0, 0.5, 1.0, 1.5, 2.0 and 2.5% of supplemented phosphorus using monosodium dihydrogen orthophosphate as the source and availability of phosphorus from this source is assumed to be 100 % to shrimps. Ingredient and proximate composition of test diets are presented in Table 1.

Table 1. Ingredient and proximate composition ($g \cdot 100g^{-1}$) of test diets 1 to 6

	Diet No					
	1	2	3	4	5	6
Ingredient						
Casein	45	45	45	45	45	45
Albumen (egg)	5	5	5	5	5	5
Bread flour	20	20	20	20	20	20
Fish oil	6	6	6	6	6	6
Lecithin	2	2	2	2	2	2
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin mixture*	2	2	2	2	2	2
Mineral mixture**	6	6	6	6	6	6
Cellulose	11.5	9.3	7.05	4.83	2.6	0.37
Guar gum	2	2	2	2	2	2
Monosodium dihydrogen orthophosphate	0.0	2.2	4.45	6.67	8.9	11.13
Total	100	100	100	100	100	100
Proximate composition:						
Moisture	6.80	6.75	6.90	7.11	7.32	7.50
Crude protein	40.46	40.89	41.47	40.64	40.34	41.19
Crude fibre	8.77	6.18	3.92	2.75	2.48	0.60
Ether extract	9.43	9.58	9.60	9.80	9.73	10.61
Total ash	6.86	7.74	8.62	10.04	11.38	12.63
NFE	27.68	28.86	29.49	29.66	28.75	27.47
Phosphorus	0.33	0.89	1.32	1.60	2.01	2.53
Calcium	1.23	1.27	1.25	1.27	1.23	1.22
Ca:P ratio	3.73:1	1.43:1	0.95:1	0.79:1	0.61:1	0.48:1

*Vitamin mix ($mg \cdot 100g^{-1}$) Vitamin A 2.0, Vitamin D 0.4, Vitamin E 12.0, Vitamin K 6.0, Choline chloride 600.0, Thiamine 18.0, Riboflavin 24.0, Pyridoxine 18.0, Niacin 108.0, Pantothenic acid 72.0, Biotin 0.2, Folic acid 3.0, Vitamin B_{12} 0.015, Inositol 150.0, Vitamin C 900.0

**Mineral mix ($g \cdot kg^{-1}$) $CaCO_3$ 28.0,$K_2SO_4$10.0 , $MgSO_4$ 12.5, $CuSO_4$ 0.2, $FeCl_3$ 0.5, $MnSO_4$0.5, KI 0.01; $ZnSO_4$ 1.0,$CoSO_4$ 0.01, Cr_2SO_4 0.05, Bread flour 7.14).

All the solid ingredients were powdered in an electrical grinder passing through 0.5mm sieve and mixed together along with additives. The diet mix was then made into soft dough by adding water (40ml for 100-g diet mix). The dough was steamed (at atmospheric pressure) for 5 min, cooled and pelletized by a hand pelletizer using 2.0 mm diameter die. The diet was dried at 40^0C to a moisture content of 6-8%. Diets were crumbled and prepared into approximately 0.5 to 1 mm size granules and stored in desiccators until used.

Proximate composition, calcium and phosphorus in diet animals, and faeces were determined following standard AOAC (1990) methods.

Feeding trial

Hatchery reared juveniles of *P. monodon* with a mean initial weight ± standard deviation of 2.54±0.28 g were randomly distributed in 18 tanks containing 100 L seawater with 15 animals per tank. There were three replicates for each treatment. Shrimps were fed at the rate of 5% of their body weight in two equally divided doses. Uneaten feed materials were collected after three hours of feeding and dried in an oven until constant weight. Actual feed intake by shrimp was calculated by subtracting the uneaten feed from the feed offered. Total water was replaced daily in each tank with a fresh batch of water. The salinity of water used in the experiments ranged from 22-25‰, pH was 7.6-8.0, temperature was 25- 28°C and phosphorus was 0.03%. Weight of shrimp was recorded fortnightly to adjust the quantity of feed. Ten days after the start of the experiment, faeces collection was commenced from all the tanks. For this purpose the shrimps were fed in the morning. After one hour, the left- over feed was removed and the bottom of the tank was cleaned. After 2 hours the faeces were siphoned out to a bolting cloth, washed gently with distilled water, collected and dried at 40° C. The faeces, collected over a period of 20 days were pooled for analysis. At the end of 45 days the animals were weighed and sacrificed for analysis.

Statistical analysis

The feeding experiment was conducted following a completely randomized design. The data obtained in this experiment were homogenous and the assumptions of ANOVA viz., normality and homogeneity of variance were satisfied. The data were subjected to Analysis of Variance and the means of treatments were compared using Duncan's multiple range test (Gomez and Gomez 1984).

Results

The total phosphorus intake by the experimental shrimps was calculated to be 17.89, 50.73, 67.52, 82.00, 102.50 and 129.64 mg respectively among the shrimp fed with the six test diets and this works out to be 0.39, 1.27, 1.56, 1.82, 2.27 and 2.88 mg of phosphorus intake per animal per day. The results of the feeding trial had shown (Table 2) that the diets with 0 and 0.5% supplementary phosphorus led to lower growth and high FCR in *Penaeus monodon*. Higher weight gain and the lowest FCR were recorded by the diets supplemented with 1.0 % and 1.5% phosphorus. Interestingly, a further increase in supplementary phosphorus had no beneficial effect, the growth and FCR showing declining trend.

Table 2. Weight gain, FCR and survival of *P. monodon* fed diets 1-6 containing different phosphorus levels (Mean ± SEM)

Diet No	Weight gain %	FCR	Survival %
1	$104.5^b \pm 3.21$	$1.82^c \pm 0.01$	87.5 ±1.3
2	$115.7^b \pm 3.56$	$1.75^{bc} \pm 0.04$	81.3 ±2.1
3	$147.3^a \pm 3.31$	$1.44^a \pm 0.02$	85.0 ±1.4
4	$154.5^a \pm 4.06$	$1.57^{ab} \pm 0.03$	87.5 ±1.2
5	$122.3^b \pm 2.91$	$1.78^c \pm 0.03$	87.5 ±1.1
6	$117.8^b \pm 2.73$	$1.77^c \pm 0.04$	81.3 ±1.5

Values bearing same superscript in the same column do not differ significantly (p <0.05)

The proximate composition of whole body of post feeding trial shrimp showed (Table 3) no significant variation except that total ash content showed marginal decrease over the initial value. Body calcium levels are however unaffected. Although the dietary calcium and phosphorus ratio varied due to increase in dietary phosphorus levels, the shrimp maintained Ca:P ratio approximately at 3:1 irrespective of phosphorus level in the diet (Fig. 1). Body phosphorus levels of shrimp fed with different diets showed marginal increase. Phosphorus excreted by the shrimp was proportional to its level in the diet showing a linear relation with dietary phosphorus (Fig. 2). The shrimp fed with zero supplemental phosphorus also excreted small amount of the element in their faeces. The faecal phosphorus is lower than the body phosphorus level up to a dietary phosphorus of

0.5% and it is almost equal at 1 and 1.5%. At higher dietary phosphorus levels, excretion in the faeces far exceeded the body phosphorus levels.

Table 3. Proximate composition, Calcium and Phosphorus levels in whole body of juvenile *P. monodon* fed diets (1-6) with different phosphorus levels.

Diet No	Moisture	Crude protein	Ether extract	Crude fiber	Total ash	NFE	Ca	P
1	75.8	62.25	8.48	3.91	16.53	8.83	2.69	0.91
2	75.8	63.72	8.85	4.11	15.45	7.87	2.71	0.94
3	76.1	63.86	8.90	4.05	15.80	7.39	2.73	0.90
4	75.1	64.41	9.05	4.12	17.18	5.24	2.85	1.01
5	76.1	62.31	9.65	4.16	16.72	7.16	2.82	0.95
6	76.1	62.23	9.50	3.98	15.83	8.46	2.82	0.94
Initial sample	75.4	61.96	8.75	4.08	17.21	8.00	2.69	0.89

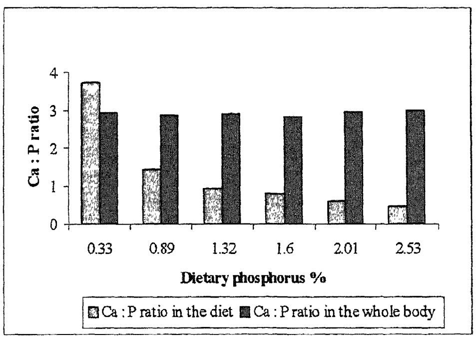

Fig. 1. Effect of dietary phosphorous on body Ca: P ratio in *P. monodon*

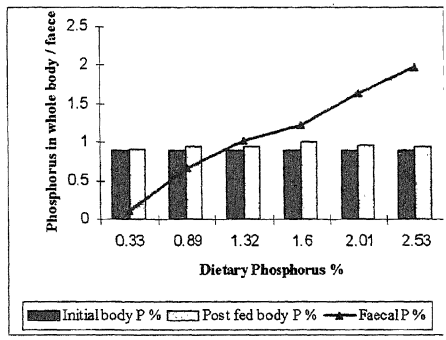

Fig. 2. Effect of dietary phosphorus on body an faecal phosphorus in *P. monodon*

Discussion

The results of the present study demonstrated the dietary essentiality of phosphorus for the shrimp *Penaeus monodon* as shown by many penaeid shrimp. However the growth of shrimp observed in this study seems to be on the lower side. This reduced growth may be due to the use of semi-purified diet and the static culture conditions. Such reduced growth in shrimp fed with semi-purified diets was observed in *P. indicus* (Ambasankar and Ahamad Ali 2002). The dietary requirement of 1.0% P shown by *P. monodon* in the present study is also in agreement with the P requirements shown by *P. japonicus* (Kitabhayashi et al. 1971; Kanazawa et al. 1984), *P. vannamei* (Davis et al. 1993), *P. indicus* (Ambasankar and Ahamad Ali 2002) and *P. monodon* (Penaflorida 1999). However *P. aztecus* had shown a lower requirement of 0.5% phosphorus in its diet (Sick et al. 1972). Diets supplemented with 1.0 and 1.5% P resulted in best growth and FCR and the optimal phosphorus intake was 1.56 and 1.82 mg per animal per day. The growth and FCR realized by these two diets are not significantly different from each other showing that shrimp can meet their phosphorus requirement from 1.0-1.5% for best performance

The dietary calcium: phosphorus ratio (Ca: P) varied from 1: 0.3 to 1: 2.1 in the present study. However, it is interesting to note that *P. monodon* fed with these diets showed a remarkable consistency in keeping body Ca: P ratio in the range of 3:1, irrespective of dietary Ca: P ratio. Similar observations were made in *P. monodon* by Penaflorida (1999). The importance of Ca: P ratio in shrimp diets has been emphasized by Kitabhayashi et al. (1971);

Deshimaru and Kuroki (1974); Hysimith et al. (1972);Huner and Colvin (1977). Diet with 1.0 and 1.5% phosphorus which gave the best results in *P. monodon* in the present study had a Ca:P ratio of 1:1 and 1:1.3 and is in agreement with the findings with the other species of penaeid shrimp. The results of the present study have also highlighted the fact that shrimp maintain a fairly constant body Ca: P ratio either by utilising the dietary calcium or by extracting it from the surrounding water (rearing medium) as suggested by many earlier investigations (Deshimaru et al. 1978; Rao et al. 1982; Dall 1981; Ahamad Ali 1999) in different penaeid shrimp species. Shrimp also seem to regulate the body phosphorus levels for maintaining the Ca: P ratio.

The dietary phosphorus had a significant effect on its excretion in *P. monodon* . The shrimp fed with zero phosphorus supplemented diet also showed 0.10% faecal phosphorus. This might be due to the constant metabolic functions of the high-energy phosphorus compounds such as ATP, ADP, AMP and cyclic AMP. The excretion of phosphorus in *P. monodon* showed linear relationship with dietary phosphorus. At lower dietary levels the percentage of phosphorus in faeces was lower than that of the whole body phosphorus levels. However, when the dietary phosphorus level exceeded the optimum required level, the faecal phosphorus level far exceeded the body phosphorus level in *P. monodon*. The excess phosphorus seems to be totally excreted out. This has an important bearing on the phosphorus levels in the practical feeds used in shrimp farming. Two factors need attention, first is the phosphorus level in feeds in excess of the optimum requirement by shrimp and second is the availability of feed phosphorus to shrimp. Phosphorus levels in the feed can be regulated through mineral mixture by taking into account the phosphorus already present in the feed ingredients used. The source of phosphorus used in the mineral mixture should be one, that makes the phosphorus available to shrimp. However, feed formulations, that contain plant ingredients having phytic acid, especially oil cakes and cereal byproducts render the feed phosphorus unavailable to shrimp (Davis et al. 1993). Such dietary phosphorus is likely to be totally excreted by shrimp. There is a need to make the feed-phosphorus available to shrimp. Phosphorus is one of the mineral elements excreted into shrimp aquaculture system. Excess levels of phosphorus released into aquaculture effluent may lead to eutrophication causing water management problems. The present study indicated that regulation of phosphorus levels in practical shrimp feeds is essential in order to make them environment friendly.

Conclusion

Juveniles of *P.monodon* have shown a dietary phosphorus requirement of 1.0 % for better growth and good FCR. Excretion of phosphorus in faeces increases linearly with dietary phosphorus and hence there is a need to regulate phosphorus levels in shrimp feeds.

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