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Dietary supplementation of microbial phytase improves growth and protein efficiency ratio of freshwater prawn (*Macrobrachium rosenbergii*)

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Abstract Phytase is a phosphatase enzyme involved in catalyzing the hydrolysis of phytic acid, which is abundant in plant-derived ingredients that are known to be best alternatives for expensive and scarce fish meal in aquafeeds. In this study, the effect of dietary supplementation of microbial phytase at 250, 500, and 750 FTU/Kg on growth, survival and body composition of freshwater prawn (*Macrobrachium rosenbergii*) was investigated. The growth and protein efficiency ratio in phytase-incorporated diet-fed prawns were significantly increased when compared to the phytase-free diet-fed prawns. The diet supplemented with 250 FTU microbial phytase-fed prawns showed best growth of 50.84 g in terms of mean weight, compared with the phytase-free diet-fed prawns showing 27.88 g in 90 days trial. In addition, a highest protein efficiency ratio of 2.12 was found in 250 FTU phytase-incorporated-diet-fed prawns, compared with 1.53 in phytase-free diet-fed prawns. Furthermore, there was no significant difference in the survival of prawns fed with phytase-supplemented and phytase-free diets. These results indicate that the dietary supplementation of microbial phytase in prawn diet would be beneficial in better utilization of plant-derived feed ingredients, which are of most promising alternatives to expensive and scarce fish meal in aqua feed formulation.

Keywords Dietary supplementation · Feed utilization · Freshwater prawn · Growth performance · Protein efficiency ratio · Phytase

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Introduction

Aquaculture, a promising industry to face the challenges of food security and economy, relies heavily on the use of fish meal that has balanced amino acid, and fatty acid profile is a costly and scarce ingredient in preparation of feed. There is always a need for the development of economical and quality fish feed using inexpensive and quality-rich ingredients to maintain the aquaculture sustainability (Murthy 2005). Additionally, the problems of increasing demand, high cost and supply of poor quality fish meal have necessitated the search for alternative protein sources. Plant-derived ingredients are the most promising alternative to fish meal, but they contain high content of phytic acid, which promotes the discharge of nitrogen and phosphorus from aquatic animals, thereby leading to water pollution (De Silva and Andersons 1995; Francis et al. 2001). Further, the use of plant-derived ingredients in aquafeeds is limited due to the presence of a wide variety of anti-nutritional substances such as trypsin inhibitor, glucosinolates and phytate (Usmani and Jafri 2002). The anti-nutritional properties of phytate can be overcome by the incorporation of microbial phytase into aquafeed, thereby increasing the bioavailability of phosphorus that leads to less discharge into the aquatic environment and minimizes the pollution.

Phytase is an enzyme chemically known as myo-inositol hexakisphosphate 3-phosphohydrolase belongs to the class 3, hydrolases, either produced by microorganisms or present in some plant ingredients. Microbial phytase is commercially available either as a dry powder or as a liquid. Natuphos® was the first commercially available phytase purified from a genetically modified *Aspergillus niger* strain. The dietary supplementation of microbial phytase has been reported to improve the utilization of phosphorus and reduced phosphorous discharge in various fishes, such as common carp (Schafer et al. 1995; Nwanna et al. 2008), rainbow trout (Rodehutscord and Pfeffer 1995; Forster et al. 1999; Vandenberg et al. 2011), channel catfish (Jackson et al. 1996; Li and Robinson 1997; Robinson et al. 2002), striped bass (Hughes and Soares 1998), African catfish (Van Weerd et al. 1999), pangas catfish (Debnath et al. 2005) Japanese flounder (Sarker and Hosokawa 2009) and tiger puffer (Laining et al. 2011). However, the role of phytase in shellfish nutrition is hardly elucidated except the work by Biswas et al. (2007), who observed that the dietary phytase supplementation had no impact on the growth of *Penaeus monodon*, but the excretion of phosphorus was significantly reduced. Hence, the present study was undertaken to determine the effects of phytase on growth, survival and body composition of freshwater prawn, *Macrobrachium rosenbergii*. The overall results of the study showed that the dietary supplementation of microbial phytase would be beneficial in improving the growth of prawn through efficient utilization of plant protein-based feed ingredients.

Materials and methods

Freshwater prawns

The post-larvae of *M. rosenbergii* produced in the freshwater prawn Hatchery of the College of Fisheries, Mangaluru, India (Murthy 1998), were reared up to juvenile stage in nursery tanks. The prawn post-larvae were fed with a practical diet containing 30 % protein and artemia during this rearing period.

Experimental diets

All the diet ingredients were procured locally except the microbial phytase (Natuphos 5000G), which is obtained from BASF India Limited, Mumbai. Phytase activity is generally expressed as phytase units or FTUs. One FTU is defined as the quantity of enzyme which liberates 1 μmol of inorganic phosphorus per minute from 0.0051 mol/L of sodium phytate at pH 5.5 and 37 °C temperature. Different levels of phytase (T_0, 0 g; T_1, 0.05 g/250 FTU; T_2, 0.1 g/500 FTU and T_3, 0.15 g/750 FTU)-incorporated test diets were formulated using square method with protein level at 35 %, and feed pellets were prepared according to Parmer et al. (2012). Briefly, the required quantities of feed ingredients such as fish meal, ground nut oil cake, rice bran and tapioca flour were weighed accurately, mixed together with water and hand-kneaded until a smooth dough consistency results. The dough was cooked under steam in a pressure cooker for 20–30 min and cooled to room temperature (RT) in an enamel tray. The required levels of vitamin–mineral premix was added, mixed uniformly and blended to get a homogeneous mixture. Then, this mixture was passed through an extruder with a die, and the resulting pellets (2 mm dia.) were dried in a hot air oven at 60 °C till the moisture levels were reduced to below 10 %. The required amount of microbial phytase was dissolved in 50 mL of distilled water and sprayed on to the finished test diets (T_1, T_2 and T_3). The control diet (T_0) was sprayed with only distilled water to maintain an equal level of moisture. The experimental diets were then packed in an airtight high-density polyethylene bags, labeled and stored at 4 °C until use.

Experimental design

The study was conducted in 12 (four treatment groups with three replicates) cement cisterns (25 m^2 size) for a period of 90 days. Aquatic floating weed, *Eichhornia* plants as shelter for the prawns, were spread on the water surface covering nearly 1/10th of each tank surface. In addition, five numbers of PVC pipes per tank (10 cm dia, 30 cm length) as hideouts were uniformly spread in all the tanks at the bottom covering 4 % of the tank area. Uniform sized juvenile prawn with an average initial weight of 2.28 ± 0.02 g were stocked at 50 numbers per tank (20,000/ha). During the experimental period, the water pH ranged from 6.38 to 7.06, water temperature from 27.3 to 31 °C, dissolved oxygen from 5.9 to 7.9 mg/L, free carbon dioxide from 0 to 0.50 ppm, total alkalinity from 36.66 to 46 mg/L of $CaCO_3$ and ammonia–nitrogen from 0.17 to 1.18 μg/L. Prawns were fed with respective diets at a rate of 10 % body weight twice daily for the first 30 days and later reduced to 5 % body weight till the end of experiment. Removal of uneaten food and fecal matter and water exchange were not performed during the study period. Prawns were weighed biweekly, and the daily ration was adjusted accordingly.

Measurement of growth parameters

During each biweekly sampling, 25 % of the total stocked prawns were collected using drag net and the length and weight of each prawn were measured. The effect of phytase-incorporated diet on the growth and survival of prawns was evaluated by computing the parameters, such as food conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER) and survival percentage using the mathematical formula provided below.

$$\mathrm{SGR} = \frac{\text{Log final weight (g)} - \text{Log initial weight (g)}}{\text{Number of days}} \times 100$$

$$\mathrm{FCR} = \frac{\text{Total feed intake}}{\text{Total weight gain}}$$

$$\mathrm{PER} = \frac{\text{Increment of body weight (g)}}{\text{Protein intake (g)}}$$

$$\text{Survial (\%)} = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$$

Biochemical analysis

The biochemical composition of wet samples of prawn and diets were analyzed at the start and end of experimental period by standard methods (AOAC 1995). Samples were dried to a constant weight at 105 °C to determine moisture level. Crude protein was determined by measuring nitrogen (Nx6.25) using the Kjeldahl method. Crude lipid was extracted with petroleum ether by the Soxhlet method, crude fiber by Fibertec system, and the ash was determined by incineration in a muffle furnace at 550 °C. All analyses were performed in triplicate. Finally, carbohydrate was calculated as nitrogen-free extract (NFE) by the difference method (Hastings 1976) using below formula.

$$\mathrm{NFE} = 100 - (\%\text{Moisture} + \%\text{Crude Protein} + \%\text{Crude fat} + \%\text{Crude fiber} + \%\text{Ash})$$

The caloric values of feed materials were calculated in terms of KJ/g using the energy values of 9 kcal/g for fat, 4 kcal/g for carbohydrate and 5 kcal/g for protein.

Statistical analysis

All statistical analyses were performed using the GraphPad Prism 6.0 for Windows. One-way ANOVA with Tukey's multiple comparison test was used to determine the significant differences between the treatment groups. A significance level of 5 % was applied in all tests.

Results

Diet formulation and proximate composition

The formulation of diets and their proximate composition are presented in Table 1. The level of phytase in the experimental diet was adjusted and formulated to the amount of rice bran added in the diet. The chemical components of diets including crude protein, crude fat, crude fiber, ash and NFE did not vary significantly between the experimental diets. However, there were slight variations in the moisture content of different experimental diets. The moisture content of diet ranged from 7.55 % in T_2 to 9.83 % in T_0. The content of crude protein ranged from 34.73 % in T_0 and T_2 to 34.79 % in T_1, crude fat ranged from 5.43 % in T_1 to 5.72 % in T_3, crude fiber ranged from 6.84 % in T_2 to 7.02 % in T_3, and the ash content ranged from 8.61 % in T_0 to 9.39 % in T_3.

Table 1 Formulation and proximate composition of various experimental diets

	Experimental diets			
	T_0	T_1	T_2	T_3
Ingredients (%)				
Fish meal	24	24	24	24
Groundnut oil cake	55	55	55	55
Rice bran	10	9.95	9.90	9.85
Tapioca flour	10	10	10	10
Vitamin–mineral mix[a]	1	1	1	1
Microbial phytase (FTU)	0.00	250 (0.05)	500 (0.10)	750 (0.15)
Proximate composition (% ±SE)				
Moisture	9.83 ± 0.03	7.92 ± 0.12	7.55 ± 0.18	9.68 ± 0.20
Crude protein	34.73 ± 0.31	34.79 ± 0.12	34.73 ± 0.34	34.78 ± 0.18
Crude fat	5.57 ± 0.19	5.43 ± 0.41	5.45 ± 0.35	5.72 ± 0.15
Crude fiber	6.90 ± 0.06	6.87 ± 0.07	6.84 ± 0.34	7.04 ± 0.01
Ash	8.61 ± 0.02	8.66 ± 0.08	9.08 ± 0.16	9.39 ± 0.51
NFE	34.36	36.33	36.35	33.39
Gross energy kJ/g	15.11	15.40	15.41	15.02

[a] Composition of vitamin–mineral mix (Supplevite™ Mumbai, India) (per 250 g): vitamin A, 5,00,000 I.U.; vitamin D3, 1,00,000 I.U.; vitamin B2, 0.2 g; vitamin E, 75 units, vitamin K, 0.1 g; calcium pantothenate, 0.25 g; nicotinamide, 1 g; vitamin B12, 0.6 mg; choline chloride, 15 g; calcium, 75 mg; manganese, 2.75 g; iodine, 0.1 g; iron, 0.75 g, zinc, 1.5 g; copper, 0.2 g; cobalt, 0.045 g

Growth performance and feed utilization

All the water quality parameters recorded during the experimental period were well within the limits recommended for the farming of freshwater prawn in all the treatments. The growth of prawn was significantly higher in the phytase-incorporated diet-fed prawns, compared with phytase-free diet-fed ones (Table 2). It was observed that the best growth of prawn, in terms of average specific growth rate (SGR) of 3.44 % per day and mean weight of 50.84 g in 90 days period, was recorded in the treatment T_1, fed on a diet incorporated with 250 FTU phytase. However, the phytase-free diet (T_0)-fed prawns recorded a lowest average SGR of 2.78 % per day and mean weight of 27.88 g. Importantly, the best average food conversion ratio (FCR) of 2.33 was recorded in treatment T_1, and the least FCR of 2.80 was observed in treatment T_0. In addition, the highest average protein efficiency ratio (PER) of 2.12 was found for treatment T_1, whereas the lowest PER of 1.53 was recorded in T_0. Furthermore, the survival of prawns did not differ significantly between phytase-incorporated and phytase-free diet-fed prawns, but the survival of prawns decreased with corresponding increase in the phytase incorporation into diet. The lowest survival of 76.66 % was recorded in treatment T_3, in which prawns were fed with highest phytase-incorporated feed. However, the highest survival of 83.33 % was recorded in treatment T_1, where in prawns were fed with lower dose of phytase incorporation.

Biochemical composition of prawn muscle

The changes observed in the biochemical composition of whole prawn are presented in Table 3. The crude protein, fat and ash contents of muscle tissue of prawn fed on phytase-

Table 2 Growth performance and feed utilization of *Macrobrachium rosenbergii* fed on different levels of phytase-incorporated diets

Parameters	Experimental diets			
	T_0	T_1	T_2	T_3
Initial weight (g)	2.28 ± 00^{a}	2.28 ± 00^{a}	2.28 ± 00^{a}	2.28 ± 00^{a}
Final weight (g)	27.88 ± 0.81^{a}	50.84 ± 4.21^{b}	43.77 ± 6.39^{c}	42.99 ± 2.00^{c}
Weight gain (g)	25.60 ± 0.81^{a}	48.55 ± 4.20^{b}	41.48 ± 6.39^{c}	40.71 ± 1.10^{c}
Specific growth rate	2.78 ± 0.03^{a}	3.44 ± 0.1^{b}	3.31 ± 0.17^{b}	3.22 ± 0.12^{b}
Food conversion ratio	2.80 ± 0.55^{a}	2.33 ± 0.59^{a}	2.63 ± 0.57^{a}	2.36 ± 0.08^{a}
Protein efficiency ratio	1.53 ± 0.22^{a}	2.12 ± 0.04^{b}	2.04 ± 0.11^{b}	1.94 ± 0.11^{b}
Survival (%)	81.33^{a}	83.33^{a}	80.00^{a}	76.66^{b}
Net production (g/25 m^2/90 days)	1133.61	2083.00	1803.84	1596.82

Values are mean ± SEM ($n = 3$)

Values within the same row with different superscripts denote significant differences ($p > 0.05$)

Table 3 Proximate composition (% wet weight) of experimental diet-fed freshwater prawn muscle tissue

Proximate composition	Initial	Experimental diets			
		T_0	T_1	T_2	T_3
Moisture (%)	79.23 ± 0.07	76.35 ± 0.15	73.45 ± 0.28	73.8 ± 0.4	74.1 ± 0.18
Dry matter (%)	20.77 ± 0.07	23.65 ± 0.15	26.45 ± 0.28	26.2 ± 0.4	25.76 ± 0.18
Crude protein (%)	13.88 ± 0.07	16.1 ± 0.35	18.2 ± 0.11	17.89 ± 0.05	17.7 ± 0.21
Crude fat (%)	1.57 ± 0.07	2.15 ± 0.1	3.5 ± 0.34	4.41 ± 0.46	4.61 ± 0.07
Ash (%)	2.47 ± 0.03	2.65 ± 0.15	2.86 ± 0.07	2.67 ± 0.24	2.45 ± 0.1
Gross energy (kJ/g)	3.97	4.63	5.44	5.6	5.63

Values are mean ± SEM ($n = 3$)

incorporated diets were higher than those of phytase-free diet-fed prawns. However, the content of carbohydrate was reduced in phytase-supplemented diet-fed prawns, compared with the control group. The highest content of crude protein of 18.20 % was found in 250 FTU phytase-incorporated diet-fed prawns, which also recorded lowest amount of crude fat (3.50 %) among the phytase-supplemented diet-fed prawns.

Discussion

The aim of this study was to evaluate the effect of phytase supplementation in diet on growth and survival of freshwater prawn. Overall, phytase-incorporated diet-fed prawns showed better growth as indicated by the significantly higher values of SGR, PER, and weight gain in all the treatments over the phytase-free diet-fed control group. The higher growth rate of prawn fed on phytase-supplemented diets could be due to the better availability of phosphorus that enhances the nitrogen retention leading to protein accretion and growth. The growth beneficial effects of supplementation of phytase in diets was reported for some fishes, such as channel catfish (Jackson et al. 1996; Li and Robinson 1997), pangas catfish (Debnath et al. 2005) and rainbow trout (Vandenberg et al. 2011). However, the dietary phytase supplementation had no effect on the growth performance and body composition of tiger shrimp (Biswas et al. 2007) and Korean rockfish (Yoo et al. 2005). In addition, the best PER of 2.12 was observed in prawns fed on 250 FTU phytase-incorporated diet, in which the best SGR was recorded. This increased PER values might have resulted from the increased digestibility of dry matter in general and protein in particular. Phytase is known to act on the protein–phytate complex and makes the protein available for biological activities. The exogenous supplementation of phytase in fish diets had shown improvement in growth and PER in nile tilapia (Abo-state et al. 2009) and rainbow trout (Vandenberg et al. 2011).

Food conversion ratio (FCR) is a measure of efficient utilization of nutrients in the feed and is dependent upon several factors, such as water temperature, size of the animal, stocking rate, quality of feed and the availability of natural food (Felt 1990). Overall, the FCR values obtained in this study were higher and could be attributed to the inadequate adjustment of feeding and non-removal of uneaten or excess food from the cisterns. However, the lower FCR of 2.33 was recorded in prawns fed on 250 FTU phytase-incorporated diet and is significantly different from phytase-free diet-fed group. Similar results of lower FCR recorded for microbial phytase-incorporated diet-fed fishes such as channel catfish (Li and Robinson 1997), mrigal (Usmani and Jafri 2002), pangas catfish (Debnath et al. 2005) and rohu (Baruah et al. 2007). The survival of prawns did not differ significantly between the treatment groups, but the maximum survival of 83.33 % was found in 250 FTU phytase-supplemented diet-fed prawns. These results indicate that there was no adverse effect of microbial phytase incorporation on survival of prawns. However, the high amount of microbial phytase (750 FTU/kg) supplementation showed lower survival (76.66 %) of prawns, and this could be because of the metabolic stress that might have resulted from the high amount of phytase enzyme incorporation into diet.

The dietary supplementation of phytase did not show significant influence on proximate composition of prawn, but the levels of crude protein and fat were higher in phytase-diet-fed groups than in phytase-free diet-fed groups. This increase in the content of muscle protein and fat might be due to the better availability of nutrients and amino acid utilization through breakdown of phytin–protein complexes in the phytase-supplemented diets, as observed in poultry (Kornegay 1995). Several fish diets incorporated with microbial phytase have reported to increase the protein and minerals digestibility (Yan et al. 2002; Cheng and Hardy 2002; Debnath et al. 2005). On the contrary, the inclusion of phytase in the soybean-based shrimp diet did not show any influence on the proximate composition of shrimp (Biswas et al. 2007). Overall, the results of this study clearly denotes that phytase supplementation in diet would influence profoundly on the efficient digestibility of nutrients, which in turn increased the growth and biochemical composition of freshwater prawn.

Conclusion

This study demonstrates that the dietary administration of phytase results in improved growth and biochemical composition of *M. rosenbergii*. The incorporation of phytase at lower levels of 250 FTU per kg feed has been found to be very effective in enhancing the growth of freshwater prawn. To determine the effective levels of dietary phytase supplementation in prawn diet, further studies are required by altering the level of dietary phytase to below 250 FTU and above 750 FTU/kg feed.

Compliance with ethical standards

Conflict of interest All the authors declare no conflict of interest.

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