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Growth Performance of Juvenile Milkfish *Chanos chanos* (Forskal) on Replacement of Fish Meal with Plant Based Diet Supplemented with Dietary Cell Bound Phytase of *Pichia anomala*

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Abstract. Results of feeding trials on juvenile milkfish with seven types of fish meal and soybean meal based diets distinctly indicated better results with fish meal based diet. Nevertheless, soybean based diet supplemented with different levels of cell bound phytase from *Pichia anomala* produced better growth performance than the soybean based diet without supplementation of phytase. The observations clearly suggested that milkfish would not show normal growth with plant based diet without phytase supplementation. Among the three concentrations of cell bound phytase of *P. anomala* used in this investigation, 1000 FTU/kg supplementation with plant based diets appeared to be favourable for the growth and survival of milkfish. The results of feed conversion efficiency, weight gain, condition factor, specific growth rate, protein efficiency ratio and survival rate at 1000 FTU/kg of phytase supplementation in soybean meal showed values comparable with the controlled diet. The biochemical profiles of the fish fed first, second, third and sixth diets were similar as compared to the fish fed fourth, fifth and seventh diets. It is suggested that commercial farming of milkfish can be practiced with soybean based diet with microbial phytase supplementation.

Keywords: fish nutrition, soybean meal, cell bound phytase, *Chanos chanos, Pichia anomala*

Introduction

Many plant protein supplements are being used in aquafeed formulations. Of all these, soybean meal has been the most extensively evaluated and commonly used in commercial feed production (Swick, 1994; Lim and Dominy, 1990).

The objective of feed formulation in aquaculture is to supply the nutrient density for optimal animal production. Feed cost and feed efficiency are the prime factors that control the farm economy. The availability of nutrients from a feed ingredient is essential in determining the nutritional value of the feed ingredient. Traditionally, the feed have been based on animal protein. However, due to cost and availability considerations, it is necessary that plant protein based feed ingredients should be utilized in the feed. Such an application of plant protein sources in feed preparation is reported from many countries (Mabahinzireki *et al.,* 2001; Naylor *et al.,* 2000). Animal protein source, the fish meal is expensive and scarce as compared to plant protein source such as soybean meal. Further, fish meal resources are limited compared to the increasing demand and the replacement of fish meal with soybean meal will become increasingly important for the sustainability of aquaculture. Soybean meal is considered a promising alternative protein source because of its ready supply and high protein content (Divakaran *et al.,* 2000). However, about two-third of phosphorus in soybean meal occurs as phytate (inositol hexaphosphate), which is not efficiently utilized by the fish (NRC, 1993). The phytate in soybean meal is unavailable to fish due to lack of an intestinal phytase for efficient phytate hydrolysis (Hughes and Soares, 1998; Pallauf and Rimbach, 1997; Raboy, 1997). The use of phytase, an enzyme chemically known as

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myo-inositolhexa-phosphate phosphohydrolase (class 3: Hydrolases), is reported to be highly effective in digesting phytate (Sajad *et al.,* 2009; Storebakken *et al.,* 1998; Vielma *et al.,* 1998). In intensive fish farming systems, poor utilization of phytates by the fish leads to detrimental effects on the aquatic environment like eutrophication (Persson, 1991). The addition of microbial phytase in the plant protein based diets has shown to increase the availability of phytate in fish. Nevertheless, the potency of the phytase depends on its nature (such as soluble or cell bound) and also on its source (Liebert and Portz, 2005). Hence it is essential to conduct feeding trials of plant protein based diets supplemented with phytase on different cultivable fish in order to assess their influence in phytate digestion and phosphorus availability for absorption.

The objective of the present experiment was to assess the suitability of soybean meal supplemented with cell bound phytase as replacement of fish meal for the juvenile milkfish. Milkfish was chosen because it is cultivated widely in Asian countries and has good prospects as an ideal fish for farming in Indian coastal waters. Further, it is commercially important and shows the faster growth and wider range of tolerance to temperature, dissolved oxygen, salinity, pH and diseases as compared to other fishes (Almendras, 1987, 1982; Villaluz, 1984; Duenas and Young, 1983; Cruz, 1981; Gerochi *et al.,* 1978; Lin, 1969).

Materials and Methods

Diet preparation. Seven experimental diets were formulated with different levels of fish meal, soybean meal and phytase (Table 1). The first diet (control) contains 61.34% of fish meal. The second and third diets each contain 36.00% of fish meal and 25.34% of soybean meal. The fourth, fifth, sixth and seventh diets contain 61.34% of soybean meal without fish meal. The diets third, fifth, sixth and seventh were supplemented with 1000, 500, 1000 and 2000 phytase activity units (FTU)/kg, respectively. The lyophilized biomass of the yeast *P. anomala* produced in cane molasses medium in Biostat C fermenter (B. Braun, Germany) has been used in this investigation (Kaur and Satyanarayana, 2005). The other contents like wheat flour, starch, fish oil, vitamins and minerals were kept constant to determine the effect of fish meal and soybean meal supplemented with cell bound microbial phytase. One FTU is defined as the amount of enzyme that generates 1 μ-mole of inorganic phosphorus per min from an excess of sodium phytate at pH 5.5 and 37 °C (Engelen *et al.,* 1994). The experiment was designed to study the effect of microbial phytase on soybean based diet and its influence on nutrient utilization and growth performance in milkfish juveniles. Diets were prepared by blending all the ingredients in 30 parts of water and then cooked. The diets were pelletized (3 mm), freeze dried and stored in a refrigerator at 20 °C until use.

Fish and experimental condition. Approximately 2500 juvenile milkfish (mean weight 5g) obtained from the Central Institute of Brackishwater Aquaculture (CIBA) Chennai, India and were randomly distributed in 24 (100 L) capacity tanks and 7 (300 L) capacity tanks, where the fish were allowed to acclimatization for two weeks. The photoperiod was set at 12 h light: 12 h dark. Tanks were supplied with filtered fresh water and the rearing tanks were aerated to maintain the oxygen level near 100% saturation. The water temperature was 29±2 °C. Fish were fed to apparent satiation with a commercial pelletized diet twice a day at 8 and 16 h during the acclimation period.

Acclimatized fish starved for 24 h and body length and weight were measured. They were subjected to a prophylactic dip in $KMnO_4$ solution (50 mg/L) and stocked into the experimental tanks of 100 L capacity. The experiments were carried out in three trials for each diet and tanks were randomly assigned to reduce tank effect. Fish were fed to apparent satiation with the experimental diets twice per day, at 8 and 16 h, for 60 days, and 50 to 70% of water was exchanged twice in a week. The number of dead fishes (if any) was recorded every day. Fish were deprived of food for 12 h before each weighing. All possible care was taken during feeding so that no uneaten food settled down on the tank bottom. Tanks were cleaned thoroughly and the fecal matter was removed from the tanks. At the end of the experiment the fish starved for 12 h, and body length and weight were measured.

Analytical procedures. The data obtained were analyzed for specific growth rate (SGR), feed conversion efficiency (FCE), protein efficiency ratio (PER), condition factor (CF), weight gain (WG), moisture, ash and survival, using the following formulae:

SGR (%) = 100 × (In average final weight – in average initial weight) / number of culture days.

FCE (%) = 100 × [wet weight gain (g) / dry feed intake (g)]

PER = total wet weight gain (g) / crude protein fed (g)

CF (%) = 100 × [wet body weight (g) / body length $(cm)^3$]

WG (%) = 100 × (final weight – initial weight) / initial weight.

Survival = 100 × (final number of fishes / initial number of fishes)

Moisture content (%) = 100 x (weight of wet sample – weight of dry sample / Weight of wet sample)

Ash (%) = 100 × (weight of ash / weight of dry sample)

The proximate composition of fish samples was determined at the beginning and at the end of the feeding trial. Similarly, proximate analysis of all the diets was carried out following the methods of AOAC (1995).

Results and Discussion

The proximate compositions of the experimental diets are presented in Table 2. The protein content of the diets ranged from 40.19 to 43.85%. Fish can absorb phosphorus from water (Lall, 1991). However, dissolved phosphorus present in water at very low levels (0.005-0.05 mg/L) is inadequate to meet their requirement (Nose and Arai, 1976). In the present experiment, phosphate level of 0.04 mg/L was recorded. Thus, it can be assumed that dissolved phosphorus did not contribute to the growth of fish.

The growth data of the present experiment showed that final body weight, FCE, PER, WG, CF, SGR and survival rate were significantly influenced by different diets (Tables 3 and 4). However, the phytase supplementation at 1000 FTU/kg improved FCE, PER, WG, CF, SGR and survival considerably as indicated by the performance data of milkfish. The replacement of fish meal with soybean meal without phytase supplementation resulted in reduced final weight, WG, PER, CF and SGR in milkfish. The 1000 FTU/kg phytase supplementation in diet six, where fish meal was completely replaced with soybean meal showed comparatively increased final weight, WG, PER, CF and SGR in milkfish than other soybean meals supplemented with phytase. In fish fed diet six, final weight, WG, PER, FCE, CF and SGR were not significantly different from fish fed diet one (control diet). However, among the three concentrations of phytase used, phytase supple-

Table 1. Composition of experimental diets for juvenile milkfish

Ingredients (%)	Diet-1	Diet-2	Diet-3	Diet-4	Diet-5	Diet-6	Diet-7
Fish meal (F)	61.34	36.00	36.00	-	-	-	-
Soybean meal (S)	-	25.34	25.34	61.34	61.34	61.34	61.34
Wheat flour	24.00	24.00	24.00	24.00	24.00	24.00	24.00
Starch	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Fish oil	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Mineral mixture	2.11	2.11	2.11	2.11	2.11	2.11	2.11
Vitamin mixture	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Kidney bean extract	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Extract of Dunalliella salina	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Phytase (Units/kg)	-	-	1000U	-	500U	1000U	2000U

Table 2. Chemical composition of experimental diets

Parameters	Diet-1	Diet-2	Diet-3	Diet-4	Diet-5	Diet-6	Diet-7
Moisture (%)	7.13	6.62	6.46	7.29	7.45	7.39	7.40
Crude protein (%)	43.85	42.61	42.79	40.64	40.19	40.63	40.60
Crude fat (%)	6.61	6.80	6.09	7.49	7.34	7.91	7.45
Total ash (%)	13.26	12.32	12.75	11.42	11.42	11.13	11.46
Carbohydrates (%)	27.72	30.17	31.01	33.21	33.33	33.01	33.39
Phosphorus (%)	2.75	2.06	2.31	1.92	1.97	1.99	1.89
Energy (kcal/100gm)	345.77	364.32	363.86	382.89	382.93	382.81	383.24

mented with 1000 FTU/kg without fish meal was found ideal in terms of final weight, WG, PER, FCE, CF and SGR for milkfish than phytase supplementation with 500 and 2000 FTU/kg. Survival of fish that ranged from 76.33±8.19 to 98.00±0.58 were recorded. Fish fed diet one showed highest survival followed by both diets six and seven. Milkfish fed diet four showed the lowest survival.

The proximate body compositions of fish fed on different diets are presented in Table 4. The fish fed diet four showed higher body moisture and ash content than fish fed diets five and seven, and six, two and three. Fish fed diet one showed lower whole body moisture and ash content than fish fed other diets. However, the fish fed diet four, where fish meal was replaced with soybean meal without phytase supplementation, showed lower whole body crude protein and crude fat than fish fed diets five and seven; and six, three and two. Fish fed diet one showed a higher body crude protein and crude fat than diets six, two and three, followed by diets seven and five.

The global production of soybean meal has continued to increase and it is the most promising alternate protein source for fish feed in terms of future availability (Hardy, 1995). Although soybean meal has frequently been assessed as an inferior protein source to fish meal, citing adverse effect on growth performance in some species (Davies and Morris, 1997; Stickney *et al.,* 1996), it has been used to replace 25-40% of the fish meal protein with no subsequent decrease in weight gain in other species (Kaushik *et al.,* 2004, 1995; Muzinic *et al.,* 2004; Vielma *et al.,* 2000). Soybean meal is the most widely used oil seed meal in animal feed formulations. It is used due to its relatively high crude protein level (44-48.5), high protein availability, good amino acid composition compared to other oilseed meals, and relatively low cost compared to fish meal.

The finding that fish meal replacement by soybean meal without phytase resulted in lower growth performance parallels the findings of Yoo *et al.* (2005) and Stickney *et al.* (1996). It has been demonstrated that dietary fish meal levels can be considerably reduced without any adverse consequence in terms of somatic growth or nutrient utilization using plant based feed with phytase supplementation (Kaushik *et al.,* 2004, 1995; Muzinic *et al.,* 2004). The suppression in overall growth performance in fish fed diet four, where fish meal was replaced with soybean meal without phytase supplementation, might be attributed to the presence of antinutritional factor in soybean meal (Linener, 1994; Vaintraub and Bulmaga, 1991) and an adverse effect of phytate on growth performance and bioavailability of various

Table 3. Growth performance of milkfish fed different experimental diets

Parameters	Diet-1	Diet-2	Diet-3	Diet-4	Diet-5	Diet-6	Diet-7
Initial weight (biomass) (g)	41.87±0.47	41.83±0.18	41.70±0.25	42.37±0.07	41.57±0.13	42.47±0.24	42.00±0.36
Final weight (biomass) (g)	99.87±0.23	80.70±0.21	68.47±2.33	52.23±0.47	57.60±0.58	83.80±0.26	67.17±0.20
Weight gain (%)	138.57±2.12	92.91±1.26	64.16±5.15	23.28±1.05	38.56±1.03	97.35±1.68	59.87±1.84
SGR (%)	96.66±0.42	64.44±0.28	44.61±3.70	16.44±0.75	26.72±0.78	68.89±0.81	41.94±0.89
FCE (%)	64.85±2.45	42.27±1.17	27.71±2.34	10.29±0.29	16.70±0.49	44.84±0.98	26.36±1.37
PER (%)	1.32±0.00	0.91±0.01	0.62±0.05	0.24±0.01	0.40±0.01	1.01±0.01	0.62±0.01
Condition factor	59.93±1.98	40.18±1.64	27.61±1.99	10.16±0.19	16.54±0.32	42.69±1.18	17.52±8.72
Survival rate (%)	98.00±0.58	96.33±1.76	94.00±2.08	76.33±8.19	80.00±8.74	96.67±0.88	96.67±1.33

Each value is a mean±SE derived from three samples.

Table 4. Proximate composition of muscle tissues of milkfish fed different experimental diets

Parameters	Diet-1	Diet-2	Diet-3	Diet-4	Diet-5	Diet-6	Diet-7
Moisture (g %)	72.39±0.75	73.58±0.78	74.19±0.37	79.59±0.34	77.25±0.16	74.09±0.91	76.78±0.36
Crude Protein (g %)	22.49±0.51	21.60±0.35	21.62±0.48	17.28±0.53	19.00±0.09	21.46±0.83	19.04±0.09
Crude Fat (g %)	3.35 ±0.13	2.97±0.17	2.83±0.16	1.31±0.27	2.11±0.12	2.94±0.10	2.34±0.22
Total Ash (g %)	1.15±0.14	1.37±0.17	1.26±0.10	1.47±0.02	1.39±0.06	1.29±0.10	1.42±0.02

Each value is a mean±SE derived from three samples.

dietary components (Satoh *et al.,* 1989; Richardson *et al.,* 1985).

The phytase supplementation in diet six (1000 FTU/kg), however, significantly improved growth performance in milkfish compared to the diets five and seven with phytase (500 and 2000 FTU/kg) supplementation, respectively. This suggests that phytase at 1000 FTU/kg diet may be effective at reducing either an antinutritional factor or adverse consequences of phytate from soybean meal. A similar level of phytase supplementation was also proposed by Liu (1997). The reduction of phytate-protein complexes in the gut and increased nutrient availability could be another explanation for this observation (Liebert and Portz, 2005). The improvement in growth performance after phytase supplementation is consistent with other studies where fish were fed with either phytase supplemented diets (Papatryphon *et al.,* 1999; Jackson *et al.,* 1996) or phytase pretreated ingredients (Vielma *et al.,* 2002). Similar patterns of phytase supplementation on growth performance have also been observed in rainbow trout *Oncorhynchus mykiss,* when canola protein concentrate was used with different dosages of phytase (500, 1500 and 4500 FTU/kg diet). Forster *et al.* (1999) demonstrated a higher SGR in fish fed phytase at 500 FTU/kg diet. Phytase dosage higher than 500 FTU/kg diet did not further increase the SGR in their observations. In Korean rock fish, although phytase supplementation in soybean meal diets at 1000 and 2000 FTU/kg did not increase growth performance, inferior results were observed when phytase was supplemented at 2000 FTU/kg diet compared to 1000 FTU/kg diet (Yoo *et al.,* 2005).

The level of phytase supplementation (1000 to 2000 FTU/kg) for maximum growth performance is in agreement with reports for Nile tilapia (Portz *et al.,* 2003; Furuya *et al.,* 2001). The growth data of fish fed the negative control diet without phytase supplementation clearly indicated the insufficiency of essential nutrients in milkfish. Therefore, reduction of phytate content in foods and feed by enzymatic hydrolysis using phytase is not only desirable but also the need of the hour (Singh *et al.,* 2006; Satyanarayana *et al.,* 2004).

Dietary phytates may form phytate-protein complexes and reduce the availability of dietary protein and amino acids (Sugiura *et al.,* 2001; Liu *et al.,* 1998). Plant protein utilization has been reported to increase (Sugiura *et al.,* 2001; Storebakken *et al.,* 1998; Vielma *et al.,* 1998;) or to remain unchanged (Yan *et al.,* 2002; Lanari *et al.,* 1998). Supplementation of microbial phytase in the diets had a positive effect on the growth performance of *Labeo rohita* juveniles (Baruah *et al.,* 2007).

Phytase treatment of soy protein concentrate was found to improve protein digestibility and retention in Atlantic salmon (Storebakken *et al.,* 1998). Microbial phytase supplementation in the diet of *Pangasius pangasius* also increased the apparent net protein utilization (Debnath, 2003). It was reported that apparent protein digestibility in the diets was significantly improved by enzyme supplementation, while non-enzyme supplementation groups showed a low digestibility (Debnath, 2003), confirming the established properties of phytate to form phytate-protein complex that are resistant to proteolytic digestion (Cheryan, 1980). Digestibility of dry matter (Papatryphon *et al.,* 1999) and crude protein (Storebakken *et al.,* 1998) were also improved by dietary phytase supplementation.

Improvement in protein digestibility by phytase supplementation has been reported in other studies (Vielma *et al.,* 2004; Storebakken *et al.,* 1998). *In vitro* studies of Ravindran *et al.* (1995) have shown that phytate-protein complexes are insoluble and consequently less digestible by proteolytic enzymes. By virtue of this chelating potential, supplemental microbial phytase may have protein-sparing effects in fish diets by releasing phytate-bound protein and improving its bioavailability.

The better performance of fish fed phytase supplemented diets implies that either the phosphorus requirement was met along with other nutrients or that phytase has other positive effects on performance. Dietary phytase had no significant effect on whole body ash content, which is in agreement with Debnath *et al.* (2005) and Sajjadi and Carter (2004). Microbial phytase hydrolysis the phytate-mineral complex and increases the availability of the minerals (Baruah *et al.,* 2005; Debnath *et al.,* 2005), leading to increased mineral utilization in the body. Similar results were also reported by Van weerd *et al.* (1999) in *Clarias gariepinus* and Vielma *et al.* (1998) in rainbow trout *O. mykiss*.

Conclusion

Cell bound microbial phytase has proved to be efficient for increased weight gain and better survival for milkfish, thereby reducing the cost of feed for aquaculture industry. In the plant based milkfish diets, the supplementation of 1000 FTU/kg of the microbial phytase resulted in growth and mineral utilization similar to control diet. Generally, more basic research is required to quantify the observed complex effects of phytase in fish diets, mainly on nutrient utilization. Conclusive studies dealing with the mechanism of phytate degradation in different fish species depending on specific characteristics of the digestive tract, different sources of phytates and varying activity from the supplemental phytase sources are needed.

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