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Use of Soybean Meal Supplemented with Cell Bound Phytase for Replacement of Fish Meal in the Diet of Juvenile Milkfish, *Chanos chanos*

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Abstract: This study was designed to determine the influence of cell bound phytase supplemented in soybean meal on growth and survival of juvenile milkfish *Chanos chanos*. Four diets were formulated with different levels of fish meal, soybean meal and phytase. The first diet (control) (F) contains 61.35% of fish meal. The fish meal was replaced by the same level of soybean meal in the second (S), third (SP_{500}) and fourth (SP_{1000}) diets, however, SP_{500} and SP_{1000} were supplemented with 500 and 1000 phytase activity units (FTU) kg^{-1}, respectively. The replacement of fish meal by soybean meal, with out phytase supplementation reduced weight gain, specific growth rate and crude protein content in the milkfish. The growth performance in fish fed diet SP_{1000} was as good as those fed diet F. Results indicated that soybean meal supplemented with 1000 FTU kg^{-1} phytase can be used for the mass culture of milkfish.

Key words: Soybean meal, phytase, *chanos chanos* and fish nutrition

INTRODUCTION

Phytase, an enzyme chemically known as myo-inositol-hexaphosphate phosphohydrolase (class 3: Hydrolases), is produced either by microorganisms or present in some plant ingredients. Presence of phytase in some animals is of microbial origin. Phytic acid is an abundant plant constituent. It is an organic form of phosphorus, which is chemically a myo-inositol-hexakis-dihydrogen phosphate. The molecular formula of phytic acid is $C_6H_{18}O_{24}P_6$ and its molecular weight is 659.86. Salts of phytic acid are called phytates. Most foods of plant origin contain 50 to 80% of their total phosphorus as phytate (Harland and Morris, 1995). Phytic acid interacts with other food ingredients due to which it acts as an antinutritional factor in several ways. Phytates reduce digestibility of proteins, starch and lipids. The amino group present on the side chain of the amino acid is one of the functional groups involved in protein-phytate interaction, thereby decreasing the digestibility of proteins. Phytate complexes with proteins, making them less soluble (Dvorakova, 1998). The salts of phytic acid are known as phytins and their availability and digestibility to monogastric animals including fish is very limited due to lack of intestinal phytase (Pointillart *et al*., 1987). Soybean products vary in their nutrient and antinutritional factors with regard to different fish species. Further, size related differences in nutrient requirements and tolerance of fish to dietary antinutritional factor is also reported (NRC, 1993). It is noteworthy, however, that these drawbacks can be minimized through the addition of phytase to the diet (Simons *et al*., 1990; Rodehutscord and Puffer, 1995). Use of phytase in feed reduces or sometimes eliminates the necessity of mineral supplementation, which also decreases the cost of feeds. Although phytase was first used for environmental reasons, it has now been discovered that there are a range of other nutritional and health benefits from using these enzymes. Usage of plant based fish feed in fish culture in lieu of fishmeal might be cost effective. A preliminary study in milk fish *C. chanos* explored the possibility of replacement of fish meal by soybean meal supplemented with phytase. Milkfish was chosen because it is one of the most ideal fish for farming in Indian coastal waters. Further it is commercially important, show faster growth rate in the first year of life and wide range of tolerance to temperature, dissolved oxygen and salinity. The aim of this study was therefore to determine the effect of phytase supplementation required in plant based diets to improve growth performance and retention efficiencies of nutrients and energy by the milk fish *C. chanos*.

MATERIALS AND METHODS

Composition and chemical analyses of the experimental diets are shown in Table 1. Four diets were formulated with different levels of fish meal, soybean meal and phytase. The first diet (control) (F) contains 61.35% of fish meal. The second (S), third (SP_{500}) and fourth (SP_{1000}) diets contain 61.35% of soybean meal and the diets third and fourth were supplemented with 500 and 1000 phytase activity units (FTU) kg^{-1}, respectively. The diets were made into pellets of 3mm diameter by a laboratory pellet machine after mixing 100 parts of ingredients with 30 parts of tap water. The diets were freeze dried and stored in a refrigerator at 20°C until used.

Table 1: Feed formulation ingredients that constituted the experimental feeds

Ingredients (%)	Diet 1 (F)	Diet 2 (S)	Diet 3 (SP_{500}U)	Diet 4 (SP_{1000}U)
Fish meal (F)	61.35	-	-	-
Soybean meal (S)	-	61.35	61.35	61.35
Wheat flour	24.00	24.00	24.00	24.00
Starch	6.00	6.00	6.00	6.00
Fish oil	6.00	6.00	6.00	6.00
Mineral mixture	2.12	2.12	2.12	2.12
Vitamin mixture	0.18	0.18	0.18	0.18
Kidney bean extract	0.30	0.30	0.30	0.30
Extract of *Dunaliella salina*	0.08	0.08	0.08	0.08
Phytase (Units/kg)	-	-	500.00U	1000.00U

Nine hundred forty juvenile milk fish *Chanos chanos* (mean weight = 9g) were obtained from the Central Institute of Brackish water Aquaculture (CIBA) Chennai, India and were randomly distributed among eight 100 l tanks and two 300 l tanks, where the fish were allowed to acclimate to the new rearing tanks for two weeks. The photoperiod was set at 12 h light: 12 h dark. Tanks were supplied with filtered tap water and the rearing tank was aerated to maintain the oxygen level near 100% saturation. The temperature was maintained at 29±1°C. Fish were fed to apparent satiation with a commercial diet twice a day at 08:00 and 16:00 h during the acclimation period.

After conditioning for two weeks, the fish were starved for 24 h and body weight was measured. The stocking density was 30 fish (mean weight 11.48 g) per tank. The experiment was designed so that duplicate tank of each treatment was randomly assigned, thus reducing tank effect. Fish were fed to apparent satiation with the experimental diets twice per day, at 08:00 and 16:00 h, 7 days per week for three weeks. Individual body weights were measured weekly. Fish were deprived of food for 24 h before each weighing. All possible care was taken during feeding so that no uneaten food settled down on the tank bottom. The fecal matter was removed from the tanks and the tanks were thoroughly cleaned.

The data obtained were analyzed for specific growth rate (SGR), feed conversion ratio (FCR), weight gain (WG) and survival, using the following formula:

SGR (%) = [100 x (In average final weight - In average initial weight) / Number of culture days].

FCR = Total dry feed intake (g)/wet weight gain (g).

WG (%) = 100 x (Final weight - Initial weight) / Initial weight.

Survival = 100 x (Final number of fishes / Initial number of fishes).

RESULTS

Growth data (Table 2) show that final body weight, feed intake (FI), feed conversion ratio (FCR) weight gain (WG) and specific growth rate (SGR) were significantly influenced by phytase supplementation. The replacement of fish meal with soybean meal resulted in reduced weight gain and SGR in milk fish *C. chanos*. However, phytase supplementation in SP_{1000} FTU kg^{-1} significantly increased weight gain and SGR in milk fish *C. chanos*. Feed consumption and FCR were highly depressed, when fish meal was replaced by soybean meal without phytase supplementation. However, both parameters were improved in fish fed diet SP_{1000} FTU kg^{-1} (Table 2). In fish fed diet SP_{1000} weight gain and SGR were as good as for fish fed diet F. However, among the two concentrations of phytase used; SP_{1000} FTU kg^{-1} was found ideal in terms of weight gain and SGR for milk fish *C. chanos* than SP_{500}FTU kg^{-1}.

The proximate body compositions of fish fed on different diets are presented in Table 3. There were no significant differences in moisture, crude fiber, dry matter and ash content among the different treatments. Fish fed diet SP_{1000} showed a higher whole body crude protein and crude fat content than those fed diets (S) and SP_{500}.

DISCUSSION

The reduced growth performance and feed consumption in fish fed diet S, where fish meal was replaced by soybean meal without phytase supplementation, confirmed the observations of a preliminary study concerning the effect of fish meal replacement by soybean meal on growth performance and feed consumption in milk fish *C. chanos*. The finding that fish meal replacement by soybean meal without phytase resulted in lower growth performance parallels the findings in other species (Pongmaneerat and Watanabe, 1992; Rumsey *et al*., 1994; Stickney *et al*., 1996; Yoo *et al*., 2005). 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*., 1995, 2004; Muzinic *et al*., 2004). The slight suppression in overall growth performance in fish fed diets SP_{500} FTU kg^{-1} might be attributed to the presence of antinutritional factor in soybean meal (Kakade *et al*., 1973; Vaintraub and Bulmaga, 1991; Linener, 1994) and an adverse effect of phytate on growth performance and bioavailability of various dietary components (Spinelli *et al*., 1983; Richardson *et al*., 1985; Satoh *et al*., 1989).

The phytase supplementation in diet SP_{1000} (1000 FTU kg^{-1}), however, significantly improved growth performance in milk fish *C. chanos* compared to the diet with phytase SP_{500} (500 FTU kg^{-1}) supplementation. This suggests that phytase at 1000 FTU kg^{-1} diet may be effective at reducing either an antinutritional factor or adverse consequences of phytate from soybean meal (Liu, 1997). The reduction of phytate-protein complexes in the gut and increased nutrient availability could be another explanation for this observation (Liebart and Portz, 2005). The improvement in growth performance after phytase supplementation is consistent with other studies, where fish were fed either phytase supplemented diets (Rodehutscord and Puffer, 1995; Jackson *et al*., 1996; Papatryphon *et al*., 1999) or

Table 2: Growth performance in milk fish *C. chanos* fed diets with different levels of fish meal, soybean meal and phytase supplementations. Each value is the mean±SE derived from three samples each of 15 juveniles

Parameter	Diet 1 (F)	Diet 2 (S)	Diet 3 ($SP_{500}U$)	Diet 4 ($SP_{1000}U$)
Initial weight (g)	11.00±1.20	11.80±0.95	11.64±1.05	11.47±1.50
Final weight (g)	15.06±1.60	12.90±1.42	13.45±1.11	14.40±2.20
Weight gain (g)	36.90±0.08	9.32±0.20	15.54±0.18	25.54±0.41
FCR	82.07	63.50	77.00	80.30
SGR (%)	19.33	5.23	8.61	13.95
Survival (%)	95	75	80	95

Table 3: Proximate composition and biological measurements in milk fish *C. chanos* under different treatments. Each value is mean derived from two samples each of three fish for each treatment

Parameters	Diet 1 (F)	Diet 2 (S)	Diet 3 ($SP_{500}U$)	Diet 4 ($SP_{1000}U$)
Moisture (%)	76.10	84.25	80.32	80.37
Dry matter (%)	23.90	15.76	19.68	19.63
Crude protein (%)	18.88	12.44	14.56	15.31
Crude fat (%)	2.63	1.80	2.19	2.20
Crude fibre (%)	0.09	0.07	0.09	0.08
Ash (%)	1.31	0.90	1.16	1.39

phytase pretreated ingredients (Vielma *et al*., 2002). Similar patterns of phytase activity 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^{-1} diet). Forster *et al*. (1999) demonstrated a higher SGR in fish fed phytase at 500 FTU kg^{-1} diet. Phytase dosage higher than 500 FTU kg^{-1} 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^{-1} did not increase growth performance, inferior results were observed when phytase was supplemented at 2000 FTU kg^{-1} diet compared to 1000 FTU kg^{-1} diet (Yoo *et al*., 2005).

Dietary phytates may form phytate-protein complexes and reduce the availability of dietary protein and amino acids (Liu *et al*., 1998; Sugiura *et al*., 2001). Francis *et al.* (2001) reviewed antinutritional factors for fish and indicated that growth and feed efficiency in commonly cultured fish species, such as carp (*Cyprinus carpio*), tilapia (*Oreochromis niloticus*), trout (*Oncorhynchus mykiss*) and salmon (*Salmo salar*), is negatively affected by the inclusion of phytate containing ingredients in the diets. *In vitro* studies have shown that phytate-protein complexes are insoluble and consequently less digestible by proteolytic enzyme (Ravindran *et al*., 1995). 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. Plant protein utilization has been reported to increase (Storebakken *et al*., 1998; Vielma *et al*., 1998; Sugiura *et al*., 2001) or to remain unchanged (Lanari *et al*., 1998; Yan and Reigh, 2002). Supplementation of microbial phytase in the diets had a positive effect on the growth performance of *Labeo rohita* juveniles (Baruah *et al*., 2007). Present study also suggests that culture of milkfish *Chanos chanos* with soybean meal is possible with phytase supplementation.

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