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Effect of Phytase Supplementation on Growth Performance and Nutrient Digestibility of *Labeo rohita* Fingerlings Fed on Corn Gluten Meal-based Diets

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ABSTRACT

Alarmingly increasing cost and reduced supply of fish meal has necessitated searching out the low cost and easily accessible alternatives for aqua-feed industry. Potential of corn gluten meal (30%) and oilseed by-product, was evaluated by comparing the corn gluten meal-based diet with a fish meal-based reference diet. The experimental diet was consisted of 70% reference diet and 30% corn gluten meal contents. The potential of corn gluten meal-based diet was enhanced with graded levels of microbial phytase enzyme to assess the dose required for optimal fish production through various growth and digestibility coefficients. The chromic oxide was included at a concentration of 1% as non-digestible marker in the feed. The fish in experimental diets performed equal or better than the reference diet. The results of the study showed that the maximum weight gain (4.68±0.017 g) in fish was observed on corn gluten meal (30%)-based test diet containing 750 FTU kg^{-1} level. Crude fat and apparent gross energy digestibility performed their best at 750 FTU kg^{-1} followed by 1000 FTU kg^{-1} level, while maximum crude protein digestibility was observed at 1000 FTU kg^{-1} level. The conclusion of the present study suggested that phytase supplementation to corn gluten meal (30%) based diets at 750 FTU kg^{-1} level, is enough to release sufficient bounded nutrients for optimal growth performance of *Labeo rohita* fingerlings. © 2011 Friends Science Publishers

Key Words: *Labeo rohita*; Phytase; Corn gluten meal; Growth performance; Nutrient digestibility

INTRODUCTION

Labeo rohita (Hamilton), commonly known as rohu, is one of the major carps species cultured in Pakistan under poly and composite-culture systems with other species of major carps and Chinese carps. The carps are traditionally fed on diet formulated by mixing only the two or three feed ingredients of plant by-products. However, the aquaculture feed industry of the world mainly depends on the use of fishmeal because it is an excellent source of fundamental nutrients such as vital amino acids, essential fatty acids, vitamins, major minerals and many growth factors (Zhou *et al*., 2004). Rising demand, high price and unstable supply of the fish meal with the extension of aquaculture makes it compulsory to search for better alternative protein sources (Gatlin *et al*., 2007; Lunger *et al*., 2007; Pham *et al*., 2008; Lim *et al*., 2011). The plant by-products are the promising sources of protein and energy (Hardy, 2000) for the formulation of economical and environment friendly aqua feeds (Cheng & Hardy, 2002). The majority of plant protein sources used in fish feeds contains phytate that may vary from of 5 to 30 g kg^{-1} (Reddy & Sathe, 2002). One of the main problems related with the use of low cost plant proteins in aqua-feed is the presence of anti-nutritional factors like phytate or phytic acid (myo-inositol 1, 2, 3, 4, 5, 6-hexaphosphate), which has deleterious effect on the morphology and physiology of digestive tract, disturbing the overall fish growth performance (Usmani & Jafri, 2002; Baruah *et al*., 2004). It is thought that about 80% of the total phosphorus (P) content in plants may be found in the form of phytate that is practically not available for agastric or mono-gastric fishes (NRC, 1993).

Phytase is an enzyme chemically known as myo-inositol hexaphosphate phosphohydrolase and belongs to Class 3: Hydrolases, that may be produced either by microorganisms or may be present in some plant ingredients. It is very specific to hydrolyze the indigestible phytate that is present in plant protein sources. Mono gastric fishes cannot produce this enzyme and can not hydrolyze the phytate. Moreover, supplementation of phytase in fish

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feeds has been generally reported to improve the bio-availability and utilization of plant phosphorus (P) by fish (Cao *et al*., 2007). Better use of nutrients also results in less discharge of P into the aquatic environment causing less aquatic pollution (Baruah *et al*., 2004). Phytase improves the nutrient digestibility and fish growth performance fed on plant in *L. rohita* fingerlings (Baruah *et al*., 2007a). Moreover, Baruah *et al.* (2005) observed better growth with supplementation of microbial phytase in combination with different protein levels in juveniles of *L. rohita*.

Corn gluten meal has also been used very successfully as the dietary protein sources in diets for tilapia (*Oreochromis niloticus*). The growth of tilapia fingerlings fed on corn gluten meal-based diet as protein source was better than of the fish fed on fish meal-based diet (Tudor *et al*., 1996). Corn gluten meal was found to be a suitable protein source in *Oreochromis niloticus* diets if the proper amino acid balance was maintained (Wu *et al*., 1995). It was found highly digestible by Nile tilapia (*Oreochromis niloticus*) and the digestion coefficients were comparable to fish meal protein (Koprucu & Ozdemir, 2005). Research is needed to study the suitability of corn gluten meal (30%) based diets with graded levels of phytase supplementation for commercially important species of carps like *L. rohita* to enhance the fish growth performance and to overcome the problems of costly fish meal and aquatic pollution caused by phytate phosphorus rich excreta.

MATERIALS AND METHODS

The experiment was carried out to study the effect of phytase on growth performance and apparent nutrient digestibility of *L. rohita* fingerlings fed on corn gluten meal (30%) based test diets with graded levels of phytase. The experiment was conducted in the Fish Nutrition Laboratory, Department of Zoology and Fisheries, University of Agriculture, Faisalabad. *L. rohita* fingerlings were obtained from Government Fish Seed Hatchery, Faisalabad and were allowed to acclimatize with experimental conditions in laboratory for two weeks in V-shaped tanks (UA system) having 70 L capacity, specially designed for the collection of fecal material from water media. During this period the fingerlings were fed once daily to apparent satiation on the basal diet used in subsequent digestibility study (Allan & Rowland, 1992). Water quality variables, particularly water temperatures, pH and dissolved oxygen were monitored through pH meter (Jenway 3510) and D.O. meter (Jenway 970). Aeration was provided round-the-clock to all the tanks through capillary system. Before starting experiment, *L. rohita* fingerlings were treated with (5 g/L) NaCl to make sure that they were free from ectoparasites and to prevent fungal infection (Rowland & Ingram, 1991).

The feed ingredients were purchased from market and analyzed for chemical composition following AOAC (1995) prior to the formulation of the experimental diets (Table I). The reference diet was prepared to supply adequate levels of required nutrients for normal fish growth. Chromic oxide was used as an inert marker at 1% inclusion level in reference diet. The test diets were composed of 70% reference diet and 30% of the test ingredient (corn gluten meal 30%) to be tested (Table II). The feed ingredients were finely grounded to pass through 0.5 mm sieve size. All ingredients were mixed in electric mixture for 10 min and fish oil was gradually added thereafter. During mixing, 10-15% water was also added to provide moisture. The floating pellets (3 mm) were prepared using Lab Extruder (SYSLG30-IV Experimental Extruder). The phytase (Phyzyme® XP 10000 FTU/g; Danisco Animal Nutrition, Fin-65101 Vaasa, Finland) solution was prepared by dissolving 2 g of microbial phytase (powder form) in 1000 ml of distilled water (Robinson *et al*., 2002). Seven test diets were then prepared by spraying graded levels of phytase to corn gluten meal (30%) based diets at 0, 250, 500, 750, 1000, 1250 and 1500 FTU/kg diet. One unit of phytase activity (FTU) is defined as the enzyme activity that liberates 1 µmol of inorganic orthophosphate min^{-1} at pH 5.5 (37°C) at a substrate concentration (sodium phosphate) of 5.1 µmol/L (Engelen *et al*., 1994).

Feeding protocol and sample collection: The fingerlings of *L. rohita* were fed twice daily (morning & afternoon) to approximate satiation. Initially, the fingerlings were fed at the rate of 2% of live wet weight on their prescribed diet and subsequently adjusted on daily feed intake. For each test diet three replicates were assigned and in each replicate fifteen fish (average weight: 7.04 g/fish) were stocked. After the feeding session of three hours, the uneaten diet was drained out from each tank by opening the valves of the tanks. The tanks were washed completely to remove the particles of diets and refilled with water. After this, feces were collected from the fecal collection tube of each tank by opening the valve I and valve II subsequently. Care was taken to avoid breaking the thin fecal strings in order to minimize nutrient leaching. Fecal material of each replicated treatment was dried in oven, ground and stored for chemical analysis. The experiment was lasted for ten weeks for the collection of 4-5 g fecal material of each replicate. Water quality variables, particularly water temperature, pH, and dissolved oxygen were monitored on daily basis using digital meters. The range of water quality parameters like temperature was 24.9-28.7ºC, pH 7.4-8.6 and dissolved oxygen 5.8-7.3 mg/L.

Chemical analysis of feed and feces: The samples of feed ingredients, test diets and feces were homogenized using a motor and pestle and analyzed following AOAC (1995): moisture was determined by oven-drying at 105°C for 12 h; crude protein (N x 6.25) by micro kjeldahl apparatus; crude fat, by petroleum ether extraction method (Bligh & Dyer, 1995) through Soxtec HT2 1045 system; crude fiber, as loss on ignition of dried lipid-free residues after digestion with 1.25% H_2SO_4 and 1.25% NaOH; ash, by ignition at 650°C for 12 hours in electric furnace (Eyela-TMF 3100) to constant weight. Total carbohydrate (N-free extract) was

calculated by difference i.e., Total carbohydrate % = 100-(Crude protein%+Ether extract%+Ash%+ CF). Gross energy was determined with the help of oxygen bomb calorimeter. For mineral estimation, the diets and feces samples were digested in a boiling nitric acid and perchloric acid mixture (2:1) according to AOAC (1995). After appropriate dilution, minerals contents were estimated using atomic absorption (Hitachi Polarized Atomic Absorption Spectrometer, Z-8200). The phosphorus was analyzed calorimetrically (UV/VIS spectrophotometer) at 720 nm absorbance. The estimation of sodium and potassium was done through flame photometer (Jenway PFP-7, UK). Chromic oxide contents in diets and feces were estimated after oxidation with molybdate reagent (Divakaran *et al.*, 2002) using UV-VIS 2001 Spectrophotometer at 370nm absorbance.

Fish in each tank were bulk weighed every 15th day during experiment to assess the growth performance of *L. rohita* fingerlings. Weight gain (%) of fingerlings was evaluated based on standard formula.

$$\text{Weight gain \%} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Feed conversion ratio of corn gluten meal (30%) based test diets was calculated by standard formula:

$$\text{FCR} = \frac{\text{Total dry feed intake (g)}}{\text{Wet weight gain (g)}}$$

Apparent nutrient digestibility coefficients (ADC) of reference and test diets were calculated as follows (NRC, 1993):

$$\text{ADC (\%)} = 100 - 100 \times \frac{\text{Percent marker in diet} \times \text{Percent nutrient in feces}}{\text{Percent marker in feces} \times \text{Percent nutrient in diet}}$$

Statistical analysis: Finally, data of growth parameters and nutrients digestibility of experimental diets was subjected to one-way analysis of variance, ANOVA (Steel *et al*., 1996.) The differences among means were compared by Tukey's honesty significant difference test and considered significant at $P<0.05$ (Snedecor & Cochran, 1991). The CoStat computer package (Version 6.303, PMB 320, Monterey, CA, 93940 USA) was used for statistical analysis.

RESULTS

The maximum weight gain (4.68±0.017 g) in fish was observed in case of corn gluten meal (30%) based test diet containing 750 FTU kg^{-1} level. However, it was not significantly different from the weight gain observed at 1000 FTU kg^{-1} level. When compared with remaining diets, these values were labeled significantly different ($P<0.05$). The weight gain (%) of the *L. rohita* fingerlings administered in the various phytase supplementation diets followed the same trend as it was observed in case of mean weight gain. The highest weight gain (13.4%) was again observed at 750 FTU kg^{-1} diet, which was higher than that of the weight gain value observed with reference diet. The minimum FCR value (1.35±0.047) was recorded at 750 FTU kg^{-1} level and it was not significantly different ($p<0.05$) from the FCR values of reference and 1000 FTU kg^{-1} levels of phytase supplementation diets. FCR values, calculated for of 0, 250, 1250 and 1500 FTU kg^{-1} levels diets were statistically ($P<0.05$) at par (Table III).

Analyzed nutrients (crude protein, crude fat & apparent gross energy) contents of reference and test diets are presented in Table IV and fecal matter in Table 5. The apparent nutrients digestibility (%) for corn gluten meal (30%) based test diets are shown in Table VI. It is obvious from the results that, in comparison with reference diet, corn gluten meal (30%) based diets supplemented with phytase enzyme released least amount of nutrients through feces at 750 and 1000 FTU kg^{-1} levels (Table V). This decrease in nutrient excretion through feces confirms that the phytase has the property of hydrolyzing the phytate-protein complexes. From the analysis of variance on the data, it is apparent that maximum crude protein digestibility (%) was observed at 1000 and 750 FTU kg^{-1} level and these values were not significantly different from each other and the values of reference diet but significantly different ($P<0.05$) from the remaining diets. The highest crude fat and apparent gross energy digestibility values of corn gluten meal-based test diet observed at 750 and the next higher digestibility was observed at 1000 FTU kg^{-1} level. These values at these levels differed from each other but not significantly however, these were significantly different ($P<0.05$) from digestibility values of reference diet and remaining test diets. It was interestingly observed that except 750 and 1000 FTU kg^{-1} levels, remaining levels of phytase supplementation in corn gluten meal (30%) based diets could not show better crude protein digestibility as compared to the reference diet. Crude fat and apparent gross energy digestibility also performed their best at 750 FTU kg^{-1} followed by 1000 FTU kg^{-1} level, while maximum crude protein digestibility was observed at 1000 FTU kg^{-1} level. Feeds treated with further higher levels of enzyme surprisingly resulted in drastic decrease in crude protein digestibility.

DISCUSSION

The growth performance of *L. rohita* fingerlings in terms of final fish weight, fish weight gain, weight gain (g fish^{-1} day^{-1}), weight gain (%), feed intake (g fish^{-1} day^{-1}) and feed conversion ratio (FCR) was significantly improved on corn gluten meal (30%) based experimental diets with graded levels of phytase supplementation upto certain limits. A significant enhancement in growth performance of *L. rohita* fingerlings was commensurate with the increase in phytase supplementation to a level of 750 FTU kg^{-1} diet, after which it was decreased. The supplementation of 1000 FTU kg^{-1} diet resulted next higher weight gain. The weight gain of *L. rohita* fingerlings at 750 FTU kg^{-1} was 13.40% higher over the weight gain of fingerlings fed with reference diet. The findings of the study provide evidence that the level of

Table I: Chemical composition (%) of feed ingredients

Ingredients	Dry matter (%)	Crude Protein (%)	Crude Fat (%)	Crude Fiber (%)	Ash (%)	Total carbohydrate (%)	Gross Energy (kcal/g)
Fish meal	91.63	48.15	7.16	0.52	26.23	17.94	3.69
Wheat flour	92.45	10.10	2.35	1.65	2.08	83.82	2.96
Corn gluten 60%	92.59	59.12	4.96	1.19	1.58	33.15	4.23
Rice polish	94.09	12.35	12.31	2.71	7.90	64.73	4.33
Corn gluten meal 30%	93.71	27.59	2.46	1.53	9.56	41.18	4.87

Table II: Ingredients composition (%) of reference and test diets

Ingredients	Reference diet	Test diets
Fish meal	20.0	14.0
Wheat flour	24.0	16.8
Corn gluten 60%	20.0	14.0
Rice polish	25.0	17.5
Fish oil	7.0	4.9
Vitamin Premix*	1.0	0.7
Minerals	1.0	0.7
Ascorbic acid	1.0	0.7
Chromic oxide	1.0	0.7
Corn gluten meal (30%)	-	30.0

Table III: Growth performance of *Labeo rohita* fingerlings fed on reference and corn gluten meal (30%)-based test diets

Parameters	Reference diet	Test diet-I	Test diet-II	Test diet-III	Test diet-IV	Test diet-V	Test diet-VI	Test diet-VII
		Phytase levels (FTU kg^{-1})						
		0	250	500	750	1000	1250	1500
Initial weight (g)	7.05±0.011	7.03±0.020	7.03±0.021	7.04±0.021	7.05±0.026	7.04±0.021	7.04±0.031	7.04±0.023
Final weight (g)	10.78±0.050	10.03±0.055	10.20±0.066	10.51±0.093	11.73±0.040	11.57±0.092	10.11±0.068	10.02±0.051
Weight gain (g)	$3.73±0.060^{b}$	$3.00±0.067^{d}$	$3.17±0.080^{d}$	$3.48±0.084^{c}$	$4.68±0.017^{a}$	$4.53±0.11^{a}$	$3.08±0.074^{d}$	$2.98±0.070^{d}$
Weight gain (%)	$52.98±0.93^{b}$	$42.72±1.03^{d}$	$45.16±1.25^{d}$	$49.41±1.15^{c}$	$66.38±0.21^{a}$	$64.43±1.73^{a}$	$43.73±1.13^{d}$	$42.35±1.12^{d}$
Weight gain ($fish^{-1}$ day^{-1}) g	0.053±0.00086	0.043±0.00095	0.045±0.0011	0.050±0.0012	0.067±0.00025	0.065±0.0016	0.044±0.0011	0.043±0.0010
Feed intake ($fish^{-1}$ day^{-1}) g	0.074±0.0025	0.0075±0.0021	0.074±0.0047	0.078±0.0050	0.090±0.0031	0.091±0.0047	0.074±0.0031	0.072±0.0035
FCR	$1.39±0.041^{ab}$	$1.74±0.046^{c}$	$1.64±0.12^{c}$	$1.58±0.067^{bc}$	$1.35±0.047^{a}$	$1.41±0.095^{ab}$	$1.68±0.030^{c}$	$1.68±0.110^{c}$

Means within rows having different superscripts are significantly different at $P < 0.05$

Data are means of three replicates ± S.D

750 FTU kg^{-1} diet was probably enough for reducing the effect of phytic acid and releasing the chelated protein and minerals of plant based diets. The present results of growth performance of *L. rohita* fingerlings on corn gluten meal (30%) based diets are in agreement with the findings of Baruah *et al*. (2007a). They demonstrated significant improvement in growth performance of *L. rohita* fingerlings when fed plant based diets supplemented with microbial phytase at 750 U kg^{-1} level. Similar results were also reported in Nile tilapia (*Oreochromis niloticus*) by Liebert and Portz (2005 & 2007) at supplementation of phytase (SP 1002) at 750 FTU kg^{-1} diet. However, the best growth performance in Nile tilapia (*Oreochromis niloticus*) fed on plants based diet was observed at 1000 FTU kg^{-1} (Riche & Garling, 2004; Ashraf & Goda, 2007; Cao *et al*., 2008). On the other hand no significant difference in weight gain of carnivorous fishes like channel catfish, *Zctulurus punctutus* (Yan & Reigh, 2002) and Atlantic salmon *Salmo salar* (Sajjadi & Carter, 2004) was reported when fed phytase supplemented plant based diets. This inconsistency in the outcome of different authors may be attributed to differences in phytic acid content in different feed ingredients, nutritional quality of plant ingredients, water quality, fish species and size and culture or experimental conditions (Ashraf & Goda, 2007).

In the present study, phytase supplementation significantly improved feed intake and feed conversion ratio (FCR) of *L. rohita* fingerlings fed on corn gluten meal (30%) based test diets. The maximum feed intake and best FCR values were observed in 750 FTU kg^{-1} diet and these values were significantly different from FCR values of reference diet and other test diets. It means that 750 FTU kg^{-1} level of phytase supplementation has increased the palatability and conversion rate of diet into flesh. Higher feed intake was reported in various fish species such as catfish, *Ictalurus punctatus* (Jackson *et al*., 1996; Li & Robinson, 1997), Atlantic salmon, *Salmo salar* (Hauler & Carter, 1997) in Nile tilapia, *Oreochromis nilotics* (Tahoun *et al*., 2009). Improved FCR were observed in rainbow trout fed on soybean meal-based diet supplemented with phytase (Wang *et al*., 2009). The findings of present study are comparable with the findings of Baruah *et al*. (2007a). They

Table IV: Analyzed composition of nutrient (%) in diets of reference and corn gluten meal (30%) based diets

Diets	Phytase levels (FTU kg^{-1})	Crude protein (%)	Crude fat (%)	Gross energy (kcal/g)
Reference diet	--	30.21±0.17	6.65±0.015	4.26±0.10
Test diet I	0	30.06±0.14	5.24±0.023	4.35±0.030
Test diet II	250	30.04±0.20	5.25±0.017	4.34±0.021
Test diet III	500	30.26±0.04	5.24±0.015	4.34±0.026
Test diet IV	750	30.16±0.10	5.24±0.015	4.33±0.025
Test diet V	1000	30.37±0.44	5.26±0.012	4.32±0.030
Test diet VI	1250	30.11±0.08	5.23±0.015	4.33±0.015
Test diet VII	1500	30.15±0.11	5.25±0.012	4.32±0.010

Table V: Analyzed composition of nutrient (%) in feces of reference and corn gluten meal (30%) based diets

Diets	Phytase levels (FTU kg^{-1})	Crude protein (%)	Crude fat (%)	Gross energy (kcal/g)
Reference diet	--	13.63±0.22	2.30±0.042	1.84±0.012
Test diet I	0	16.69±0.32	2.02±0.075	2.36±0.042
Test diet II	250	16.57±0.29	1.82±0.031	2.18±0.030
Test diet III	500	16.03±0.07	1.72±0.026	1.85±0.032
Test diet IV	750	13.17±0.12	1.57±0.053	1.74±0.031
Test diet V	1000	12.33±0.06	1.67±0.057	1.92±0.036
Test diet VI	1250	15.31±0.07	1.80±0.101	2.11±0.021
Test diet VII	1500	16.51±0.40	1.89±0.087	2.11±0.021

Table VI: Apparent nutrient digestibility (%) of reference and corn gluten meal (30%)-based diets

Diets	Phytase levels (FTU kg^{-1})	Crude protein (%)	Crude fat (%)	Apparent gross energy (%)
Reference diet	--	58.88 ±1.02^{a}	69.02±0.64^{bc}	61.05±0.89^{b}
Test diet I	0	48.67±1.83^{b}	64.39±1.39^{d}	51.62±0.90^{d}
Test diet II	250	50.41±1.84^{b}	68.91±0.66^{bc}	53.69±2.34^{cd}
Test diet III	500	51.50±1.30^{b}	69.97±0.41^{bc}	62.53±0.21^{ab}
Test diet IV	750	61.77±1.38^{a}	73.76±1.01^{a}	65.62±1.76^{a}
Test diet V	1000	63.77±0.47^{a}	71.58±1.43^{ab}	63.88±1.73^{ab}
Test diet VI	1250	53.03±2.47^{b}	68.27±1.45^{c}	56.71±1.00^{c}
Test diet VII	1500	50.27±3.23^{b}	67.30±0.46^{c}	56.64±1.74^{c}

also noticed improved FCR for *L. rohita* fed plant based diet supplemented with microbial phytase. In present study the improvement in weight gain, feed intake and feed conversion ratio of *L. rohita* fingerlings was due to enhanced release of nutrients of plant based diets by breaking down the bonds between phytate-protein and phytate-minerals (Vielma *et al*., 1998).

Phytate binds with trypsin thus reduces the bioavailability of a protein components and overall performance of fish reduces (Tacon, 1997). It can also form phytate-protein and phytate-mineral-protein complexes that create resistance to proteolytic digestion (Cheryan, 1980). Furthermore, phytate may chelate with amino acids in the stomach of different fish species and reduces availability of amino acid (Usmani & Jafri, 2002). In the present study, protein digestibility by *L. rohita* fingerlings fed without or low dose of phytase supplementation confirms the above mentioned theories. However, the poor performance of fish with reference to protein digestibility at higher doses of phytase supplementation is surprising and difficult to explain. The highest apparent crude protein digestibility (%) of *L. rohita* fingerlings fed on corn gluten meal (30%) based diet was observed with 1000 FTU kg^{-1} diet, while the next higher digestibility was observed at 750 FTU kg^{-1} diet. An increasing trend of crude protein digestibility was reported by Baruah *et al*. (2007a), while Liebert and Portz, (2007) also achieved optimal protein digestibility at almost similar phytases (SP 1002 & Ronozyme® P) supplementation. The higher apparent Digestibility Coefficient (ADC) of crude protein in soybean meal-based diets supplemented with microbial phytase, observed in the present research work, clearly indicated that the acceptability of the alternative plant protein based test diets supplemented with phytase enzyme has increased as reported by Vielma *et al*. (2004); Liebert and Portz (2005), and Ashraf and Goda, (2007), Nwanna *et al*. (2008), Laining *et al*. (2010), Wang *et al*., 2009. However, Yan and Reigh (2002); Sajjadi and Carter (2004); Dalsgaard *et al*. (2009) could not see any significant effect on protein digestibility. A decrease in protein digestibility by phytase supplementation was also reported by Hossain and Jauncey (1993) and Teskeredzic *et al*. (1995). This discrepancy, observed in several studies for nutrient digestibility, can be linked to variation in protein quality of feed ingredients, pH of fish stomach and drying procedures (Wang *et al*., 2009). Generally, the impact of phytase supplementation on nutrient digestibility depend on a variety of factors such as concentration and source of phytate in the diet, concentration and source of protein in the diet (Selle *et al*., 2000), digestibility of protein source, levels of calcium and phosphorus (Sugiura *et al*., 2001). Additionally, methods used for phytase addition during diet manufacturing, such as pretreatment of feed ingredient or direct phytase supplementation to the diet, which may also have influences on feed utilization efficiency and fish growth performance (Ashraf & Goda, 2007).

Like the crude protein, the significantly highest values of crude fat and gross energy digestibility were observed at 750 FTU kg^{-1} diet as compared with reference diet and other remaining phytase levels based test diets. Portz and Liebert (2004), and Ashraf and Goda (2007) noticed optimal fat and gross energy digestibility values of diets similar or a bit higher doses (1000-2000 FTU kg^{-1}) of phytase supplementation. Nwanna (2005) suggested that a very high dose of phytase (8000 FTU kg^{-1} level) supplementation is required for optimal gross energy digestibility in Nile tilapia. In contrary, the doses of phytase above 1000 FTU kg^{-1} resulted in significant decrease in fat digestibility coefficients might be due to limited amount of fat contents of experimental diets. As reported by Wang *et al.* (2009). However, Dalsgaard *et al*. (2009) and Lanari *et al*. (1998) observed no effect on the crude fat and gross energy digestibility. This recommends that the effect of phytase supplementation on the gross energy utilization may be dependent on ingredient source, phytase dosage and fish size. In the present findings, supplementation of phytase

enhanced gross energy digestibility in all ingredients, while the effect on sunflower meal was the most significant as compared with other plant ingredients.

A large number nutritional factors such as concentration and source of phytic acid in the fish diet, source and amount of protein in the that diet (Selle *et al*., 2000), digestibility levels of protein source, levels of calcium and phosphorus seems to be involved (Sugiura *et al*., 2001). Moreover, the methods used for phytase incorporation during fish diet manufacturing, such as direct enzyme supplementation on diet ingredient or pretreatment with enzyme phytase could also have influence on nutrient digestibility, growth performance of fish and efficiency of feed utilization. Variations in the conclusions of various authors may also be attributed to difference in phytate content in different feed ingredients, fish species used and a range of other inherent characteristics of feed ingredients (Debnath *et al*., 2005).

CONCLUSION

Phytase supplementation to corn gluten meal (30%)-based diets at 750 FTU kg^{-1} level, is enough to release sufficient bounded nutrients for optimal growth performance of *L. rohita* fingerlings.

REFERENCES

Allan, G.L. and S.J. Rowland, 1992. Development of an experimental diet for silver perch (*Bidyanus bidyanus*). *Austasia Aqua*., 6: 39–40

Ashraf, M. and A.S. Goda, 2007. Effect of dietary soybean meal and phytase levels on growth, feed utilization and phosphorus discharge for Nile tilapia (*Oreochromis niloticus* L.). *J. Fish. Aquat. Sci*., 2: 248–263

Association of Official Analytical Chemists (AOAC), 1995. *Official Methods of Analysis*, 15th edition, p: 1994. Association of Official Analytical Chemists, Washington DC

Baruah, K., A.K. Pal, P.S. Narottam, K.K. Jain, S.C. Mukherjee and D. Debnath, 2005. Dietary protein level, microbial phytase, citric acid and their interactions on bone mineralization of *Labeo rohita* (H) juveniles. *Aquat. Res*., 36: 803–812

Baruah, K., K.A.K. Pal, P.S. Narottam and D. Debnath, 2007a. Microbial Phytase supplementation in rohu, *Labeo rohita*, diets enhances growth performance and nutrient digestibility. *J. World Aquat. Soc*., 38: 129–137

Baruah, K., N.P. Sahu, A.K. Pal and D. Debnath, 2004. Dietary Phytase: An ideal approach for cost effective and low-polluting aquafeed. *NAGA, World Fish Center Quat.*, 27: 15–19

Baruah, K., N.P. Sahu, A.K. Pal, K.K. Jain, D. Debnath and S.C. Mukherjee, 2007b. Dietary microbial phytase and citric acid synergistically enhances nutrient digestibility and growth performance of *Labeo rohita* (Hamilton) juveniles at sub-optimal protein level. *Aquat. Res*., 38: 109–120

Bligh, E.G. and W.J. Dyer, 1995. A rapid method of total fat extraction and purification. *Canadian J. Biochem. Physiol*., 37: 911–917

Cao, L., W. Wang, C. Yang, Y. Yang, J. Diana, A. Yakupitiyage, Z. Luo and D. Li, 2007. Application of microbial phytase in fish feed. *Enz. Microb. Technol*., 14: 342–362

Cao, L., Y. Yang, W.M. Wang, A. Yakupitiyage, D.R. Yuan and J.S. Diana, 2008. Effects of pretreatment with microbial phytase on phosphorous utilization and growth performance of Nile tilapia (*Oreochromis niloticus*). *Aquacult. Nutr*., 14: 99–109

Cheng, Z.J. and R.W. Hardy, 2002. E ect of microbial phytase on apparent nutrient digestibility of barley, canola meal, wheat and wheat middlings, measured in vivo using rainbow trout (*Oncorhynchus mykiss*). *Aquacult. Nutr*., 8: 271–277

Cheryan, M., 1980. Phytic acid interactions in food systems. CRC; *Crit. Rev. Food Sci. Nutr*., 13: 297–335

Dalsgaard, J., K.S. Ekmann, P.B. Pedersen and V. Verlhac, 2009. Effect of supplemented fungal phytase on performance and phosphorus availability by phosphorus-depleted juvenile rainbow trout (*Oncorhynchus mykiss*) and on the magnitude and composition of phosphorus waste output. *Aquaculture*, 286: 105–112

Debnath, D., A.K. Pal, P.S. Narottam, K.K. Jain, S. Yengkokpam and S.C. Mukherjee, 2005. Effect of dietary microbial phytase supplementation on growth and nutrient digestibility of *Pangasius pangasius* (Hamilton) fingerlings. *Aquacult. Res*., 36: 180–187

Divakaran, S., G.O. Leonard and P.F. Ian, 2002. Note on the methods for determination of chromic oxide in shrimp feeds. *J. Agric. Food Chem*., 50: 464–467

Engelen, A.J., F.C. Van Der Heeft, P.H.G. Randsdrop and E.L.C. Smith, 1994. Simple and rapid determination of phytase activity. *JAOAC*, 77: 760–764

Gatlin, I.I.I., D.M., F.T. Barrows, P. Brown, K. Dabrowski, T.G. Gaylord, R.W. Hardy, E. Herman, G. Hu, A. Krogdahl, R. Nelson, K. Overturf, M. Rust, W. Sealey, D. Skonberg, E.J. Souza, D. Stone, R. Wilson and E. Wurtele, 2007. Expanding the utilization of sustainable plant products in aqua feeds: a review. *Aquacult. Res*., 38: 551–579

Hardy, R.W., 2000. New developments in aquatic feed ingredients and potential of enzyme supplements. *In*: Cruz-Suarez, L.E., D. Ricque-Marie, M. Tapia-Salazar, M.A. Olvera-Novoa and R. Civera-cerece-do (eds.), *Advances en Nutricion Acuicola V. Memorias del V Simposium Internacional de Nutricion Acuicola, Merida*, Yucatan, Mexico, pp: 216–226

Hauler, R.C. and C.G. Carter, 1997. Phytase stimulates appetite in Atlantic salmon *Salmo salar* parr fed soybean meal. *Proc. Nutr. Soc. Australia*, 21: 139–148

Hossain, M.A. and K. Jauncey, 1993. The effects of varying dietary phytic acid, calcium and magnesium levels on the nutrition of common carp, *Cyprinus carpio*. *In*: *Fish Nutrition in Practice. 4th International Symposium on Fish Nutrition and Feeding, Biarritz, France*, pp: 705–715. June 24-27, 1993. INRA, Paris, France

Jackson, L., M.H. Li and E.H. Robinson, 1996. Use of microbial phytase in channel catfish *Ictalurus punctatus* diets to improve utilization of phytate phosphorus. *J. World Aquacult. Soc*., 27: 309–313

Koprucu, K. and Y. Ozdemir, 2005. Apparent digestibility of selected feed ingredients for Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, 250: 308–316

Laining, A.M. Ishikwa, K. Kyaw, J. Gao, N.T. Binh, S. Koshio, S. Yamaguchi, S. Yokoyama and J. Koyama, 2010. Dietary calcium/phosphorous ratio influences the efficacy of microbial phytase on growth, minerals digestibility and vertebral mineralization in juvenile tiger puffer, *Takifugu rubripes. Aquacult. Nutr.*, 17: 267–277

Lanari, D., E.D. Agaro and C. Turri, 1998. Use of nonlinear regression to evaluate the effects of phytase enzyme treatment of plant protein diets for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 161: 345–356

Li, M.H. and E.H. Robinson, 1997. Microbial phytase can replace inorganic phosphorus supplements in channel catfish *Ictalurus punctatus* diets. *J. World Aquacult. Soc*., 28: 402–406

Liebert, F. and L. Portz, 2005. Nutrient utilization of Nile tilapia *Oreochromis niloticus* fed plant based low phosphorus diets supplemented with graded levels of different sources of microbial phytase. *Aquaculture*, 248: 111–119

Liebert, F. and L. Portz, 2007. Different sources of microbial phytase in plant based low phosphorus diets for Nile tilapia *Oreochromis niloticus* may provide different effects on phytate degradation. *Aquaculture*, 267: 292–299

Lim, S.J., S. Kim, G. Ko, J. Song, D. Oh, J. Kim and K. Lee, 2011. Fish meal replacement by soybean meal in diets for Tiger puffer, *Takifugu rubripes*. *Aquaculture*, 313: 165–170

Lunger, A.N., E. McLean and S.R. Craig, 2007. The effects of organic protein supplementation upon growth, feed conversion and texture quality parameters in juvenile cobia (*Rachycentron canadum*). *Aquaculture*, 264: 342–352

National Research Council (NRC), 1993. *Nutrient Requirements of Fish*, p: 114. Washington, DC, National Academy Press

Nwanna, L.C., 2005. Effect of Toasting and Incubation of Soybean Meal Supplemented with Phytase in Practical Diets on the Growth and Mineral Deposition in Nile Tilapia. *J. Anim. Vet. Adv*., 4: 234–239

Nwanna, L.C., C.A. Oishi and M.P. Filho, 2008. Use of phytase to improve the digestibility of alternative feed ingredients by Amazon tambaqui, *Colossoma Macropomum*. *Sci. Asia*, 34: 353–360

Pham, M.A., K.J. Lee, T.M. Dang, S.J. Lim, G.Y. Ko, J. Eo and D.H. Oh, 2008. Improved apparent digestibility coefficient of protein and phosphorus by supplementation of microbial phytase in diets containing cottonseed and soybean meal for juvenile olive flounder (*Paralichthys olivaceus*). Asian-Australas. *J. Anim. Sci.,* 21: 1367–1375

Portz, L. and F. Liebert, 2004. Growth, nutrient utilization and parameters of mineral metabolism in Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) fed plant based diets with graded levels of microbial phytase. *J. Anim. Physiol. Anim. Nutr*., 88: 311–320

Reddy, N. and S.K. Sathe, 2002. *Occurrence, Distribution, Content and Dietary Intake of Phytate, Food Phytates*, pp: 25–52. CRC Press, New York

Riche, M. and D.L. Garling, 2004. Effect of phytic acid on growth and nitrogen retention in tilapia *Oreochromis niloticus* L. *Aquacult. Nutr*., 10: 389–400

Robinson, E.H., M.H. Li and B.B. Manning, 2002. Comparison of microbial phytase and dicalcium phosphate or growth and bone mineralization of pond-raised channel catfish, *Ictalurus punctatus*. *J. Appl. Aquacult.*, 12: 81–88

Rowland, S.J. and B.A. Ingram, 1991. *Diseases of Australian Native Fishes*. In: Fisheries Bulletin 4 NSW Fisheries, Sydney, NSW, Australia

Sajjadi, M. and C.G. Carter, 2004. E ect of phytic acid and phytase on feed intake, growth, digestibility and trypsin activity in Atlantic salmon (*Salmo salar* L.). *Aquacult. Nutr*., 10: 135–142

Selle, P.H., V. Ravindran, R.A. Caldwell and W.L. Bryden, 2000. Phytate and phytase: Consequences for protein utilization. *Nutr. Res. Rev*., 13: 255–278

Snedecor, G.W. and W.G. Cochran, 1991. *Statistical Methods*, 8th edition, p: 503. Iowa State University Press, Ames. Iowa

Steel, R.G.D., J.H. Torrie and D.A. Dickey, 1996. *Principles and Procedures of Statistics*: *A Biomaterical Approach*, 3rd edition, pp: 336–352. McGraw Hill International Book Co. Inc., New York

Sugiura, S.H., J. Gabaudan, F.M. Dong and R.W. Hardy, 2001. Dietary microbial phytase supplementation and the utilization of phosphorus, trace minerals and protein by rainbow trout *Oncorhynchus mykiss* (W) fed soybean meal-based diets. *Aquacult. Res*., 32: 583–592

Tacon, A.G.J., 1997. Fish Meal Replacers: Review of Anti nutrients Within Oilseeds and Pulses-a Limiting Factor for the Aquafeed Green Revolution? *In*: Tacon, A. and B. Basurco (eds.), *Feeding Tomorrow's Fish*, pp: 153–182. Proceedings of the workshop of the CIHEAM Network on Technology of Aquaculture in the Mediterranean (TECAM), jointly organized by CIHEAM, FAO and IEO Mazarron, Spain, 24-26 June 1996, CIHEAM, Apodo, Spain

Tahoun, A.M., H.A. Abo-State and Y.A. Hammouda, 2009. Effect of adding commercial phytase to DDGS based diets on the performance and feed utilization of Nile tilapia (*Oreochromis niloticus*) fingerlings. *American-Eurasian J. Agric. Environ. Sci*., 5: 550–555

Teskeredzic, Z., D.A. Higgs and B.S. Dosanjh, 1995. Assessments of undephytinized and dephytinized rapeseed protein concentrate as sources of dietary protein for juvenile rainbow trout (*Onchorhynchus mykiss*). *Aquaculture*, 131: 261–277

Tudor, K.W., R.R. Rosati, P.D. O'Rourke, Y.V. Wu, D. Sessa and P. Brown, 1996. Technical and economic feasibility of on farm fish feed production using fishmeal analogs. *Aquacult. Eng.*, 15: 53–65

Usmani, N. and A.K. Jafri, 2002. Influence of dietary phytic acid on the growth, conversion efficiency and carcass composition of *Cirrhinus mrigala* (H) fry. *J. World Aquacult. Soc.*, 33: 199–204

Vielma, J., K. Ruohonen, J. Gabaudan and K. Vogel, 2004. Top-spraying soybean meal-based diets with phytase improves protein and mineral digestibilities but not lysine utilization in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquacult. Res*., 35: 955–964

Vielma, J., S.P. Lall and J. Koskela, 1998. Effects of dietary phytase and cholecalciferol on phosphorus bioavailability in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 163: 309–323

Wang, F., Y.H. Yang, Z.Z. Han, H.W. Dong, C.H. Yang and Z.Y. Zou, 2009. Effects of phytase pretreatment of soybean meal and phytase-sprayed in diets on growth, apparent digestibility coefficient and nutrient excretion of rainbow trout (*Oncorhynchus mykiss* Walbaum). *Aqua. Int*., 17: 143–157

Wu, Y.V., R.R. Rasati, D. Sessa and P. Brown, 1995. Utilization of corn gluten feed by Nile tilapia. *Prog. Fish Cult*., 57: 305–309

Yan, W. and R.C. Reigh, 2002. Effects of fungal phytase on utilization of dietary protein, minerals, and dephosphorylation of phytic acid in the alimentary tract of channel catfish *Zctulurus punctutus* fed an All-Plant-Protein Diet. *J. World Aqua. Soc.*, 33: 10–22

Zhou, Q.C., B.P. Tan, K.S. Mai and Y.J. Liu, 2004. Apparent digestibility of selected feed ingredients for juvenile cobia *Rachycentron canadum*. *Aquaculture*, 241: 441–451

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