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EFFICACY OF PHYTASE SUPPLEMENTATION ON MINERAL DIGESTIBILITY IN *LABEO ROHITA* FINGERLINGS FED ON CORN GLUTEN MEAL (30%) BASED DIETS

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This study was conducted to assess the effects of phytase supplementation on minerals digestibility to *Labeo rohita* fingerlings that were fed corn gluten meal (30%) based diet. The experimental diet was consisted of 70% reference diet and 30% test ingredient i.e. corn gluten meal contents. The chromic oxide was included at a concentration of 1% as non-digestible marker in the feed. The results of the study showed that the high digestibility (%) of P, Mg, Na, K, Cu and Zn was observed at 750 FTU kg^{-1} level phytase supplementation and differed significantly ($p<0.05$) from the reference and other test diets. The present study suggests that the supplementation of feed with phytase enzyme may reduce the need for supplementing minerals, resulting not only lower feed cost but mineral discharges through feces into the aquatic ecosystem.
Keywords: *Labeo rohita*, phytase, corn gluten meal, mineral digestibility

INTRODUCTION

The aquaculture feed industry mainly uses fishmeal because of its source of amino acids, essential fatty acids, vitamins, minerals and growth factors (Zhou *et al*., 2004). However, rising demand, high prices and unstable supply of the fishmeal with the extension of aquaculture makes sense to search and introduce alternate protein sources (Lim *et al*., 2011). The plant byproducts are the promising sources of protein and energy (Hardy, 2000) and can be used for the formulation of economical and environment friendly aqua-feeds (Cheng and Hardy, 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 disturbs the physiology of digestive tract, limiting the overall fish growth (Baruah *et al*., 2004). Approximately 80% of the total phosphorus (P) contents in plants found in the form of phytate is practically not available for agastric and mono-gastric fishes (NRC, 1993). Phytic acid can chelate with essential minerals and decrease their bioavailability to fish. The phytic acid, in cereal grains and oilseeds, makes phytate-mineral complexes that chelates with other divalent and trivalent cations such as calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn). Recently, various efforts have been made to liberate phosphorous from phytate (Hotz and Gibson, 2005) but the best results were obtained by enzymatic hydrolysis of phytate through phytase supplementation (Silva *et al*., 2005). Phytase hydrolyzes the anti-nutritional phytate and enhances the concentration of Ca, Mg, Mn and Zn in bone, plasma and the whole body (Vielma *et al*., 1998). Nwanna (2005) reported that Ca, P, Fe, Mg, Zn and Mn composition in fish fed phytase supplemented diets was better than in fish fed without phytase supplemented diet. There is little information on the use of microbial phytase in stomach-less fish like *Labeo rohita*. Corn gluten meal has also been used successfully as the dietary protein source in diets for tilapia (*Oreochromis niloticus*) and has been found to be a suitable protein source in diets with the proper amino acid balance (Wu *et al*., 1995). It was also found to be highly digestible by Nile tilapia (*Oreochromis niloticus*) with digestion coefficients comparable to fishmeal protein (Koprucu and Ozdemir, 2005). The major objective of present study was to investigate the effect of phytase supplementation on minerals digestibility/availability in *Labeo rohita* fingerlings fed corn gluten meal (30%) based diet and to formulate cost-effective and environment friendly feed for indigenous culture able fish species.

MATERIALS AND METHODS

The experiment was conducted in the Fish Nutrition Laboratory, Department of Zoology and Fisheries, University of Agriculture, Faisalabad, Pakistan.

Fish and experimental condition: *Labeo rohita* fingerlings were obtained from Government Fish Seed Hatchery, Faisalabad and acclimatized with experimental conditions in laboratory for two weeks in V-shaped tanks of 70L, specially designed for the collection of fecal material. Fingerlings were fed once daily to apparent satiation on the basal diet used in subsequent digestibility study (Allan and Rowland, 1992). Water temperature, pH and dissolved oxygen (DO) were measured by using thermometer, pH

meter (Jenway 3510) and DO meter (Jenway 970). Aeration was provided continuously to all the tanks through capillary system. Before starting the experiment, *Labeo rohita* fingerlings were treated with 5g/L of NaCl to ensure they were free from ectoparasite and fungal infection (Rowland and Ingram, 1991).

***Feed ingredients and experimental diets*:** The feed ingredients purchased from a commercial feed mill were analyzed for chemical composition following AOAC (1995) prior to the formulation of the experimental diets (Table 1). 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 diet was composed of 70 % reference diet and 30 % of the corn gluten meal (Table 2). The feed ingredients were grounded to pass through 0.5 mm sieve size. All ingredients were mixed in electric mixture for 10 minutes and fish oil was gradually added. During mixing 10-15 percent water was added for moisture. The floating pellets (3 mm) were prepared using Lab Extruder (model SYSLG30-IV). The phytase (Phyzyme® XP 10000 FTU/g; Danisco Animal Nutrition, Fin-65101 Vaasa, Finland) solution was prepared by dissolving 2g of microbial phytase (powder form) in 1000 ml of distilled water (Robinson *et al*., 2002). Seven test diets were prepared by spraying graded levels of phytase to sunflower meal based diets at 0, 250, 500, 750, 1000, 1250 and 1500 FTU kg^{-1} 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 and 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 *Labeo rohita* were fed twice daily (morning and afternoon) to approximate satiation. For each test diet three replicates were conducted and in each replicate fifteen fish were stocked. After the feeding session of two hours, the uneaten diet was drained out from each tank by opening the valves of the tanks. The tanks were washed completely to remove any particles of diets and refilled with water. Fecal material was collected from the fecal collection tube of each tank. Care was taken to avoid breaking the thin fecal strings to minimize nutrient leaching. Fecal material of each replicated treatment was dried in oven, ground and stored for chemical analysis. The experiment lasted for ten weeks for the collection of 4-5 g fecal material of each replicate.

***Minerals Analysis of Feed and Feces*:** 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). Calcium, Mg, Fe, Cu, Zn and Mn were measured using Atomic Absorption Spectrophotometer (Model Hitachi Polarized, Z-8200). The phosphorus (P) was analyzed calorimetrically (UV-VIS 2001 spectrophotometer) at 720nm absorbance. The estimation of sodium (Na) and potassium (K) was done through Flame photometer (Jenway PFP-7, UK). Chromic oxide contents in diets and fecal material were estimated after oxidation with molybdate reagent (Divakaran *et al.*, 2002) using Spectrophotometer (UV-VIS 2001) at 370nm absorbance.

Table 1. 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 2. 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

Calculation of digestibility

Apparent nutrient digestibility coefficients (ADC) of test diets and test ingredients were calculated by the following formula reported in NRC (1993):

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

Statistical Analysis

Minerals availability data 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 and Cochran, 1991). The CoStat computer software program (Version 6.303, PMB 320, Monterey, CA, 93940 USA) was used for statistical analysis.

RESULTS

The percentage minerals composition in reference diet, corn gluten meal (30%) based test diets and feces are presented in Table 3 and 4. Minerals availability/digestibility (%) on corn gluten meal (30%) based test diets is presented in Table 5. Phytase supplementation increased the minerals availability to fish. Digestibility data show that 750 FTU kg^{-1} level of corn gluten test diet (diet-IV) proved best and produced high digestibility coefficient values for fingerlings when compared to reference and other phytase supplement diets. The digestibility values of P (82.9%), Mg (68.7%), Na (77%), K (80%), Cu (72%) and Zn (81%), respectively, at 750 FTU kg^{-1} level differed significantly ($p<0.05$) when compared to the reference and other phytase supplement test diets. However, the digestibility of Mn (74%) was highest at 1000 FTU kg^{-1} and significantly different ($p<0.05$) from reference and other phytase treated corn gluten meal based diets. The Fe digestibility (65%) recorded at 750 FTU kg^{-1} level was higher than phytase performance observed at 500 FTU kg^{-1} level. The Ca digestibility at 750 and 1000 FTU kg^{-1} levels were significantly differed ($p<0.05$) from the reference and other phytase supplemented test diets.

DISCUSSION

Phytate chelates with minerals and makes them unavailable to fish by reducing their digestibility (Sandberg *et al*., 1993), while breakdown of phytate may improve leaching of essential nutrients. Wise (1983) indicated that presence of phytate in plant based diets may chelate with minerals such as Ca, Mg, Fe, Cu, Zn and Mn. Reduction in fish growth may be correlated with scarcity of essential minerals (Lall, 2002). In the present study the maximum digestibility values of most of the minerals for *Labeo rohita* fingerlings fed on corn gluten meal (30%) based test diets, were at 750 FTU kg^{-1} diet followed by 1000 FTU kg^{-1} diet, and differed significantly from reference and other phytase levels based test diets used. These results are in agreement with Sugiura *et al*. (2001) and Wang *et al*. (2009). However Debnath *et al*. (2005) suggested that 500 FTU kg^{-1} level of phytase supplementation is enough for optimal digestibility of minerals. Laining *et al*. (2010) found a higher (2000 FTU kg^{-1}) level of phytase supplementation for improving minerals digestibility. The differences in optimal doses may be due to the variation in experimental species and types of major plant sources of the experimental diets. The digestibility of Ca, Mg, Na, K, Fe, Cu, Zn and Mn in *Labeo rohita* fed on corn gluten meal (30%) based diets may have been enhanced with phytase supplementation.

The phosphorous (P) availability/digestibility may have enhanced the availability of Ca, Mg, Na, K, Fe, Cu, Zn and Mn. The role of phytase liberating phosphorous and other minerals for different fish species has been addressed by Baruah *et al*. (2007). Gao *et al*. (2006) evaluated the impact of phytase supplementation in the diet of grass carp. They concluded that 500 to 1000 FTU kg^{-1} levels of phytase supplementation enhances the phosphorous availability to fish and probably reduces the phosphorous excretion through feces as suggested by Cao *et al*. (2008).

CONCLUSION

Phytase supplementation having 750 FTU kg^{-1} level in corn gluten meal (30%) based diets may prove highly beneficial in developing cost effective and environment friendly aqua feed for major carps.

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Table 3. Analyzed minerals composition (%) of reference and corn gluten meal (30 %) based test diets

Diets	Phytase (FTU kg^{-1})	Ca (%)	P (%)	Mg (%)	Na (%)	K (%)	Fe (%)	Cu (%)	Zn (%)	Mn (%)
Reference diet	**---**	0.23±0.015	3.74±0.015	0.091±0.0020	1.03±0.015	1.65±0.035	0.11±0.0070	0.098±0.0025	0.15±0.0058	0.079±0.0015
Test diet-I	**0**	0.25±0.015	2.72±0.015	0.072±0.0015	0.84±0.015	1.39±0.015	0.077±0.0015	0.068±0.0032	0.12±0.015	0.071±0.0012
Test diet-II	**250**	0.25±0.012	2.74±0.017	0.074±0.0017	0.82±0.0058	1.47±0.021	0.075±0.0015	0.071±0.0021	0.13±0.012	0.073±0.0006
Test diet-III	**500**	0.26±0.0058	2.73±0.015	0.073±0.0015	0.86±0.012	1.45±0.015	0.077±0.0010	0.070±0.0006	0.13±0.015	0.072±0.0026
Test diet-IV	**750**	0.26±0.010	2.75±0.015	0.075±0.0015	0.84±0.020	1.43±0.021	0.078±0.0031	0.071±0.0015	0.12±0.015	0.072±0.0015
Test diet-V	**1000**	0.25±0.015	2.72±0.012	0.072±0.0012	0.85±0.015	1.47±0.015	0.076±0.0015	0.073±0.0015	0.11±0.010	0.072±0.0015
Test diet-VI	**1250**	0.24±0.021	2.73±0.021	0.073±0.0021	0.82±0.015	1.40±0.021	0.075±0.0015	0.071±0.0021	0.13±0.017	0.074±0.0015
Test diet-VII	**1500**	0.24±0.015	2.73±0.015	0.073±0.0015	0.74±0.0058	1.42±0.058	0.075±0.0021	0.072±0.0010	0.12±0.021	0.070±0.0021

Data are means of three replicates ± S.D.

Table 4. Analyzed minerals composition (%) of feces of reference and corn gluten meal (30%) based test diets

Diets	Phytase (FTU kg^{-1})	Ca (%)	P (%)	Mg (%)	Na (%)	K (%)	Fe (%)	Cu (%)	Zn (%)	Mn (%)
Reference diet	**---**	0.12±0.010	2.13±0.020	0.064±0.0050	0.61±0.035	0.73±0.040	0.063±0.0040	0.054±0.0031	0.058±0.0030	0.048±0.0031
Test diet-I	**0**	0.13±0.012	1.06±0.064	0.043±0.0032	0.48±0.031	0.62±0.023	0.048±0.0031	0.041±0.0020	0.051±0.0040	0.045±0.0025
Test diet-II	**250**	0.098±0.0025	0.92±0.055	0.040±0.0015	0.39±0.015	0.54±0.050	0.040±0.0021	0.036±0.0026	0.054±0.0042	0.041±0.0020
Test diet-III	**500**	0.089±0.0020	0.87±0.065	0.039±0.0020	0.36±0.026	0.52±0.031	0.037±0.0032	0.032±0.0035	0.048±0.0031	0.035±0.0023
Test diet-IV	**750**	0.058±0.0050	0.54±0.055	0.027±0.0026	0.22±0.026	0.31±0.025	0.031±0.0025	0.023±0.0015	0.024±0.0025	0.023±0.0015
Test diet-V	**1000**	0.076±0.0035	0.76±0.055	0.038±0.0030	0.36±0.031	0.46±0.035	0.046±0.0036	0.031±0.0015	0.035±0.0035	0.021±0.0021
Test diet-VI	**1250**	0.090±0.0011	1.02±0.095	0.048±0.0051	0.48±0.035	0.72±0.031	0.053±0.0032	0.044±0.0026	0.057±0.0061	0.028±0.0040
Test diet-VII	**1500**	0.100±0.0068	1.05±0.038	0.057±0.0015	0.53±0.025	0.80±0.021	0.057±0.0045	0.050±0.0021	0.051±0.0081	0.038±0.0023

Data are means of three replicates ± S.D.

Table 5. Minerals availability/digestibility (%) of reference and corn gluten meal (30%) based test diets

Minerals	Reference diet	Phytase levels (FTU kg^{-1})						
		0 (Test diet-I)	**250** (Test diet-II)	**500** (Test diet-III)	**750** (Test diet-IV)	**1000** (Test diet-V)	**1250** (Test diet-VI)	**1500** (Test diet-VII)
Ca	52.82±0.97^{de}	52.51±3.40^{e}	64.38±1.82^{bc}	68.24±1.01^{bc}	80.28±2.66^{a}	72.43±3.89^{ab}	64.49±4.58^{bc}	60.74±2.42^{cd}
P	48.41±1.41^{f}	63.91±1.96^{de}	69.90±2.66^{bcd}	70.74±2.25^{bc}	82.92±2.07^{a}	75.21±1.67^{b}	65.47±2.86^{cde}	61.90±2.87^{e}
Mg	39.48±1.78^{d}	44.64±3.38^{cd}	51.82±2.16^{bc}	51.31±2.21^{bc}	68.68±2.32^{a}	53.12±3.98^{b}	40.20±2.40^{d}	29.05±3.67^{e}
Na	47.21±3.27^{c}	47.73±3.60^{c}	57.05±2.32^{b}	61.58±1.55^{b}	77.11±2.38^{a}	62.43±2.72^{b}	45.88±2.11^{c}	42.23±0.88^{c}
K	59.21±1.06^{c}	59.09±0.91^{c}	66.91±3.13^{b}	67.27±2.60^{b}	80.72±2.47^{a}	72.29±2.93^{b}	52.14±3.61^{cd}	48.78±2.94^{d}
Fe	50.37±2.10^{bc}	42.25±2.53^{cd}	52.63±3.58^{bc}	58.39±4.08^{ab}	64.95±3.34^{a}	46.04±6.80^{bc}	34.07±3.87^{de}	30.49±1.57^{e}
Cu	48.93±1.35^{cd}	44.52±1.42^{de}	54.20±3.58^{bc}	58.83±3.92^{b}	71.85±3.11^{a}	62.61±3.37^{b}	43.06±3.36^{de}	37.41±3.65^{e}
Zn	64.18±1.57^{bc}	59.03±3.99^{c}	61.42±1.24^{c}	65.29±3.69^{bc}	81.42±4.14^{a}	71.29±2.25^{b}	59.11±3.70^{c}	60.47±3.36^{c}
Mn	45.50±3.42^{d}	42.06±4.22^{d}	49.78±1.99^{cd}	54.99±4.07^{c}	71.46±2.78^{ab}	74.28±2.21^{a}	65.07±2.96^{b}	50.96±2.98^{cd}

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