Efficacy of phytase supplementation in improving mineral digestibility in *Labeo rohita* fingerlings fed on canola meal-based diets

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Abstract

A feeding trial of ten weeks was conducted to evaluate the effect of microbial phytase supplementation on mineral digestibility in *Labeo rohita* fingerlings fed on canola meal-based diets. The experiment consisted of a reference diet and a basal diet. The reference diet was prepared according to the requirements of *L. rohita* and was used as a standard diet. The basal diet was made by replacing 30% reference diet with canola meal which was used as the test ingredient in this experiment. Seven test diets were prepared by spraying graded levels (0, 250, 500, 750, 1000, 1250 and 1500 FTU kg^{-1}) of phytase on canola meal-based basal diet to assess the optimal dose required to achieve best performance in terms of mineral digestibility coefficients. Chromic oxide was incorporated as an indigestible marker in the diets. Phytase supplementation significantly increased mineral digestibility in *L. rohita* fingerlings at 750 FTU kg^{-1} followed by that at 1000 FTU kg^{-1} as compared to the reference diet. The results of our study suggested that phytase supplementation in canola meal-based diet at the rate of 750 FTU kg^{-1} is optimum to release sufficient chelated minerals in *L. rohita*.

Keywords: *Labeo rohita*, Phytase, Mineral digestibility, Canola meal

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Introduction

Labeo rohita (rohu) is one of the important major carp species cultured in Pakistan. It is grown under poly-culture system with other species of major and Chinese carps (Hussain *et al*., 2011a). Regional culture practices are largely based on semi-intensive culture systems. No cost effective feed is available to local fish farmers. However, various crude formulations in the form of mesh feeds are used to further enhance the fish production.

Use of fish meal in fish feed formulation is very effective. However, as fish meal is available in a limited quantity, its price has been increasing considerably with the fast growth of worldwide aquaculture (Tacon and Metian, 2008; FAO, 2009; Hardy, 2010; Shapawi *et al*., 2013). Its increasing demand, rising price and unstable supply has forced the researchers to look for alternate protein sources to meet the demand of the rapidly developing aquaculture feed industry (Pham *et al*., 2008; Lim *et al*., 2011; Lech and Reigh, 2012; Shapawi *et al*., 2013). Agriculture industry based plant by-products are the promising sources of protein and energy which can be used for the development of economical and environmental friendly aqua-feeds (Cheng and Hardy 2002; Hussain *et al*., 2011b). However, the majority of such protein sources contain high phytate or phytic acid contents (Reddy and Sathe, 2002). Phytic acid is an organic form of phosphorous that is abundantly found in plant materials such as oilseeds, cereals and legumes (Rao *et al*., 2009). It is estimated that about 80% of the total phosphorous contents in plants may be present in the form of phytate which is almost not available for agastric or mono-gastric fishes (NRC, 1993). Phytic acid itself may chelate most of the essential minerals and decrease their bioavailability to fish. Phytic acid present in cereal grains and oilseeds makes phytate-mineral complex structures with divalent and trivalent cations such as; Ca, Mg, Fe, Zn, Cu and Mn, which in turn decreases the bioavailability of these nutrients to fish as well (Francis *et al*., 2001; Helland *et al*., 2006). Wise (1983) indicated that the presence of phytate in plant based diets may chelate with minerals such as Ca, Mg, Fe, Cu, Zn and Mn. The breakdown of phytate may improve the release of essential nutrients.

Phytase is an enzyme that is very specific and effective in hydrolyzing the indigestible phytate in plant protein sources. Monogastric and agastric fishes do not produce this enzyme so they are unable to hydrolyze the phytate contents (Cao *et al*., 2007). Supplementation of phytase in fish feed is well established as an efficient and practical method of improving mineral digestibility in monogastric or stomachless fishes (Sajjadi and Carter, 2004; Liu *et al*., 2013). Hydrolysis of phytate through phytase supplementation in plant-based diets consequently liberates phosphorus from phytate complexes. This freely available phosphorous plays a pivot role in improving fish performance

(Baruah *et al*., 2004; Biswas *et al*., 2007; Cao *et al*., 2007; Pham *et al*., 2008). Moreover, the supplementation of phytase in plant by-product based fish feeds increases the bioavailability of minerals to fish making it cost effective and environmentally friendly (Gabriel *et al.,* 2007). Many plant by-products have been successfully used in aquaculture diets without declining the feed pellet quality. The most important product of canola is its oil content whereas canola meal is also a valuable protein source for use in animal feed. Some researchers have intended to assess the nutritional value of canola meal for a variety of animals (Ahmadauli *et al*., 2008; Newkirk, 2009; Enami and Safafar, 2010). Canola meal is commonly being used in aqua-feeds for various fish species such as trout, salmon, catfish, tilapia, bass, perch, sea bream and turbot (Enami, 2011). The amino acid profile of canola meal protein is similar to that of herring meal protein and superior to soybean meal protein (Shafaeipour *et al*., 2008). The cost of canola meal is usually lower than the cost of both fish meal and soybean meal, which are the major protein sources being used in aqua feeds (Higgs *et al*., 1995; Sajjadi and Carter, 2004). Less information is available on phytase supplementation in canola meal based diets for stomach-less fish such as *L. rohita.* The major objective of our study was to evaluate the efficacy of phytase supplementation on mineral digestibility in *L. rohita* fingerlings fed on canola meal-based diets and to formulate cost effective and environmentally friendly feeds for the indigenous cultured fish species.

Materials and methods

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

Experimental design

Canola meal was selected to formulate the experimental diet. The experimental diet was comprised of 30% test ingredient (Canola meal) and 70% reference diet (Table 2). The experimental diet was then further divided into seven test diets and sprayed with graded levels (0, 250, 500, 750, 1000, 1250 and 1500 FTU kg^{-1}) of phytase. Seven phytase level based test diets of canola meal and one reference diet were fed to eight fish groups stocked in specially designed V-shaped tanks. The experiment lasted ten weeks until 4-5 g of fecal material was collected from each tank. Three replicate tanks were used for each treatment. The experimental diets with seven phytase levels were labeled I to VII. The fish performance in each experimental group in relation to various minerals digestibility parameters was evaluated and compared with each other and with the group fed the reference diet using Completely Randomized Design (CRD). The relationship between the mineral digestibility parameters and phytase supplementation levels were established by using quadratic regression analysis.

Fish and experimental conditions

L. rohita fingerlings (average weight 7.04 ± 0.011g fish^{-1}) were obtained from Government Fish Seed Hatchery, Faisalabad and allowed to acclimatize with experimental conditions in the laboratory for two weeks in twenty four tanks (1.7 square feet in V shape) each with a capacity of 70L, which were specially designed for the collection of fecal material from water media. Fingerlings were kept under 12/12 light cycle throughout the trial. During this period the fingerlings were fed on the reference diet (Table 2) once daily to apparent satiation, used in subsequent digestibility studies (Allan and Rowland, 1992). Water quality variables, particularly water temperature, pH, dissolved oxygen (DO) and electrical conductivity (EC) were monitored throughout the study period twice daily (morning and evening). pH was measured with pH meter (Jenway 3510) and it fluctuated between 7.4 and 8.6. Dissolved oxygen and temperature were recorded by D.O. meter (Jenway 970) and values varied from 5.8 to 7.3mg/L and 24.9^{o}C to 28.7^{o}C, respectively. Electrical conductivity was determined with electrical conductivity meter (HANNA: HI. 8633) and its values varied from 1.30 dSm^{-1} to 1.52 dSm^{-1}. Aeration (24hours) was provided to all the tanks through capillary system. Before starting the experiment, *L. rohita* fingerlings were treated with (5g/L) NaCl for 1-2 minutes to make them free of any infection (Rowland and Ingram, 1991).

Feed ingredients and formulation of experimental diets

The feed ingredients were purchased from the local poultry and grain markets and analyzed for chemical composition (AOAC, 1995) prior to the formulation of the reference and experimental diets (Table 1). The feed ingredients were finely ground and passed through 0.5 mm sieve size. All feed ingredients were mixed in an electric mixer for 10 minutes. Fish oil was gradually added while the mixing process of ingredients continued for 5 minutes. While mixing, 10-15% water was also added to produce a suitable texture. The diets were extruded into floating pellets (3mm) through Lab Extruder (model SYSLG30-IV Experimental Extruder). The above procedure was followed to formulate reference and canola meal based experimental diets. The required concentrations (0, 250, 500, 750, 1000, 1250 and 1500 FTU kg^{-1}) of phytase (Phyzyme® XP 10000 FTU g^{-1}; Danisco Animal Nutrition, Fin-65101 Vaasa, Finland) were prepared in 25ml distilled water and sprayed on the seven test diets (Robinson *et al*., 2002). The 0 FTU kg^{-1} level test diet was also sprayed with 25mL of distilled water only to maintain an equal level of moisture. All the diets were stored at 4°C until use.

Table 1: Chemical composition (%) of feed ingredients (Dry matter basis).

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
Canola meal	94.12	38.10	1.52	1.39	9.26	49.73	3.13

Table 2: Ingredients composition (%) of reference and test diets (As fed basis).

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	16.6
Fish oil	7.0	4.9
Vitamin Premix**	1.0	1.0
Mineral Premix***	1.0	1.0
Ascorbic acid	1.0	1.0
Chromic oxide	1.0	0.7
Canola meal	-	30.0
Total	**100.0**	**100.0**

**Each Kg of Vitamin premix contains

Vitamin A	15 M.I.U.	Vitamin D_3	3 M.I.U.
Vitamin B_1	5000 mg	Vitamin E	6000 IU
Vitamin B_2	6000 mg	Vitamin K_3	4000 mg
Vitamin B_6	4000 mg	Folic acid	750 mg
Vitamin B_{12}	9000 mcg	Calcium pantothenate	10000mg
Vitamin C	15000mg	Nicotinic acid	25000mg

***Each Kg mineral granules contains

Ca (Calcium)	155gm	Mn (Manganese)	2000mg
P (Phosphorous)	135gm	Cu (Copper)	600mg
Mg (Magnesium)	55gm	Co (Cobalt)	40mg
Fe (Iron)	1000 mg	I (Iodine)	40mg
Zn (Zinc)	3000 mg	Se (Selenium)	3mg
Na (Sodium)	45gm		

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^oC) at a substrate concentration (sodium phosphate) of 5.1 mmol/L (Engelen *et al*., 1994).

Chemical analysis of feed ingredients

The samples of feed ingredients were homogenized using a motor and pestle and analyzed by standard methods (AOAC, 1995): moisture was determined by oven-drying at 105°C for 12 h; crude protein (N × 6.25) by micro Kjeldahl apparatus; crude fat, by petroleum ether extraction method (Bligh and Dyer, 1959) 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 an electric furnace (Eyela-TMF 3100) to constant weight. Total carbohydrate (N-free extract) was calculated by difference, i.e., Total carbohydrate % =100-(Moisture% + Crude Protein%+ Ether extract%+Crude fiber %+Ash %). Gross energy was determined by oxygen bomb calorimeter.

Collection of fecal material and analysis of minerals

L. rohita fingerlings were fed twice daily (morning and 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 to daily feed intake. For each test diet, three replicates were assigned and in each replicate fifteen fish were stocked. After the feeding session of two hours, the uneaten diet was collected and water 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. Fecal material of each replicated treatment was dried in the oven, ground and stored for chemical analysis. For mineral estimation, the diets and feces samples were digested in boiling nitric acid and perchloric acid mixture (2:1) by following standard methods (AOAC, 1995). After appropriate dilution, mineral contents (calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn) and manganese, (Mn) were estimated using atomic absorption (Hitachi Polarized Atomic Absorption Spectrometer, Z-8200). Calibrated standards for mineral estimation were prepared from commercially available standards (AppliChem® Gmbh Ottoweg4, DE-64291 Darmstadt, Germany). The estimation of sodium (Na) and potassium (K) was done through flame photometer (Jenway PFP-7, UK). Phosphorus (P) was analyzed calorimetrically (UV/VIS spectrophotometer) using ammonium molybdate as reagent at 720 nm absorbance through standard methods (AOAC, 1995).

Estimation of chromic oxide

Chromic oxide was used as an inert marker in diets assuming that the amount of the marker in the feed and feces remains constant throughout the experimental period and the entire ingested marker appears in the feces.

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. The apparent digestibility of minerals such as Ca, P, Mg, Na, K, Fe, Cu, Zn and Mn was determined indirectly at the end of the experiment using chromic oxide as the inert marker.

Calculation of apparent nutrient digestibility coefficients (ADC %) of test diets

Apparent nutrient digestibility coefficients (ADC) of test diets were calculated with the help of formula described in NRC (1993):

$$ADC\ (\%) = 100 - 100 \times \frac{Marker\ in\ diet\ \times Nutrient\ in\ feces}{Marker\ in\ feces\ \times Nutrient\ in\ diet}$$

Statistical analysis

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

Results

The analyzed mineral composition of reference diet, canola meal-based test diets and feces are presented in Tables 3 and 4, respectively. Mineral digestibility of canola meal-based test diets is presented in Table 5. Table 4 makes it clear that phytase enzyme supplementation played a very important role in increasing the mineral digestibility and minimum amount of minerals was excreted at 750 and 1000 FTU kg^{-1} levels, indicating that at these levels more minerals were available to *L. rohita* fingerlings as compared to the reference diet and remaining levels of phytase supplementation based diets. The maximum digestibility values (%) of P, Fe, Zn and Mn were found at 750 FTU kg^{-1} diet which differed significantly ($p<0.05$) from the next higher digestibility level (1000 FTU kg^{-1}) and the mineral contents of reference diet and remaining test diets having different levels of phytase supplementation (Table 5). The digestibility value of Cu was highest at 1000 FTU kg^{-1} supplementation level in canola meal-based diet. This value also differed significantly ($p<0.05$) from the reference and other phytase supplemented test diets. The digestibility values of Ca, Mg, Na and K were close enough to each other at 750 and 1000 FTU kg^{-1} levels. However these values were significantly different from those in reference and other phytase supplemented test diets. Comparing the digestibility of various minerals the highest values were observed for zinc followed by phosphorous while the minimum digestibility value was noted for Mn.

Table 3: Analyzed mineral composition of reference and canola meal-based test diets (dry weight basis).

Diets	Phytase levels ($FTUkg^{-1}$)	Ca (%)	P (%)	Mg (%)	Na (%)	K (%)	Fe (%)	Cu (%)	Zn (%)	Mn (%)
Reference diet	---	0.23	3.74	0.091	1.03	1.65	0.11	0.098	0.15	0.079
Test diet-I	**0**	0.22	3.22	0.082	0.82	1.61	0.092	0.063	0.13	0.053
Test diet-II	**250**	0.22	3.23	0.082	0.84	1.61	0.094	0.062	0.12	0.057
Test diet-III	**500**	0.22	3.22	0.080	0.80	1.61	0.091	0.064	0.13	0.052
Test diet-IV	**750**	0.24	3.26	0.083	0.85	1.63	0.094	0.066	0.14	0.056
Test diet-V	**1000**	0.23	3.22	0.083	0.83	1.66	0.093	0.065	0.13	0.054
Test diet-VI	**1250**	0.21	3.25	0.082	0.82	1.61	0.091	0.068	0.13	0.053
Test diet-VII	**1500**	0.22	3.22	0.081	0.81	1.64	0.093	0.068	0.13	0.053
PSE		0.0069	0.0102	0.0009	0.0087	0.0131	0.0017	0.0011	0.0078	0.0009
P		0.2069	0.0000	0.0000	0.0000	0.0685	0.0002	0.0000	0.1419	0.0000

Data are means of three replicates.
PSE = pooled SE = √MSE/n (where MSE= mean-squared error).

Table 4: Analyzed mineral composition of feces of *Labeo rohita* fed on reference and canola meal-based test diets (dry weight basis).

Diets	Phytase levels ($FTUkg^{-1}$)	Ca (%)	P (%)	Mg (%)	Na (%)	K (%)	Fe (%)	Cu (%)	Zn (%)	Mn (%)
Reference diet	---	0.120^{c}	2.13^{f}	0.064^{e}	0.61^{e}	0.73^{d}	0.063^{d}	0.054^{f}	0.058^{c}	0.048^{d}
Test diet-I	**0**	0.110^{bc}	1.51^{de}	0.058^{de}	0.56^{de}	0.69^{cd}	0.55^{cd}	0.043^{de}	0.040^{b}	0.035^{c}
Test diet-II	**250**	0.110^{bc}	1.42^{cd}	0.051^{cd}	0.45^{bc}	0.59^{bc}	0.043^{b}	0.032^{bc}	0.031^{ab}	0.032^{bc}
Test diet-III	**500**	0.100^{bc}	1.26^{bc}	0.039^{b}	0.36^{ab}	0.53^{ab}	0.043^{b}	0.030^{b}	0.034^{b}	0.026^{ab}
Test diet-IV	**750**	0.070^{a}	0.85^{a}	0.029^{a}	0.28^{a}	0.45^{a}	0.032^{a}	0.026^{ab}	0.022^{a}	0.018^{a}
Test diet-V	**1000**	0.090^{ab}	1.16^{b}	0.036^{ab}	0.34^{a}	0.54^{ab}	0.044^{b}	0.018^{a}	0.031^{ab}	0.028^{bc}
Test diet-VI	**1250**	0.100^{bc}	1.42^{cd}	0.043^{bc}	0.43^{bc}	0.63^{bcd}	0.052^{ab}	0.038^{cd}	0.039^{b}	0.032^{bc}
Test diet-VII	**1500**	0.110^{bc}	1.62^{e}	0.062^{e}	0.47^{cd}	0.72^{d}	0.058^{ab}	0.049^{ef}	0.054^{c}	0.035^{c}
PSE		0.0052	0.0381	0.0018	0.0179	0.0218	0.0020	0.0017	0.0020	0.0016
P		0.0003	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

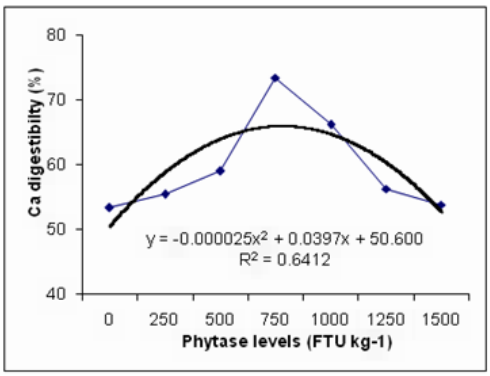
Data are means of three replicates
PSE = pooled SE = √MSE/n (where MSE= mean-squared error).

Improved growth and feed performance was also observed in response to phytase supplementation. Again, optimum performance was recorded at 750 FTU kg^{-1} phytase level (Data not shown). Quadratic regression analysis indicated that optimum mineral digestibility for Ca, P, Mg, Na, K, Fe, Cu, Zn and Mn occurred at 794, 739, 755, 794, 736, 683, 743, 665 and 726 FTU kg^{-1} levels, respectively (Fig.1).

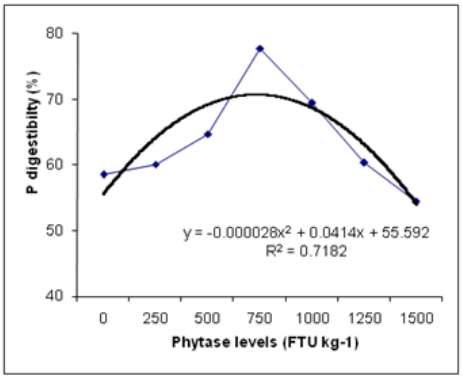
Table 5: Mineral digestibility (%) of reference and canola meal-based test diets.

Mineral	Reference diet	Phytase levels (FTU kg^{-1})							PSE	*p*
		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^{c}	55.38^{c}	55.42^{c}	59.02^{bc}	73.50^{a}	66.27^{ab}	56.31^{c}	53.85^{c}	1.653	0.0000
P	48.41^{e}	58.61^{d}	60.14^{cd}	64.75^{bc}	77.80^{a}	69.50^{b}	60.36^{cd}	54.46^{d}	1.232	0.0000
Mg	39.48^{ef}	37.15^{ef}	43.86^{de}	56.52^{bc}	70.19^{a}	63.62^{ab}	52.33^{cd}	30.58^{f}	2.123	0.0000
Na	47.21^{de}	40.56^{e}	51.60^{cd}	59.18^{bc}	71.43^{a}	65.64^{ab}	52.33^{cd}	46.65^{de}	1.916	0.0000
K	59.21^{e}	62.58^{de}	66.76^{bcd}	64.96^{bc}	76.48^{a}	72.11^{ab}	64.87^{cde}	60.40^{de}	1.312	0.0000
Fe	50.37^{cde}	47.90^{e}	58.79^{bc}	57.24^{bcd}	71.00^{a}	59.32^{b}	48.73^{de}	43.31^{e}	1.819	0.0000
Cu	48.93^{d}	39.24^{e}	53.17^{cd}	58.18^{bc}	65.82^{b}	76.11^{a}	49.05^{d}	34.24^{e}	1.765	0.0000
Zn	64.18^{c}	72.46^{b}	75.73^{b}	75.93^{b}	86.27^{a}	79.10^{b}	73.57^{b}	62.81^{c}	1.462	0.0000
Mn	45.50^{c}	42.23^{c}	48.83^{bc}	55.32^{b}	68.23^{a}	56.19^{b}	45.67^{c}	39.77^{c}	1.883	0.0000

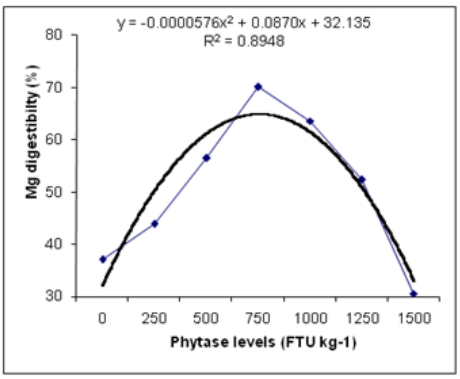
Means within rows having different superscripts are significantly different at $p<0.05$.
Data are means of three replicates.
PSE = pooled SE = √MSE/n (where MSE= mean-squared error).



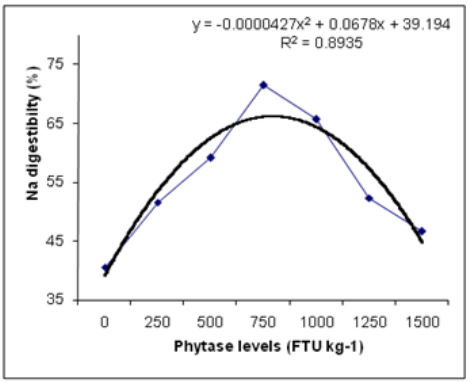
Ca (*794 **66.36)



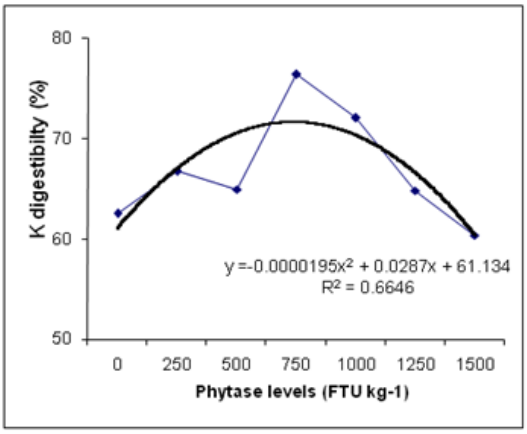
P (*739 **70.89)



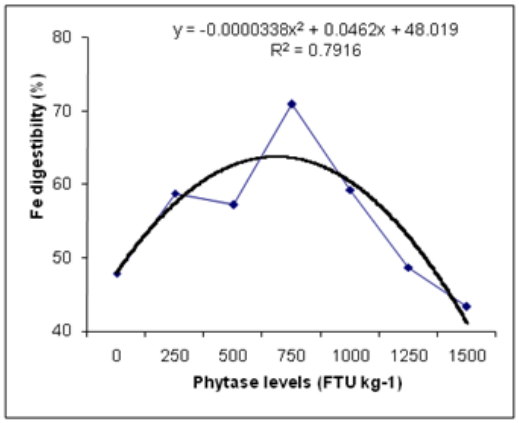
Mg (*755 **64.99)



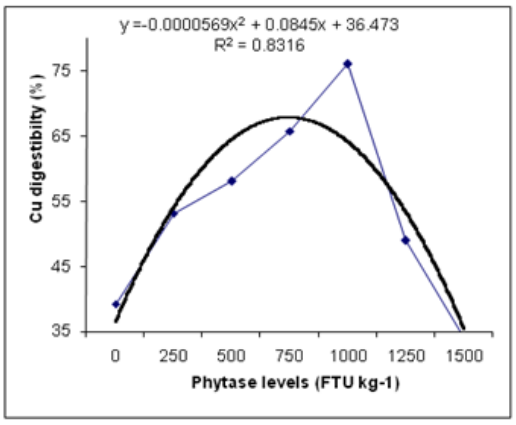
Na (*794 **66.11)



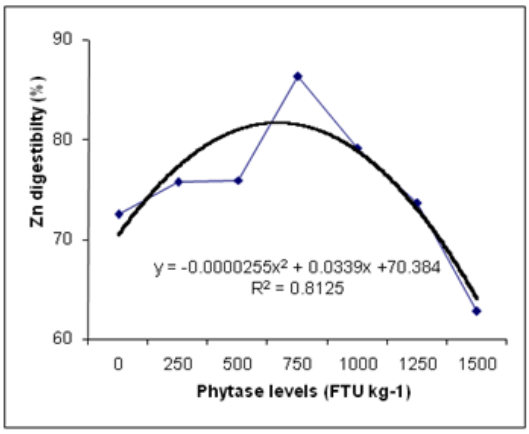
K (*736 **71.69)



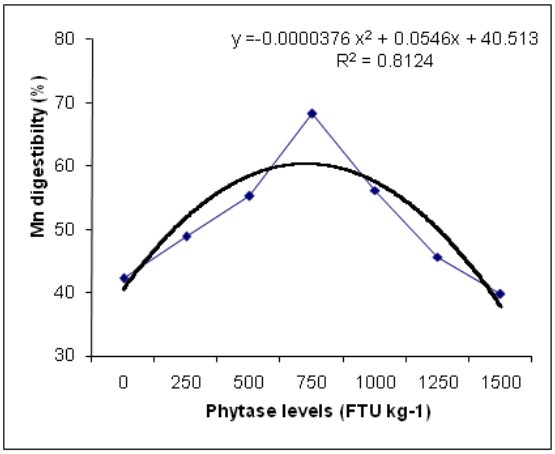
Fe (*683 **63.81)



Cu (*743 **67.84)



Zn (*665 **81.65)



Mn (*726 **60.33)

(*Optimal phytase level FTU kg^{-1} ** Mineral digestibility %)

Figure 1: The quadratic relationship between mineral digestibility (%) of reference, canola meal-based test diets and phytase levels (FTU kg^{-1}).

Discussion

In the present study the observed and quadratic regression analyses based on optimal calculated values for most of the minerals fluctuated narrowly around 750 FTU kg^{-1} level of phytase supplementation while next higher level of mineral digestibility was at or around 1000 FTU kg^{-1} diet. The values of minerals at these levels were significantly different from those in the reference diet and remaining phytase levels based test diets. However, the lower (0 and 250 FTU kg^{-1}) and higher (1250 and 1500 FTU kg^{-1}) levels of phytase supplementation performed almost similarly and were not found to be significantly effective in increasing the digestibility of these minerals. In general, the results showed that mineral digestibility started increasing at 250 FTU kg^{-1} level and reached its maximum at 750 FTU kg^{-1} level of phytase supplementation. So, it was concluded that 750 FTU kg^{-1} was the optimum level at which the maximum utilization of these minerals occurred in the fish body and minimum minerals were excreted into the aquatic environment through feces. Reduced mineral excretion through fish feces was probably due to the hydrolysis of phytate contents by the phytase enzyme supplementation and so more minerals were utilized by *L. rohita* fingerlings. Similar results were observed in our earlier study when *L. rohita* fingerlings were fed on corn gluten (30%) meal-based diet supplemented with phytase. The maximum mineral digestibility with phytase supplementation was observed at level of 750 FTUkg^{-1} whereas the next higher level of mineral digestibility was 1000 FTUkg^{-1} (Hussain *et al*., 2011b). The phytase supplementation probably broke down the phytate, liberating more chelated minerals. In this way mineral utilization was increased (Wang *et al*., 2009; Hussain *et al*., 2011b). However, Baruah *et al*. (2007) suggested that supplementation of dietary microbial phytase at 500 FTUkg^{-1} level improves the absorption of minerals such as Na, K, P, Mg, Mn and Fe. However for fingerlings of *Pangasius pangasius*, Debnath *et al*. (2005) also reported improved absorption and retentions of minerals such as Na, P, Fe, Mg and Mn concentrations in the whole body (except for body Mn, Mg and K contents) at 500 FTU kg^{-1} level of phytase supplementation. In other studies phytase supplementation at 500 FTU kg^{-1} level was found optimum in improving mineral utilization in Japanese seabass *Lateolabrax japonicas* (Ai *et al.*, 2007) and in *Cyprinus carpio* L. fed on plant based diets (Sardar *et al* 2007).

Positive results of phytase supplementation in terms of higher mineral digestibility were also reported by Sugiura *et al*. (2001) in rainbow trout. Phytase supplementation on plant based diets fed to sex-reversed red tilapia, confirmed a great increase in serum zinc (Phromkunthonge and Gabaudan, 2006) and phosphorous digestibility (Tudkaew *et al*., 2008). In the present study the higher mineral digestibility (Ca, Mg, Na, K, Fe, Cu, Zn

and Mn) for the *L. rohita* fingerlings fed on phytase supplemented canola meal-based diet has confirmed the hydrolysis of the anti-nutritional factor like phytate. Use of phytase probably reduced the phytate contents of the plant based diets, increasing bioavailability of minerals for *L. rohita* fingerlings. As the mineral digestibility increased with phytase supplementation, consequently fish excreted lower amount of minerals through feces into the aquatic environment. The amount of mineral discharge through feces of fish fed on the reference diet was comparatively higher than that of the fish fed phytase supplemented canola meal-based diets. This reduced mineral excretion with phytase supplementation in the present study verified the fact that the mineral digestibility increases with phytase supplementation. Similar findings were also reported by Nwanna *et al*. (2007). They found that most of the phytase action was focused on the phytate degradation which resulted in the liberation of more minerals resulting in increased mineral digestibility.

In the present research work, the mineral digestibility of *L. rohita* fingerlings was lower in group fed on reference diet and 0 FTU kg^{-1} level as compared to other phytase treated diets. When phosphorous (P) digestibility was improved, the digestibility of minerals such as Ca, Mg, Na, K, Fe, Cu, Zn and Mn were also amplified. The positive role of phytase in liberation of phosphorous and other minerals for different fish species has been reported by many authors (Sugiura *et al*., 2001; Vielma *et al*., 2004; Baruah *et al*., 2007; Dalsgaard *et al*., 2009). Gao *et al*. (2006) evaluated the impact of phytase supplementation in the diets of grass carp. They found that phytase supplementation between 500 and 1000 FTU kg^{-1} levels had significantly increased phosphorous availability to fish and reduced the phosphorous excretion through feces. In comparison with the control or reference diet, phytase supplementation in plant based diets reduced the phosphorus excretion into aquatic environment (Cao *et al*., 2008). In a study conducted by Nwanna and Schwarz (2007), higher digestibility of phosphorous resulted in reduced phosphorous discharge in all the fish groups supplemented with phytase as compared to that of the control group. Phytase supplementation in plant based diets may prove very beneficial in developing cost effective and environment friendly feed of *L. rohita* by improving nutrient digestibility and reducing the nutrient excretion into aquatic environments and it is expected that it will be helpful in reducing aquatic pollution (Baruah *et al*., 2004; Nwanna *et al*., 2005; Ashraf and Goda, 2007; Gabriel *et al*., 2007; Hussain *et al*., 2011b).

The present study provided evidence that fish feed supplemented with phytase enzyme increases the mineral digestibility. It may also decrease the need for mineral supplementation in fish feed, which will reduce the feed cost and mineral discharge through feces into the aquatic environment.

Absorption and utilization of most important minerals such as Ca, P, Mg, Na, K, Fe, Cu, Zn and Mn were found superior in fish groups fed with canola meal based diet having 750 FTU kg^{-1} level of phytase supplementation.

It was concluded that 750 FTU kg^{-1} is the optimum level of phytase supplementation that has a significant role in improving mineral digestibility in *L. rohita* fingerlings fed on canola meal-based diets. It was also concluded that phytase supplementation in plant meal-based diets is very helpful in developing cost effective and environment friendly aqua-feed for major carps.

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