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Efficacy of Phytase Supplementation on Growth Performance and Mineral Digestibility of *Labeo rohita* Fingerlings Fed on Cottonseed Meal Based Diet

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Abstract.- Phytate, present in plant feed ingredients, act as anti-nutritional factor by chelating indispensable minerals and other nutrients making them unavailable to the fish. These undigested nutrients are excreted out causing pollution in the water bodies. The present study was conducted to evaluate the efficacy of phytase supplementation on growth performance and minerals digestibility of *Labeo rohita* fingerlings. Reference diet was made by replacing 30% of reference diet with cottonseed meal as test ingredient. Seven cottonseed meal based test diets were prepared by spraying graded levels of phytase (0, 250, 500, 750, 1000, 1250 and 1500 FTU kg^{-1}) to the basal diet. Chromic oxide was included as inert marker in the diets to assess the minerals digestibility. The results of present study showed improved growth and feed performance of fingerlings in response to phytase supplementation. Maximum performance was obtained by the fish fed on test diet-IV having 750 FTU kg^{-1} phytase level. Similarly, minerals digestibility performance was also positively affected by phytase supplementation. Again, maximum response of minerals absorption was recorded at the phytase level of 750 FTU kg^{-1} diet. It was concluded that the phytase supplementation to cottonseed meal based diet at 750 FTU kg^{-1} level is optimum to release adequate chelated minerals for maximum growth performance of *L. rohita* fingerlings. These results suggest that phytase supplementation to cottonseed meal based diet can help in the development of sustainable aquaculture by reducing the feed cost and minerals discharge through feces in the aquatic ecosystem.

Keywords: Phytase, cottonseed meal, *Labeo rohita*, growth performance, mineral digestibility

INTRODUCTION

The aquaculture industry is developing more efficiently than other food production sectors. However, economic factors such as cost of fish feed are limiting development (Yıldırım *et al*., 2014). Aquaculture feed mainly depends on the use of fishmeal due to its high nutritional and palatability value (NRC, 2011). It is rich in essential amino acids and fatty acids required for optimum fish growth. However, its availability has alarmingly reduced in the recent years due to over exploitation of natural fishing grounds resulting in tremendous increase in its prices. These increasing fishmeal prices motivated the scientists to identify the cost-effective alternatives of fishmeal (Pham *et al*., 2008; Lech and Reigh, 2012; Shapawi *et al*., 2013)

Plant by-products are the promising sources of protein and energy and may be used for the formation of cost effective and environment friendly aqua-feed (Cheng and Hardy, 2002; Hussain *et al*., 2011a; Khan *et al*., 2011; Hussain *et al*., 2015a,b). One of the main problems related with the use of economical plant proteins in fish feed is the presence of anti-nutritional factors like phytate or phytic acid (myo-inositol 1, 2, 3, 4, 5, 6-

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hexaphosphate), which has harmful effect on the morphology and physiology of digestive tract, affecting fish growth performance (Usmani and Jafri, 2002; Baruah *et al*., 2004). It is considered 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 mono-gastric or agastric fishes (NRC, 1993). Phytase is an enzyme that is produced either by some microorganisms or present in some plant ingredients. This enzyme is very specific to hydrolyze the indigestible phytate complexes that are present in plant ingredients. Monogastric and agastric fish species such as *Labeo rohita* also do not have intrinsic phytase activity, hence inclusion of such phytate rich plant ingredients in fish feed, is imperative (Baruah *et al*., 2004; Cao *et al*., 2007; Hussain *et al*., 2011b). Microbial phytase is now being used as feed supplement. Many plant by-products have been successfully used in aquaculture without affecting the feed quality. Efforts are required to study the suitability of locally available plant by-products to enhance the fish production (Gabriel *et al*., 2007; Hussain *et al*., 2011a). Cottonseed meal (CSM) has long been used as valuable ingredient for diets of both terrestrial animals (Colin-Negrete *et al*., 1996) as well as aquatic species, because it is easily available, inexpensive and rich in protein (Pham *et al*., 2008). It has also been successfully used in various fish species such as rainbow trout (Cheng and Hardy, 2002), tilapia (Mbahinzireki *et al*., 2001), sunshine bass (Rawles and Gatlin, 2000), olive flounder (Pham *et al*., 2008) and parrot fish (Lim and Lee, 2009). However, less information is available for the formulation of artificial feeds for commercially important stomach-less fish like *Labeo rohita* (Iqbal *et al*., 2014). The objective of the present study is to investigate the effect of phytase supplementation on growth performance and mineral digestibility of *L. rohita* fingerlings fed on CSM-based diets that may lead to the development of cost effective and environment friendly fish feed.

MATERIALS AND METHODS

Experimental design

CSM was selected as test ingredient to formulate basal diet which comprised 30% cottonseed meal and 70% reference diet. Basal diet was then further divided into seven test diets and sprayed with graded levels of phytase (0, 250, 500, 750, 1000, 1250 and 1500 FTU kg^{-1}). These seven test diets and one reference diet were fed to eight fish groups in three replicates, stocked in specially designed V-shaped tanks. The experiment lasted till the collection of 4-5 g fecal material of each replicate separately.

In this study, there is only one factor with different levels and all replicates and treatments were applied randomly to the experimental units *i.e.* tanks and all experimental units were provided homogenous conditions; hence Completely Randomized Design (CRD) was used. Due to slow growth rate and less amount of fecal matter collection, both growth and digestibility trials were conducted simultaneously. The trial consisted of two overlapping sections: (i) assessment of growth performance in terms of weight gain and feed conversion ratio (FCR), (ii) assessment of the mineral digestibility of the test and reference diets which was determined indirectly using chromic oxide as inert marker.

Fish and experimental conditions

Experimental fish (*L. rohita*) fingerlings were procured from a local public sector hatchery and allowed to acclimate for two weeks in V-shaped tanks (70 L capacity), designed for the collection of fecal material. During acclimation period the fingerlings were fed on reference diet as described by Allan and Rowland (1992). pH, dissolved oxygen and electrical conductivity were monitored through pH meter (Jenway 3510), D.O. meter (Jenway 970) and electrical conductivity meter (HANNA: HI. 8633), respectively. The range of water quality parameters such as temperature was 24.9-28.7ºC, pH 7.4-8.6, dissolved oxygen 5.8-7.3 mg L^{-1} and electrical conductivity 1.30-1.52 dSm^{-1}. Compressed air was supplied from an air compressor through capillary system and air stones in the form of micro bubbles to all the experimental tanks. The fingerlings were treated with 0.5% saline solution to kill and remove any pathogen if present (Rowland and Ingram, 1991).

Table I.- 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
Cottonseed meal	92.90	41.38	2.63	1.23	6.76	48.00	3.71

Feed ingredients and formulation of experimental diets

The feed ingredients were procured from the local poultry feed market and analyzed chemically following the standards methods (AOAC, 1995) prior to the diet preparation (Table I). The compositions of reference and basal diet have been given in Table II. Reference and CSM based basal diet were prepared by mixing appropriate amount of finely ground (< 0.5 mm particle size) ingredients in electric mixer for 10 min. Later on, fish oil was added gradually while mixing was continued for further five min. Afterwards, 10-15% water was also added to prepare suitable dough (Lovell, 1989). The diets were extruded into floating pellets (3mm) through Lab Extruder (model SYSLG30-IV Experimental Extruder). 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 25 ml distilled water and sprayed on 1 kg of each test diet (Robinson *et al*., 2002). The reference diet was also sprayed with a similar amount of distilled water to maintain an equal level of moisture. The diets were stored at 4°C till further use.

Growth study

Labeo 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 on daily feed intake. For each test diet three replicates were assigned and in each replicate fifteen fish (average weight: 7.04 g $fish^{-1}$) 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. Fish in each tank were bulk weighed every 15th day during experiment to assess the growth performance of *Labeo rohita* fingerlings. Weight gain (%) and feed conversion ratio (FCR) of fingerlings was evaluated based on standard formulae.

Table II.- Ingredients composition (%) of reference and basal diet (As fed basis).

Ingredients	Reference diet	Basal diet
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**	1.0	1.0
Ascorbic acid	1.0	1.0
Chromic oxide	1.0	0.7
Cottonseed meal	-	30.0

*****Each Kg of vitamin premix containes Vitamin A, 15 M.I.U. ; Vitamin B_1, 5000 mg ; Vitamin B_2, 6000 mg ; Vitamin B_6, 4000 mg ; Vitamin B_{12}, 9000 mcg ; Vitamin C, 15000mg ; Vitamin D_3, 3 M.I.U. ; Vitamin E, 6000 IU ; Vitamin K_3, 4000 mg ; Folic acid, 750 mg ; Calcium pantothenate, 10000mg ; Nicotinic acid, 25000mg

Each Kg mineral granules contains Ca, 155g; P, 135g; Mg, 55g; Fe, 1000 mg; Zn, 3000 mg;** Na, 45g ; Mn, 2000mg ; Cu, 600mg ; Co, 40mg ; I, 40mg; Se, 3mg

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

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

Chemical analysis of feed and feces

The samples of feed ingredients, test diets and feces were homogenized using a mortar 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) was determined by Micro Kjeldahl Apparatus and crude fat by petroleum ether extraction method 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 whereas ash by ignition at 650°C for 12 h in electric furnace (Eyela-TMF 3100) to constant weight. Total carbohydrate (N-free extract) was calculated by difference, *i.e.*, total carbohydrate % =100- (CP%+ EE%+CF%+Ash %). 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, mineral contents were estimated using Atomic Absorption Spectrophotometer (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 and potassium was done through Flame Photometer (Jenway PFP-7, UK). The phosphorus was analyzed calorimetrically (UV/VIS spectrophotometer) using ammonium molybdate as reagent at 720 nm absorbance (AOAC, 1995).

Digestibility studies

Chromic oxide was used as an inert marker at 1% inclusion level in reference diet assuming that the amount of the marker in the feed and feces remains constant throughout the experimental period and that all of the ingested marker will appear in the feces.

After the completion of feeding session, feces were collected from the fecal collecting tube of each tank. Care was taken to avoid breaking the thin fecal strings in order to minimize the nutrient leaching. Fecal material of each replicated treatment was dried in oven, grinded and stored for chemical analysis. Chromic oxide contents in diets and feces were estimated after oxidation with molybdate reagent (Divakaran *et al.*, 2002) using UV-VIS 2001 Spectrophotometer at 370 nm absorbance. The apparent digestibility of minerals *i.e.*, calcium (Ca), phosphorous (P), magnesium (Mg), sodium (Na), potassium (K), iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn) of test diets was determined indirectly at the end of the experiment using chromic oxide as inert marker.

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

ADC% of experimental diets were calculated by the formula reported in NRC (1993):

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

Statistical analysis

The growth and mineral digestibility data for each variable was statistically analyzed by using one way analysis of variance (ANOVA) followed by Tukey's Honesty Significant Difference Test (Steel *et al.*, 1996; Snedecor and Cochran, 1991) by using COSTAT (Version 6.303, PMB 320, Monterey, CA, 93940 USA) software package.

RESULTS

The growth performance of the *L. rohita* fingerlings have been summarized in Table III. The highest weight gain of fish fed CSM based diet was observed at 750 FTU kg^{-1} phytase level. This was not only significantly different ($P<0.05$) from the weight gain in case of reference diet but also from all other phytase supplemented test diets. Maximum feed intake was also visualized at 750 FTU kg^{-1} phytase level followed by phytase level of 1000 FTU kg^{-1} The best FCR value (1.27) was also observed in the diet containing phytase level of 750 FTU kg^{-1}. It was significantly ($P<0.05$) different from the FCR values noted at 0, 250, 1250 and 1500 FTU kg^{-1} levels of phytase supplementation. However, it did not differ significantly when compared with 500 and 1000 FTU kg^{-1} phytase supplemented diets. Quadratic regression analysis

Table III.- Growth performance of *Labeo rohita* fingerlings fed on reference and cottonseed meal-based test diets.

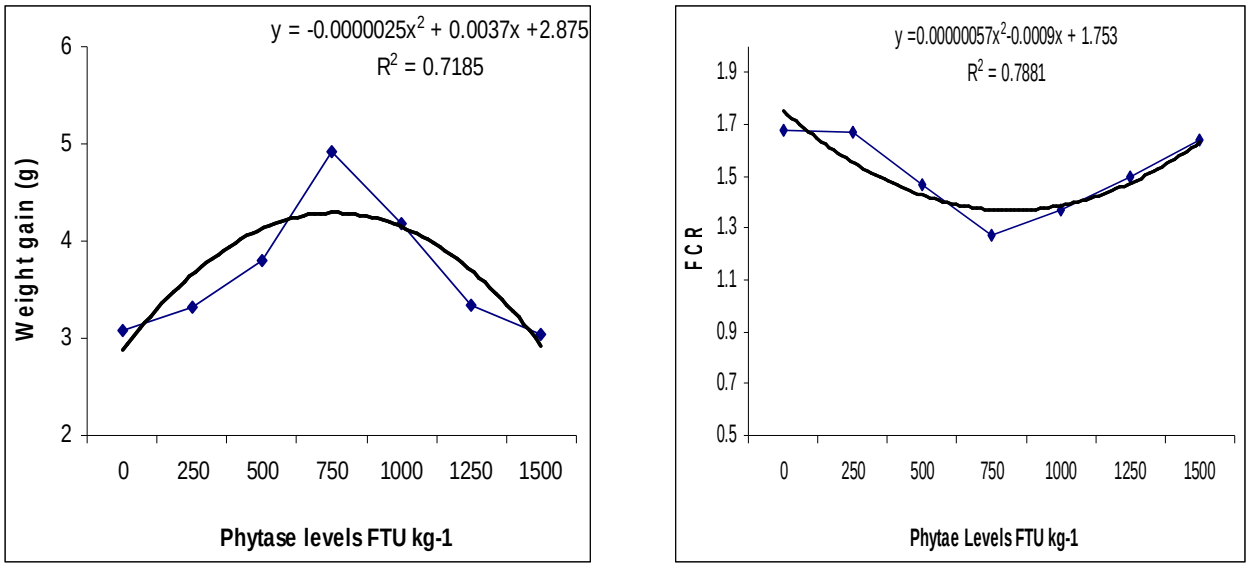
Parameters	Reference diet	Phytase levels (FTU kg-1) 0 Test diet I	250 Test diet II	500 Test diet III	750 Test diet IV	1000 Test diet V	1250 Test diet VI	1500 Test diet VII	PSE	*p* value
Initial weight (g)	7.05	7.03	7.06	7.05	7.05	7.04	7.04	7.06	0.0082	<0.425
Final weight (g)	10.78^{c}	10.12^{e}	10.39^{d}	10.85^{c}	11.97^{a}	11.22^{b}	10.40^{d}	10.09^{e}	0.0355	<0.001
Weight gain (g)	3.73^{c}	3.09^{e}	3.33^{d}	3.80^{c}	4.92^{a}	4.18^{b}	3.35^{d}	3.04^{e}	0.0329	<0.001
Weight gain (%)	52.98^{c}	43.93^{e}	47.24^{d}	53.87^{c}	69.71^{a}	59.37^{b}	47.61^{d}	43.03^{e}	0.4605	<0.001
Weight gain ($fish^{-1}$ day^{-1}) g	0.053^{c}	0.044^{e}	0.048^{d}	0.054^{c}	0.070^{a}	0.060^{b}	0.048^{d}	0.043^{e}	0.0005	<0.001
Feed intake (fish-1 day-1) g	0.074^{bc}	0.074^{bc}	0.079^{abc}	0.080^{abc}	0.089^{a}	0.082^{ab}	0.072^{bc}	0.071^{c}	0.002	<0.001
FCR	1.39^{ab}	1.68^{c}	1.67^{c}	1.47^{abc}	1.27^{a}	1.37^{ab}	1.50^{bc}	1.64^{c}	0.0452	<0.001

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

Table IV.- Analyzed mineral composition (%) of reference and cottonseed meal-based test diets.

Diets	Phytase levels (FTU kg^{-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	0.26	2.41	0.092	0.93	1.37	0.082	0.072	0.13	0.047
Test diet-II	250	0.27	2.44	0.095	0.93	1.33	0.084	0.073	0.14	0.042
Test diet-III	500	0.24	2.49	0.093	0.96	1.38	0.086	0.076	0.14	0.044
Test diet-IV	750	0.27	2.49	0.096	0.97	1.42	0.089	0.077	0.14	0.045
Test diet-V	1000	0.26	2.46	0.090	0.95	1.39	0.084	0.073	0.14	0.044
Test diet-VI	1250	0.22	2.46	0.095	0.94	1.40	0.083	0.072	0.13	0.041
Test diet-VII	1500	0.24	2.43	0.093	0.93	1.38	0.084	0.074	0.13	0.043
PSE		0.0073	0.0123	0.0013	0.0100	0.0105	0.0018	0.0009	0.0078	0.0011
P		0.0024	0.0000	0.0681	0.0000	0.0000	0.0000	0.0000	0.2960	0.0000

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



Cottonseed meal-based diet (*740 4.24) FCR (*790 **1.40)

(*Optimal phytase level FTU kg^{-1} ** Weight gain ** FCR)

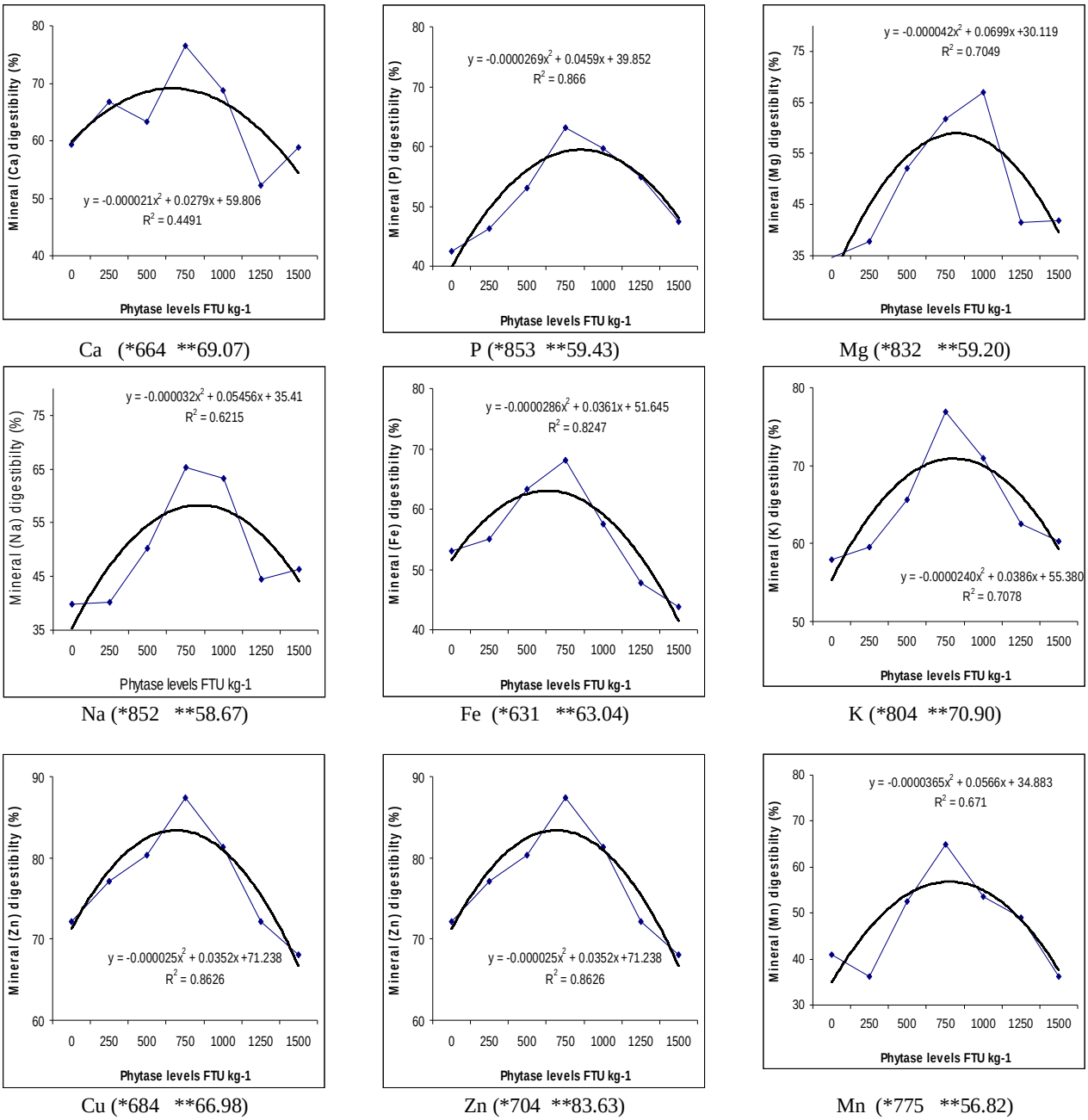
Fig. 1. The relationship between growth performances of *Labeo rohita* fingerlings in terms of weight gain (g) and FCR and phytase levels (FTU kg^{-1}).

indicated that phytase supplementation in cottonseed meal based diet provided optimum weight gain and FCR at 740 FTU kg^{-1} and 790 FTU kg^{-1} levels, respectively (Fig. 1).

The phytase incorporation in CSM based diet increased the mineral digestibility of *L. rohita* fingerlings in response to phytase supplementation (Table VI). Highest digestibility (%) values of Ca, P, K, Cu, Fe and Zn were noticed in fingerlings fed on diet supplemented with 750 FTU kg^{-1} phytase level which differed significantly (P<0.05) from all other treatments. However, Mg digestibility was found highest at 1000 FTU kg^{-1} phytase diet. The digestibility values of Na at 750 and 1000 FTU kg^{-1} levels were found to be statistically at par (P<0.05) but these values significantly differed from reference and all other phytase treated diets. For all the observed minerals, next higher digestibility values were observed in the diet containing 1000 FTU kg^{-1} phytase except Fe which showed next highest digestibility at 500 FTU kg^{-1} phytase level. Quadratic regression analysis indicated that optimum digestibility of Ca, P, Mg, Na, K, Fe, Cu, Zn and Mn occurred at 664, 853, 832, 852, 804, 631, 684, 704 and 775 FTU kg^{-1} phytase levels, respectively (Fig. 2).

DISCUSSION

CSM is a rich source (40%) of protein. It has been successfully used as major ingredient of feed for various fish species. However, its very high content of phytic acid limits its use as major ingredient in diets of stomach less fish species (Cao *et al*., 2007). The concentration of phytic acid in diet inversely relates with the growth performance of fish as it act as anti-nutritional factor (Sajjadi and Carter, 2004). Spinelli *et al*. (1983) also noticed decrease in growth rate and feed efficiency with the increase in phytate concentration in feed of rainbow trout. The growth performance of *L. rohita* fingerlings in terms of weight gain and FCR was significantly improved in CSM-based diets supplemented with phytase. The maximum weight gain and best FCR were observed at 750 FTU kg^{-1} phytase supplementation level. A linear increase in growth performance was observed with the increase in phytase supplementation dose upto 750 FTU kg^{-1}, however interestingly, higher doses causes decrease in growth performance resulting in overall quadratic



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

Fig. 2. The relationship between mineral digestibility (%) by *L. rohita* fingerlings and phytase levels (FTU kg^{-1}).

trend. The findings of the present study provide evidence that phytase supplementation at 750 FTU kg^{-1} level is sufficient to minimize the effect of phytic acid while using cottonseed meal as major feed ingredient in diet of *L. rohita*. The present results of growth performance of *L. rohita* fingerlings fed on phytase supplemented diets are in agreement with the findings of Baruah *et al*. (2007) and Hussain *et al*. (2011c). Negative response of growth performance at higher levels of phytase

Table V.- Analyzed mineral composition (%) of feces of reference and cottonseed meal-based test diets.

Diets	Phytase levels (FTUkg^{-1})	Ca (%)	P (%)	Mg (%)	Na (%)	K (%)	Fe (%)	Cu (%)	Zn (%)	Mn (%)
Reference diet	---	0.120b	2.13^{e}	0.064^{d}	0.61^{b}	0.73^{d}	0.063^{f}	0.054^{d}	0.058^{e}	0.048^{d}
Test diet-I	0	0.120^{b}	1.55^{d}	0.068^{d}	0.62^{b}	0.65^{cd}	0.043^{cd}	0.036^{bc}	0.039^{cd}	0.031^{c}
Test diet-II	250	0.097^{ab}	1.43^{c}	0.064^{d}	0.61^{b}	0.58^{bc}	0.041^{bc}	0.034^{bc}	0.034^{bc}	0.029^{bc}
Test diet-III	500	0.100^{ab}	1.32^{bc}	0.050^{bc}	0.54^{b}	0.54^{bc}	0.036^{ab}	0.032^{b}	0.032^{b}	0.024^{ab}
Test diet-IV	750	0.073^{a}	1.08^{a}	0.043^{ab}	0.39^{a}	0.38^{a}	0.033^{a}	0.023^{a}	0.021^{a}	0.019^{a}
Test diet-V	1000	0.100^{ab}	1.22^{b}	0.037^{a}	0.43^{a}	0.50^{ab}	0.044^{cd}	0.031^{ab}	0.031^{b}	0.025^{bc}
Test diet-VI	1250	0.120^{b}	1.25^{b}	0.062^{d}	0.59^{b}	0.59^{bc}	0.049^{de}	0.041^{c}	0.042^{d}	0.023^{ab}
Test diet-VII	1500	0.110^{b}	1.44	0.061^{cd}	0.56^{b}	0.62^{cd}	0.053^{d}	0.041^{c}	0.045^{d}	0.031^{c}
PSE		0.0069	0.0252	0.0022	0.0182	0.0234	0.0014	0.0017	0.0015	0.0013
P		0.0026	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

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

Table VI.- Mineral digestibility (%) of reference and cottonseed meal-based test diets.

		Phytase levels (FTU kg-1)								
		0	250	500	750	1000	1250	1500		
Mineral	Reference diet	Test diet I	Test diet II	Test diet III	Test diet IV	Test diet V	Test diet VI	Test diet VII	PSE	*p* value
Ca	52.82^{cd}	59.38^{bcd}	66.82^{ab}	63.24^{bc}	76.55^{a}	68.70^{ab}	52.16^{d}	58.91^{bcd}	2.138	< 0.001
P	48.41^{cde}	42.50^{e}	46.20^{de}	53.10^{bcd}	63.15^{a}	59.67^{ab}	54.92^{bc}	47.38^{de}	1.431	< 0.001
Mg	39.48^{c}	34.56^{c}	37.81^{c}	52.14^{b}	61.84^{ab}	67.06^{a}	41.47^{c}	41.93^{c}	2.005	< 0.001
Na	47.21^{bc}	39.90^{c}	40.19^{bc}	50.16^{b}	65.25^{a}	63.33^{a}	44.46^{bc}	46.29^{bc}	2.05	< 0.001
K	59.21^{cd}	57.96^{d}	59.56^{cd}	65.67^{bc}	76.89^{a}	70.92^{ab}	62.56^{cd}	60.32^{cd}	1.529	< 0.001
Fe	50.37^{cde}	53.00^{cd}	54.96^{cd}	63.30^{ab}	68.03^{a}	57.55^{bc}	47.73^{de}	43.88^{e}	1.585	< 0.001
Cu	48.93^{d}	55.55^{cd}	57.44^{bcd}	62.35^{bc}	74.68^{a}	66.12^{ab}	50.11^{d}	50.19^{d}	1.975	< 0.001
Zn	64.18^{e}	72.14^{cd}	77.13^{bc}	80.30^{b}	87.43^{a}	81.35^{ab}	72.20^{cd}	68.02^{de}	1.355	< 0.001
Mn	45.50^{bcd}	40.94^{cd}	36.29^{d}	52.55^{b}	64.88^{a}	53.61^{b}	49.08^{bc}	36.29^{d}	2.064	< 0.001

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

supplementation in the present study is difficult to explain. However, inferior results were also observed in case of Korean rockfish, *Sebastes schlegeli* fed on soybean meal based diet when phytase was supplemented at 2000 FTU kg^{-1} level as compared to 1000 FTU kg^{-1} level (Yoo *et al.*, 2005). Liebert and Portz (2007) have concluded that supplementation of phytase at 750 FTU kg^{-1} is sufficient for maximum degradation of phytate in plant based diet resulting in higher growth performance of Nile tilapia (*Oreochromis nilotics*). Nwanna *et al.* (2007) also reported significant increase in growth performance at 750 and 1000 FTU kg^{-1} levels of phytase supplementation in diets of *Cyprinus carpio*.

In the present study, maximum weight gain % (70%) was observed at 750 FTU kg^{-1} level of phytase supplementation whereas literature revealed more increase for the similar time duration in fish feeding trials with juvenile fish (Debnath *et al*., 2005; Baruah *et al*., 2007; Sardar *et al*., 2007). It indicates that fish did not respond well to the experimental feed. The poor growth performance might be partially due to less intake of feed as the fish could not be domesticated properly, partially because of the experimental water salinity, which was higher as compared to its natural riverine habitats. It is also evident that pond fisheries has also been affected alarmingly, probably due to the rapidly deteriorating quality of ground waters of this region as the same was used in the present study.

It is well known that phytate chelates with minerals and makes them unavailable to fish by reducing their digestibility (Cao *et al*., 2007; Hussain *et al*., 2011a). Reduction in fish growth may correlate with the scarcity in availability of essential minerals (Lall, 2002). In the present study, phytase supplementation improved mineral digestibility to the fingerlings. Quadratic regression analysis indicated an optimum dose for mineral digestibility around 750 FTU kg^{-1} phytase supplemented level. The digestibility of minerals at this level was significantly different from reference diet and remaining phytase sprayed diets. Higher growth performance observed in the present study may attributed to the improved mineral digestibility. Positive results of phytase supplementation in terms of higher mineral digestibility were also reported by Sugiura *et al*. (2001) in rainbow trout and Hussain *et al*. (2011a) in *L. rohita*. Improved mineral absorption in against to phytase supplementations was also reported by other researchers (Baruah *et al*., 2007; Ai *et al*., 2007; Sardar *et al*., 2007).

In conclusion, the present study provided sufficient evidence that 750 FTU kg^{-1} level of phytase supplementation had a significant effect on the mineral digestibility resulting in higher growth performance by *L. rohita* fingerlings fed on cottonseed meal-based diets. Phytase supplementation in plant based diets may decrease the need for supplementing minerals, which will reduce the cost of fish feed and minerals discharge through feces into the aquatic ecosystem resulting in environment friendly aquaculture.

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