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Effect of phytase supplementation on growth performance and mineral digestibility in *Labeo rohita* (Hamilton, 1822) fingerlings fed on sunflower meal based diet

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ABSTRACT

Present study was conducted to evaluate the efficacy of phytase supplementation on growth performance and mineral digestibility of *Labeo rohita* fingerlings fed sunflower meal based diet. Control diet I without inclusion of sunflower meal and phytase, and control diet II with inclusion of sunflower meal but without supplementation of phytase were prepared. Six experimental diets were prepared by spraying graded levels (250, 500, 750, 1000, 1250 and 1500 FTU kg^{-1}) of phytase in the control diet II. All the diets were isonitrogenous (30.63%) and isoenergetic (4.28 kcal g^{-1}). Chromic oxide was used as inert marker and mixed in the diets at 1% level. Total duration of the experiment was ten weeks (70 days). Initially, the fingerlings were fed at the rate of 2% of live wet weight on their prescribed diet twice daily and subsequently adjusted on daily feed intake. The experiment was run under completely randomised design (CRD). The results of our study showed increased growth performance of fingerlings in response to phytase supplementation. Maximum growth was observed in fingerlings fed on test diet supplemented with phytase at 750 FTU kg^{-1}. Maximum digestibility of minerals such as Ca, P, Mg, Na, K, Fe, Cu and Mn was also noticed in fish fed diet supplemented with phytase at 750 FTU kg^{-1} level. It was concluded that the phytase supplementation in sunflower meal based diet at 750 FTU kg^{-1} level is optimal to release adequate levels of chelated minerals for maximum growth performance of *L. rohita* fingerlings. These results also suggest that phytase supplementation in sunflower meal based diets may decrease the need for supplementing minerals, which will reduce the cost of fish feed and mineral discharge through faeces into the aquatic environment resulting in ecofriendly aquaculture.

Keywords: Growth performance, *Labeo rohita*, Mineral digestibility, Phytase, Sunflower meal

Introduction

The aquaculture sector is developing more efficiently than other food production industries. However, economic factor such as cost of fish feed is limiting its development (Yildırım *et al*., 2014). Fish feed mainly depends on the use of fishmeal due to its high nutritional and palatability value (NRC, 2011). Fish meal 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 overutilisation of natural fishing grounds leading to tremendous increase in price. The increasing fish meal price motivated researchers to identify economical alternatives (Lech and Reigh, 2012; Shapawi *et al*., 2013). Plant byproducts are promising sources of protein and energy and may be used for the development of cost effective and environment friendly aquafeed (Cheng and Hardy, 2002; Hussain *et al*., 2011a; 2014; 2015a, b). The problem related with the use of plant byproducts in fish feed is the presence of anti-nutritional factors like phytate or phytic acid. This has harmful effect on the morphology and physiology of digestive tract. Moreover, due to negative charge on the phytate, it chelates positively charged amino acids, proteins, fatty acids and mineral cations leading to their deficiency in fish which affect fish growth performance (Usmani and Jafri, 2002; Baruah *et al*., 2004). About 80% of the total phosphorus content in plants may be found in the form of phytate that is practically not available for monogastric or agastric fishes (NRC, 1993).

Phytase is an enzyme that is produced either by certain microorganisms or present in some plant ingredients.

It is very specific to hydrolyse the indigestible phytate complexes that are present in plant ingredients. Agastric fish species such as *Labeo rohita* also do not have natural phytase activity, hence use of phytate rich plant ingredients in fish feed, fail to contribute positively and ultimately reduces the fish growth performance (Baruah *et al.,* 2004; Cao *et al*., 2007; Hussain *et al*., 2011b). Sunflower meal plays significant role as alternative and economical source of important nutrients. World over, it is the fourth largest source of protein after soybean meal, cottonseed meal and canola meal (Anjum *et al*., 2014). Sunflower meal has approximately 40% protein content (Mushtaq *et al*., 2006). However, like other plant ingredients, sunflower meal also contains phytate (0.2%) which make its use difficult in fish feed formulations. Inclusion of 0.5 or 1% phytate in purified diets for agastric common carp (*Cyprinus carpio*) caused significant reduction in growth and feed efficiency (Hossain and Jauncey, 1993). Specific growth rate of rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) was significantly decreased when phytate was included @ >1% of total diet (Usmani and Jafri, 2002). However, many studies reported that addition of phytase to diets enhanced growth performance. An increase in weight gain was reported in channel catfish fed phytase supplemented diets containing only plant protein or a combination of plant and animal protein sources (Jackson *et al*., 1996). In the study of Li and Robinson (1997), fish fed diets containing phytase at 250 FTU kg^{-1} or above consumed more feed, gained more weight and had a lower FCR in comparison to fish fed on basal diet containing no phytase. It is also known that the distraction of cell wall matrix of sunflower meal by supplementation of exogenous microbial enzymes lead to easy access of the endogenous proteolytic enzymes to digest the chelated proteins (Choct and Kocher, 2000). The objective of the present research work was to investigate the effect of phytase supplementation on growth performance and mineral digestibility of *L. rohita* fingerlings fed on sunflower meal based diets, with an aim to develop cost effective and environment friendly fish feed.

Materials and methods

Experimental fish

Labeo rohita fingerlings were procured from the Government Fish Seed Hatchery, Satiana Road, Faisalabad, Pakistan and allowed to acclimate for two weeks in V-shaped tanks (70 l capacity), specially designed for collection of faecal material. The experiment was conducted in the Fish Nutrition Laboratory, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad. During acclimation period, the fingerlings were fed on control diet. Initially, the fingerlings were fed at the rate of 2% of live wet weight on their prescribed diet twice daily and subsequently adjusted on daily feed intake. pH, dissolved oxygen and electrical conductivity were monitored using pH meter (Jenway 3510), D.O. meter (Jenway 970) and electrical conductivity meter (HANNA: HI. 8633) respectively. The water quality parameters recorded were in the optimum range: temperature 24.9-28.7°C, pH 7.4-8.6, dissolved oxygen 5.8-7.3 mg l^{-1} and electrical conductivity 1.30-1.52 dS m^{-1}. Tanks were provided with proper aeration. Fingerlings were treated with 0.5% saline solution initially, as a means of disinfection (Rowland and Ingram, 1991).

Preparation of experimental diets

Feed ingredients were procured from the local poultry feed market and analysed chemically following standard methods (AOAC, 1995) prior to diet preparation (Table 1). Biochemical composition of the experimetnal diets are given in Table 2. Diets were prepared by mixing appropriate amount of finely ground (<0.5 mm particle size) ingredients in an electric mixer for 10 min. Later on, fish oil was added gradually while mixing was continued for further five minutes. Subsequently, 10-15% water was added to prepare a dough of required consistency (Lovell, 1989). The diets were extruded into floating pellets (3 mm) in a 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 dissolved in 25 ml distilled water and sprayed on 1 kg of each test diet (Robinson *et al*., 2002). The diets without phytase supplementation were also sprayed with same quantity of distilled water alone, in order to maintain an equal level of moisture. After drying, the diets were stored at 4°C until further use.

Table 1. Chemical composition (%) of feed ingredients (Drymatter basis)

Ingredients	Drymatter (%)	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Ash (%)	Nitrogen free extract (%)	Gross energy (kcal g^{-1})
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
Sunflower meal	94.13	42.91	3.27	1.74	10.90	41.18	3.63

Table 2. Ingredients composition (%) of experimental diets (as fed basis) and biochemical composition (on drymatter basis) of experimental diets

Parameters	Control diet I	Control diet II	Test diet I	Test diet II	Test diet III	Test diet IV	Test diet V	Test diet VI
Fish meal	20.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0
Wheat flour	24.0	16.8	16.8	16.8	16.8	16.8	16.8	16.8
Corn gluten (60%)	20.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0
Rice polish	25.0	16.6	16.6	16.6	16.6	16.6	16.6	16.6
Fish oil	7.0	4.9	4.9	4.9	4.9	4.9	4.9	4.9
Vitamin Premix*	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mineral**	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Ascorbic acid	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Chromic oxide	1.0	0.7	1.0	0.7	1.0	0.7	1.0	0.7
Sunflower meal	-	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Phytase(FTU kg^{-1})	-	0	250	500	750	1000	1250	1500
Proximate analysis								
Crude Protein (%)	30.21	30.07	30.78	30.78	31.38	30.92	30.74	30.23
Crude Fat (%)	6.65	5.67	5.63	5.66	5.68	5.63	5.67	5.65
Gross energy (kcal g^{-1})	4.26	4.26	4.31	4.27	4.32	4.29	4.24	4.29

**Each Kg of Vitamin premix containes			
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 B6	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		

Experimental design

The eight experimental diets were fed to eight different experimental groups having three replicates in each group (Table 2). Fifteen acclimatised fish (average weight: 7.04 g $fish^{-1}$) each were randomly stocked in the V-shaped experimental tanks (70 l capacity). Proper aeration was provided in the experimental tanks. Experiment was conducted under completely randomised design. Total duration of the experiment was ten weeks (70 days) during which 4-5 g faeces were collected from each replicate for proximate analysis. *Labeo rohita* fingerlings were fed twice daily (morning and afternoon) and feed consumption record was maintained. Water was drained out from each tank daily and the tanks were cleaned and refilled. Chromic oxide was included at 1% level as an inert marker in the diet for conducting the digestibility trial for 70 days. During this period also the fish were fed with respective diets. After completion of experimental feeding, faecal matter were collected from the faecal collecting tube of each tank. Care was taken to avoid breaking the thin faecal strings in order to minimise nutrient leaching. Faecal material from each replicated treatment was dried in an oven, ground and stored for chemical analysis.

Sampling for growth performance

After the 2 h feeding session, the uneaten diet was collected. Fish in each tank were bulk weighed every 15th day during the experiment to assess the growth performance. Weight gain (%) and feed conversion ratio (FCR) were evaluated using standard formulae:

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

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

Chemical analysis

The samples of feed ingredients, diets and faeces were homogenised using mortar and pestle and analysed for proximate composition as well as gross energy following standard methods (AOAC, 1995). Moisture was determined by oven drying at 105°C for 12 h. Crude protein (N×6.25) was determined in Micro Kjeldahl Apparatus and crude fat by petroleum ether extraction method using Soxtec HT2 1045 system. Crude fiber was determined as loss on ignition of dried lipid-free residues

after digestion with 1.25% H_2SO_4 and 1.25% NaOH. Ash content was estimated by ignition at 650°C for 12 h in an electric muffle furnace (Eyela-TMF 3100) to constant weight. Nitrogen free extract (NFE) was calculated as, NFE % = 100 - (CP%+ EE%+CF%+Ash%). Gross energy was determined using Oxygen Bomb Calorimeter.

For estimation of minerals such as calcium (Ca), phosphorous (P), magnesium (Mg), sodium (Na), potassium (K), iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn), the diets and faecal 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 Polarised 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 in a Flame Photometer (Jenway PFP-7, UK). Phosphorus was analysed calorimetrically (UV/VIS spectrophotometer) using ammonium molybdate as reagent at 720 nm absorbance (AOAC, 1995).

Chromic oxide content in diets and faeces were estimated after oxidation with molybdate reagent (Divakaran *et al.*, 2002) using UV VIS Spectrophotometer at 370 nm. The apparent digestibility of minerals such as Ca, P, Mg, Na, K, Fe, Cu, Zn and Mn of experimental diets was determined using the following formula (NRC, 1993):

$$\text{Apparent nutrient digestibility coefficient, ADC (\%)} = \frac{100 - 100 \times \text{Percent marker in diet} \times \text{Percent nutrient in faces}}{\text{Percent marker in faces} \times \text{Percent nutrient in diet}}$$

Statistical analysis

Mean values of the growth and mineral digestibility data of all experimental groups were statistically analysed using one way analysis of variance (ANOVA) followed by Tukey's Honesty Significant Difference Test (Snedecor and Cochran, 1991; Steel *et al.,* 1996) using COSTAT (Version 6.303, PMB 320, Monterey, CA, 93940 USA) software package.

Results and discussion

Phytase supplementation in sunflower mealbased test diets increased the nutritional quality by hydrolysing the phytate complexes. A significant ($p<0.05$) increase in weight gain was observed with increase in phytase concentration up to a level of 750 FTU kg^{-1} after which weight gain of fish decreased with further increase in phytase levels (1000, 1250 and 1500 FTU kg^{-1}). The highest weight gain (5.21g and 74%) of *L. rohita* fingerlings was observed in case of fish fed sunflower meal based experimental diet with 750 FTU kg^{-1} level of phytase followed by fish fed diets supplemented with phytase at 1000 and 500 FTU kg^{-1}. The values were found to be significantly different ($p<0.05$) from the weight gain of fish fed control diet and other experimental diets (Table 3). Among the fish groups fed control diets, fish fed control diet I showed better weight gain as compared to those fed control diet II. Significantly high ($p<0.05$) feed intake and feed conversion ratio (FCR = 1.21) was noted in fish fed diet supplemented with phytase at 750 FTU kg^{-1} level in comparison to other groups. Though feed intake did not vary significantly between the fish of control I and II, fish of control I showed better FCR than control II. Quadratic regression analysis indicated that phytase supplementation in sunflower mealbased diet provided optimum weight gain at 663 FTU kg^{-1} level (Fig. 1).

Table 3. Growth performance of *Labeo rohita* fingerlings fed on different experimental diets

Parameters	Phytase levels (FTU kg^{-1})								PSE	p
	Control diet I	Control diet II	Test diet I	Test diet II	Test diet III	Test diet IV	Test diet V	Test diet VI		
		0	250	500	750	1000	1250	1500		
Initial weight (g)	7.05	7.04	7.05	7.05	7.04	7.05	7.07	7.05	0.0078	0.2921
Final weight (g)	10.78^{c}	10.14^{e}	10.40^{d}	10.92^{c}	12.25^{a}	11.11^{b}	10.48^{d}	10.11^{e}	0.0398	0.0000
Weight gain (g)	3.73^{c}	3.10^{e}	3.35^{d}	3.87^{c}	5.21^{a}	4.06^{b}	3.41^{d}	3.06^{e}	0.0406	0.0000
Weight gain (%)	52.98^{c}	44.08^{e}	47.49^{d}	54.84^{bc}	73.97^{a}	57.56^{b}	48.18^{d}	43.38^{e}	0.5901	0.0000
Weight gain (g fish^{-1} day^{-1})	0.053^{c}	0.044^{e}	0.048^{d}	0.055^{bc}	0.074^{a}	0.058^{b}	0.049^{d}	0.044^{e}	0.0006	0.0000
Feed intake (g fish^{-1} day^{-1})	0.074^{b}	0.073^{b}	0.076^{b}	0.079^{ab}	0.090^{a}	0.078^{b}	0.070^{b}	0.070^{b}	0.0025	0.0007
FCR	1.39^{ab}	1.65^{c}	$1.60b^{c}$	1.42^{abc}	1.21^{a}	1.34^{a}	1.44^{abc}	1.60^{bc}	0.0514	0.0002

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)

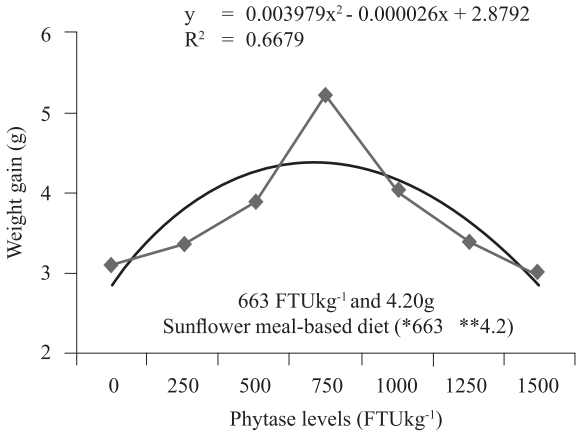

Fig. 1. Relationship between growth performance of *Labeo rohita* fingerlings in terms of weight gain (g) and phytase levels (FTU kg^{-1})

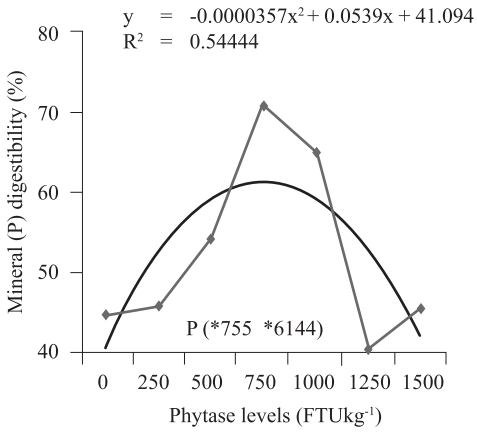

Fig. 2. The quadratic relationship between P digestibility (%) of experimental diets and phytase levels (FTU kg^{-1})

The digestibility of different experimental diets increased significantly with increasing levels of phytase supplementation with significantly ($p<0.05$) high mineral digestibility in fish fed diet supplemented with phytase at 750 FTU kg^{-1} level followed by 1000 FTU kg^{-1} level. Mineral digestibility did not improve with further increase in phytase level (Table 4). There was no significant difference in mineral digestibility between the fish of control I and control II groups but numerically better digestibility of minerals was observed in the fish of control I (Table 4). Comparing the digestibility of various minerals at optimal phytase supplementation level, K, Ca, Zn and P showed higher digestibility values as compared to Na, Fe, Cu and Mn, with the minimum digestibility value for Na. Quadratic regression analysis indicated optimum P digestibility at 755 FTU kg^{-1} level (Fig. 2).

Sunflower meal is a rich source of protein (Mushtaq *et al*., 2006). But the main limitation in using sunflower meal in fish feed is its higher polyphenol and phytate contents. Phytate reduces the availability of protein by binding with trypsin (Spinelli *et al*., 1983). Phytate not only reduces the availability of P (Storebakken *et al*., 1998), it also decreases the availability of other minerals including Zn, Mg and Ca to the fish (Denstadli *et al*., 2006). However, sunflower meal has been successfully used as a major ingredient in the feed for various fish species after removal of anti-nutritional factors (Cao *et al*., 2007). In the present study we supplemented exogenous phytase in sunflower meal based diet for removal of harmful effects of phytate and tried to standardise the optimum dose of phytase supplementation in the diet of rohu fingerlings. Inclusion of sunflower meal in the diet reduced growth performance and mineral digestibility in *L. rohita.* Growth performance of *L. rohita* fingerlings in terms of weight gain and feed conversion ratio (FCR) was significantly improved ($p<0.05$) in sunflower meal based

Table 4. Mineral digestibility (%) of *Labeo rohita* fingerlings fed on different experimental diets

Minerals	Control I	Phytase levels (FTU kg^{-1})							PSE	p
		Control diet II	Test diet I	Test diet II	Test diet III	Test diet IV	Test diet V	Test diet VI		
		0	250	500	750	1000	1250	1500		
Ca	52.82^{c}	49.63^{c}	48.78^{c}	56.73^{bc}	76.43^{a}	70.84^{a}	60.94^{b}	54.41^{bc}	2.138	0.0000
P	48.41^{bc}	44.92^{cd}	46.10^{cd}	54.44^{b}	70.75^{a}	64.94^{a}	40.89^{d}	45.80^{cd}	1.431	0.0000
Mg	39.48^{b}	40.30^{b}	45.33^{b}	53.41^{a}	58.91^{a}	54.11^{a}	39.87^{b}	37.62^{b}	2.005	0.0000
Na	47.21^{cde}	40.56^{e}	49.86^{cd}	51.82^{bc}	69.99^{a}	56.35^{b}	47.67^{cde}	42.06^{de}	2.050	0.0000
K	59.21^{d}	59.90^{d}	63.67^{cd}	67.00^{bc}	79.12^{a}	70.7^{8b}	65.64^{bcd}	60.36^{cd}	1.529	0.0000
Fe	50.37^{cd}	47.36^{d}	49.55^{d}	57.03^{bc}	69.82^{a}	60.32^{b}	50.88^{cd}	48.93^{d}	1.585	0.0000
Cu	48.93^{b}	47.04^{b}	51.35^{b}	51.83^{b}	64.57^{a}	60.74^{a}	46.59^{b}	50.08^{b}	1.975	0.0000
Zn	64.18b^{c}	57.67^{c}	61.30^{c}	63.60^{c}	71.35^{ab}	74.75^{a}	62.59^{c}	60.99^{c}	1.355	0.0000
Mn	45.50^{de}	45.12^{de}	52.18^{bcd}	56.42^{bc}	69.49^{a}	61.08^{ab}	48.60^{cde}	42.96^{e}	2.064	0.0000

Means within the same row 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)

diets supplemented with phytase which might be due to better utilisation of phytate bound proteins and minerals. A linear increase in growth performance was observed with increase in phytase supplementation with the maximum weight gain and lowest FCR noted in fish fed diet supplemented with phytase at 750 FTU kg^{-1} followed by 1000 FTU kg^{-1}. However, interestingly further higher doses of phytase supplementation caused reduction in growth performance of fish. The findings of the present study showed that phytase supplementation at 750 FTU kg^{-1} level is sufficient to minimise the effect of phytic acid while using sunflower meal as major feed ingredient in the diet of *L. rohita*. The present results on 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*. (2011b; 2015). Liebert and Portz (2007) found that phytase supplementation at 750 FTU kg^{-1} level 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*. Different studies reported that the optimum dosage of phytase supplementation varies with fish species. Better growth performance was observed in Nile tilapia (*Oreochromis niloticus*) fed on plant based diet supplementd with phytase at 1000 FTU kg^{-1} (Ashraf and Goda, 2007; Cao *et al*., 2008). On contrary, Ai *et al*. (2007) observed no significant effect on feed efficiency ratio (FER) and growth performance of Japanese seabass, (*Lateolabrax japonicus*) by phytase supplementation at 500 FTU kg^{-1} diet. Similarly, Yoo *et al*. (2005) in juvenile Korean rock fish *Sebastes schlegeli,* Lim and Lee (2009) in parrot fish *Oplegnathus fasciatus*, Masumoto *et al*. (2001) in Japanese flounder *Paralichthys olivaceus*, Yan and Reigh (2002) in channel catfish *Ictalurus punctutus* and Sajjadi and Carter (2004) in Atlantic salmon *Salmo salar* L. did not find significant effect of phytase supplementation in plant based diets, on weight gain. This inconsistency in the results by different workers might be attributed to differences in phytic acid content in different feed ingredients, nutritional quality of plant ingredients, water quality, fish species and size as well as culture/ experimental conditions (Ashraf and Goda, 2007). In the present study, maximum weight gain (74%) was observed at 750 FTU kg^{-1} level of phytase supplementation, whereas higher values have been reported for similar duration of fish feeding trials with juvenile fish (Debnath *et al*., 2005; Baruah *et al*., 2007; Sardar *et al*., 2007). It might be due to the variation of experimental conditions as well as species differences. The poor growth performance might be partially due to less intake of feed with higher FCR as the fish could not be domesticated properly, because of the experimental water salinity, which was higher as compared to its natural riverine habitats. It is evident that pond fisheries has been affected alarmingly, probably due to the rapidly deteriorating quality of ground waters in Faisalabad District, Punjab, and the same water was used in the present study. Poor growth performance recorded at higher levels of phytase supplementation in the present study is difficult to explain (Hussain *et al*., 2015a). 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).

Phytase supplementation significantly improved feed intake and feed conversion ratio (FCR) of *L. rohita* fingerlings fed on sunflower meal based diets. The maximum feed intake and most superior FCR values were observed in fish fed on diet supplemented with phytase at 750 FTU kg^{-1} diet indicating phytase supplementation increased the palatability and conversion rate of diet into fish flesh. Higher feed intake was reported in various fish species by different authors *viz*., channel catfish *I. punctatus* (Li and Robinson, 1997), Atlantic salmon *S. salar* (Hauler and Carter, 1997), Nile tilapia *Oreochromis niloticus* (Tahoun *et al*., 2009) and *L. rohita* (Hussain *et al*., 2011; 2014). Lower values of FCR were found in rainbow trout fed on soybean meal based diet supplemented with phytase (Wang *et al*., 2009; Hussain *et al*., 2011; 2014).

Increased growth performance observed in the present study could be attributed to the improved mineral digestibility. Phytate present in plant ingredients, chelates minerals (Ca, Na, K, Mg, Zn, Cu, Mn and Fe) and makes them unavailable to fish by reducing their digestibility (Cao *et al*., 2007). Phytase supplementation helps to hydrolyse phytate and thereby leading to better mineral availability. Positive results of phytase supplementation in terms of higher mineral digestibility were reported in rainbow trout (Sugiura *et al*., 2001) and in *L. rohita* (Hussain *et al*., 2011a; 2014; 2015a). Improved mineral absorption with phytase supplementations was also reported by other researchers (Ai *et al*., 2007; Baruah *et al*., 2007; Sardar *et al*., 2007). In the present study, the observed as well as quadratic regression analyses based optimal calculated values of phytase supplementation level for P digestibility fluctuated narrowly around 750 FTU kg^{-1} followed by 1000 FTU kg^{-1} diet. However, Baruah *et al*. (2007) suggested that supplementation of dietary microbial phytase at 500 FTU kg^{-1} level improves the absorption of minerals such as Na, K, P, Mg, Mn and Fe. Whereas, 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 in the whole body (except for body Mn, Mg and K contents) at 500 FTU kg^{-1} level of phytase supplementation. Phytase supplementation at 500 FTU kg^{-1} level was found optimum for improving mineral utilisation in Japanese seabass *Lateolabrax japonicus* (Ai *et al*., 2007) and in *Cyprinus carpio* L. fed on plant based diets (Sardar *et al*., 2007).

In the present study, the higher mineral digestibility (Ca, Mg, P, Na, K, Fe, Cu, Zn and Mn) observed in *L. rohita* fingerlings fed on phytase supplemented sunflower meal based diet, has confirmed the hydrolysis of the anti-nutritional factor, phytate resulting in increased bioavailability of minerals and consequently fish excreted lower amount of minerals through faeces in to the aquatic environment. This finding was in corroboration with the observations of Hussain *et al*. (2011b; 2014; 2015a). Similar findings were also reported by Nwanna *et al*. (2007) who observed that most of the phytase action focused on 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 the group fed on control diet I and II as compared to phytase supplemented 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 in different fish species has been reported by many authors (Baruah *et al*., 2007; Hussain *et al*., 2011b; 2014; 2015a). In comparison with control 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 phosphorus discharge in all the fish groups supplemented with phytase as compared to that of the control group. Thus, phytase supplementation in plant based diets might prove beneficial in developing cost effective and environment friendly feed for *L. rohita* by improving nutrient digestibility and reducing the nutrient excretion into aquatic environment and it is expected that it will be helpful in reducing aquatic pollution (Baruah *et al*., 2004; Ashraf and Goda, 2007; Gabriel *et al*., 2007; Hussain *et al*., 2011b; 2014; 2015a, b).

The present study provided sufficient evidences that 750 FTU kg^{-1} level of phytase supplementation had significant effect on improved feed intake, better feed conversion and higher mineral digestibility resulting in higher growth performance of *L. rohita* fingerlings fed on sunflower meal based diets. Phytase supplementation in plant based diets could help to reduce the need for supplementing minerals, which will reduce the cost of fish feed and mineral discharge through faeces into the aquatic environment resulting in ecofriendly aquaculture.

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