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RESEARCH ARTICLE

Role of Phytase Supplementation in Improving Mineral Digestibility of Dry Bread Meal Based Diet Fed to *Labeo rohita* Fingerlings

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ABSTRACT

A feeding trial was conducted to study the effect of phytase supplementation on apparent digestibility of mineral in *Labeo rohita* fingerlings fed on dry bread meal (DBM) based diet. Eight experimental diets consisting of one reference diet and seven test diets, designated as DBM_1 (0 FTU/kg), DBM_2 (250 FTU/kg), DBM_3 (500 FTU/kg), DBM_4 (750 FTU/kg), DBM_5 (1000 FTU/kg), DBM_6 (1250 FTU/kg) and DBM_7 were prepared. Each test diet was consisted of 70% reference diet and 30% test ingredient i.e. dry bread. Apparent digestibility coefficient (ADC) of each mineral was estimated by using chromic oxide as an inert marker. Highest ADC for Ca, P, Fe, Cu, Zn, Na, K and Mn was observed in the DBM_4 diet which was supplemented with phytase level of 750 FTU/kg. Results from the present study indicated that phytase supplementation may be useful to develop cost effective and environment friendly aqua feed for *L. rohita* fingerlings.

INTRODUCTION

In aquafeeds, fishmeal due to its high protein content, balanced amino acid profile and high digestibility is being used as a major protein source (Hardy, 1995). However, because of increasing demand, unstable supply and high price of fishmeal (Lunger et al., 2007) attempts are being made to replace fishmeal with plant protein sources (Cain and Garling, 1995). One of the major problem associated with the use of these plant proteins is the presence of anti-nutritional factors like phytate (*myo*-inositol-1,2,3,4,5,6-hexaphosphates). Almost 80% P in plants is found in phytate form (NRC, 1993) which is biologically not available to mono-gastric or agastric species including fish. Phytate not only reduces the availability of P but it also makes insoluble chelate complexes with divalent and trivalent cations including calcium (Ca), zinc (Zn), sodium (Na), potassium (K), iron (Fe), copper (Cu), magnesium (Mg), manganese (Mn) and other trace elements and reduces their bioavailability.

Various attempts have been made for phytate hydrolysis to release P and other chelated mineral to make them biologically available (Hotz and Gibson, 2005). Enzymatic hydrolysis through phytase supplementation in feed has been proven best for maximum dephosphorylation of phytate (Silva et al., 2005). Phytase (PHY) (*myo*-inositol-hexaphosphate phosphohydrolase) is an enzyme that catalyzes the hydrolysis of phytate (Nagai and Funahashi, 1962) resulting in the release of bounded mineral. Phytase supplementation enhanced the apparent digestibility coefficient (ADC) of Ca, P, N, Zn, Mg, Cu and Fe in Rainbow trout, *Oncorhynchus mykiss* (Sugiura et al., 2001). Similarly, improved ADC for P and Mg was also observed in PHY supplemented group compare to other groups in Common carp, *Cyprinus carpio* (Nwanna et al., 2007). Vielma et al. (1998) reported increased concentration of Ca, Zn, Mg and Mn in plasma, bone and whole body of Rainbow trout, *O. mykiss* fed on phytase supplemented diet.

Different wheat products have been investigated in different feed trials for their possible inclusion in the fish diet. Extruded wheat (Kaushik et al., 1989), wheat gluten (Storebakken et al., 1998) and fermented wheat (Skrede et al., 2002) have been used in Atlantic salmon diet and improved ADC of nutrients was observed. However, there is scarcity of literature on the applications of dry bread meal (DBM) (a wheat based product) based diet in fish feed. Therefore, major objective of present study was to investigate the mineral digestibility in a DBM based diet supplemented with graded levels of phytase fed to Rohu, *L. rohita*, fingerlings.

MATERIALS AND METHODS

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

Fish and experimental conditions

Fingerlings were obtained from Public Sector Fish Hatchery. They were acclimatized to laboratory conditions for two weeks in V-shaped tanks (70 L) which were specially designed to collect the fecal material. Fingerlings were fed daily twice to appropriate satiation on the reference diet used in subsequent digestibility study (Allan and Rowland, 1992). Water temperature, dissolved oxygen (DO) and pH were measured by using thermometer, DO meter (Jenway 970) and pH meter (Jenway 3510) correspondingly. Continuous aeration was provided by using capillary system. Prior to the experiment, fingerlings were treated with NaCl solution (5g/L) to avoid ectoparasites and fungal infections (Rowland and Ingram, 1991).

Feed ingredients and experimental diets

Feed ingredients were purchased from the markete. Their chemical composition was determined following AOAC (1995) before the formulation of reference and test diets. The composition of reference diet is given in table 1. Test diets were prepared by taking 70% of reference diet and 30% of test ingredient i.e dry bread (table 1). To determine the apparent nutrient digestibility of ingredients, chromic oxide was added in each experimental diet. Phytase was sprayed according to the Robinson et al. (2002). Seven test diets were prepared by spraying graded levels (0, 250, 500, 750, 1000, 1250 and 1500 FTU/kg) of phytase to dry bread meal (DBM) based diet.

Feeding protocol and sample collection

Three replicates (each having fifteen fishes) were made for each experimental diet. Feeding to the fingerlings and collection of samples were carried out as described by Hussain et al. (2011).

Mineral analysis of feed and feces

For analysis of mineral, samples of diets and feces were digested according to AOAC (1995). Atomic Absorption Spectrophotometer (Model Hitachi Polarized, Z-8200) was used to measure the Mg, Ca, Cu, Zn, Fe and Mn while P was analyzed using spectrophotometer (UV-VIS 2001) at 720 nm absorbance. Flame photometer (Jenway PFP-7, UK) was used to estimate the Na, and K. After oxidizing the samples with molybdate reagent (Divakran et al., 2002), chromic oxide contents were measured using spectrophotometer (UV-VIS 2001) at 370 nm absorbance. Apparent digestibility coefficient (ADC) for each mineral was calculated by the following formula reported in NRC (1993).

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

Statistical analysis

Data was subjected to one-way analysis of variance (ANOVA) (Steel et al., 1996). The difference among means was compared by Tukey's honesty significant difference test and considered significance at $p<0.05$ (Snedecor and Cochran, 1991). The CoStat computer software program (Version 6.303, PMB 320, CA, 93940 USA) was used for the said statistical analysis.

RESULTS

The results of chemical analysis of minerals of reference and test diets are presented in table 2. Minerals analysis of feces of these experimental diets is summarized in table 3. In the present study, it has been observed that phytase supplementation improved the absorption of minerals in DBM based diet fed to *Labeo rohita* fingerlings (Table 4). Highest ADC% for all observed minerals was recorded at 750 FTU/kg phytase level in DBM_4 diet. Digestibility values of Ca, P, Fe, Cu, Zn, Na, K and Mn were 59.03%, 74.96%, 68.46%, 79.44%, 68.66%, 79.41%, 75.48% and 77.14% respectively in DBM_4 diet. However, among these minerals, a significant ($p<0.05$) difference between reference and DBM_4 diet was observed for Zn, Cu, Fe, Na and Mn while rest of the minerals showed non-significant increase in absorption. The maximum digestibility values were recorded for Cu and Na which increased from 57.8% and 61.3% respectively (Reference diet) to 79.4% (750 FTU/kg phytase), however, relationship between ADC of these minerals and phytase levels was not linear.

DISCUSSION

Phytate reduces the bioavailability of minerals to mono-gastric and agastric animals including fishes in several ways. It contains up to 80% of the total P in plants which is unavailable to the animals (NRC, 1993). Furthermore, phytate chelates with several cations such as Mg, Fe, Zn, Cu, Na, K and Mn and make them unavailable to the fish (Nwanna *et al.,* 2008).

The results of present study showed that phytase incorporation to DBM based test diets has positive

Table 1: Composition (%) of reference and test diets

	Reference diet	Test diets						
		DBM_1	DBM_2	DBM_3	DBM_4	DBM_5	DBM_6	DBM_7
Ingredients								
Fish meal	20	14	14	14	14	14	14	14
Wheat flour	24	15.6	15.6	15.6	15.6	15.6	15.6	15.6
Corn gluton (60%)	20	14	14	14	14	14	14	14
Rice polish	25	17.5	17.5	17.5	17.5	17.5	17.5	17.5
Fish oil	7	4.9	4.9	4.9	4.9	4.9	4.9	4.9
Vitamin premix*	1	1	1	1	1	1	1	1
Mineral mixture**	1	1	1	1	1	1	1	1
Ascorbic acid	1	1	1	1	1	1	1	1
Chromic oxide	1	1	1	1	1	1	1	1
Dry bread	-	30	30	30	30	30	30	30
Phytase (FTU/kg)	-	-	250	500	750	1000	1250	1500

***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 mg; Calcium pantothenate, 10000 mg; Vitamin C, 15000 mg; Nicotinic acid, 25000mg; **Each Kg mineral granules contains Ca, 155gm; Mn, 2000mg; P, 135gm; Cu, 600mg; Mg, 55gm; Co, 40mg; Fe, 1000 mg; I, 40mg; Zn, 3000 mg; Se, 3mg; Na, 45gm.

Table 2: Analyzed mineral composition (%) of reference and test diets

Mineral	Reference diet	Test diets						
		DBM_1	DBM_2	DBM_3	DBM_4	DBM_5	DBM_6	DBM_7
Ca	0.210±0.010	0.220±0.020	0.210±0.020	0.210±0.020	0.240±0.010	0.240±0.050	0.230±0.010	0.210±0.020
Fe	0.095±0.007	0.095±0.021	0.086±0.004	0.095±0.007	0.110±0.000	0.105±0.007	0.089±0.001	0.085±0.001
K	0.160±0.007	0.150±0.028	0.150±0.007	0.170±0.014	0.180±0.000	0.170±0.007	0.140±0.014	0.140±0.021
Cu	0.028±0.001	0.026±0.001	0.028±0.001	0.029±0.001	0.037±0.002	0.036±0.002	0.025±0.004	0.023±0.001
Mn	0.120±0.014	0.100±0.028	0.130±0.028	0.100±0.007	0.100±0.014	0.130±0.035	0.100±0.007	0.090±0.021
Zn	0.012±0.001	0.011±0.002	0.007±0.004	0.012±0.003	0.016±0.011	0.013±0.001	0.012±0.001	0.012±0.003
Na	0.059±0.002	0.033±0.001	0.044±0.006	0.068±0.004	0.077±0.002	0.070±0.007	0.050±0.006	0.060±0.002
P	0.012±0.001	0.011±0.001	0.006±0.004	0.012±0.002	0.012±0.004	0.012±0.001	0.012±0.001	0.017±0.006

Table 3: Analyzed mineral composition (%) of feces of reference and test diets

Mineral	Reference diet	Test diets						
		DBM_1	DBM_2	DBM_3	DBM_4	DBM_5	DBM_6	DBM_7
Ca	0.100±0.014	0.110±0.007	0.110±0.000	0.100±0.014	0.110±0.021	0.120±0.028	0.160±0.021	0.130±0.014
Fe	0.060±0.001	0.065±0.006	0.070±0.001	0.058±0.001	0.040±0.007	0.052±0.003	0.076±0.001	0.072±0.004
K	0.056±0.004	0.055±0.001	0.059±0.001	0.048±0.001	0.051±0.002	0.070±0.001	0.072±0.007	0.071±0.000
Cu	0.013±0.001	0.014±0.001	0.018±0.007	0.010±0.001	0.009±0.000	0.010±0.000	0.013±0.003	0.018±0.001
Mn	0.032±0.001	0.035±0.001	0.034±0.006	0.029±0.001	0.026±0.000	0.027±0.001	0.032±0.004	0.031±0.001
Zn	0.004±0.000	0.004±0.000	0.003±0.002	0.005±0.001	0.003±0.000	0.004±0.001	0.007±0.000	0.006±0.001
Na	0.025±0.001	0.026±0.000	0.032±0.002	0.023±0.007	0.018±0.001	0.020±0.001	0.029±0.001	0.026±0.002
P	0.004±0.000	0.004±0.000	0.003±0.002	0.005±0.001	0.003±0.001	0.004±0.001	0.007±0.000	0.006±0.001

effects on digestibility of minerals in *L. rohita* fingerlings. Maximum ADC values for most of the studied minerals were observed at test diet DBM_4, supplemented with phytase level of 750 FTU/kg. This improved minerals digestibility in DBM based diet could be attributed to the hydrolysis of the phytate present in the diet resulting in release of bounded minerals. Our results are in line with the findings of Hussain et al. (2011). They reported significantly ($p<0.05$) improved digestibility of minerals in response to graded levels of phytase supplementation with best results at 750 FTU/kg in *L. rohita* fingerlings. Similarly, Baruah et al. (2007) also suggested an optimum level of 750 U/kg phytase for maximum digestibility of minerals in *L. rohita* fingerlings. In a recent study, Liu et al. (2013) also reported significantly improved digestibilities of Ca and P in response to phytase supplementation in grass carp, *Ctenopharyngodon idellus*. Improved P apparent digestibility had also been reported in tra catfish juveniles having phytase supplemented soybean meal based diet (Hung et al., 2014). Sugiura et al. (2001) and Wang et al. (2009) also reported enhanced minerals digestibility in response to phytase supplementation in Rainbow trout, *O. mykiss*. However, there are inconsistent reports on optimum phytase level in literature. A low level of phytase supplementation (500 FTU/kg) for optimal digestibility of minerals was reported in *Pangasius pangasius* (Debnath et al., 2005), while, a higher level of 2000 FTU/kg phytase was suggested optimum for maximum mineral digestibility in Tiger puffer,

Table 4: Apparent digestibility coefficients (ADC%) of mineral of reference and test diets

Mineral	Reference diet	Test diets						
		DBM_1 (0 FTU/kg)	DBM_2 (250 FTU/kg)	DBM_3 (500 FTU/kg)	DBM_4 (750 FTU/kg)	DBM_5 (1000 FTU/kg)	DBM_6 (1250 FTU/kg)	DBM_7 (1500 FTU/kg)
Ca	56.82±7.85[a]	53.17±9.41[ab]	53.06±6.16[ab]	57.83±0.73[a]	59.03±6.82[a]	58.33±0.12[a]	34.01±1.22[b]	46.45±0.90[ab]
Fe	42.91±1.40[bcd]	38.53±9.18[cde]	25.80±0.28[def]	45.30±4.14[bc]	68.46±1.12[a]	58.71±0.41[ab]	20.63±4.40[f]	24.37±5.44[ef]
K	68.97±4.07[ab]	66.78±5.45[abc]	65.21±3.56[abc]	74.64±2.01[a]	75.48±1.45[a]	66.41±0.92[abc]	52.05±2.97[c]	65.12±6.70[bc]
Cu	57.82±7.79[bc]	53.26±5.40[bcd]	40.85±1.77[de]	68.42±3.21[ab]	79.40±1.53[a]	77.13±1.26[a]	52.13±4.66[cd]	30.70±1.65[e]
Mn	75.84±1.14[b]	73.40±7.00[c]	75.71±1.62[b]	75.08±0.81[b]	77.14±2.21[a]	76.75±8.45[a]	68.43±2.62[d]	72.91±5.05[c]
Zn	66.24±0.44[c]	65.08±1.04[c]	55.06±6.39[e]	61.08±1.75[d]	68.66±24.38[a]	67.15±2.61[b]	41.64±0.59[f]	55.18±4.63[e]
Na	61.29±1.32[b]	31.39±0.63[d]	33.15±3.02[d]	69.35±1.31[ab]	79.41±1.16[a]	75.98±4.03[a]	49.87±2.21[c]	63.52±4.35[b]
P	67.63±2.41[a]	65.15±5.33[ab]	55.06±6.39[ab]	62.90±0.82[ab]	74.96±12.29[a]	67.15±2.61[a]	41.64±0.59[b]	65.49±8.74[ab]

Means within rows having different superscripts are significantly different at $P<0.05$; Data are means of three replicates ± S.D

Takifugu rubripes (Laining et al., 2010). Variations in optimal level of phytase may be due to the difference in plant ingredients used in the diet and in experimental species (Hussain et al., 2011).

In conclusion, present study demonstrated that DBM based diet supplemented with 750 FTU/kg phytase level improved minerals digestibility/absorption in Rohu, *L. rohita* fingerlings. Increased bioavailability of naturally occurring minerals in plant ingredients reduces the requirements of mineral supplements and decreases the excretion of undigested minerals in natural water bodies. Thus, phytase supplementation can be expected to develop cost effective and environment friendly aqua feed for Rohu, *L. rohita* fingerlings.

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