INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 10, No. 6, p. 124-132, 2017
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Phytase supplementation improves growth performance and crude protein digestibility in *Labeo rohita* fingerlings fed barley meal based diet

Mahroze Fatima*[1], Muhammad Afzal[2], Syed Zakir Hussain Shah[3], Syed Makhdoom Hussain[4]

[1]*Department of Zoology, Govt. College Women University, Faisalabad, Pakistan*

[2]*Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan*

[3]*Department of Zoology, University of Sargodha, Pakistan*

[4]*Department of Zoology, Government College University, Faisalabad, Pakistan*

Article published on June 23, 2017

Key words: Growth, Graded phytase levels, *Labeo rohita,* Nutrients digestibility

Abstract

Barley is a staple cereal grain, full of digestible proteins and energy, can be effectively used to replace fishmeal. Phytase is an enzyme used to hydrolyze phytate (anti-nutrient) present in plant proteins. Therefore, this study was designed to evaluate the optimum level of phytase supplementation for maximum growth and nutrient digestibility in *Labeo rohita* fingerlings fed barley meal based diet. Fish (average body weight 14.28±0.14 g) were fed on 8 experimental diets including 1 reference and 7 test diets. Reference diet was used as standard diet and formulated to provide all necessary nutrients required for normal fish growth. Test diets were consisted of 70% reference and 30% barley meal and were supplemented with graded levels of phytase (0, 250, 500, 750, 1000, 1250, 1500 FTUkg^{-1}). Chromic oxide was used as inert marker to assess nutrient digestibility. Fingerlings having 750 FTU kg^{-1} phytase supplemented diet showed significantly (p<0.05) improved growth and feed performance as compared to reference and other test diets. Similarly, digestibility data also showed maximum absorption of crude protein and gross energy at 750 FTUkg^{-1} phytase level. In conclusion, phytase supplementation at the level of 750 FTUkg^{-1}diet can improve growth performance and nutrient digestibility of *L. rohita* fingerlings to its maximum level in barley meal based diet. However, higher levels of its supplementation were not helpful to further improve the growth and digestibility performance.

***Corresponding Author:** Mahroze Fatima ✉ mahrozef@gmail.com

Introduction

High quality protein makes fishmeal a preferred protein source for aquaculture feed industry. Due to insufficient worldwide supply of fishmeal and its high cost, plant by-products are used in formulation of economical fish feed (Hussain *et al.,* 2011). Barley is a staple cereal grain and full of dietary fibers and proteins for energetic and healthy metabolism. Majorobstacle in use of plant-derived protein ingredients is the presence of anti-nutritional factors which reduces the nutrients and mineral uptake, hence increasing discharge of these nutrients and minerals in feces (Higgs *et al*., 1995; Hardy, 1995). Phytic acid an anti-nutritional factor which stores much of phosphorus of plant protein sources in it (Cheryan and Rackis, 1980). Barley grains store about 60-80% of total phosphate as phytic acid (Rasmussen and Hatzack, 1998). It forms insoluble complexes by interacting with multivalent cations of minerals and proteins, hence rendering their decreased bioavailability (Cheryan and Rackis, 1980).

Fish and other mono-gastric animals usually lack enzymes to hydrolyze this phytic acid (Baruah *et al*., 2004). In plant protein based diets, supplementation of phytase, a phytate hydrolyzing enzyme, for liberation of phosphorus and other bound nutrients from phytic acid is becoming a common practice (Pham *et al*., 2008; Lim and Lee, 2009; Shah *et al.,* 2015). Phytic acid have phosphorus in its inositol ring in the form of orthophosphate groups, phytase cleaves these groups and liberates free phosphorus and reduces its binding affinity to different cations (Lei *et al*.,1993). Phytase is considered an eco-friendly feed additive because of its ability to minimize the discharge of P and other nutrients in the water bodies (Cao *et al*., 2008).

Use of phytase in aqua feed enhanced the net utilization and digestibility of protein (Debnath, 2003), feed efficiency ratio (Ai *et al*., 2007), nutrient digestibility (Papatryphon *et al*., 1999; Portz and Liebert, 2004) and growth of fish (Shah *et al.,* 2016).

Feces analysis of rainbow trout provided with phytase in diet clearly showed decreased nutrient contents leading to their reduced concentrations in water (Vielma *et al*., 2000). A lot of literature is available on phytase addition in the plant based diets of fish, but no report is available regarding use of barley meal and phytase in the diet of Indian major carps. Therefore, the purpose of this study was to optimize the phytase supplementation in barley meal based diet fed to for *Labeo rohita* fingerlings.

Materials and methods

The experiment was conducted in Fish Nutrition Laboratory, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan. *Labeo rohita* fingerlings were acquired from Government Fish Seed Hatchery, Faisalabad and were treated with 0.5% w/v solution of NaCl to safeguard them from fungal infection and ectoparasites (Rowland and Ingram, 1991).

For acclamation to laboratory conditions fish were fed once daily in water tanks particularly schemed for fecal collection as mentioned by Hussain *et al.* (2011). pH meter (Jenway 3510), thermometer and DO meter (Jenway 970) were used to monitor water pH (7.4-8.6), temperature (24.9-28.7°C) and dissolved Oxygen (DO) (5.8-7.3 mg/L), respectively. Continuous aeration was provided with the help of capillary system throughout the experiment.

Experimental diets and design

Feed ingredients were procured from commercial feed market and chemical composition was analyzed following AOAC (1995) before formulation of experimental diet. Reference diet was prepared by keeping essential nutrient requirements of fish for normal growth in mind. Chromic oxide at the inclusion level of 1% was used as an inert marker for crude protein and gross energy digestibility studies. Experimental diets consisted of 70% reference diet and 30% barley meal as test ingredient (Table 1). The feed ingredients were ground and passed from 0.05mm sieve to obtain required size.

Feed ingredients were mixed for 10 minutes using electric mixer with gradual addition of fish oil. Water (10-15%) was added to moisturize the diet while mixing. Floating pallets of 3 mm size were made with the help of Lab extruder (model SYSLG30-IV Experimental Extruder). Phytase was sprayed at the level of 0, 250, 500, 750, 1000, 1250 and 1500 FTU $kg^{-1}$to the barley meal based experimental diet resulting in the formulation of seven test diets.

Fish and feeding protocol

Fish were fed in the morning and afternoon daily to approximate feed requirement of fish. Fish were fed to apparent satiation, twice a day. After feeding period of three hours, uneaten diet was collected for determination of FCR and tanks were washed and refilled with filtered fresh water. After two hours of washing feces were collected from each tank. Nutrient leaching from feces was minimized by avoiding breakage of fecal strings.

The collected fecal material was oven dried, ground and stored separately for chemical analysis. Weight gain of fish was recorded using electrical balance fortnightly. Feeding and fecal collection experiment last for 90 days.

Chemical analysis

The samples (feed ingredients, reference and test diets and fecal material) were homogenized in mortar and pestle and were analyzed following AOAC (1995): moisture contents were determined by oven drying for 12 hours at 105°C; crude protein by micro Kjeldahl apparatus (N x 6.25); crude fat, through petroleum ether extraction method by using Soxtec HT2 1045 system. Grossenergy was estimated using adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline. USA). Molybedate reagent oxidation of test diets and feces was done for determination of chromic oxide contents (Divakaran *et al.*, 2002) with the help of UV-VIS 2001 Spectrophotometer at 370nm absorbance.

Growth performance was assessed by bulk weight of fish every fortnight and then weight gain% and specific growth rate (SGR) were calculated using following formulas reported by Mohseni *et al.* (2009)

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

$$\text{SGR} = \frac{100(\ln \text{average final weight} - \ln \text{average initial weight})}{\text{Number of culture days}}$$

Feed conversion ratio (FCR) of reference and test diets (30% barley meal based) was evaluated with the help of standard formula.

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

Standard NRC (1993) formula was followed for calculation of apparent nutrient digestibility coefficients (ADC) of reference and test diets.

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

Statistical analysis

Experiment was conducted under completely randomized design (CRD). One-way analysis of variance (ANOVA) was applied for calculation of growth and nutrient digestibility data while Tukey's Honestly Significant Difference test was used for estimation of differences between means at significance level of $p<0.05$. Whole of this statistical analysis was done using Co-Stat computer package (Version 6.303, PMB 320, Monterey, CA, 93940 USA).

Results and discussion

Phytic acid being an antinutrional factor decreased the availability of nutrients. Its effects on growth are proportional to its amount present in the diet (Sajjadi and Carter, 2004). Alvi (1994) reported decreased growth performance of *L. rohita* by feeding 1% or more phytic acid in the diet.

Growth performance

The findings of the present study (Table 2) shows growth in terms of weight gain, weight gain%, weight gain fish^{-1} day^{-1}, specific growth rate (SGR) and feed conversion ratio (FCR) ascertain very clearly that phytase incorporation in the diet improved the growth performance of *L. rohita* fingerlings. Growth performance showed linear increase with the increasing concentrations of phytase up to the level of 750 FTU kg^{-1} diet, and to somehow, up to 1000 FTU kg^{-1}, however, further increase in phytase concentration (1250 to 1500 FTU kg^{-1}) caused a significant decrease in growth performance (Fig. 1). Weight gain at 750 FTUkg^{-1} level differed significantly from all other levels of phytase. Although weight gain% and SGR were also highest at this level but there was no statistical difference in them on all phytase levels. Improved FCR (1.39±0.030) was observed at 750 FTU kg^{-1}phytase level which differed non-significantly from 1000 FTU kg^{-1} phytase diet but significantly from all other test diets and reference diet. Phytase supplementation enhanced nutrients liberation from phytate bonding, which consequently lead to improved growth of fish fingerlings (Shah *et al.*, 2016).

These positive effects of phytase supplementation on the growth performance of the fingerlings in the present study are consistent with the results reported by Baruah *et al.* (2007) and Shah *et al.* (2016). Similarly, enhanced growth performance in *Cyprinus carpio* (Nwanna and Schwarz, 2007) was also observed by the supplementation of phytase in plant based diets. Improved growth performance in present study may attributed to i) increased fish apatite due to phytase addition which lead to enhanced feed intake (Li and Robinson, 1997) and ii) release of chelated nutrients from phytate which become available to fish (Lim and Lee, 2009).

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

Ingredients	Reference diet	Test diet 1	Test diet 2	Test diet 3	Test diet 4	Test diet 5	Test diet 6	Test diet 7
Fish meal	20	14	14	14	14	14	14	14
Wheat flour	24	16.8	16.78	16.77	16.76	16.75	16.74	16.73
Corn gluten 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	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Mineral mixture[2]	1	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Chromic oxide[3]	1	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Barley meal	-	30	30	30	30	30	30	30
Phytase	-	0	0.0125	0.0250	0.0375	0.0500	0.0625	0.0750
Phytase (FTUkg^{-1})[4]	-	0	250	500	750	1000	1250	1500
Total	100	100	100	100	100	100	100	100
Analysed Composition								
Crude Protein (%)	32.29	32.81	31.17	31.45	31.99	31.45	31.17	31.99
Gross Energy (Kcal/g)	3.85	3.62	3.64	3.83	4.13	4.00	3.5	3.4

[1]Each Kg of Vitamin premix contains: Vitamin A, 15 MIU; Vitamin D3, 3 MIU; Nicotinic acid, 25000 mg; Vitamin B1, 5000 mg; Vitamin E, 6000 IU; Vitamin B2, 6000 mg; Vitamin K3, 4000 mg; Vitamin B6, 4000 mg; Folic acid, 750 mg, Vitamin B12, 9000 mcg; Vitamin C, 15000 mg; Calcium pantothenate, 10000 mg.

[2]Each kg of mineral mixture contains; Ca (Calcium) 155 gm, P (Phosphorous) 135gm, Mg (Magnesium) 55gm, Na (Sodium) 45gm, Zn (Zinc) 3000 mg, Mn (Manganese) 2000 mg, Fe (Iron) 1000 mg, Cu (Copper) 600 mg, Co (Cobalt) 40 mg, I (Iodine) 40mg, Se (Selenium) 3mg.

[3]Chromic oxide as digestibility marker

[4]The 0.05 g of PHY provides 1000 FTU, where, the FTU is one phytase activity unit that liberates 1 µmol of inorganic orthophosphate/min from 5.1mmol/L substrate (sodium phosphate) at 5.5 pH and 37 ○C temperature.

Feed conversion ratio

This study showed that 750 FTU kg^{-1} phytase concentration is enough for improving FCR, hence, making diet palatable and efficiently converting it into flesh of fish. These results are in accordance with Hussain *et al.* (2011), and Baruah *et al.* (2007) who also observed decreased FCR value in plant meal based diet fed *L. rohita* at 750 FTU kg^{-1} phytase level.

Table 2. Growth performance of *Labeo rohita* fingerlings fed reference and barley meal based test diets.

Growth Parameters	Reference diet	Test diet 1	Test diet 2	Test diet 3	Test diet 4	Test diet 5	Test diet 6	Test diet 7	PSE	*p* value
Initial weight (g)	14.18	14.47	14.23	14.49	14.24	14.09	14.21	14.23		
Final weight (g)	18.05^{bc}	17.90^{cd}	17.73^{cd}	19.32^{bc}	19.02^{a}	18.37^{ab}	17.62^{ab}	17.40^{d}		<0.001
Weight gain (g)	3.87^{bc}	3.43^{cd}	3.50^{cd}	3.83^{bc}	4.78^{a}	4.28^{ab}	3.41^{ab}	3.17^{d}	0.09	<0.001
Weight gain (%)	27.55	21.50	25.09	22.31	33.70	27.46	23.14	21.49	2.09	0.0954
Weight gain $fish^{-1}$ day^{-1} (g)	0.043^{bc}	0.038^{cd}	0.039^{cd}	0.042^{bc}	0.053^{a}	0.047^{ab}	0.037^{cd}	0.035^{d}	1.01	<0.001
FCR	2.15^{d}	2.84^{a}	2.33^{cd}	1.77^{e}	1.39^{f}	2.1^{ef}	2.42^{bc}	2.11^{ab}	0.03	<0.001
SGR (%)	0.27	0.22	0.24	0.22	0.32	0.27	0.23	0.22	1.80	0.0879

Data are means of three replicates

Means within rows having different superscript are significantly different

PSE= Pooled SE= √MSE/n (where MSE=Mean-squared error).

Table 3. Apparent digestibility.

Experimental Diets	Crude Protein	Gross Energy
Reference	54.23^{c}	51.88^{ab}
Test diet 1	54.65^{c}	44.66^{cd}
Test diet 2	63.75^{abc}	44.36^{cd}
Test diet 3	62.29^{bc}	48.16^{bc}
Test diet 4	73.46^{a}	57.91^{a}
Test diet 5	69.68^{ab}	48.44^{bc}
Test diet 6	64.31^{abc}	38.47^{d}
Test diet 7	61.34^{bc}	31.94^{e}
PSE	1.53	0.93
P value	<0.001	<0.001

Coefficients (ADCs%) of crude protein and gross energy by *Labeo rohita* fingerlings fed reference and barley meal based test diets.

Data are means of three replicates

Means within columns having different superscript are significantly different

PSE= Pooled SE= √MSE/n (where MSE=Mean-squared error).

Crude protein digestibility

Present study showed highest digestibility of proteins (73.46±0.142) at phytase level of 750 FTU kg^{-1} and next higher value (69.68±0.800) was observed at 1000 FTU kg^{-1}phytase level. Gross energy digestibility was also observed highest (57.91±0.130) at 750 FTU kg^{-1} which differed significantly from reference diet (51.88±1.305) (Table 3). Trend line analysis of parameters has showed clearly that there is increase nutrients digestibility up to 750 FTUkg^{-1} and to somehow, up to 1000 FTUkg^{-1} phytase levels and any further increase in phytase concentration resulted in decreased digestibility of crude protein and gross energy, which suggests that phytase increase up to a certain level is considered efficient after which it leads towards lowered efficiency (Fig. 1).

Phytate binds to proteins and inhibits the activities of digestive enzymes (Liener, 1994) leading to reduced digestibility of proteins (Kumar *et al.,* 2011).

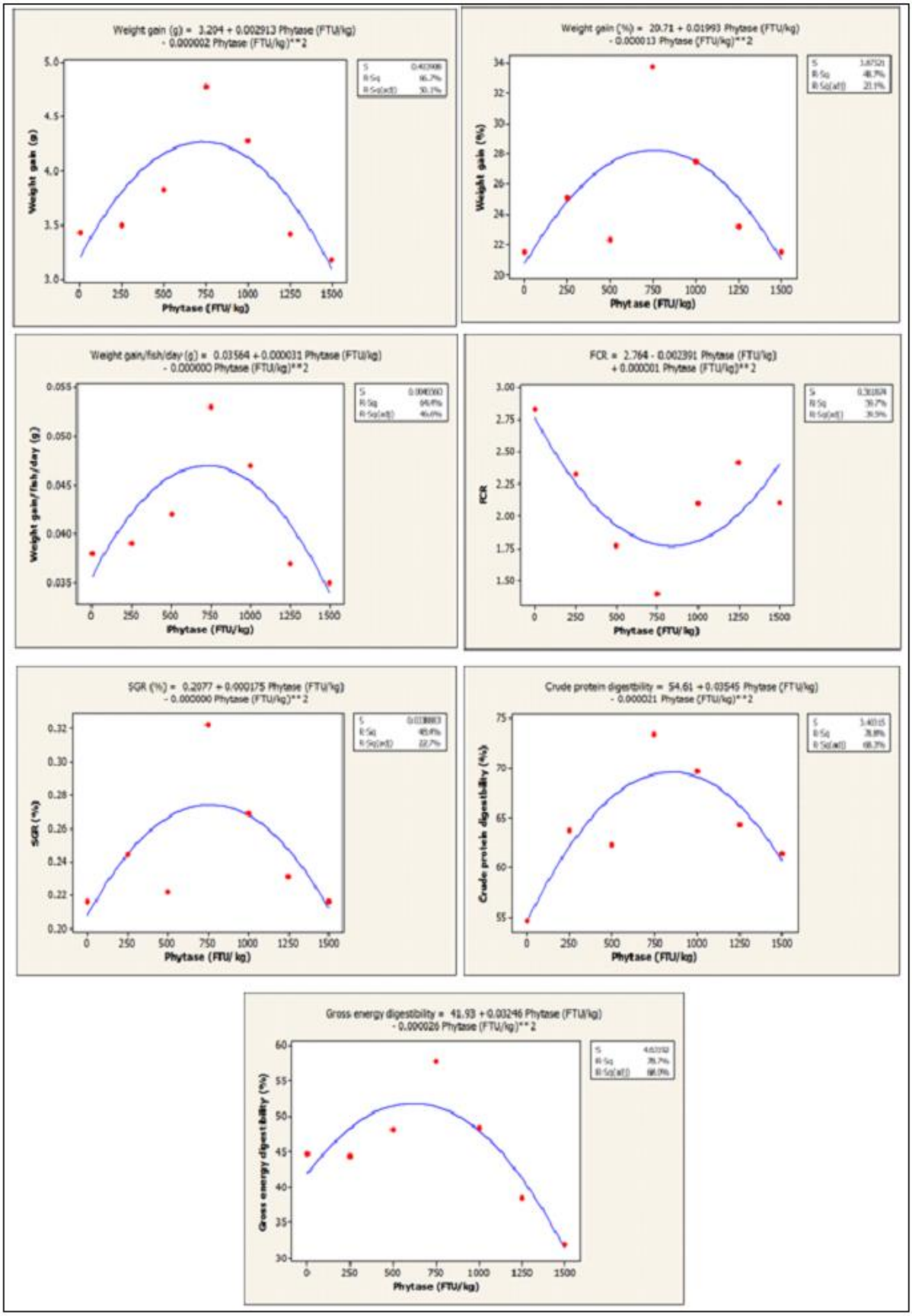

Fig. 1. Trend line showing growth and apparent digestibility coefficients (ADC%) of crude protein and gross energy by *Labeo rohita* fingerlings fed reference and barley meal based test diets.

Hussain *et al.* (2015) reported 1000 FTU kg^{-1} phytase as optimum dose for enhancing crude protein digestibility of corn gluten meal based diet for *L. rohita*. Several other studies had showed improved crude protein digestibility against phytase supplementation in Nile tilapia (Portz and Liebert, 2004), *Pangasius pangasius* (Debnath *et al.,* 2005), *Cyprinus carpio* (Sardar *et al.,* 2007), rainbow trout (Wang *et al.,* 2009) and Japanese flounder (Sarker *et al.,* 2006) fed on different plant based diets.

Gross energy digestibility

The highest gross energy digestibility observed at 750 FTU kg^{-1} phytase levels in the present study, is clearly supported by Hussain *et al.* (2011) as they also observed highest gross energy digestibility at 750 FTU kg^{-1} phytase level for *Labeo rohita* feeding on corn gluten meal based diet. Phytase supplementation also had enhanced the gross energy digestibility of Nile tilapia (Portz and Liebert, 2004) and rainbow trout (Cheng and Hardy, 2002) in plant based diets.Increased nutrients digestibility refers towards increased deposition of these nutrients in fish body and decreased aquatic environmental pollution as well as reduced need of supplementation of these nutrients (Vielma *et al.,* 2000).

Conclusion

In short, present study clearly indicated that phytase supplementation at the level of 750 FTU kg^{-1} is effective in improving growth performance and nutritional status of *Labeo rohita*in barley meal based diet. The demands for millions of tons of fish taken from the ocean annually for production of fish diet can be reduced using barley proteins in commercial fish feeds instead of fish meal.

References

Ai Q, Mai K, Zhang W, Xu W, Tan B, Zhang C, Li H. 2007. Effects of exogenous enzymes (phytase, non-starch polysaccharide enzyme) in diets on growth, feed utilization, nitrogen and phosphorus excretion of Japanese seabass, *Lateolabrax japonicus*. Comparative Biochemistry and Physiology **147,** 502- 508. http://dx.doi.org/10.1016/j.cbpa.2007.01.026.

Alvi AS. 1994. Adventitious toxins in plant origin feedstuffs: Quantification and tolerance level in fish. Masters dissertation, Aligarh Muslim University, Aligarh, India.

AOAC (Association of Official Analytical Chemists). 1995. Official Methods of Analysis. 15th Ed., Association of Official Analytical Chemist, Washington, D.C. USA, p.1094

Baruah, K, Pal AK, Sahu NP, Debnath D. 2007. Microbial phytase supplementation in rohu, *Labeo rohita*, diets enhances growth performance and nutrient digestibility. Journal of the World Aquaculture Society **38,** 129- 137. http://dx.doi.org/10.1111/j.1749-7345.2006.00081.x

Baruah K, Sahu NP., Pal AK, Debnath D. 2004. Dietary Phytase: An ideal approach for cost effective and low-polluting aquafeed. NAGA, World Fish Center Quarterly **27,** 15- 19. http://www.worldfishcenter.org/Naga/na_2314.pdf.

Cao L, Yang Y, Wang WM, Yakupitiyage A, Yuan DR Diana JS. 2008. Effects of pretreatment with microbial phytase on phosphorous utilization and growth performance of Nile tilapia (*Oreochromis niloticus*). Aquaculture Nutrition **14,** 99- 109. http://dx.doi.org/10.1111/j.1365-2095.2007.00508.x.

Cheng ZJ, Hardy RW. 2002. Effect of microbial phytase on apparent nutrient digestibility of barley, canola meal, wheat and wheat middlings, measured *in vivo* using rainbow trout (*Oncorhynchus mykiss*). Aquaculture Nutrition **8,** 271- 277. http://dx.doi.org/10.1046/j.13652095.2002.00219.x.

Cheryan M, Rackis JJ. 1980. Phytic acid interactions in food systems. Critical Reviews in Food Science and Nutrition **13,** 297- 335. http://dx.doi.org/10.1080/10408398009527293.

Debnath D. 2003. Effect of dietary microbial phytase supplementation on growth performance and body composition of *Pangasius pangasius* fingerlings. Central institution of fisheries education, versova, Mumbai, India. M.F.Sc. thesis.

Debnath D, Pal AK, Sahu NP, Jain KK, Yengkokpam, Mukherjee SC. 2005. Effect of dietary microbial phytase supplementation on growth and nutrient digestibility of *Pangasius pangasius* (Hamilton) fingerlings. Aquaculture **36,** 180- 187. http://dx.doi.org/10.1111/j.1365-2109.2004.01203.x.

Divakaran S, Leonard GO, Ian PF. 2002. Note on the methods for determination of chromic oxide in shrimp feeds. Journal of Agriculture and Food Chemistry **50,** 464- 467.

Higgs DA, Dosanjh BS, Prendergast AF, Beames R., Hardy RW, Riley W, Deacon G. 1995. Chapter 11. Use of rapeseed/canola protein products in finfish diets. In: Sessa, D, Lim C, Eds. AOCS monograph entitled "Nutrition and utilization technology in aquaculture". AOCS Press, Champaign, IL, 130- 156 P.

Hardy RW. 1995. Current issues in salmonid nutrition. In: Lim CE *et al.* Eds. Nutrition and utilization technology in aquaculture. AOCS Press, Champaign, 26- 35 P.

Hussain SM, Afzal M, Javid A, Aslam N, Hussain M, Shah SZH, Azmat H, Mubarik MS. 2015. Role of phytase supplementation in improving nutrient digestibility in *Labeo rohita* (Hamilton, 1822) fingerlings fed on cottonseed meal based diet. Indian Journal of Fisheries **62,** 78- 84.

Hussain SM, Afzal M, Rana SA, Javid A, Iqbal M. 2011. Effect of phytase supplementation on growth performance and nutrient digestibility of *Labeo rohita* fingerlings fed on corn gluten meal-based diets. International Journal of Agriculture and Biology **13,** 916- 922.

Kumar V, Sinha AK, Makkar HPS, Deboeck G, Becker K. 2011. Phytate and phytase in fish nutrition. Journal of Animal Physiology and Animal Nutrition **3,** 335- 364. http://dx.doi.org/10.1111/j.1439-0396.2011.01169.x.

Li MH, Robinson EH. 1997. Microbial phytase can replace inorganic phosphorous supplements in channel catfish *Ictalurus punctatus* diets. Journal of the World Aquaculture Society **28,** 402- 406. http://dx.doi.org/10.1111/j.17497345.1997.tb00287.x.

Liener IE. 1994. Implications of antinutritional components in soybean foods. Critical Reviews in Food Science and Nutrition **34,** 31- 67. http://dx.doi.org/10.1080/10408399409527649.

Lei XG, Ku PK, Miller ER, Ullrey DE, Yokoyama MT. 1993. Supplemental microbial phytase improves bioavailability of dietary zinc to weanling pigs. Journal of Nutrition **123,** 1117- 1123.

Lim SJ, Lee KJ. 2009. Partial replacement of fish meal by cottonseed meal and soybean meal with iron and phytase supplementation for parrot fish *Oplegnathus fasciatus*. Aquaculture **290,** 283- 289. http://dx.doi.org/10.1016/j.aquaculture.2009.02.018

Mohseni M, Bahmani M, Pourali H, Poudeaghani M, Bae JY, Bai SC. 2009. Effect of soybean meal as a fishmeal replacement dietary lysine and methionine supplementation in great sturgeon *Huso huso*. In: 6th International Symposium on Sturgeon, Hubei Province, China, 25- 31 P.

NRC (National Research Council). 1993. Nutrient Requirements of Fish. National Academic Press, Washington, DC, 114.

Nwanna LC, Schwarz FJ. 2007. Effect of supplemental phytase on growth, phosphorus digestibility and bone mineralization of common carp (*Cyprinus carpio* L.). Aquaculture Research **38,** 1037- 1044. http://dx.doi.org/10.1111/j.1365-2109.2007.01752.x.

Papatryphon E, Howell RA, Soares JH. 1999. Growth and mineral absorption by striped bass *Morone saxatilis* fed a plant feedstuff based diet supplemented with phytase. Journal of the World Aquaculture Society **30,** 161- 73. http://dx.doi.org/10.1111/j.17497345.1999.tb00863.x

Pham MA, Lee KJ, Dang TM, Lim SJ, Ko GY, Eo J, Oh DH. 2008. Improved apparent digestibility coefficient of protein and phosphorus by supplementation of microbial phytase in diets containing cottonseed and soybean meal for juvenile olive flounder (*Paralichthys olivaceus*). Asian-Australasian Journal of Animal Sciences **21,** 1367- 1375.

Portz L, Liebert F. 2004. Growth, nutrient utilization and parameters of mineral metabolism in Nile tilapia, *Oreochromis niloticus* fed plant based diet with graded levels of microbial phytase. Journal of Animal Physiology and Animal Nutrition **88,** 311- 320.
http://dx.doi.org/10.1111/j.1439-0396.2004.00486.x.

Rasmussen SK, Hatzack F. 1998. Identification of two low-phytate barley (*Hordeum vulgare* L.) grain mutants by TLC and genetic analysis. Hereditas **129,** 107-112.
http://dx.doi.org/10.1111/j.1601-5223.1998.00107.x.

Rowland SJ, Ingram BA. 1991. Diseases of Australian native fishes. In: Fisheries Bulletin 4 NSW Fisheries, Sydney, NSW, and Australia.

Sajjadi M, Carter CG. 2004. Effect of phytic acid and phytase on feed intake, growth, digestibility and trypsin activity in Atlantic salmon (*Salmo salar* L.). Aquaculture Nutrition **10,** 135- 142.
http://dx.doi.org/10.1111/j.1365-2095.2003.00290.x.

Sardar P, Randhawa HS, Abid M, Prabhakar SK. 2007. Effect of dietary microbial phytase supplementation on growth performance, nutrient utilization, body compositions and haemato-biochemical profiles of *Cyprinus carpio* (L.) fingerlings fed soy protein-based diet. Aquaculture Nutrition **13,** 444- 456.
http://dx.doi.org/10.1111/j.1365-2095.2007.00497.x.

Sarker PK, Fukada H, Hosokawa H, Masumoto T. 2006. Effect of phytase with inorganic phosphorus supplement diet on nutrient availability of Japanese flounder (*Paralichthys olivaceus*). Aquaculture Science **54,** 391- 398.
http://doi.org/10.11233/aquaculturesci1953.54.391.

Shah SZH, Afzal M, Akmal A, Fatima M, Hussain SM. 2016. Effect of citric acid and phytase on growth performance and mineralization of *Labeo rohita* juveniles fed soybean meal based diet. International Journal of Agriculture and Biology **18,** 111- 116.
http://dx.doi.org/10.17957/IJAB/15.0071.

Shah SZH, Afzal M, Fatima M, Hussain SM, Bhatti MA, Hayat HF. 2015. Supplementation of phytase and citric acid to soybean meal based diet enhance muscle mineralization of rohu, *Labeo rohita*, juveniles. Journal of Science **5,** 796- 800.

Vielma J, M-Kinen T, Ekholm P, Koskela J. 2000. Influence of dietary soy and phytase levels on performance and body composition of large rainbow trout, *Oncorhynchus mykiss* and algal availability of phosphorus load. Aquaculture **183,** 349- 362.
http://dx.doi.org/10.1016/S0044-8486(99)00299-9.

Wang F, Yang Y, Han Z, Dong H, Yang C, Zou Z. 2009. Effects of phytase pretreatment of soybean meal and phytase-sprayed in diets on growth, apparent digestibility coefficient and nutrient excretion of rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquaculture International **17,** 143- 157.
http://dx.doi.org/10.1007/s10499-008-9187-5.