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# Growth Performance and Nutrient Digestibility of *Cirrhinus mrigala* Fingerlings Fed Phytase Supplemented Sunflower Meal Based Diet

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**This study is part of M.Phil thesis of TH who collected data and compiled the results. SMH planned the research and provided facilities for conducting the research work. MA helped in writing, revising and improvement of manuscript. AJ and MH analysed the data statistically and interpreted the results. NS provided help and facilities for chemical. MZHA collected and analysed samples. MMS helped in experiments and date collection.**

**Key words**
**Sunflower meal, *Cirrhinus mrigala*, Growth, Nutrient digestibility, Phytase.**

## ABSTRACT

A feeding trial was conducted to study the effects of graded levels (0, 500, 1000, 1500 and 2000 FTU $kg^{-1}$) of microbial phytase on growth performance and nutrient digestibility of *Cirrhinus mrigala* fingerlings fed sunflower meal based diet. The chromic oxide was added as inert marker in the diets. Three replicate groups of 15 fish (Average weight 5.05±0.013 g $fish^{-1}$) were fed at the rate of 5% of live wet weigh and feces were collected twice daily. The results of present study showed improved growth performance of *Cirrhinus mrigala* fingerlings in response to phytase supplementation. Maximum growth performance was observed by the fish group fed on test diet-III having 1000 FTU $kg^{-1}$ level. Similarly, nutrient digestibility was also significantly increased ($p<0.05$) by 1000 FTU $kg^{-1}$ level of phytase supplementation. Digestibility coefficients for sunflower meal based diet increased 15.76%, 17.70% and 12.70% for crude protein, crude fat and apparent gross energy as compared to the reference diet, respectively at 1000 FTU $kg^{-1}$ level. It was concluded that the phytase supplementation to sunflower meal based diet at 1000 FTU $kg^{-1}$ level is optimum to release adequate chelated nutrients for maximum growth performance of *C. mrigala* fingerlings. Our results also suggested that phytase supplementation to sunflower meal based diet can help in the development of sustainable aquaculture by reducing the feed cost and nutrient discharge through feces into the aquatic ecosystem.

## INTRODUCTION

*Cirrhinus mrigala* is bottom feeder fish species, feed on decaying organic matter and vegetable debris (Britz *et al*., 1997; Azeredo *et al*., 1998). Aquaculture industry has been developing more efficiently than any other food producing sector (Yildirım *et al*., 2014). Fish meal is being used by aqua feed industry as a potential source of basic nutrients such as amino acids, fatty acids, vitamins, minerals and growth factors (Zhou *et al*., 2004; Rahim *et al*., 2017). It is major source of protein in fish feeds (Drew *et al*., 2007). However, increasing demand, limited, unstable supply and high cost of fish meal with the expansion of aquaculture made it necessary to search for alternative protein sources (Pham *et al*., 2008; Lim *et al*., 2011). Plant by-products are considered as best alternative protein and energy sources for fish growth (Gatlin *et al*., 2007; Hussain *et al*., 2011a, b, 2015). Plant meal based aqua-feed has anti-nutritional factors and these factors affect the morphology and physiology of digestive tract and disturbs the overall fish growth, one of them is phytate or phytic acid (Baruah *et al*., 2004). Phytate is a major storage form of phosphorus and it is estimated that 80% of total phosphorus is chelated in plant seeds, which is practically not available for agastric fish species (NRC, 1993). Phytic acid is a strong chelator and forms complexes with lipids and its derivatives along with other nutrients (Vohra and Satanarayana, 2003). It can chelate with proteins and

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reduce its availability for fish (Helland *et al*., 2006). Phytase is an enzyme chemically known as myo-inositol hexa-phosphate phosphohydrolase can be obtained from microbes and may be from some plant ingredients. This enzyme hydrolyzes the non-digestible phytic acid contents present in plant based fish feeds. Mono-gastric fish lack the ability to hydrolyze the phytate present in plant based diet due to absence of intrinsic phytase enzyme. This enzyme has been used as supplement in fish feed to increase the bioavailability and utilization of phosphorus (Baruah *et al*., 2007; Cao *et al*., 2007). It is now widely being used as supplement in plant based diets for increasing the digestibility and availability of protein which results in the better fish growth and reduce aquatic pollution (Farhangi and Carter, 2007; Lin *et al*., 2007; Soltan, 2009; Hussain *et al*., 2015).

Sunflower meal is considered as most promising alternative to fishmeal and an economical source of important nutrients. In all over the world, it is the fourth largest plant based source of protein contents after soybean meal, cottonseed meal and canola meal (Anjum *et al*., 2014). It has approximately 40% protein content that mainly depends on the oil extraction and de-hulling process (Mushtaq *et al*., 2006). It has been recognized that the disruption of cell wall matrix of sunflower meal by supplementation of exogenous microbial enzymes result in endogenous pro-teolytic enzymes to digest the chelated proteins (Choct and Kocher, 2000). It was also noted that it has more ability of degradation than soybean meal and canola meal. It contains 8-10% lignin, 20-25% cellulose and 30-34% protein (NRC, 1996). The present study was designed to search out the better and cost effective protein sources for *Cirrhinus mrigala* fingerlings to improve the fish growth performance and to reduce the aquatic pollution caused by phytic acid.

## MATERIALS AND METHODS

The experiment was conducted in the Fish Nutrition Lab, Department of Zoology, Government College University, Faisalabad, Pakistan.

*Experimental design*

Sunflower meal was selected as test ingredient to formulate basal diet which comprised of 30% sunflower meal and 70% reference diet. Basal diet was then further divided into one reference diet and five test diets and sprayed with graded levels of phytase (0, 500, 1000, 1500 and 2000 FTU $kg^{-1}$). These five test diets and one reference diet were fed to six fish groups in three replicates, stocked in specially designed V-shaped tanks. The experiment lasted for 60 days till the collection of 4-5 g fecal material from each replicate separately.

In this study, phytase with different levels of treatments was used to check its efficacy on growth and nutrient digestibility of fish by using Completely Randomized Design (CRD). 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) the first section was comprised of assessment of growth performance in terms of weight gain and feed conversion ratio (FCR) (ii) Second section was consisted of assessment of the nutrient digestibility of the test and reference diets which was determined indirectly by using chromic oxide as an inert marker.

*Fish and experimental conditions*

*C. mrigala* 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 (Allan and Rowland, 1992). Dissolved oxygen, pH 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 for 1-2 min 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 (%) | Carbohydrates (%) | Gross energy ($kcalg^{-1}$) |
|---|---|---|---|---|---|---|---|
| Fish meal | 91.63 | 48.15 | 7.16 | 1.07 | 26.73 | 16.89 | 2.69 |
| Wheat flour | 92.45 | 10.10 | 2.35 | 2.65 | 2.08 | 82.82 | 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 | 13.54 | 12.70 | 10.18 | 51.23 | 3.33 |
| Sunflower meal (test ingredient) | 93.80 | 41.93 | 3.74 | 1.97 | 10.83 | 37.99 | 3.54 |

**Table II.- Ingredients composition (%) of reference and test diets (as fed basis).**

| Ingredients | Reference diet | Test diets |
|---|---|---|
| Fish meal | 20.0 | 14.0 |
| Wheat flour | 24.0 | 16.8 |
| Corn gluten 60% | 20.0 | 14.0 |
| Rice polish | 25.0 | 17.5 |
| Fish oil | 7.0 | 4.9 |
| Vitamin premix | 1.0 | 0.7 |
| Minerals | 1.0 | 0.7 |
| Ascorbic acid | 1.0 | 0.7 |
| Chromic oxide | 1.0 | 0.7 |
| Sunflower meal (Test ingredient) | - | 30.0 |

*Feed ingredients and formulation of experimental diets*

The feed ingredients were obtained 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 diet and sunflower meal based basal diets were prepared by mixing appropriate amount of finely ground (< 0.5 mm particle size) ingredients in electric mixer for 10 minutes. Later on, fish oil was added gradually while mixing was continued for further five minutes. Afterwards, 10-15% water was also added to prepare suitable dough (Lovell, 1989). The diets were extruded into pellets (3mm) through Lab Extruder (model SYSLG30-IV Experimental Extruder). The required concentrations (0, 500, 1000, 1500 and 2000 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*

*C. mrigala* fingerlings were fed twice daily (morning and afternoon) at the rate of 5% 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: 5.05±0.013g $fish^{-1}$) 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 15$^{th}$ day during experiment to assess the growth performance of *C. mrigala* fingerlings. Weight gain (%) and feed conversion ratio (FCR) of fingerlings was evaluated based on standard formulae:

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

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

*Chemical analysis of feed and feces*

The samples of feed ingredients, test diets and feces were homogenized using a motor and pestle and analyzed by standard methods (AOAC, 1995). Moisture was determined by oven-drying at 105°C for 12 h. Crude protein (N × 6.25) 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.

*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 nutrient digestibility 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*

Apparent nutrient digestibility coefficients (ADC %) of experimental diets were calculated by using standard formula (NRC, 1993):

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

**Table III.- Growth performance of *Cirrhinus mrigala* fingerlings fed reference diet and phytase supplemented sunflower meal-based test diets.**

| Experimental diets | Phytase levels (FTU kg$^{-1}$) | Initial weight (g) | Final weight (g) | Weight gain (g) | Weight gain (%) | Weight gain (fish$^{-1}$day$^{-1}$)g | Feed intake (fish$^{-1}$day$^{-1}$)g | FCR |
|---|---|---|---|---|---|---|---|---|
| Reference diet | - | 5.05±0.010 | 10.58±0.332 | 5.53±0.326$^{cd}$ | 109.50±6.342$^{cd}$ | 0.08±0.005 | 0.12±0.014 | 1.50±0.10$^{bcd}$ |
| Test diet I | 0 | 5.05±0.015 | 10.40±0.103 | 5.35±0.095$^{d}$ | 105.87±1.783$^{d}$ | 0.08±0.001 | 0.13±0.012 | 1.72±0.17$^{cd}$ |
| Test diet II | 500 | 5.04±0.012 | 10.92±0.095 | 5.88±0.087$^{bc}$ | 116.74±1.587$^{bc}$ | 0.08±0.001 | 0.12±0.006 | 1.42±0.05$^{abc}$ |
| Test diet III | 1000 | 5.04±0.012 | 12.25±0.049 | 7.21±0.044$^{a}$ | 142.96±0.792$^{a}$ | 0.10±0.001 | 0.12±0.005 | 1.15±0.04$^{a}$ |
| Test diet IV | 1500 | 5.05±0.017 | 11.11±0.075 | 6.06±0.078$^{b}$ | 120.07±1.694$^{b}$ | 0.09±0.001 | 0.12±0.007 | 1.34±0.07$^{ab}$ |
| Test diet V | 2000 | 5.06±0.012 | 10.48±0.042 | 5.41±0.046$^{d}$ | 106.91±1.055$^{d}$ | 0.08±0.001 | 0.14±0.011 | 1.77±0.13$^{d}$ |

Means within rows having different superscripts are significantly different. Data are means of three replicates.

*Statistical analysis*

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

**Table IV.- Analyzed nutrients composition (%) of reference and sunflower meal-based test diets.**

| Experimental diets | Phytase levels (FTU kg$^{-1}$) | Crude protein | Crude fat | Apparent gross energy |
|---|---|---|---|---|
| Reference diet | - | 31.20±0.16 | 5.64±0.02 | 4.71±0.02 |
| Test diet I | 0 | 30.45±0.31 | 4.61±0.02 | 4.14±0.01 |
| Test diet II | 500 | 30.32±0.09 | 4.58±0.04 | 4.17±0.02 |
| Test diet III | 1000 | 30.34±0.07 | 4.59±0.02 | 4.13±0.01 |
| Test diet IV | 1500 | 30.43±0.01 | 4.59±0.01 | 4.13±0.02 |
| Test diet V | 2000 | 30.33±0.15 | 4.64±0.02 | 4.14±0.01 |

Data are means of three replicates.

## RESULTS

A significant (P<0.05) increase in weight gain was commensurate with increase in phytase concentration to a level of 1000 FTU kg$^{-1}$ after which further increase in phytase level decreased the weight gain of fish. The maximum weight gain of *Cirrhinus mrigala* fingerlings was observed when fingerlings were fed sunflower meal based test diet having 1000 FTU kg$^{-1}$ level of phytase supplementation and this value was significantly different (P<0.05) from the weight gain on reference diet and other phytase supplementation based test diets. The best FCR value (1.15±0.04) was also observed in the diet containing phytase level of 1000 FTU kg$^{-1}$. It was significantly ($P$<0.05) different from the FCR values noted at 0, 500 and 2000 FTU kg$^{-1}$ levels of phytase supplementation. However, it did not differ significantly when compared with 1500 FTU kg$^{-1}$ phytase supplemented diet. The comparatively lower values of FCR with sunflower meal based diet showed that fish palatability was improved by phytase supplementation. The minimum value of FCR at 1000 FTU kg$^{-1}$ revealed that at this level maximum feed was converted into flesh.

**Table V.- Analyzed nutrients composition (%) of feces of *Cirrhinus mrigala* fingerlings fed on reference and sunflower meal-based test diets.**

| Experimental diets | Phytase levels (FTU kg$^{-1}$) | Crude protein | Crude fat | Apparent gross energy |
|---|---|---|---|---|
| Reference diet | - | 14.33±0.34 | 2.60±0.11 | 2.32±0.15 |
| Test diet I | 0 | 14.18±0.15 | 2.22±0.10 | 1.97±0.14 |
| Test diet II | 500 | 13.33±0.52 | 1.93±0.12 | 1.88±0.04 |
| Test diet III | 1000 | 10.10±0.02 | 1.49±0.12 | 1.68±0.11 |
| Test diet IV | 1500 | 12.78±0.20 | 1.80±0.20 | 1.75±0.11 |
| Test diet V | 2000 | 14.26±0.07 | 2.22±0.19 | 1.97±0.06 |

Data are means of three replicates.

The data presented in Table VI makes it clear that phytase enzyme supplementation played a significant role in increasing nutrient digestibility and minimum amount of nutrients was discharged through feces at 1000 and 1500 FTU kg$^{-1}$ phytase levels (Table V). It is also obvious from the results that in comparison with reference diet and phytase supplemented sunflower meal-based diets, 1000 FTU kg$^{-1}$ level showed maximum values of crude protein (74%), crude fat (77%) and gross energy (68%)

digestibility (Table VI). It was observed that gross energy digestibility value at 1000 FTU $kg^{-1}$ level did not differ significantly from phytase level of 1500 FTU $kg^{-1}$ but varied ($P<0.05$) from the reference diet and remaining phytase supplemented diets. In general, the results showed that nutrient digestibility started increasing from 500 FTU $kg^{-1}$ level of phytase supplementation and reached to its maximum at 1000 FTU $kg^{-1}$ level.

**Table VI.- Apparent nutrient digestibility (%) of *Cirrhinus mrigala* fingerlings fed reference diet and sunflower meal-based test diets.**

| Exp. diets | Phytase levels | Crude protein | Crude fat | Apparent gross energy |
|---|---|---|---|---|
| Reference diet | - | 58.33±1.57$^{c}$ | 59.15±0.89$^{c}$ | 55.63±2.20$^{c}$ |
| Test diet I | 0 | 54.62±1.92$^{c}$ | 53.05±0.41$^{d}$ | 53.71±1.45$^{c}$ |
| Test diet II | 500 | 59.62±2.92$^{bc}$ | 61.38±1.13$^{c}$ | 58.66±1.57$^{bc}$ |
| Test diet III | 1000 | 74.09±1.93$^{a}$ | 76.85±2.21$^{a}$ | 68.33±2.04$^{a}$ |
| Test diet IV | 1500 | 64.05±1.63$^{b}$ | 66.57±2.78$^{b}$ | 63.84±2.45$^{ab}$ |
| Test diet V | 2000 | 59.43±1.90$^{bc}$ | 58.72±1.58$^{c}$ | 59.03±2.32$^{bc}$ |

Means within rows having different superscripts are significantly different. Data are means of three replicates

## DISCUSSION

In present study, *Cirrhinus mrigala* fed on sunflower meal based diets supplemented with graded levels (0, 500, 1000, 1500 and 2000 FTU $kg^{-1}$) of phytase showed improved weight gain, weight gain (%) and feed conversion ratio (FCR) as compared to reference diet. It indicates that phytase has potential to be an alternative protein source of fish meal. The highest increase in weight gain was observed at 1000 FTU $kg^{-1}$ phytase supplementation level. It was 33.46% higher over the weight gain of fingerlings fed on reference diet. Similar growth performance was also reported in Nile tilapia when fed on plant based diet supplemented with 1000 FTU $kg^{-1}$of phytase compared with the phytase control diet (Riche and Garling, 2004; Ashraf and Goda, 2007; Cao *et al*., 2008). After reviewing the literature related to the dose response studies, it was concluded that the optimal responses of phytase supplementation ranged between 250-1500 FTU $kg^{-1}$ levels depending upon the sources and origin of phytases, experimental fish species, diet formulation technology and studied response parameters. Thus the level of phytase supplementation should have been adjusted based on considerations of earlier impactors (Cao *et al*., 2007; Kumar *et al*., 2011). However, there were limited data available for comparative studies on phytase supplementation in different fish diets, mainly in plant-based diets. Liebert and Portz (2005) compared nutrient utilization of Nile tilapia (*Oreochromis niloticus*) fed plant based low phosphorous diets supplemented with graded levels (500, 750, 1000 and 1250 FTU $kg^{-1}$) of two different sources of phytase were used: phytase A (SP 1002 CT) and phytase B (Ronozyme P5000). It was found that phytase A supplementation of at least 750 FTU $kg^{-1}$ diet was adequate to improve growth, FCR, protein deposition, while supplementation of at least 1000 FTU $kg^{-1}$ from phytase B resulted in intermediate growth as compared to supplementation of phytase A. This result indicated that different phytase sources might lead to different effects. On the other hand, Furuya *et al*. (2001) found that supplementation of phytase at 700 FTU $kg^{-1}$ level was sufficient to maintain growth of Nile tilapia fed on plant-based diets. The effect of phytic acid on growth depends largely on its quantity in the diet (Sajjadi and Carter, 2004). Spinelli *et al*. (1983) also concluded increase in phytate concentration decreases the growth rate and feed efficiency in rainbow trout. The findings of the present study provide evidence that phytase supplementation at 1000 FTU $kg^{-1}$ level was sufficient for minimizing the effect of phytic acid and releasing the chelated nutrients of plant based diets. A significant increase in growth performance of fingerlings was proportionate with the increase in phytase supplementation to a level of 1000 FTU $kg^{-1}$, after which it decreased. The next higher weight gain was noted at 1500 FTU $kg^{-1}$ level. The present results of growth performance of *C. mrigala* fingerlings on plant based diets were in agreement with the findings of Baruah *et al*. (2007). However, Biswas *et al*. (2007) recorded significant increase in growth performance in red sea bream (*Pagrus major*), when compared to the control diet, at 2000 FTU $kg^{-1}$ level of phytase supplementation. It indicated that phytase supplementation at 2000 FTU $kg^{-1}$ diet might be effective in reducing an anti-nutritional factor or adverse consequences of phytate from soybean meal (Liu, 1997). Phytase dosage higher than 2000 FTU $kg^{-1}$ level, however, did not further increase the growth performance in red sea bream. Similar patterns of phytase activity on growth performance have also been observed in many studies such as those by Forster *et al*. (1999), Yoo *et al*. (2005), Liebert and Portz (2007) and Baruah *et al*. (2007b). When canola protein concentrate was used for rainbow trout (*Oncorhynchus mykiss*) with different levels (500, 1500 and 4500 FTU $kg^{-1}$) of phytase, Forster *et al*. (1999) found a higher specific growth rate (SGR) in rainbow trout fed on phytase supplemented diet at 500 FTU $kg^{-1}$ level. Phytase supplementation, higher than 500 FTU $kg^{-1}$ level did not further increase the SGR. In Korean rockfish, although supplementation

of phytase in soybean meal diets at 1000 and 2000 FTU $kg^{-1}$ levels did not increase growth performance, inferior results were observed 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) reported that supplementation of phytase (SP 1002) at 750 FTU $kg^{-1}$ was sufficient for maximum degradation of phytate resulting higher growth performance of Nile tilapia (*Oreochromis nilotics*). Nwanna and Schwarz (2008) also documented an increase in growth parameters at 750 and 1000 FTU $kg^{-1}$ levels of phytase (RonozymeP(CT) Lot HB 950009) supplementation in diets of common carp (*Cyprinus carpio*). Baruah *et al*. (2007b) observed that the growth promoting effect was higher in *Labeo rohita* juveniles fed with a sub-optimum protein (25%) diet containing both 3% citric acid and 500 FTU $kg^{-1}$ level of phytase supplementation than those which were fed with 35% crude protein diets. For their better results, they argued that citric acid provided the most favorable environment for phytase activity by lowering pH of the fish intestine.

**Table VII.- Comparison of different fish species to show the optimum level of phytase supplementation.**

| Optimum dose of Phytase (FTU $kg^{-1}$) | Fish species | Phytase supplementation in fish diets improved following parameters | References |
|---|---|---|---|
| 250 | Channel catfish | Growth performance | Robinson *et al*. (2002) |
| 250 | Channel catfish | Improved weight gain, feed intake and FCR | Li and Robinson (1997) |
| 400 | Rainbow trout | Nutrient and mineral digestibility | Cheng and Hardy (2003) |
| 500 | *Labeo rohita* | Nutrient and mineral digestibility | Baruah *et al*. (2007b) |
| 500 | Rainbow trout | Amino acids, protein and mineral digestibility | Cheng and Hardy (2004) |
| 500 | Rainbow trout | Protein, gross energy and mineral digestibility | Cheng and Hardy (2002) |
| 500 | *Cyprinus carpio* (L.) | Growth, nutrient and mineral digestibility | Sardar *et al*. (2007) |
| 500 | *P. pangasius*) | Growth and protein digestibility | Debnath *et al*. (2005) |
| 500 | Rainbow trout | Growth and nutrient digestibility | Forster *et al*. (1999) |
| 500-1000 | Common carp | Weight gain | Schafer *et al*. (1995) |
| 700 | Nile tilapia | Growth performance improved | Furuya *et al*. (2001) |
| 750 | *Oreochromis niloticus* | Growth performance, Protein effciency ratio (PER), phosphorous (P) and energy utilization | Liebert and Portz (2005) |
| 750 | *Labeo rohita* | Growth, protein and mineral digestibility | Baruah *et al*. (2007a) |
| 750 | *Clarias garipinus* | Growth performance | Van Weerd *et al*. (1999) |
| 750 | *Labeo rohita* | Growth Performance, Nutrient digestibility, Minerals availability | Hussain *et al*. (2011) |
| 750 | *Labeo rohita* | Growth Performance, Nutrient digestibility, Minerals availability | Hussain *et al*. (2014) |
| 750 | *Labeo rohita* | Growth Performance, Nutrient digestibility, Minerals availability | Hussain *et al*. (2015) |
| 800 | *Cyprinus carpio* | Growth performance | Bai *et al*. (2003) |
| 1000 | *Carassius carassius* | Growth performance | Yu and Wang (2000) |
| 1000 | *Oreochromis niloticus* | PER, P digestibility | Cao *et al*. (2008) |
| 1000 | *Oreochromis niloticus* | Growth performance and mineral digestibility | Portz and Liebert (2004) |
| 1000 | Red tilapia | Serum phosphorous (P) digestibility | Phromkunthong and Gabaudan (2006) |
| 1000 | Channel catfish | Growth parameters and phosphorus digestibility | Jackson *et al*. (1996) |
| 1000 | *Chanos chanos* | Growth rate and nutrient digestibility | Hassan *et al*. (2009) |
| 1000 | Striped bass | Growth and mineral digestibility | Papatryphon *et al*. (1999) |
| 1000-2000 | *Sebastes schlegeli* | P digestibility | Yoo *et al*. (2005) |
| 4000 | sex-reversed red tilapia | Growth performance | Tudkaew *et al*. (2008) |
| 4000 | *Colossoma macropomum* | Growth, protein and fat digestibility | Nwanna *et al*. (2008) |
| **Phytase supplementation in fish diets could not improve following parameters** | | | |
| 0-1200 | *Oncorhynchus mykiss* | No effect was observed on weight gain and feed efficiency of fish | Vielma *et al*. (2000) |
| 2000-3000 | *Oncorhynchus mykiss* | Protein digestibility increased, SGR and FCR was not improved, but negative effect on lipid digestibility | Wang *et al*. (2009) |
| 500-8000 | Channel catfish | No effect on weight gain and protein digestibility | Yan and Reigh (2002) |

This inconsistency in the outcome could be attributed to differences in phytic acid content in different feed ingredients, nutritional quality of plant ingredients, water quality, fish species, size and culture and varying experimental conditions (Ashraf and Goda, 2007).

Sunflower meal is a rich source 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 stomach less fish species (Farhangi and Carter, 2007). The growth performance of *C. mrigala* fingerlings in terms of weight gain and feed conversion ratio (FCR) was significantly improved in sunflower meal-based diets supplemented with phytase. The maximum weight gain and best FCR were observed at 1000 FTU $kg^{-1}$ phytase supplementation level. A steadily increase in growth performance was observed with the increase in phytase supplementation dose up to 1000 FTU $kg^{-1}$, however interestingly, higher doses (2000 FTU $kg^{-1}$) causes decrease in growth performance. The findings of the present study provide evidence that phytase supplementation at 1000 FTU $kg^{-1}$ level is sufficient to minimize the effect of phytic acid while using sunflower meal as major feed ingredient in diet of *C. mrigala*. The present results of growth performance of *C. mrigala* fingerlings fed on phytase supplemented diets are similar to the findings of Baruah *et al*. (2007) and Hussain *et al*. (2011b).

Phytic acid can non-selectively chelates to proteins and also inhibit enzymes (pepsin, trypsin and alpha-amylase) activities (Liener, 1994) resulting in reduced protein digestibility (Kumar*et al*., 2011). The treatment of fish feed with phytase resulted in the improvement of protein digestibility and retention in fishes (Cheng and Hardy, 2002; Vielma *et al*., 2004; Debnath *et al*., 2005; Baruah *et al*., 2005; Ai *et al*., 2007; Liebert and Portz, 2007). In present study, the maximum apparent crude protein digestibility (%) of *Cirrhinus mrigala* fingerlings fed on sunflower meal was observed at 1000 FTU $kg^{-1}$ of phytase. It was also evident that further increase in phytase level resulted in a decline in the digestibility of nutrients. In a comparative study with two different types of phytase enzyme (Wang *et al*., 2009) determined that 750 FTU $kg^{-1}$ level of phytase A (SP 1002) supplementation was enough for increasing nutrient digestibility resulting in optimally increased fish growth. Similarly, the phytase B (Ronozyme® P) supplemented at 1000 FTU $kg^{-1}$ diet was found adequate for maximum phytate degradation resulting in increased nutrient digestibility. However, Baruah *et al*. (2007) found highest digestibility of crude protein for *Labeo rohita* juveniles fed soybean meal based diet at 750 FTU $kg^{-1}$ level whereas it decreased at 1000 FTU $kg^{-1}$ level. Protein digestibility was also increased in rainbow trout fed on soybean meal based diet supplemented with phytase at 500-1000 FTU $kg^{-1}$ levels (Dalsgaard *et al*., 2009). The maximum value of crude fat digestibility of sunflower meal was also observed at 1000 FTU $kg^{-1}$ level and this value was significantly different ($p<0.05$) from digestibility values of reference diet and left over test diets. Further higher phytase supplementation levels resulted in significant decrease in fat digestibility. Ashraf and Goda (2007) also observed similar trend. They found that phytase supplementation increased the apparent digestibility coefficient of lipid up to 88.3% at 1000 FTU $kg^{-1}$ level and at higher levels the lipid digestibility started decreasing (87% to 84%). In contrast, Wang *et al*. (2009) reported reduced lipid digestibility by phytase supplementation. They claimed that phytase supplementation might inhibit lipase activity which in turn minimized the lipase hydrolysis efficiently for lipid, resulted in reduced lipid digestibility in phytase supplemented diets. However, Dalsgaard *et al*. (2009) found no significant effect on fat digestibility in rainbow trout (*Oncorhynchus mykiss*) fed on plant based test diets supplemented with phytase. Higher digestibility of gross energy (68.33%) was observed at 1000 FTU $kg^{-1}$ level. The present study is in accordance with Ashraf and Goda (2007). They found maximum gross energy digestibility (73%) on 1000 FTU $kg^{-1}$ level and after this optimum level, it decreased to 71.8% on 1500 FTU $kg^{-1}$and 71% on 2000 FTU $kg^{-1}$ level. Portz and Liebert (2004) determined significant improvement in gross energy digestibility in diets supplemented with 1000 and 2000 FTU $kg^{-1}$ levels. In general, phytase supplementation improves the protein bioavailability and this may lead to further increases in gross energy digestibility resulting in maximum fish growth performance (Liebert and Portz, 2005). Cheng and Hardy (2002) reported that phytase supplementation in diets having canola protein concentrate enhanced apparent gross energy digestibility for rainbow trout, while Lanari *et al*. (1998) did not find any positive response for rainbow trout fed soybean meal based test diets. Differences in results of different researchers may be due to changes in phytic acid contents in feed ingredients, fish species used and a range of other inherent characteristics of feed ingredients (Vielma *et al*., 2004).

## CONCLUSION

In conclusion, the present study confirmed that sunflower meal-based diet supplemented with 1000 FTU $kg^{-1}$ level of phytase significantly released chelated nutrients and increased the nutrient digestibility for improving growth performance of *Cirrhinus mrigala* fingerlings. It was also found that phytase supplementation

played a significant role in developing environment friendly and economical feed for indigenous culture able major carp *Cirrhinus mrigala*.

*Statement of conflict of interest*

Authors have declared no conflict of interest.

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