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# Dietary Phytase Supplemented *Moringa oleifera* Leaf Meal Improved Growth and Nutrient Digestibility in *Cirrhinus mrigala*

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**Key words**
***Cirrhinus mrigala, Moringa oleifera,* Phytase, Growth performance, Nutrient digestibility, Aquaculture**

## ABSTRACT

The current study was conducted to provide a sustainable and affordable feed for fingerlings of *Cirrhinus mrigala* by using *Moringa oleifera* leaf meal (MOLM), as well as to examine the effects of phytase supplementation on growth and digestibility of nutrients. MOLM was used as basal diet. Total six test diets were prepared with 0, 200, 400, 600, 800, and 1000 FTUkg$^{-1}$ of phytase supplementation. For every treatment, 15 fingerlings were distributed triplicate tanks. The fingerlings were given feed at 5% of their daily wet weight every day. It was noted that test diet-V (800 FTU kg$^{-1}$ phytase) resulted in maximum weight gain (WG) (17.95±0.1g), weight gain percentage (WG%) (248.78±2.84) and minimum feed conversion ratio (FCR) (1.13±0.01). In the case of nutrient digestibility, best results were given by Test diet-V as crude protein (CP) (78.06±0.22%), gross energy (GE) (67.68±0.26%) and ether extract (EE) (81.19±0.85%) were highest in that group. Therefore, this study concluded that the phytase supplemented MOLM resulted in improved growth and nutrient digestibility of *C. mrigala* and optimal level of phytase supplementation was 800 FTU kg$^{-1}$.

## INTRODUCTION

A significant food supply is necessary to support the growing world population. The productivity of aquaculture is increasing to meet some of this growing food demand. Contrary to the industries of beef, pork, and poultry, aquaculture has been rising tremendously over time and today, it accounts for around half of the animal protein consumed globally. Aquaculture, being the main animal protein industry, is expanding more quickly than other areas of food production. Fishery products and fish being farmed account almost 40% (FAO, 2020). However, for growth, this industry needs a substantial amount of fish feed ingredients that are sustainable, cheap, and good for the environment (Tacon, 2020). Issues concerning nutrition, particularly those pertaining to global food security, have emerged as a consequence of rapidly growing population (FAO, 2018). Indian major carps account for 87% of the India's total aquaculture output. Around 70% of the world's Indian major carp is cultured in India (Ramakrishna *et al*., 2013). One of the three Indian major carps, *C. mrigala*, is commonly farmed in Southeast Asian countries. Its production is attributed to faster growth of mrigal specie. India is regarded as the largest producer of *C. mrigala* globally (FAO, 2020).

Fishmeal (FM) has long been the primary protein source in the aquafeed production due to its high protein content. FM is mostly implemented in aquaculture feed since it has a high nutritional value and is extremely palatable (NRC, 2011). FM is rich in minerals, vitamins and essential fatty acids. Aquaculture is facing fierce competition as a result of the significant global production limits and rising demand for FM. FM has recently risen to the top spot as an expensive protein source in feeds for both livestock and aquaculture (El-Sayed, 2020). FM has been substituted with less expensive, locally accessible

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protein sources (Hardy, 2010).

In this context, the use of plant components has quickly drawn the attention of fish nutritionists since they are inexpensively and locally accessible (Napier *et al*., 2020). For herbivorous fish feed formulation many plant sources are being used including soybean meal (Hassaan *et al*., 2015), corn meal (Khalifa *et al*., 2018), sunflower seed meal (Hassaan *et al*., 2018) and jatropha meal (Hassaan *et al*., 2017) etc. Currently, plant-based sources like *Moringa olifera*, soybean, and many others are used to substitute protein in FM either entirely or in part. Plant by-products are regarded as a good FM substitute in fish diets due to their low cost and year-round accessibility (Hussain *et al*., 2018). Furthermore, it has been observed that plant meals had improved fish growth performance (Hussain *et al*., 2011). The miracle tree, the horseradish tree, and the ben oil tree are some of the common names for *M. olifera*. It is a well-known and widely distributed plant of the Moringaceae family with a wide range of therapeutic and dietary advantages everywhere in the world (Luqman *et al*., 2012). The moringa leaves contain flavonoids, polyphenols, carotenoids and ascorbic acid, which are sources of minerals, vitamin A, B, C, and amino acids (Anwar *et al*., 2007).

Phytate is one of many anti-nutritional components found in moringa leaves that cannot be eliminated by soaking or heating. Higher concentrations of phytic acid in plant protein sources have a negative impact on growth, nutritional retention, and mineral absorption (notably cationic minerals, phosphorus) (Gatlin *et al*., 2007). Researches had shown that about 60–80% phosphorus in plant by products are in the form of chelated compound phytate (Lei *et al*., 2013). Phosphorous cannot be metabolized by mono and a-gastric fish, therefore this flow of nutrients causes aquatic pollution.

The phytate complex can be broken down by the enzyme phytase, also known as myo-inositol hexakisphosphatephospho-hydrolase. Mono-gastric fish cannot hydrolyze phytate because they lack the ability to produce the phytase enzyme. Phytase supplementation in the diet is a very effective way to increase fish growth and nutrient digestion. Additionally, it reduces water pollution by ensuring that phosphorus is properly digested and absorbed by fish (Hussain *et al*., 2016).

This study aims to investigate the effects of dietary phytase supplementation in *M. oleifera* leaf meal (MOLM), on the growth parameters and nutrient digestibility of *C. mrigala.*

## MATERIALS AND METHODS

The recent research was conducted to observe the impacts of phytase supplemented of MOLM based diet on growth rate and digestibility of nutrients in *C. mrigala* fingerlings in Fish Nutrition Laboratory, Department of Zoology, Government College University, Faisalabad.

*Experimental design*

MOLM was used as major ingredient to formulate experimental diets. Fingerlings of *C. mrigala* were purchased from Government Fish Seed Hatchery, Faisalabad. The fingerlings used in this experimental trial had already spent two weeks being raised in cement tanks under test conditions. There was acclimatization period of two weeks. On daily basis, the fingerlings were fed once until they appeared satisfied. 0.5% saline solution was used to treat fingerlings to set them free from ectoparasites before feeding trial (Rowland, 1991). For each treatment, triplicate tanks were used. 15 fingerlings were kept in each replicate. Air pumps were used to maintain $O_2$ level. Temperature and pH were also monitored using digital meters on daily basis.

*Feed ingredients and experimental diets*

Garden of University of Agriculture, Faisalabad provided moringa leaves. The leaves were processed initially by soaking in tap water for 3 days at room temperature. After those leaves were dried completely. Before being added to experimental diets, processed ingredients were ground separately and sieved to the desired particle size. Grinded ingredients were analyzed for chemical composition following AOAC (1995) protocol, prior to the formulation of the experimental diets.

*Formation of feed pellets*

The materials for the feed, including FM, wheat flour, canola meal, and others, were grinded to pass via 0.5 mm sieve. After 5 min of thorough mixing of all ingredients in a mixer, fish oil was progressively added. Then 10-15% water was added to form suitable dough. After that, an extruder was used to treat it to create pellets (Lovell, 1989). The six MOLM based test diets were prepared by spraying graded levels (0, 200, 400, 600, 800 and 1000 FTU $kg^{-1}$) of phytase. For preparing stock solution, 1000ml of distilled water was used to dissolve 2g phytase (Phyzyme, Fermic Mexico, and 10,000 Units/g). The corresponding phytase supplemented diets were prepared as described above by adding more water and the varying volumes of stock solution needed for each test diet.

*Feeding protocol and sample collection*

On daily basis, recommended diets were given to fingerlings at rate of 5% of their biomass twice a day. The tanks were washed thoroughly after two h to remove

remaining feed particles and refilled with water. Then feces were collected after 2-3 h from the fecal collection tube. Fecal material of each replicated treatment was dried in oven at 105°C, ground and stored for chemical analysis. Water quality variables i.e., water temperature, pH, and dissolved oxygen were monitored on daily basis using digital meters. Through a capillary system, all tanks are continuously aerated.

*Growth study*

At the conclusion of the trial, fish in each tank were bulk weighed to measure growth rate. Standard formulae were used to measure growth performance of fingerlings. Weight gain % (WG%), feed conversion ratio (FCR) and Specific growth rate (SGR) were measured by using values of initial weight (IW), final weight (FW) and feed intake (FI).

WG % = (FW– IW)/IW × 100

FCR =Total dry FI(g)/ Wet WG(g)

SGR% = [ln(FW)-ln(IW)/ Trial days] × 100

*Chemical analysis of feed and feces*

Motor and pestle was used to homogenize the samples of entire body, experimental diets and feces separately. Standard methods given by AOAC (1995) were followed to analyze these samples. Moisture was measured by drying the sample in oven at 105°C for almost 12h. Micro Kjeldahl apparatus was used to measure protein content (N × 6.25). Soxtec HT2 1045 system was used to measure lipid content through ether extraction (EE) method. After being digested with 1.25% sulfuric acid and 1.25% sodium hydroxide, dried lipid-free residues were used to calculate the content of crude fibre (CF). Ash produced by ignition for 12 h in an electric furnace at 650°C. Bomb calorimeter was used to measure gross energy (GE). Protocol given by AOAC (1995) was used for mineral estimation of whole body, feces and feed samples. For this reason, each was digested in a separate boiling solution of $HNO_3$ and $HCLO_4$ (2:1).

*Chromic oxide estimation*

Ash samples from feed and faeces were first oxidized with $HCLO_4$ reagent, and the concentration of Cr2O3 was then determined using the acid digestion method given by Divakaran *et al*. (2002).

*Calculation of digestibility*

For experimental diets, apparent digestibility coefficients (ADCs) were evaluated using the formulae described by NRC (1993).

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

*Statistical analysis*

Finally, one-way Analysis of Variance (ANOVA) was applied on data of growth, digestibility of nutrients and mineral status of experimental groups (Steel *et al*., 1996). Tukey's honesty test was used to evaluate the differences between the treatments at significant value of ($p<0.05$) (Snedecor and Cochran, 1991). For statistical analysis, the CoStat Computer Package was employed.

## RESULTS

*Growth performance*

Table III and Figures 1 and 2 displays the results of growth performance of fingerlings of *C. mrigala* fed phytase supplemented MOLM-based diets. The recent study showed that the phytase supplementation considerably enhanced growth performance of *C. mrigala* when compared to control diet (without phytase supplementation). Fingerlings fed test Diet V (800 FTU kg-1 phytase supplementation) showed the best growth performance, with WG (g), WG (%), and FCR as 17.95±0.1, 248.78±2.84, and 1.13±0.01 correspondingly. Second best growth performance was noticed in the case of 600 FTU $kg^{-1}$ phytase supplementation. However, minimum growth performance was shown by fingerlings fed control diet (without phytase supplementation). Weight gain, weight gain % and FCR of fingerlings fed control diet were 7.23±0.08g, 101.29±0.94% and 2.26±0.04, respectively.

**Table I. Chemical composition (%) of feed ingredients (dry matter basis).**

| Ingredients | Dry matter (%) | Crude protein (%) | Crude fat (%) | Crude fiber (%) | Ash (%) | Gross energy (Kcal/g) | Carbohydrates (%) |
|---|---|---|---|---|---|---|---|
| Fish meal | 91.23 | 46.21 | 6.12 | 1.11 | 24.13 | 4.03 | 18. 23 |
| Wheat flour | 92.34 | 10.34 | 2.32 | 2.53 | 2.79 | 2.82 | 78.81 |
| Corn gluten | 94.34 | 12.45 | 12.71 | 11.44 | 10.86 | 4.32 | 47.34 |
| MOLM | 93.49 | 37.21 | 1.31 | 1.32 | 8.22 | 3.11 | 48.65 |

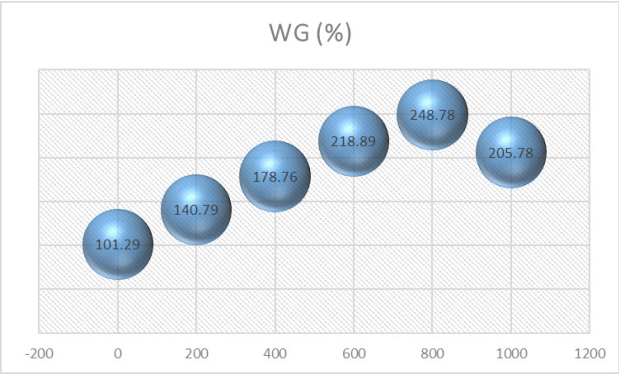


Fig. 1. Weight gain (%) of *C. mrigala* fingerlings fed phytase supplemented MOLM based diets.

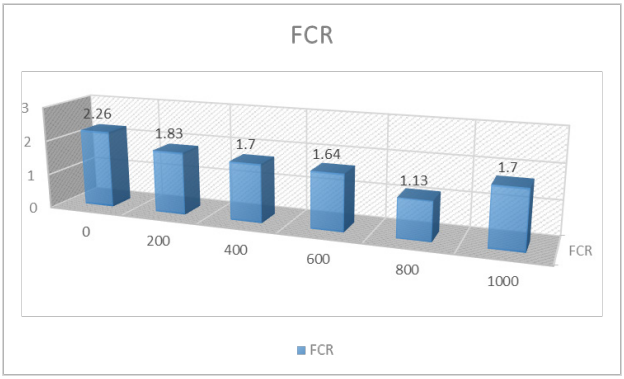


Fig. 2. FCR of *C. mrigala* fingerlings fed phytase supplemented MOLM based diets.

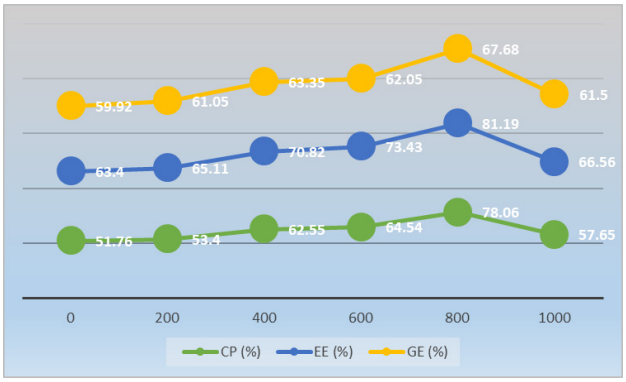


Fig. 3. Effect of MOLM based phytase supplemented test diets on nutrient digestibility of *C. mrigala* fingerlings.

*Nutrient digestibility*

Findings of nutrient digestibility (CP, EE and GE) of *C. mrigala* fingerlings fed phytase supplemented MOLM-based diets are presented in Table IV and Figure 3. The best nutrient digestibility results were shown by test diet-V, with CP, EE and GE values as 78.06±0.22, 81.19±0.85 and 67.68±0.26, respectively. This indicated maximum nutrients were absorbed in fish body and least nutrients removed through feces at phytase supplementation of 800 FTU $kg^{-1}$. However, minimum nutrient digestibility was noticed in case of control with no phytase supplementation. The values of CP, EE and GE for control diet were 51.76±1.55, 63.40±2.87 and 59.92±3.64, respectively.

**Table II. Ingredients composition (%) of experimental diet.**

| Ingredients | Test Diet I (control) | Test Diet II | Test Diet III | Test Diet IV | Test Diet V | Test Diet VI |
|---|---|---|---|---|---|---|
| MOLM | 35 | 35 | 35 | 35 | 35 | 35 |
| Fish meal | 10 | 10 | 10 | 10 | 10 | 10 |
| Canola meal | 20 | 20 | 20 | 20 | 20 | 20 |
| *Wheat flour | 17 | 17 | 17 | 17 | 17 | 17 |
| Rice polish | 8 | 8 | 8 | 8 | 8 | 8 |
| Fish oil | 6 | 6 | 6 | 6 | 6 | 6 |
| Vitamin premix | 1 | 1 | 1 | 1 | 1 | 1 |
| Mineral premix | 1 | 1 | 1 | 1 | 1 | 1 |
| Ascorbic acid | 1 | 1 | 1 | 1 | 1 | 1 |
| Chromic oxide | 1 | 1 | 1 | 1 | 1 | 1 |

MOLM, *Moringa oleifera* leaf meal (Phytase enzyme was used at the expense of * wheat flour).

## DISCUSSION

This study's findings indicated that supplementing with phytase at a level of 800 FTU/kg was appropriate for getting the maximum growth performance. Similar to our findings, by using phytase (1000 FTU/kg) in soybean meal (SBM) based diet, Rachmawati and Samidjan (2016) observed improved growth performance in *Penaeus monodon*. In a study by Rachmawati and Samidjan (2018), all growth metrices were increased at phytase concentrations between 943 and 1100 FTU/kg. Contrary to our findings, Qiu and Davis (2017) observed that phytase had no noticeable impact on growth of *Litopenaeus vannamei*. Debnath *et al*. (2005) noticed the maximum growth rate at level of 500 FTU kg/1 phytase supplementation. It indicated that this level was optimum for increasing the bioavailability of nutrients in *Pangasius pangasius*. Numerous factors may be responsible for these variations in phytase activity and outcomes, including different concentrations of phytase, feed component types, feed processing procedures, lack or presence of stomach, pH of stomach, species, and feed drying techniques (Wang *et al*., 2009).

**Table III. Effect of graded levels of phytase supplemented MOLM based diets on the growth performance of *C. mrigala* fingerlings.**

| Growth parameters / Phytase FTU kg$^{-1}$ | Test diet-I (Control diet) 0 | Test diet-II 200 | Test diet-III 400 | Test diet-IV 600 | Test diet-V 800 | Test diet-VI 1000 |
|---|---|---|---|---|---|---|
| IW(g) | 7.29±0.08$^{a}$ | 7.24±0.01$^{a}$ | 7.33±0.01$^{a}$ | 7.34±0.09$^{a}$ | 7.22±0.01$^{a}$ | 7.32±0.01$^{a}$ |
| FW(g) | 14.54±0.10$^{e}$ | 17.43±0.24$^{d}$ | 20.43±0.19$^{c}$ | 23.41±0.42$^{b}$ | 25.17±0.16$^{a}$ | 22.37±0.44$^{c}$ |
| WG(g) | 7.23±0.08$^{d}$ | 10.19±0.2$^{c}$ | 13.10±0.1$^{c}$ | 16.07±0.3$^{b}$ | 17.95±0.1$^{a}$ | 15.06±0.4$^{b}$ |
| FI | 0.24±0.00$^{c}$ | 0.25±0.01$^{bc}$ | 0.26±0.01$^{ab}$ | 0.27±0.01$^{a}$ | 0.28±0.01$^{a}$ | 0.23±0.01$^{c}$ |
| WG (fish$^{-1}$ day$^{-1}$) g | 0.10±0.01$^{d}$ | 0.13±0.01$^{c}$ | 0.15±0.00$^{b}$ | 0.16±0.01$^{b}$ | 0.25±0.00$^{a}$ | 0.13±0.01$^{c}$ |

a-d Means within rows having different superscripts are significantly different at p<0.05; Data are means of three replicates (± shows Standard Deviations). IW, initial weight; FW, final weight; WG, weight gain; FI, feed intake

**Table IV. Analyzed composition (%) of CP, CF and GE of feed and feces of *C. mrigala* fed on graded levels of phytase (FTUkg$^{-1}$) supplemented MOLM based diets.**

| Experimental diets | Phytase | CP (%) | EE (%) | GE (%) |
|---|---|---|---|---|
| **Diets** | | | | |
| Test diet-I (Control diet) | 0 | 30.95±0.03$^{a}$ | 7.30±0.02$^{b}$ | 3.20±0.03$^{ab}$ |
| Test diet-II | 200 | 30.81±0.10$^{ab}$ | 7.52±0.04$^{a}$ | 3.25±0.09$^{a}$ |
| Test diet-III | 400 | 30.87±0.05$^{ab}$ | 7.27±0.05$^{b}$ | 3.31±0.07$^{a}$ |
| Test diet-IV | 600 | 30.64±0.09$^{b}$ | 7.30±0.02$^{b}$ | 3.29±0.06$^{a}$ |
| Test diet-V | 800 | 30.82±0.17$^{ab}$ | 7.29±0.07$^{b}$ | 3.23±0.06$^{a}$ |
| Test diet-VI | 1000 | 30.86±0.04$^{ab}$ | 7.29±0.04$^{b}$ | 3.05±0.01$^{b}$ |
| **Feces** | | | | |
| Test diet-I (Control diet) | 0 | 15.59±0.44$^{a}$ | 2.79±0.22$^{a}$ | 1.34±0.11$^{a}$ |
| Test diet-II | 200 | 14.90±0.08$^{b}$ | 2.79±0.08$^{a}$ | 1.34±0.11$^{a}$ |
| Test diet-III | 400 | 12.42±0.09$^{d}$ | 2.28±0.03$^{b}$ | 1.34±0.10$^{a}$ |
| Test diet-IV | 600 | 11.53±0.13$^{e}$ | 2.06±0.05$^{b}$ | 1.22±0.06$^{a}$ |
| Test diet-V | 800 | 7.37±0.08$^{f}$ | 1.49±0.07$^{c}$ | 1.14±0.02$^{a}$ |
| Test diet-VI | 1000 | 13.94±0.15$^{c}$ | 2.40±0.06$^{a}$ | 1.25±0.03$^{a}$ |

Data are means of three replicates (± shows Standard Deviations); CP, Crude protein; EE, Ether Extract (crude fat); GE, Gross energy; a-f Means within column having different superscripts are significantly different at p<0.05; Data are means of three replicates (± shows Standard Deviations).

In contrast to a control diet and other phytase-supplemented diets, Hussain *et al*. (2017) found that *C. catla* displayed the best growth performance when fed a diet including phytase supplemented (900 FTU kg$^{-1}$) moringa by-products. Hussain *et al*. (2015) found that rohu at 750 FTU kg-1 produced somewhat different results. When Rohu was given SBM with 1000 FTU kg/1, Bano and Afzal (2018) discovered that Rohu had the greatest value of fat digestibility (80%).

The study's findings showed that adding phytase to MOLM-based meals considerably improved *C. mrigala* ability to digest nutrients when compared to diets without it. The maximum ADC% of CP (78%) and GE (67%) values was observed in fish fed MOLM-based diet supplemented with phytase (800 FTU kg/1). MOLM based diet contains protein, fat, and other essential nutrients. However, an anti-nutritional component called phytate or phytic acid is included in MOLM diets (Gopalakrishnan *et al*., 2016). In plant-based diets, phytic acid binds to and prevents the absorption of nutrients necessary for the best possible growth of fish (Kumar *et al*., 2012). When phytic acid was added to their diet, rainbow trout had decreased protein digestibility, demonstrating that phytic acid combines with protein to decrease fish availability (Spinelli *et al*., 1983). Another study showed that supplementation of feed with phytase (500-1000 FTUkg/1) improved the digestion of protein (Rachmawati and Samidjan, 2018). Storebakken *et al*. (1998) also stated that supplementation of enzymes improves digestibility and retention of protein in fish. Debnath *et al*. (2005) also noted that addition of phytase enzyme in feed of atlantic salmon enhanced its digestibility coefficients at optimum dose of 500 FTUkg/1. Husain *et al*. (2014) further stated that phytase can dissociate anti-nutrients in feed, such as phytic acid and trypsin inhibitor, and that's why it increased the digestion of nutrients.

The phytase enzyme transforms these anti-nutritional compounds into simple, easily absorbed minerals (Sokrab *et al*., 2012). Shahzad *et al*. (2017) and Hussain *et al*. (2017) reached similar conclusions. When fingerlings of *C. catla* were given a diet containing 900 FTU kg/1, they showed the highest digestibility of CP and GE. According to Hussain *et al*. (2016), CP and GE digestibility were improved in *C. mrigala* fingerlings fed an SBM-based test diet supplemented with phytase at 1000 FTU kg$^{-1}$, which

is essentially identical to our findings. Maas *et al*. (2019) observed enhanced growth performance in Nile tilapia fed a diet based on sunflower meal with 1000 FTU $kg^{-1}$ phytase supplementation.

The results published by Hussain *et al*. (2015) were very similar to those observed in recent research, which revealed that increased nutritional digestibility at 800 FTU $kg^{-1}$ of phytase supplementation. Moreover, they noticed that feeding on a diet based on MOLM resulted in greater development due to enhanced protein and fat digestion. Supplementing with phytase improved nutritional digestibility; however, differences persist due to environmental factors or differences in fish species. In another study, Danwitz *et al*. (2016) found that rapeseed protein concentrated with phytase supplementation impaired the digestibility of CP in *Psetta maxima* (turbots) when compared to controls.

## CONCLUSION

This study concluded that the *C. mrigala* fingerlings fed phytase supplemented MOLM resulted in improved growth and nutrient digestibility. For both parameters of *C. mrigala*, the optimum level of phytase supplementation was found to be 800 FTU $kg^{-1}$.

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*Ethical statement*

All the procedures and methods used in this study followed the ethical guidelines provided by Government College University Faisalabad.

*Statement of conflict of interest*

The authors have declared no conflict of interest.

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