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Role of Phytase Supplementation in Improving Growth Parameters and Mineral Digestibility of *Catla catla* Fingerlings Fed Moringa by-Products Based Test Diet

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Abstract

A 90-days feeding trial was conducted to determine the influence of phytase on growth and mineral availability to *Catla catla* fingerlings fed mixture of *Moringa oleifera* leaf meal (MOLM) and *M. oleifera* seed meal (MOSM) based diets. Due to the presence of anti-nutritional factors in plant by-products based diets, reduced mineral availability to fish results in poor fish growth performance. Phytase enzyme is beneficial to decrease these anti-nutritional effects of plant based diets. MOLM+MOSM mixture was used to prepare six test diets that were supplemented with graded levels (0, 300, 600, 900, 1200 and 1500 FTU kg^{-1}) of phytase. Fingerlings were fed at the rate of 4% live wet weight twice a day. On the basis of results it was noted that addition of phytase showed significant (*$p<0.05$*) enhancement in bioavailability of minerals, resulting in improved growth performance (weight gain 229%, SGR 1.32 and FCR 1.3). Maximum (ADC %) of minerals was noted at 900 FTU kg^{-1} level of phytase supplemented MOLM+MOSM based diet. On the basis of these results it was suggested that phytase supplementation at 900 FTU kg^{-1} level is helpful to develop an eco-friendly and cost effective fish feed by using moringa by-products based diet.

Keywords: MOLM+MOSM, *Catla catla*, mineral digestibility, growth performance, phytase

Introduction

Catla catla is commonly known as Thaila. It is surface feeder and being cultured in Pakistan with other fish species (Aslam *et al.*, 2016). The reported production of this fish has been increased during the first decade of 21st century and in 2012 was about 2.8 million tons per annum (FAO, 2015).

In human diet, fish is being used as a quality protein supplement and to resolve the problem of food shortage (Sheikh and Sheikh, 2004). Demand for fish consumption is constantly increasing as a food source and for health benefits (Tiamiyu *et al.*, 2016). As fish is the most important source of protein, it also needs a high amount of protein in its own diet. Nearly 40% of shellfish and fish that is being eaten by human are reared in aquaculture sector (Chabi *et al.*, 2015). Fish meal (FM) is a major protein source in aqua feeds, for different fish species because it is an excellent source of essential minerals, indispensable amino acids, essential fatty acids, and attractants (Dawood *et al.*, 2015). Feed primarily accounts for 50 to 60% of total cost in fish culture (Essa *et al.*, 2004). The main objective

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for most fish farmers is to produce high quality fish feed at low cost. However, increasing demand, unstable supply and high price of the fish meal with the expansion of aquaculture made it necessary to search for alternative protein sources (Hardy, 2010; FAO, 2014). Many efforts have been conducted to reduce fishmeal consumption and utilization of alternative protein sources in diets (Barnes *et al.*, 2012; Dedeke *et al.*, 2013). Plant proteins, which are usually considered as low cost as FM, have been utilized as a substitute in diets for fresh water fishes (Hussain *et al.*, 2014; Hussain *et al.*, 2015a; Wang *et al.*, 2015).

The use of plant by products singly or in combination of two or more than two plant by products appears to be economically beneficial in terms of the cost (Enterria *et al.*, 2011). Plant by products contains several anti-nutritional factors, which limit its utilization in fish diets (Plaipetch and Yakupitiyage, 2014; Hussain *et al.*, 2015a). Some anti-nutritional factors can be partially removed by proper heat treatment, soaking and extraction procedures (Liener, 1994), except phytate, a cyclic inositol compound containing six phosphate groups, is relatively heat stable and cannot be efficiently removed without enzymatic reactions (Vielma *et al.*, 2000). A high content of phytic acid also has an adverse impact on growth and reduces the digestibility minerals (Hussain *et al.*, 2011; 2015a; Dawood *et al.*, 2015). Phytic acid and its salt form phytate, represent 60-80% of total phosphorus in plant based feeds (Lei *et al.*, 2013). Phytate-bound phosphorus is not used by monogastric animals such as fish and causes water pollution (NRC, 1993). Furthermore, phytate may interfere with the availability of other minerals (Liener, 1994; Hussain *et al.*, 2011) and can bind with trypsin and decrease protein availability in fish (Spinelli *et al.*, 1983). There is a need of a specific enzyme that can breakdown the phytate and decreases the problems of digestibility for fish. Phytase is an enzyme that is used for hydrolyses of phytic acid or its salt phytate to myo-inositol and phosphorus (Lei *et al.*, 2013). Phytase supplementation in plant by products is being extensively used to get free phosphorus from phytate complexes (Lim and Lee, 2009). Supplemental dietary phytase is an effective method to improve the mineral digestibility, FCR and decreases the water pollution by proper digestion and absorption of phosphorus (Liu *et al.*, 2013; Hussain *et al.*, 2011; 2015a).

M. oleifera is a promising protein source when included in fish diets at low levels (Chiseva, 2006). *M. oleifera* leaf meal (MOLM) have a relatively high crude protein content which varies from 25% (Makkar and Becker, 1996) to 32% (Soliva *et al.*, 2005). Leaves of moringa are the best source of high number of nutrients and minerals (Bosh, 2004; Grubben and Denton, 2004). *M. oleifera* seed meal (MOSM) contains essential minerals such as Ca, K, Fe, Mg, Cu and Zn etc. (Anjorin *et al.*, 2010). It is also a good source of protein (332.50 to 383.00 g kg^{-1}), important vitamins, essential amino acids i.e. methionine, cystine, tryptophan (Makkar and Becker, 1996).

Therefore, the present research was focused on to find out the best and least cost protein sources using phytase supplemented MOLM+MOSM based diet for commercially important specie *C. catla* to enhance its production and to overcome the problems of expensive fish meal.

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Materials and Methods

The current experimental work was conducted to explore the effects of phytase on growth performance and ADC% of minerals to *C. catla* fingerlings fed MOLM+MOSM based test diets. The study trial was performed in Fish Nutrition Lab, Department of Zoology, Government College University Faisalabad, Pakistan.

Fish and experimental conditions

Fingerlings of *C. catla* were procured from the Government Fish Seed Hatchery, Satiana Road, Faisalabad. For fourteen days, fish fingerlings were acclimatized to the laboratory conditions before the start of experiment. Fingerlings were stocked in specially designed V-shaped tanks having 70 L water capacity. Fish fingerlings were fed once a day on basal diet during the acclimatization period (Allan and Rowland, 1992). Water quality parameters such as dissolved oxygen (DO), pH as well as temperature were observed on daily basis. Air pump was used to supply air by capillary system through-out the study period. Prior to the start of experimental work, fingerlings were bathed for 1 to 2 minutes with 0.5% saline solution to kill the pathogens if present (Rowland and Ingram, 1991).

Experimental design

MOLM and MOSM were used as major experimental feed ingredients to prepare six test diets and supplemented with graded phytase levels (0, 300, 600, 900, 1200 and 1500 FTU kg^{-1}). Six fish groups were stocked in water tanks. They were fed on control diet (0 FTU kg^{-1}) and five phytase supplemented MOLM+MOSM based test diets. Triplicate tanks were used for each treatment and in each replicate 15 fingerlings were stocked. Experimental duration of trial was 90-days. Each MOLM+MOSM based diet supplemented with phytase was compared with other diets and control diet to determine growth performance and ADC% of minerals by using Completely Randomized Design (CRD).

Processing of *M. oleifera* by-products

Fresh moringa leaves were collected from the local garden and washed to remove the dirt and dust particles. The leaves were drained appropriately and dried for six days under shady place to avoid the damage of vitamins by photo-dynamic oxidation or damage. Dried leaves of moringa were separated from the stalks to decrease crude fibers in the diet (Madalla *et al.*, 2013). *M. oleifera* seeds were obtained from local market of Faisalabad. Seeds were air-dried and de-fatted by press method (Weiss, 1971; Salem and Makkar, 2009).

Formation of Feed Pellets

Ingredients used for preparation of fish feed were procured from market and were pressed by grinding method with size of 0.3 mm. Before the experimental diet preparation, chemical composition of feed ingredients was analyzed (Table 1) by following standard methods (AOAC, 1995). Cr_2O_3 was used as inert marker at the rate of 1% in all the test diets. Feed mixer was used to mix all feed ingredients for 5-10 minutes whereas fish oil was also added during

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this process. Suitably textured dough was prepared by slowly blending of feed ingredients in mixer after adding 10-15% of tap water. The prepared dough was further processed through pelleting machine to formulate feed pellets (Lovell, 1989). One control and five phytase supplemented test diets were prepared by using MOLM+MOSM by spraying different phytase levels (0, 300, 600, 900, 1200 and 1500 FTU kg^{-1}). The required concentrations of phytase enzyme were formulated in 25 mL of distilled H_2O and sprayed on each experimental diet (Robinson *et al.*, 2002). Control diet (0 FTU kg^{-1} level) was also sprayed with similar quantity of H_2O to conserve the equivalent amount of moisture. After drying, diets were stored at 4°C until use.

Feeding protocol and sample collection

C. catla fingerlings were weighed and fed on their prescribed diet at the rate of 4% of body weight twice a day. Uneaten diet was collected to estimate FCR. The water was drained out after two hours of feeding period to eliminate the uneaten diet particles. Faeces were collected by opening fecal collecting tube of each tank. Fecal material was collected carefully to avoid breakage of faeces for minimizing the leaching of minerals in water. Feces were dried at 65ºC in oven, stored for further analysis.

Growth study

Fifteen fingerlings of average weight (8.07±0.041g $fish^{-1}$) were stocked in each replicate. The fish were bulk weighed in each tank on fortnightly basis throughout the whole experimental period to evaluate the growth performance of *C. catla* fingerlings. Growth parameters such as weight gain (g), FCR (feed conversion ratio), SGR (specific growth rate) and weight gain (%) of fingerlings were calculated by using standard formulae (NRC, 1993).

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

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

$$\text{SGR\%} = \frac{\text{(ln. final wt. of fish – ln. initial wt. of fish)}}{\text{Trial day}} \times 100$$

Chemical analysis of minerals

1g of the sample (feed and feces) was weighed for mineral estimation. Weighed samples were taken in open mouth conical flask. Before putting on hot plate, HNO_3 (20ml) was added in the flask. 10ml of per-chloric acid was added before placing it on hot plate. Heat the mixture until it left only 1ml then diluted by 50 mL of H_2O after removing from hot plate. Filter the solution to eliminate all particles remained in digestion solution before the mineral analysis. Atomic Absorption Spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan) was used to estimate minerals from diluted mixture using AOAC (1995) methods. Na and K were estimated by flame

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photometer (Jenway PFP-7, UK). UV/VIS spectrophotometer at 720 nm absorbance was used to determine phosphorous contents in the experimental diets and feces (AOAC, 1995).

Calculation of mineral's apparent digestibility coefficient (ADC%)

ADC% of minerals was estimated by using standard formula (NRC, 1993).

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

Statistical analysis

Finally, data of growth and ADC% of minerals (K, Ca, Fe, Na, Cu, Mn, Zn, P, Mg, Al, Cr, Sr, Pb, Ba, Cd, Co, Mo and Ni) were subjected to one-way ANOVA (Steel *et al.*, 1996). Tukey's Honesty Significant Difference Test was used to compare the differences among treatments and was considered significant at $p<0.05$ (Snedecor and Cochran, 1991). For statistical analysis, CoStat Computer Package (Version 6.303, PMB 320, Monterey, CA, 93940 USA) was used.

Results

In the present study, different levels of microbial phytase (0, 300, 600, 900, 1200 and 1500 FTU kg^{-1}) were used in MOLM+MOSM based test diets, to determine the effects of this enzyme on growth performance of *C. catla* fingerlings. Maximum weight gain (18g), WG% (229%) and SGR (1.32) of *C. catla* fingerlings fed mixture of moringa by-products (MOLM+MOSM) based test diets was observed at 900 FTU kg^{-1} level based diet. Next higher WG (16g), WG% (203%) and SGR (1.23) was observed in fish fed 600 FTU kg^{-1} level based diet. These were significantly ($p<0.05$) different from the lowest (12g) weight gain found in fish fed on control and other phytase supplemented test diets (Table 3). It was noted that WG and WG% was started increasing from 300 FTU kg^{-1} level and reached to maximum at 900 FTU kg^{-1} level based diet. Interestingly further phytase supplementation could not increase the WG, WG% and SGR of fish. In term of FCR values, lowest FCR (1.30) was again noted in fish fed on test diet III (900 FTU kg^{-1} level based diet) followed by (1.38) at 600 FTU kg^{-1} level. These FCR values were statistically ($p<0.05$) different from the values observed for control and each other test diet. Whereas again highest FCR (1.68) was noted in fish fed 0 FTU kg^{-1} level based diet (Table 3).

All the observed minerals such as Ca, Na, K, Fe, Cu, Zn, Mn, P, Mg, Al, Cr, Sr and Pb were statistically ($p<0.05$) similar in control and phytase supplemented test diets (Table 4). Whereas some of the minerals (Ba, Cd, Co, Mo and Ni) were very low from the range (<0.0001) in diets and could not be noted samples, so ADC% of these minerals could not be determined. It was observed that there was highest mineral discharge through feces when *C. catla* fed control diet that was significantly ($p<0.05$) different from each test diet. Decrease in mineral excretion through fish faeces was noted after the phytase supplementation from 300 FTU kg^{-1} level and reached its minimum at 900 FTU kg^{-1} level based diet then it again started to increase till 1500 FTU kg^{-1} level based diet (Table 5). Lowest mineral

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discharge at 900 FTU kg^{-1} level based diet showed that this is the most optimum level of phytase supplementation in MOLM+MOSM based diet which resulted into environment friendly aquaculture by lowering mineral's excretion into water.

Table 6 shows that mineral digestibility was started to increase after supplementation of phytase from 300 FTU kg^{-1} level based diet and reached to its maximum when fish fed 900 FTU kg^{-1} level based diet. It was also noted that further increase in phytase supplementation could not improve the ADC% of minerals till 1500 FTU kg^{-1} level. ADC% of minerals shows that maximum digestibility values of Ca (77%), Na (72%), Fe (68%), Cu (68%), Mn (75%), P (67%) and Mg (67%) were calculated for fish fed on 900 FTU kg^{-1} level based diet followed by 600 and 1200 FTU kg^{-1} level based diets. These values were significantly (*p<0.05*) different from values observed from test diets whereas Mg and P were statistically (*p<0.05*) similar with digestibility values observed for test diet-IV (1200 FTU kg^{-1} level). On the other hand maximum K (75%), Al (62%), Cr (65%) and Sr (54%) digestibility was observed for fish fed at 1200 FTU kg^{-1} level based diet in which values of Al, Cr and Sr were statistically (*p<0.05*) similar with the digestibility values calculated on 900 FTU kg^{-1} level based diet. In contrary to these, highest Zn (76%) and Pb (55%) digestibility values were noted at 600 FTU kg^{-1} level based diet and it was significantly (*p<0.05*) different from control and other test diets. It showed that phytase has released the chelated minerals from phytate in between 600 FTU kg^{-1} level to 1200 FTU kg^{-1} levels.

Discussion

Presence of phytate in feed reduces fish growth performance in terms of weight gain and FCR (Spinelli *et al.*, 1983). Maximum weight gain WG (18g), WG% (229%) and SGR (1.32) was observed in fish fed on 900 FTU kg^{-1} level in MOLM+MOSM based diets. Nearly similar results were found in terms of WG and WG%, when common carp fingerlings were fed at 800 FTU kg^{-1} supplemented plant-meal based diet (Bai *et al.*, 2003). Nwanna *et al.* (2007) also found a significant (*p<0.05*) increase in overall *Cyprinus carpio* WG and WG% at 750 and 1000 FTU kg^{-1} levels based diets. In another study, positive results were found by Hussain *et al.* (2015b) but on a little different level (750 FTU kg^{-1}) of phytase supplementation. They found maximum weight gain (5g), weight gain % (68%) in *L. rohita* fingerlings fed canola meal based diet. This difference in findings for growth indices may be linked with many factors such as types of feed ingredients used, varying levels of phytase, processing methods of feed, pH of stomach and methods used for feed drying (Wang *et al.*, 2009).

In present study maximum weight gain (%) was found when *C. catla* fingerlings were fed on 900 FTU kg^{-1}level based diet. Nearly similar results were observed in a study conducted by Hussain *et al.* (2014), they found maximum weight gain and weight gain % of *C. mrigala* fingerlings fed soybean meal based diet supplemented with phytase at 1000 FTU kg^{-1} level. Similarly, Yu and Wang (2000) also found the same results when fish was fed at 1000 FTU kg^{-1} level based diet. In contrast, non-significant (*p<0.05*) effect of phytase supplementation was reported in case of *O. mykiss* growth when fingerlings were fed phytase supplemented plant meal based diet (Vielma *et al.*, 2000). In contradiction to the present findings many other researchers such as Robinson *et al.*(2002), Baruah *et al.* (2007a), Lim and Lee (2009) and Wang *et al.* (2009) did not found any significant (*p<0.05*) effect of phytase

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supplementation on fish growth performance, when these fish species were fed with or without phytase supplemented plant meal based diets. This variability in findings may be due to type of phytase enzyme, fish species, process of feed preparation, fish gut pH and age of fish (Kumar *et al.*, 2011; Dersjant-Li *et al.*, 2015). Furthermore, Kumar *et al.* (2011) suggested that for better results, phytase enzyme should be supplemented on the basis of earlier published information.

Current study showed lowest FCR value (1.30) of *C. catla* fingerlings fed on 900 FTU kg^{-1} level in MOLM+MOSM based diet. The FCR value obtained at 900 FTUkg^{-1} level was found best as compared to FCR of fish fed on control diet and other phytase supplemented test diets. Similar to our results Riche and Garling (2004); Ashraf and Goda (2007) and Cao *et al.* (2008) observed maximum SGR and FCR values when *O. niloticus* (Nile tilapia) fed plant meal based diets with 1000 FTU kg^{-1} level of phytase supplementation. In contrast to our findings, higher FCR values of *Monorone saxatilix* (stripped seabass) were observed when fed with a little higher dose (at 1300 FTU kg^{-1} level) of phytase supplementation in plant meal based diet (Hughes and Soares, 1998). On the other hand, Hussain *et al.* (2011) observed maximum improvement in FCR of *L. rohita* fingerlings when they fed 750 FTU kg^{-1} level based diet that was close to the optimum level (900 FTU kg^{-1}) found in present study. In contrary, phytase supplementation could not enhance the overall growth performance of *O. mykiss* fed phytase supplemented canola meal based diets (Vielma *et al.*, 2000). Non-significant ($p<0.05$) difference was observed in term of growth performance when Korean rock fish (Yoo *et al.*, 2005), parrot fish (Lim and Lee, 2009) Japanese flounder (Masumoto *et al.*, 2001), channel catfish (Yan and Reigh, 2002) and Atlantic salmon (Sajjadi and Carter, 2004) fed on different plant meal based diets supplemented with phytase at different levels. Similarly, Yoo *et al.* (2005) concluded that *S. schlegeli* (Korean rockfish) could not improve its growth indices when fed soybean meal based diets supplemented with phytase at 1000 and 2000 FTU kg^{-1} levels supplemented diets. This controversy in results may be due to use of different fish species, phytase type, feed ingredient, methods of feed preparation etc. (Baruah *et al.*, 2017b).

Phytate commonly exists in plant based ingredients that usually binds with divalent cations and is known as a major anti-nutritional factor (Soetan and Oyewole, 2009). Breakdown of complex chelated structure of phytate enhances the release and utilization of essential minerals. Many researchers indicated that phytate present in plant by-products may chelate with some of the important minerals such as Fe, Ca, Mn, Cu, Ni, Cr, Na, K, P, Mg and decreases their availability to mono-gastric fish (Cao *et al.*, 2007; Dersjant-Li *et al.*, 2015; Hussain *et al.*, 2015a,b). Present results showed that supplementation of phytase in moringa by-products (MOLM+MOSM) based diets is much beneficial for improving mineral digestibility in *C. catla* fingerlings as compared to fish fed on control diet (without phytase supplementation), because of the release of chelated minerals form phytate complex. From present study, it was noted that 900 FTU kg^{-1} is the most optimum level of phytase supplementation that can decrease excretion of these essential minerals in water through fish feces and increase mineral digestibility. Whereas, some of the minerals showed maximum digestibility for fingerlings when they fed on 600 FTU kg^{-1} level based diet and remaining were found maximum at 1200 FTU kg^{-1} level based diet. Literature reviewed that phytase can influence the mineral digestibility from 250 to 1500 FTU kg^{-1} levels in different fish species in different environmental conditions (Cao *et al.*, 2007). Similar to our findings, Hussain *et al.* (2015b) found that anti nutritional factors in soybean meal based

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diets such as phytate played negative role in mineral digestibility, whereas phytase supplementation at 1000 FTU kg^{-1} level improved mineral digestibility by breaking down the chelated phytate-minerals complex resulting in maximum utilization of essential minerals by fish and decreased mineral discharge in water. Increased mineral utilization was also observed by Cheng and Hardy (2002), when they supplemented plant meal based diets with phytase that liberated chelated minerals from phytate present in plant feed stuffs. Zhu *et al.* (2014) noted significantly decreased mineral contents in feces after phytase supplementation. Hussain *et al.* (2015a) also found positive effects of phytase supplementation at 750 FTU kg^{-1} level in improving mineral digestibility of *L. rohita* fingerlings when fed with canola meal based diets as compared to control (0 FTU kg^{-1} level) and other phytase supplemented diets. Almost similar with present findings, Van-Weerd *et al.* (1999) found that phytase addition at 1000 FTU kg^{-1} level in soybean meal based diet showed maximum ADC% of P in *Clarias gariepinus*. While, Hussain *et al.* (2016) found that 750 FTU kg^{-1} is the optimum level for maximizing ADC% of minerals in *L. rohita* fingerlings. In another study, *C. mrigala* fingerlings were fed phytase supplemented sunflower meal based diets and showed maximum nutrient digestibility at 1000 FTU kg^{-1} level (Hussain *et al.*, 2014). In contrary to current results, Baruah *et al.* (2007a) found maximum mineral digestibility values in *L. rohita* fingerlings fed on 500 FTU kg^{-1} level in plant meal based diet. They concluded that minerals such as Fe, Mg, K, Mn, P and Na showed highest ADC% at 500 FTU kg^{-1} level based diet as compared to control and other phytase supplemented test diets. Similar to these findings, Debnath *et al.* (2005) also noted highest ADC% of minerals (Mn, Fe, Na, Mg and P) in *Pangasius pangasius* at 500 FTU kg^{-1} level based diet. Variations in optimal level of phytase supplementation may be due to difference in plant ingredients used in diet formulation and experimental fish species (Dersjant-Li *et al.*, 2015). In contrary to these findings and present results, Laining *et al.* (2010) observed highest mineral digestibility and absorption in *Takifu gurubripes* (tiger puffer), when fed with phytase supplementation at 2000 FTU kg^{-1} in soybean meal based diet. In another study Nwanna and Bello (2014) suggested that phytase supplementation played a non-significant role in term of mineral digestibility in *Oreochromis niloticus*, Nile tilapia fingerlings. They found little improvement in mineral digestibility at a very high dose (8000 FTU kg^{-1}) of phytase supplementation. Their results were not in specific range of phytase supplementation (250 to 1500 FTU kg^{-1} level) as narrated by Cao *et al.* (2007). Reasons for these variations in results of different studies may be the use of different quality and quantity of phytase enzyme, feed formulation technology, feed drying methodology or process used for phytase supplementation (Baruah *et al.*, 2007b).

Conclusion

It was concluded that the phytase supplementation played very important role in improving growth performance and mineral digestibility of *C. catla* fingerlings and ultimately resulted in decreasing water pollution when fed on phytase supplemented MOLM+MOSM based diets. Best values in term of maximum growth performance and mineral digestibility to fish body indicated that fish fed phytase supplemented diet became healthier as compared to fish fed on control diet (0 FTU kg^{-1}). It was also concluded that supplementation of phytase at 900 FTU kg^{-1} level is

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the optimum level that significantly improved the growth parameters and minerals digestibility to fish fed on mixture of MOLM+MOSM based diet.

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

Ingredients	MOLM+MOSM (Mixture)	Fish meal	Rice polish	Wheat flour	Corn gluten 60%
Dry matter (%)	92.13	91.67	94.06	92.4	92.34
Crude Protein (%)	32.22	48.17	12.38	10.15	59.51
Crude Fat (%)	4.02	7.12	13.46	2.3	4.58
Gross Energy (kcal/g)	14.05	2.65	3.18	2.95	4.35
Crude Fiber (%)	9.27	1.12	12.74	2.67	1.23
Ash (%)	3.98	24.66	10.17	2.06	1.36
Carbohydrates	36.46	16.28	48.07	79.87	28.97

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Table 2. Ingredients composition (%) of control and test diets (Dry matter basis)

Ingredients	Test Diet-I (Control)	Test Diet-II	Test Diet-III	Test Diet-IV	Test Diet-V	Test Diet-VI
	Phytase Level (FTU kg^{-1})					
	0	300	600	900	1200	1500
MOLM+MOSM	35	35	35	35	35	35
Fish meal	15	15	15	15	15	15
Soybean meal	15	15	15	15	15	15
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	1.0	1.0	1.0	1.0	1.0	1.0
Chromic oxide	1.0	1.0	1.0	1.0	1.0	1.0
Ascorbic acid	1.0	1.0	1.0	1.0	1.0	1.0
Mineral mixture	1.0	1.0	1.0	1.0	1.0	1.0

MOLM= *M. oleifera* leaf meal, MOSM= *M. oleifera* seed meal
*Phytase enzyme was used at the expense of wheat flour

Table 3. Growth performance of *C. catla* fingerlings fed graded levels of phytase supplemented moringa by-products (MOLM and MOSM) mixture based diets

Growth parameters	Test Diet –I (Control diet)	Test Diet –II	Test Diet –III	Test Diet –IV	Test Diet –V	Test Diet –VI
	Phytase levels (FTU kg^{-1})					
	0	300	600	900	1200	1500
IW (g)	8.12±0.03	8.10±0.01	8.10±0.06	8.05±0.03	8.05±0.02	8.04±0.03
FW (g)	20.18±0.1^{f}	21.42±0.2^{e}	24.59±0.1^{b}	26.49±0.1^{a}	23.50±0.1^{c}	22.20±0.1^{d}
WG (g)	12.06±0.11^{f}	13.32±0.21^{e}	16.49±0.11^{b}	18.44±0.09^{a}	15.45±0.08^{c}	14.16±0.13^{d}
WG (%)	148.57±1.8^{f}	164.40±2.4^{e}	203.50±0.2^{b}	229.16±1.4^{a}	192.01±1.4^{c}	176.08±2.1^{d}
FI	0.23±0.003^{d}	0.24±0.004^{c}	0.25±0.003^{b}	0.27±0.002^{a}	0.25±0.001^{b}	0.24±0.002^{c}
WG (fish^{-1} day^{-1})g	0.13±0.001^{f}	0.15±0.002^{e}	0.18±0.001^{b}	0.20±0.001^{a}	0.17±0.001^{c}	0.16±0.001^{d}
FCR	1.68±0.01^{f}	1.59±0.003^{e}	1.38±0.01^{b}	1.30±0.003^{a}	1.44±0.001^{c}	1.52±0.004^{d}
SGR	1.01±0.01^{f}	1.08±0.01^{e}	1.23±0.001^{b}	1.32±0.005^{a}	1.19±0.01^{c}	1.13±0.01^{d}

Means within rows having different superscripts are significantly different at $P < 0.05$
Data are means of three replicates
IW= Initial Weight, FW= Final Weight, WG= Weight gain, FI= Feed Intake, SGR= Specific Growth Rate, FCR= Feed Conversion Ratio

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Table 4. Analyzed mineral composition (%) of MOLM+MOSM mixture test and control diets

Minerals	Test Diet –I (Control diet)	Test Diet –II	Test Diet –III	Test Diet –IV	Test Diet –V	Test Diet –VI
	Phytase levels (FTU kg^{-1})					
	0	300	600	900	1200	1500
Ca	1.31±0.04	1.32±0.06	1.32±0.05	1.31±0.05	1.32±0.05	1.33±0.05
Na	0.049±0.007	0.052±0.007	0.051±0.007	0.051±0.007	0.051±0.009	0.050±0.005
K	0.97±0.07	0.95±0.04	0.95±0.09	0.94±0.07	0.95±0.06	0.94±0.06
Fe	0.038±0.004	0.040±0.004	0.040±0.005	0.040±0.005	0.039±0.004	0.041±0.006
Cu	0.0091±0.0004	0.0093±0.0005	0.0092±0.0007	0.0092±0.0005	0.0092±0.0005	0.0091±0.0004
Zn	0.0235±0.001	0.0233±0.001	0.0250±0.004	0.0250±0.004	0.0253±0.002	0.0243±0.004
Mn	0.035±0.003	0.035±0.003	0.036±0.005	0.037±0.004	0.036±0.004	0.035±0.005
P	1.58±0.06	1.55±0.07	1.57±0.06	1.55±0.07	1.56±0.04	1.57±0.05
Mg	0.0098±0.0004	0.0095±0.0003	0.0093±0.0005	0.0097±0.001	0.0097±0.0005	0.0094±0.0003
Al	0.00041±0.00006	0.00038±0.00006	0.00039±0.00002	0.00038±0.00003	0.00038±0.00006	0.00038±0.00004
Cr	0.068±0.005	0.065±0.003	0.066±0.002	0.065±0.004	0.068±0.002	0.066±0.003
Sr	0.00018±0.00001	0.00018±0.00001	0.00018±0.00001	0.00018±0.00001	0.00018±0.00001	0.00018±0.00001
Pb	0.0036±0.0003	0.0034±0.0003	0.0036±0.0003	0.0036±0.0003	0.0036±0.0005	0.0036±0.0002
Ba	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cd	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Co	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Mo	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Ni	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Data are means of three replicates

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Table 5. Analyzed mineral composition (%) in faeces of *C. catla* fed MOLM+MOSM mixture based diets

Minerals	Test Diet –I (Control diet)	Test Diet –II	Test Diet –III	Test Diet –IV	Test Diet –V	Test Diet –VI
	Phytase levels (FTU kg^{-1})					
	0	300	600	900	1200	1500
Ca	0.64±0.04^{a}	0.58±0.03^{a}	0.41±0.03^{b}	0.32±0.02^{c}	0.48±0.03^{b}	0.61±0.03^{a}
Na	0.027±0.003^{a}	0.024±0.003^{ab}	0.018±0.002^{bc}	0.015±0.002^{c}	0.019±0.004^{abc}	0.024±0.003^{ab}
K	0.48±0.04^{a}	0.43±0.02^{ab}	0.35±0.04^{bc}	0.33±0.03^{cd}	0.25±0.02^{d}	0.41±0.03^{abc}
Fe	0.024±0.002^{a}	0.021±0.002^{ab}	0.017±0.002^{bc}	0.014±0.002^{c}	0.014±0.001^{c}	0.017±0.002^{bc}
Cu	0.0054±0.0002^{a}	0.0049±0.0002^{ab}	0.0034±0.0002^{c}	0.0031±0.0001^{c}	0.0036±0.0002^{c}	0.0046±0.0002^{b}
Zn	0.012±0.0004^{a}	0.011±0.0002^{ab}	0.0061±0.00075^{c}	0.0087±0.001^{bc}	0.010±0.0007^{ab}	0.011±0.0017^{ab}
Mn	0.02±0.002^{a}	0.016±0.002^{ab}	0.013±0.002^{bc}	0.01±0.001^{c}	0.012±0.001^{bc}	0.015±0.002^{ab}
P	0.91±0.02^{a}	0.74±0.03^{b}	0.63±0.02^{c}	0.55±0.02^{d}	0.56±0.01^{d}	0.70±0.02^{b}
Mg	0.0067±0.0004^{a}	0.0056±0.0003^{b}	0.0042±0.0003^{c}	0.0035±0.0002^{de}	0.0033±0.0001^{e}	0.0041±0.0002^{cd}
Al	0.00025±0.00003^{a}	0.00022±0.00003^{ab}	0.00016±0.00001^{bc}	0.00016±0.00002b^{c}	0.00015±0.00002^{c}	0.00017±0.00002^{bc}
Cr	0.047±0.003^{a}	0.038±0.001^{b}	0.036±0.001^{b}	0.026±0.002^{d}	0.026±0.001^{d}	0.031±0.001^{c}
Sr	0.00014±0.000004^{a}	0.00012±0.00001^{ab}	0.00011±0.00001^{b}	0.000094±0.000003^{c}	0.000087±0.000004^{c}	0.000091±0.00001^{c}
Pb	0.0027±0.0002^{a}	0.0022±0.0002b	0.0017±0.0001c	0.0020±0.0002^{bc}	0.0021±0.0002bc	0.0024±0.0001ab

Means within rows having different superscripts are significantly different at $P< 0.05$
Data are means of three replicates

Table 6. Apparent mineral digestibility (%) of *C. catla* fingerlings fed MOLM+MOSM mixture based diets

Minerals	Test Diet –I (Control diet)	Test Diet –II	Test Diet –III	Test Diet –IV	Test Diet –V	Test Diet –VI
	Phytase levels (FTU kg^{-1})					
	0	300	600	900	1200	1500
Ca	53.46 ± 0.85^{e}	58.47 ± 0.89^{d}	70.24 ± 0.74^{b}	77.05 ± 0.76^{a}	65.82 ± 0.89^{c}	56.38 ± 0.78^{d}
Na	48.86 ± 0.9^{d}	56.67 ± 0.85^{c}	66.77 ± 0.92^{b}	72.35 ± 0.54^{a}	64.62 ± 0.93^{b}	54.51 ± 0.77^{c}
K	52.69 ± 0.61^{e}	57.32 ± 0.92^{d}	65.02 ± 0.99^{c}	67.61 ± 0.91^{b}	74.75 ± 0.77^{a}	58.63 ± 0.86^{d}
Fe	41.43 ± 0.78^{e}	49.51 ± 0.94^{d}	59.6 ± 0.91^{c}	68.31 ± 0.88^{a}	65.30 ± 0.92^{b}	60.6 ± 0.71^{c}
Cu	44.33 ± 0.86^{e}	50.27 ± 0.84^{d}	64.28 ± 0.89^{b}	68.37 ± 0.67^{a}	63.45 ± 0.57^{b}	52.57 ± 0.72^{c}
Zn	51.37 ± 0.79^{e}	57.40 ± 0.81^{d}	76.52 ± 0.95^{a}	67.59 ± 0.88^{b}	61.53 ± 0.8^{c}	56.16 ± 0.98^{d}
Mn	46.42 ± 0.61^{d}	57.29 ± 0.84^{c}	66.62 ± 0.91^{b}	74.71 ± 0.95^{a}	67.90 ± 0.99^{b}	58.37 ± 0.75^{c}
P	45.59 ± 0.8^{e}	54.75 ± 0.69^{d}	61.98 ± 0.77^{b}	67.35 ± 0.65^{a}	65.96 ± 0.97^{a}	57.92 ± 0.98^{c}
Mg	35.21 ± 0.92^{d}	44.34 ± 0.83^{c}	57.41 ± 0.93^{b}	66.61 ± 0.85^{a}	68.21 ± 0.87^{a}	58.51 ± 0.83^{b}
Al	41.57 ± 0.73^{e}	46.39 ± 0.67^{d}	59.52 ± 0.83^{bc}	61.78 ± 0.97^{ab}	62.54 ± 0.86^{a}	57.76 ± 0.87^{c}
Cr	35.45 ± 0.95^{e}	44.5 ± 0.78^{d}	48.19 ± 0.99^{c}	63.45 ± 0.92^{a}	64.7 ± 0.87^{a}	54.39 ± 0.98^{b}
Sr	26.29 ± 0.48^{e}	35.72 ± 0.74^{d}	40.62 ± 0.97^{c}	52.53 ± 0.98^{ab}	54.04 ± 0.5^{a}	51.35 ± 0.99^{b}
Pb	27.37 ± 0.76^{e}	38.36 ± 0.8^{d}	55.22 ± 0.75^{a}	49.55 ± 0.92^{b}	43.34 ± 0.88^{c}	36.55 ± 0.75^{d}

Means within rows having different superscripts are significantly different at P< 0.05
Data are means of three replicates