Potential of phytase supplemented *Moringa oleifera* leaf meal based diet on mineral digestibility of *Oreochromis niloticus* fingerlings

Sarfraz Q.[1]; Hussain S.M.[1*]; Sharif A.[1]; Selamoglu Z.[2]; Bashir F.[1]; Ahsan S.[1]

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Abstract

This current study was planned to evaluate effect of exogenous phytase supplemented*Moringaoleifera* leaf meal (MOLM) diet on mineral digestibility of *Oreochromis niloticus* fingerlings. To conduct the experiment, six experimental diets based on MOLM with 0, 200, 400, 600, 800 and 1000 FTU kg^{-1} phytase level wereformulated. Chromic oxide (1%) was added in the feed as indigestible marker. Completely Randomized Design (CRD) with three replicates was adopted to accomplish the experiment. Stocking density in each V-shaped triplicate tank was 15 fingerlings; fed at the rate of 5% of live wet weight. Results predicted that the fish group fed MOLM based diet supplemented with 600 FTU kg^{-1} phytase showed highest Apparent Digestibility Coefficient (ADC) % of minerals and minimum quantity was discharged through feces. Hence, to release chelated minerals in MOLM, phytase at the level of 600 FTU kg^{-1}proved very effective.By viewing above results, it became clear that phytase supplementation to MOLM based diet is helpful in formulation of cost effective and environment friendly feed for*O. niloticus* fingerlings.

Keywords: Aquaculture, MOLM, Mineral absorption, Exogenous phytase, Juveniles, Supplementation

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1-Fish Nutrition Lab, Department of Zoology, Government College University, Faisalabad, Pakistan

2-Nigde ÖmerHalisdemir University, Faculty of Medicine, Department of Medical Biology, Nigde, Turkey

*Corresponding author's Email:drmakhdoom90@gmail.com

Introduction

The fish gaining popularity among aqua-culturists around the world is *O. niloticus*; as it owes outstanding growth and reproductive potential even in varied cultural conditions and compensates handling stress effectively (Tsadik and Bart, 2007). Its farming trend is increasing and consequently earning capital amount; the profit belonging to rearing of 5.67 million metric ton of Nile tilapia in 2015 is six billion dollars as stated by Food and Agriculture Organization (FAO, 2017).

Aquaculture sector is paying heed to the continuous and cheap source of protein to meet human nutritional requirements globally (Gobi *et al.*, 2016)and succeeded in enhancing world fish production from 29-38% (FAO, 2016). But the challenge faced by this industry is in terms of fish feed formulation cost. Fish Meal (FM) is rich protein ingredient for fish;butvery expensive and inaccessible to the fish farmers due to short supply (Rivas-Vegaa*et al.*, 2006; Wang *et al.*, 2006). This problem has opened new insights for the researchers to check potential of innovative natural plant by-products in place of fish meal (Dawood *et al.*, 2014; Gerzhova*et al.*, 2015;Yan*et al.*, 2017).

Until now various plant protein replacements have been examined in order to fulfil fish meal scarcity and to increase budget of fish feed. For instance, corn protein concentrate (Khalifa*et al.*, 2018), fermented soybean meal (Hassaan*et al.*, 2015), okara meal (El-Saidy, 2011), Jatropha meal (Hassaan*et al.*, 2017), soy protein essence (Ribeiro *et al.*, 2016) and fermented sunflower meal (Hassaan*et al.*, 2018) accounted for partial substitution of fish meal.Being nutritionally rich, *M. oleifera* belongs to Moringaceae family and can be easily found in tropical and subtropical regions with much applications in food and medicinal purposes (Makkarand Becker, 1999; Djissou*et al.*, 2016).Leaves of this plantare regarded as source of proteins, ascorbic acid, carotenoids, iron and vitamins. Substitution of fish meal with MOLMas protein source was achieved up to 10% in the diet of *Labeorohita* and *Clariasgariepinus* (Arsalan*et al.*, 2016; Ezekiel *et al.*, 2016; Mehdi *et al.*, 2016). Furthermore, success was met by feeding seed meal of *M. oleifera* as protein source to *O. niloticus* (Hashem *et al.*, 2017).

However, complete sparing of fish meal with plant meal leadtoimbalance of essential amino acids, unavailability of cation minerals and phosphorus (P) and reduced growth (Geurden*et al.*,2013) due to less palatability and several Anti-Nutritional Factors (ANFs).Phytate or phytic acid is a chief source of phosphorus. Monogastric and a-gastric fishes are devoid of endogenous phytase, hence cannot utilize 50-80% of P; aggregated in chelate form.In order to liberate P and associated minerals from phytate, we supplement plant meal withenzymes. Phytase is a microbial enzymewith chemical name myo-inositol hexa-phosphate

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phosphohydrolase and capable of hydrolysing indigestible phytate.Exogenous phytase functions in minimizing water pollution by effective absorption of P and maximizing mineral (P, N, Mg, Ca, Cu, Zn, and Fe) digestibility (Hussain *et al.*, 2011; Liu *et al.*, 2013). Harmful effects of phytic acid can be effectively reduced by the addition of phytase (Hussain *et al.*, 2015). It can be utilized in fish feed as itimproves growth, nutrients and mineral availability and helps in reduction of P pollution in the water (Kumar *et al.*, 2012). Hence, it was hypothesized that addition of phytase in MOLM will result in better ADC % of mineralsto*O. niloticus* juveniles (Table 1).

Table 1: Chemical Formulation of Control and Test Diets prepared for *O. niloticus* fingerlings based on MOLM

Ingredients	Test Diet-I	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	1.0	1.0	1.0	1.0	1.0	1.0
Mineral Premix	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
Chromic oxide	1.0	1.0	1.0	1.0	1.0	1.0
Phytase Level(FTU kg^{-1})	0	200	400	600	800	1000

MOLM=*Moringa oleifera*Leaf Meal
Phytase enzyme was used at the expense of wheat flour.

Materials and methods

Supplementation of exogenous phytase in MOLM based diet was carried out in Fish Nutrition Laboratory, Department of Zoology, and Government College University Faisalabad; in order to study impact onADC % of mineralsin*O.niloticus*juveniles.

Fish and experimental conditions

Nile tilapia fingerlings were sampled and collected from Government Fish Seed Hatchery, Satiana Road, Faisalabad and kept in triplicate fish rearing tanks for acclimatization to the laboratory environment over 2 weeksperiod. Stocking density was 15 fingerlings in each tank. In order to achieve apparent satiation, fish were fed upon basal diet once daily(Allan and Rowland, 1992).To free fish from external parasites and fungal infection, saline bath (NaCl 5g L^{-1}) was given to the fish. Continuous supply of oxygen was ensured in each tank all through the feeding period.

Feed ingredients and experimental diets

To formulate test diet, all the feed constituents were purchased from University of Agriculture, Faisalabad. Sorting out of *M. oleifera* leaves by

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dipping in tap water for three days was carried out in Fish Nutrition lab at Government College University Faisalabad. Prior to the formulation of the experimentaldiet,standard methods of Association of Official Analytical Chemists (AOAC, 1995) werefollowed to determine chemical composition ofingredients. Indigestible marker used in test diet was Cr_2O_3 (1%). For experimental trial, six isoproteic, isolipidic and isocaloric sub-diets were prepared based on MOLM. Particle size of pellets was standardized up to 0.5mm, keeping in mind the 10-15% moisture level in the feed, floating pellets were processed by operating extruder (Lovell, 1989). Pellets were sprinkled with six graded levels of phytase; as 0, 200, 400, 600, 800 and 1000 FTU kg^{-1} (Table 2).

Table 2: Percentage chemical analysis of feed ingredients (Dry matter basis).

Ingredients	Dry matter (%)	Crude Protein (%)	Crude Fat (%)	Crude Fiber (%)	Ash (%)	Gross Energy (kcal/g)	Carbohydrates (%)
Fish meal	91.53	46.17	6.15	1.13	24.23	4.07	18.25
Wheat flour	92.53	10.54	2.36	2.59	2.81	2.86	78.84
Rice polish	94.78	12.56	12.75	11.54	10.89	4.36	47.81
Canola meal	93.52	37.10	1.35	1.39	8.27	3.15	48.74
MOLM	91.83	28.95	2.83	19.45	8.91	3.84	36.02

Feeding practiceforsample collection

Experimental diet was offered to the *O. niloticus* fingerlings once in morning (8:00 am) and then in afternoon (2:00 pm). After two hours of diet inoculation in the tank, unconsumed diet and feces were collected for chemical analysis and dried in oven at 60^{o}C.To minimize discharge of minerals, feces were handled very carefully. Tanks were refilled with fresh water. Duration of this feeding trial was 2 months. Water quality parameters were maintained in each tank as temperature (20-30°C), dissolved oxygen (8.68 -10.92mg/L) and pH (6.7-7.7).

Chemical analysis for mineral estimation

To assess minerals in diet and feces, 0.5g sample was added in the open mouth conical flask. Prior to its installation on hot plate, 30ml HNO_3 was also added inthe sample. Once the solution started to boil, 10ml perchloric acid was mixed in conical flask and waited until 1ml of the solution left behind. Dilution of the sample was done by addition of 50ml distilled water, after removing from hot plate. Before mineral analysis (AOAC, 1995), solution was filtered with the aid of filter paper to remove any particulate substance. Mineral contents from diluted mixturewere analysed by using Atomic Absorption Spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan) following the conditions described by (AOAC, 1995).Flame photometer was used to analyse Na and K (Jenway PFP-7, UK). To determine phosphorus contents in the sample, UV/VIS spectrophotometer at 720 nm

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absorbance was used (AOAC, 1995). Chromic oxide determination in feed and feces was brought about by oxidation with molybdate reagent (Divakaran*et al.*, 2002) at 370nm absorbance in UV-VIS 2001 spectrophotometer.

Determination of Apparent Mineral Digestibility

In order to calculate ADC% of test diets and feces, formula stated by National Research Council (NRC, 1993) was applied.

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

Data analysis

To analyse observed results, CoStat Computer Package (version 6.303, PMB 320, Monterey, CA, 93940 USA) wasused. One-way Analysis of Variance (ANOVA) was used in order to determine difference among treatments (Steel*et al.*, 1996). Means were compared by Tukey's Honestly Significant Difference test (Snedecor and Cochran, 1991) at $p<0.05$ significance level.

Results

Data illustrated in Table 3 regarding mineral composition of experimental diets and control diet was almost similar. By the supplementation of phytase enzyme in MOLM, phytate complex underwent degradation and resulted in release of essential minerals. All the experimental diets were comparable to one another with reference to mineral composition. The values presented in Table 4 showed the mineral contents present in the feces. Data regarding mineral digestibility (%) in Table 5 represented significant differences ($p<0.05$)between experimental diets and reference diet.Minimal amount ofminerals was excreted through fecesby the fish group treated with test-diet IV and resulted in maximum mineral availability to the fish. Optimum values of minerals such as Mg (65%), Na(61%), K(77%), Cu(63%), Zn(81%), Fe(72%), Cr(83%) and Ca(54%) were noticedby applyingtest-diet IV (600 FTU kg^{-1}) succeeded by test diet-V with 800 FTU kg^{-1}. Negligible differences regarding mineral digestibility were present between control diet and test-diet II with 200 FTU kg^{-1} phytase. Further, mineral rich feces were excreted by the fish group treated with control diet. In case of mineral utilization, our result showed increase in mineral digestion withincreasing phytase dose up-to 600 FTU kg^{-1}. While, after this concentration,phytase did not play any significant role in improving digestibility. It was concluded that 600 FTU kg^{-1}dose of phytase in MOLM based diet was the most suitable among control and other test diets for maximum ADC% of minerals to *O. niloticus* fingerlings.

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Table 3: Analysed composition of minerals (%) in the diet of *O. niloticus* fingerlings fed on phytase supplemented MOLM 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	200	400	600	800	1000
Mg	0.095±0.001	0.094±0.001	0.094±0.001	0.095±0.001	0.096±0.001	0.095±0.001
Na	0.095±0.001	0.094±0.001	0.094±0.001	0.095±0.001	0.096±0.001	0.095±0.001
K	1.33±0.01	1.33±0.01	1.34±0.01	1.33±0.01	1.323±0.01	1.32±0.01
Cu	0.088±0.001	0.088±0.002	0.087±0.001	0.088±0.001	0.088±0.001	0.088±0.001
Zn	0.12±0.001	0.13±0.001	0.12±0.001	0.13±0.001	0.13±0.001	0.13±0.00
Fe	0.07±0.001	0.07±0.001	0.07±0.001	0.07±0.001	0.07±0.001	0.07±0.001
Cr	0.095±0.001	0.094±0.001	0.094±0.001	0.095±0.001	0.096±0.001	0.095±0.001
Ca	0.24±0.01	0.24±0.02	0.24±0.02	0.22±0.01	0.25±0.01	0.27±0.01

Data are means of three replicates.

Table 4: Analysed composition (%) of minerals in the feces of *O. niloticus* fingerlings fed on phytase supplemented MOLM 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	200	400	600	800	1000
Mg	$0.067±0.001^{a}$	$0.053±0.001^{b}$	$0.043±0.001^{c}$	$0.036±0.002^{d}$	$0.057±0.001^{b}$	$0.058±0.001^{b}$
Na	$0.75±0.001^{a}$	$0.66±0.01^{b}$	$0.58±0.01^{c}$	$0.52±0.01^{d}$	$0.57±0.02^{c}$	$0.65±0^{b}$
K	$0.67±0.02^{a}$	$0.57±0.01^{b}$	$0.53±0.01^{bc}$	$0.33±0.01^{d}$	$0.46±0.01^{c}$	$0.48±0.06^{c}$
Cu	$0.048±0.001^{a}$	$0.042±0.001^{b}$	$0.036±0.001^{c}$	$0.035±0.001^{c}$	$0.037±0.001^{c}$	$0.042±0.001^{b}$
Zn	$0.07±0.01^{a}$	$0.05±0.001^{b}$	$0.04±0.001^{b}$	$0.03±0.001^{c}$	$0.03±0.001^{c}$	$0.05±0.001^{b}$
Fe	$0.05±0.001^{a}$	$0.04±0.001^{c}$	$0.04±0.001^{d}$	$0.02±0.001^{e}$	$0.05±0.001^{b}$	$0.05±0.001^{a}$
Cr	$0.06±0.001^{a}$	$0.037±0.001^{d}$	$0.043±0.001^{c}$	$0.017±0.001^{e}$	$0.036±0.001^{d}$	$0.058±0.001^{b}$
Ca	$0.18±0.01^{a}$	$0.15±0.01^{b}$	$0.14±0.01^{b}$	$0.11±0.01^{c}$	$0.15±0.01^{b}$	$0.19±3.39^{a}$

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

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Table 5: Apparent digestibility coefficient (%) of minerals for *O. niloticus* fingerlings fed on phytase supplemented MOLM 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	200	400	600	800	1000
Mg	39.79±2.46^{d}	48.79±3.15^{c}	57.18±1.84^{b}	65.52±1.75^{a}	46.43±1.22^{c}	43.42±0.78^{cd}
Na	47.24±0.86^{c}	51.07±0.78^{c}	56.01±1.85^{b}	61.38±1.07^{a}	57.83±2.35^{ab}	50.96±0.47^{c}
K	54.36±0.063^{d}	61.33±0.096^{c}	63.41±1.22^{bc}	76.88±0.46^{a}	68.62±1.26^{b}	65.87±5.43^{bc}
Cu	40.98±0.568^{c}	57.08±0.72^{b}	61.88±0.48^{a}	63.25±2.80^{a}	62.04±1.90^{a}	55.72±0.21^{b}
Zn	54.61±3.53^{e}	64.26±1.79^{cd}	65.22±0.71^{c}	81.30±0.82^{a}	75.83±1.07^{b}	60.15±1.25^{d}
Fe	32.35±1.72^{d}	44.25±2.64^{c}	53.53±2.07^{b}	71.78±1.22^{a}	49.34±1.40^{b}	43.42±1.52^{c}
Cr	37.01±1.24^{e}	64.07±1.37^{b}	57.18±1.84^{c}	83.07±0.58^{a}	66.17±0.67^{b}	43.42±0.78^{d}
Ca	33.28±3.99^{c}	40.96±1.72^{bc}	45.28±4.79^{ab}	54.21±3.54^{a}	44.66±0.18^{b}	34.62±3.38^{c}

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

Discussion

Being predominant constituent of nucleic acids and plasma membrane, P is regarded as a major component in skeletal muscles of the fish. It plays a key role in ATP production (Jobling, 2012), so P is considered necessary for growth and reproduction of fish (Hardy and Shearer, 1985). A sufficient amount of research is done in aquaculture to enhance P availability in fish by supplementing feed additives. Phytase addition in diet is considered much helpful to fulfil dietary P needs of the fish. In current study, *O. niloticus* fingerlings fed on MOLM based diet supplemented with six graded levels (0, 200, 400, 600, 800, 1000 FTU kg^{-1}) of phytase experienced statistically significant results with regard to minerals digestibility.

Present findings showed that the supplementation of phytase at the level of 600 FTU kg^{-1} is optimum for the minerals (Ca, Na, K, Mg, Zn, Fe, Cu and Cr) digestibility. Similar to our findings, Yan *et al*. (2002) described role of graded levels of phytase in different plant by-productsbased meals such as corn, soybean and wheat middling's on channel catfish (*Ictalurus punctatus*) fingerlings. They found that the fish group fed on diet supplemented with 1000 FTU kg^{-1} have highly mineralized (Ca, P, Mg, Mn) bones as compared to the control diet. Sardar *et al.* (2007) stated maximum digestibility of major minerals at 500 FTU kg^{-1}of phytase level. Almost similar to our results, (Baruah*et al.*, 2007) and (Hussain *et al.*, 2015) reported a significant ($p<0.05$) effect of phytase at 750 FTU kg^{-1} and 1000 FTU kg^{-1}on

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minerals absorption in *L. rohita* fingerlings fed on cotton seed meal-based diet. Because it aided in breakdown of chelated minerals and resulted in maximum absorption and decreased mineral excretion through body. Phytase supplementation also lead to the excretion of less amount of phosphorus mass through feces. From the results, it was clear that phytase supplementation increased the mineral bioavailability by hydrolysing the bonds between phytate and numerous minerals, which increased the mineral digestibility and resultantly deposited in the bones. However, maximum mineral digestibility in *Takifugu rubripes* was achieved by offering soybean meal diet at 2000 FTU kg^{-1}phytase level (Laining*et al.*, 2011). Zhu *et al.* (2014) narrated phytase supplementation brought about dramatic decrease in mineral contents of feces. In addition to it (Hung *et al.*, 2015) claimed the improved ADC% of phosphorus in *Pangasianodon hypophthalmus*, upon feeding phytase at 1500 FTU kg^{-1}level in soybean meal based diet. So, the results of the present work are supported by above studies and variations may be due to fish species, environmental conditions or differences among diet composition.

In contrast to our findings, Nwanna and Bello (2014) reported insignificant effects of phytase on ADC% of minerals in *O. niloticus* fingerlings, when supplemented in plant by-products based meal at very high concentration (8000 FTU kg^{-1}). The reason behind this diverted result is explained by (Cao *et al.*, 2007) as he narrated that phytase supplementation is effective within specific range of 250 to 1500 FTU kg^{-1}. Baruah *et al.* (2007) and Dersjant-Li*et al.* (2015) described that various factors are responsible for dissimilar results such as feed processing techniques, quality and quantity of exogenous phytase and feed drying technology. Overall, this study concluded that deleterious effects of phytic acid are efficiently reduced at 600 FTU kg^{-1} dose of phytase, when supplemented in MOLM based diet. In addition to it, a noticeable (p<0.05) improvement in mineral absorption was found at the same (600 FTU kg^{-1}) phytase level as compared to the control and other experimental diets.

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