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Improvement in Mineral Digestibility and Whole Body Composition of *Catla catla* Fingerlings Fed Phytase Supplemented MOSM Based Diet

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Authors' Contribution
MMS conducted the research and wrote the manuscript. SMH planned and supervised the research. FJ provided the research facilities. MIH helped in chemical analysis of minerals. AJ helped in statistical analysis and interpretation of the results. MA helped in manuscript preparation. MZHA helped in conducting feeding trial and feces collection.

Key words
MOSM, *Catla catla*, Mineral digestibility, Phytase, Carcass composition.

ABSTRACT

Moringa oleifera seed meal (MOSM) was used as test ingredient to formulate six test diets and were supplemented with graded levels (0, 300, 600, 900, 1200 and 1500 FTU kg^{-1}) of phytase. The fingerlings were fed at the rate of 4% of live fish body weight twice a day and faeces were collected from each tank. Present research work was carried out to determine the effect of phytase supplementation on mineral digestibility and carcass composition of *Catla catla* fingerlings fed MOSM based diets. Phytate in plant by-products decreases the bioavailability of minerals and deposition of nutrients in fish body, resulting in poor fish growth. Results demonstrated that phytase supplementation showed significant ($p<0.05$) improvement in ADC% of minerals and carcass composition of fish. Maximum digestibility of minerals and improved carcass composition of *C. catla* fingerlings was noted at 900 FTU kg^{-1} level of phytase supplemented MOSM based test diet. It was further noted that phytase supplementation decreases the discharge of minerals through faeces resulting in reduced aquatic pollution. It was concluded that phytase supplementation at 900 FTU kg^{-1} level was helpful to develop an eco-friendly and cost effective fish feed by using MOSM based diet.

INTRODUCTION

Moringa 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, vitamins and essential amino acids EAA (Makkar and Becker, 1996). These EAA are comparatively less in other plant ingredients such as soybean meal (Ferreira *et al*., 2008). The seed protein contents are higher (33-38%) than those reported for important grain legumes and soybean varieties (Ferreira *et al*., 2008). Analysis of the proximate composition of moringa seeds have also shown the higher levels of crude fat, fiber, protein, carbohydrates and minerals (Abdulkarim *et al*., 2005).

Over population has resulted in increasing demand for food and nutritional quality in under developed countries throughout the world (Abdulkadir *et al*., 2016). Now a day in food producing sectors, aquaculture is one of the rapidly flourishing industries in recent decades because of the need for high quality fish protein to meet human's nutritional requirements (Tacon and Metian, 2013). Nearly 40% of shellfish and fish that is being eaten by human are reared in aquaculture sector (Chabi *et al*., 2015). As fish is the most important source of protein, it also needs a high amount of protein in its own diet. Aquaculture feed industry usually depends on the use of fishmeal (FM) as it is major source of protein and contains important vitamins, minerals and nutrients (Dawood *et al*., 2015). Fish feed mainly accounts for 60% of total cost of fish culture system (Essa *et al*., 2004). Whereas, unstable supply, higher demand and increasing cost of the fish meal made it compulsory to search for unconventional and low cost sources of protein (Rana *et al*., 2009; FAO, 2014). The best alternatives of protein sources are plant by-products because of their low cost and easy availability throughout the year. Use of plant ingredients as protein sources singly or in mixture of two or more plant by-products appears economically more useful (Enterria *et al*., 2011). A number of researchers have

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found positive effects on mineral digestibility and carcass compositions when different fish species were fed on plant by-products based diets (Chu *et al*., 2015; Hussain *et al*., 2015a, b; Liu *et al*., 2015; Muin *et al*., 2015). Plant by-products contain several anti-nutritional factors such as phytate, Tannins, trypsin inhibitors, saponins, oxalates and cyanide contents. Majority of these anti-nutritional factors can be easily removed by heat, soaking or by extraction methods (Liener, 1994), whereas phytic acid or phytate cannot be removed by these methods (Plaipetch and Yakupitiyage, 2014). Phytate is chemically known as myo-inositol hexaphosphate that is chelated form of phosphorus. Availability of minerals especially cation minerals, protein and lipids are adversely affected by higher concentration of phytate or phytic acid in plant by-products or oilseeds meals based diets (Cao *et al*., 2007). It is estimated that 60-80% of total P is present in chelated form that is known as phytic acid in plant by-products based diets (Lei *et al*., 2013). This chelated phosphorous cannot be utilized by mono-gastric and a-gastric fishes, resulting in higher mineral discharge into water media causing aquatic pollution (NRC, 1993). Moreover, phytate prevents absorption of divalent minerals such as Fe, Ca, Mg, Zn, Mn and Cu in fish digestive system. It is also capable of binding with proteins and starch (Jondreville *et al*., 2005; Noureddini and Dang, 2009). Phytate complex only can be broken down by some enzymatic reactions because it is a stable compound (Vielma *et al*., 2000).

Phytase is chemically known as myo-inositol hexa-kis-phosphate phospho-hydrolase, belongs to class III hydrolases. Fermentation of genetically modified microorganisms, phytase can be produced at low cost (Yao and Fan, 2000). Phytase supplementation in plant by-products is being extensively used to get free P from phytic acid complexes (Lim and Lee, 2009). Supplemental dietary phytase is an effective method to improve the availability of minerals and carcass composition of fish. It also decreases water pollution by proper digestion and absorption of P in fish body (Liu *et al*., 2013; Hussain *et al*., 2015a). Use of phytase can efficiently decrease the adverse effects of phytic acid that is present in plant by-products based diets (Hussain *et al*., 2016; 2017). Phytase deficiency may cause three main problems due to less availability of minerals *i.e.* 1) water pollution caused by minerals discharge through fish feces 2) need of extra dietary P supplementation 3) as well as the depletion of rock P deposits (Lei *et al*., 2013). Supplementation of dietary phytase in plant by-products based diet significantly improves the fish carcass composition (Hung *et al*., 2015). It also improves protein absorption and increase digestibility of minerals such as P, N, Mg, Ca, Cu, Zn, and Fe in oilseed meal based diets (Christopher *et al*., 2011; Hussain *et al*., 2016).

C. catla commonly known as Thaila, is being cultured in Pakistan with other carps (Aslam *et al*., 2016). The reported production of *C. catla* has been increased during the first decade of 21st century and in 2012 it was about 2.8 million ton/annum (FAO, 2015). Research is needed to enhance the fish production as well as to overcome the problems of costly fish meal and water pollution caused by excretion of chelated phosphorus through fish feces. Therefore, the purpose of this study was to examine the effects of phytase supplementation on mineral availability and carcass composition of *C. catla* fingerlings fed MOSM based test diets.

MATERIALS AND METHODS

The present research work is performed to investigate the impact of phytase supplementation on mineral digestibility and carcass composition of *C. catla* fingerlings fed MOSM based test diets. The experiment was conducted in Fish Nutrition Laboratory, Department of Zoology, Government College University Faisalabad, Pakistan.

Fish and experimental conditions

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

Experimental design

Moringa oleifera seed meal (MOSM) was used as test ingredient to formulate six test diets and supplemented with graded levels (0, 300, 600, 900, 1200 and 1500 FTU kg^{-1}) of phytase enzyme. One control and five phytase supplemented MOSM based test diets were fed to six fish groups stocked in water tanks. Triplicate tanks were used for each treatment and in each replicate 15 fingerlings were stocked. Duration of the experiment was 90-days. Each MOSM based diet supplemented with phytase was compared with other as well as control diet to determine ADC% of minerals and carcass composition by using completely randomized design (CRD).

Table I.- 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
MOSM	35	35	35	35	35	35
Wheat flour*	17	17	17	17	17	17
Fish meal	15	15	15	15	15	15
Soybean meal	15	15	15	15	15	15
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

MOSM= *M. oleifera* seed meal. *Phytase enzyme was used at the expense of wheat flour. **vitamin premix/kg: Vitamin D_3, 3,000,000 IU; Vitamin A, 15,000,000 IU; Vitamin E, 30000 IU; Vitamin B_1, 3000 mg; Vitamin B_6, 4000 mg; Vitamin B_{12}, 40 mg; Vitamin B_2, 7000 mg; Vitamin C, 15,000 mg; Vitamin K_3, 8000 mg; Folic acid, 1500 mg; Calcium pantothenate, 12,000 mg; Nicotinic acid, 60,000 mg. ***mineral premix/kg: Mn(Manganese), 2000 mg; Ca (Calcium), 155 gm; Zn (Zinc), 3000 mg; Cu (Copper), 600 mg; Co (Cobalt), 40 mg; I (Iodine), 40 mg; P (Phosphorous), 135 g; Fe (Iron), 1000 mg; Mg (Magnesium), 55 gm; Se (Selenium), 3 mg; Na (Sodium), 45 gm.

Table II.- Chemical composition (%) of feed ingredients (dry matter basis).

Ingredients	MOSM	Fish meal	Rice polish	Wheat flour	Corn gluten meal (60%)
Dry matter (%)	93.74	91.67	94.06	92.4	92.34
Crude protein (%)	34.41	48.17	12.38	10.15	59.51
Crude fat (%)	4.28	7.12	13.46	2.3	4.58
Crude fiber (%)	2.03	1.12	12.74	2.67	1.23
Ash (%)	9.63	24.66	10.17	2.06	1.36
Gross energy (kcal/g)	4.17	2.65	3.18	2.95	4.35
Carbohydrates	45.48	16.28	48.07	79.87	28.97

Processing of Moringa seeds and formation of feed pellets

Feed ingredients (Table I) were obtained from local market of Faisalabad. *M. oleifera* seeds were air-dried and de-fatted by press method (Weiss, 1971; Salem and Makkar, 2009). Ingredients were finely grinded to pass through 0.3 mm sieve size. Feed ingredients were analyzed for chemical composition (Table II) by following standard methods of AOAC (2000). Cr_2O_3 was used as inert marker at the rate of 1% in all the test diets. All feed ingredients were thoroughly mixed in a feed mixer for about 5-10 min whereas fish oil was also added during this process. Feed ingredients were blended slowly into the mixer after adding 10-15% of tap water, resulting in suitably textured dough and were processed through pelleting machine to formulate pellets (Lovell, 1989). One control and five phytase supplemented test diets were prepared using MOSM based diet by spraying graded levels (0, 300, 600, 900, 1200 and 1500 FTU kg^{-1}) of phytase. The required concentrations of phytase enzyme were prepared in 25 mL distilled water and sprayed on each test diet (Robinson *et al*., 2002). Control diet (0 FTU kg^{-1} level) was sprayed with a similar amount of water to maintain the equivalent amount of moisture. All diets were dried and stored at 4°C until use.

Feeding protocol and sample collection

C. catla fingerlings were fed at the rate of 4% of live wet fish body weight on their prescribed diet twice daily. After the feeding session of two hours, the uneaten diet was drained out from each tank by opening the valves of the tanks. The tanks were washed completely to remove the feed particles and refilled with tap water. Feces were collected by the opening fecal collecting tube of each replicated tank. Fecal material was collected carefully to avoid the breakage of faeces to minimize the leaching of minerals in water. Faeces were dried in oven at 65°C and stored for further chemical analysis.

Table III.- Analyzed mineral composition (%) of MOSM based 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	0.064±0.0018	0.064±0.0012	0.066±0.00057	0.064±0.0038	0.064±0.0034	0.063±0.0018
Na	0.0074±0.0003	0.0074±0.0005	0.0076±0.0005	0.0073±0.0006	0.0075±0.0005	0.0075±0.0008
K	1.33±0.05	1.34±0.06	1.35±0.04	1.33±0.06	1.34±0.06	1.35±0.07
Fe	0.053±0.001	0.052±0.002	0.055±0.003	0.053±0.002	0.055±0.003	0.054±0.005
Cu	0.0033±0.00003	0.0033±0.0001	0.0033±0.0001	0.0034±0.00003	0.0033±0.00004	0.0033±0.0001
Zn	0.047±0.0005	0.047±0.001	0.047±0.001	0.048±0.0004	0.047±0.001	0.047±0.0005
Mn	0.021±0.001	0.021±0.001	0.021±0.001	0.021±0.001	0.021±0.001	0.021±0.001
P	2.11±0.02	2.1±0.02	2.1±0.04	2.1±0.03	2.12±0.02	2.11±0.03
Mg	0.0088±0.0003	0.0090±0.0002	0.0089±0.0006	0.0090±0.0003	0.0089±0.0004	0.0090±0.0004
Al	0.00053±0.00001	0.00054±0.00001	0.00053±0.00001	0.00053±0.000004	0.00053±0.00001	0.00054±0.00001
Cr	0.032±0.003	0.031±0.004	0.032±0.004	0.032±0.004	0.031±0.01	0.031±0.003
Sr	0.00009±0.000002	0.00009±0.000003	0.0001±0.000004	0.00009±0.000003	0.00009±0.000004	0.00009±0.000005
Pb	0.0028±0.0001	0.0028±0.0001	0.0029±0.0001	0.0029±0.0001	0.0029±0.0001	0.0029±0.0001
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.

Chemical analysis of fish carcass

After 90 days of feeding trial five fingerlings from each tank were selected randomly, sacrificed and dried at room temperature for carcass study. Moisture contents were calculated after oven-drying of homogenised samples at 105°C for 12 h. Micro Kjeldahl Apparatus (InKjel M behr Labor Technik GmbH D-40599 Dusseldorf) was used to determine the crude protein (CP) contents (N × 6.25) whereas Soxhlet system (Soxhlet Extraction Heating Mantels, 250 ml 53868601) was used to analyse the crude fat (EE) by the help of ether extraction method. Crude fiber (CF) contents were calculated as loss on ignition of dried lipid-free residues after digestion with 1.25% H_2SO_4 and 1.25% NaOH whereas ash was determined by ignition at 650°C for 12 h in electric furnace (Naberthern B170) to constant weight. Total carbohydrates (N-free extract) were calculated by using standard formula *i.e.* Total carbohydrates (%) =100- (EE % + CP % + Ash % + CF %). Oxygen bomb calorimeter was used to estimate the gross energy of samples.

Estimation of minerals

For estimation of minerals, 1g of the sample (experimental feed and faeces) was weighed and put into an open mouth conical flask. Nitric acid was added (20ml) in the flask and placed it on hot plate. When the mixture began to boil, 10ml perchloric acid was added and again placed on hot plate and heated it until 1ml of the mixture was left behind. The flask was removed and diluted by adding distilled water until the volume (50ml) became crystal clear. This final solution was filtered by using filter paper to remove all particulate matter in the digestion solution prior to the analysis of minerals (AOAC, 2000). After appropriate dilution, mineral contents were estimated by using Atomic Absorption Spectrophotometer. The estimation of Na and K was done through flame photometer (Jenway PFP-7, UK).

Determination of phosphorus (P)

UV/VIS spectrophotometer at 720 nm absorbance was used to determine P contents in the experimental diets and feces. Ammonium molybdate (2.5g) in 100ml of distilled H_2O was used to prepare the ammonium molybdate reagent. Prepared solution was titrated against pure H_2SO_4 till it became clear. Sodium meta-bisulphite (7.5g) was dissolved in 50ml of distilled H_2O to prepare the amino-nepthol-sulphonic acid reagent and then added 0.125g of amino nepthol in that prepared reagent. The reagent was titrated against Na_2SO_4 (20%) till 0 turbidity level. Standard P solution was prepared by dissolving 0.351g potassium dihydrogen P in 100ml of distilled H_2O. Then the test tubes were marked as standard, test sample

and blank and filled them with samples as marked for P analysis. Test tubes were shaken well and permitted to settle down for five minutes. Blue colour appeared in the solution containing P. At 720 nm, the absorbance was noted on spectrophotometer after setting the zero with blank.

Calculation of minerals ADC

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

ADC (%) = 100 –100 × (% marker in diet × % minerals in faeces / % marker in faeces × % minerals in diet)

Statistical analysis

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

RESULTS

Current results showed that there was a similar amount of minerals in all type of diets (Table III) but significant ($p<0.05$) difference was recorded in mineral contents during analysis of faeces (Table IV). Overall lowest amount of minerals in *C. catla* fingerling faeces was observed when fed at 900 FTU kg^{-1} level followed by 600 FTU kg^{-1} level supplemented diet, significantly ($p<0.05$) different from control and other phytase supplemented test diets. Whereas some of the minerals were noted the minimum in faeces at 600 and others were at 1200 FTU kg^{-1} level supplemented diet. However mineral discharge through faeces was recorded maximum when fish was fed with control diet (0 FTU kg^{-1} level). Results indicated that mineral discharge was decreased with increase in phytase addition and reached to its minimum at 900 FTU kg^{-1} level. It was noted that mineral excretion was increased with higher phytase supplementation upto 1500 FTU kg^{-1} level. Results showed that digestibility of Ca (76%), Na (69%), K (76%), Fe (70%), Cu (70%), Zn (75%), P (73%), Mg (75%) and Pb (54%) were maximum at 900 FTU kg^{-1} level. Whereas, higher Mn (72%) digestibility was observed in fish fed at 600 FTU kg^{-1} level based diet and it was statistically ($p<0.05$) different from control and other test diets. On contrary, minerals such as Al (61%), Cr (69%) as well as Sr (51%) were recorded highest at 1200 FTU kg^{-1} level and were statistically ($p<0.05$) different from control and other test diets. However, other minerals such as Ba, Cd, Co, Mo and Ni were not calculated as they were very

Table IV.- Analyzed mineral composition (%) in faeces of *C. catla* fed phytase supplemented MOSM based 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	0.030±0.001a	0.025±0.0004b	0.020±0.0004c	0.017±0.001d	0.021±0.001c	0.027±0.001b
Na	0.0038±0.001a	0.0035±0.0005ab	0.0028±0.0004cd	0.0025±0.001d	0.003±0.001bc	0.0036±0.001ab
K	0.77±0.04a	0.67±0.02b	0.45±0.03d	0.34±0.02e	0.57±0.03c	0.68±0.03b
Fe	0.032±0.001a	0.028±0.001b	0.024±0.001c	0.018±0.0004d	0.022±0.001c	0.029±0.002ab
Cu	0.0019±0.0001a	0.0017±0.0006b	0.0015±0.00004c	0.0011±0.0001e	0.0014±0.00004d	0.0017±0.0001b
Zn	0.022±0.0001a	0.019±0.0005b	0.014±0.0003d	0.013±0.0003d	0.015±0.001c	0.022±0.001a
Mn	0.011±0.0004a	0.01±0.0004b	0.0063±0.0002d	0.0076±0.0002c	0.01±0.0002b	0.011±0.0003a
P	1.26±0.04a	1.08±0.03b	0.78±0.02d	0.61±0.02e	0.91±0.01c	1.13±0.04b
Mg	0.0061±0.0003a	0.0052±0.0002b	0.0038±0.0003c	0.0025±0.0002d	0.0032±0.0002cd	0.0050±0.0004b
Al	0.00036± 0.00001^{a}	0.00031± 0.000003^{b}	0.00028± 0.00001^{c}	0.00024± 0.000003^{d}	0.00022± 0.00001^{e}	0.00027± 0.000002^{c}
Cr	0.020±0.002^{a}	0.015±0.001^{bc}	0.0094±0.001^{d}	0.011±0.002^{cd}	0.012±0.001^{bcd}	0.015±0.001^{ab}
Sr	0.00007± 0.000002^{a}	0.000067± 0.000002^{ab}	0.000063± 0.000002^{b}	0.000055± 0.000002^{c}	0.000047± 0.000003^{d}	0.000046± 0.000003^{d}
Pb	0.0023±0.0002^{a}	0.0021±0.0001^{ab}	0.0019±0.0001^{bc}	0.0014±0.00003^{d}	0.0014±0.00004^{d}	0.0018±0.00002^{c}

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

Table V.- Apparent mineral digestibility (%) of *C. catla* fingerlings fed MOSM based test and control diets.

Minerals	Test diet –I (control)	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	55.46±0.92^{f}	63.94±0.19^{d}	71.08±0.1^{b}	76.05±0.4^{a}	67.89±0.3^{c}	60.25±0.47^{e}
Na	51.22±0.67^{e}	56.29±0.47^{d}	65.76±0.54^{b}	69.05±0.74^{a}	61.19±0.9^{c}	54.71±0.77^{d}
K	45.62±0.87^{e}	53.08±0.63^{d}	68.94±0.84^{b}	76.50±0.95^{a}	59.02±0.53^{c}	52.47±0.65^{d}
Fe	43.10±0.05^{e}	49.18±0.59^{d}	59.73±0.92^{d}	69.57±0.36^{a}	62.24±0.73^{b}	50.08±0.68^{d}
Cu	46.58±0.45^{e}	50.41±0.28^{d}	56.88±0.61^{c}	70.33±0.93^{a}	59.59±1.25^{b}	51.57±0.54^{d}
Zn	54.90±0.7^{e}	61.68±0.31^{d}	73.23±0.22^{b}	75.51±0.53^{a}	68.64±0.57^{c}	56.23±0.8^{e}
Mn	48.11±0.71^{d}	56.20±0.46^{c}	71.93±0.22^{a}	66.73±0.46^{b}	55.3±0.95^{c}	48.39±0.85^{d}
P	43.76±0.72^{f}	51.81±0.78^{d}	65.65±0.87^{b}	73.28±0.54^{a}	58.67±0.37^{c}	49.70±0.21^{e}
Mg	35.22±0.56^{e}	45.40±0.58^{d}	60.22±0.67^{c}	74.75±0.94^{a}	65.94±0.74^{b}	47.26±0.7^{d}
Al	35.98±1.43^{e}	46.07±0.89^{d}	51.67±0.46^{c}	58.64±0.48^{b}	61.15±0.54^{a}	52.68±0.95^{c}
Cr	39.48±0.97^{d}	55.38±0.67^{c}	66.76±0.95^{b}	67.76±0.94^{ab}	69.37±0.99^{a}	53.92±0.93^{C}
Sr	26.12±1.06^{e}	31.06±0.47^{d}	37.39±0.93^{c}	43.68±0.78^{b}	50.98±0.85^{a}	52.84±0.98^{a}
Pb	23.83±0.99^{e}	29.02±0.98^{d}	37.51±0.63^{c}	54.49±0.79^{a}	52.28±0.82^{a}	40.70±0.96^{b}

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

Table VI.- Proximate composition (%) of *C. catla* carcass fed phytase supplemented MOSM based diet after 90-days feeding trial.

Carcass parameters	Test diet –I (control)	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
Crude fat	10.44±0.42^{d}	11.70±0.39^{c}	12.82±0.29^{b}	13.92±0.13^{a}	11.83±0.27^{c}	11.01±0.25^{cd}
Crude protein	55.47±0.45^{e}	57.67±0.38^{c}	60.59±0.36^{b}	62.35±0.19^{a}	58.40±0.25^{c}	56.44±0.35^{d}
Carbohydrate	17.49±1.16^{a}	16.12±0.68^{a}	13.89±0.67^{bc}	12.53±0.59^{c}	15.75±0.33^{ab}	16.67±0.49^{a}
Ash	8.41±0.35^{a}	7.27±0.44b^{c}	6.61±0.36^{cd}	5.88±0.28^{d}	7.13±0.24^{bc}	7.99±0.13^{ab}
Crude fiber	1.25±0.02^{a}	1.18±0.05^{a}	1.07±0.06^{b}	0.93±0.03^{c}	1.17±0.04^{ab}	1.21±0.02^{a}
Moisture	6.94±0.17^{a}	6.06±0.28^{b}	5.01±0.13^{c}	4.39±0.34^{d}	5.71±0.14^{b}	6.68±0.09^{a}

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

low in diet (Table V). Lowest ADC% of all the minerals observed for fish fed on control diet (0 FTU kg^{-1} level). Whereas ADC% of minerals was decreased at the higher (1500 FTU kg^{-1}) phytase supplementation in MOSM based diet.

Whole body analysis of fish fed phytase supplemented MOSM based diet is shown in Table VI. Least EE (10%) and CP (55%) retention in fish body was observed when fed with test diet-I (0 FTU kg^{-1} level). Whereas on the other hand maximum values of EE (14%) and CP (62%) were observed in fish fed on test diet-IV (900 FTU kg^{-1} level) based diet followed by fish that fed at test diet-III (600 FTU kg^{-1} level) *i.e.*13% and 61%, respectively. These values were significantly ($p<0.05$) different from the fish fed on other phytase supplemented test diets as well as control diet (without phytase supplemented MOSM based diet). From these results it was found that improvement in fat and protein retention for *C. catla* fingerlings was started to improve at 300 FTU kg^{-1} level and reached to its maximum when fingerlings were fed with 900 FTU kg^{-1} supplemented MOSM based test diet, whereas further increase in phytase supplementation (1200 and 1500 FTU kg^{-1} level) could not enhance nutrient retention in fish body. Minimum amount of carbohydrates (13%) and crude fiber (1%) contents were analyzed in fish fed at 900 FTU kg^{-1} level supplemented diet as compared to other test diets and control diet (0 FTU kg^{-1} level). However maximum values of carbohydrate (17%) and crude fiber (1%) were observed

in fish fed at 0 FTU kg^{-1} level based diet (control diet) that was statistically ($p<0.05$) similar with the fish that fed at 300 FTU kg^{-1} level supplemented diet. Similarly minimum moisture (4%) and ash (6%) contents were recorded in fish fed at 900 FTU kg^{-1} level supplemented based diet that was significantly ($p<0.05$) different from the fish which fed phytase supplemented and non-supplemented MOSM based diets (control diet). However maximum moisture (7%) and ash (8%) contents were found in the fish fed at 0 FTUkg^{-1} level supplemented diet (control) that was statistically ($p<0.05$) similar with the fish fed on 1500 FTUkg^{-1} level supplemented MOSM based diets. These findings indicate that a decline in carbohydrates, crude fiber, moisture and ash contents was observed when fish fed on phytase supplemented test diets at 300 FTUkg^{-1} level. Lowest values for these parameters were noted in the fish fed at 900 FTU kg^{-1} level supplemented based diet and started to increase with the increase in phytase supplementation in fish feed up to 1500 FTU kg^{-1} level.

These findings showed that phytase supplementation is compulsory for the higher mineral digestibility and nutrient (CP and EE) retention in fish body resulting in improved fish performance. Improvement in mineral digestibility and carcass composition was started from 300 FTU kg^{-1} level and reached to the maximum at 900 FTU kg^{-1} level after which it gradually decreased after the further increase in phytase supplementation upto 1500 kg^{-1} level. It was found that 900 FTU kg^{-1} level of phytase supplementation is the most suitable level for the higher ADC% of minerals and maximum deposition of protein and lipids in fish body that are important for fish performance.

DISCUSSION

Phytate commonly exists in plant based ingredients that usually binds with divalent cations and is known as a major anti-nutritional factor. Phytate makes minerals unavailable to fish and decrease their digestibility due to the binding between phytate complex and major minerals (Oh *et al*., 2004; Nwanna *et al*., 2007). Breakdown of complex chelated structure of phytate enhances the release and utilization of essential minerals. Researchers indicated that phytate present in plant by-products may chelate with some of the important minerals *i.e.* Fe, Ca, Mn, Cu, Ni, Cr, Na, K, P and Mg (Cao *et al*., 2007; Dersjant-Li *et al*., 2015; Hussain *et al*., 2015a, b). From present study, it was noted that 900 FTU kg^{-1} is the most optimum level of phytase supplementation that can increase mineral digestibility for *C. catla* fingerlings fed MOSM based diet. Whereas, some of the minerals showed maximum digestibility for fingerlings when they were fed with 600 FTU kg^{-1} level and remaining were fed at 1200 FTU kg^{-1} level supplemented diet. It was found that phytase influences the mineral digestibility from 250 to 1500 FTU kg^{-1} levels in different fish species at different environmental conditions (Cao *et al*., 2007). 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. Similar to our findings, Hussain *et al*. (2015b) noted that anti nutritional factors in soybean meal based 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. Almost similar with present results, Van-Weerd *et al*. (1999) concluded that phytase addition at 1000 FTU kg^{-1} level in soybean meal based diet showed maximum ADC% of P in *Clarias gariepinus*. Our results are also supported by the findings of Hussain *et al*. (2015a). They reported significantly ($p<0.05$) higher digestibility values of minerals for *L. rohita* fingerlings fed cottonseed meal based diet supplemented with 750 and 1000 FTU kg^{-1} level. While, Hussain *et al*. (2016) found that 750 FTU kg^{-1} is the optimum level for maximizing ADC% of minerals in *L. rohita* fingerlings. On the other hand, maximum ADC% of P had been claimed in tra cat fish juveniles fed soybean meal based diet supplemented at higher level of phytase e.g. 1500 FTU kg^{-1} (Hung *et al*., 2015). Variations in optimal level of phytase supplementation may be due to difference in plant ingredients used in diet formulation and experimental fish species (Baruah *et al*., 2007a). In contrary to current results, Baruah *et al*. (2007b) found maximum mineral digestibility values in *Labeo rohita* fingerlings fed at 500 FTU kg^{-1} level in plant meal based diets. They concluded that minerals such as Fe, Mg, K, Mn, P and Na showed highest ADC% at 500 FTU kg^{-1} level as compared to control and other phytase supplemented test diets. 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 at the level of 2000 FTU kg^{-1} in soybean meal based diet. In another study Nwanna and Olusola (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). Hussain *et al*. (2016) reported maximum minerals utilization by *L. rohita* fingerlings fed at 400 FTU kg^{-1} level and 4% citric acid

supplemented cottonseed meal based test diet. However, it was noted that this difference in phytase supplementation levels may be dependent on the amount of phytate in plant meal based diet, pH of digestive system, fish species, experimental conditions feed processing methods, feed drying methods, quality and type of phytase and methods used for feed preparation (Baruah *et al*., 2007a; Dersjant-Li *et al*., 2015). According to these studies and current results, higher mineral digestibility and utilization in fingerlings resulted in minimum water pollution and maximum performance of fish after the use of phytase in fish feed.

It was found that phytate is a chelated complex compound that usually binds with important nutrients such as CP, EE and other nutrients making them unavailable to fish, resulting in poor fish carcass composition (Cao *et al*., 2007). Maximum retention of CP (62%) and EE (14%) were recorded in *C. catla* fingerlings fed at 900 FTU kg^{-1} level as compared to control and remaining phytase supplemented test diets. However, lowest values of CP% and EE% in fish were observed at 0 FTU kg^{-1} level (control diet). Similarly, different researchers also found that phytase supplementation improves the protein retention in fish body when fed on plant by-products based diets (Storebakken *et al*., 1998; Khajepour *et al*., 2012). Increasing nutrient bioavailability would positively affect body composition and bone strength when fish fed on phytase supplemented soybean meal based diet (Sardar *et al*., 2007). Very close findings to our results were reported by Cheng *et al*. (2015). They found maximum protein retention in yellow catfish (*Pelteobagrus fulvidraco*), when fed phytase supplemented plant meal based diet supplemented with 1000 FTU kg^{-1}. Whereas, in contradiction, microbial phytase supplementation at 500 FTU kg^{-1} level in the diet of *Pangasius pangasius* increased the apparent protein retention in fish body (Debnath *et al*., 2005). Soybean meal based phytase supplemented diets enhanced nutrient retention in *C. gariepinus* at levels ranging from 750 FTU kg^{-1} and 1000 FTU kg^{-1} levels and the values obtained at these levels were significantly different from the values obtained from the diet without phytase supplementation (Akpoilih *et al*., 2016). In current study 900 FTU kg^{-1} level was found as the best level for increasing nutrient retention in fish body, that was in optimal ranges (250-1500 FTU kg^{-1}) reported by Cao *et al*. (2007), whereas Olusola and Nwanna (2014) found a very high level of phytase dose (8000 FTU kg^{-1} level) for improvement in protein retention in *O. niloticus* fingerlings fed processed soybean meal based diets. Nearly similar results were observed by Yoo and Bai (2014). They found maximum crude fat in *Paralichthys olivaceus* (Olive Flounder) when fed phytase supplemented soybean meal based diet supplemented at 1000 FTU kg^{-1} level. Highest body lipid contents were recorded when tiger puffer fed phytase supplemented plant protein of soybean, corn and wheat meal based diet. This divergence in enzyme doses may be due to ingredient composition, the presence or absence of the stomach, fish species as well as types of phytase (Baruah *et al*., 2007a).

Lowest body crude fiber (1%), ash (6%) and moisture (4%) contents were observed in the *C. catla* fingerlings when they fed at 900 FTU kg^{-1} level supplemented MOSM based diets. Similar to present results, increase in CP contents, resulted in decreased moisture and crude ash values were noted in the red sea bream fed phytase supplemented plant meal based diet (Hossain *et al*., 2007). Moisture contents were also recorded lower in catfish when fed phytase supplemented plant protein based diet as compared to control diet (Hung *et al*., 2015). Similar to our results, Vielma *et al*. (2004) found minimum fish carcass ash and also significantly reduced nutrient contents in fish when fed with phytase deficient diets. Phytase supplemented plant protein based diets of *Ictalurus punctatus* (channel catfish) showed highest ash contents as compared to control diet (Jackson *et al*., 1996). Pham *et al*. (2008) also observed an increase in ash contents of *Paralichthys olivaceus* (Olive Flounder) fed phytase supplemented cottonseed meal and soybean meal based diets. On contrary, Cheng *et al*. (2015) found no difference in moisture contents of whole fish body fed with or without phytase supplemented diets. In contrast, higher contents of moisture were recorded in *C. carpio* when fed at 0 FTU kg^{-1} level supplemented based diet (Sardar *et al*., 2007).

CONCLUSION

Current research work provided sufficient evidences about phytase supplementation that breakdown the phytate complex present in MOSM based diets. From these findings, it was concluded that supplementation of phytase at 900 FTU kg^{-1} level played a major role in improving ADC% of minerals and carcass composition of *C. catla* fingerlings as compared to control diet (without phytase supplementation). Addition of phytase also decreases the cost of fish feed by improving the quality of plant meal based diet as compared to costly fish meal.

Statement of conflict of interest

Authors have declared no conflict of interest.

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