



Use of phytase at graded levels for improving nutrient digestibility, growth and hematology of *Catla catla* fingerlings fed *Moringa oleifera* seed meal (MOSM) based diet

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ABSTRACT

Present research work was conducted to evaluate the effect of phytase supplementation on nutrient digestibility, growth performance and hematological indices of *Catla catla* (Hamilton, 1822) fingerlings fed *Moringa oleifera* seed meal (MOSM) based diet. Existence of phytate in plant byproducts decreases the bioavailability of nutrients to fish, resulting in low nutrients availability, poor fish growth and hematological indices in fish body. *Moringa* byproducts such as *M. oleifera* seed meal (MOSM) was used as test ingredient to formulate experimental diet. Experimental diet was divided into 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 wet weight twice a day in V-shaped indoor tanks. Phytase supplementation showed significant ($p < 0.05$) improvement in bioavailability of nutrients, growth indices and hematological parameters. Results showed that maximum growth performance, digestibility of nutrients and improved hematological values of *C. catla* fingerlings were noted at 900 FTU kg⁻¹ level of phytase supplementation in MOSM based test diet. It was concluded that phytase supplementation at 900 FTU kg⁻¹ level was helpful to develop an eco-friendly and cost effective fish feed incorporating moringa seed meal.

Keywords: *Catla catla*, Growth, Hematology, *Moringa oleifera* seed meal, Nutrient digestibility, Phytase

Introduction

Catla catla (Hamilton, 1822) commonly known as 'thaila', is a surface feeder being cultured widely along with other freshwater fish species in Pakistan (Aslam *et al.*, 2016). Production of this species increased during the first decade of 21st century and in 2012, world production was about 2.8 million t (FAO, 2015). Demand for fish consumption is constantly increasing as a source of food and for health benefits (Abdulkadir *et al.*, 2016; Tihamiyu *et al.*, 2016). Fishmeal is a major source of protein used in formulation of feed for different fish species as it contains important vitamins, attractants, nutrients, fatty acids and amino acids (Dawood *et al.*, 2015). However, unstable supply, higher demand and increasing cost of fish meal led to search for alternative cost effective sources of protein (Hardy, 2010; Lim *et al.*, 2011). The best alternative protein sources are plant byproducts because of their low cost and easy availability throughout the year. A number of researchers have found positive effects on fish growth and

nutrient digestibility when different fish species were fed plant byproducts based diets (Hussain *et al.*, 2015a; Liu *et al.*, 2017). One of the cost effective plant protein sources is *Moringa oleifera* (family Moringaceae) known as miracle tree (Yuangsoi *et al.*, 2014) and locally called 'sohanjana' or 'sanjana' in Pakistan and found common in southern Punjab. *M. oleifera* seed meal (MOSM) is a good source of protein (33 to 38%), important vitamins, essential amino acids *viz.*, methionine, cystine and tryptophan (Makkar and Becker, 1996). *M. oleifera* seed is also virtuous source of protein, fat as well as other nutrients. However, MOSM contains the anti-nutritional factor phytate or phytic acid (Worku, 2016). Presence of phytate in plant based diets can cause adverse effects on nutrient digestibility in fish (Hussain *et al.*, 2015a, b). Moreover, it is capable of binding with essential amino acids in different fish species that decreases bioavailability of nutrients, especially protein (Usmani and Jafri, 2002). Further, growth and hematological parameters are also adversely

affected by higher concentrations of phytate/phytic acid in plant byproducts or oilseed meals based diets (Cao *et al.*, 2007). Chelated phosphorous cannot be utilised by mono-gastric fishes, resulting in increased nutrient discharge into water media causing aquatic pollution (NRC, 1993). Phytate complex can be broken down only by some enzymatic reactions because it is a stable compound (Vielma *et al.*, 2000). Phytase, chemically known as myo-inositol hexa-kisphosphatephospho-hydrolase, belongs to class III hydrolases. Supplemental dietary phytase is an effective method to improve the nutrients availability and feed conversion ratio (FCR) of fish. It also decreases water pollution by proper digestion and absorption of nutrients in fish body (Hussain *et al.*, 2011; Liu *et al.*, 2013; Hussain *et al.*, 2015a). It was noted that growth parameters of Indian major carps was significantly decreased when phytic acid was incorporated in feed (Alvi, 1994). Phytase supplementation improved growth performance and nutrient digestibility of *Labeo rohita* fed on plant byproducts based diets (Baruah *et al.*, 2007a; Hussain *et al.*, 2015b). The purpose of the present study was to examine the effects of phytase supplementation on nutrient digestibility, growth performance and hematological indices of *C. catla* fingerlings fed MOSM based diets.

Materials and methods

The experiment was conducted in the 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

initiation of the experiment, fingerlings were acclimatised to the laboratory conditions for fourteen days in specially designed V-shaped tanks having 70 l water holding capacity. During this period, the fingerlings were 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 for aeration by capillary system throughout the experimental period. Before starting experimental feeding, *C. catla* fingerlings were treated for 1 to 2 min with 0.5% saline solution to remove the pathogens if any present (Rowland and Ingram, 1991).

Experimental design

M. oleifera seed meal (MOSM) was used as the major protein source in the basal diet. Six test diets were prepared by supplementing the basal diet with graded levels (0, 300, 600, 900, 1200 and 1500 FTU kg⁻¹) of phytase enzyme. The diets were fed to six groups of fishes, with each group having triplicate tanks, each stocked with 15 fingerlings per tank (8.06±0.042 g fish⁻¹). Duration of the experiment was 90 days.

Processing of *M. oleifera* seeds and preparation of experimental diets

M. oleifera seeds were obtained from local market in Faisalabad. Seeds were air dried and defatted by press method (Weiss, 1971; Salem and Makkar, 2009). Processed moringa seeds were ground to powder.

The feed ingredients procured from local commercial market, finely ground and passed through 0.3 mm sieve size (Table 1). Prior to the formulation of the experimental diets, the feed ingredients were analysed for proximate composition (Table 2) following standard methods

Table 1. 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
MOSM	35	35	35	35	35	35
Fish meal	15	15	15	15	15	15
Soybean meal	15	15	15	15	15	15
Rice polish	8	8	8	8	8	8
Wheat flour*	17	17	17	17	17	17
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
Phytase level (FTU kg ⁻¹)	0	300	600	900	1200	1500

MOSM = *M. oleifera* seed meal

* Phytase enzyme was used at the expense of wheat flour

** Vitamin premix per kg: Vitamin D₃: 3,000,000 IU, Vitamin A: 15,000,000 IU, Vitamin E: 30000 IU, Vitamin B₁: 3000 mg, Vitamin B₆: 4000 mg, Vitamin B₁₂: 40 mg, Vitamin B₂: 7000 mg, Vitamin C: 15,000 mg, Vitamin K₃: 8000 mg, Folic acid: 1500 mg, Calcium pantothenate: 12,000 mg, Nicotinic acid: 60,000 mg.

*** Mineral premix per kg: Mn (Manganese): 2000 mg, Ca (Calcium): 155 g, 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 g, Se (Selenium): 3 mg, Na (Sodium): 45 g.

Table 2. Proximate composition (%) of feed ingredients (Dry matter basis)

Parameter	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 ⁻¹)	4.17	2.65	3.18	2.95	4.35
Carbohydrates (NFE)	45.48	16.28	48.07	79.87	28.97

(AOAC, 1995). Cr₂O₃ 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. Feed ingredients were blended slowly into the mixer after adding 10-15% of tap water to form suitably textured dough which was pelleted using a pelleting machine (Lovell, 1989). Five phytase supplemented test diets were prepared by spraying graded levels (300, 600, 900, 1200 and 1500 FTU kg⁻¹) of phytase. The required concentrations of phytase enzyme (Phyzyme® XP 10000 FTU g⁻¹; Danisco Animal Nutrition, Fin-65101 Vaasa, Finland) were prepared in 25 ml distilled water and sprayed on 1 kg of each test diet (Robinson *et al.*, 2002). Control diet (0 FTU kg⁻¹ level) was sprayed with an equal amount of distilled water to maintain equivalent level of moisture. All the diets prepared 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 body weight on respective diet, twice daily. After the feeding session of 2 h, 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. Fecal material was collected carefully to avoid the breakage of faeces for minimising the leaching of nutrients in water. Faeces were dried in oven at 65°C and stored for further chemical analysis.

Chemical analysis of feed and faeces

Moisture content of test diets and faeces 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) content (N × 6.25). Soxhlet system (Soxhlet Extraction Heating Mantels, 250 ml 53868601) was used to analyse the crude fat (EE). Crude fiber content was estimated by digestion with 1.25% H₂SO₄ and 1.25% NaOH. The difference in weight after calcination indicates the quantity of fibre present. 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 using standard formula:

$$\text{Total carbohydrates (\%)} = 100 - (\text{EE \%} + \text{CP \%} + \text{Ash \%} + \text{CF \%}).$$

Oxygen bomb calorimeter was used to estimate the gross energy (GE) of samples. Chromic oxide contents in the diets and faeces were estimated after oxidation with molybdate reagent using a UV-VIS 2001 Spectrophotometer at 370 nm (Divakaran *et al.*, 2002).

Apparent nutrient digestibility coefficient (ADC) of test diets was calculated using the standard formula (NRC, 1993):

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

Evaluation of growth

The fish were bulk weighed in each tank at start and at end of the study period to evaluate the growth performance of *C. catla* fingerlings. Growth parameters such as weight gain (g), FCR, specific growth rate (SGR) and weight gain % of fingerlings were calculated using standard formulae (NRC, 1993):

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

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

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

Hematological study

Blood samples were collected from the caudal vein using heparinised syringe and were analysed for hematological indices at Molcare Lab, Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan. Micro-hematocrit technique was used to determine hematocrit/packed cell volume (PCV) value as per Brown (1980). Red blood cells (RBC) and white blood cells (WBC) counts were analysed using a Neubauer counting chamber (Blaxhall and Daisley, 1973).

Hemoglobin (Hb) concentration was determined as described by Wedemeyer and Yastuke (1977). Mean corpuscular hemoglobin concentration (MCHC); mean corpuscular hemoglobin (MCH) and mean cell volume (MCV) were calculated using the following formulae:

$$\text{MCHC} = \text{Hb}/\text{PCV} \times 100$$

$$\text{MCV} = \text{PCV}/\text{RBC} \times 10$$

$$\text{MCH} = \text{Hb}/\text{RBC} \times 10$$

Statistical analysis

Data of ADC of nutrients (CP, EE and GE), growth parameters as well as haematological indices were subjected to one way analysis of variance (Steel *et al.*, 1996). The differences among treatments were compared by Tukey's Honest 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 analyses.

Results

Nutrient levels were approximately similar in control as well as all the phytase supplemented MOSM based test diets (Table 3).

Nutrients in faeces were significantly ($p < 0.05$) different between fish fed on control and phytase supplemented MOSM based test diets (Table 4). It was found that lower amount of nutrients was excreted through faeces of fingerlings fed on phytase supplemented MOSM based diets as compared to fishes fed on control diet. Lowest amount of nutrients was discharged through

faeces when fingerlings were fed on diet supplemented with phytase at 900 FTU kg^{-1} followed by 600 FTU kg^{-1} level.

It was found that 900 FTU kg^{-1} level showed maximum digestibility values of crude fat *i.e.* EE (80%), crude protein *i.e.* CP (72%) and gross energy *i.e.* GE (74%). These values were significantly ($p < 0.05$) different from control diet as well as remaining phytase supplemented test diets. Lowest ADC of nutrients (EE 49, CP 52 and GE 53%) was observed when fish were fed on control diet (0 FTU kg^{-1} level) as compared to phytase supplemented MOSM based diets (Table 5). From these results it was also noted that increasing ADC of nutrients resulted in decreased nutrient discharge through faeces in water.

Results of fish growth parameters *viz.*, weight gain, FCR and SGR are shown in Table 6. Phytase supplementation was found to significantly ($p < 0.05$) improve growth performance of *C. catla* fingerlings fed MOSM based diets as compared to control diet. Growth parameters of *C. catla* fingerlings was found to increase from 300 FTU kg^{-1} level and reached maximum at 900 FTU kg^{-1} level of phytase supplementation.

Maximum weight gain (20 g) and SGR (1.37) were observed in fingerlings fed phytase supplemented diet at 900 FTU kg^{-1} level followed by fish fed on test diet III supplemented with 600 FTU kg^{-1} level (weight gain 17 g and SGR 1.27). These values were significantly ($p < 0.05$) higher as compared to weight gain (13 g) and SGR (1.04%) of fish fed on control diet (without phytase).

Table 3. Apparent crude protein (CP), crude fat (EE) and gross energy (GE) levels in the experimental diets

Experimental diets	Phytase levels (FTU kg^{-1})	CP (%)	EE (%)	GE (kcal g^{-1})
Test diet I (Control diet)	0	32.99±0.17	8.05±0.09	3.56±0.12
Test diet II	300	32.89±0.17	8.08±0.05	3.61±0.11
Test diet III	600	33.20±0.36	8.03±0.06	3.61±0.07
Test diet IV	900	33.23±0.58	8.03±0.09	3.59±0.09
Test diet V	1200	32.84±0.53	8.01±0.11	3.59±0.13
Test diet VI	1500	33.59±0.35	8.02±0.05	3.57±0.06

Values are Mean±SD

Table 4. Composition (%) of apparent CP, EE and GE in faeces of *C. catla* fingerlings fed on experimental diets

Experimental diets	Phytase levels (FTU kg^{-1})	CP (%)	EE (%)	GE (kcal g^{-1})
Test diet I (Control diet)	0	16.92±0.17 ^c	4.31±0.09 ^c	1.80±0.04 ^c
Test diet II	300	14.06±0.10 ^c	3.75±0.05 ^d	1.61±0.07 ^{cd}
Test diet III	600	12.10±0.36 ^b	2.44±0.12 ^b	1.37±0.05 ^b
Test diet IV	900	9.97±0.06 ^a	1.69±0.12 ^a	0.99±0.05 ^a
Test diet V	1200	14.92±0.18 ^d	3.16±0.16 ^c	1.54±0.07 ^c
Test diet VI	1500	14.24±0.27 ^c	3.86±0.07 ^d	1.6±0.08 ^{de}

Values are Mean±SD

Means within same columns having different superscripts are significantly different ($p < 0.05$)

Minimum FCR (1.15) of fingerlings was found at 900 FTU kg⁻¹ followed by 600 FTU kg⁻¹ (1.26). It was noted that FCR values at these levels were significantly ($p < 0.05$) different from that of control diet and remaining levels of phytase supplemented diets (Table 7). Highest (1.60) FCR was observed in fish fed on control diet (0 FTU kg⁻¹ level).

Phytase supplementation played a significant ($p < 0.05$) role in improving hematological indices of

C. catla fingerlings as compared to the fish fed on control diet (0 FTU kg⁻¹ level). Maximum values of WBCs (7.91×10^3 mm⁻³), RBCs (3.02×10^6 mm⁻³) and Hb (9.03 g 100 ml⁻¹) were noted in fish fed MOSM based diet supplemented with 900 FTU kg⁻¹ level of phytase and were statistically ($p < 0.05$) similar with the fish fed at 600 FTU kg⁻¹ level. On the other hand, minimum values of WBCs (7.11×10^3 mm⁻³), RBCs

Table 5. Apparent nutrient digestibility (%) of MOSM based diets fed to *C. catla* fingerlings

Experimental diets	Phytase levels (FTU kg ⁻¹)	CP (%)	EE (%)	GE (%)
Test diet I (Control diet)	0	51.62±0.29 ^e	49.47±0.91 ^e	52.62±0.61 ^d
Test diet II	300	60.002±0.32 ^e	56.54±0.71 ^d	58.46±0.87 ^e
Test diet III	600	66.01±0.48 ^b	71.63±0.99 ^b	64.71±0.99 ^b
Test diet IV	900	72.23±0.58 ^a	80.45±0.80 ^a	74.44±0.81 ^a
Test diet V	1200	56.31±0.47 ^d	62.61±0.77 ^c	59.32±0.78 ^e
Test diet VI	1500	60.13±0.17 ^c	54.86±0.83 ^d	53.68±0.89 ^d

Values are Mean±SD

Means within same columns having different superscripts are significantly different ($p < 0.05$)

Table 6. Growth performance of *C. catla* fingerlings fed graded levels of phytase supplemented MOSM 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)	8.04±0.015	8.04±0.015	8.037±0.02	8.07±0.04	8.06±0.02	8.15±0.03
FW (g)	20.56±0.09 ^e	22.02±0.11 ^d	25.24±0.16 ^b	27.81±0.18 ^a	23.49±0.22 ^c	22.58±0.18 ^d
WG (g)	12.52±0.08 ^e	13.98±0.01 ^d	17.20±0.17 ^b	19.74±0.14 ^a	15.43±0.21 ^c	14.43±0.2 ^d
WG (%)	155.66±0.91 ^e	173.77±1.29 ^d	214.06±2.22 ^b	244.57±1.37 ^a	191.32±2.60 ^c	171.17±2.32 ^d
WG (fish ⁻¹ day ⁻¹) (g)	0.14±0.001 ^e	0.16±0.001 ^d	0.19±0.002 ^b	0.22±0.002 ^a	0.17±0.002 ^c	0.16±0.002 ^d
FI	0.22±0.002 ^d	0.23±0.002 ^c	0.24±0.003 ^b	0.25±0.002 ^a	0.23±0.002 ^c	0.23±0.002 ^{cd}
FCR	1.60±0.005 ^f	1.49±0.004 ^e	1.26±0.004 ^b	1.15±0.002 ^a	1.36±0.01 ^c	1.42±0.02 ^d
SGR	1.04±0.004 ^e	1.12±0.01 ^d	1.27±0.01 ^b	1.37±0.004 ^a	1.19±0.01 ^c	1.13±0.01 ^d

Values are Mean±SD (n=3)

Means within same columns having different superscripts are significantly different ($p < 0.05$)

IW= Initial weight, FW = Final weight, WG = Weight gain, FI = Feed intake, SGR = Specific growth rate, FCR = Feed conversion ratio

Table 7. Hematological parameters of *C. catla* fingerlings fed MOSM based control and phytase supplemented test diets

Hematological indices	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
WBC (10 ³ mm ⁻³)	7.11±0.08 ^e	7.30±0.07 ^b	7.77±0.06 ^a	7.91±0.08 ^a	7.49±0.06 ^b	7.34±0.06 ^b
RBC (10 ⁶ mm ⁻³)	1.47±0.07 ^d	1.80±0.06 ^e	2.83±0.05 ^a	3.02±0.05 ^a	2.33±0.09 ^b	1.82±0.14 ^c
Hb (g 100 ml ⁻¹)	7.11±0.09 ^e	7.43±0.10 ^d	8.66±0.06 ^b	9.03±0.12 ^a	8.00±0.21 ^c	7.51±0.09 ^d
PCV (%)	21.23±0.27 ^e	22.02±0.16 ^d	23.83±0.09 ^c	26.02±0.18 ^b	29.04±0.36 ^a	24.07±0.17 ^c
PLT	55.89±0.54 ^d	61.39±0.42 ^c	62.32±0.22 ^c	65.79±0.53 ^b	67.89±0.39 ^a	68.76±0.24 ^a
MCV (fl)	104.32±0.96 ^c	91.19±0.27 ^f	182.67±0.26 ^b	158.58±0.35 ^c	127.63±0.28 ^d	201.43±0.29 ^a
MCH (pg)	28.90±0.25 ^e	25.60±0.32 ^f	39.65±0.28 ^c	46.85±0.36 ^b	35.65±0.09 ^d	59.90±0.21 ^a
MCHC (%)	25.58±0.53 ^e	27.74±0.24 ^d	33.84±0.12 ^c	35.25±0.30 ^b	28.53±0.31 ^d	37.31±0.29 ^a

Values are Mean±SD (n=3)

Means within rows having different superscripts are significantly different ($p < 0.05$)

WBC = White blood cells, RBC = Red blood cells, PCV = Packed cell volume, Hb = hemoglobin concentration, PLT = Platelet, MCV = Mean corpuscular volume, fl = Femtoliters, MCH = Mean corpuscular hemoglobin, pg = Pictograms, MCHC = Mean corpuscular hemoglobin concentration

($1.47 \times 10^6 \text{ mm}^{-3}$) and Hb (7.11 g 100 ml⁻¹) were observed in fish fed on control diet without phytase supplementation. The highest PCV (29.04%) and platelet (PLT, 63.95) values were noted in fish fed at 1200 FTU kg⁻¹ diet followed by values of 26.02 and 65.79%, respectively in fish fed on 900 FTU kg⁻¹ level diet. MCH (59.90 pg) and MCHC (37.31%) were highest in fish fed on diets supplemented with 1500 FTU kg⁻¹ of phytase.

Discussion

According to the results of the current study, it was found that addition of phytase in MOSM based diets was useful for improvement in apparent digestibility coefficient (ADC) of nutrients in *C. catla* fingerlings. Highest values for ADC% of CP (72%), EE (80%) and GE (74%) were found when fish were fed diet supplemented with phytase at 900 FTU kg⁻¹ level. Similarly, Ashraf and Goda (2007) observed that optimal level of phytase enzyme for highest CP digestibility was 1000 FTU kg⁻¹. They also found that ADC% of CP could not be further improved on higher supplementation levels of this enzyme. Nearly similar to our results, Hussain *et al.* (2015a) found higher CP digestibility in *C. mrigala* fingerlings when fed soybean meal based test diet supplemented with phytase at 1000 FTU kg⁻¹ level. Maximum CP digestibility was observed in *L. rohita* fingerlings fed phytase supplemented soybean meal based diet (Baruah *et al.*, 2007a), cottonseed and sunflower meal based diets (Hussain *et al.*, 2011; 2015b) at 750 FTU kg⁻¹ level. In contrast, non-significant ($p < 0.05$) values of protein digestibility were reported after phytase supplementation in plant meal based diets (Cheng and Hardy, 2002; Yan and Reigh, 2002; Dalsgaard *et al.*, 2009). In another study, decreased CP digestibility was found in *Psetta maxima* (turbot) fed phytase supplemented rapeseed protein concentrate as compared to control diet (Danwitz *et al.*, 2016). Similar to present findings, Hussain *et al.* (2014) found maximum EE and GE digestibility at 1000 FTU kg⁻¹ level when *C. mrigala* fingerlings were fed soybean meal based diet. On the other hand, 750 FTU kg⁻¹ level was suggested as the most suitable level of supplementation for higher fat digestibility in *L. rohita* fingerlings (Baruah *et al.*, 2007b). Higher EE digestibility was found in Nile tilapia when fed on 1000 and 2000 FTU kg⁻¹ of phytase supplemented diets (Portz and Liebert, 2004). Hydrolysis of chelated phytic acid resulted in lower EE excretion in faeces that ultimately showed higher utilisation of EE by fish fingerlings resulting in higher digestibility (Hussain *et al.*, 2014; 2015a,b). In contrary, Dalsgaard *et al.* (2009) observed non-significant ($p < 0.05$) results of fat digestibility in *Oncorhynchus mykiss* fed phytase supplemented soybean meal based diets. However, decreased EE digestibility was found in *O. mykiss* fed phytase supplemented soybean

meal based diet. The probable reason for this reduction in fat digestibility might be that the phytase supplementation inhibited lipase activity which led to decreased lipase hydrolysis efficiency for lipids, resulting in reduced lipid digestibility (Wang *et al.*, 2009).

Many researchers observed increased GE digestibility values for *L. rohita* (Hussain *et al.*, 2015a; 2016), *C. mrigala* (Hussain *et al.*, 2014), *O. mykiss* (Forster *et al.*, 1999) and Nile tilapia (Ashraf and Goda, 2007) when fish were fed with phytase supplemented plant protein based diet. Hussain *et al.* (2011) observed maximum GE digestibility in *L. rohita* fingerlings when fish were fed phytase supplemented canola meal based diet at 750 FTU kg⁻¹ level. According to another similar study, highest ADC of GE was recorded at 1000 FTU kg⁻¹ level which decreased on further increase (1500 FTU kg⁻¹) in phytase supplementation (Ashraf and Goda, 2007). However, Lanari *et al.* (1998) noted that there was no optimistic response in rainbow trout fed phytase supplemented soybean meal based diets. They argued that it was due to various nutritional factors, source or type of phytase enzyme as well as source and amount of phytic acid (Selle *et al.*, 2000).

Presence of phytate in feed reduces fish growth performance in terms of weight gain, FCR and SGR (Spinelli *et al.*, 1983). Maximum growth was observed in fish fed on 900 FTU kg⁻¹ level of phytase in MOSM based diet. Nearly similar results were found when common carp fingerlings were fed with 800 FTU kg⁻¹ supplementation in plant-meal based diet (Bai *et al.*, 2003). Nwanna *et al.* (2007) also found a significant ($p < 0.05$) increase in overall WG and WG% in *C. carpio* when fed with diets supplemented with phytase at 750 and 1000 FTU kg⁻¹ levels. In another study, positive results were found by Hussain *et al.* (2015b), but on a slightly different (750 FTU kg⁻¹) level of phytase supplementation. In contrast, non-significant ($p < 0.05$) effect of phytase supplementation was reported in case of *O. mykiss* when fingerlings were fed phytase supplemented plant meal based diets (Vielma *et al.*, 2000). Similarly, in contradiction to the present findings, many other researchers did not observe any significant ($p < 0.05$) effect of phytase supplementation on fish growth performance, when these fish species were fed with or without phytase supplemented plant meal based diets (Robinson *et al.*, 2002, Baruah *et al.*, 2007a, Lim and Lee, 2009, Wang *et al.*, 2009). This disparity in results for growth indices may be linked with many factors such as types of feed ingredients used, varying levels of phytase, processing methods of feed, fish species, stomach pH and methods used for feed drying (Wang *et al.*, 2009).

Current study showed lowest FCR value (1.15) and maximum SGR (1.37) in *C. catla* fingerlings fed on 900 FTU kg⁻¹ phytase supplemented diet. Similar to our results, Riche and Garling (2004); Ashraf and Goda (2007) and Cao *et al.* (2008) observed maximum SGR and lowest FCR values when *O. niloticus* were fed plant meal based diets supplemented with phytase at 1000 FTU kg⁻¹ level. On the other hand, maximum SGR in *O. mykiss* was observed when they were fed on phytase supplemented plant based diets at 500 FTU kg⁻¹ level (Forster *et al.*, 1999). In contrast to current findings, higher FCR values for stripped seabass *Morone saxatilis* were observed when fed with a higher dose (1300 FTU kg⁻¹) of phytase in plant meal based diet (Hughes and Soares, 1998). Statistically non-significant ($p > 0.05$) difference was observed in terms of growth performance when parrot fish were fed on different plant meal based diets supplemented with phytase at different levels (Lim and Lee, 2009). Activity of phytase supplementation depends on the quantity of phytate present in plant meal based diets, environmental conditions, experimental fish species and source of phytase enzyme (Kumar *et al.*, 2011).

Toxic constituents present in fish feed can affect the blood formation. Hematological parameters provide important information in monitoring stress response of fish and in the improvement of fish health (Hrubec *et al.*, 2000). As phytate chelates with iron, that is essential for formation of RBCs, it can lead to lowered oxygen carrying capacity of blood (Spinelli *et al.*, 1983). In the present study, maximum values of WBCs, RBCs and Hb were found at 900 FTU kg⁻¹ level phytase supplemented MOSM based diet. Phytase supplementation is suggested as a powerful stimulator of immune system in fish, resulting in higher number of monocytes (macrophages) and higher blood cell production in monogastric animals (Ehsani and Torki, 2010). In contrary to present findings, Baruah *et al.* (2009) found non-significant ($p > 0.05$) effects on RBCs, WBCs hematocrit and hemoglobin in *L. rohita* fingerlings fed phytase supplemented (500 FTU kg⁻¹ level) soybean meal based diet. They argued that fish were fed a diet containing both phytase and citric acid, which would have led to release of higher amount of Cu and Fe from chelated phytate which resulted in improving hematological parameters of fish. Similarly, the values of RBCs, WBCs and Hb were found higher in *Cyprinus carpio* fed diets supplemented at 500 FTU kg⁻¹ level of phytase in soya protein based diet (Sardar *et al.*, 2007). Encouraging results were found when phytase was used in *Gadus morhua* (Atlantic cod) feed which resulted in higher number of WBCs as compared to fish fed control diet (Lazado *et al.*, 2010). Hemoglobin (Hb) level in

fish fed phytase supplemented diet was slightly higher than that of fish fed a diet without phytase, but was not significantly ($p < 0.05$) different from that of other dietary treatments (Yoo and Bai, 2014).

Supplementation of phytase played a major role in improving ADC of nutrients, growth performance and hematological indices of *C. catla* fingerlings as compared to control diet in the present study. Furthermore, it was also found that 900 FTU kg⁻¹ is the optimum level of phytase supplementation in MOSM based diet for the maximum improvement in fish performance.

References

- Abdulkadir, A. R., Zawawi, D. D. and Jahan, M. S. 2016. Proximate and phytochemical screening of different parts of *Moringa oleifera*. *Russian Agri. Sci.*, 42: 34-36.
- Allan, G. L. and Rowland, S. J. 1992. Development of an experimental diet for silver perch (*Bidynus bidyanus*). *Austasia Aqua.*, 6: 39-40.
- Alvi, A. S. 1994. *Adventitious toxins in plant origin feedstuffs: Quantification and tolerance level in fish*. Masters dissertation, Aligarh Muslim University, Aligarh, India.
- AOAC 1995. *Official methods of analysis, 15th edn*. Association of Official Analytical Chemists, Washington, D.C., USA., p. 1094.
- Ashraf, M. and Goda, A. S. 2007. Effect of dietary soybean meal and phytase levels on growth, feed utilisation and phosphorus discharge for Nile tilapia (*Oreochromis niloticus* L.). *J. Fish. Aquatic Sci.*, 2: 248-263.
- Aslam, S., Abbas, S., Kalhoro, M. A. and Shoaib, A. 2016. Anchor worms (lernaecid parasites), *Lernaea polymorpha* Yu and *Lernaea cyprinacea* (copepode: lernaecidae) on major carps at different fish farms in Punjab, Pakistan. *Sci. Int.*, 28: 295-298.
- Bai, D. Q., Qiao, X. T., Wei, D., Guo, L. and Qi, H. L. 2003. Effects of phytase on utilisation ratio of nutrient composition (calcium, phosphorus) of Carp (*Cyprinus carpio* L.). *J. Tianjin Agric. Coll.*, 10: 6-11 [in Chinese].
- Baruah, K., Pal, A. K., Sahu, N. P., Debnath, D., Yengkokpam, S., Norouzitallab, P. and Sorgeloos, P. 2009. Dietary crude protein, citric acid and microbial phytase interacts to influence the hemato-immunological parameters of rohu, *Labeo rohita* juveniles. *J. World Aquac. Soc.*, 40: 824-831.
- Baruah, K., Pal, A. K., Narottam, P. S. and Debnath, D. 2007a. Microbial phytase supplementation in rohu, *Labeo rohita*, diets enhances growth performance and nutrient digestibility. *J. World Aquac. Soc.*, 38: 129-137.
- Baruah, K., Sahu, P. N., Pal, A. K., Jain, K. K., Debnath, D. and Mukherjee, C. S. 2007b. Dietary microbial phytase and

- citric acid synergistically enhances nutrient digestibility and growth performance of *Labeo rohita* (Hamilton) juveniles at sub-optimal protein level. *Aquac. Res.*, 38: 109-120.
- Blaxhall, P. C. and Daisley, K. W. 1973. Routine haematological methods for use with fish blood. *J. Fish Biol.*, 6: 771-781.
- Brown, B. A. 1980. Routine hematology procedures. *Hematology: Principles and procedures*, p. 71-112.
- Cao, L., Wang, W., Yang, C., Yang, Y., Diana, J., Yakupitiyage, A., Luo, Z. and Li, D. 2007. Application of microbial phytase in fish feed. *J. Enzyme Microb. Tech.*, 40: 497-507.
- Cao, L., Yang, Y., Wang, W. M., Yakupitiyage, A., Yuan, D. R. and Diana, J. S. 2008. Effect of pre-treatment with microbial phytase on phosphorus utilisation and growth performance of Nile tilapia (*Oreochromis niloticus*). *Aquac. Nutr.*, 14: 99-109.
- Cheng, Z. J. and Hardy, R. W. 2002. Effect of microbial phytase on apparent nutrient digestibility of barley, canola meal, wheat and wheat middlings, measured *in vivo* using rainbow trout (*Oncorhynchus mykiss*). *Aquac. Nutr.*, 8: 271-277.
- Dalsgaard, J., Ekmann, K. S., Pedersen, P. B. and Verlhac, V. 2009. Effect of supplemented fungal phytase on performance and phosphorus availability by phosphorus depleted juvenile rainbow trout (*Oncorhynchus mykiss*) and on the magnitude and composition of phosphorus waste output. *Aquaculture*, 286: 105-112.
- Danwitz, A. V., Bussel, C. G. J. V., Klatt, S. F. and Schulz, C. 2016. Dietary phytase supplementation in rapeseed protein based diets influences growth performance, digestibility and nutrient utilisation in turbot (*Psetta maxima*). *Aquaculture*, 450: 405-411.
- Dawood, M. A. O., Koshio, S., Ishikawa, M. and Yokoyama, S. 2015. Effects of partial substitution of fish meal by soybean meal with or without heat-killed *Lactobacillus plantarum* (LP20) on growth performance, digestibility and immune response of Amberjack, *Seriola dumerili* juveniles. *BioMed Res. Inter.*, Article ID 514196. <http://dx.doi.org/10.1155/2015/514196>
- Divakaran, S., Leonard, G. O. and Lan, P. F. 2002. Note on the methods for determination of chromic oxide in shrimp feeds. *J. Agric. Food Chem.*, 50: 464-467.
- Ehsani, M. and Toriki, M. 2010. Effects of dietary inclusion of guar meal supplemented by β -mannanase on performance of laying hens, egg quality characteristics and diacritical counts of white blood cells. *American J. Anim. Vet. Sci.*, 5: 237-243.
- FAO 2015 *Catla catla* (Hamilton, 1822) *Cultured Aquatic Species Information Programme*, Fisheries and Aquaculture Department, Food and Agriculture Organisation, Rome.
- Forster, I., Higgs, D. A., Dosanjh, B. S. and Rowshandeli, M. 1999. Potential for dietary phytase to improve the nutritive value of canola protein concentrate and decrease phosphorus output in rainbow trout (*Oncorhynchus mykiss*) held in 11° C freshwater. *Aquaculture*, 179: 109-125.
- Hardy, R. W. 2010. Utilisation of plant proteins in fish diets: effects of global demand and supplies of fishmeal. *J. Aquac. Res.*, 41: 770-776.
- Hrubec, T. C., Cardinale, J. L. and Smith, S. A. 2000. Haematology and plasma chemistry reference intervals for cultured tilapia (*O. niloticus*). *Vet. Clin. Path.*, 29: 7-12.
- Hughes, K. P. and Soares, J. H. 1998. Efficacy of phytase on phosphorus utilisation in practical diets fed to striped bass, *Morone saxatilis*. *Aquac. Nutr.*, 4: 133-140.
- Hussain, S. M., Afzal, M., Javid, A., Hussain, A. I., Ali, Q., Mustafa, I., Chatha S. A. S., Shah S. Z. H., Hussain, M. and Ullah, M. I. 2015b. Efficacy of phytase supplementation on growth performance and mineral digestibility of *Labeo rohita* fingerlings fed on cottonseed meal based diet. *Pakistan J. Zool.*, 47: 699-709.
- Hussain, S. M., Afzal, M., Nasir, S., Javid, A., Azmat, H., Makhdoom, S. M. and Iqbal, M. 2016. Role of phytase supplementation in improving nutrient digestibility and growth performance for *Labeo rohita* fingerlings fed on canola meal-based diet. *J. Appl. Anim. Res.*, p. 1-7.
- Hussain, S. M., Afzal, M., Rana, S. A., Javed, A. and Iqbal, M. 2011. Effect of phytase supplementation on growth performance and nutrient digestibility of *Labeo rohita* fingerlings fed on corn gluten meal-based diets. *Int. J. Agric. Biol.*, 13: 916-922.
- Hussain, S. M., Shahzad, M. M., Afzal, M., Javid, A., Mubarik, M. S., Shah, S. Z. H., Hussain, M., Ahmad, S., Arsalan, M. Z. H., Manzoor, R. and Riaz, D. 2015a. Efficacy of phytase enzyme for increasing mineral digestibility of *Cirrhinus mrigala* fingerlings fed on soybean meal-based diet. *Pakistan J. Zool.*, 47: 1807-1816.
- Hussain, S. M., Shahzad, M. M., Jabeen, F., Nasir, S., Afzal, M., Javid, A., Ahmad, S., Arsalan, M. Z. H., Riaz, D., Khichi, T. A. A., Ahmad, A. W. and Furqan, M. 2014. Growth performance and nutrient digestibility of *Cirrhinus mrigala* fingerlings fed on soybean meal-based diet supplemented by phytase. *Int. J. Biosc.*, 5: 212-221.
- Kumar, V., Sinha, A. K., Makkar, H. P. S., De-Boeck, G. and Becker, K. 2011. Phytate and phytase in fish nutrition. *J. Anim. Physiol. Anim. Nutr.*, 96: 335-364.
- Lanari, D., Agaro, E. D. and Turri, C. 1998. Use of non-linear regression to evaluate the effects of phytase enzyme treatment of plant protein diets for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 161: 345-356.

- Lazado, C. C., Caipang, C. M. A., Gallage, S., Brinchmann, M. F. and Kiron, V. 2010. Responses of Atlantic cod *Gadus morhua* head kidney leukocytes to phytase produced by gastrointestinal-derived bacteria. *Fish Physiol. Biochem.*, 36: 883-891.
- Lim, S. J. and Lee, K. J. 2009. Partial replacement of fish meal by cottonseed meal and soybean meal with iron and phytase supplementation for parrot fish *Oplegnathus fasciatus*. *Aquaculture*, 290: 283-289.
- Lim, S. J., Kim, S., Ko, G., Song, J., Oh, D., Kim, J. and Lee, K. 2011. Fish meal replacement by soybean meal in diets for tiger puffer, *Takifu gurubripes*. *Aquaculture*, 313: 165-170.
- Liu, H., Jin, J., Zhu, X., Han, D., Yang, Y. and Xie, S. 2017. Effect of substitution of dietary fish meal by soybean meal on different sizes of gibel carp (*Carassius auratus gibelio*): digestive enzyme gene expressions and activities and intestinal and hepatic histology. *Aquac. Nutr.*, 23: 129-147.
- Liu, L. W., Su, J. M., Zhang, T., Liang X. Z. and Luo, Y. L. 2013. Apparent digestibility of nutrients in grass carp diet supplemented with graded levels of phytase using pre-treatment and spraying methods. *Aquac. Nutr.*, 19: 91-99.
- Lovell, R. T. 1989. *Nutrition and feeding of fish*. Van Nostrand-Reinhold, New York, 260 pp.
- Makkar, H. P. S. and Becker, K. 1996. Nutritional value and antinutritional components of whole and ethanol extracted *Moringa oleifera* leaves. *Anim. Sci. Tech.*, 63: 211-228.
- NRC 1993. *Nutrient requirements of fish*, National Research Council, National Academy Press, Washington, DC USA.
- Nwanna, L. C., Eisenreich, R. and Schwarz, F. J. 2007. Effect of wet-incubation of dietary plant feedstuffs with phytases on growth and mineral digestibility by common carp *Cyprinus carpio* L. *Aquaculture*, 271: 461-468.
- Portz, L. and Liebert, F. 2004. Growth, nutrient utilisation and parameters of mineral metabolism in Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) fed plant based diets with graded levels of microbial phytase. *J. Anim. Physiol. Anim. Nutr.*, 88: 311-320.
- Riche, M. and Garling, D. L. 2004. Effect of phytic acid on growth and nitrogen retention in tilapia *Oreochromis niloticus* L. *Aquac. Nutr.*, 10: 389-400.
- Robinson, E. H., Li, M. H. and Manning, B. B. 2002. Comparison of microbial phytase and dicalciumphosphate on growth and bone mineralisation of pond raised channel catfish, *Ictalurus punctatus*. *J. Appl. Aquac.*, 12: 81-88.
- Rowland, S. J. and Ingram, B. A. 1991. Diseases of Australian native fishes. *Fisheries Bulletin 4*, NSW Fisheries, Sydney, NSW, Australia.
- Salem, H. B. and Makkar, H. P. S. 2009. Defatted *Moringa oleifera* seed meal as a feed additive for sheep. *Anim. Feed Sci. Tech.*, 150: 27-33.
- Sardar, P., Randhawa, H. S., Abid, M. and Prabhakar, S. K. 2007. Effect of dietary microbial phytase supplementation on growth performance, nutrient utilisation, body compositions and haemato-biochemical profiles of *Cyprinus carpio* (L.) fingerlings fed soyprotein-based diet. *Aquac. Nutr.*, 13: 444-456.
- Selle, P. H., Ravindran, V., Caldwell, A. and Bryden, W. L. 2000. Phytate and phytase: consequences for protein utilisation. *Nutr. Res. Rev.*, 13: 255-278.
- Snedecor, G. W. and Cochran, W. G. 1991. *Statistical methods*. 8th edn. Iowa State University. Press, Ames., USA, 503 pp.
- Spinelli, J., Houle, C. R. and Wekell, J. C. 1983. The effects of phytates on the growth of rainbow trout (*Salmo gairdneri*) fed purified diets containing varying quantities of calcium and magnesium. *Aquaculture*, 30: 71-83.
- Steel, R. G. D., Torrie, J. H. and Dickey, D. A. 1996. *Principles and procedures of statistics*, 3rd edn. McGraw Hill International Book Co., Inc., New York, USA, p. 336-352.
- Tiamiyu, L. O., Okomoda, V. T. and Aende, A. 2016. Growth performance of *Oreochromis niloticus* fingerlings fed *Moringa oleifera* leaf as replacement for soybean meal. *J. Aquac. Engin. Fish. Res.*, 2: 61-66.
- Usmani, N. and Jafri, A. K. 2002. Influence of dietary phytic acid on the growth, conversion efficiency and carcass composition of *Cirrhinus mrigala* (H) fry. *J. World Aquac. Soc.*, 33: 199-204.
- Vielma, J., Makinen, T., Ekholm, P. and Koskela, J. 2000. Checked the Influence of dietary soy and phytase levels on performance and body composition of large rainbow trout *Oncorhynchus mykiss* and algal availability of phosphorus load. *Aquaculture*, 183: 349-362.
- Wang, F., Yang, Y. H., Han, Z. Z., Dong, H. W., Yang, C. H. and Zou, Z. Y. 2009. Effects of phytase pretreatment of soybean meal and phytase-sprayed in diets on growth, apparent digestibility coefficient and nutrient excretion of rainbow trout (*Oncorhynchus mykiss* Walbaum). *Aquac. Int.*, 17: 143-157.
- Wedemeyer, G. A. and Yastuke, W. T. 1977. *Clinical methods for the assessment of the effects of environmental stress on fish health*. Fish and Wild Life Service Technical paper 89, USDI, Washington DC, USA.
- Worku, A. 2016. *Moringa oleifera* as a potential feed for livestock and aquaculture industry. *African J. Agric. Sci. Tech.*, 4: 666-676.
- Yan, W. and Reigh, R. C. 2002. Effects of fungal phytase on utilisation of dietary protein and minerals and dephosphorylation of phytic acid in the alimentary tract of channel catfish *Ictalurus punctatus* fed an all-plant protein diet. *J. World Aquac. Soc.*, 33, 10-22.

- Yoo, G. and Bai, S. C. 2014. Effects of the dietary microbial phytase supplementation on bioavailability of phosphorus in juvenile olive flounder *Paralichthys olivaceus* fed soybean meal based diets. *Fish Aquac. Sci.*, 17: 319-324.
- Yuangsoi, B., Klahan, R. and Charoenwattanasak, S. 2014. Partial replacement of protein in soybean meal by moringa seed cake (*Moringa oleifera*) in bocourti's catfish (*Pangasius bocourti*). *Songklanakarin J. Sci. Technol.*, 36(2): 125-135.