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Improvement in Nutrient Digestibility and Growth Performance of *Catla catla* Fingerlings Using Phytase in *Moringa oleifera* Leaf Meal Based Diet

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Authors' Contribution
MMS conducted feeding trial, collected data and prepared manuscript. SMH planned and supervised the research and provided all materials for the research. AMA compiled the results. AJ helped in statistical analysis. AC and MH helped in manuscript preparation. SZHS helped in chemical analysis.

Key words
MOLM, *Catla catla*, Growth performance, Nutrient digestibility, Phytase

ABSTRACT

The present research work was conducted to evaluate the effect of phytase supplementation on growth and nutrient digestibility of the *Catla catla* fingerlings fed *Moringa oleifera* leaf meal (MOLM) based diet. The phytic acid's presence in plant by-products decreases the bioavailability of nutrients to fish, resulting in poor fish growth and low nutrient digestibility in the body. Experimental diet was divided into six groups and were supplemented with graded levels (0, 300, 600, 900, 1200 and 1500 FTU kg^{-1}) of phytase. Cr_2O_3 was incorporated in all diets at the rate of 1% as a non-digestible marker. The fingerlings were fed at the rate of 4% of live wet weight twice a day and faeces were collected from each tank. On the basis of results it was noted that phytase supplementation showed significant ($p<0.05$) improvement in growth indices (WG%, FCR, SGR) and digestibility of nutrients (i.e. CP, EE and GE) when *C. catla* fingerlings were fed at 900 FTU kg^{-1} level in MOLM based diet. It was further noted that phytase supplementation decreased the discharge of nutrients through faeces resulting in reduced eco-pollution. On the basis of results it was concluded that phytase supplementation at 900 FTU kg^{-1} level was helpful to develop a cost-effective as well as eco-friendly fish feed by using MOLM based diet.

INTRODUCTION

World population is continuously growing, thus great pressure is being imposed on availability of resources including food supply. Food scarcity is a serious problem these days, especially in the form of protein (Khan *et al*., 2015). The world has incredibly relied on fish as a fundamental supplier of nutrients, particularly protein on account of its cost (Agbo *et al*., 2014). So, higher production of fish is required to meet ever increasing needs of growing population. Feed, a major input in aquaculture production, is a fundamental challenge facing the development and growth of aquaculture (Gabriel *et al*., 2007). *Catla catla* commonly known as Thaila, is being cultured in Pakistan with other fish species and are surface feeders (Aslam *et al*., 2016). The reported production of this fish has increased during the first decade of 21st century and in 2012 was about 2.8 million tons per annum (FAO, 2015). Aquaculture feed mainly relies upon the utilization of fishmeal (FM) because of its high palatability and nutritious esteem (NRC, 2011). Whereas, unstable supply, higher demand and increasing cost of the FM made it necessary to search for alternative sources of protein (Rana *et al*., 2009; Hardy, 2010). These sources should be of low cost with higher protein contents (Lim *et al*., 2011). Use of different plant protein sources instead of FM was suggested by the researchers (Barnes *et al*., 2012; Dedeke *et al*., 2013; Hussain *et al*., 2016, 2019; Shahzad *et al*., 2016). Researchers have found positive effects on overall fish performance when different fish species were fed plant by-products based diets (Hussain *et al*., 2014, 2016; Chu *et al*., 2015; Muin *et al*., 2015; Liu *et al*., 2015; Shahzad *et al*., 2017). One of the better and cost effective plant protein sources is *M. oleifera* also known as miracle

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tree. Moringa belongs to the Moringaceae family. It is fast growing plant with several economically important feed supplements and medicinal uses. Moringa is a promising protein source when it was included in fish diets at low levels (Chiseva, 2006). High crude protein contents are present in moringa leaves which vary from 25% (Makkar and Becker, 1996) to 32% (Soliva *et al*., 2005). Leaves of moringa are important source of high amount of nutrients (Bosh, 2004; Grubben and Denton, 2004) and rich source of essential vitamins (Soliva *et al*., 2005).

But moringa leaves contain several anti-nutritional factors such as phytate that cannot be removed by soaking or heating (Liener, 1994). Growth, nutrient retention, absorption of minerals especially cation minerals, protein and phosphorus (P) are adversely affected by higher phytate or phytic acid concentrations in plant by-products or oilseeds meal based diets (Cao *et al*., 2007; Gatlin *et al*., 2007). It is estimated in plant by-products based diets, 60-80% of total P is present in chelated form that is known as phytic acid (Lei *et al*., 2013). This chelated phosphorous cannot be utilized by mono-gastric and a-gastric fishes, resulting in nutrient discharge into water media causing aquatic pollution (NRC, 1993). It is also capable of binding with proteins and starch (Jondreville *et al*., 2005; Noureddini and Dang, 2009). Furthermore, phytate binds with trypsin and other amino acids, decreases protein digestibility in mono-gastric and a-gastric fishes (Singh and Krikorian, 1982; Spinelli *et al*., 1983). Phytate complex only can be broken down by some enzymatic reactions because it is a stable compound (Vielma *et al*., 2000). Phytase that is known as myo inositol hexa-kisphosphatephospho-hydrolase, can breakdown the phytate complex enzymatically. Mono-gastric fishes cannot hydrolyze the phytate because their body cannot produce phytase enzyme. Supplemental dietary phytase is very effective to improve the nutrient digestibility and higher growth performance of fish. It also decreases water pollution by proper digestion and absorption of phosphorus in the fish body (Hussain *et al*., 2011; Liu *et al*., 2013; Hussain *et al*., 2016). It was noted that phytase improved nutrient digestibility and growth of *C. catla* and *C. mrigala* fed plant by-products based diets (Hussain *et al*., 2011, 2016; Shahzad *et al*., 2017). Therefore, the present research was focused on to find out the best and least cost protein sources for commercially important fish specie i.e. *C. catla* to enhance its production and to overcome the problems of expensive fish meal using phytase supplemented MOLM based diet.

MATERIALS AND METHODS

The present research work was carried out to evaluate the effects of phytase supplementation in MOLM based diet on nutrient digestibility and growth performance of *C. catla* fingerlings. The trial was performed in Fish Nutrition Lab, Department of Zoology, Government College University, Faisalabad. *C. catla* fingerlings were procured from the Government Fish Seed Hatchery, satiana Road, Faisalabad. Fingerlings were stocked in specially designed V-shaped water tanks (70 L water capacity) for two weeks and were fed on basal diet (Allan and Rowland, 1992). Parameters related to water quality were monitored using specific apparatus i.e. EC meter (HANNA: HI. 8633), pH meter (Jenway 3510) and DO meter (Jenway 970). Through capillary system, aeration was provided round the clock to all the experimental tanks. Before the start of trial with fingerlings of *C. catla,* all the pathogens were killed by 0.5 % NaCl saline solution treatment (Rowland and Ingram, 1991).

Experimental design

Moringa by-product such as *M. oleifera* leaf meal (MOLM) was used as test ingredient to formulate experimental diets by the supplementation of phytase with graded levels (0, 300, 600, 900, 1200 and 1500 FTU kg^{-1}). One control diet and five phytase supplemented MOLM 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. The fingerlings were fed at the rate of 4% of live wet body weight. The duration of the experiment was 90-days. Test diets supplemented with phytase were compared with control diet to determine growth performance parameters and nutrient digestibility by using completely randomized design (CRD).

Processing of M. oleifera *leaves*

M. oleifera fresh leaves were collected from a local garden and washed to remove the dirt and dust particles. The leaves were drained appropriately and dried under a shady place for six days to avoid the damage of vitamins by photo-dynamic oxidation or damage. Dried leaves of moringa were separated from the stalks to decrease crude fiber contents in the diet (Madalla *et al*., 2013). Processed moringa seeds and leaves were ground to make a powder.

Formation of feed pellets

The feed ingredients were procured from a commercial feed mill and were analysed for chemical composition (Table I) following AOAC (1995) prior to the formulation of the experimental diet. The feed ingredients were finely ground to pass through 0.3mm sieve size. All ingredients

were mixed at appropriate concentration (Table II) in an electric mixer for 10 minutes and fish oil was gradually added. During mixing of ingredients 10–15% water was also added to prepare suitable texture dough and processed through pelleting machine to formulate pellets (Lovell, 1989). One control and five phytase supplemented test diets were prepared using each moringa by-product by spraying graded levels (0, 300, 600, 900, 1200 and 1500 FTU kg^{-1}) of phytase as shown in Table III. The required concentrations of phytase enzyme (Phyzyme® XP 10000 FTU g^{-1}; Danisco Animal Nutrition, Fin-65101 Vaasa, Finland) were prepared in 50 mL distilled water and sprayed on one kg of each test diet (Robinson *et al*., 2002). Control diet (0 FTU kg^{-1} level) was sprayed with a similar amount of distilled water to maintain the equivalent amount of moisture. All the sprayed diets were dried and stored at 4°C until use.

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

Ingredients	MOLM	FM	Rice polish	Wheat flour	SBM
Dry matter (%)	91.83	91.67	94.06	92.4	92.98
Crude protein (%)	28.95	49.17	12.38	10.15	40.72
Crude fat (%)	2.83	7.12	11.46	2.35	4.53
Crude fiber (%)	19.95	1.12	12.74	2.67	8.67
Ash (%)	10.87	24.66	15.17	2.96	12.16
Gross energy (kcal/g)	3.84	2.65	3.18	2.95	3.91
Carbohydrates	37.4	17.93	48.25	81.87	33.92

MOLM, *M. oleifera* leaf meal; FM, fish meal; SBM, Soybean meal.

Feeding protocol and sample collection

C. catla fingerlings were fed at the rate of 4% of live wet body weight on their prescribed diet twice daily. After the feeding session of two hours, the left over 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 water. Faeces were collected by the opening valve-I and valve-II subsequently from the fecal collecting tube of each replicated tank. Fecal material was collected carefully to avoid the breakage of faeces for minimizing the leaching of nutrients in water. Faeces were dried in an oven at 65°C for 3-4 h and stored for further chemical analysis.

Growth study

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

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

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

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

Table II. Ingredients composition (%) of experimental diet (dry matter basis).

Ingredients	Test diet composition
MOLM	35
FM	15
Soybean meal	15
Rice polish	8
Wheat flour*	17
Fish oil	6
Vitamin Premix**	1.0
Chromic oxide	1.0
Ascorbic acid	1.0
Mineral mixture ***	1.0

MOLM, *M. oleifera* leaf meal; * Phytase enzyme was used at the expense of wheat flour;
** Vitamin premix/kg, Vitamin D_3, 3000000 IU; Vitamin A, 15000000 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, 15000 mg; Vitamin K_3, 8000 mg; Folic acid, 1500 mg; Calcium pantothenate, 12,000 mg; Nicotinic acid, 60000 mg.
*** Mineral premix/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.

Chemical analysis of feed and faeces

After 90 days of feeding trial, moisture contents 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) contents (N × 6.25) whereas Soxhlet system (Soxhlet Extraction Heating Mantels, 250 ml 53868601) was used to analyse the crude fat by the help of petroleum ether extraction (EE) 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)

Table III. Effect of MOLM based phytase supplemented test diets on the compositions of nutrients in diets and faeces of *C. catla* fingerlings.

Experimental diets	Phytase (FTU kg^{-1})	CP (%)	EE (%)	GE (kcal g^{-1})
Diet				
Test diet –I (Control diet)	0	28.28±0.15	6.91±0.09	3.00±0.08
Test diet –II	300	28.31±0.09	6.91±0.02	3.01±0.06
Test diet –III	600	28.35±0.11	6.92±0.04	2.98±0.05
Test diet –IV	900	28.39±0.27	6.92±0.06	3.01±0.06
Test diet –V	1200	28.36±0.30	6.93±0.06	3.01±0.07
Test diet –VI	1500	28.41±0.02	6.92±0.04	2.96±0.06
Faeces				
Test diet –I (Control diet)	0	17.69±0.31^{e}	4.08±0.07^{a}	1.73±0.06^{e}
Test diet –II	300	15.61±0.21^{d}	3.57±0.06^{b}	1.55±0.10^{d}
Test diet –III	600	14.28±0.13^{c}	3.15±0.07^{c}	1.33±0.04^{c}
Test diet –IV	900	9.53±0.13^{a}	2.53±0.11^{d}	0.93±0.04^{a}
Test diet –V	1200	11.64±0.07^{b}	2.01±0.07^{e}	1.13±0.05^{b}
Test diet –VI	1500	13.68±0.19^{c}	3.28±0.07^{c}	1.46±0.02^{cd}

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

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 (GE) of samples. Chromic oxide as an inert marker was added in test diets to determine nutrient digestibility. Chromic oxide content after oxidation of the ash samples of feces and experimental diets with perchloric reagent was estimated by acid digestion method (Divakaran, Leonard, and Lan, 2002) through UV-VIS 2001 spectrophotometer at 350nm.

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

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

Statistical analysis

Finally, data of ADC% of nutrients (CP, EE and GE) and growth parameters (WG, WG%, SGR and FCR) 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

Results showed that, there was an equal amount of nutrients in all the MOLM based diets including the control diet (Table III). Whereas, this table also shows maximum nutrients discharged in water through faeces when fish was fed on the control diet (0 FTU kg^{-1} level based diet) and minimum nutrients (CP and GE) were discharged at 900 FTU kg^{-1} level followed by 1200 FTU kg^{-1} level based diet. This reduction in nutrients excretion through faeces indicated that phytate complex was being broken-down by phytase addition. It was observed that further increase in phytase supplementation (1500 FTU kg^{-1}), increased the nutrient excretion. Primary data confirms that highest ADC% was observed at 900 FTU kg^{-1} level when we talk about CP and GE. Maximum values of CP (69%) and GE (72%) digestibility were found in fish fed at 900 FTU kg^{-1} level of phytase supplemented MOLM based diet followed (CP 62% and GE 66%) by 1200 FTU kg^{-1} level based diet. Whereas lowest ADC% of CP (42%) and GE (47%) were recorded in fish fed on the control diet as shown in Table IV. Results of CP and GE were significantly ($p<0.05$) different from ADC% values observed for control and each other phytase supplemented test diet. On the contrary, minimum apparent EE contents were discharge through faeces at 1,200 FTU kg^{-1} followed by 900 FTU kg^{-1} diet (Fig. 1). Similarly, the maximum ADC% for EE (74%) was observed at 1,200 FTU kg^{-1} level followed by (67%)

when fingerlings were fed on test diet IV supplemented with 900 FTU kg^{-1} level. These values were significantly ($p<0.05$) different from all the phytase supplemented test and control diets. Lowest (45%) ADC% was observed for fish fed on control diet (0 FTU kg^{-1} level based diet).

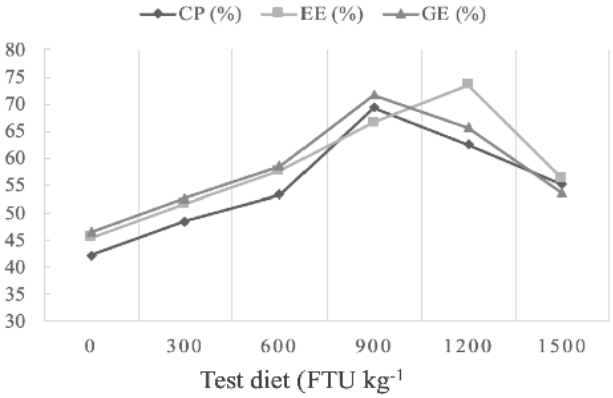

Fig. 1. Apparent nutrient digestibility of *C. catla* fingerlings fed MOLM based diets.

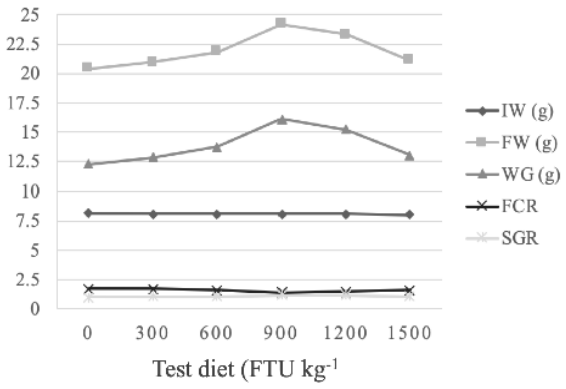

Fig. 2. Growth performance of *C. catla* fingerlings fed graded levels of phytase supplemented MOLM based diets.

Table IV. Effect of MOLM based diets on the apparent nutrient digestibility of *C. catla* fingerlings.

Experimental diets	Phytase levels (FTU kg^{-1})	CP (%)	EE (%)	GE (%)
Test diet –I (Control diet)	0	42.22±0.10^{f}	45.43±0.98^{e}	46.57±0.86^{e}
Test diet –II	300	48.51±0.46^{e}	51.67±0.65^{d}	52.73±0.62^{d}
Test diet –III	600	53.38±0.35^{d}	57.64±0.80^{c}	58.54±0.92^{c}
Test diet –IV	900	69.33±0.39^{a}	66.58±0.96^{b}	71.67±0.97^{a}
Test diet –V	1200	62.50±0.11^{b}	73.54±0.96^{a}	65.63±0.72^{b}
Test diet –VI	1500	55.15±0.33^{c}	56.19±0.93^{c}	53.63±0.97^{d}

$^{a-f}$ Means within column having different superscripts are significantly different at $P<0.05$; Data are means of three replicates (± shows Standard Deviations).

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

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)	8.13±0.03	8.1±0.04	8.09±0.03	8.08±0.05	8.09±0.03	8.06±0.02
FW (g)	20.42±0.11^{e}	20.93±0.03^{d}	21.85±0.10^{c}	24.22±0.14^{a}	23.32±0.1^{b}	21.12±0.11^{d}
WG (g)	12.29±0.13^{e}	12.84±0.06^{d}	13.76±0.13^{c}	16.14±0.17^{a}	15.23±0.11^{b}	13.06±0.12^{d}
WG (%)	151.13±1.95^{e}	158.55±1.46^{d}	170.02±2.1^{c}	199.80±2.93^{a}	188.14±1.71^{b}	162.10±1.54^{d}
FI	0.239±0.002	0.24±0.002	0.241±0.002	0.254±0.002	0.252±0.002	0.236±0.003
WG ($fish^{-1}$ day^{-1})g	0.14±0.001^{e}	0.14±0.001^{d}	0.15±0.001^{c}	0.18±0.002^{a}	0.17±0.001^{b}	0.15±0.001^{d}
FCR	1.75±0.004^{a}	1.69±0.01^{b}	1.58±0.001^{d}	1.42±0.005^{f}	1.49±0.002^{e}	1.63±0.01^{c}
SGR	1.02±0.01^{e}	1.06±0.01^{d}	1.09±0.01^{c}	1.22±0.01^{a}	1.18±0.01^{b}	1.07±0.01^{ed}

$^{a-d}$ Means within rows having different superscripts are significantly different at $p<0.05$; Data are means of three replicates (± shows Standard Deviations). IW, Initial Weight; FW, Final Weight; WG, Weight gain; FI, Feed Intake; SGR, Specific Growth Rate; FCR, Feed Conversion Ratio.

Mean overall growth performance (WG, WG%, FCR and SGR) of *C. catla* fingerlings fed on MOLM based test diets is presented in Table V. These results clearly indicate that phytase supplemented MOLM based diet enhanced the growth parameters of *C. catla* fingerlings as compared to the control diet (without phytase supplementation) yet the initial weight of fish was statistically ($p<0.05$) similar in all the diets. From the current findings it was noted that all the parameters related to growth performance of *C. catla* fingerlings fed on MOLM based diet started to increase from 300 FTU kg^{-1} diet to its maximum at 900 FTU kg^{-1} level based diet. It was also found that MOLM based test diets having further higher levels (1,200 and 1,500 FTU kg^{-1} levels) of phytase supplementation resulted in decreased growth performance. Results showed that *C. catla* fingerlings fed MOLM based diet supplemented at 900 FTU kg^{-1} level significantly increased ($p<0.05$) growth performance when compared to the control (without phytase) and other phytase supplemented test diets.

Figure 2 shows that the highest WG (16g) and WG% (200%) were recorded in fingerlings fed MOLM based phytase supplemented (900 FTU kg^{-1} diet) followed (15g and 188%) by 1,200 FTU kg^{-1} diet. These values were significantly higher ($p<0.05$) than the control (12g and 151%) and remaining phytase supplemented test diets. Lowest FCR value (1.42) was noted in fish fed on test diet IV (900 FTU kg^{-1} level) followed by (1.49) at 1200 FTU kg^{-1} level based diet. These values were significantly ($p<0.05$) different from the values observed for fish that fed on the control and remaining phytase supplemented test diets. However highest FCR value (1.75) was found in fish that fed on the control diet (without phytase supplementation). Lowest FCR showed highest feed conversion into fish flesh. Similarly, *C. catla* fingerlings showed maximum SGR (1.22) at 900 FTU kg^{-1} level based diet followed by (1.18) when the fish fed at 1200 FTU kg^{-1} level and was significantly ($p<0.05$) higher from other test diets as well as control diet. Results showed that SGR of fish fed at 600 FTU kg^{-1} level based diet was statistically ($p<0.05$) at par with the SGR value of fish that fed at 300 FTU kg^{-1} level and 1500 FTU kg^{-1} based diet. Whereas minimum SGR (1.02) was found for fish fed on the control diet (0 FTU kg^{-1} level based diet).

From these findings it was clear that supplementation of phytase in MOLM based diet significantly improved ADC% of nutrients as well as growth performance for fingerlings fed 900 FTU kg^{-1} as well as 1200 FTU kg^{-1} diets ($p<0.05$). These results indicate that ADC% of CP and GE and growth parameters increased from 300 FTU kg^{-1} diet and reached its maximum at 900 FTU kg^{-1} diet. Further phytase supplementation (1,200 and 1,500 FTU kg^{-1}) did not improve the ADC% of CP and GE and growth. Whereas ADC% of EE started to improve from 300 FTU kg^{-1} diet and reached its maximum at 1,200 FTU kg^{-1} diet, additional phytase supplementation to 1,500 FTU kg^{-1} diet decreased percent ADC. It was concluded that the 900 FTU kg^{-1} diet supplemented had maximum nutrient digestibility, growth and reduced nutrient's discharge in water through faeces resulting in decreased aquatic pollution.

DISCUSSION

M. oleifera leaf meal (MOLM) based diet is virtuous sources of protein, fat as well as other nutrients. But in MOLM based diets, an anti-nutritional factor i.e. phytate or phytic acid is present (Gopalakrishnan *et al*., 2016; Worku, 2016). The presence of phytate in plant based diets can cause adverse effects on nutrient digestibility in fish (Hussain *et al*., 2016; Shahzad *et al*., 2018). According to results of the current study, it was found that addition of phytase in MOLM based diet was useful for the improvement in apparent digestibility coefficient (ADC %) of nutrients for *C. catla* fingerlings when compared with the control diet (without phytase supplementation). Highest values for ADC% of CP (69%) and GE (72%) were observed when fish were fed at the 900 FTU kg^{-1} of phytase supplemented MOLM based diet. Similarly, Ashraf and Goda (2007) observed that optimal level of phytase enzyme for highest CP and GE digestibility was 1000 FTU kg^{-1} level. They also found that ADC% of CP and GE could not be further improved on higher supplementation levels of enzyme. Hussain *et al*. (2017) and Shahzad *et al*. (2018) also found similar results. They found maximum CP and GE digestibility in *C. catla* when fingerlings were fed at 900 FTU kg^{-1} level based diet. Nearly similar to our results, Hussain *et al*. (2016) found higher CP and GE digestibility in *Cirrhinus mrigala* fingerlings when fed soybean meal based test diet supplemented with phytase at 1000 FTU kg^{-1} diet. Whereas, maximum CP and GE digestibility was observed in *Labeo rohita* fingerlings fed phytase supplemented soybean meal based diet (Baruah *et al*., 2007a) and soybean meal based diets (Hussain *et al*., 2014) at 750 FTU kg^{-1} level. In contrast to these findings, 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). Whereas, 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). This variation in results is difficult to explain but the possible reasons may be due to the different fish species, feed ingredients, different experimental conditions, different sources of phytase enzyme, pH of the stomach,

feed processing methods or feed drying methods (Liu *et al*., 2013).

Present results indicated that maximum ADC% of EE i.e. 73% was observed in fish fed on MOLM based phytase supplemented (1200 FTU kg^{-1} level) diet followed by (67%) when fingerlings were fed at 900 FTU kg^{-1} level based diet. Nearly similar to present findings, Hussain *et al*. (2014) found maximum EE digestibility at 1000 FTU kg^{-1} level when *C. mrigala* fingerlings fed soybean meal based diet. They also concluded that further phytase addition (1500 and 2000 FTU kg^{-1}) leads to a decline in ADC% of EE. In contrary to these, 750 FTU kg^{-1} diet was suggested as the most suitable level for higher fat digestibility in *L. rohita* fingerlings (Baruah *et al*., 2007b). Whereas, higher EE digestibility was found in Nile tilapia when it fed at 1000 and 2000 FTU kg^{-1} level based test diets (Portz and Liebert, 2004). Reasons for these variations may be type or source of phytase and amount of phytate in plant ingredients. From these findings, it was clear that different levels of phytase supplementation showed maximum EE digestibility because of the different fish species, different protein source and experimental conditions. On contrary, Dalsgaard *et al*. (2009) observed non-significant ($p>0.05$) results of fat digestibility in *Oncorhynchus mykiss* (rainbow trout) fed phytase supplemented soybean meal based diets. Whereas, decreased EE digestibility was found in *O. mykiss* (rainbow trout) fed phytase supplemented soybean meal based diet (Wang *et al*., 2009). Cao *et al*. (2007) explained that the 250-1,500 FTU kg^{-1} levels of phytase supplementation are sufficient to release the crude lipid from phytate complex for higher fat digestibility.

The presence of phytate in feed results reduced fish growth performance in terms of weight gain and FCR (Spinelli *et al*., 1983). Maximum WG (16g) and WG% (200%) were observed in fish fed on 900 FTU kg^{-1} diet in MOLM based diets. These values of WG and WG% were much higher as compared to other phytase supplemented test diets and control diet (0 FTU kg^{-1} diet). Nearly similar results were found in terms of WG and WG%, when common carp fingerlings fed at 800 FTU kg^{-1} supplemented plant-meal based diet (Bai *et al*., 2003). Similar findings were discussed by Shahzad *et al*. (2017, 2018) in which they found maximum growth performance in the *C. catla* fingerlings fed 900 FTU kg^{-1} levels based diets as compared to control and other test diets. Nwanna *et al*. (2007) also found a significant ($p<0.05$) increase in overall *Cyprinus carpio* WG and WG% at 750 and 1,000 FTU kg^{-1} levels based diets. Nearly similar results were observed in a study conducted by Hussain *et al*. (2014), they found maximum weight gain and weight gain % of *C. mrigala* fingerlings fed soybean meal based diet supplemented with phytase at 1,000 FTU kg^{-1} level. Similarly, Yu and Wang (2000) also found the same results when fish was fed at 1,000 FTU kg^{-1} level based diet. In contrast, non-significant ($p<0.05$) effect of phytase supplementation was reported in case of *O. mykiss* growth when fingerlings fed phytase supplemented plant meal based diets (Vielma *et al*., 2000). In contradiction to the present findings many other researchers such as Robinson *et al*. (2002), Baruah *et al*. (2007a), Lim and Lee (2009) and Wang *et al*. (2009) did not find any significant effect ($p>0.05$) of phytase supplementation on fish growth performance, when these fish species were fed with or without phytase supplemented plant meal based diets. It was obvious from the previous literature that maximum growth performance increment in different fish species was observed when fed plant meal based diets supplemented with microbial phytase ranging between 250 to 1500 FTU kg^{-1} diet (Cao *et al*., 2007). This controversy 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, stomach pH and methods used for feed drying (Wang *et al*., 2009).

The current study showed lowest FCR (1.42) and highest SGR (1.22) values of *C. catla* fingerlings fed at 900 FTU kg^{-1} level based diet in MOLM based diet. Similar to our results Riche and Garling (2004), Ashraf and Goda (2007), Cao *et al*. (2008) observed maximum SGR and minimum FCR values when *O. niloticus* (Nile tilapia) fed plant meal based diets with phytase supplementation at 1,000 FTU kg^{-1} level. Hussain *et al*. (2017) also observed highest SGR (1.37) and lowest FCR (1.15) in *C. catla* fingerlings when they were fed on *M. oleifera* seed meal based diet with phytase supplementation at 900 FTU kg^{-1} level based diet. Nwanna and Schwarz (2008) found improvement in growth parameters when *C. carpio* (common carp) fingerlings fed phytase supplemented plant meal based diet at 750 and 1000 FTU kg^{-1} levels. On the other hand, Hussain *et al*. (2011) observed maximum improvement in FCR of *L. rohita* fingerlings when they fed 750 FTU kg^{-1} level based diet that was close to the optimum level (900 FTU kg^{-1}diet) found in present study. In contrast to current findings, higher FCR values of *Monorone saxatilix* (stripped seabass) were observed when fed with a little higher dose (at 1300 FTU kg^{-1} level) of phytase supplementation in plant meal based diet (Hughes and Soares, 1998). Similar results were found when *C. catla* fingerlings were fed on phytase supplemented moringa by-products based diets at 900 FTU kg^{-1} level based diet (Shahzad *et al*., 2018). They found minimum FCR (1.30) and maximum SGR (1.32) in *C. catla* fingerlings fed on moringa by-products based diet supplemented with 900 FTU kg^{-1} level. Another close result was also recorded in a study, when *C. mrigala* fingerlings fed phytase supplemented soybean meal based

diets (Hussain *et al*., 2014). They found that fingerlings showed maximum growth performance in terms of WG (11g), WG% (155%) and FCR (1.43) when fish fed at 1,000 FTU kg^{-1} level based diet as compared to control and other levels of phytase supplemented test diets. It was noted that phytase supplementation improves growth performance but on varying levels. 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). In contrary, phytase supplementation cannot enhance the overall growth performance of *O. mykiss* fed phytase supplemented canola meal based diets (Vielma *et al*., 2000). Statistically, no significant ($p<0.05$) difference was observed in term of growth performance when Korean rock fish (Yoo *et al*., 2005), parrot fish (Lim and Lee, 2009) Japanese flounder (Masumoto *et al.*, 2001), channel catfish (Yan and Reigh, 2002) and Atlantic salmon (Sajjadi and Carter, 2004) fed on different plant meal based diets supplemented with phytase at different levels. Researchers argued that these variations in results conducted by different researchers (on different fish species, different time interval and in different geographical areas) may be due to the methodology of feed preparation, type of phytase, species of fish and ingredients used in feed preparation (Baruah *et al*., 2007b; Cao *et al*., 2007).

Literature showed that there was not a single study conducted on supplementation of phytase in MOLM based diet. But, many researchers performed their respective trials to replace FM with leaf meal and found positive effects on nutrient digestibility in fish (Madalla *et al*., 2013; Bello and Nzeh, 2013; Ganzon-Naret, 2014; Abo-State *et al*., 2014; Ncha *et al*., 2015; Mehdi *et al*., 2016). Nwanna *et al*. (2008) performed a study to investigate effects of phytase supplemented Brazil nut and Leucaena leaf meal mixture based diet on nutrient digestibility in Amazon tambaqui which resulted in non-significant fish performance. The findings of present study showed that the supplementation of phytase in MOLM based diets resulted in reduced excretion of nutrients through faeces into water, which in turn enhanced nutrient digestibility as compared to fish fed at 0 FTU kg^{-1} diet. The utilization of nutrients in fish body and less discharge in water is very useful in improving growth performance and reducing the pollution in aquatic environment (Hussain *et al*., 2016). From these findings, it was concluded that phytase supplementation at 900 FTU kg^{-1}level in MOLM based diet is essential for improving fish growth performance by higher nutrient digestibility as compared to control diet (without phytase supplementation).

Statement of conflict of interest

Authors have declared no conflict of interest.

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