



Original article

Improvement in overall performance of *Catla catla* fingerlings fed phytase included low cost plant by products-based diet

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ABSTRACT

Phytic acid's presence in low-cost Moringa by-products effect the availability of important nutrients, diminishing the fish quality and blood composition in fish. Phytate having chelating effects with nutrients and minerals, can be reduced by the supplementation of phytase enzyme. Without the use of enzyme, plant meal may cause water pollution and decrease the fish health that results in higher culture cost. Therefore, current study was designed to check improvement in overall performance of *Catla catla* fingerlings fed Moringa by product-based diets supplemented with phytase (0, 300, 600, 900, 1200 and 1500, FTU/kg). All diets were integrated with non-digestible marker (Cr_2O_3) at the rate of 1%. The fingerlings were fed couple of times a day (4% of live wet weight). Results showed significant ($p < 0.05$) improvement in nutrient digestibility (i.e. EE, CP and GE), carcass composition and hematological parameters (i.e. RBCs, PLT and Hb) at 900, FTU/kg of phytase in contrast with other treatments. Moreover, phytase addition improves the water quality by reducing the nutrients leaching through feces at low cost. Current results indicated that, using mixture of Moringa seed meal and Moringa leaf meal based diet supplemented with phytase at 900, FTU/kg concentration is the most optimum level to develop a cost-effective as well as eco-friendly fish feed with maximum absorption of important nutrients and minerals in fish body resultantly high higher fish performance.

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1. Introduction

Labeo rohita i.e. raho, *C. catla* i.e. thaila and *Cirrhinus mrigala* i.e. mori are Indian carps, predominating the public as well as administrative sector in Pakistan (FAO, 2017). *Catla* ordinarily recognized as Thaila is column feeder and is popular in polyculture system and cultured with other species in Pakistan (Aslam et al., 2016). It has been described that *Catla* production has augmented through the initial decennium of 21st century and was nearly 2.8 millions ton in 2012 per year (FAO, 2015).

The rapid expansion of human population created nutrition issues especially food security concerns throughout the world (FAO, 2018). Aquaculture is rapidly flourishing at present due to the need of good quality fish protein (FAO, 2014). Intensification of aquaculture resulted in a high demand of better-quality feed development for both complete as well as supplementary feeding in tanks and in ponds (Hernandez et al., 2012). Among all aquaculture fishes, *C. catla* is of peculiar importance because they contribute to the low cost as well as best sources of protein for human diet (Khan et al., 2012). It contains high quality nutrients such as vitamins, good amino acid profile, minerals and fatty acids (Zhou et al., 2004; Dawood et al., 2015). However, high cost, rising demand and dwindling fish meal (FM) supply compels to find alternate source of protein (Hardy, 2010; FAO, 2014). These alternatives should be inexpensive and ameliorate quality protein (Lim et al., 2011). Many researchers have recommended the usage of other better-quality sources of proteins to reduce FM consumption (Shahzad et al., 2017, 2018; Hussain et al., 2019). By products of plants are considered as an excellent replacer of fish meal due to

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less-cost and easy availability around the year in fish diet by many research workers since last two decades (Wang et al., 2015; Hussain et al., 2018, 2019). Beneficial effects of plant meal on the growth-performance (GP) of fish were also examined (Hussain et al., 2011). Amidst these, one of the most cost effective plant by products based protein source is *M. oleifera*. It is the member of the Moringaceae family. It is an invasive species with various economically significant feed additives and therapeutic uses. Moringa is a propitious source of protein, incorporated in fish feed (Shahzad et al., 2016). Moringa leaves contain higher contents of crude protein, varying from 25% to 32% (Hassan et al., 2018; Soliva et al., 2005). Moringa leaves are significant source of vital nutrients (Grubben & Denton, 2004) and opulent source of essential vitamins (Soliva et al., 2005). *M. oleifera* seed meal (MOSM) is an excellent protein source constituting almost 33 to 38%, amino acids i.e. tryptophan, cysteine, methionine, and considerable vitamins (Hassan et al., 2018; Liang et al., 2019), various phenolic compounds and beta carotene (Anwar et al., 2007; Compaore et al., 2011). Composition of Moringa seed-cake has shown high amount of amino acids, as described in leaves, that are relatively lower in other plant-based diets (Ferreira et al., 2008).

But Moringa by-products comprise of different anti-nutritional factors suchlike phytate that cannot be isolated by heating or soaking (Olagbemide and Alikwe, 2014). Negative effects of phytate containing plant-based diets were observed on nutrient retention, blood composition and nutrients digestibility (Cao et al., 2007; Gatlin et al., 2007). It is predicted that in plant by-products-based diets, maximum P amount (60–80%) in the form of chelated phytate complex is present (Lei et al., 2013). Mono-gastric and a-gastric fishes cannot utilize chelated form of phosphorous that releases into water, resulting in aquatic pollution and algal blooms. Moreover, phytate binding with trypsin and other amino acids, diminishes protein digestibility in a-gastric fishes and mono gastric fishes (Singh and Krikorian, 1982; Spinelli et al., 1983). Phytate complex only can be hydrolyzed by some enzymatic reactions for it is a stable compound (Vielma et al., 2000). Phytate plays a detrimental role in overall fish performance. Hematological indices such as WBCs, RBCs and Hb, provides crucial information for researchers in tracking stress response of fish and in the advancement of fish health (Hrubec et al., 2000). Phytase that is noted as myo inositol-hexa kispophosphatephosphohydrolase, can hydrolyze the phytate complex. Mono-gastric fishes cannot breakdown the phytate because phytase enzyme is not being produced in their body. Addition of dietary phytase is very efficient way to enhance the nutrient digestibility and increased GP of fingerlings. Phytase also reduces pollution in water by proper assimilation and retention of P in fish (Liu et al., 2013; Hussain et al., 2016). Renowned feature of phytase is that it improved nutrient digestibility and hematology of thaila and mori, fed plant-based protein sources (Hussain et al., 2011, 2016; Shahzad et al., 2017). Henceforth, the major focus of this research was to determine the best and economically feasible sources of protein for commercially substantial fish species such as *C. catla* for higher production and to decrease issues of high-priced fish meal using phytase included MOSM + MOLM based diet.

2. Material and methodology

Current study was design to test the improvement in overall performance of *C. catla* fingerlings with particular focus on GP and Hematological indices of fish when fed on phytase included Moringa by-products-based diet. The trial was accomplished in Zoology Department, GCUF. Fingerlings of *C. catla* were brought from local Hatchery and were accustomed for two weeks to the trial conditions. Especially constructed water tanks with 70 L water

capacity were used for keeping fingerlings. During the period of acclimatization, the fingerlings were fed on basal diet once in a day (Allan and Rowland, 1992). On the daily basis, water quality parameters including pH, temperature and DO were analyzed. By using air pump, oxygen was supplied round the clock through capillary system. Due to the possibility of microbial presence, fingerlings of *C. catla* were treated (0.5% of saline solution).

2.1. Experimental design

In order to formulate test diets (TSDs), mixture of *M. oleifera* seed meal (MOSM) and *M. oleifera* leaf-meal i.e. MOLM was used as basic component. Experimental diet was partitioned into one control and five TSDs and these diets were added with different phytase levels (0, 300, 600, 900, 1200 and 1500, FTU/kg). For each treatment triplicate tanks were used. Fifteen fingerlings of experimental fish, with an average weight (8.07 ± 0.041 g fish⁻¹) were kept in triplicate tanks and fed 4% of their live wet body weight for 90-days. All TSDs were compared with non-supplemented and other TSDs to evaluate nutrients digestibility, carcass composition and hematological parameters by utilizing Completely Randomized Design (CRD).

2.2. Processing of Moringa by-products and formation of feed pellets

Procured seeds and collected leaves were dried by placing shadowy place for at least six days to prevent the denaturation of vitamins by photo dynamic reactions and were ground to produce fine powder. Other ingredients for feed, procured from feed mill and were examined for their composition (Table 1) as per AOAC (1995) methods before the diet formulation. All the ingredients, were finely ground (0.3 mm sieve size) and mixed up at appropriate concentration and distilled water (DW) was added for preparation of apposite texture dough for formulation of pellets (Lovell, 1989). To prepare the required phytase enzyme concentrations 50 ml DW was used and then sprayed on each TSD (Robinson et al., 2002). In order to maintain equivalent amount of moisture, similar quantity of DW was sprayed on Control diet. After drying all the sprayed diets were stored (4 °C) until use.

2.3. Fingerlings feeding and collection of sample

After approximately two hours of feeding, drained the remaining feed and refill the tanks. Fecal collection was done from each triplicate cautiously to prevent wreckage of thin fecal strings to reduce the nutrients leaching. Feces were oven dried for 3–4 h at 65 °C and homogenized to store for further chemical analysis.

2.4. Chemical analysis of feed, feces and carcass

Afterward, 90 days of feeding trial, moisture contents of TSDs, feces and carcass of fish were checked by oven drying at 105 °C for about 12 h. Analysis of EE i.e. crude fat (Soxhlet system) and CP i.e. crude protein (Micro Kjeldahl Apparatus) was done as approved methods. Loss on ignition of dried defatted samples by digestion (with 1.25% H₂SO₄ and 1.25% NaOH) method was used for the analysis of crude fibres (CF). For carbohydrates estimation formula was used i.e. CH₂O (%) = 100 – (EE% + CP% + Ash% + CF%). The gross energy (GE) was estimated by using oxygen bomb-calorimeter.

ADC% of TSDs was estimated by the formula (NRC, 1993).

ADC (%) = 100 – 100

$$\times \frac{\% \text{ marker in TSDs} \times \% \text{ nutrient in faeces}}{\% \text{ marker in faeces} \times \% \text{ nutrient in TSDs}}$$

Table 1
Physical and chemical ingredients composition (%) of diet (Dry matter basis).

Physical composition (%) of test diet		Chemical composition (%) of ingredients					
Ingredients	TSD composition	CP (%)	EE (%)	CF (%)	Ash (%)	GE (kcal/g)	Carbohydrates
MOSM + MOLM	35	32.22	4.02	14.05	9.27	3.98	36.46
FM	15	48.17	7.12	1.12	24.66	2.65	16.28
SYBM	15	32.51	4.58	1.23	7.36	4.35	49.97
Rice polish	8	12.38	13.46	12.74	10.17	3.18	48.07
Wheat flour	17	10.15	2.3	2.67	2.06	2.95	79.87
Fish-oil	6						
Vitamin-mix*	1						
Chromic oxide	1						
Vitamin-C	1						
Mineral-mix**	1						

MOLM = *M. oleifera* leaf meal MOSM = *M. oleifera* seed meal FM = fish meal SYBM = Soybean meal CP = crude protein EE = Ether Extract CF = Crude fiber GE = Gross energy. * Vitamin premix/kg: Vitamin D₃: \$3,000,000 IU, Vitamin E: 30000 IU, Vitamin A: 15,000,000 IU, Vitamin B₁: \$3000 mg, Vitamin B₂: 7000 mg, Vitamin B₆: 4000 mg, Vitamin B₁₂: 40 mg, Vitamin C: \$15,000 mg, Vitamin K₃: 8000 mg, Nicotinic acid: 60,000 mg, Calcium pantothenate: 12,000 mg, Folic acid: \$1500 mg. ** Mineral premix/kg: Zinc (Zn): \$3000 mg, Manganese (Mn): \$2000 mg, Calcium (Ca): 155 g, Copper: 600 mg, Iron (Fe): 1000 mg, Cobalt (Co): 40 mg, Selenium (Se): \$3 mg, Sodium (Na): 45 g, Iodine (I): 40 mg, Phosphorous (P): 135 g, Magnesium (Mg): 55 g.

2.5. Haematological study

Fish fingerlings from each trial were immobilized using clove oil (60 mg/L) for at least 05 min. Being less soluble, oil was first solubilized in alcohol (Peake, 1998; Coyle et al., 2004). Blood was obtained from anaesthetised fish (caudal vein) by heparinized syringe. Afterward, samples were shifted in Lab for analysis of haematological parameters. Capillary tubes in Micro-haematocrit techniques were used to estimate haematocrit (Brown, 1980). For the measurement of WBCs and RBCs were counted haemocytometer (Neubauer count chamber) was used (Blaxhall and Daisley, 1973). Hb was estimated as illustrated by Wedemeyer and Yastuke (1977). To measure mean corpuscular haemoglobin concentration (MCHC); mean cell volume (MCV) and mean corpuscular haemoglobin (MCH) following equations were used:

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

$$\text{MCH} = \text{Hb/RBC} \times 100$$

$$\text{MCV} = \text{PCV/RBC} \times 100$$

2.6. Statistical analysis

Finally, statistics of ADC% of nutrients (CP, EE and GE), hematological parameters (RBCs, WBCs, Hb, PLT etc.) and carcass of *C. catla* were prone to one-way Analysis of Variance. For the comparison of difference among all the treatments and considerably significant at $p < .05$ Tukey's-Honesty-Significant Difference-test was applied (Snedecor and Cochran, 1991). The Co-Stat-Computer software was utilized for statistical analysis.

3. Results

It was notable that all the diets prepared by using Moringa by-products i.e. MOSM and MOLM mixture contain an equal amount

of nutrients i.e. EE, CP and GE (Table 2). Nevertheless, analyzed composition of nutrients in feces, excreted by *C. catla* fingerlings was statistically ($p < .05$) different, when fingerlings were fed on MOSM and MOLM mixture-based diets (Table 3). Results indicated that there was a minimum nutrient discharge through feces and maximum nutrients were retained in fish body, when fingerlings were fed the 900, FTU/kg, accompanied by 600, FTU/kg level-based diet. Though, feeding on non-supplemented feed has resulted in maximum nutrient discharge in water via feces. Nutrient discharge through feces in water began to decrease from 300, FTU/kg to 900, FTU/kg level, then incremented at higher doses (1200 and 1500 FTU/kg diet) of phytase (Table 3).

Furthermore, it was noted that the fish fed on phytase included Moringa by-products-based diets showed improved ADC% of nutrients in contrast with the non-supplemented diet. Lowest values of nutrients digestibility were observed when fingerlings were fed at 0 FTU/kg diet resulted in lowest ADC% of nutrients (i.e. EE 49%, CP 47%, and GE 53%). The ADC% of nutrients were enhanced with increased phytase supplementation at 300, FTU/kg level-based diet and were found maximum at 900, FTU/kg diet. It was found that percent ADC of nutrients could not augment on further phytase addition (1200 and 1500 FTU/kg levels). Results showed that there was maximum ADC of nutrients (GE 71%, CP 71% and EE 78%) observed at 900, FTU/kg followed through 600, FTU/kg level-based diet with percent CP, GE and EE of 65%, 67% and 69%, respectively (Table 4). These average values were statistically higher ($p < .05$) in contrast with non-supplemented and other TSDs. These results provide strong evidence that lower nutrient discharge resulted in higher percentage of ADC in term of nutrients when fingerlings were fed on Moringa by-products-based diets supplemented with phytase. Moreover, highest nutrients ADC% and lowest discharge was documented in fish fed at 900, FTU/kg, that is the optimal phytase level addition in Moringa by-products-based diets.

Table 2
Analyzed compositions of diets for *C. catla* fed on MOSM + MOLM mixture based phytase added TSDs.

TSDs	Phytase levels (FTU/kg)	CP (%)	EE (%)	GE (kcal g ⁻¹)
TSD-I (non-supplemented)	0	31.33 ± 0.01	7.51 ± 0.05	3.21 ± 0.03
TSD-II	300	31.25 ± 0.02	7.51 ± 0.02	3.18 ± 0.04
TSD-III	600	31.37 ± 0.02	7.50 ± 0.04	3.17 ± 0.05
TSD-IV	900	31.39 ± 0.02	7.50 ± 0.08	3.16 ± 0.04
TSD-V	1200	31.27 ± 0.04	7.49 ± 0.09	3.17 ± 0.04
TSD-VI	1500	31.29 ± 0.03	7.50 ± 0.05	3.19 ± 0.05

Data are means of 3 duplicates (±shows Standard Deviations).

Means within column having similar superscripts presenting that diets are isoenergetic and isocaloric.

Table 3
Analyzed compositions of nutrients in *C. catla* fingerlings feces fed on phytase added MOSM and MOLM mixture based TSDs.

Experimental diets	Phytase levels (FTU/kg)	CP (%)	EE (%)	GE (kcal g ⁻¹)
TSD-I (non-supplemented)	0	17.43 ± 0.11 ^e	4.07 ± 0.04 ^a	1.64 ± 0.05 ^e
TSD-II	300	14.62 ± 0.11 ^d	3.48 ± 0.09 ^b	1.42 ± 0.07 ^{cd}
TSD-III	600	11.63 ± 0.18 ^b	2.45 ± 0.07 ^d	1.12 ± 0.5 ^b
TSD-IV	900	9.72 ± 0.11 ^a	1.74 ± 0.09 ^e	0.99 ± 0.03 ^a
TSD-V	1,200	13.65 ± 0.13 ^c	2.87 ± 0.11 ^c	1.32 ± 0.04 ^c
TSD-VI	1,500	14.95 ± 0.08 ^d	3.36 ± 0.06 ^b	1.47 ± 0.03 ^d

Data are means of 3 duplicates (±shows Standard Deviations).

^{a-e}Means inside the column with dissimilar superscripts are significantly dissimilar at $p < 0.05$.

Table 4
Apparent nutrient digestibility of *C. catla* fingerlings on phytase added MOSM and MOLM mixture based TSDs.

Experimental diets	Phytase levels (FTU/kg)	CP (%)	EE (%)	GE (%)
TSD-I (Control diet)	0	47.26 ± 0.62 ^e	48.59 ± 0.88 ^e	51.52 ± 0.89 ^e
TSD-II	300	55.64 ± 0.45 ^d	56.03 ± 0.92 ^d	57.57 ± 0.97 ^d
TSD-III	600	64.76 ± 0.59 ^b	68.93 ± 0.75 ^b	66.55 ± 0.90 ^b
TSD-IV	900	71.19 ± 0.14 ^a	78.39 ± 0.73 ^a	70.96 ± 0.61 ^a
TSD-V	1200	58.61 ± 0.15 ^c	63.73 ± 0.75 ^c	60.61 ± 0.68 ^c
TSD-VI	1500	54.55 ± 0.24 ^d	57.43 ± 0.93 ^d	56.15 ± 0.97 ^d

Data are means of 3 duplicates (±shows Standard Deviations).

^{a-e}Means inside the column with dissimilar superscripts are significantly dissimilar at $p < 0.05$.

Our results make it clear, that phytase addition played major role in improving carcass composition in fish fed phytase included Moringa by-products-based diets. These results indicated that there were lowest contents of ash (7%), crude fibre (1%) and carbohydrates (12%) in fish body at 900, FTU/kg, was statistically higher ($p < .05$) from the values examined at non-supplemented and other TSDs. Whilst the highest crude ash (10%), crude fiber (2%) and carbohydrate (21%) values were noticed in fish which fed on TSD-I (without phytase addition). In similar way, highest moisture contents (8%) were recorded at 0 FTU/kg diet.

Highest values of nutrient digestibility (EE 13% and CP 61%) in whole fish body were examined at 900, FTU/kg level of phytase included MOSM + MOLM mixture based diet. It was found that the said values were statistically higher ($p < .05$), from the values of other fish carcass followed (58% and 12% respectively) at 600, FTU/kg level based diet. While, lowest EE (9%) and CP (49%) in whole fish body were examined in fish that fed on TSD-I (without phytase supplementation). It was observed that EE and CP contents in fish increased when fingerlings fed 300, FTU/kg diet, reached its ultimatum value at 900, FTU/kg level mixture-based TSD (See Table 5). Furthermore, increased supplementation (1200 and 1500 FTU/kg diet) did not further improve the nutrient (CP and EE) contents in *C. catla* fingerlings body. These results indicate that mixture of MOLM and MOSM based diet supplemented with 900, FTU/kg phytase level is optimum supplemented for highest CP and EE contents in fish body for better and improved growth.

Table 5
Carcass composition (%) of fingerlings carcass fed MOSM and MOLM mixture-based phytase added diets after ninety days feeding trail.

Carcass parameters	TSD-I (non-supplemented)	TSD-II	TSD-III	TSD-IV	TSD-V	TSD-VI
	Phytase Levels (FTU/kg)	300	600	900	1,200	1,500
Fat	9.50 ± 0.07 ^f	10.14 ± 0.07 ^e	12.34 ± 0.08 ^b	13.49 ± 0.09 ^a	11.54 ± 0.06 ^c	10.71 ± 0.06 ^d
Protein	49.13 ± 0.21 ^e	52.87 ± 0.13 ^d	58.48 ± 0.15 ^b	61.04 ± 0.12 ^a	56.30 ± 0.14 ^c	53.07 ± 0.17 ^d
Carbohydrate	21.01 ± 0.21 ^a	17.90 ± 0.38 ^b	13.59 ± 0.15 ^d	11.66 ± 0.18 ^e	14.60 ± 0.11 ^c	18.20 ± 0.38 ^b
Ash	9.92 ± 0.04 ^a	9.29 ± 0.07 ^b	7.74 ± 0.06 ^c	6.90 ± 0.04 ^f	8.49 ± 0.05 ^c	8.15 ± 0.06 ^d
Crude fiber	2.15 ± 0.13 ^a	1.95 ± 0.08 ^{ab}	1.26 ± 0.08 ^d	1.09 ± 0.07 ^d	1.51 ± 0.07 ^c	1.79 ± 0.09 ^b
Moisture	8.29 ± 0.06 ^a	7.84 ± 0.08 ^c	6.59 ± 0.08 ^e	5.82 ± 0.07 ^f	7.56 ± 0.0 ^d	8.06 ± 0.08 ^b

Data are means of 3 duplicates (±shows Standard Deviations).

^{a-f}Means inside the rows with dissimilar superscripts are statistically dissimilar at $p < 0.05$.

Table 6 demonstrate that there were significant effects of phytase enzyme on hematology of fingerlings fed mixture of MOSM and MOLM based diet in contrast to the fish that were fed on the non-supplemented diet. According to results, statistically ($p < .05$) maximum values of WBCs ($8.01 \times 10^3 \text{mm}^{-3}$), RBCs ($2.88 \times 10^6 \text{mm}^{-3}$) and Hb (8.92 g/100 ml) found in fish fed at 900, FTU/kg diet and second higher Hb (8.54 g/100 ml), RBCs ($2.54 \times 10^6 \text{mm}^{-3}$) and WBCs ($7.55 \times 10^3 \text{mm}^{-3}$) values were found in fingerlings fed the 600, FTU/kg level based diet. Whereas the lowest WBCs ($6.95 \times 10^3 \text{mm}^{-3}$), Hb (6.99 g/100 ml) and RBCs ($1.27 \times 10^6 \text{mm}^{-3}$) were found in fish that fed on the non-supplemented diet, was statistically differ from fingerlings fed the other experimental TSDs. These results also indicate that the values of WBCs, RBCs as well as Hb were began to increase when fish were fed at 300, FTU/kg diet and reached highest in fish that fed on 900, FTU/kg diet. Furthermore, it was also noted that further supplementation of phytase (1200 and 1500 FTU/kg level) did not enhance the number of WBCs, RBCs and Hb in fish. Although, maximum PLT (64.47) and PCV (26.15%) were found in fish fed the 1200 FTU/kg diet followed by (25.18% and 63.92, respectively) at 900, FTU/kg diet and was prominently higher ($p < .05$) from the fish that fed on non-supplemented diet. Nevertheless, least number of PCV (20.2%) and PLT (54.07) was examined in fingerlings fed with non-supplemented feed. It was also recognized that maximum MCV (199.7 fl), MCH (56.42 pg) and MCHC (36.65%) values were observed in fingerlings fed the 1500 FTU/kg level-based diet. These

Table 6
Hematological parameters of fingerlings fed MOSM + MOLM mixture-based non-supplemented and phytase included TSDs.

Hematological indices	TSD-I (non-supplemented) Phytase Levels (FTU/kg)	TSD-II	TSD-III	TSD-IV	TSD-V	TSD-VI
	0	300	600	900	1200	1500
WBC (10^3 mm^{-3})	6.95 ± 0.13 ^d	7.19 ± 0.10 ^{cd}	7.55 ± 0.12 ^b	8.01 ± 0.13 ^a	7.44 ± 0.09 ^{bc}	7.21 ± 0.08 ^{cd}
RBC (10^6 mm^{-3})	1.27 ± 0.04 ^e	1.73 ± 0.06 ^d	2.54 ± 0.14 ^b	2.88 ± 0.20 ^a	2.14 ± 0.10 ^c	1.86 ± 0.11 ^{cd}
Hb (g/100 ml)	6.99 ± 0.11 ^d	7.34 ± 0.06 ^{cd}	8.54 ± 0.08 ^b	8.92 ± 0.12 ^a	7.70 ± 0.12 ^c	7.42 ± 0.24 ^c
PCV (%)	20.20 ± 0.22 ^e	21.43 ± 0.06 ^d	26.06 ± 0.14 ^a	25.18 ± 0.20 ^b	26.15 ± 0.19 ^a	23.71 ± 0.11 ^c
PLT	54.07 ± 0.18 ^f	59.43 ± 0.13 ^d	61.47 ± 0.13 ^c	63.92 ± 0.17 ^b	64.47 ± 0.09 ^a	58.55 ± 0.14 ^e
MCV (fl)	102.42 ± 0.25 ^e	90.95 ± 0.08 ^f	156.52 ± 0.13 ^b	153.03 ± 0.16 ^c	149.58 ± 0.22 ^d	199.66 ± 0.21 ^a
MCH (pg)	27.40 ± 0.29 ^e	23.52 ± 0.16 ^f	36.57 ± 0.28 ^c	44.93 ± 0.09 ^b	34.74 ± 0.21 ^d	56.42 ± 0.06 ^a
MCHC (%)	24.35 ± 0.11 ^e	22.30 ± 0.17 ^f	31.11 ± 0.21 ^c	34.11 ± 0.15 ^b	27.11 ± 0.21 ^d	36.65 ± 0.10 ^a

Data are means of 3 duplicates (±shows Standard Deviations).

^{a-f}Means inside the rows with dissimilar superscripts are statistically dissimilar at $p < 0.05$.

hematological indices revealed that 900, FTU/kg diet is the most appropriate level for the improved hematological indices and resulted in better fish growth as well as improved immune system due to higher count of WBCs.

From these findings it could be inferred that phytase supplementation is crucial for the improvement of hematological indices, nutrient digestibility and fish carcass in contrast to non-supplemented feed. Maximum improvement in all the said parameters was observed in fingerlings that were fed at 900, FTU/kg level based diet.

4. Discussion

The existence of phytic acids compound in oilseed meal based diets may account for adverse effects on nutrient digestibility in fish (Hussain et al., 2015a, 2015b). In addition to, it can bind with essential amino acids in different species of fish, that mitigate bioavailability of nutrients, especially proteins (Usmani and Jafri, 2002). Current study has established a strong evidence, that phytase supplementation in MOSM + MOLM based diet was practically useful for enhancing the ADC% of nutrients for *C. catla* when compared with non-supplemented feed. According to the present results, level of 900, FTU/kg was the optimal phytase inclusion for the maximum amelioration in the nutrient digestibility in contrast with other test and non-supplemented diet. Likewise, in one of the studies, maximum improvement in nutrient digestibility was examined in *C. catla* fingerlings fed phytase substituted MOSM based diet (Hussain et al., 2017; Shahzad et al., 2018) and MOLM based diet (Shahzad et al., 2020). They found maximum CP (72%), GE (74%) and EE (80%) digestibility when the fingerlings were fed at 900, FTU/kg level-based diet. Almost similar to our results, Hussain et al. (2015a) found higher digestibility of EE, CP and GE in mori fingerlings when they fed soybean meal (SYBM) based TSD with phytase inclusion at 1000, FTU/kg of diet. Whereas, maximum CP, GE and EE digestibility was observed in raho fingerlings fed phytase included SYBM based diet (Baruah et al., 2007a), canola, cottonseed and sunflower meal based diets (Hussain et al., 2011, 2015a) at approximately 750, FTU/kg level. Analogous to present findings, they also observed a decline in digestibility values at higher doses of phytase addition. Whereas, Sardar et al. (2007) found maximum ADC% of gross energy, fat and protein in fish fed on a low level of phytase substituted plant-based diet. They suggested that the 500, FTU/kg level is optimal for the improvement of ADC% of CP, EE and GE and amino acid profile in *Cyprinus carpio*. On the other hand, Nwanna and Bello (2014) found a high dose i.e. 8000, FTU/kg as the optimal phytase level for maximal digestibility of EE, GE and CP in tilapia fed SYBM-based diet. Conversely, non-significant effects of phytase addition on EE, GE and CP digestibility were examined in salmon (Sajjadi and Carter, 2004), rainbow trout (Dalsgaard et al., 2009). Generally,

250–1500 FTU/kg diet of phytase addition in oilseed by-products is studied as optimum level for higher percentage of ADC of EE, CP and GE in fish. However, variability of optimum phytase supplementation level depends on composition of ingredients, presence or absence of stomach, feed formulation, feed processing technology, pH, type of phytase, methods of using phytase, along with fish species. Therefore, phytase supplementation in plant by-products-based diet should be utilized after considering these factors (Cao et al., 2007).

It was observed that phytate was chelated complex binds with fatty acids, protein contents and gross energy etc. making them inaccessible to fingerlings causing poor composition of body (Cao et al., 2007). Present results indicate that phytase supplementation is crucial for the breakdown of phytate complex resulting in higher nutrient retention in *C. catla* in-contrast to fingerlings fed on non-supplemented diet. Highest retaining of EE and CP was found in fingerlings fed on 900, FTU/kg level-based diets in-contrast to non-supplemented and other supplemented TSDs. Though, lowest EE% and CP% in fish were recorded at 0 FTU/kg level-based diet as evaluated by Shahzad et al. (2018). Similarly, various scientists found higher protein retaining capacity of fish when they were fed phytase included oilseed by-products-based diets (Khajepour et al., 2012). In contrast to present results, Nwanna et al. (2008) observed non-significant ($p < .05$) differences in concentrations of proteins in *Colossoma macropomum* (Amazon tambaqui) body when fed on mixture of Brazil nut and leucaena leaf meal. The improved retention of nutrients with phytase included diets can also enhance fish body mineralization peculiarly in the developmental period (Cao et al., 2007). However, phytase addition at higher levels may decrease the carcass composition in contrast with the fish fed on non-supplemented diet. Advancing nutrient biodigestibility would positively affect body composition and bone strength meanwhile the fish fed on phytase included SYBM-based diet (Sardar et al., 2007). Inclusion of phytase can hydrolyze chelated phytate structure and may enhance the nutrients retention in fish body, resulting in better fish muscle composition (Liebert and Portz, 2005).

Results of current study showed that EE and CP in fish body began to increase from TSD-II (300, FTU/kg) and attains highest value when fingerlings fed on 900, FTU/kg level in Moringa by-products-based diets. Besides this optimum level, it could not increase with increasing level of enzyme addition upto 1500, FTU/kg level. Similar positive findings were also observed in catfish (Kim and Hung, 2007); raho (Baruah et al., 2007a) and common carp (Phromkunthong et al., 2010) when these species were fed on phytase included diet. Akpoilih et al. (2016) found that, phytase included (from 750, FTU/kg to 1000, FTU/kg levels) SYBM-based diets resulted higher nutrient retention in *C. gariepinus* in contrast with non-supplemented diet. Whereas, Hung et al. (2015) found that inclusion of dietary inclusion of phytase (1500, FTU/kg) in

SYBM based diet statistically ($p < .05$) increased the EE and CP retention in *Pangasianodon hypophthalmus* (Tra catfish).

While, maximum EE in body of rainbow trout, *O. mykiss* was recorded at lower dose (500, FTU/kg) when compared to current findings when fingerlings fed plant-based diet (Cheng, 2004). Ai et al. (2007) documented higher level of EE contents in whole body of Japanese seabass (*Lateolabrax japonicus*) when fed on phytase included diet but it didn't differ statistically ($p < .05$) from that fish which were fed on non-supplemented diet. In present study, it was noteworthy that further increase of phytase addition at 1200, and 1500, FTU/kg levels in fish feed could not enhance CP and crude lipid retention in fingerlings body that was in optimal ranges (250–1500, FTU/kg) as reported by Cao et al. (2007). In comparison to our results, Olusola and Nwanna (2014) found that crude fiber was recorded minimum at elevated dose i.e. 2000, FTU/kg level-based diet. This difference among the results was may be due to the different phytase type, fish species or plant by-products used in diet (Baruah et al., 2004). Reason for this non-improvement is intricate to explain nevertheless, it can be proposed that care should be taken during phytase supplementation in fish feed. This discrepancy in phytase inclusion levels might be because of the ingredient composition, stomach presence, fish species as well as characteristics and types of phytase (Baruah et al., 2007b).

In general, chelated structure of phytate present in plant by-products influences fish performance, including fish hematology (Ehsani and Torki, 2010). As phytate chelates with iron, that is essential for RBCs, causes reduced oxygen carrying capacity in body (Spinelli et al., 1983). Nutrients digestibility has potential influence on almost all features of the fish hematology including immune system (Kubena and McMurray, 1996). Supplementation of phytase in plant-based diet improves the minerals in animal blood (Singh et al., 2003). It was on record in many studies that immune system played vital role for defense system against infectious pathogens and was markedly affected by absorption of nutrients and minerals (Shiau and Su, 2003; Baruah et al., 2009). According to present results, maximum values of WBCs, RBCs and Hb were found at 900, FTU/kg level in Moringa by-products-based diet. Although, lowest values of WBCs, RBCs and Hb were found in blood of fish when fed on non-supplemented diet prepared by using Moringa by-products only (0 FTU/kg). It was found that phytase included fish feed improves the hematological parameters of *C. catla* fingerlings. Phytase supplementation is recommended as a powerful stimulator of immune system in fish, resulting in improved number of monocytes (macrophages) and resulting in high number of blood cells production in monogastric animals (Ehsani and Torki, 2010). On contrary to current study findings, Baruah et al. (2009) found non-significant ($P > .05$) effects on WBCs and RBCs in rahu fingerlings fed on phytase included (500, FTU/kg level) SYBM-based diet. Although, RBCs and WBCs were found higher in *C. carpio* at 500, FTU/kg level supplemented in soya-protein based diet (Sardar et al., 2007). In another study, it was found that when phytase was used in *Gadus morhua* (Atlantic cod) feed, resulted in higher number of WBCs of fish in contrast with fish fed on non-supplemented diet (Lazado et al., 2010). Hemoglobin (Hb) level in fish, fed phytase included diet was relatively higher than the fish fed a diet without phytase but significantly ($p < .05$) not variable from other dietary treatments (Yoo and Bai, 2014). Sardar et al. (2007) also examined maximum values of Hb and hematocrit at 500, FTU/kg level that was in normal range of these mentioned indices. Maximum RBCs and WBCs were counted in *O. niloticus* fed on phytase treated SYBM at 500, FTU/kg level-based diet when compared to fish fed on Jatropha meal-based diet at similar level of phytase addition (Kumar et al., 2010). There are not sufficient evidences in literature about influential effect of phytase on hematological responses of *C. catla* fingerlings. Current results depicted that highest values of PLT and

PCV were maximum in fish fed at 1200, FTU/kg level-based MOSM + MOLM based diets. Baruah et al. (2009) concluded that, fish were fed a diet containing both citric acid and phytase, released higher amount of Fe and Cu from chelated phytate, which resulted in optimal increase in hemoglobin and hematocrit values of fish.

Present work provides enough evidences, that inclusion of phytase breakdown the phytic acid present in Moringa by-products-based diets and augment overall performance of *C. catla* fingerlings. Moreover, it was also found that 900, FTU/kg is the optimum level of phytase inclusion in Moringa by-products-based diets for the maximum improvement in fish performance when compared to other phytase included TSDs and non-supplemented diet and is an ideal approach towards cost effective and environment friendly feed. It was also observed that phytase addition improves the feed quality as compared to expensive FM by using low-cost moringa by-products-based diet.

Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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