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Carcass composition and hematological study of *Catla catla* fingerlings fed on phytase supplemented *Moringa oleifera* leaf meal (MOLM) based diet

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Abstract

Present study was conducted to investigate carcass composition and hematological parameters of *Catla catla* fingerlings fed on phytase supplemented *Moringa oleifera* leaf meal (MOLM) based diet. Graded levels (0, 300, 600,900, 1200 and 1500 FTU kg^{-1}) of phytase enzyme were used to prepare one control and five test diets. *Catla catla* fingerlings were fed twice a day at the rate of 4% of wet weight. After 90 days trial, four fish from each tank were sacrificed after taking blood from caudal vein to analyze hematological parameters. Fish fed MOLM based test diet supplemented with phytase at 900 FTU kg^{-1} level showed maximum retention of crude protein and crude fat in fish body as compared to fish fed on control diet (without phytase supplementation). Carbohydrate, crude fiber, moisture and ash values were highest at control diet whereas lowest contents were recorded at 900 FTU kg^{-1} level. Results of hematological study also showed that the maximum values of RBCs (2.66±0.08 $10^6 mm^{-3}$), WBCs (7.68±0.09 $10^3 mm^{-3}$) and Hb (8.74±0.17 g/100ml) were found at 900 FTU kg^{-1} level of phytase supplementation. Whereas highest PCV and PLT count was observed at 600 FTU kg^{-1}. It was concluded that use of phytase at 900 FTU kg^{-1} level improved carcass composition and hematological parameters of *C.* catla fingerlings without any side effect on fish performance.

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Introduction

Fish is the most important source of protein and it also needs a higher amount of protein in diets. As protein is the most expensive component of fish feed. Therefore, efforts to reduce feed cost have resulted in increased use of plant proteins as alternatives for expensive fish meal (FM) protein in diet (Ping *et al.*, 2010). FM is a major protein source in aqua feeds for different fish species because it is a main source of vital nutrients (Dawood *et al.*, 2015). Whereas increasing demand, unstable supply and high cost of the FM with the development of aquaculture made it necessary to search for alternative protein sources (Tacon and Metian, 2008; FAO, 2014). Use of plant protein sources based diets has been proposed as alternatives of FM (Tiamiyu *et al.*, 2016). *M. oleifera* constitutes of an excellent source of vitamins, minerals and amino acids. (Thiam *et al.*, 2015).

Different studies have been conducted by using various plant leaf meal protein and showed that plant leaf meal may be good source of protein i.e. on cassava leaf meal (Ng and Wee, 1989); on *Alfalfa* (Yousif *et al.*,1994) and on *Carica papaya* (Reyes and Fermin, 2003). Moringa leaves have a relatively high crude protein content which varies from 25% (Makkar and Becker, 1996) to 32% (Soliva *et al.*, 2005).

These are the best source of high amount of nutrients i.e. crude protein, crude fat and gross energy etc. (Bosh, 2004; Grubben and Denton, 2004). A high proportion of this protein is potentially available for digestion due to a higher level of pepsin soluble nitrogen (82-91%) and low proportion (1-2%) of acid detergent insoluble protein (Makkar and Becker, 1996).

The use of plant by-products is still much less (up to 25% of substitution) because of the large amount of indigestible carbohydrates and presence of anti-nutritional factors i.e. phytate (Laining *et al.,* 2011). Higher phytate contents results in an adverse impact on carcass composition and retention of nutrients, especially protein (Soltan 2009).

The most efficient way to reduce the effect of phytate is the use of phytase enzyme (Hussain *et al.*, 2016). Use of dietary phytase is an effective method to improve the carcass composition and decreases the aquatic pollution by maximum digestion and absorption of nutrients in fish body (Danwitz *et al.*, 2016). Phytase supplem-entation is necessary for the improvement in carcass composition when monogastric or agastric fish fed on plant based diet (Yoo and Bai, 2014).

Phytase supplemented plant by-product meal based diet enhanced the deposition of nutrients in fish body (Vielma *et al.*, 1998). Complete hydrolysis of phytic acid in plant based diet after phytase supplementation improves overall fish performance (Storebakken *et al.*, 1998). An appropriate recommendation of suitable inclusion of phytase in diet is needed to provide the actually required nutrients for the animals to avoid or minimize the side effects of enzyme supplementation (Lei and Porres, 2003),

Hematological techniques, including erythrocyte count, Hb concentration, haematocrit and RBCs count, have provided valuable knowledge for fish Zoologists in the development of fish health and in monitoring stress responses (Hrubec *et al.,* 2000) whereas WBCs counts can be applied as a measure of general immune response (Blaxhall 1972; Soivio and Oikari 1976).

In past, most of the studies were conducted to determine the normal range of hematological parameters and to elaborate the physiological process related with blood components when fish fed on plant based diet (Rainza-paiva *et al.,* 2000). *Catla catla* commonly called Thaila, one of the indigenous carp specie of south Asia that is cultured in government and private sector under composite as well as poly-culture system. Less research work is reported on this important fish in past. Study of carcass composition and hematological parameters provide us information about the improvement in fish health. Phytate is anti-nutritional factor that plays a negative impact on

carcass composition and nutrients retention in fish body thus there is need to investigate the optimal phytase supplementation in fish feed to promote nutrient utilization in fish. Therefore, the present study was conducted to determine the effect of phytase supplementation in MOLM based diet on carcass composition and hematological parameters of *C. catla* fingerlings.

Materials and methods

The present research work was conducted to study the carcass composition and hematological parameters of *Catla catla* fingerlings fed on phytase supplemented *Moringa oleifera* leaf meal (MOLM) based diet. Experimental work was performed in the Fish Nutrition Laboratory, Department of Zoology, Government College University Faisalabad, Pakistan.

Fish and experimental conditions

C. catla fingerlings were acquired from the local fish seed hatchery and were acclimatized to the lab conditions in V-shaped fish tanks (having 70 L water capacity) for two weeks. Fingerlings were treated with saline solution for 5-7 minutes to kill the pathogens (Rowland and Ingram, 1991).

During acclimatization period the fingerlings were fed once a day on basal diet (Allan and Rowland, 1992). Parameters related to water quality were monitored using specific apparatus. Air pump was used to supply the oxygen by capillary system throughout the experimental period.

Experimental design

Moringa by-product such as MOLM was used as test ingredient to formulate experimental diet. Experimental diet was supplemented with graded levels (0, 300, 600, 900, 1200 and 1500 FTU kg^{-1}) of phytase to formulate one control (0 FTU kg^{-1}) and five test diets. 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 weight. Fish fed on different phytase supplemented MOLM based test diets were compared with each other and control fish to study carcass composition and hematological parameters by using Completely Randomized Design (CRD).

Processing of Moringa oleifera leaves

Moringa oleifera leaves were collected from the local garden and were washed to remove the dirt and dust particles. The leaves were drained appropriately and dried under 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 fibers in the MOLM based diet. Processed moringa leaves were grinded in the form of powder to pass through 0.3 mm sieve size and analyzed for chemical composition following (AOAC, 1995).

Formation of pellets

The feed ingredients (Table 1) that were procured from private feed mill, finely grinded to pass through 0.3 mm sieve size and analyzed for chemical composition following (AOAC, 1995) prior to the formulation of the experimental diets (Table 2).

All ingredients were mixed in a mixer for 5 minutes and fish oil was gradually added thereafter. Feed was blended slowly into the mixer after adding 10-15% of water, resulting in suitably textured dough and was processed by experimental extruder (SYSLG30-IV Experimental Extruder) to make floating pellets (Lovell, 1989). One control and five test diets were prepared using MOLM by spraying graded levels (0, 300, 600, 900, 1200 and 1500 FTU kg^{-1}) of phytase.

The required concentrations (300, 600, 900, 1200 and 1500 FTU kg^{-1}) of phytase (Phyzyme® XP 10000 FTU g–1; Danisco Animal Nutrition, Fin-65101 Vaasa, Finland) were prepared in 25 mL of 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 a similar amount of distilled water to maintain the equivalent amount of moisture. All the sprayed diets were dried in shady place and stored at 4°C until use.

Table 1. Chemical composition (%) of feed ingredients.

Ingredients	Dry matter (%)	Crude Protein (%)	Crude Fat (%)	Crude Fiber (%)	Ash (%)	Gross Energy (kcal/g)	Carbohydrates
MOLM*	91.83	28.95	2.73	19.45	8.91	3.98	35.98
Fish meal	91.67	48.17	7.12	1.12	24.66	2.65	16.29
Rice polish	94.06	12.38	13.46	12.74	10.17	3.18	48.07
Wheat flour	92.40	10.15	2.3	2.67	2.06	2.95	79.87
Corn gluten 60%	92.34	59.55	4.58	1.23	1.36	4.35	29.07

*MOLM= *Moringa oleifera* leaf meal

Table 2. Ingredients composition (%) of test diets.

Ingredients	Control diet	Test Diet-I	Test Diet-II	Test Diet-III	Test Diet-IV	Test Diet-V
MOLM	35	35	35	35	35	35
Phytase Level (FTU kg^{-1})	0	300	600	900	1200	1500
Fish oil	6	6	6	6	6	6
Rice polish	8	8	8	8	8	8
Fish meal	15	15	15	15	15	15
Soybean meal	15	15	15	15	15	15
Vitamin C	1.0	1.0	1.0	1.0	1.0	1.0
Wheat flour*	17	17	17	17	17	17
Vitamin Premix	1.0	1.0	1.0	1.0	1.0	1.0
Mineral Premix	1.0	1.0	1.0	1.0	1.0	1.0
Chromic oxide	1.0	1.0	1.0	1.0	1.0	1.0

*Phytase enzyme was used at the expense of wheat flour

Feeding protocol

The fingerlings of *C. catla* were fed at the rate of 4% of live wet weight on their prescribed diet twice a day. After the feeding session of two hours, the uneaten diet was drained out from each tank by opening the valves of the tanks. The tanks were washed completely to remove the particles of diets and refilled with water.

Chemical analysis of whole fish body

After 90 days trial four fish from each tank were sacrificed and dried at room temperature. The dried samples of fish from each treatment were homogenized separately using a mortar and pestle to analyze using standard methods (AOAC, 1995). Oven-drying method was used to determined moisture in whole fish body at 105°C for 12 h. Crude protein (Nx6.25) of whole body sample was analyzed by using Micro Kjeldahl`s (InKjel M behr Labor Technik GmbH D-40599 Dusseldorf) method and crude fat was determined by petroleum ether using Soxhlet system (Soxhlet Extraction Heating Mantels, 250 ml 53868601). Crude fiber contents were determined as loss on ignition of dried lipid-free residues after digestion with 1.25% sodium hydroxide and 1.25% H_2SO_4 whereas ash by ignition in electric furnace (Naberthern B170) at 650°C for 12 hours to constant weight. Total carbohydrates (N-free extract) were calculated by difference, *i.e.*, total carbohydrates % =100-(CP%+ EE%+ CF%+ Ash % +Moisture %).

Blood samples and hematological assay

Blood was taken from caudal vein using heparinized syringe and blood samples were taken to the Molcare Lab, Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan for hematological analysis. Micro-hematocrit technique (Brown, 1980) was used to determined hematocrit by the help of capillary tubes. RBC (Red Blood Cells) and WBC (White Blood Cells) counts were determined with a haemo-cytometer with approved Neubauer counting chamber (Blaxhall and Daisley, 1973). Hb (Hemoglobin) concentration estimates were determined as described by Wedemeyer and Yastuke (1977). The following parameters were used to calculate: MCHC (mean corpuscular hemoglobin concentration); MCH (mean corpuscular hemoglobin) and MCV (mean cell volume) by using the following formulae.

MCHC = Hb/PCV x 100

MCV = PCV/RBC x 10

MCH = Hb/RBC x 10

Statistical Analysis

Finally data of fish carcass and hematology were subjected to one-way analysis of variance (ANOVA) (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

Carcass composition of fish fed phytase supplemented *Moringa oleifera* leaf meal (MOLM) based diet is presented in table 3. Supplementation of phytase enzyme played a significant role in improving nutrient retention in fish when fish fed on MOLM based phytase supplemented diet as compared to the fish fed on control diet. Maximum contents of crude protein (56.45±0.25) and crude fat (13.76±0.12) in fish were found at 900 FTU kg^{-1} level of phytase supplemented MOLM based diet that was significantly different ($P>0.05$) from the fish fed on control as well as other phytase supplemented test diets.

Minimum crude protein and crude fat in fish was observed in fish fed control diet. It was observed that protein and fat retention in fish was started from 300 FTU kg^{-1} level and reached to maximum when fingerlings fed 900 FTU kg^{-1} level of phytase supplemented MOLM based test diet, whereas further increase in phytase supplementation (1200 and 1500 FTU kg^{-1}) resulted in a decreased nutrient retention in fish body.

Highest amount of carbohydrate (24.25±0.55), ash (10.27±0.14), crude fiber, (2.39±0.15) and moisture (8.69±0.20) was recorded in fish fed control diet (0 FTU kg^{-1}) that was significantly ($P>0.05$) different from the fish fed on other test diets (Table 3).

The lowest contents of carbohydrate (16.89±0.15), ash (6.37±0.08), crude fiber, (1.32±0.05) and moisture (5.22±0.19) were found in the fish fed on test diet-III at 900 FTUkg^{-1} level. It was found that 900 FTU kg^{-1} level of phytase supplementation is the most suitable level for the increased deposition of most compulsory nutrients i.e. protein and fat in fish body.

According to results maximum values of RBCs ($2.26\times10^{6}mm^{-3}$), WBCs ($7.68\times10^{3}mm^{-3}$) and Hb (8.74 g/100ml) were noted with MOLM based diet supplemented at 900 FTU kg^{-1} level and it was significantly different ($P>0.05$) from fish fed on control and other test diets (Table 4). The minimum values of RBCs, WBCs and Hb were observed in fish fed on control diet. Highest PCV (27.05±0.16), PLT (63.95±0.19) were noted in fish fed on 600 FTU kg^{-1} level based diet whereas lowest values were observed in the fish fed on control diet (0 FTU kg^{-1}). On the other hand highest values of MCV, MCH and MCHC were observed in the fish fed on 1500 FTU kg^{-1} level and significantly different from the fish fed on control and others test diets. The hematology showed that 900 FTU kg^{-1} level is optimum for healthy fish growth, monitoring stress response and WBCs counts can be applied as a measure of immune response.

Table 3. Proximate composition of *Catla catla* carcass fed phytase supplemented MOLM based diet after 90-days feeding trial.

Diets	Control diet	Test Diet–I	Test Diet –II	Test Diet –III	Test Diet –IV	Test Diet –V
Phytase levels (FTU kg^{-1})	0	300	600	900	1200	1500
Carbohydrate	24.25 ± 0.55^{a}	23.23 ± 0.43^{b}	21.24 ± 0.25^{c}	16.89 ± 0.15^{e}	19.10 ± 0.21^{d}	22.69 ± 0.39^{b}
Protein	44.89 ± 0.28^{f}	46.47 ± 0.24^{e}	49.58 ± 0.34^{c}	56.45 ± 0.25^{a}	52.86 ± 0.22^{b}	47.78 ± 0.23^{d}
Fat	9.51 ± 0.19^{e}	10.47 ± 0.19^{d}	11.49 ± 0.22^{c}	13.76 ± 0.12^{a}	12.53 ± 0.32^{b}	10.10 ± 0.16^{d}
Ash	10.27 ± 0.14^{a}	9.93 ± 0.17^{ab}	8.72 ± 0.14^{c}	6.37 ± 0.08^{e}	7.71 ± 0.13^{d}	9.52 ± 0.22^{b}
Crude fiber	2.39 ± 0.15^{a}	2.03 ± 0.10^{b}	1.87 ± 0.09^{bc}	1.32 ± 0.05^{d}	1.48 ± 0.06^{d}	1.77 ± 0.04^{c}
Moisture	8.69 ± 0.20^{a}	7.87 ± 0.15^{b}	7.09 ± 0.15^{c}	5.22 ± 0.19^{e}	6.32 ± 0.10^{d}	8.14 ± 0.22^{b}

Table 4. Hematological parameters of *Catla catla* fingerlings fed phytase supplemented MOLM based test diets.

Diets	Control diet	Test Diet–I	Test Diet –II	Test Diet –III	Test Diet –IV	Test Diet –V
Phytase levels FTU kg^{-1}	0	300	600	900	1200	1500
WBC (10^3mm^{-3})	6.79 ± 0.09^{d}	6.92 ± 0.05^{d}	7.15 ± 0.08^{bc}	7.68 ± 0.09^{a}	7.31 ± 0.08^{b}	6.98 ± 0.06^{cd}
RBC (10^6mm^{-3})	1.13 ± 0.06^{d}	1.51 ± 0.08^{c}	1.76 ± 0.09^{c}	2.66 ± 0.08^{a}	2.26 ± 0.11^{b}	1.19 ± 0.18^{d}
PCV (%)	18.81 ± 0.21^{e}	20.41 ± 0.08^{d}	27.05 ± 0.16^{a}	24.95 ± 0.23^{b}	21.96 ± 0.12^{c}	22.23 ± 0.18^{c}
Hb(g/100ml)	6.89 ± 0.08^{c}	7.23 ± 0.11^{c}	8.16 ± 0.17^{b}	8.74 ± 0.17^{a}	7.85 ± 0.12^{b}	6.98 ± 0.12^{c}
PLT	51.98 ± 0.15^{f}	55.08 ± 0.10^{d}	63.95 ± 0.19^{a}	61.96 ± 0.24^{b}	58.55 ± 0.08^{c}	54.27 ± 0.37^{e}
MCV (fl)	99.02 ± 0.28^{e}	89.02 ± 0.59^{f}	118.16 ± 0.20^{d}	151.05 ± 0.13^{c}	169.32 ± 0.17^{b}	195.77 ± 0.15^{a}
MCH (pg)	23.39 ± 0.13^{e}	20.93 ± 0.10^{f}	32.90 ± 0.24^{d}	43.06 ± 0.14^{b}	35.92 ± 0.20^{c}	49.55 ± 0.28^{a}
MCHC (%)	22.88 ± 0.13^{f}	24.86 ± 0.11^{e}	27.11 ± 0.21^{d}	32.49 ± 0.33^{b}	29.00 ± 0.17^{c}	34.70 ± 0.24^{a}

WBC =White blood cell, RBC =Red Blood Cell, PCV =Packed cell volume, Hb =hemoglobin concentration, PLT =Platelet, MCV =Mean corpuscular volume, MCH =Mean corpuscular hemoglobin, MCHC =Mean corpuscular hemoglobin concentration

Discussion

In past fish meal (FM) was being used in fish feed as a major protein source because it contain highest protein proportion and other necessary nutrients for fish growth. But low supply due to limited stock and high cost make it compulsory to search inexpensive and easily available protein sources.

Therefore, plant protein meal, such as *Moringa oleifera* is being increasingly used in fish diets for the development of low-cost and easily available fish feed (Bello *et al.*, 2012; Dienye and Olumuji *et al.*, 2014). MOLM is being successfully used in fish feed but presence of anti-nutritional factor i.e. phytate limits nutrients retention in fish body (Tagwireyi *et al.*, 2014). Usually 250 to 1500 FTU kg^{-1} of phytase supplementation in plant by-products is considered as optimum level for nutrient retention in different fish species. However, variability of nutrient retention depends on ingredients composition, feed formulation, presence or absence of stomach, feed processing technology as well as fish species. So phytase supplementation in plant by-products based diet should be used after considering these factors (Cao *et al.*, 2007). According to our results protein and lipid retention in fish body started increasing from 300 FTU kg^{-1} level and reached to maximum when fingerlings were fed 900 FTUkg^{-1} level of phytase supplemented MOLM based test diet, whereas further increase in phytase supplementation (1200 and 1500 FTU kg^{-1}) resulted a decrease in fish body. These observation shows that 900 FTU kg^{-1} level in MOLM based diet is optimum for maximum nutrients retention in *Catla catla*.

Our results indicated that highest crude protein retention (56%) in whole fish body was recorded in the fish fed on 900 FTU kg^{-1} phytase level in MOLM based diet as compared to control diet (45%). Similarly many researchers also found phytase supplementation improves the protein retention in fish body fed on plant by-products based diet (Lanari *et al.*, 1998; Debnath *et al.*, 2005b; Khajepour *et al.*, 2012). Contrary to these, Nwanna *et al.* (2008) observed non-significant differences in protein concentrations in *Colossoma macropomum* (Amazon tambaqui) body fed on Brazil nut and leucaena leaf meal. Nearly similar to our results, maximum protein retention in yellow catfish (*Pelteobagrus fulvidraco*) carcass was found in juveniles when fed phytase supplemented plant based meal at 1000 FTU kg^{-1} (Cheng *et al.*, 2015). Whereas in contradiction, microbial phytase supplementation at 500 FTU kg^{-1} level in the diet of *Pangasius pangasius* increased the apparent protein retention in fish body (Debnath *et al.*, 2005a). Higher muscle protein level was recorded in *Cyprinus carpio* fed on plant based diet supplemented with 500 FTU kg^{-1} level as compared to fish fed control diet (Sardar *et al.*, 2007; Khajepour *et al.*, 2012). On the other side Hung *et al.* (2015) found that supplementation of dietary phytase at 1500 FTU kg^{-1} level in soybean meal based diet significantly improves the crude protein retention in *Pangasianodon hypophthalmus* (Tra catfish). In our study 900 FTU kg^{-1} level was found best level for increasing nutrient retention in fish body that was in optimal ranges (250-1500 FTU kg^{-1}) reported by Cao *et al.* (2007), whereas Olusola and Nwanna (2014) found a very high level of phytase dose (8000 FTU kg-^{1} level) for maximum protein retention in

Oreochromis niloticus fed on processed soybean meal based diet. This divergence in enzyme doses may be due to ingredient composition, the presence or absence of the stomach, fish species as well as characteristics and types of phytase (Baruah *et al.*, 2004).

Our results showed that crude fat in fish body started increasing form 300 FTU kg^{-1} level based diet and reached to its maximum (14%) at 900 FTU kg^{-1} level in MOLM based diet. Furthermore, it started decreasing as level of enzyme supplementation started increasing upto 1500 FTU kg^{-1}. Very close findings to our results were observed by Yoo and Bai (2014). They found maximum crude fat in *Paralichthys olivaceus* (Olive Flounder) when fed phytase supplemented soybean meal based diet at 1000 FTU kg^{-1} level. In our study, it was interesting to note that further increase of phytase supplementation at 1200 and 1500 FTU kg^{-1} levels in fish diet resulted in decrease of protein and fat retention in fingerlings body. Reason for this decrease is difficult to explain however it can be proposed that care should be taken during phytase supplementation in fish feed. On the other hand Hung *et al.* (2015) noticed a little bit higher dose (1500 FTU kg^{-1} level) of phytase supplementation is sufficient for maximum increase in fat contents in *Pangasianodon hypophthalmus* (Tra catfish) when fed soybean meal based diet. In contrast, Nwanna *et al.* (2008) found non-significant difference in fish lipid contents when Amazon tambaqui fed on Brazil nut and leucaena leaf meal based diet with or without phytase. Interestingly, Khajepour *et al.* (2012) observed lowered lipid value at 500 FTU kg^{-1} level of phytase supplementation in muscles of *Cyprinus carpio* fed on plant based diet.

Lowest body ash (6%), crude fiber (1%) and moisture contents (5%) were observed in the fish fed on 900 FTU kg^{-1} phytase supplemented MOLM based diet. Similar to our results increase in crude protein contents, resulted in decreased moisture and crude ash values was noted in the red sea bream fed plant based diet (Hossain *et al.,* 2007). Similar to our results, moisture contents were recorded lower in catfish fed on phytase supplemented plant protein based diet as compared to control diet (Hung *et al.*, 2015). Cheng *et al.* (2015) found no significant difference in moisture contents of whole fish body fed with or without phytase supplemented diets. In contrast higher contents of moisture were recorded in *Cyprinus carpio* when fed at 0 FTU kg^{-1} phytase level (Sardar *et al.*, 2007). Contrary to our results Olusola and Nwanna (2014) found that crude fiber was recorded minimum at 2000 FTU kg^{-1} level based diet. This difference among the results was may be due to the fish type, phytase type, plant type used in diet (Baruah *et al.*, 2004).

Our results indicate highest values of RBCs, WBCs and Hb were noted at 900 FTU kg^{-1} level of phytase supplementation. Supplementation of phytase in fish feed enhanced the blood cells. Phytase enzyme supplementation is suggested as a powerful stimulator of immune system of fish, resulting in higher number of monocytes (macrophages) and resultant higher blood cells production in mono-gastric animals (Ehsani and Torki, 2010). RBCs and WBCs were found higher in *Cyprinus carpio* at 500 FTU kg^{-1} level of soya-protein based phytase and dicalcium phosphates supplemented diet (Sardar *et al.*, 2007). Phytase was used in *Gadus morhua* (Atlantic cod) feed and resulted in higher number of WBCs of fish (Lazado *et al.*, 2010).

Hemoglobin (Hb) level in fish fed on phytase supplemented diet was little higher than the fish fed on a diet without phytase but significantly not different from other dietary treatments (Yoo and Bai *et al.*, 2014). Highest values of RBCs, WBCs, Hb and Hematocrit were calculated at 500 FTU kg^{-1} level of phytase that was in normal range of these parameters (Sardar *et al.*, 2007). The area of hematological study is least explored by researchers and it needs an intensive research work to search out the possible interaction of phytase enzyme on hematological parameters.

Conclusion

These results showed a positive effect of phytase supplementation on fish carcass composition and hematology. Best values in term of maximum nutrient

retention and hematological parameters in whole fish body indicated that fish fed phytase supplemented diet became healthier as compared to fish fed on control diet (0 FTU kg^{-1}). It was also concluded that supplementation of phytase at 900 FTU kg^{-1} level is the optimum level that significantly improved the carcass composition and hematological indices of fish fed on MOLM based diet.

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