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EFFECT OF PHYTASE SUPPLEMENTED MORINGA BY-PRODUCTS BASED DIETS ON The PERFORMANCE OF *OREOCHROMIS NILOTICUS* FINGERLINGS

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ABSTRACT

Increasing human population and lower food availability motivated the researchers to search out the alternative and low cost feed sources for fish that is the basic protein source in human diet. For this purpose, study was performed to develop an eco-friendly and low-cost feed for 'Nile tilapia' fingerlings using Moringa derived products supplemented with phytase (PHY) enzyme. *Moringa oleifera* seed meal and leaf meal were used as the major components to make seven iso-nitrogenous and iso-caloric test diets (TD). These Moringa based test diets were developed by using different PHY levels (0 control, 500, 650, 800, 950, 1100 and 1250 FTU per kg). Seven groups (in three replicates) of tilapia fingerlings were fed at the rate of 4% of their live weight two times a day. After 70 days feeding trials, blood samples from each replicate were collected for the analysis of hemato-immunological parameters. Then these fish were sacrificed and dried for carcass analysis. After the application of one-way ANOVA, maximum retention of protein (18.26%) and fat (8.92) were found at 950 FTU per kg in comparison to other test diets. Study of hematological indices revealed that the maximal values of RBCs (2.92×10^6 mm^{-3}), PLT (64) and Hb (8.19 g/100ml) were recorded at 950 FTU per kg level. Similarly, highest MCH, PCV, MCV and Ht. were also observed at 950 FTU per kg level. For the study of expression of growth genes, gel electrophoresis method was performed. It was concluded that PHY added diet at 950 FTU per kg showed maximum improvement in hemato-immunological parameters and body composition of tilapia with no side effects on fish health and non-differentiable results regarding growth gene expression.

Keywords: Carcass, hematology, Nile tilapia, Phytase, *Moringa oleifera.*

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INTRODUCTION

In food-producing industries, one of the developing and advance industry is the aquaculture (Yildirim *et al.* 2014). Many problems faced by aquaculture now a days and feed is one of them that limits profitability. Feed that provided the fish should be stable, environment-friendly, normally available and cost effective (Shahzad *et al.* 2016;2019). Fishmeal (FM) in feed costs about 40-60% of total production charges in farms (Foidl *et al.* 2001). Plant meal-based diets proved to be suitable and economically profitable for aquaculture by many researchers (Mahmoud *et al.* 2016). Various feed ingredients from plants are used as the alternate source of FM on trial basis. Using plant derived products as a source of food for aquaculture may have certain major problems. Moringa seeds are enriched with phytate (an anti-nutritional agent) and it cannot be catabolized by fish, consequently discharged into surrounding water and cause water contamination (NRC 1993).

In Pakistan, *Moringa oleifera* is commonly called 'sohanjana' or 'sanjana' and located in Southern Punjab, is an excellent protein-rich plant and proved to be a cost-effective source for fish feed (Chiseva 2006). *M. oleifera* seeds are excellent source of protein (33 to 38%). Certain important amino acids e.g., methionine, cysteine and glycine and some essential minerals are also present in seeds (Hassan *et al*. 2018; Liang *et al*. 2019). Leaves of Moringa have high quantity of protein varying from 23% (Hassan *et al*. 2018) 32% (Soliva *et al.* 2005) and higher content of minerals and nutrients (Liang *et al*. 2019). Minerals that Moringa seeds carry include Na, P, Zn, Mg, K, Fe and Ca etc. (Anjorin *et al.* 2010).

There are three common problems with Moringa feed that are caused by the presence of phytic acid in Moringa based diets and deficiency of PHY in fish digestive system; undigested phosphorous remains unabsorbed and excreted through faeces (Lei *et al.* 2013). The gene expression level of fatty acid synthase (FAS), the regulatory element binding protein of sterol and growth genes were negatively regulated by the excess

phosphorus level in fish body (Ji *et al.* 2017). Adverse effects of phytate in the plant-based diet can be minimized by using PHY enzyme (Hussain *et al.* 2015; Shahzad *et al.* 2018). After the breakdown of phytic acid by PHY enzyme, bounded cationic minerals become free for the consumption of organism and also minimize the release of phosphorus in the environment through excretions (Vohra *et al.* 2006).

Plant ingredients in feed were also reported negatively in immune functions of fish due to the presence of phytic acid (Sitjà-Bobadilla *et al.* 2005; Kokou *et al.* 2012). Erythrocyte (RBC) count, leucocyte (WBC) count, Hb concentration and hematocrit provide important knowledge about development, health and stress responses of fish for ichthyologists (Hrubec *et al.* 2000). WBC count also proves helpful for the monitoring of immune responses (Blaxhall 1972; Soivio and Oikari 1976). Monocytes, neutrophil, eosinophil and lymphocytes are the basic parameters for the demonstration of immunity of fish. Less research work was reported in past on hematology and immunity of Nile tilapia. Therefore, this research work was conducted to produce higher quality meat of fish at lowest cost by using PHY added Moringa derived products and also to improve hemato-immunological indices of tilapia.

MATERIALS AND METHODS

This work was performed from November 2018 to May 2019to study the body composition, hematology, immunity and growth gene expression of Nile tilapia fed on Moringa derived product based diet. Research work was performed in the Fish Nutrition Lab, DSNT, University of Education, Lahore, Pakistan (31.4537° N, 74.2990° E).

Fish and experimental treatments: Tilapia were purchased from Manawan Fish Hatchery, Lahore and stocked in V-shaped fish tanks for two-week acclimatization period. Saline solution was used to treat fish for 5-7 minutes to kill disease causing micro-organisms (Rowland and Ingram 1991). Different parameters like pH, temperature and dissolved oxygen were monitored for the quality of water on daily basis. For respiration, air is supplied by capillary system using air pump.

Experimental design: For the preparation of test diets, derived products of Moringa were used as feed ingredients. Moringa derived products based diets were developed by using various levels of supplementation of PHY (0, 500, 650, 800, 950, 1100 and 1250 FTU per kg); one control (without PHY) and six test diets. To check the effect of these test diets, eighteen fingerlings were kept in each triplicate tank (total 21 tanks). Fingerlings (Average weight 6.86 g $fish^{-1}$Average length 7cm) were fed two times in a day with 4% of feeding rate of their live weight. In each triplicate, fish were fed on control and test diets of specific graded levels of PHY. Total duration of the experimental trial was conducted following a completely randomized design (CRD) for a period of 70 days. *C. carpio* fingerlings fed with test diets were compared with control as well as between test groups to assess body composition, hematology and immunological parameters.

Pellets Formation and feeding: Collected Moringa leaves and seeds were crushed and ground as fine powder after their processing as described by Shahzad *et al*. (2019). Proximate chemical composition of all feed ingredients was done (AOAC 1995) before pellet formation (Table 1). All feed items in specific proportion were mixed for approximately 5 minutes. The fish oil was poured into the mixer very slowly. Distilled water (15-20%) was added in the feed; consequently, textured dough was produced. Hand pelleting machine was used for further processing of dough to make feeding pellets (Lovell, 1989). There were seven diets; one control and six test diets. Solutions with different PHY concentrations (500, 650, 800, 950, 1100 and 1250 FTU per kg) were sprayed on test diets having weight of one kilogram each (Robinson *et al.* 2002). To maintain normal moisture contents of feed, equal volume of distilled water was sprayed on control diet. Freshly prepared diets were dried normally in shady and cool place and stored at 4°C in air tight jars until use. Tanks were washed after one hour of feeding with fresh water thoroughly to remove remaining particles of feed to decrease the contamination of water and tanks were filled with fresh water.

Analysis of carcass: Three fish were brought out from each tank and sacrificed after feeding trial. The fish carcass samples were dried at room temperature (for 2 days till the samples got dried) and crushed into fine powder separately using pestle and mortar (AOAC 1995). For the removal of moisture, oven-drying method was used for about 12 hours at 105°C. For the analysis of crude fat, petroleum ether extraction (EE) method was used with Soxhlet apparatus. Crude protein (CP) was determined with the use of Micro Kjeldahl's apparatus. After digestion with 1.25% NaOH and 1.25% H_2SO_4, crude fiber (CF) contents were checked by ignition of lipid-free dried residue forming ash in electric furnace (Naberthern B170) for about 10 hours at 650°C to constant weight. Total carbohydrate contents were calculated using following formula (FAO 2003):
Total carbohydrates (%) = 100- (Moisture % + CP % + CF%+ EE%+ Ash %)

Analysis of hematological parameters: After feeding trial, blood samples (randomly selected fingerling; one from each replicate) from each replicate were collected for analysis. The samples were collected with

anticoagulant i.e. 10% ethylene diamine tetraacetate (EDTA). These collected blood samples were taken to Hematological Lab for analysis. Hematocrit was analyzed by the help of capillary tube method using Micro-hematocrit technique (Brown 1988). RBC's and WBC's were counted with the help of haemo-cytometer using Neubauer Counting Chamber method (Blaxhall and Daisley 1973). Concentration of hemoglobin (Hb) in different samples was checked as explained by Wedemeyer and Yastuke (1977). To calculate MCH (mean corpuscular hemoglobin); MCHC (mean corpuscular hemoglobin concentration); and MCV (mean cell volume), the following mathematical relations were used:

MCHC = 100(Hb/Packed Cell Volume)
MCH = 10(Hb/RBC)
MCV = 10(PCV/RBC)

Blood samples for the analysis of immunological indices were collected without anticoagulant. For the analysis of immunological indices, these samples were stored in a freezer at -20ºC. Smears of blood samples were prepared for the determination of lymphocytes, eosinophils, monocytes and neutrophils with Neubauer differential counting method. Centrifugation method was used for the separation of serum samples (Schalm 1986).

Collection of muscle sample for gel electrophoresis: In clean Eppendorf tubes, the muscle samples, from four Tilapia of each group, were collected separated and preserved at -80ºC till use at the end of experiment and FAS and LPL were analyzed (Norag *et al.* 2018).Gel electrophoresis was performed at one hundred and twenty volt for 30 minutes and results observed under UV iluminator. Bands of amplified cDNA of FAS and LPL genes were compared to differentiate the level of gene expression in Tilapia.

Statistical Analysis: The data obtained were statistically analyzed by One-Way ANOVA using Co-Stat computer software package to study the effect of different PHY treated diets on fish fingerlings. The variations among different parameters were compared by using Tukey's Honesty Significant Difference Test. These differences were considered significant at $P<0.05$ (Snedecor and Cochran 1991).

RESULTS AND DISCUSSION

Composition of fish body fed Moringa based diets with the addition of PHY is given in Table 2. PHY added diets showed significant improvements in fish body composition by the maximum absorption of nutrients in comparison to control diet. Maximum contents of crude protein (18.26%), crude fat (8.92%) and gross energy (2.21 kcal/g) were observed at 950 FTU per kg PHY added Moringa derived based diets. These values were different significantly from the values found in fingerlings fed with control and other test diets. Fish with control diet gave least contents of crude protein (12.86%), crude fat (6.45%) and gross energy (1.06 kcal/g). Moringa derived products based diets with the addition of PHY improved the retention of protein according to many aqua-culturists (Lanari *et al.* 1998; Debnath *et al.* 2005; Khajepour *et al.* 2012). Closely similar to our results, maximum retention of protein, fat and gross energy were recorded at 900 FTU per kg addition of PHY in *Catla catla* fed Moringa by-products meal based diets (Shahzad *et al.* 2016,2018). Cheng *et al.* (2015) recorded that plant based diets at 1000 FTU per kg addition of PHY show maximum protein and fat in *Pelteobagrus fulvidraco* (yellow catfish). However, Olusola and Nwanna (2014) strongly disagreed with these findings. They found maximum retention of protein in Nile tilapia was recorded at very high addition of PHY at (8000 FTU per kg) fed on soy-bean plant based diets. A little disagreement was observed by Yoo and Bai (2014). Highest fat contents in carcass of Olive Flounder (*Paralichthy solivaceus)* were found at 1000 FTU per kg addition of PHY in soybean based diets. Hung *et al.* (2015) noted that dietary inclusion of PHY enzyme at 1500 FTU per kg in soybean plant based diets significantly improves the retention of crude protein in *Pangasianodon hypophthalmus* (Tra catfish). In the light of above-mentioned results, we can argue that the possible explanations for dissimilarities in values of results may be the quantity, quality (Baruah *et al*., 2007; Dersjant-Li *et al*., 2015) and dissimilar sources of PHY, feed components, methods of diet formulation or changed fish species (Liu *et al*., 2013).

Highest contents of ash (6.03%) and moisture (72.51%) were found in fish fed on diet without addition of PHY and were different significantly from other replicates with PHY addition. While increasing the level of PHY caused gradual decrease in ash and moisture contents till 950 FTU per kg (5.12% and 64.53% respectively) and then gradual increase was occurred from 1100 FTU per kg to 1250 FTU per kg. It was observed that improvement of protein and lipids in fish body starts from 500 FTU per kg level and reached to its highest when tilapia were fed at 950 FTU per kg PHY added Moringa derived based diet. However, more increase in PHY from 1100 to 1250 FTU per kg resulted a decrement in retention of nutrients in fish body. Results of carcass parameters showed that 950 FTU per kg PHY in Moringa based diets was very suitable for the maximum retention of nutrients by fish. Similarly, Hossain *et al.* (2007) observed that Red sea bream fed with plant derived products based diet shows decreasing trends in crude ash and moisture values and improvement in crude protein values. Sardar *et al.* (2007) also agreed with the current findings and noted higher values of moisture at PHY level (0 FTU per kg) in *Cyprinus*

carpio. Shahzad *et al.* (2016;2018;2020) strongly agreed with the current results of the study and observed least contents of body ash, crude fiber and moisture in *Catla catla* were found at 900 FTU per kg addition of PHY in Moringa leaf meal based diets. Whereas, non-significant differences were observed by Cheng *et al.* (2015) in fish body carcass irrespective of PHY addition. Nwanna *et al.* (2008) in contrast found that, leucaena leaf meal and Brazil nut based diets irrespective of PHY addition showed non-significant differences in lipid contents of *Colossoma macropomum,* Amazon tambaqui fish. The contradictions and variations in results are considered to occur because of the type and source of PHY enzyme used, numerous nutritional factors, along with the source and quantity of phytic acid (Selle *et al*. 2000) and protein sources in fish diets (Sugiura *et al*. 2001).

Based on the present findings, improvement in hematological parameters such as RBC (2.92 10^6 mm^{-3}), PLT (63.77), hemoglobin (8.19 g/100ml), PCV (26.39) and Ht. (32.91) was observed in fish fed on test diet IV (950 FTU per kg). Second highest values (RBC 2.70 10^6 mm^{-3}, PLT 62.77, PCV 25.75 and Ht. 31.60) were noted in fish fed on 800 FTU per kg level based diet. Similarly Ehsani and Torki (2010) also found that the addition of PHY stimulates the immune system of fish producing high number of macrophages. With agreement of current study, Shahzad *et al.* (2019;2020) also noted closely similar results. They observed high contents of RBCs and Hb in *Catla catla* were observed in diet having 900 FTU per kg PHY level. Whereas, in contradiction to our results, highest counts of WBCs and RBCs were observed at 500 FTU per kg in *C. carpio* fed diet consisting of soya-bean plant added with PHY and dicalcium phosphate (Sardar *et al.* 2007).On the other hand, lowest values of the said parameters were found in the fingerlings that were fed with control diet. Whereas, highest value of WBCs (6.91 10^3 mm^{-3}) was observed at control diet and started to decrease from test diet 2. Lowest WBCs (6.48 10^3 mm^{-3}) were noted at diet IV. The Overall values of WBCs were not significantly different from the fingerlings fed on other test diets. Maximum number of Lymphocytes (25.97%) and Monocytes (2.94%) were found in the fingerlings that were fed at test diet 4 supplemented with 950 FTU per kg of phytase as compared to other test diets. Whereas higher Eosinophils (1.63%) were found in the fingerlings fed on test diet 6 and Neutrophils (79.60%) at control diet. Low count of Eosinophile (1.16) and Neutrophile (69.92) was observed in fish fed diet IV (950 FTU per kg). Different immune responses were observed in fish fed with different diets having different PHY levels but fish fed with diet IV produced slightly better results compared to other test diets. WBCs count played very important role in innate immunity of fish and their count can be used as tool to check the health status of fish. WBC count will increase in conditions of stress i.e. dietary imbalance, certain infection etc. (Roberts 1978). Whereas, some researchers found positive results by using real time PCR techniques to study the expression of genes (lipoprotein lipase i.e. LPL and fatty acid synthase i.e. FAS) of fish fed PHY added diets. They concluded that PHY played an important role in positive regulation of genes expression in fish body (Norag *et al.* 2018). A little research work has been performed to study the impact of PHY on growth genes such as LPL and FAS of fish. In another experiment Norag *et al*. (2018) studied the influence of low phosphorus diet with or without PHY addition on the expression of LPL and FAS genes of tilapia. They concluded that normal phosphorus was helpful in positive regulation of (LPL) mRNA expression and negative regulation of (FAS) mRNA in the liver of tilapia. They use PCR technique to check the impact of PHY on gene expression. Whereas, current outcomes indicate that comparative gene expression was not differentiable. The optimum range of PHY is 250 to 1500 FTU per kg in plant diets as described by many aqua-culturists in different fishes (Cao *et al.* 2007; Shahzad *et al.* 2016).These contradictory values of results as above-mentioned might be due to the source of PHY, feed components, methods of diet formulation or changed fish species (Cao *et al*., 2007).

Table 1. Physical and chemical Constituents composition (%) of diet (dry matter).

Physical composition (%) of test diet		Chemical composition (%) of feed constituents						
Constituents	**TD composition**	**Dry matter (%)**	**Crude Protein (%)**	**Crude Fat (%)**	**Crude Fiber (%)**	**Ash (%)**	**Gross Energy (kcal per g)**	**Carbohydrates**
MOSM+MOLM	36	91.24	33.31	3.91	13.56	10.07	4.02	39.15
Fishmeal	17	92.39	49.31	6.99	1.29	24.66	2.23	17.75
Corn Gluten (60%)	13	91.78	58.97	4.96	1.23	1.41	4.21	33.43
Wheat flour	16	93.09	9.43	2.41	2.88	2.06	3.09	83.22
Rice polish	7.5	92.2	13.02	12.76	13.06	11.17	3.03	49.99
Fish oil	6.5							
Mineral Premix	1							
Chromic oxide	1							

Vitamin Premix	1
Ascorbic acid	1
Total	100

Nutrients in test and control diet supplemented with PHY fed *O. niloticus*

Diets	TD-I (Control diet)	TD –II	TD –III	TD –IV	TD –V	TD –VI	TD –VII
PHY level	0	500	650	800	950	1100	1250
CP in diet	31.57	31.56	31.58	31.59	31.56	31.59	31.56
EE in diet	6.16	6.19	6.20	6.18	6.18	6.17	6.20
GE in diet	3.52	3.53	3.50	3.51	3.50	3.51	3.53

MOSM= *M. oleifera* seed meal MOLM= *M. oleifera* leaf meal

Mineral premix/kg:

Zinc(Zn):3000 mg, Manganese(Mn):2000 mg, Calcium (Ca): 155 g,(Cu) Copper: 600 mg, Iron (Fe): 1000 mg, Cobalt (Co): 40 mg, Selenium(Se):3mg,Sodium (Na): 45 g, Iodine (I): 40 mg, Phosphorous (P): 135 g, Magnesium (Mg): 55 g

Vitamin premix/kg:

VitaminD_3:3,000,000IU,Vitamin E:30000 IU, Vitamin A: 15,000,000 IU,VitaminB_1:3000 mg, VitaminB_2: 7000 mg, Vitamin B_6: 4000 mg, Vitamin B_{12}: 40 mg,VitaminC:15,000mg,VitaminK_3: 8000mg,Nicotinicacid:60,000 mg,Calciumpantothenate:12,000 mg,Folicacid:1500 mg.

Table 2. Proximate analysis of tilapia carcass fed PHY added Moringa derived products meal based diets.

Diets	PHY level (FTU per kg)	Crude Protein	Crude Fat	Gross energy	Ash	Crude fiber	Moisture
Control diet	0	12.86^{e}	6.45^{d}	1.06^{c}	6.03^{a}	1.08^{a}	72.51^{a}
TD 1	500	13.98^{d}	6.99^{cd}	1.40^{bc}	5.89^{ab}	1.15^{a}	70.59^{b}
TD 2	650	15.20^{c}	7.67^{bc}	1.64^{ab}	5.72^{abc}	1.09^{a}	68.67^{c}
TD 3	800	16.72^{b}	8.18^{ab}	1.86^{ab}	5.52^{bcd}	1.14^{a}	66.58^{d}
TD 4	950	18.26^{a}	8.92^{a}	2.21^{a}	5.12^{d}	0.97^{a}	64.53^{e}
TD 5	1100	17.33^{b}	8.12^{ab}	1.86^{ab}	5.39^{cd}	1.01^{a}	66.29^{d}
TD 6	1250	13.94^{d}	7.13^{cd}	1.56^{bc}	5.69^{abc}	1.14^{a}	70.54^{b}
SE		0.41962	0.18458	0.08489	0.07054	0.02138	0.59933

$^{a-d}$ Means within column having dissimilar superscripts are quietly different at $p<0.05$. Data are means of three replicates (SE=Standard Error)

Table 3. Hematological indices of Nile Tilapia fed PHY added Moringa based diets.

Diets	Control diet	TD 1	TD 2	TD 3	TD 4	TD 5	TD 6	
PHY Levels (FTU/kg)	0	500	650	800	950	1100	1250	**SE**
RBC (10^6 mm^{-3})	1.12^{e}	1.87^{d}	2.11^{cd}	2.71^{ab}	2.92^{a}	2.46^{bc}	1.96^{d}	0.12730
WBC (10^3 mm^{-3})	6.91^{a}	6.81^{ab}	6.59^{ab}	6.66^{ab}	6.48^{b}	6.72^{ab}	6.78^{ab}	0.04060
PLT	55.49^{e}	58.79^{d}	61.51^{bc}	62.61^{ab}	63.77^{a}	60.54^{cd}	54.68^{e}	0.73089
Hb (g/100ml)	4.97^{f}	5.69^{ef}	6.68^{cd}	7.24^{bc}	8.19^{a}	7.57^{ab}	5.87^{de}	0.24429
PCV (%)	21.47^{c}	23.80^{b}	24.09^{b}	25.75^{a}	26.39^{a}	23.43^{b}	21.62^{c}	0.40004
MCHC (%)	27.90^{e}	30.46^{d}	32.48^{bc}	35.34^{a}	34.22^{ab}	33.32^{bc}	31.55^{cd}	0.52848
MCH (pg)	31.43^{f}	38.49^{e}	42.41^{d}	48.92^{b}	49.26^{b}	54.62^{a}	44.71^{c}	1.59813
MCV (fl)	101.32^{f}	88.23^{g}	129.44^{e}	197.48^{a}	165.52^{b}	160.80^{c}	148.37^{d}	7.89728
Hematocrit (Ht) %	22.76^{d}	28.28^{b}	29.90^{b}	31.60^{a}	32.91^{a}	29.39^{b}	24.92s^{c}	0.75214

$^{a-d}$ Means within rows having dissimilar superscripts are quietly different at $p<0.05$. Data are means of three replicates (SE= Standard Error).

Table 4. Immunological parameters of Nile Tilapia fed PHY added Moringa based diets.

Diets	Control diet	TD 1	TD 2	TD 3	TD 4	TD 5	TD 6	
PHY Levels (FTU/kg)	0	500	650	800	950	1100	1250	**SE**
Lymphocyte %	15.78^{d}	18.47^{c}	19.83^{c}	24.95^{a}	25.97^{a}	22.16^{b}	18.82^{c}	0.77237
Eosinophil %	1.48^{ab}	1.41^{ab}	1.35^{ab}	1.29^{ab}	1.16^{b}	1.24^{ab}	1.63^{a}	0.04201
Monocyte %	3.15^{a}	2.79^{ab}	2.34^{bc}	2.05^{cd}	2.94^{a}	1.75^{d}	1.77^{d}	0.12201
Neutrophil %	79.60^{a}	77.32^{b}	76.48^{bc}	71.72^{d}	69.92^{e}	74.84^{c}	77.77^{b}	0.72607

$^{a-d}$ Means within rows having dissimilar superscripts are quietly different at $p<0.05$. Data are means of three replicates (SE= Standard Error).

Conclusion: It was revealed from the results that addition of PHY gave positive results regarding fish carcass composition and hemato-immunological parameters. Fish that were fed with PHY added Moringa derived products meal-based diets showed improvement in nutrient retention and hematological parameters in whole fish body as compared to fish fed on control diet (0 FTU per kg). Results revealed that diet IV (950 FTU per kg) is most suitable level that significantly enhanced the hemato-immunological indices and carcass of tilapia. But unfortunately, expression of genes was not differentiable by gel electrophoresis technique.

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