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Growth Responses of Striped Catfish *Pangasianodon hypophthalmus* (Sauvage, 1878) to Exogenous Enzyme Added Feed

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Authors' Contribution
HA designed and supervised the work. MK executed the experimental work. NK and HA performed statistical analysis. AH and MK did hematological parameters. SMH, HA and SA wrote the manuscript. AJ, AH and AU analyzed microbial load.

Key words
Exogenous enzyme, Striped catfish, Fish growth, Microbial load, Hematological parameters.

ABSTRACT

A feeding trial was conducted for 90 days in glass aquaria to check the effect of exogenous enzyme (α-amylase) added feed on growth performance, health and hematological analysis of *Pangasianodon hypophthalmus* (initial average weight 32.46±0.36 g). The 40% crude protein diet was prepared by adding feed ingredients along with 0.75, 0.50, 0.25, 0.00 g kg^{-1} of α-amylase as T1, T2, T3 and T4 (control). At the end of feeding trial, 100% survival rate was recorded. Growth rate was significantly increased in fish fed with enzyme supplemented diets in comparison with control group. The maximum increase in growth rate was recorded in treatment # 2. Highest protein contents 68.18% was observed in treatment # 3 (0.75g kg^{-1} α-amylase). Specific growth rate (SGR), feed conversion ratio (FCR) and condition factor (CF) were higher in all enzyme supplemented groups as compared to control group ($p<0.05$). Hematological parameters were recorded among all groups and concentration of red blood cells (RBCs), white blood cells (WBCs), hematocrit (HCT) and numbers of lymphocytes were highest in treatment # 1 with the mean values of 1.29×10ˆ12 L^{-1}, 54.9×10ˆ9 L^{-1}, 18% and 48.7×10ˆ9 L^{-1}. Microbial load was also determined inside and outside of fish as highest microbial load was observed in control group fish on both media nutrient agar (NA) and eosin methylene blue (EMB) *viz*. 1.8×10^3 and 1.56×10^3 (cfu ml^{-1}) in gut contents whereas on skin only total bacterial count (0.36×10^3) was observed and no coliform bacteria were present while least CFU's were counted in group 2 among skin 0.14×10^3 cfu ml^{-1} and gut 0.10×10^3 cfu ml^{-1} on nutrient agar. The results suggested that the enzyme supplementation improved the growth and health of *P. hypophthalmus*.

INTRODUCTION

Carps and other cyprinids contribute significantly towards total global aquaculture production (Naz *et al*., 2012). In several marginal areas fish meat is widely utilized and considered as a main source of nutrition. It devotes a healthy diet by providing nutrients (vitamins and minerals) and high-value amino acids and is a magnificent source of essential omega-3 fatty acids that are associated with health and welfare (Domingo *et al*., 2006).

Fish are largely used to evaluate the quality of aquatic communities and some of their physiological changes can also be considered as biological indicators of environmental pollution (Dautremepuits *et al*., 2004).

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The aquaculture feed industry depends on the fishmeal, which is the most liked protein source for fish feed owing to magnificent amino acid and fatty acid profile. Its use for viable farming is confined due to limited supply, high cost and stationary production level (New and Wijkstro, 2002; Baruah *et al*., 2004). Therefore research is being directed towards concluding quality alternative protein sources that, ideally, are less expensive and easily available as replacement for fishmeal (Currie, 2000).

Presently fish feed formulation is heavily dependent on fish meal for herbivorous fishes in general and for carnivorous in specific. Quality of fish meal is always variable and quantity is never certain due to declining catches from wild sources (Ogello *et al*., 2014). Its low production and its competition with other livestock doubts its availability and escalates its prices which ultimately affects the cost of fish feed and sustainability of fish culture. Replacement of fish meal with cost effective plant products in fish feed is a major challenge for aquaculturists

to make this business economical and profitable (Ai and Xie, 2005). Exhaustive efforts have been expended on this highly important aspect with partial success. Plant products have their own limitations and constraints which hinder their total substitution with animal protein sources (Francis *et al.,* 2001). Among others poor digestibility of carbohydrates is the major one. Fish has very simple stomach which either has no carbohydrases or quantity is too low to digest complex carbohydrates. Therefore some sort of intervention is inevitable to circumvent this problem.

Addition of enzymes in the feed of different animals has been practiced to enhance digestive processes (Yildirim and Turan, 2010), however, not much work has been done in fish due to aquatic media. Literature is available that shows that addition of digestive enzymes in artificial feed like other animals can enhance the digestive processes in fish (Sunde *et al*., 2004). Compared to herbivorous fish varieties omnivorous species appear to digest starch components of plant materials more effectively than carnivorous fishes. Specific activity of α-amylase is higher in herbivorous fish, followed by that of omnivorous and carnivorous fishes (Al-Tameemi *et al*., 2010).

Exogenous enzymes are now extensively used throughout the world as additives in animal feed. Protease enzyme is used to improve the digestibility of protein in pigs (O-Doherty and Forde, 1999), cattle (Zobell *et al*., 2000) and poultry (Ghazi *et al*., 2003). Certain enzymes provide a powerful tool that can enhance the digestibility of plant based ingredients by in time release of required nutrients from feed and its ingredients (Tameemi *et al*., 2010). Though endogenous amylases help fish in breakdown and digestion of complex carbohydrates to some extent but additional input of carbohydrates hamper digestive processes badly making intact excretion of these compounds from the digestive tract. Although the efficacy of enzyme supplementation in improving protein digestibility is known, however, very less reports are available on the addition of enzymes to increase the digestibility of carbohydrates in fish.

The effects of enzyme supplementation on growth and survival of several cultured fish species have been demonstrated by several authors such as Salmon by Refstie *et al*. (1999), Pangasius by Debnath *et al*. (2005), Tilapia by Drew *et al*. (2005), Lin *et al*. (2007) and Yigit and Olmez (2011) and Catfish by Yildrim and Turan (2010), but there are very few published reports on effects of exogenous enzymes on feed utilization and growth performance of major carps *viz*. *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*.

Addition of exogenous enzymes (fungal xylanase, β-glucanase, pentosonase, β-amylase, fungal β-glucanase, hemicellulase, pectinase, cellulase and cellubiase) in catfish feeds has improved growth and feed efficiency which highlights their fruitful utility (Yildirim and Turan, 2010).

Striped catfish *Pangasianodon hypophthalmus* is a fast rising species that gain market size within a period of 8 months and its feed can vary from farm-made feed to formulated pellets (Hung and Huy, 2007). This exotic species (Pakistan) increased much reputation due to its rapid growth, calm culture system, great disease resistance and patience to an extensive variety of environmental change (Sarkar *et al*., 2007). Striped catfish is farmed typically in earthen ponds, up to 4 m deep, in nine provinces in the Mekong Delta in South Vietnam (Phan *et al*., 2009).

Keeping in view all these aspects, the present study was conducted to evaluate the effect of different dosses of the feed additives containing exogenous enzymes on the growth performance, health and hematological parameters of *P. hypophthalmus* considering that α-amylase will improve all these parameters.

MATERIALS AND METHODS

Fish collection

Individuals of *P. hypophthalmus* (Striped catfish) for experiment were obtained from fish hatchery located at Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences, Lahore (Ravi Campus, Pattoki). Fish were kept in holding tanks supplied with semi-static flow through aerated water system for at least 2 weeks for the purpose of acclimatization to the lab before execution of feeding trials. Experimental trail was conducted in 80 liter glass aquaria placed inside the fish hatchery having dimensions 90cm×38cm×45cm (Length × Width × Depth).

Diet preparation

Isonitrogenous (40% crude protein) and isocaloric diets were prepared from locally available feed ingredients including fish meal, 25%; soy bean meal, 25%; guar meal, 20%; maiza glutan meal, 10%; canola meal, 17%; linceed oil, 2%; vitamin D, 0.5% and vitamin premix, 0.5%. The enzymes were obtained from Deerland Enzymes Company, USA. The 40% protein feed was prepared in bulk and then distributed in to four equal parts. Group # 1 was received α-amylase @ 0.25g kg^{-1}, Group # 2 α-amylase @ 0.50 g kg^{-1}, group # 3 α-amylase @ 0.75 g kg^{-1} and group # 4 was served as control and was free of any exogenous enzyme. All these groups were here after designated as treatment # 1, treatment # 2 and treatment # 3 and control, respectively. All feed ingredients with their respective enzyme were

thoroughly mixed in a mixer. Well mixed and pelleted feed was sun dried till constant weight. The dry pellets were refrigerated in hygienically safe plastic containers.

Experimental procedure

Prior to distribution, 10 individuals of *P. hypophthalmus* were randomly collected from bulk stock. After measurement and weight for the base line data, the fish were distributed in all the treatments and control with three replicates of each. Fish were fed twice a day with fixed hours and the feeding trial was continued for 12 weeks at ambient temperature.

Fish growth

Fish growth parameters *viz*. average increase in wet weight (g), fork length (mm), total length (mm), condition factor (CF), feed intake (FI) (g), feed conversion ratio (FCR), specific growth rate (SGR) and fish survival rate (%) was measured and recorded on fortnightly basis throughout the experimental period.

Proximate analysis

At the end of feeding trial, five fish from each treatment were sacrificed for the determination of proximate analysis. Proximate analysis include moisture, dry matter, ash, fat, protein, fiber and carbohydrate contents following the protocol of AOAC.

Hematological parameters

Two fishes from each treatment were randomly selected for the collection of blood. Blood was collected with the help of syringes in vaccutainers, in which EDTA (anticoagulant) is already present. Blood sample was used for calculation of the following parameters: white blood cells, lymphocytes, red blood cells, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, platelet count, mean platelet volume, platelet distribution width, procalcitonin test and platelet large cell ratio.

Microbial load

Microbial load was checked in both outside (skin) and inside (intestine) of fish. For this purpose two types of media were used: nutrient agar (NA) and eosin methylene blue (EMB).

Nutrient agar

Nutrient agar is used for the growth of bacteria; it allows all type of bacteria to grow in the form of colonies. First of all media was prepared by mixing nutrient agar and distilled water as (20g L^{-1}) and then autoclaved the media. Sterilized petri plates were used for pouring of media. Sample from gastrointestinal tract (GIT) and skin was taken in proper sanitized containers. By pour plate method, sample was poured and then media. Mixed both first clock wise and then anti clock wise after pouring. When the plates solidified, these were transferred to incubator at 37 °C for 24 h.

Eosin methylene blue

EMB agar is used specifically for the growth of coliforms that are disease causing agents in fish and also in humans. Both skin and GIT samples were taken in proper sanitized conditions. Pour plate method was used. Media was prepared by mixing EMB agar and distilled water as (36g L^{-1}) and then autoclaved the media. Sample was poured and then media. Mixed both first clock wise and then anti clock wise after pouring. When the plates solidified, these are transferred to incubator at 37 °C for 24 h.

Physicochemical parameters

Water temperature (^oC) and pH were kept constant with the mean values of 30±1 ^oC and 7.5±0.5, respectively. Physicochemical parameters *viz*. dissolved oxygen (mgL^{-1}), electrical conductivity ($mScm^{-1}$), total dissolved solids (TDS) (mgL^{-1}) and salinity (mgL^{-1}) were recorded twice a day throughout the experimental period. These parameters recorded by using digital meters.

Statistical analysis

Data was subjected to ANOVA for its statistical significance among treatments. Mean values were compared to assess their intensity of significance among treatment groups by Duncan's multiple range test. Probability level was set at $P<0.05$. The SAS (Statistical Analysis Software) version 9.1 was used for all statistical analyses due to its wider applications.

RESULTS

During research trial, *P. hypophthalamus* were grown under controlled conditions with four different feed strategies *viz*. treatment # 1 with 0.25 g kg^{-1} enzyme, treatment # 2 with 0.50 g kg^{-1} enzyme, treatment # 3 with 0.75 g kg^{-1} enzyme and control group without enzyme added feed, for a period of 12 week. There was no statistical difference at the start of experiment between control and experimental groups, initial weights were similar indicating that the fish health and condition was similar. After 90 days of trial there were statistical differences in growth parameters, microbial load, hematological parameters and physicochemical parameters.

Table I.- Growth responses of *Pangasius hypophthalamus* when fed with exogenous enzyme added feed.

Growth parameters	Treatments				
	Control	T1	T2	T3	Means
Total Wt. gain	21.72±0.33d	30.17±0.06c	42.70±0.51a	36.71±0.35b	32.82±8.99
Total F.L gain	43.39±0.29a	28.20±0.10d	40.27±0.22b	35.90±0.20c	36.94±6.58
Total L. gain	30.36±0.15d	35.43±0.09c	58.61±0.12a	47.40±0.10b	42.95±12.65
Feed intake	84.3±1.00d	111.21±0.23c	161.83±0.20a	136.74±0.51b	123.52±33.32
FCR	1.26±0.01a	1.21±0.01b	1.26±0.01a	1.22±0.01b	1.23±0.02
CF	1.56±0.04a	1.51±0.01a	1.43±0.14a	1.26±0.03b	1.44±0.13
SGR	3.75±0.12d	4.55±0.03c	5.60±0.01a	5.24±0.04b	4.78±0.81

Means with same letters in a single column are statistically similar at $p< 0.05$.
Total Wt. gain, total weight gain; Total F.L gain, total fork length gain; Total L. gain, total length gain; FCR, feed conversion ration; CF, condition factor; SGR, specific growth rate.

Growth parameters

Growth parameters, such as average wet weight gain, average fork length gain, average total length gain, FCR, CF, SGR and survival rate were recorded every fortnight (Table I). Survival rate of fish during trial was recorded 100% throughout the experimental period which indicates that no external stress was given to fish.

Table II.- Proximate composition of *Pangasius hypophthalamus* when fed with exogenous enzyme added feed.

Parameters	Treatments			
	Control	T1	T2	T3
Moisture	83.83%	81.76%	78.27%	83.96%
Dry matter	16.17%	18.24%	21.73%	16.04%
Fat	5.78%	4.84%	7.35%	4.50%
Ash	25%	22%	21%	22%
Fiber	0.86%	0.90%	0.87%	0.69%
Protein	67.81%	63.43%	61.25%	68.18%
Carbohydrates	0.55%	8.83%	9.35%	4.63%

The average values obtained for all growth parameters during all three treatments and control are presented in Table I. The table showed significant differences for all parameters among all treatments. Overall increase in weight gain was recorded maximum in treatment # 2 with average value of 42.70±0.51 g during 12 week of experimental period whereas the minimum average weight gain was observed in control treatment (21.72±0.33 g). Maximum increase in average fork length was observed in control treatment (43.39±0.29 mm) and minimum gain was recorded in treatment # 1 (28.20±0.10 mm). Average total length increased maximally in treatment # 2 (58.61±0.12 mm) and minimally in treatment # 1 (35.43±0.09 mm). Maximum feed intake was during treatment # 2 and minimum feed intake was observed in control with the mean values of 161.83±0.20 and 84.3±1.00 g, respectively. Among all the treatments, better FCR was shown in treatment # 1 (1.21±0.01) and higher in treatment # 2 and control (1.26±0.01). The average feed conversion ratio for the experimental trial for all the treatments was recorded as 1.23±0.02. CF was observed maximum in treatment # 3 (1.26±0.03) while minimum in control group (1.56±0.04). The SGR was observed maximum with the mean values of 5.60±0.01 in treatment # 2 whereas it was found minimum in control treatment with the mean value of 3.75±0.12. The overall average of SGR for all the treatments and control was recorded as 4.78±0.81.

Proximate analysis

The proximate composition parameters *viz*. moisture, dry matter, crude fat, ash, crude protein, crude fiber and carbohydrates of striped catfish (*P. hypophthelmus*) was calculated at the end of experimental trial and presented in Table II. Moisture contents were recorded as 81.76%, 78.27%, 83.96% and 83.83% for treatment # 1, 2, 3 and control trial, respectively. Dry matter values for Treatment # 1, 2, 3 and control were recorded as 18.24%, 21.73%, 16.04% and 16.17%, respectively. Highest quantity of fat contents were observed in treatment # 2 when fed with enzyme added feed @ 0.50 g kg^{-1} (7.35%) and lowest was observed in treatment # 3 (4.50%) when fed with 0.75 g kg^{-1} enzyme added feed. Ash was calculated as 22%, 21%, 22% and 25% for Treatment # 1, 2, 3 and control group. Fiber contents in all treatments were less than 1% and the recorded values are 0.90%, 0.87%, 0.69% and 0.86% for treatment # 1, 2, 3 and control, respectively. Crude protein was present in fish with the mean values of 63.43%, 61.25%, 68.18% and 67.81% for treatment # 1, 2, 3 and

control group. Carbohydrate contents were calculated by collected all as fat, ash, fiber and protein and subtracted it with 100. Carbohydrate contents were computed as 8.83%, 9.35%, 4.63% and 0.55% for treatment # 1, 2, 3 and control group, respectively.

Microbial load

The data obtained on microbial load of experimental fish during the present study for all three treatments and control group is presented in Table III. During this study, the microbial load of fish, *P. hypophthalmus*, was determined and recorded. Microbial load was recorded differently among treatments. Growth of bacteria on nutrient agar occurred after 24 h while on EMB agar after 48 h. Colonies of bacteria were counted in colony forming units (cfu). Highest microbial load was observed in gut contents of control group on both media, nutrient agar (NA) and eosin methylene blue (EMB), with the mean values of 1.8×10^3 and 1.56×10^3 cfu whereas, on skin only total bacterial count (0.36×10^3 cfu) was observed and no coliform bacteria were present. Bacterial load among skin and gut in treatment # 1 was observed as 0.08×10^3 (NA) and 0.26×10^3 (EMB), 0.34×10^3 (NA) and 0.00 (EMB) cfu, respectively. In treatment # 2 and 3, only total bacterial count was observed on nutrient agar media in both skin and gut contents whereas no coliforms bacterial growth was observes on EMB agar media. The mean values of growth of overall bacterial count on NA in treatment # 2 in gut and skin were recorded as 0.14×10^3 and 0.10×10^3 cfu, respectively, whereas the same values were recorded for treatment # 3 were 0.18×10^3 and 0.04×10^3 cfu.

Table III.- Microbial load of *Pangasius hypophthalamus* when fed with exogenous enzyme added feed.

	Treatments			
	Control	**T1**	**T2**	**T3**
Gut contents (cfu/ml)				
Nutrient agar	1.8×10^3	0.34×10^3	0.14×10^3	0.18×10^3
EMB agar	1.56×10^3	Nil	Nil	Nil
Skin (cfu/ml)				
Nutrient agar	0.36×10^3	0.08×10^3	0.10×10^3	0.04×10^3
EMB agar	Nil	0.26×10^3	Nil	Nil

Hematological parameters

Hematological parameters such as white blood cells, lymphocytes, red blood cells, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, platelet count, mean platelet volume, platelet distribution width, procalcitonin test and platelet large cell ratio were recorded and presented in Table IV. Concentration of RBC's, WBC's , HCT and numbers of lymphocytes were highest in treatment 1 with the average values of 1.29×10^{12} L^{-1}, 54.9×10^9 L^{-1}, 18% and 48.7×10^9 L^{-1}. However, PDW and P_LCR were present only in treatment 4 *viz*. 13.3fL and 22.5%. Highest concentration of platelets was shown in treatment 3 and control, 40×10ˆ9 L^{-1}. GRAN# and MCHC were highest in treatment 2 blood sample respectively, 5.2×10ˆ9 L^{-1} and 38.3 g dL^{-1}.

Table IV.- Hematological parameters of *Pangasius hypophthalmus* when fed with exogenous enzyme added feed.

Parameters	**Control**	**T1**	**T2**	**T3**
WBC, $\times10^9$/L	35.8	54.9	41.3	44.3
LYM, %	90.9	88.7	83.3	92.7
MID, %	2.4	2.8	4.1	2.5
GRAN, %	6.7	8.5	12.6	4.8
LYM, $\times10^9$/L	32.6	48.7	34.4	41.1
MID, $\times10^9$/L	0.9	1.6	1.7	1.1
GRAN, $\times10^9$/L	2.3	4.6	5.2	2.1
RBC, $\times10^{12}$/L	1.06	1.29	1.27	1.15
HGB, g/dL	8.4	6.7	6.1	5.8
HCT, %	13.7	18.0	15.9	15.2
MCV, fL	130.0	140.3	125.6	132.5
MCH, Pg	79.2	51.9	48.0	50.4
MCHC, g/dL	61.3	37.2	38.3	38.1
RDW_CV, %	11.5	8.8	10.4	9.4
RDW_SD, fL	55.6	61.6	54.5	54.5
PLT, $\times10^9$/L	40	29	19	40
MPV, fL	6.4	6.4	6.3	11.4
PDW, fL	**.*	**.*	**.*	13.3
PCT, %	0.02	0.01	0.01	0.04
P_LCR, %	0.0	0.0	0.0	22.5

WBC, white blood cells; LYM, lymphocytes; MID, mid range; GRAN, granulocytes; RBC, red blood cells; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; PLT, platelet count; MPV, mean platelet volume; PDW, platelet distribution width; PCT, procalcitonin test; P_LCR, platelet large cell ratio.

Physicochemical parameters

The mean values of physic-chemical parameters are presented in Table V. The temperature and pH of test media of all three treatments and control was kept constant at 30°C and 7.5, respectively. All other parameters *viz*. Electrical conductivity, dissolved oxygen, salinity and total dissolved solids were recorded twice a day throughout the experimental period. The pH value of all the treatment showed non-significant differences throughout the experimental period of 12 weeks. Mean values of pH were recorded for treatment 1, 2, 3 and control as 7.54±0.43, 7.42±0.52, 7.53±0.24 and 7.49±0.55, respectively.

Table V.- Physicochemical parameters recorded during the study period.

Parameters	Treatments				
	Control	T1	T2	T3	Means
pH	7.49±0.55a	7.54±0.43a	7.42±0.52a	7.53±0.24a	7.43±0.13a
E.C (mScm^{-1})	2216.78±224.20a	2189.55±205.57a	2213.75±207.27a	2165.25±189.30a	2196.33±24.03a
Temperature (°C)	29.52±0.56a	29.45±0.59a	29.44±0.59a	29.42±0.86a	29.45±0.04a
TDS (mg/l)	1120.00±176.82a	1116.50±164.85a	1128.75±189.00a	1105.13±166.01a	1117.59±9.77a
D.O (mg/l)	6.61±7.86a	5.31±0.63a	5.25±0.43a	5.04±0.45a	5.55±0.71a
Salinity (mg/l)	1.00±0.00a	1.00±0.00a	1.00±0.00a	1.00±0.00a	1.00±0.00a

Means with same letters in a single column are statistically similar at $p< 0.05$.
EC, electrical conductivity; TDS, total dissolved solids; D.O, dissolved oxygen.

Electrical conductivity values were recorded non-significant for all groups. Temperature of water in all the treatments and their replicates showed non-significant. Mean value of temperature was recorded for treatment 1, 2, 3 and control with the mean values of 29.45±0.59, 29.44±0.59, 29.42±0.86 and 29.52±0.56 (°C), respectively. TDS was measured and non-significant results showed in treatment 1, 2, 3 and control with the mean values of 1116.50±164.85, 1128.75±189.00, 1105.13±166.01 and 1117.59±9.77 (mgL^{-1}), respectively. DO was measured and all values were non-significant in all treatments, 1, 2, 3 and control. Salinity remains non-significant during whole experimental period with the constant value 100±0.00 mgL^{-1} in all treatments.

DISCUSSION

Fish growth response and feed utilization were improved with enzyme supplementation, suggesting that the negative effects of plant ingredients were compensated to some extent by addition of the enzymes (Tahoun *et al*., 2011). The present results are in agreement with the previous studies on growth responses of fish by feeding exogenous enzyme added feed. Many researchers conducted experiments on exogenous enzymes added feed to determine the growth responses of various fish species *viz*. Drew *et al*. (2005) conducted experiment on *Oncorhynchus mykiss*, Debnath *et al*. (2005) on *Pangasius pangasius* and Lin *et al*. (2007) on *Oreochromis niloticus*. Mehboob *et al*. (2017) determined positive effects on growth responses of stripped cat fish when fed with fenugreek seeds added feed. Present results are in-line with these studies and revealed that the enzyme additive feed exhibited higher growth and SGR than that of free enzymes diet (control) indicated that enzyme is beneficial for the growth of catfish. The enzymes were added in the moist feed mass and no heat was used in the extrusion. During the present study, growth was enhanced significantly in treatment 2, fed with 0.50 g kg^{-1} enzyme supplemented diet with an average wet weight (g) and length (mm) of 42.70±0.51 g and 58.61±0.12 mm, respectively, during 90 days of trail. Addition of α-amylase in feed promotes growth of fish by causing effective digestion. According to Yildirim and Turan (2010) supplemented enzyme complex group can significantly improve growth performance and feed utilization in African catfish. Vielma *et al*. (2002) also recorded improved growth of rainbow trout by exogenous supplementation of phytase enzyme. Jackson *et al*. (1996) observed improved growth rate in channel catfish when fed with exogenous enzyme added feed. These results indicated that exogenous enzyme supplemented feed can promote fish growth. The proximate composition of present study shows similar results among protein values, crude fats and percent carbohydrates in all treatments and control. Begum *et al*. (2012) also determined proximate analysis of *Pangasionodon hypphthalmus* and recorded the values of moisture, protein, fat and ash (%) of the edible portion as 78.29 ±0.22, 12.78 ±0.16, 16.55 ± 1.52 and 1.78 ±0.19, respectively.

Microbial studies within the GIT of the fish have received great attention due to recent studies. These microbial populations give defense against pathogens to the GIT and aid host digestive function via the production of exogenous enzyme and vitamins (Dimitroglou *et al*., 2011). During the present research trial, total bacterial count for coliforms in itestine was highest in control treatment and was recorded non-significant among treatments. GIT is considered as one of most important and intimate sites of interaction with the external world and is on the major site for pathogenic invasion in fish (Ringo *et al*., 2007). For present results, treatment # 1 showed significant results of total bacterial count on skin (0.26×10^3 cfu ml^{-1}), remaining all were non-significant which indicates that enzymes additive feed have no negative impact on fish health and it may be used in feed to improve fish health and growth enhancement. High ratio of bacterial count is related with an increase in suitable attachment sites (Ringo *et al*., 1995) which results in histological and functional development of fry and improved internal environmental conditions for bacterial growth (Vine *et al*., 2006). As compared to the

terrestrial animals, which have flora in their GIT, aquatic animals have most short lived microbes (Panigrahi *et al*., 2004) and are affected by the conditions of surrounding water (Gomez-Gill *et al*., 2000).

RBC's, WBC's and lymphocytes were significantly higher in enzyme supplemented group, treatment 1 with 0.25 g kg^{-1} α-amylase and other treatments containing enzyme shown better results than control group. On contrary to present findings, Kumar *et al*. (2005) concluded that haematological parameters are not affected due to dietary carbohydrate in fish except for WBC, which is related to immunological response in *L. rohita* juveniles. Waagbø *et al*. (1994) found a negative correlation between haemoglobin and increasing dietary carbohydrate level in Atlantic salmon.

CONCLUSION

The present study indicated that enzymes additive feed exhibited beneficial effect on growth performance, feed conversion rate and microbial load. *P. hypophthalmus* showed best growth on a diet containing 40% protein contents and 0.50 g kg^{-1} α-amylase and formulated to provide best efficiency. Other possible effects of the enzyme supplements, such as an action on intestinal microbial flora or an improvement in growth by the release of a growth enhancing factors, cannot be disregarded. On the other hand, enzyme supplementation also effects on WBC's, RBC's, lymphocytes and platelets. The experimental trail on catfish with enzyme added feed was conducted first time in Pakistan and beneficial results were observed.

Statement of conflict of interest

Authors have declared no conflict of interest.

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