

Improved Growth and Nutritional Quality of Pangas Catfish, *Pangasius hypophthalmus* (Sauvage, 1878) by Enzyme Supplementation

Palash Bala¹, Md. Ghulam Mustafa², Shankar Chandra Mandal^{2,*} 

¹District Fisheries Office, Jashore, Bangladesh.

²University of Dhaka, Department of Fisheries, Dhaka 1000, Bangladesh.

Article History

Received 19 May 2020
Accepted 02 October 2020
First Online 07 October 2020

Corresponding Author

Tel.: +8801913129746
E-mail: shankar@du.ac.bd

Keywords

Enzyme supplementation
Flesh yield
Hepatosomatic index
Length-weight relationship

Abstract

Pangas catfish, *Pangasius hypophthalmus* is one of the most widely cultured fish species in Asia. The growth and flesh quality of fish depend largely on the quality and composition of feed. Inclusion of exogenous enzyme can improve the palatability and digestibility of feed. However, there is a lack of research on the use of exogenous enzymes in fish diet for improved growth and nutritional quality of fish. Thus, in the current study, the effects of enzyme inclusion in fish diet were evaluated using pangas catfish as an experimental animal. The study was conducted in a laboratory condition with a flow-through system where 12 plastic tanks with 750 L water capacity were used where the tanks were grouped randomly. Each tank was stocked with 15 fingerlings of pangas catfish. Three different concentrations (3, 4 and 5% of the total feed) of Zymex, a commercially available multi-enzyme were used for treatment groups along with a control group with no enzyme supplementation. The length-weight relationship of the experimental fish using the initial and final data was established where a significantly positive relationship was found ($P < 0.05$). Growth performance including condition factor, specific growth rate and feed conversion ratio was significantly improved by 3% enzyme supplementation ($P < 0.05$). Average daily gain did not vary significantly between treatments. Three percent enzyme supplementation in the diet significantly increased crude protein and lipid contents and reduced moisture content ($P < 0.05$). Significantly higher ash content was found in fish fed both 3 and 5% enzyme contained diet ($P < 0.05$). In case of body indices, 5% enzyme supplementation provided significantly higher viscerosomatic index ($P < 0.05$) while 3% provided significantly higher flesh yield ($P < 0.05$). However, hepatosomatic index did not vary significantly between treatments. From the findings it is concluded that enzyme supplementation at 3% of the diet can improve the growth performance and nutritional quality of pangas catfish.

Introduction

Aquaculture plays a significant role to fulfill the demand of a growing world population by a steady growth of aquaculture production (FAO, 2018). The rate of increment of fish production is double the world population growth rate (FAO, 2014). The production of

aquaculture food products including fish, crustaceans, and mollusks has been recorded at 82 million tons (excluding aquatic plants) in 2018 (FAO, 2020). Aquaculture food fish contributed a record over 46% of the total capture fisheries and aquaculture in 2018 contributing to the increment of per capita fish consumption from 9 kg in the 1961 to 20.5kg in 2018

(FAO, 2020). This sector has great potential to expand and to meet the growing protein demand of an increasing global population. It is expected that, aquaculture shall provide about 59 percent of the fish available for human consumption by 2030. (FAO, 2020).

Pangas catfish (*Pangasius hypophthalmus*) is one of the commercially cultured fish species which contributed around 3% of total global aquaculture production in 2016 where Viet Nam shares the majority of the world's pangas production (FAO, 2018). Several other countries including Thailand and Bangladesh also produce a significant amount of pangas. Pangas, for example in Bangladesh, contributed more than 10% of the total country's aquaculture production in 2017-2018 amounting to nearly half a million metric tons (DoF, 2018). Thus, a high percentage of protein supply comes from pangas aquaculture. Sarker (2000) reported pangas as one of the best-suited aquaculture species due to its easy culture system, favorable weather condition for culture, good survival rates, fast growth and ability to survive at high stocking densities and high market demand which have attracted many fish farmers to switch to pangas aquaculture.

Aquaculture production largely depends on feed which is one of the important aspects of aquaculture where the poor-quality feed can decrease the production rate to a significant level. Thus, the use of quality feed reduces the production cost as well as wastages of feed ingredients by increasing the digestibility. Digestibility can be increased by the inclusion of digestive enzymes in the feed. The quality of feed ingredients determines the rate of digestibility (Felix & Selvaraj, 2004). Improved growth performance has been reported by the exogenous enzyme supplementation (Farhangi & Carter, 2007; Lin, Mai & Tan, 2007; Soltan, 2009). The effects of exogenous and digestive enzyme supplementation in diets on growth and feed utilization of several cultured fish and shellfish have been demonstrated elsewhere. For example, Buchanan, Sarac, Poppi & Cowan (1997) have investigated the effects of an additional enzyme with canola meal on tiger shrimp (*Penaeus monodon*) resulting in a significantly higher weight gain. Jackson, Li & Robinson (1996) have shown the weight gain of channel catfish, *Ictalurus punctatus* by feeding a diet with the inclusion of microbial phytase. Nutritional quality has been reported to be affected by endogenous and exogenous factors where endogenous factors include sex and life cycle of the fish and exogenous factors include diet composition, water temperature, salinity etc. (Shearer, 1994). Growth and flesh composition of fish have also been reported by the same researcher to be affected by those factors. The inclusion of digestive enzymes in the feed has been reported to improve the growth and nutritional quality of fish (Lin *et al.*, 2007; Rahman & Sarker, 2019; Yigit, Bahadir, Didinen & Diler, 2018). Supplementation with enzyme can help to eliminate the effects of anti-nutritional factors and

improve the utilization of dietary energy and amino acids, resulting in improved performance of fish (Farhangi and Carter, 2007; Lin and Tan, 2007; Soltan, 2009). Multi-enzyme complexes have the potential to achieve high catalytic efficiency for sequence reactions due to their advantages in eliminating product inhibition, facilitating intermediate transfer and in situ regenerating co-factors (Wang, Zhang, Wang, Jiang and Fang, 2017). On the contrary of this sentences, some studies have noted no significant increase in growth performance with enzyme supplemented diet in rainbow trout, *Oncorhynchus mykiss* (Yigit *et al.* 2018; Shakoori, Hoseinifar, Paknejad, Jafari & Safari, 2018). Therefore, enzyme supplementation can be pointed as one of the an important elements in the cultural practice. Thus, the current study hypothesized that the enzyme supplementation will increase the growth performance and nutritional quality of pangas catfish. Therefore, this study aimed to investigate the impacts of exogenous enzyme supplementation on some aspects of the growth performance and nutritional quality of pangas catfish.

Materials and Methods

Experimental Fish and Enzyme

Fingerlings of pangas catfish, *Pangasius hypophthalmus*, a fast-growing omnivorous fish and tolerant to high stocking densities were used in this study. A commercially available multi-enzyme complex (Zymex) containing Amylase, Xylanase, Protease, B. Glucanase, Phytase and Pectinase was used. Zymex enzyme complex contains digestive enzymes which accelerate healthy gastrointestinal (GI) activity by maintaining health condition of animal by increasing the digestion of low-quality products by reducing nutrients loss through excreta allowing the reduction of recommended nutritional levels in the diet but also reducing the environmental pollution by minimizing the production of excreta (Khedr, Kamelia, Tahja and Elramy, 2016).

Experimental Design

The fingerlings were collected from a hatchery of Mymensingh, Bangladesh. The fingerlings were first acclimatized at the experimental site using tap water. One hundred and eighty fingerlings (Initial length: 9.06 ± 0.10 ; weight: 5.51 ± 0.17 g) were distributed randomly into twelve 750 L tanks each tank receiving 15 fingerlings. Continuous aeration was supplied throughout the experiment. The experiment consisted of one control group and three levels of enzyme supplementation groups. Three levels of enzyme mixture were 3, 4 and 5 % of the total feed. No enzyme was supplemented into diet for control group. Fish were cultured for 60 days in the laboratory condition. Daily

water exchange was adjusted to avoid the decomposition of fish excreta and excess feed. Daily monitoring of some water quality parameters was performed where pH was measured using Orion field pH meter (model 210A, Orion Laboratories) while dissolved oxygen (DO) along with water temperature was measured using a portable HACH DO meter (model DO175). The temperature, dissolved oxygen and pH of the culture units were 27.25 ± 1.20 °C, 8.26 ± 0.27 mg/l and 8.05 ± 0.12 respectively. A locally available feed was mixed with different concentrations of experimental enzyme. Fish were fed twice a day at a rate of 5% of body weight.

Sample Preparation and Measurements

Individual length and weight of every fish were measured at the beginning of the experiment and end of each month with a measuring board and electric balance to calculate the condition factor (K), average daily gain (ADG) and specific growth rate (SGR). Sample fish (5 individuals) were captured with a fine mesh scoop net and total body weight was measured followed by the extraction and weighting of viscera, liver and flesh content separately from the fish to evaluate the viscerosomatic index (VSI), hepatosomatic index (HSI) and flesh yield (FY) at the beginning and the end of the experiment.

Growth Analysis

The initial and final length and weight of individual fish were measured for determination of growth performance. Length-weight relationship of experimental fish (initial and final) was determined following the method describe by Imam, Bala, Balarabe, & Oyeyi (2010). With the previously recorded data, the condition factor (K) was calculated by the equation, $K = (W/L^3 \times 100)$, where W = Fish weight (g) and L = Fish length (cm). Average daily gain (ADG) is simply the rate of weight gain per day over a specified period and was determined as, $ADG = (Final\ weight - Initial\ weight) / Duration\ (day)$, where length and weight of every fish of each treatment were used. The specific growth rate (SGR) is the percentage of body weight increase per day. It was calculated as, $SGR = (LnWT - LnWt) / (T - t) \times 100$ where, LnWT = Natural log of weight at time T and LnWt = Natural log of weight at initial time t, T-t = duration in day. Feed conversion ratio (FCR) was calculated from the amount of feed (kg) that were used to produce 1 Kg of fish by the following formula: $FCR = Feed\ consumed\ (g) / Weight\ gain\ (g)$. Body indices like viscerosomatic index (VSI) and hepatosomatic index (HSI) along with flesh yield (FY) are the proportion of viscera, liver and flesh weight to the total body weight respectively. They were calculated with the following formula:

$$VSI = (Viscera\ Weight \times 100) / Body\ Weight;$$

$$HSI = (Liver\ Weight \times 100) / Body\ Weight;$$

$$FY = (Flesh\ Weight \times 100) / Body\ Weight.$$

Proximate Composition Analysis

The oven drying method was used to determine the moisture content following the method of AOAC (1990). The sample for moisture content was pre-weighted and dried for 6 hours in an oven at 105 °C. The loss of weight was calculated as moisture content. Samples for the determination of ash content were burned in a muffle furnace for 16 hours at 550 °C. The weight of the residue was calculated as ash content. The standard micro-Kjeldahl method was used to determine crude protein content. The amount of nitrogen was first determined which was finally multiplied by 6.25 (Protein conversion factor) to convert the nitrogen into crude protein. An automated lipid extractor (Soxhlet system) was used to determine the lipid content.

Statistical Analysis

Data were analyzed using Graph Pad Prism version 8.0 and results were presented as mean \pm SEM. Tukey's multiple comparison test of one-way ANOVA was used to compare the data between treatments. Any significant difference was determined at $P < 0.05$. The normality test was done using the Shapiro-Wilk test at $P < 0.05$ (Ghasemi & Zahediasl, 2012) before the analysis.

Results

Length-weight Relationship of the Experimental Fish

Initial and final length (cm) and weight (g) of the experimental fish were recorded and were plotted to test the normality. The data passed the normality test and hence length-weight relationship was determined. Both the initial and final data showed a significant positive correlation between the length and weight of the fish ($r^2 = 0.87$ and 0.86 respectively; $P < 0.05$; Figure 1). Moreover, the initial and final length and weight of the experimental fish of each of the treatments were determined (Figure 2). Final length in fish fed with 3% enzyme mixed feed was significantly higher than other groups ($P < 0.05$) while the final weight in fish fed with 3% enzyme mixed feed was significantly higher than the control group ($P < 0.05$).

Growth Performance

Growth performance in terms of daily weight gain, specific growth rate, feed conversion ratio and condition factor of pangas catfish fed with exogenous enzyme mixed in supplementary feed was determined. Feeds were mixed with the enzyme at 3, 4 and 5%. Average daily gain (ADG) was calculated after 30 days and 60

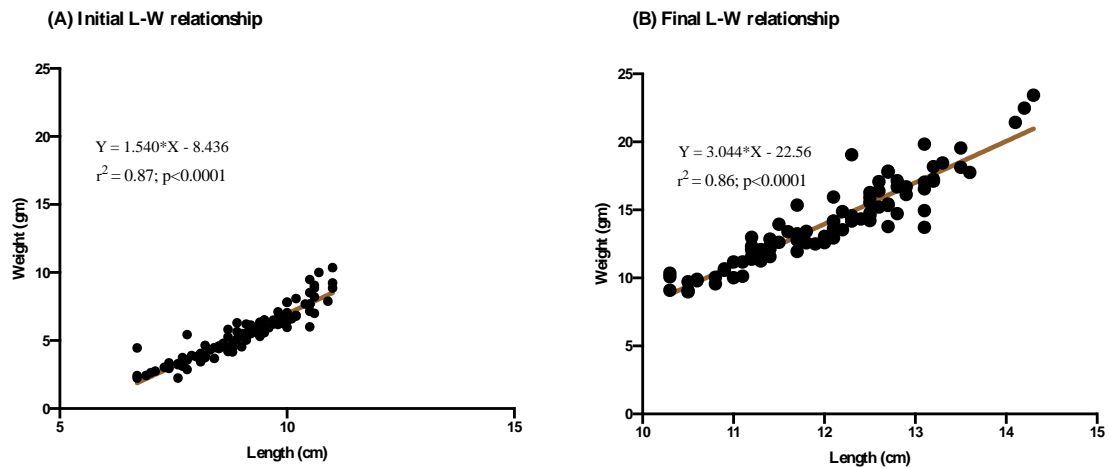


Figure 1. Length-weight relationship of the experimental fish (A) before starting the experiment and (B) at the end of the experiment.

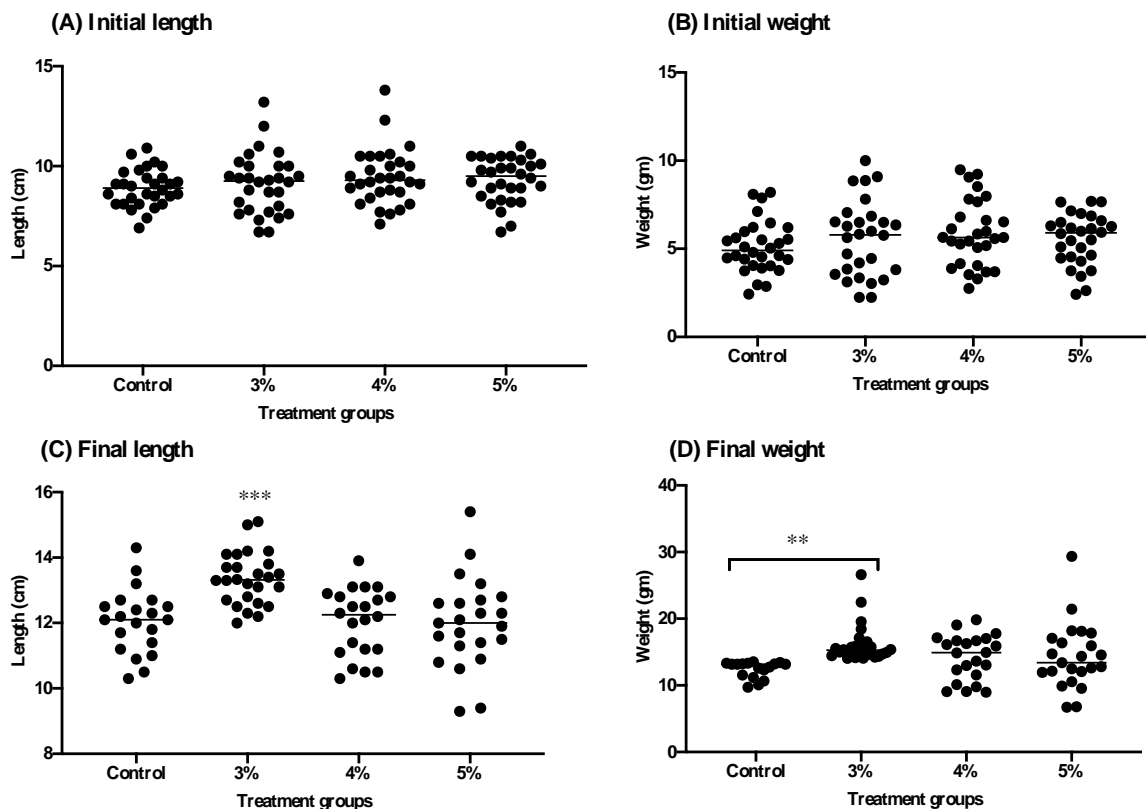


Figure 2. The initial and final length and weight of the experimental fish. Length and weight of individual fish of each of the treatment group were plotted and data were analyzed using Graph Pad Prism version 8.0 where multiple comparison of one-way ANOVA was employed at $P < 0.05$.

days of the rearing period. No significant difference between the control group and enzyme supplemented groups at both 30 and 60 days of culture (Figure 3A). ADG in fish of different treatment groups ranged between 0.18 and 0.20g at day 30 and day 60. The specific growth rate (SGR) was calculated after 30 days and 60 days of the rearing period. Significantly higher SGR was found in the fish fed with 3% enzyme mixed supplemented feed (1.70 ± 0.02 and 1.72 ± 0.05 %,

respectively at 30 and 60 day) than that of control fish (1.36 ± 0.06 and 1.42 ± 0.09 %, respectively at 30 and 60 day) and fish fed with 4% (1.3 ± 0.12 and 1.27 ± 0.08 %, respectively at 30 and 60 day) and 5% (1.35 ± 0.07 and 1.36 ± 0.01 %, respectively at 30 and 60 day) enzyme supplement feed at both 30 and 60 day culture period (Figure 3B; $P < 0.05$). However, the control group and 4 and 5% enzyme supplemented group showed similar SGR at both duration ($P > 0.05$).

Condition factor (K) was measured at the first day of the rearing period and at the end of the 30 and 60-day rearing period (Figure 3C). Significantly higher K value was found in 3% enzyme supplemented group at both 30 and 60 day of culture than that of other groups ($P < 0.0001$). However, all other treatment groups including the control group (no enzyme supplemented group) showed similar K values ($P > 0.05$). Feed conversion ratio (FCR) of fish at both 30 and 60 day culture periods, in the 3% enzyme supplemented group gave significantly lower FCR (2.20 ± 0.02 and 2.30 ± 0.02 , respectively at 30 and 60 day) than that of other groups ($P < 0.0001$) where FCR in fish of the control group (2.80 ± 0.05 and 2.80 ± 0.03 , respectively at 30 and 60 day) had significantly lower FCR than that of 4% ($P < 0.05$) and 5% ($P < 0.0001$) enzyme mix feed (Figure 3D). Furthermore, FCR in fish fed with 4% enzyme mixed feed (around 2.90 at both 30 and 60 day) was significantly lower than that of 5% enzyme mixed feed (around 3.0 at both 30 and 60 day) in both duration ($P < 0.05$).

Body Indices

Body indices including the viscerosomatic index (VSI), hepatosomatic index (HSI) and flesh yield (FY) were calculated at the end of the experiment (day 60 of culture period). Significantly higher VSI was found in fish fed with supplemented feed containing 5% enzyme mix than that of other groups (Figure 4A, $P < 0.01$). However, VSI in control fish and fish fed supplemented feed containing 3 and 4% enzyme mix groups did not vary significantly ($P > 0.05$). VSI in control, 3, 4 and 5% enzyme supplemented groups was 8.61 ± 0.07 , 8.25 ± 0.15 , 8.45 ± 0.17 and $8.99 \pm 0.23\%$, respectively. Moreover, the hepatosomatic index (HSI) presented insignificant differences between treatments (Figure 4B) where the values were 2.28 ± 0.07 , 2.40 ± 0.06 , 2.36 ± 0.03 and $2.40 \pm 0.05\%$, respectively. In the case of flesh yield (FY), a significantly higher value was found in fish fed with feed containing a 3% enzyme mix ($49.72 \pm 0.34\%$) than that of other groups ($P < 0.001$) where similar flesh yield was observed (47.13 ± 0.34 , 47.29 ± 0.26 and $46.59 \pm 0.21\%$, respectively in control, 4 and 5% enzyme mixed diet) (Figure 4C).

Nutritional Quality

In this study, moisture, ash, protein and lipid content were determined as indicators of the nutritional quality of the experimented fish. At the end of the experiment, the proximate composition of the fish body was determined. These nutritional parameters were compared between treatments for the final proximate composition data obtained at the end of the experiment (60-day experiment). Among proximate composition, crude protein content in fish fed 3% enzyme mixed feed was significantly higher ($26.54 \pm 0.12\%$) than that of the control group ($24.63 \pm 0.09\%$) ($P < 0.0001$) and 4% (25.18

$\pm 0.32\%$) and 5% ($25.22 \pm 0.09\%$) enzyme inclusion groups ($P < 0.001$) (Figure 5A). However, similar protein content was found in fish fed 4 and 5% enzyme mixed feed ($P > 0.05$). Significantly lower moisture content was found in fish fed with 3% enzyme included feed than that of control fish (Figure 5B; $P < 0.01$). However, moisture content did not vary significantly in fish fed 3, 4 and 5% enzyme mixed feed. Moisture content in fish in control, 3, 4 and 5% enzyme mixed groups were 62.26 ± 0.58 , 59.26 ± 0.67 , 60.23 ± 0.40 and $60.89 \pm 0.23\%$, respectively. Lipid content was significantly increased in enzyme supplemented groups than that of the control group ($P < 0.001$) where increasing enzyme percentage did not increase lipid content significantly (Figure 5C). The lipid content in fish in control, 3, 4 and 5% enzyme mixed groups were 11.70 ± 0.05 , 12.11 ± 0.02 , 12.13 ± 0.07 and $12.33 \pm 0.06\%$, respectively. Inclusion of enzyme mix in the diet increased the ash content in fish significantly from the control fish ($P < 0.01$; Figure 5D). Five percent enzyme inclusion did give the highest ash content which was similar to that of 3% enzyme mixed group. Fish fed 4% enzyme mixed feed had similar ash content to 3% enzyme mix group ($P > 0.05$) but significantly lower than 5% enzyme mix group ($P < 0.01$). The ash content found in fish in control, 3, 4 and 5% enzyme mixed groups were 1.20 ± 0.01 , 1.26 ± 0.01 , 1.21 ± 0.01 and $1.28 \pm 0.02\%$, respectively.

Discussion

Growth Performances

Several indicators of growth performance were improved by enzyme supplementation in pangas catfish. Improved growth performance in terms of SGR and FCR has been reported in rohu, *L. rohita* feed with exogenous multi-enzyme supplemented feed (Rahman & Sarker, 2019). Carp, *Cyprinus carpio* fingerlings have also been shown to respond positively to enzyme supplemented feed by increased weight gain and nutritional quality (Bogut, Opacak & Stevic, 1995). Improved growth performances have also been reported in Nile tilapia, *Oreochromis niloticus* by enzyme inclusion in the diets (Maas, Verdegem, Dersjant & Schrama, 2018). However, the addition of enzyme mix in the supplemented feed has not been found to improve growth performance and body composition of rainbow trout, *O. mykiss* as reported by Yigit *et al.* (2018). Another study with rainbow trout, *O. mykiss* fry feeding with enzyme-containing feed showed no significant changes in growth performance (Shakoori, Hoseinifar, Paknejad, Jafari & Safari, 2018). ADG in the present study changed significantly with enzyme supplementation. However, several studies have shown similar ADG in different fishes including pangas. Sangrattanakhul (1989) has shown ADG of *Anabas testudineus* ranging from 0.10 to 0.12g while Shahjahan (1997) has found ADG of GIFT tilapia, *O. niloticus* ranging

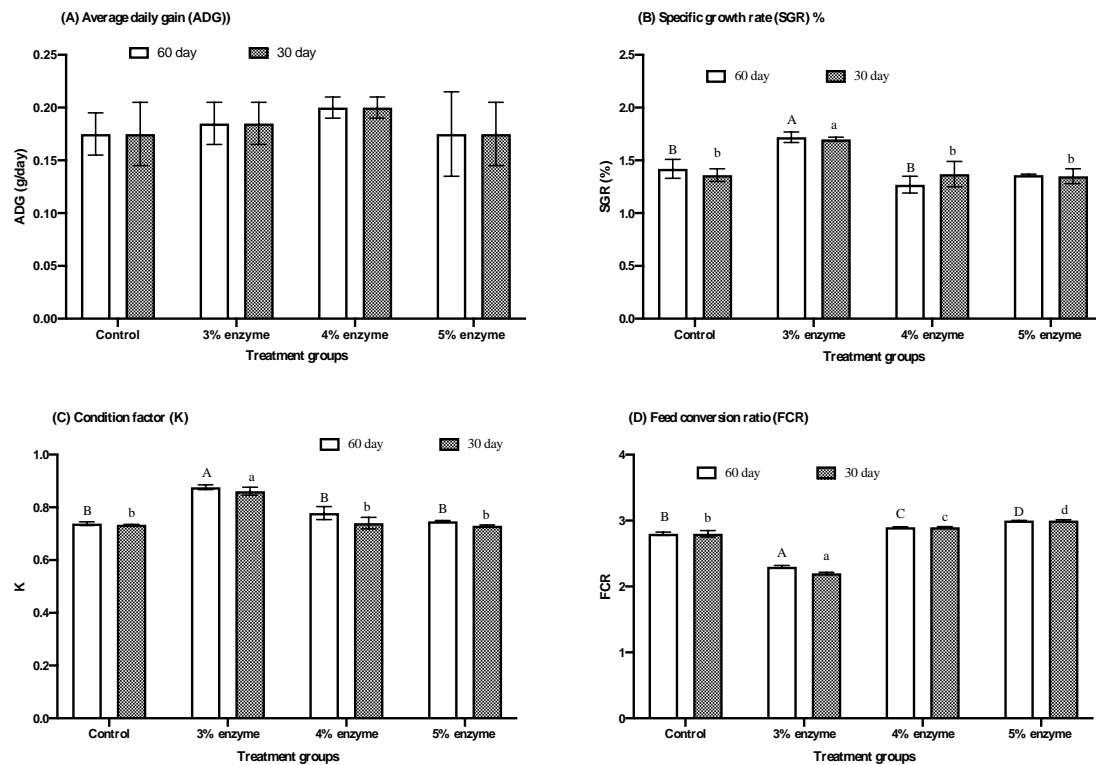


Figure 3. Growth performance of pangas catfish fed with enzyme mix with the supplemental feed for 30 and 60 days. Different growth parameters including (A) average daily gain, (B) specific growth rate, (C) condition factor and (D) feed conversion ratio were determined. Data were analyzed using Graph Pad Prism version 8.0. Multiple comparison of one-way ANOVA was employed to compare the growth performance between treatments. Data were presented as mean \pm SEM. Bar with same patterns different letters are significantly different at $P < 0.05$. Capital and small letters denote significantly different between treatments at day 30 and 60 respectively.

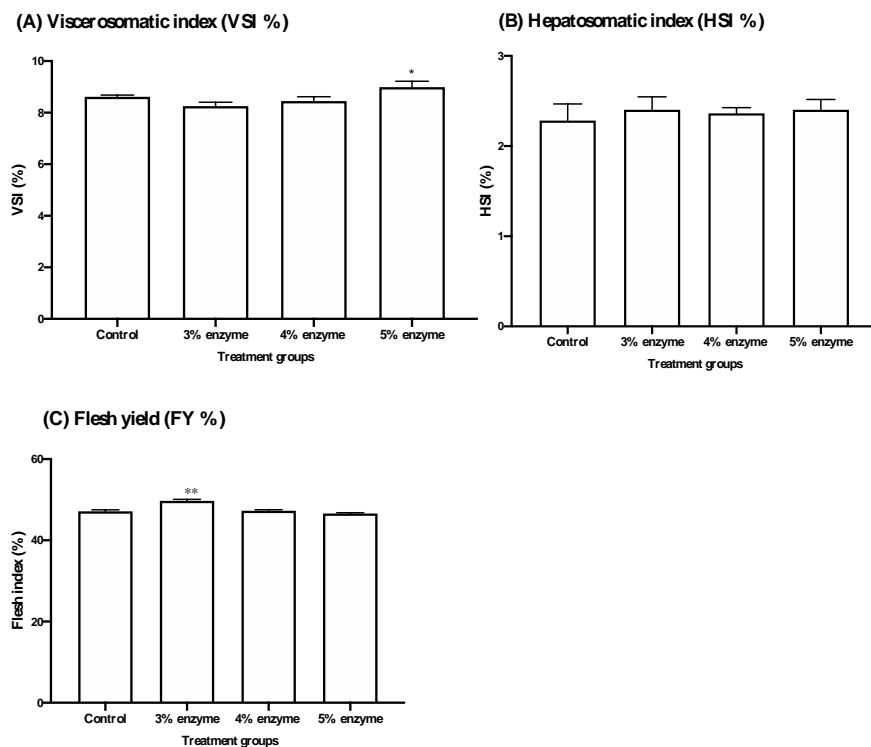


Figure 4. Body indices of pangas catfish fed with enzyme mix with the supplemental feed. Different body indices including (A) viscerosomatic index, (B) hepatosomatic index, and (C) flesh yield were determined at the end of the 60-day laboratory experiment. Data were analyzed using Graph Pad Prism version 8.0. Multiple comparison of one-way ANOVA was employed to compare the body indices between treatments. Data were presented as mean \pm SEM. Bar with * and ** are significantly different at $P < 0.01$ and $P < 0.001$ respectively.

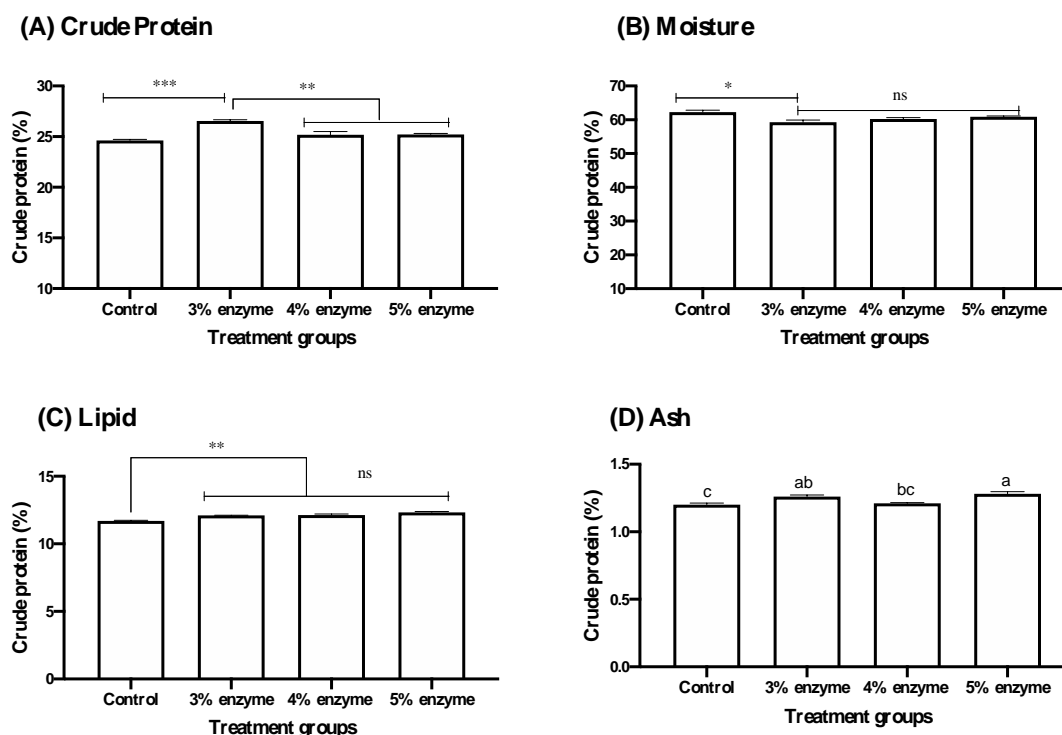


Figure 5. Nutritional quality of pangas catfish (muscle, dry matter basis) fed a supplemental diet containing enzyme mix. Different nutrient components including (A) crude protein, (B) moisture, (C) lipid and (D) ash were determined at the end of the 60-day laboratory experiment. Data were analyzed using Graph Pad Prism version 8.0. Multiple comparisons of one-way ANOVA was employed to compare the body indices between treatments. Data were presented as mean \pm SEM. Bar with *, ** and *** are significantly different at $P < 0.01$, $P < 0.001$ and $P < 0.0001$ respectively (A-C) while bars with different letters are significantly different at $P < 0.01$.

from 0.14 to 0.175g. Higher ADG of 1.87 ± 0.21 also reported by Ahmed, Chakma, Shamsuddin, Minar, Islam & Majumdar (2013). Mandal, Hossain, Islam & Mirza (2002) have reported ADG differs significantly with the stocking density. In the present study, the ADG of pangas catfish were similar to the findings of Sangrattanakhul (1989) and Shahjahan (1997). Yildirim & Turan (2010) have reported significantly higher specific growth rate of African catfish (*Clarias gariepinus*) fed a diet containing 0.75g enzyme complex per Kg feed ranging between 1.09 and 1.23 which are in agreement with the findings of the current study where 3% enzyme supplementation provided improved growth of pangas catfish. Moreover, exogenous xylanase has been shown to increase body weight and SGR in grass carp, *Ctenopharyngodon idella* (Jin *et al.*, 2020), juvenile Jian carp, *Cyprinus carpio* var. Jian (Jiang *et al.*, 2014), juvenile Gibel carp, *Carassius auratus gibelio* (Liu *et al.*, 2018).

Each fish has a characteristic range of condition factor (K), which reflects their body conformation. In this study, significantly higher condition factor was found at 3% enzyme supplemented feed which was nearly 0.88. In a study, Saha, Mollah & Roy (1998) have demonstrated K value nearly 1.0 in the case of *C. batrachus* fed on formulated diets. Rahman, Bhadra, Begum & Hussain (1997) have shown K value between 0.81 and 0.87 in catfish fed selected supplemental diet

which supports the findings of the present study. Feed conversion ratio (FCR) of pangas catfish fed with 3% enzyme supplemented feed in this study has been the lowest. The improved feed conversion ratio due to enzyme supplementation in the present study is supported by the findings of Huang *et al.* (2020) who have reported a significantly lower FCR in Japanese seabass, *Lateolabrax japonicus* using multi-enzyme supplemented diet (Huang *et al.*, 2020). Tahoun, Mabroke, El-Haroun & Suloma (2011) have also reported a positive effect on FCR of fish when supplemented with multi-enzyme complex at 0.5g per Kg feed. Ahmed *et al.* (2013) have reported FCR from 1.93 ± 0.30 to 2.34 ± 0.12 in pangas. Yildirim & Turan (2010) have also reported the best FCR in African Catfish, *Clarias gariepinus* fed with diet containing enzyme of 0.75g per Kg feed.

Body Indices

To evaluate the effects of exogenous enzyme supplementation on body indices of pangas catfish were also determined in the current study. Five percent enzyme supplementation provided significantly higher viscerosomatic index (VSI) in the present study which was around 9% of the total body weight. However, the hepatosomatic index (HSI) did not vary in control and treated fish which was around 2.5% of the total body

weight. In a study, the exogenous enzyme has been found to reduce the HSI while VSI remained stable in mullet, *Mugil cephalus* (Travagli, Zanardi, Bernini, Nepi, Tenori & Bocci, 2010). Phumee, Hashum, Aliyu-Paiko & Shu-Chien (2009) have reported maximum VSI as 7.79% and HSI as around 2% in pangas catfish. In another study, Aliyu-Paik, Hashim & Shu-Chien (2010) have reported VSI between 3.5 and 4.8% and HSI between 2.1 and 2.6% in *Channa striatus* feeding with different percentage of protein and lipid contained diets where different lipid levels have had significantly different VSI and HSI. Ranjan, Sahu, Deo & Kumar (2018) reported significantly higher HSI in *L. rohita* fed enzyme supplemented diet. Ighwela, Ahmad & Abol Munafi (2014) have reported no variation of VSI in Nile tilapia feed different diets while shown differences in HSI between treatments. However, they have shown a lower level of VSI (around 1.5%) and HSI (around 0.55%). The flesh yield was significantly higher in fish fed 3% enzyme mixed diet than that of fish fed the control diet. The highest flesh yield in this study ranged between 46 and 48%. Torry Research Station (1989) has reported fish flesh yield between 28 and 57% of the total body weight in different fish and shellfish which corresponds to the flesh yield of pangas catfish found in the current study.

Nutritional Quality

The nutritional quality of the experimental fish was determined in control fish and fish fed enzyme contained diets to evaluate the effects of the enzyme on proximate composition of fish. Protein is one of the elements of fish body composition playing an important role in fish growth performance and development (Gatlin III, 2003). In the present study, a 3% enzyme mixed feed was found to provide significantly higher crude protein content than control fish and fish fed higher than 3% enzyme contained feed. Significantly higher crude protein level has also been reported in African catfish, *C. gariepinus* (Yildirim & Turan, 2010) and in *L. rohita* (Ranjan *et al.*, 2018) by enzyme supplementation which supports the findings of the current study. Enzyme supplementation might facilitate the digestion of protein in the diet and subsequent absorption. Enzyme supplementation was found to negatively impact on the moisture content in the experimental fish. The decrease in moisture content in the current study might be related to the lipid content where a dose-dependent increase in lipid content was found in this study. Moisture content in fish is inversely correlated with lipid content. However, several studies have reported no significant changes in moisture content in different fishes, for example, Siti-Norita, Arbakariya, Noor-Azlina & Ibrahim (2015) in tilapia fed with diets containing B-mannose enzyme, Ranjan *et al.* (2018) in *L. rohita* using phytase, xylanase and cellulase supplemented diets, and Yildirim & Turan (2010) in African catfish fed exogenous enzyme complex

(xylanase, β -glucanase, pentosonase, β -amilase, hemicellulase, pectinase, cellulase and cellulase) supplemented diets. These studies have also reported significantly higher lipid content in fish fed enzyme contained feed which supports the findings of the present study. The difference in moisture content found in the present study and previous studies as mentioned here might be because of the composition of the multi-enzymes supplemented in the diet. Another reason might be the difference in fish species. Ash is another important component that helps fish for the formation of tissues, osmoregulation and lots of different other metabolic functions (Lall, 2002). Ash content was significantly higher in pangas catfish fed 3 and 5% enzyme contained diet than that of control fish in this study. Enzyme addition in feed might help in mineralization of feed that increases the percentage of ash content in the proximate composition. An increase in ash content in *L. rohita* has also been reported by Rahman & Sarker (2019) which supports the findings of this study.

Conclusion

To keep pace with the population growth rate, aquaculture production should be increased using modern technologies. Use of exogenous enzyme can play a role in increasing aquaculture production. The addition of multi-enzyme in feed can improve growth performance, feed utilization and nutritional quality of pangas catfish. Based on the promising results of the current study, and previous findings, more attention should be devoted for this field to optimize the dose and combination of exogenous enzymes for each species as well as to understand the underlying mechanism of how multi-enzyme work on fish.

References

- Ahmed, G.U., Chakma, A., Shamsuddin, M., Minar, M.H., Islam, T., & Majumdar, M.Z. (2013). Growth performance of Thai pangas *Pangasianodon hypophthalmus* using prepared and commercial feed. *International Journal of Life Sciences Biotechnology and Pharma Research*, 2, 92–102.
- Aliyu-Paiko, M., Hashim, R., & Shu-Chien, A.C. (2010). Influence of dietary lipid/protein ratio on survival, growth, body indices and digestive lipase activity in Snakehead (*Channa striatus*, Bloch 1793) fry reared in recirculating water system. *Aquaculture Nutrition*, 16(5), 466–474. <https://doi.org/10.1111/j.1365-2095.2009.00683.x>
- AOAC. (1990). *Official Methods of Analysis of the Association of Official Analytical Chemists*. Ed. Kenneth Helrich. Association of Official Agricultural Chemists. Arlington, Virginia, USA., 1230 pp.
- Bogut, I., Opačak, A., & Stević, I. (1995). The influence of polyzymes added to the food on the growth of carp fingerlings (*Cyprinus carpio* L.). *Aquaculture*, 129(1–4), 252. [https://doi.org/10.1016/0044-8486\(95\)91971-w](https://doi.org/10.1016/0044-8486(95)91971-w)

- Buchanan, J., Sarac, H.Z., Poppi, D., & Cowan, R.T. (1997). Effects of enzyme addition to canola meal in prawn diets. *Aquaculture*, 151(1-4), 29-35. [https://doi.org/10.1016/S0044-8486\(96\)01478-0](https://doi.org/10.1016/S0044-8486(96)01478-0)
- DoF. (2018). Yearbook of Fisheries Statistics of Bangladesh 2017-18. Fisheries Resources Survey System (FRSS), Department of Fisheries. Bangladesh: Ministry of Fisheries. Volume 35 : p. 129.
- Torry Research Station. (1989). Yield and nutritional value of the commercially more important fish species. (FAO Fisheries Technical Paper. No. 309). Rome, FAO., 187 pp.
- FAO. (2014). The state of world fisheries and aquaculture. Food and Agriculture Organization of the United Nations, Rome., 223 pp.
- FAO. (2018). The state of world fisheries and agriculture 2018 - meeting the sustainable development goals. Food and Agriculture Organization of the United Nations, Rome., 210 pp.
- FAO. 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome. <https://doi.org/10.4060/ca9229en>
- Farhangi, M., & Carter, C.G. (2007). Effect of enzyme supplementation to dehulled lupin-based diets on growth, feed efficiency, nutrient digestibility and carcass composition of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture Research*, 38(12), 1274-1282. <https://doi.org/10.1111/j.1365-2109.2007.01789.x>
- Felix, N., & Selvaraj, S. (2004). Enzymes for sustainable aquaculture. In A review of global tilapia farming practices A review of global tilapia farming practices. *Aquaculture Asia*, IX(1), 1-14.
- Gatlin III, D.M. (2003). Nutrition and fish health. In J.E. Halver & R.W. Hardy, (Eds.), *Fish nutrition* (pp. 671-702). San Diego, California, USA. Academic Press Inc., 824 pp.
- Ghasemi, A., & Zahediasl, S. (2012). Normality tests for statistical analysis: A guide for non-statisticians. *International Journal of Endocrinology and Metabolism*, 10(2), 486-489. <https://doi.org/10.5812/ijem.3505>
- Huang, Z., Li, Z., Xu, A., Zheng, D., Ye, Y., & Wang, Z. (2020). Effects of exogenous multienzyme complex supplementation in diets on growth performance, digestive enzyme activity and non-specific immunity of the Japanese seabass, *Lateolabrax japonicus*. *Aquaculture Nutrition*, 26(2), 306-315. <https://doi.org/10.1111/anu.12991>
- Ighwela, K.A., Terengganu, K., Ahmad, A.B., & Abol-Munafi, A.B. (2014). The selection of viscerosomatic and hepatosomatic indices for the measurement and analysis of *Oreochromis niloticus* condition fed with varying dietary maltose levels. *International Journal of Fauna and Biological Studies IJFBS*, 1(13), 18-20.
- Imam, T.S., Bala, U., Balarabe, M.L., & Oyeyi, T.I. (2010). Length-weight relationship and condition factor of four fish species from Wasai Reservoir in Kano, Nigeria. *African Journal of General Agriculture*, 6(3), 125-130.
- Jackson, L.S., Li, M.H., & Robinson, E.H. (1996). Use of microbial phytase in channel catfish *Ictalurus punctatus* diets to improve utilization of phytate phosphorus. *Journal of the World Aquaculture Society*, 27(3), 309-313. <https://doi.org/10.1111/j.1749-7345.1996.tb00613.x>
- Jiang, T.T., Feng, L., Liu, Y., Jiang, W.D., Jiang, J., Li, S. H., Tang, L., Kuang, S.Y., & Zhou, X.Q. (2014). Effects of exogenous xylanase supplementation in plant protein-enriched diets on growth performance, intestinal enzyme activities and microflora of juvenile Jian carp (*Cyprinus carpio* var. Jian). *Aquaculture Nutrition*, 20(6), 632-645. <https://doi.org/10.1111/anu.12125>
- Jin, Y.L., Zhou, X. Q., Jiang, W.D., Wu, P., Liu, Y., Jiang, J., Zeng, Y.Y., Kuang, S.Y., Tang, L., Zhang, Y.A., & Feng, L. (2020). Xylanase supplementation in plant protein-enriched diets improves growth performance by optimizing the intestinal microflora and enhancing the intestinal immune function in grass carp grow-out (*Ctenopharyngodon idella*). *Aquaculture Nutrition*, 26(3), 781-800. <https://doi.org/10.1111/anu.13038>
- Khedr, M. N., Kamelia, M. Z., Tahia, E., & Elramy, S. M. (2016). Comparison between effects of Complex enzyme and Multi enzyme supplementation on the Productive Performance of Broiler. *Benha Veterinary Medical Journal*, 31(1), 78-84
- Lall, S.P. (2002). The Minerals. In J.E. Halver & R.W. Hardy, (Eds.), *Fish nutrition* (pp. 259-308). San Diego, California, USA. Academic Press Inc., 824 pp.
- Lin, S., Mai, K., & Tan, B. (2007). Effects of exogenous enzyme supplementation in diets on growth and feed utilization in tilapia, *Oreochromis niloticus* x *O. aureus*. *Aquaculture Research*, 38(15), 1645-1653. <https://doi.org/10.1111/j.1365-2109.2007.01825.x>
- Maas, R.M., Verdegem, M.C., Dersjant-Li, Y., & Schrama, J.W. (2018). The effect of phytase, xylanase and their combination on growth performance and nutrient utilization in Nile tilapia. *Aquaculture*, 487, 7-14.
- Mandal, S., Hossain, M.A., Islam, M A., & Mirz, M.J.A. (2002). Larval rearing and fry raising of *Pangasius sutchi* (Fowler). *Bangladesh Journal of Fisheries Research*, 6(2), 183-186.
- Phumee, P., Hashim, R., Aliyu-Paiko, M., & Shu-Chien, A.C. (2009). Effects of dietary protein and lipid content on growth performance and biological indices of iridescent Shark (*Pangasius hypophthalmus*, Sauvage 1878) fry. *Aquaculture Research*, 40(4), 456-463.
- Rahman, M.A., Bhadra, A., Begum, N., & Hussain, M.G. (1997). Effects of some selective supplemental feeds on the survival and growth of catfish (*Clarias batrachus* Lin.) fry. *Bangladesh Journal of Fisheries Research*, 1(July), 55-58.
- Rahman, M., & Sarker, S. (2019). Effects of Exogenous Multi Enzyme on the Growth, Blood Parameters and Flesh Compositions of *Labeo rohita* (H). *IOSR Journal of Agriculture and Veterinary Science*, 12(8), 43-48.
- Ranjan, A., Sahu, N.P., Deo, A.D., & Kumar, S. (2018). Comparative Growth Performance, in vivo Digestibility and Enzyme Activities of *Labeo rohita* Fed with DORB Based Formulated Diet and Commercial Carp Feed. *Turkish Journal of Fisheries and Aquatic Sciences*, 18, 1025-1036. https://doi.org/10.4194/1303-2712-v18_9_02
- Saha, M.R., Mollah, M.F.A., & Roy, P.K. (1998). Growth and survival of *Clarias batrachus* (Lin.) larvae fed on formulated diets. *Progressive Fish-Culturist*, 2(2), 151-158. [https://doi.org/10.1577/1548-8640\(1998\)060<0293:GASOLS>2.0.CO;2](https://doi.org/10.1577/1548-8640(1998)060<0293:GASOLS>2.0.CO;2)
- Sangrattanakul, C. (1989). Effect of Pelletized diets containing various levels of protein on growth and survival of Climbing Perch, *Anabas testudineus* (Bloch), (Master Thesis). Kasetsart University, Bangkok, Thailand.
- Sarker, M.T. (2000). *Pangus Chash Babosthapana* (Management of Pangus culture) (In Bengali). Department of Fisheries, Bangladesh. pp. 25.

- Shahjahan, M.A.Y. (1997). Culture of genetically improved farmed tilapia (GIFT) in cages (Master Thesis). Bangladesh Agricultural University, Mymensingh, Bangladesh.
- Shakoori, M., Hossein Hoseinifar, S., Paknejad, H., Jafari, V., & Safari, R. (2018). The effects of dietary lysozyme on growth performance and haematological indices of rainbow trout (*Oncorhynchus mykiss*) fingerling. *International Journal of Aquatic Biology*, 6(1), 31–36. <https://doi.org/10.22034/ijab.v6i1.432>
- Shearer, K.D. (1994). Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. *Aquaculture*, 119(1), 63–88. [https://doi.org/10.1016/0044-8486\(94\)90444-8](https://doi.org/10.1016/0044-8486(94)90444-8)
- Siti-Norita, M., Arbakariy, A., Azlina, I.N. and Ibrahim, C.O. (2015). Effect of B-Mannanase Supplementation on the Growth and Apparent Digestibility of Red Tilapia Fed Formulated Diets Containing Palm Kernel Cake. *Global Advanced Research Journal of Agricultural Science*, 4(2), 075-088.
- Soltan, M.A. (2009). Effect of dietary fish meal replacement by poultry by-product meal with different grain source and enzyme supplementation on performance, feces recovery, body composition and nutrient balance of Nile tilapia. *Pakistan Journal of Nutrition*, 8(4), 395–407.
- Tahounl, A.A.M., Mabroke, R.S., El-Haroun, E.R., & Suloma, A. (2011). Effect of exogenous enzyme supplementation on reproductive performance of broodstock Nile tilapia reared in a hapa-in-pond hatchery system. *Egyptian Journal of Aquatic Biology and Fisheries*, 15(3), 61–73.
- Travagli, V., Zanardi, I., Bernini, P., Nepi, S., Tenori, L., & Bocci, V. (2010). Effects of ozone blood treatment on the metabolite profile of human blood. *International Journal of Toxicology*, 29(2), 165–174. <https://doi.org/10.1177/1091581809360069>
- Wang, S., Zhang, Y., Ren, H., Wang, Y., Jiang, W., & Fang, B (2017) Strategies and perspectives of assembling multi-enzyme systems. *Critical Reviews in Biotechnology*, 37(8), 1024-1037, DOI: 10.1080/07388551.2017.1303803
- Yigit, N.O., Koca, S.B., Didinen, B.I., & Diler, I. (2018). Effect of protease and phytase supplementation on growth performance and nutrient digestibility of rainbow trout (*Oncorhynchus mykiss*, Walbaum) fed soybean meal-based diets. *Journal of Applied Animal Research*, 46(1), 29–32. <https://doi.org/10.1080/09712119.2016.1256292>
- Yildirim, Y.B., & Turan, F. (2010). Effects of exogenous enzyme supplementation in diets on growth and feed utilization in African catfish, *Clarias gariepinus*. In *Journal of Animal and Veterinary Advances*, 9(2): 327-331. <https://doi.org/10.3923/javaa.2010.327.331>