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Effect of Enzyme Supplementation in Diet for the Growth of Channel Catfish

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Abstract: Channel catfish has practically proven to be one of the species with virtually remarkable performance in fresh water pond culture. Due to better growth rate, good adaptation and acceptability towards artificial feed channel catfish was introduced to Pakistan. A study was conducted to see the role of dietary enzyme Laccasse supplementation in a diet (35% CP) on growth of fingerlings of channel catfish. For this purpose a six week nutrition study was conducted in Aquaria of Aquaculture and Fisheries program (NARC). Fish was fed a locally prepared (35% CP) diet incorporated with three different concentration of Laccasse enzyme i.e., at the rate of 1, 2 and 3 mL/kg. The dietary pellets were prepared and air dried and fed to the fish at the rate of three percent of wet body weight twice daily. Prior to the start of study fish were acclimatized for a period of two weeks on experimental diets. Weights of the fish were recorded at the start and end of trial fortnightly in between. At the end result was deduced through the data of weight gain. The results revealed that Laccasse supplemented at a concentration of 2 mL/kg has higher growth, however it is insignificant ($p>0.05$) with a concentration of 3 mL/kg. So addition of laccasse in higher concentration or lower concentrations serve no purpose. We concluded that further detailed studies are added to evaluate the role of laccasse enzyme in feed utilization and growth enhancement of channel catfish juveniles.

Keywords: Aquaculture, cat channel fish, laccasse enzyme

INTRODUCTION

Pakistan is rich in freshwater endowment with immense potential for fisheries and aquaculture development (Basavaraga *et al*., 1999). In Pakistan aquaculture is spawned over 16,000 ha, with a production of 30,000 mt and carp and inland aquaculture is about 20% of it (Anonymous, 1998). In order to facilitate consumer's demands as well as to supplement the fish farmer's income, there is a large scope for catfish culture in our fish farming systems. Worldwide various varieties of catfish have been tested for their suitability under different culture conditions. Fish farming is practiced in the Punjab, Northwest Frontier and Sindh Provinces on a limited scale as regards the species and investment is concerned, as only introduced species such as trout, common carp, grass carp, silver carp and other carp species are being cultured, along with the native Indian carp. Inland fisheries and aquaculture have received increasing attention in recent years and the government has established several fish hatcheries and training facilities for fish farmers in the country (FAO, 2005).

Out of catfish's species, channel catfish (*I. punctatus)* have received much attention of the grower's worldwide (Bush *et al*., 1981). The channel catfish (*I. punctatus)* has practically proven to be a species with remarkable performance in fresh water pond culture (Buentrllo and Gatlin, 2000). Catfish farming is an important agricultural industry in the United States, with more than 60,000 acres of water devoted to catfish production. Channel catfish grow well where water temperatures are above 20°C for at least four months each year.

Channel catfish can feed during day or night and they will eat a wide variety of both plant and animal material. Channel catfish usually feed near the bottom in natural waters but will take some food from the surface (Collins and Delmondo, 1979).

Fish meal has been widely used as a protein source for many years. It is basically produced from fishery waste (salmon, tuna, etc.) that are associated with the processing of various edible human fishery products. Fishmeal of high quality provides a balanced amount of all essential amino acids, phospholipids and fatty acids (e.g., DHA or docosahexaenoic acid and EPA or eicosapentaenoic acid) for optimum development, growth and reproduction, especially of larvae and brood stock also aid in disease (Yamanda and Wise, 1981).

Nutrition can affect many mechanisms controlling development of fish fingerlings. The development process of fish fingerlings can be affected by several

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genetic and environmental factors, in particular the availability and composition of feeds during early stages of larval life. Moreover, enzyme supplementation can significantly improve growth performance and feed utilization in juvenile fish fingerlings (Robinson, 1994; Hoehne-Reitan and Kjørsvik, 2004).

Interest in the enzymes has grown significantly in recent years ranging from food applications to bioremediation processes. One of the enzyme Laccases get peculiar attention in this aspect (Govoni *et al*., 1986). Laccases are currently of interest in baking due to its ability to cross-link biopolymers in both flour and gluten dough have described the potential applications of laccase in different aspects of the food industry such as beverage processing, ascorbic acid determination, sugar beet pectin gelation, baking and as a biosensor.

This study was designed with the aim to see the role of dietary enzyme laccasse in the growth of channel catfish juveniles. For this purpose a diet (35% CP) was prepared from locally available feed ingredients. Three different concentration of enzyme laccasse were used i.e., 1 mL, 2 mL and 3 mL/kg of diet. These were fed to triplicate group of fish. The aim of the study to evaluate the effect of different concentration of laccasse enzyme on growth of channel catfish fingerlings when incorporated in a locally prepared diet.

MATERIALS AND METHODS

Production of enzymes in liquid culture: The media used for enzyme production (Laccase) consists of soy meal (30 gm/L), maltose (15 gm/L) and mycological peptone (15 gm/L) as described by Bumpus *et al*. (1987). In the present research work batch cultures for enzyme production were set up in shake flasks (120 rpm) at 30°C for 168 h in the productive media at pH 5 using *Aspergillus flavus* SA2 as an inoculum source. After the fermentation period enzyme was extracted by filtration and used for enzyme assay.

Measurement of laccase activity: Laccases activity was measured by using 5 mM 2, 6-dimethoxyphenol (DMP) as substrate in 100 mM tartarate buffer 4.5 pH. Laccase activity was measured following the method of Muzariri *et al*. (2001).

PROTEIN ESTIMATION OF CRUDE ENZYME

The estimation of protein was carried out by taking Bovine Serum Albumin (BSA) as a standard by adopting the procedure of Lowry *et al*. (1951).

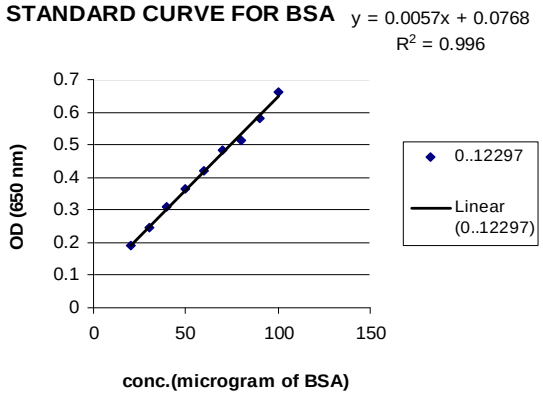

Fig. 1: Showing standard curve for BSA

Four solutions were prepared with the following composition, for protein estimation.

Solution A: Na_2CO_3 (2.00 g), NaOH (0.40 g), Na K Tartarate (1.0 g) and dissolved in distilled water (100 mL)

Solution B: $CuSO_4$. $5H_2O$ (0.5 g) and dissolved in distilled water (100 mL)

Solution C: 25 mL of Solution A, 0.5 mL of solution B (Solution 'C' was prepared freshly).

Solution D: Folin phenol and distilled water in 1:1 ratio (1.5 mL Folin phenol, 1.5 mL of distilled water). This solution 'D' was prepared freshly.

Procedure: 1 mL of sample (crude enzyme) was taken and then 1.0 mL of solution 'C' was added in the tube. After 10 min of incubation at ambient condition 0.1 mL of solution 'D' was added and mixed well. Mixture was incubated at 37°C for 30 min. After 30 min, the O.D was measured at 650 nm. Figure 1 was made for standard curve. Then the amount of sample protein in mg/mL was calculated.

BSA standard curve: Different dilutions of BSA i.e., 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µ gm were made from the stock solution of concentrations 0.1 µg BSA/100 mL of distilled water. After standardization, optimal dilution was selected for further study. The volume was made up to 10 mL by adding distilled water, except in blank. Then 1 mL of solution 'C' was added in each test tube. After 10 min, kept at ambient condition. The 0.1 mL of solution 'D' was added, mixed well and incubated at 37°C for half an hour and O.D was recorded at 650 nm.

Calculations: Graph of standard curve was prepared using BSA as standard and amount of sample protein

was estimated. Concentration of the protein present in the sample was determined by the following equation:

$$y = mx+b$$

$$\text{Protein (mg/mL)} = \frac{\text{Optical Density of Sample}}{\text{Optical Density of Standard}} \times \frac{\text{Concentration of Standard x1}}{\text{mL of sample used}}$$

Determination of specific activity of crude enzyme: The specific activity of crude extract (enzyme units/mL/mg of protein/mL) was determined by dividing the enzyme activity of crude extract by its protein content (mg/mL).

DETERMINATION OF TOXIN IN CRUDE ENZYME

Aflatoxin detection in crude enzyme was tested by using Neogen ELISA kit (Veratox for Aflatoxin HS Product No. 8031).

Study site: The present study was conducted at the Aquaria Labs of the Aquaculture and Fisheries Programme (AFP), National Agricultural Research Centre (NARC), Islamabad for a period of 6 weeks.

Experimental animal: Juvenile channel catfish (*Ictalurus punctatus*) was used as experimental animals. The fish of approximately same size (i.e., weight and length) was sampled from the raceways containing locally bred fingerlings of channel catfish. These were then acclimatized on experimental diets for two weeks prior to the start of study.

Experimental diets: Fish were fed with diets having three different concentration of dietary enzyme laccasse. The diet (35% C.P) was prepared from locally available feed ingredients (Table 1). Laccasse was added as three concentrations 1 mL, 2 mL, 3 mL/kg to the diet. Each treatment was fed to triplicate group of fish. Diet containing no enzyme was used as control feed. Feed was grinded in electric grinder and pellets were made by mincing machine and these were then air dried and was kept in airtight containers at cool place until use.

Table 1: Ingredients and composition of diet (35% CP) used in the study

Ingredients	Composition (%)
Soybean meal	28.55
Canola meal	5.0
Fish meal	8.0
Gluten	44.37
Wheat bran	10.00
Dicalcium phosphate	0.32
Cod liver oil	1.50
Carboxymethyl Cellulose	2.00
Mineral Premix	0.26
Total	100

Experimental design: Fish were kept in 40 L capacity aquaria and were fed diets twice daily at the rate of 3% of their wet body weight. Aquaria were provided with fresh air through Hi Blow air pump with the help of stones aerators. Weight of the fish was recorded at the start and end of experiment and fortnightly during the experiment. At the end, result was analyzed on the basis of weight gain.

Physico-chemical analysis of water: Physio-chemical characteristics of water such as alkalinity and hardness were recorded once in a week and other parameters such as temperature, Dissolved Oxygen (DO), Electrical Conductivity (EC) and were recorded on daily basis.

Data analysis: At the end of trial all the data was added in MS Excel 2003 software. The data was analyzed by applying ANOVA. Means with significant difference were compared through C software using DMRT.

RESULTS AND DISCUSSION

In the present study laccase production was carried out in the production medium with the inoculation of *Aspergillus flavus* SA2 under sterilized condition. After the fermentation period (2 weeks), extracted crude extra cellular enzyme was analyzed for protein content and enzyme units (Table 2).

For the commercial application of laccase it is necessary that it should be of good quality and free of toxin compounds. As we know that different fungal strains previously been reported are well known for their mycotoxin or aflatoxin production that may limit the use of enzyme for industrial application. In order to check the toxicity of the crude laccase, produced during the present study in production media, enzyme linked immuno assay was conducted for aflatoxin detection. Our results showed that the concentration of aflatoxin produced by selected fungal strain was found to be under permissible limits (Table 3).

These results were in agreement with earlier findings that proved, *A. flavus* and *A. parasiticus* strains population invading wheat grains were all non aflatoxigenic ones (Rashid *et al*., 2008). These results support the application of laccase enzyme for commercial or industrial use with low cost production

Table 2: Protein estimation of crude laccases

Strain	Total activity of crude extract (U)	Total protein of crude extract (mg)	Specific activity (U/mg)
Aspergillus flavus SA2	1.17914	67.832	0.0174

Table 3: Aflatoxin detection in crude Laccase by ELISA

Strains	Toxin (ppb)	Permissible limits
Aspergillus flavus SA2	0.27	<20 ppb

Table 4: Physico-chemical parameters of water recorded during the study period

Laccasse Treatments	Temp°C	pH	EC (µs/cm)	Alkalinity (mg/L)	Hardness (mg/L)	DO (mg/L)
1mL/kg	24.0±2.5	7.7±0.5	898.8±114.9	177.6±37.3	240.4±15.0	6.5±2.3
2 mL/kg	24.2±2.6	7.5±0.5	882.0±115.5	172.8±30.7	230.2±11.5	5.8±1.8
3 mL/kg	24.1±2.8	7.7±0.4	887.3±116.2	161.6±25.9	237.0±10.5	6.6±2.0
Control	24.5±2.9	7.6±0.5	891.2±117.0	165.0±27.0	240.0±13.5	6.4±1.6

Values are mean ± S.D; EC = Electrical Conductivity; DO = Dissolved Oxygen

Table 5: Weight gain of channel catfish fry when fed on a 35% CP diet supplemented with different ratios of laccase enzyme

	Laccase		
Control	1 mL/kg	2 mL/kg	3 mL/kg
0.26 ± 0.09^{b}	0.24 ± 0.13^{b}	0.42 ± 0.06^{a}	0.38 ± 0.00^{a}

a: $p<0.05$; b: $p<0.01$

because utility of enzyme without any extra investment on their purification could be possible. It was also stated that highly competitive atoxigenic strains might be applied to agricultural fields as biocompetitive agents (Egel *et al*., 1994; Cotty and Cardwell, 1999; Hornand Dorner, 1999). The enzyme subject to a series of toxicological tests to document its safety in use was reported by Brinch and Pedersen (2002).

Results of water quality during the experimental trial are given in Table 4. The maximum temperature recorded during the trial was 24.5±2.9°C and minimum temperature was 24.0±2.5°C. Maximum pH was 7.7±0.5 and minimum was 7.5±0.5. Maximum EC was 898.8 µs/cm and minimum was 882.0 µs/cm. Maximum alkalinity was 177.6 mg/L and minimum was 161.6 mg/L. Maximum DO was 6.6 mg/L and minimum was 5.8 mg/L. Maximum Hardness was 240.0 mg/L and minimum was 230.2 mg/L. All these parameters recorded during the study were found normal for channel catfish culture (Durburow, 2000) except electrical conductivity values which were above than the normal ranges.

Weight gain of channel catfish fry when fed on a 35% CP diet supplemented with different ratios of Laccasse enzyme is given in Table 5. The results revealed that fish fed a diet supplemented with laccasse enzyme at a concentration of 2 mL/kg has significantly ($p<0.05$) higher weight gain (i.e., 0.42±0.06) as compared to the rest of treatments. However this treatment was insignificant to those fish fed a diet supplemented with laccasse enzyme at a concentration of 3 mL/kg (0.38± 0.00). Weight gain by fish when fed on a 35% CP normal diet was calculated to be (0.26±0.13). So we can conclude that feed supplemented with dietary enzyme has not produced much profound effect on the growth of channel catfish, however when enzyme was added at the rate of 2 mL/kg then weight gain was observed.

The Fig. 2 clearly depicts that growth has accelerated from first week of experiment when the fish weight was almost 0.45 g and at the end of experiment i.e., after 6 weeks, fish has attained the weight of 0.70 g by 1 mL/kg concentration of enzyme while with 2

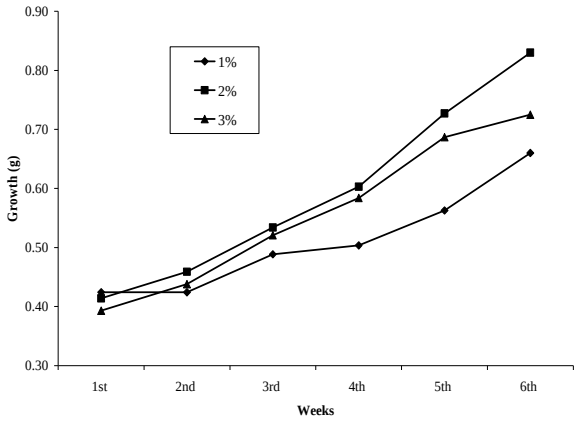

Fig. 2: Growth (g) of channel catfish fry when fed on a 35% CP diet supplemented with different ratios of Laccase enzyme

mL/kg it was 0.85 g and by 3 mL/kg it was recorded to be 0.65 g. Thus in our results increase of laccasse supplementation in a diet either results insignificant growth or reduction in growth as well as a smaller concentration of laccasse also serve no purpose in the diet.

Our results are in agreement with Golterman *et al*. (1978) who conducted experiments to study the effects of enzyme supplementation to a fish and found no significant ($p>0.05$) difference between control and experimental groups but our results do not agree with the results of Robinson (1994) who suggested that enzyme supplementation can significantly improve growth performance and feed utilization in fish fingerlings. Our results are however in agreement with the results of Webster and Reed (1994) who performed feeding trial and found that the dietary enzyme when added in a ratio of 1.5 to 2.5 was found suitable for best growth of channel catfish.

The effects of dietary enzymes on fish growth, feed intake, nutrient utilization, plasma and body mineral concentration were reported in literature (Robinson, 1994; Webster and Reed, 1994; Bardócz *et al*., 1993; Barros *et al*., 1985; Balmer and Blomhoff, 2002; Golterman *et al*., 1978).

We concluded that further detailed studies are added to evaluate the role of laccasse enzyme in feed utilization and growth enhancement of channel catfish juveniles. However it seems that laccasse is not a promising enzyme for dietary supplementation in order to enhance the growth of the fish.

CONCLUSION

Laccase enzyme can serve as a good, nutritive and cheap alternative source for fish feed and contribute well in reducing the constraint caused by higher prices and low availability of nutritious sources for fish feed as it give the fruitful results.

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