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Original Article

Studies on amylase and lipase activity in fishes fed with diet containing different feed ingredients

Mirfa Murtaza[1], Sajid Abdullah[1], Wardah Hassan[1*], Khalid Abbas[1], Huma Naz[1], Muhammad Anjum Zia[2]

[1]Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad
[2]Department of Biochemistry, University of Agriculture, Faisalabad

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Abstract
The activity of digestive enzymes was determined in *Hypophthalmichthys molitrix, Catla catla* and *Cyprinus carpio* fed with different feed ingredients. There were two glass aquaria (3×2×2 ft) for each species and ten fish in each aquarium. Fish in one aquarium were fed on rice polish and fish in second aquarium were fed on corn gluten @ 3 % of their wet biomass for 70 days. Results showed that fish wet weight varied significantly between the rice polish and corn gluten fed fishes. The corn gluten fed fish showed significantly better growth performance than fish fed with rice polish. The activity of amylase and lipase was higher in all fish species fed with corn gluten. Liver showed higher amylase activity than the intestine, while the activity of lipase was higher in intestine. It was concluded that different fish species respond differently to various feed ingredients. Therefore it is important to follow the acceptability and digestibility standards during the selection of ingredients and for preparation of feed for different fish species.
Key Words: Digestive enzymes, rice polish, corn gluten, fish

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INTRODUCTION

It is expected that all over the world the human population in 2050 will exceed 10 to 12 billion people due to continuous increase in population (Welch and Grahm, 1999). This situation is considered as a main challenge to increase food production to fulfill the feed demand of increasing population. To overcome this situation aquaculture sector has potential to provide good quality and healthy food stuff (Diana, 2009).Due to decrease in capture fishery production the aquaculture is getting substantial importance to increase the fish production all over the world (FAO, 2009). Nutritional requirements of the cultured fish species in the large scale fish production can be fulfilled by the intensification of suitable feeding protocols. In the development of sustainable fish production feed cost and supply are among the vital tasks. So, for fish feed formulation, aquaculture sector is searching for less expensive feed ingredients (Stone, 2003). Insufficient knowledge about different digestive processes in the digestive tract of fish is also a limitation to appropriate feed preparation for certain fish species. Selection of different ingredients from the market is really a difficult task which can confirm good growth and improved health of fish, eventually sustaining the economics of the fish business (Falcon-Hidalgo *et al*., 2010).

Increasing price and demand of fish oil and meal are major issues to maintain long term fish production. So, other feed sources alternative to fish oil and meal can be used (Kristofersson and Anderson, 2006). The different types of alternative feed sources such as animals, microbes and plants can be used (Yun *et al*., 2013). The ability to digest and metabolize the carbohydrate fraction of dietary components is species specific (Hemre *et al*., 2002). The digestion and metabolism of feed ingredients depends upon fish species, sources and quantity of the ingredients (Stone, 2003; Krogdahl *et al*., 2005).The digestive enzymes of fish are being extensively studied. It is important for studying the mechanism of feed digestion and ability of organisms to adapt the alterations

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*Corresponding author: wardahhassan1@hotmail.com

in the feed composition (Sunde *et al*., 2004). Recent studies have concentrated on assessing the organisms potential to digest, absorb and assimilate the major nutrients. By studying the activity of digestive enzymes these processes can be primarily monitored (Guzman *et al*., 2005). The quantity and quality of digestive enzymes are responsible for digestion and absorption of nutrients from a given diet in the fish. For the preparation of commercial fish feed, it is necessary to understand the digestive physiology of cultured fish species which are yet to be established (Seenappa and Devaraj, 1995). Several earlier studies have revealed that the production and enzymes activity within the stomach depends on the feeding behavior of organisms. In fish species, the different functional and anatomical features reflect the high adaptability in their feeding habits. Fishes improve the adaptations to changing environmental conditions to utilize the wide range of food resources (Lundstedt *et al*., 2004). The current study was conducted to determine the effect of different feed ingredients on growth and digestive enzymes activity in different fish species.

MATERIALS AND METHODS

The present project was conducted with fish species *viz. Hypophthalmichthys molitrix, Catla catla* and *Cyprinus carpio* in the wet laboratory at Fisheries Research Farms, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad (Pakistan). Fish stock of desired weight (6 g) and age (60 days) were obtained from the Fish Seed Hatchery, Faisalabad. They were brought to the wet laboratory and acclimated to laboratory conditions for 14 days. During this period the fingerlings were fed with basal diet (Table I). After acclimatization fish were transferred in glass aquaria for growth and enzymatic studies.

Physico-chemical variables of the test media *viz.* temperature, pH, total hardness, dissolved oxygen, electrical conductivity, calcium and magnesium were made at 12-hr intervals throughout the study period by following the methods of APHA (1998). Water temperature and dissolved oxygen were determined by electronic meter HANNA HI-9143 and pH by HI-8520. Before the start of experiment, fish were treated with pinch of $KMnO_4$.Fishes were fed with rice polish and corn gluten@3% of their body weight two times a day throughout the experimental trial. The wet weight of *H. molitrix, C. catla* and *C. carpio* fingerlings were measured and recorded on weekly basis throughout the experiment. At the end of experimental trial fish was randomly collected, two fish from each replicate. The fish was dissected and organs viz. intestine and liver were removed for enzymes analysis. Removed organs were covered with aluminium foil and kept at 1℃.

Table I: Percent composition of basal diet

Sr. No.	Ingredients	Percentage
1	Fish meal	50.00
2	Corn gluten	34.27
3	Rice polish	05.51
4	Wheat flour	03.00
5	Oil (sun flower)	03.22
6	Vitamin & mineral mixture	04.00

Digestible protein (DP) =40%
Digestible energy (DE) =3.10 K calg^{-1}

Enzyme Analysis:

The stored samples were used for enzyme analysis. The organs of fish were homogenized in chilled tris HCl (50 mM) and taken in centrifuge tubes. Then samples were centrifuged (6000 rpm) at 4˚C for 15 minutes. The supernatant was collected and stored at 0˚C. Three replicates were used for each sample.

Amylase assay:

Amylase activity was determined by using the starch as substrate (Bernfeld, 1955). Properly diluted sample (1ml) and of 1% starch substrate (1 ml) was incubated for 3 minutes at 37 ˚C. By the addition of 3.5-dinitrosalicylic acid reagent (2 ml) the reaction stopped. The solution was heated in boiling water for 5 minutes, chilled and then added distilled water (20 ml). Absorbance was checked against blank at 540 nm wavelength.

Enzyme activity (U/ml) =

$$\frac{\Delta A\ enzyme - \Delta A\ blank}{incubation\ time\ x\ dilution\ factor} \text{ x standard factor}$$

Standard factor calculated for amylase is 6.5

Lipase assay:

In a glass flask 3.5 ml phosphate buffer (0.2 M, pH 6.9), 1 ml sample and 0.5 ml olive oil were added. After that it was agitated for 30

minutes at 37˚C in water bath. Then added 1 ml acetic acid in it and titrated with NaOH (10 mM) till the pH of solution became 10.

Enzyme activity (U/ml) =

$$\frac{NaOH\ vol.\ x\ Molarity\ of\ NaOH\ x\ 1000\ x\ 2\ x\ df}{volume\ of\ sample\ used}$$

Statistical analyses:

The data on the different parameters of the fish growth and digestive enzymes were subjected to statistical analyses by following Steel *et al*. (1997). The data obtained were statistically analyzed using Minitab 17.

RESULTS

Fish species, *Hypophthalmichthys molitrix, Catla catla* and *Cyprinus carpio* showed significant growth increment when fed with rice polish and corn gluten. However, higher growth performance was recorded in all fish species fed with corn gluten as compared to rice polish. Among fish species *C. catla* showed better growth than *H. molitrix* and *C. carpio* (Table II).

Table II: Growth (g) comparison of fish species fed with different feed ingredients

Fish species/ Treatments	Rice polish	Corn gluten
Hypophthalmichthys molitrix	$1.16^{b}\pm$ 0.15	$1.51^{a}\pm$ 0.68
Catla catla	$1.20^{b}\pm$ 0.33	$1.58^{a}\pm$ 0.65
Cyprinus carpio	$1.14^{b}\pm$ 0.19	$1.48^{a}\pm$ 0.47

Means with different letters in a single row for each experiment are statistically different at p< 0.01

Enzyme studies

Feed ingredients (corn gluten and rice polish) significantly influenced the activity of digestive enzymes.

Table III: Amylase activity (U/ml) in fish species fed with different feed ingredients

Feed ingredients	*Hypophthalmichthys molitrix*		*Catla catla*		*Cyprinus carpio*	
	Intestine	Liver	Intestine	Liver	Intestine	Liver
Rice polish	$1.58^{b}\pm$ 0.03	$3.28^{b}\pm$ 0.05	2.08^{b} ±0.01	$4.18^{b}\pm$ 0.03	$2.59^{a}\pm$ 0.22	$3.59^{b}\pm$ 0.06
Corn gluten	$1.71^{a}\pm$ 0.08	$3.41^{a}\pm$ 0.08	$2.72^{a}\pm$ 0.19	$5.45^{a}\pm$ 0.07	$2.43^{b}\pm$ 0.02	$4.26^{a}\pm$ 0.09

Means with different letters in a single column for each experiment are statistically different at p< 0.01

Table IV: Lipase activity (U/ml) in fish species fed with different feed ingredients

Feed ingredients	*Hypophthalmichthys molitrix*		*Catla catla*		*Cyprinus carpio*	
	Intestine	Liver	Intestine	Liver	Intestine	Liver
Rice polish	$1218^{b}\pm$ 2.30	$597.66^{b}\pm$ 5.36	$1143^{b}\pm$ 1.76	$546^{b}\pm$ 1.15	$1179^{b}\pm$ 1.87	$596.11^{b}\pm$ 1.54
Corn gluten	$1225^{a}\pm$ 2.45	$605.33^{a}\pm$ 6.35	$1216^{a}\pm$ 1.15	$592^{a}\pm$ 1.17	$1275^{a}\pm$ 2.16	$601^{a}\pm$ 1.93

Means with different letters in a single column for each experiment are statistically different at p< 0.01

All the fish species showed better amylase activity when fed with corn gluten as compared to rice polish. Among fish species amylase activity was maximum in liver of *C. catla*. However, organs of fish species i.e. liver and intestine also showed difference in the amylase activity. Better amylase activity was observed in the liver of fish as compared to intestine. It has been shown in Table III. Lipase activity was also higher in the fish species fed with corn gluten than rice polish. Among fish species lipase activity was maximum in intestine

of *C. carpio* fed with corn gluten however, intestine of *H. molitrix* showed greater lipase activity in corn gluten and rice polish fed medium. Better lipase activity was observed in the intestine of fish as compared to liver (Table IV).

Water Quality Parameters

The water quality parameters were within the tolerable limits for fish. Water temperature ranged from 28-31°C; pH, from 7.8 to 8.8; dissolved oxygen, from 5.2 to 6.5 ppm and total hardness (TH), from 220 to 224 mg/L (Table V).

Table V: Range of water quality parameters during experimental trial

Sr. No.	Water quality parameters	
1.	Dissolved oxygen	5.2-6.5 mg/L
2.	Temperature	28-31℃
3.	pH	7.8-8.8
4.	Electrical conductivity	1.26-1.80 mS/cm
5.	Total hardness	220-224 mg/L
6.	Calcium	13.1-21.7 mg/L
7.	Magnesium	35.25-45.43 mg/L

DISCUSSION

In the present study fishes showed gradual increment in wet weights when fed with different feed ingredients i.e. rice polish and corn gluten (plant based feed ingredients). The results of this experiment revealed that all fish species fed with corn gluten had maximum growth rate. *C. catla* showed significantly maximum growth rate than *H. molitrix* and *C. carpio* when fed on corn gluten as compare to rice polish. Rao *et al*. (2006) reported elevated specific growth rate of *L. rohita* when fed with medicinal plants as compared to control. In fish, growth rate and survival data are important parameters to estimate the effects of feed quality on growth and related metabolic reactions (Wang *et al*., 2006).

Corn gluten meal successfully replaces fish meal protein because of its high protein and low fiber contents (Pereira and Oliva-Teles, 2003). Ashraf *et al*. (2008) also observed different growth rate in *C. mrigala* when fed with feeds containing different ingredients. *H. molitrix* showed reduced growth as compared to *C. mrigala* and *C. catla* when fed on soybean meal and cotton seed meal (Ismat *et al*., 2013).

The activity of amylase and lipase observed in the digestive tract and liver of our experimental fishes is in accordance with many previous studies. Activity is different in different regions of fish as described by many scientists. In fishes amylase activity was lower in intestine and higher in liver fed with different feed ingredients while lipase activity was higher in intestine as compared to liver. Klahan *et al*. (2008) concluded that different fish had different levels of enzymes activity. The protease and lipase activities were higher in small sized fish while amylase activity was higher in the large sized fish. The protease and lipase activities appeared to be high in the intestine while amylase activity was higher in the liver. Pavasovic *et al.* (2007) documented higher amylase activity in fish when fed with diets containing plant-based ingredients.

Conclusion

From this study it can be concluded that animal based protein in fish diet could be replaced by plant based protein without compromising growth in fish. Furthermore, corn gluten could be a better feed ingredient for fish growth than rice polish.

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