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Comparative study of α-amylase activity in three Cyprinid species of different feeding habits from Southern Iraq

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Abstract

This work provides a comparative data of α-amylase activity in the gut extract of three Cyprinid species inhabiting Garma River (Basrah Governorate, Southern Iraq). Those species are bunny *Barbus sharpeyi* (herbivorous), common carp *Cyprinus carpio* (omnivorous), and shilik *Aspius vorax* (carnivorous). The study also investigate the discrepancy between the activity of the enzyme in common carp collected from Garma river with those reared in ponds. The specific activity of α-amylase has apparently been influenced by feeding habits of the three studied species. Values of α-amylase activity averaged 1.84 U mg^{-1} protein in the herbivorous bunny. It showed a significant ($P<0.01$) superiority to that of the omnivorous common carp (1.33 U mg^{-1} protein) which, in turn, showed a significant ($P<0.01$) superiority to that of the carnivorous shilik (0.76 U mg^{-1} protein). Omnivorous common carp which was collected from fish ponds showed a significantly ($P<0.01$) higher value of α-amylase specific activity which reached 1.92 U mg^{-1} protein when compared with the value recorded in common carp collected from Garma River which reached 1.33 U mg^{-1} protein.

Keywords: Cyprinid fish, feeding habits, α-amylase activity.

Güney Irak'ta Farklı Beslenme Alışkanlıkları Olan Üç Sazangil Türünde α-amilaz Aktivitesi Üzerine Karşılaştırmalı Çalışma

Özet

Bu çalışma, Garma Nehrinde (Basrah, Güney Irak) yaşayan üç Sazangil türünün ait mide özütündeki α-amilaz aktivitesine ait karşılaştırmalı verileri göstermektedir. Bu türler, Bunni *Barbus sharpeyi* (otobur), pullu sazan *Cyprinus carpio* (etotobur) ve shilik *Aspius vorax* (etobur). Çalışma aynı zamanda Garma Nehrinden toplanan ile havuzda yetiştirilmiş pullu sazanların enzim aktivitesi arasındaki farklılığı da araştırmaktadır. Özgül α-amilaz aktivitesi, araştırılan üç türün beslenme alışkanlıklarından belirgin bir şekilde etkilenmiştir. α-amilaz aktivitesine ait değerler, otobur bunni'de ortalama protein 1,84 U mg^{-1} olmuştur. Etotobur pullu sazanınkiyle karşılaştırıldığında anlamlı bir üstünlük ($P<0,01$) göstermiştir. Buna karşılık pullu sazan ise etobur shilik'inden (0,76 U mg^{-1} protein) daha anlamlı bir üstünlük ($P<0,01$) göstermiştir. Garma Nehrin'den toplanan ve 1,33 U mg^{-1} protein seviyesine ulaşmış pullu sazanda kaydedilen değer ile karşılaştırıldığında, balık havuzundan toplanan etotobur pullu sazan, 1,92 U mg^{-1} protein seviyesine ulaşarak anlamlı derecede ($P<0,01$) yüksek bir özgül α-amilaz aktivitesi göstermiştir.

Anahtar Kelimeler: Sazangil balıklar, beslenme habitatı, α-amylase aktivitesi.

Introduction

The study of digestive enzymes in fish has a wide range of potential interest. Study of digestive enzymes is an essential step towards understanding the mechanism of digestion and how the organism adapts to changes in the nutritional environment (Sunde *et al*., 2004). On the other hand, the assessment of the activity of digestive enzymes in cultured species may be helpful in the selection of feed ingredients (Lan and Pan, 1993). Recent investigations on digestive processes have focused on evaluating the ability of organisms to hydrolyze, absorb and assimilate the principal dietary nutrients, these processes can be initially examined by analyzing the activity of digestive enzymes (total proteinases, trypsin, chymotrypsin α-amylase and lipase) (Guzman *et al*., 2005).

Herbivorous and omnivorous fishes appear to digest starchy components of plant materials more effectively than carnivorous fishes. The current understanding of digestive enzymes activity in fishes indicates a strong correlation with diet. In herbivorous (*Boops boops*) and omnivorous (*Cyprinus carpio*, *Carassius auratus*, *Tinca tinca* and *Pagellus erythrinus*), carbohydrase activity (e.g. α-amylase) is higher than in carnivores (*Oncorhynchus mykiss*, *Sparus aurata*, *Anguilla anguilla* and *Diplodus annularis*) fishes (Fernandez *et al*., 2001; Hidalgo *et al*., 1999). Several studies indicate the impact of feeding habits on α-amylase activity and found that herbivorous and omnivorous fishes showed higher amylase activity compare with carnivorous fishes (Horn *et al.*, 2006; Drewe *et al.*, 2004; Fernandez *et al.*, 2001; Hidalgo *et al.*, 1999; Kuzmina, 1996). While other studies such as Chakrabarti *et al.* (1995) doesn't indicate such effect.

The aim of the present study was to evaluate the differences in α-amylase activity in the gut extracts prepared from bunny (*Barbus sharpeyi*), common carp (*Cyprinus carpio*) and shilik (*Aspius vorax*) which have different feeding habits and investigate the discrepancy between the activity of the enzyme in common carp living in rivers with those reared in ponds.

Materials and Methods

Fish

Adults of three Cyprinid fish species: bunny *Barbus sharpeyi* (herbivorous), common carp *Cyprinus carpio* (omnivorous) and shilik *Aspius vorax* (carnivorous) were collected from Garma river and Marine Science Center fish ponds, Basrah Governorate, Southern Iraq (47°46′ E; 30°35′ N) in July and August, 2006. Fishes were collected by cast net early in the morning and then transported live directly to the laboratory and kept in glass aquaria. Ten specimens of fish representing each species were transferred to (45 liter) laboratory glass aquaria (30x60x25 cm) under the following conditions:

Water Temperature: 22.0–24.0°C
pH: 7.8–8.2
Dissolved Oxygen: 7.5-8.5 mg/L
Water salinity: 0.9-1.2 g/L

Fish were kept there for a few hours under continuous artificial aeration before analysis.

Preparation of Extracts

Ten specimens of each species were used for analysis, fish were killed by a blow on the head, total weight of fish were measured (Table 1), specimens were dissected and the digestive tract of each fish was excised on a cutting board kept on ice and emptied by squeezing out the food, The activities of α-amylase was assayed in the gut tissue immediately, each tissue sample was ground separately in a pre-chilled, ground-glass homogenizer kept on ice with 9 volumes of 50 mM Tris (pH 7.2) buffer. The homogenates were put in freezer for cooling and then centrifuged at 5,500 rpm for 20 min. The supernatants were removed and kept in refrigerator for use in the assays at the same day (Drewe *et al*., 2004).

Assay α-Amylase

Starch was used as the substrate in the determination of amylase activity (Bernfeld, 1955) where 1ml of properly diluted gut extract was incubated for 3 min at 37°C with 1ml of 1% starch substrate (1 g soluble starch and 0.0067 M NaCl in 100 ml 0.02 M NaH_2PO_4, pH 6.9). The reaction was stopped by the addition of 2 ml 3.5- dinitrosalicylic acid reagent. The solution was then heated for 5 min in boiling water, cooled and 20 ml distilled water added. The absorbance at 540nm was read and a standard curve was established with maltose (0.1–1.0 mg ml^{-1} distilled water), to convert readings into mg of maltose. The amylase specific activity is defined as 1 mg of maltose produced per min per mg protein at 37°C. The amount of soluble protein in the gut extracts was determined by the Lowry method (Lowry *et al*., 1951) using bovine serum albumin as a standard protein. 0.1 ml of gut extract sample or the standard was added to 0.1 ml of 2 N NaOH. The mixture was then hydrolyzed at 100°C for 10 min in boiling water bath, then cooled to room temperature and 1 ml of freshly mixed complex-forming reagent was added. After 10 minutes, 0.1 ml of Folin reagent was added and mixed using a vortex mixer. The absorbance then read at 550 nm after 30 minutes.

Statistical Analysis

All data were expressed as Mean ± SD. Data were analyzed by ANOVA followed by Duncan's multiple range test for multi-group comparisons. A probability level of $P<0.01$ was considered statistically significant. The SPSS (Statistical Package for the Social Sciences) version 16.0 was used.

Table 1. Body weight of Common carp *Cyprinus carpio* (omnivorous), Bunny *Barbus sharpeyi* (herbivorous) and Shilik *Aspius vorax* (carnivorous) collected from Garma river and Marine Science Center ponds

Species	Body weight (g)
Common carp (from Marine Science Center fish ponds)	72.05±7.79
Common carp (from Garma river)	84.43±8.42
Bunny (from Garma river)	78.10±9.04
Shilik (from Garma river)	77.25±9.93

Mean±S.D., (n = 10)

Results

The specific activity of α-amylase has apparently been influenced by feeding habits of the three studied species. The specific activity (Figure 1A) as well as activity per body weight (Figure 1B) of α-amylase (1.84 U mg^{-1} protein, 0.023 U g^{-1} body weight respectively) recorded by herbivorous bunny showed a significant (P<0.01) superiority to that of the omnivorous common carp (1.33 U mg^{-1} protein, 0.017 U g^{-1} body weight respectively) which, in turn, showed a significant (P<0.01) superiority to that of the carnivorous shilik (0.76 U mg^{-1} protein, 0.011 U g^{-1} body weight respectively).

Omnivorous common carp which was collected from Marine Science Center fish ponds showed a significant (P<0.01) higher value of specific activity and activity per body weight of α-amylase which reached 1.92 U mg^{-1} protein, 0.026 U g^{-1} body weight respectively when compared to the value recorded by common carp collected from Garma River which reached 1.33 U mg^{-1} protein. Soluble protein values (Figure 1C) in the gut extracts were very close and not significantly different (P>0.01) among the three species ranged between 0.98 to 1.10 mg ml^{-1}.

Discussion

Digestive processes in fish aren't well known as in mammals, although the data obtained in fish so far show that the digestive enzymes studied are qualitatively similar to those observed in other vertebrates. A comparative study of the activity of digestive proteolytic enzymes and amylase can reveal the capacity of different species to use protein and carbohydrates (Hidalgo *et al*., 1999). Chan *et al*. (2004) mentioned that the activity of α-amylase follows a pattern influenced more by phylogeny than

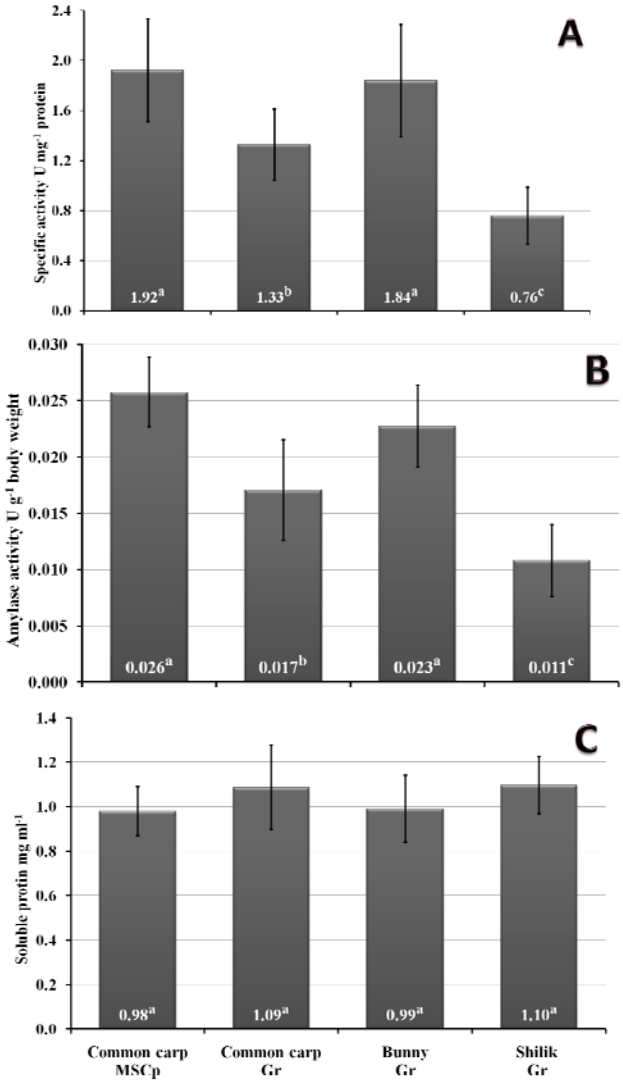

Figure 1. Specific activity of α-amylase (A), α-amylase activity per body weight (B) and soluble protein (C) in the gut extract of the Common carp *Cyprinus carpio* (omnivorous), Bunny *Barbus sharpeyi* (herbivorous) and Shilik *Aspius vorax* (carnivorous) collected from Garma river (Gr) and Marine Science Center ponds (MSCp), means with different superscripts are significantly different (P<0.01).

by diet in prickleback fishes. On contrary, Fernandez *et al*. (2001) pointed out that the adaptations of the digestive system of different species (*Pagrus pagrus*, *Pagellus erytrhinus*, *Pagellus bogaraveo*, *Boops boops* and *Diplodus annularis*) exhibit closer correlation with their diet rather than on their taxonomic category. This has also confirmed by results of Kuzmina (1996) which indicate that changes in digestive enzyme activity is affected by feeding behaviour and biochemical composition of food.

Results of the current work showed that the specific activity of α-amylase was significantly ($P<0.01$) higher in herbivorous bunny, followed by omnivorous common carp then carnivorous shilik. These results are comparable to those of other studies which indicate that the amylase activity largely depends on the feeding habits. Furthermore, herbivorous and omnivorous fishes showed higher amylase activity compared with carnivorous fishes in these studies (Horn *et al*., 2006; Drewe *et al*., 2004; Fernandez *et al*., 2001; Hidalgo *et al*., 1999; Kuzmina, 1996). Saoud (2004) when studying the food component of these three species in the same environment found that bunny food components consisting mainly of aquatic plants (90.01%) and algae (9.99%), which makes it a herbivorous fish, while occurrence of snails (61.49%), organic materials (27.26%) and aquatic plants (10.15%) in the food of common carp indicates its omnivorous feeding habit. Shilik food components were only from animal origins such as fish, crustaceans and insects (57.14%, 33.33% and 9.25% respectively), putting it in the carnivores category.

Cultured common carp reared in fish ponds recorded significantly ($P<0.01$) higher amylase specific activity compared with those in natural freshwater environment (Garma river). This might be due to differences in their food components in both habitats. Cultured fishes feed on mixture of ingredients such as grains, wheat bran, and small proportions of soybean and fishmeal. This mean that they fed on diet containing a higher proportion of carbohydrate compared with naturally raised carp. The present results are in agreement with many studies (Keshavanath *et al*., 2002; Appleford and Anderson, 1996; Kawai and Ikeda, 1972) mentioned that common carp had ability to increase their amylase activity with the increasing of carbohydrate in their diet.

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