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EFFECT OF STARVATION AND RE-FEEDING ON α–AMYLASE ACTIVITY IN COMMON CARP, *CYPRINUS CARPIO* L.

Ali H. H. Al-Gharrawi[1], Dhamyaa O. A. Al-Saadi[2] and Mohannad H. Alasha[3]

[1]Al-Mammon University College, Iraq.
[2]Department of Animal Production, College of Agricultural Engineering Sciences, University of Baghdad, Iraq.
[3]Agricultural Researches Directorate, Ministry of Science and Technology, Iraq.

Abstract

This research was designed to assess the effect of starvation and re-feeding periods, on the activity of *Cyprinus carpio* L. intestine amylase. Fish were divided into four feeding groups. The control group was fed to satiation twice a day throughout the experiment. The other three group were deprived of feed for (2, 4 and 6 day) respectively,and then fed to satiation during the re-feeding period. The experiments revealed that significant changes were observed in amylase activity was measured after 2 day and 4 days of starvation, the recorded value were (1.663, 1.600, 1.614 and 1.443 U/ml g.protein) for T_o, T_2, T_4 and T_6, respectively. Whereas, the activities of amylase were increase with the onset of starvation, then decrease with day starvation. There is a correlation between starvation and relative activity of the digestive enzyme.

Key words : Starvation, Common carp, Amylase, Digestive enzyme.

Introduction

Common carp *Cyprinus carpio* L. is one of the most widely cultured species in Iraq, this species is omnivores, highly tolerant to wide fluctuations in environmental conditions and is preferred for culture by many farms (Abd-Ali, 2006). Production of *C. carpio* L. depends mainly on artificial feeding, thus detailed studies of digestive enzymes of great importance (Lukasz *et al*., 2009). The activities of digestive enzymes depends on fish age (Alvarez-Gonzalez *et al*., 2010); food habit (Charkarbarti *et al*., 1995) and genetics (Ruibin *et al*., 2010) and starvation and Re- feeding ((Mukherjee and Maitra, 2015; Abolfath *et al*., 2012; Jiaojiao *et al*., 2017). The efficiency of food to growth ratiodepends on physiological capacities in fish to digest and transform ingested nutrients (Furne *et al*., 2005). Hence, digestion mechanism in fish have been practically studied in the last two decades, so that the amylase of digestive enzyme activities in an easy and reliable biochemical method that can provide insight into the digestive physiology in fish and their nutritional conditions (Bolasina *et al*., 2006). Digestive and absorption of nutrients depend on the activity of the digestive enzymes, in particular those located in the rush border section of the intestine, which are responsible for the final stage of break down and assimilation of the food (Hassanatabar *et al*., 2013). The enzyme production is suitable amount and the enzyme distribution along the gut change with feeding habit (Tengjaroenkul *et al*., 2002). Amylase level is affected by filling degree of the gut and the nutritional condition (Bitterlich, 1985). Higher level amylase were detected when the fish was not starved (Munilla–Moran and Stark, 1990). Herbivorous and omnivorous species have been reported to have more amylase activity than carnivorous species (Sabapathy, and Teo, 1993).Higher enzymatic level have also been reported for younger fish than adults (Kawai and Ikeda, 1971). In the recent years, the cultivation scale developed rapidly and as a result, more studies have done about digestive enzymes (Yonghua *et al*., 2015). The aim of this experiment is to studythe assess of changes in amylase activity of *C. carpio* L. during periods of starvation and Re–feeding.

Materials and Methods

Experiment design

C. carpio L. (average weight 40±4.5g) were obtained from a local commercial farm, transported to the lab. at AL-Mammon Univ. Coll. The experiment was set up as; one control group and three handling groups (T_2, T_4 and T_6) successively, each treatment was done in duplicate. starvation period were performed as ; 0 day(T_0 for control group), 2 days for T_2, 4 days for T_4, 6 days for T_6. After starvation were feed for 30 days on commercial diets containing 24% protein, twice a day, such that, each feeding last for 30 min (Chen *et al*., 2013). Before (pre) each, the aquaria were cleaned from anyleft bait and excretion debris, by changing the water using siphon tube with fresh water.

Sample preparation and assessment of amylase activity

Four fish samples were collected out of each group (before starvation, after starvation and after re-feeding at periods of 2,4 and 6 days. Each fish was weighed and dissected; the intestines were eliminated by scraping with a sharp razor and washed out with glacial physiological saline, wiped with absorbent cotton, weighed and stored at –15°C. Each sampled has grounded with a blender ; and homogenized for 6 h in ice bath at 4°C with stirring; the homogenized samples was mix with 4 of 0.2 M K–phosphate buffer (pH 6.7) containing 6% NaCl. After that, the mixture was filtered from tissue debris a piece of fabric; centrifuged at 4°C for 30 min (12000 rpm) using cooled centrifuge. The supernatant was taken for the assessment of amylase activity. Total amylase activity was measured using soluble starch as a substrate according to (Miller, 1959); optical density was followed at 540 µm for 20 sec.

Statistical analysis

The statistical analysis (SAS, 2012) was used to analyze data to the effect of days, study parameters (Duncan, 1955) multiple range to significant compare is on between means.

Results

During starvation, amylase activity of *Cyprinus carpio* L. of intestinal had a wavy change in different time: it declined first, picked up later and finally declined again (table 1). Being starvation for 2 days, the amylase activity had a linear decrease (P≤0.05); after 4 days starvation, amylase activity had gone back to the control–group (P≤0.05). When starvation last 6 days, the amylase activity had an apparent decrease (table 2).

Table 2 shows that, there were no significant difference in amylase activity between (T_0, T_2, and T_4) compared withcontrol group.While there were significant difference between during starvation andbefore starvation for all treatments, the value were (0.501, 0.499, 0.501 and 0.532 U/mlg protein) for during starvation and (1.621, 1.602, 1.632 and 1.611 U/ml g protein) before starvation. Also there are significant difference in amylase activity between T6 (6 days starvation) compared with all treatments, the value were (1.663, 1.600, 1.614 and 1.443 U/ml g protein) after re-feeding for T_0, T_2, T_4 and T_6, respectively.

Table 1 : Amylase activity of *Cyprinus carpio* L.

Tissue	Protein (U/ml g)			
	T_0	T_2	T_4	T_6
Intestine	1.50± 0.04 A	1.01± 0.02	1.42 ±0.05 A	0.80± 0.02 b
Level of sig	0.296*			

*Means having with the different letters in same column differed significantly (P≤ 0.05). T_0 control group, T_2 fish starved 2 day, T_4 fish starved 4 day, T_6 fish starved 6 day.

Table 2 : Amylase activity during starvation and re-feeding periods.

Group	Organ	Before starvation	Amylase activity	
			During starvation	After re-feeding
T0 (control)	Intestine	1.621a A	0. 501b A	1. 663a A
T 2	Intestine	1.602a A	0.499b A	1.600a A
T4	Intestine	1. 632a A	0.501b A	1. 614a A
T6	Intestine	1.611a A	0.532b A	1.443a B

Means having with the different letters in same row, capital letter in same column differed significantly (P<0.05). T_0 (control group), T_2 fish starved 2 day, T_4 fish starved 4 day, T_6 fish starved 6 day.

Discussion

When fish is threatened by starvation, its body's metabolism would have some changes to adapt with the situation; one of these adaption mechanisms is adjusting enzyme activity by change to use the substances in body reasonably and to keep alive. Where the (Wang *et al*., 2005) reference to that, the abilities of adjustment are differ to; somatotype, breeding and degree of starvation. The experiments showed that, starvation has different effects on the enzyme activity; such that , at the onset of starvation, the activity of amylase gradually increased after 2–days after starvation , and reached the maximum at the 4th day (table 2). This result is similar to that found by Zhang *et al*. (2010). As a result being starved for 4

days *Cyprinus carpio* L. activity of amylase in intestinal tract has increased. After short stress response, starved fish were in persistent state of starvation, and its whole digestive system didn't get mechanical stimulation from food, and simultaneously used sebum as the main energy source, which make it as a new interesting issue in fish diet and breeding. Fish secretion and activity of digestive enzymes are related to the digestive enzyme act or substrate in the alimentary canal. The increase of substrate will affect digestive and secretion. In the present work, after 2days of re–feeding fish; amylase activity highly increased, compared with control group .Before re- feeding the amylase activity went down to control values gradually. Starving for up to 4 days, increasing fish appetite largely by devouring the largest amount of food , which in turn reflected the enzyme's effectiveness .Depending on the finding of the study , it is recommend that ; productivity of fish farmers, would increased by 2 days starvation followed by 2 days re-feeding may lead to good harvests and decreasing in expenditure.

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