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Effect of different dietary proteins and fats on the digestive enzymes activities in the common carp fingerlings (*Cyprinus carpio* L.) reared in floating cages

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Abstract - The digestive enzyme activities of the common carp fed with different diets of protein sources were investigated during 150 days trial in order to obtain a theoretical basis for artificial diet preparation for the culture of carp fingerlings (*Cyprinus carpio* L.). The results showed that the fish at treatment 3 (protein 13.82%, fat 4.76% and carbohydrate 67.81%) had high levels of alkaline protease activity. Acid protease activity was high at the treatment 1 (protein 23.68%, fat 16.28% and carbohydrate 43.68%) ($p<0.05$). In general, the acid protease activity was higher than alkaline protease during the course of the study. Amylase activity reached a highest value in fish fed with none animal protein sources (treatment 3). Lipase activity showed a high level in fish fed with high lipid contents (treatment 1).

Keywords: digestive enzymes, nutrition, common carp, fish meal, float cages.

Introduction

The activity of digestive enzymes is considered as a significant indicator of digestive physiology in fish species, and the capacity of digestion and absorption of nutrients, which affects the development and the growth in fish were determined by the level of digestive enzyme activity (Wei *et al.*, 2010). However, research on the digestion of fish has mainly focused on the occurrence and activity changes of digestive enzymes during larvae and juveniles of some fishes (Chen and Zhang, 2004).

There are some differences between digestive enzymes in fish species which have different dietary behavior due to their different diets and digestive system structure.

Regarding amylase activity, the omnivorous species demonstrated higher activity than the carnivores. There were differences in growth and survival rates between fish fed on live and compound diets. These differences were attributed to the nutritional value of the feed, food digestion, nutrient absorption, and metabolic factors (Segner and Rosch, 1992; Hidalgo *et al.* 1999). However, digestive enzymes activities affect feed utilization by fish, and its understanding is essential to optimize diet formulation.

However, digestive enzyme activities of fish are affected by the diet composition and feeding habit of the fish (Lundstedt *et al.* 2004; Perrin *et al.* 2004; Corre'a *et al.* 2007; Debnath *et al.* 2007; Santigosa *et al.* 2008; Chatzifotis *et al.* 2008; Cedric 2009), the nutritional value of the feed, food digestion, nutrient absorption and metabolic factors (Segner and Rosch 1992; Chakrabarti *et al.*, 1995), fish age, pH and temperature (Kuz´mina, 1990; 1996). However, Watanabe and Kiron, (1994) suggested that a commercial diet, which was found to develop weaning efficiency and to decrease the feeding cost, would be of enormous benefit in finfish farming.

The aim of this study is to assess the digestive enzyme activities from the full gut of common carp fingerlings fed with different protein sources. Results from this work will provide a theoretical basis for artificial diet preparation for carp aquaculture.

Materials and Methods

Experimental diets:

Fingerlings of the common carp (*C. carpio*) (12.71 to 15.62 g and 7.61 to 9.80 cm) were kept at outdoor farm (25 per cage) at the Marine science center, Basrah University from January to June 2012. The fish were kept for about 14 days for acclimation. Fish of each cage: 1, 2 and 3 were fed with three different feed ingredients T1, T2 and T3, respectively (Table 1).

Fish were fed with pellets twice a day. Feeding rate was adjusted every two weeks at 3-5% of the fish biomass. Feed quantity was also re-adjusted every two weeks depending on fish weight. However, fish weights were taken every four weeks throughout the period of the study. Temperature, dissolve oxygen (Do), pH and salinity were also recorded daily.

Table 1. Ingredient and proximate composition of the experimental diets (%) used for C. *carpio*.

Treatment	T1	T2	T3
Fish meal	10	20	0
Wheat flour bran	20	20	20
Wheat Flour	20	20	20
Soybean meal	30	10	0
Ground corn	5	5	10
Rice particles	5	5	20
Rice bran	5	10	10
Barley	5	10	10
Broad bean	0	0	10
Total	100	100	100
Crude protein	23.68	19.86	13.82
Crude fat	16.28	5.84	4.76
Carbohydrate	43.68	57.62	67.81
Ash	8.19	8.82	3.48
Moisture	8.17	7.86	10.13

Preparation of crude enzyme solution:

Gut crude digestive enzyme extraction was done by rinsing the gut with cold distilled water and homogenized with tissue homogenizer in cold (4°C) phosphate buffer with pH 7.2 (1:10, w:v). The sample was then centrifuged (10,000 rpm/10 min at 4°C) and the supernatant was kept at -20°C for enzyme analysis (Chovatiya *et al*., 2011).

Measurement of enzyme activity:

Acid protease assay:

Solution of 6.2 ml was prepared from 1 ml of 3% casein solution, 1 ml of KCl-HCl buffer solution (pH 2.6), 4 ml of distilled water, and 0.2 ml of enzyme solution. The solution was incubated at 37°C water bath for 20 min. 3 ml of 10% trichloroacetic acid was added to the solution in order to prevent the enzyme reaction. The solution was then centrifuged at 5000 r/min for 20 minutes and enzyme activity was assessed at 280 nm wavelengths by using spectrophotometer (Whitaker, 1958).

Alkaline protease assay:

The same procedure of assessing acid protease was employed to assess alkaline protease assay, except by replacing KCl-HCl buffer solution with buffer of Glysine-NaOH (pH 10.0) (Whitaker, 1958). The amount of enzyme that would give an increase in absorbance at 280 nm of 0.01 under assay condition as a unit activity was used during this study (Whitaker, 1958).

α-Amylase assay:

The activity of α-amylase assay was estimated following Wilson and Ingledew (1982) as: the substrate of 0.2% soluble starch dissolved in boiling 0.05 M NaH_2PO_4 buffer (pH 6.9) and cooled to 40°C. 1 ml of stock solution (0.5% I2 in 5.0% KI) was diluted by adding it to 500 ml of distilled water containing 5 ml of 5N HCI to a prepare a fresh iodine reagent. 1.0 ml of enzyme solution was placed in a test tube and warmed up to 40°C in water bath. At 10 min after the addition of 2.0 ml of starch substrate, the reaction was stopped by removing a 0.2-ml sample and adding it to 5.0 ml of iodine reagent. The absorbance at 620 nm was measured against a blank (0.2 ml of water in 5 ml of iodine reagent). One unit of α-amylase is known as the quantity of enzyme that will hydrolyze 0.1 mg of starch during 10 min at 40°C when 4.0 mg of substrate is present.

Lipase Assay:

Measurement of lipase activity was assessed by estimating fatty acids released from enzymatic hydrolysis of triglycerides in a stabilized emulsion of olive oil (Borlongan, 1990). One unit of lipase activity (U) was known as the amount in micromoles of 0.05M NaOH needed to neutralize the fatty acids released during 1 min of incubation with the substrate and after correction by an appropriate blank (Borlongan, 1990).

Data analysis:

Analysis of variance (ANOVA) was used to calculate the effect of dietary protein and fat levels on digestive enzymes activity of the fish. If ANOVA

showed significant effects, the least significant difference (LSD) test was applied to estimate differences between individual treatment means (Snedecor and Cochran, 1989). Differences were considered significant at ($P<0.05$). The SPSS Statistics software (version 17) was employed for the data analysis.

Results

Water parameters through the experiment were within the acceptable ranges for health protection and growth of *C. carpio* during the study period. Water temperature ranged from 11.7°C to 25.6°C, dissolved oxygen from 2.51 to 7.9 $mg.l^{-1}$, salinity from 1.5 to 1.7 and pH from 7.5 to 7.9. However, these parameters did not show any statistical differences during the study period. The activities of the digestive enzymes in the gut of *C. carpio* are shown in Table (2).

Table 2. Digestive enzyme activity of the experimental fishes at the end of the 150-day feeding experiment.

Treatment	Protease pH2.6	Protease pH10.0	Amylase	Lipase
1	11.0717 ±0.84c	16.5330 ±2.17a	29.214 ±0.16c	0.6845 ± 0.03 a
2	13.9467 ±0.52b	9.4790 ±1.94b	33.444 ±0.20b	0.2707 ± 0.03 b
3	15.0497 ±0.63b	13.7247 ±0.16ab	34.785 ±0.36a	0.3748 ± 0.04 b

Different superscripts in the same column signify statistical differences ($P<0.05$) (mean ± S.D.).

Acid proteases:

Acid proteases activity was at the lowest level at T1 diet (11.0717 ± 0.84) and increased at T2 reaching the highest value at T3 (15.0497 ± 0.63) ($P<0.05$) (Fig. 1).

Alkaline protease:

The highest level of alkaline protease activity was recorded at T1 (16.5330 ± 2.17) and the lowest level was observed at T2 (9.4790 ± 1.94). The value of alkaline protease activity (13.7247 ± 0.16) was recorded at T3 (Fig. 2).

α-Amylase:

Amylase activity at T3 (34.785 ± 0.36) was significantly higher ($p<0.05$) than that at T1 (29.214 ± 0.16) and T2 (33.444 ± 0.20) (Fig. 3).

Lipase:

The highest lipase activity (0.6845 ± 0.03) was observed at T1 diet ($P<0.05$), followed by T2 and T3 diets (Fig. 4).

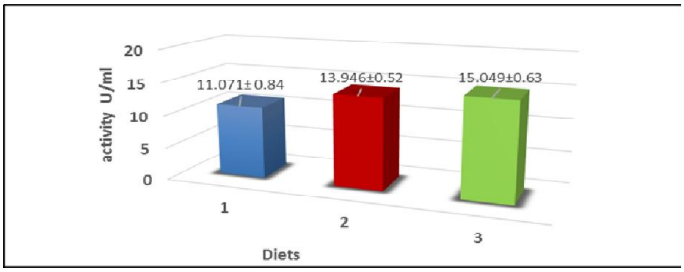

Figure 1. Acid protease activity of common carp *C. carpio* fingerlings fed three different diets.

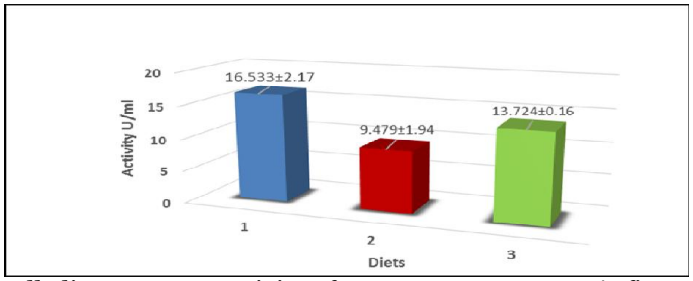

Figure 2. Alkaline protease activity of common carp *C. carpio* fingerlings fed three different diets.

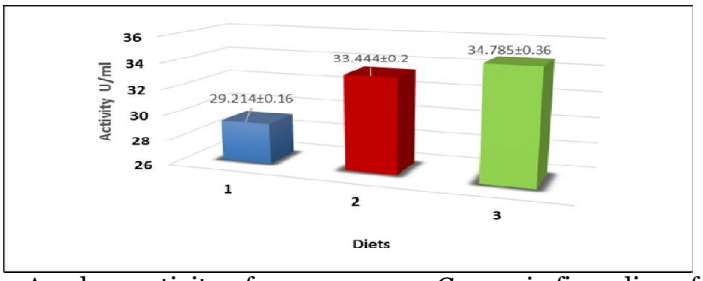

Figure 3. Amylase activity of common carp *C. carpio* fingerlings fed three different diets.

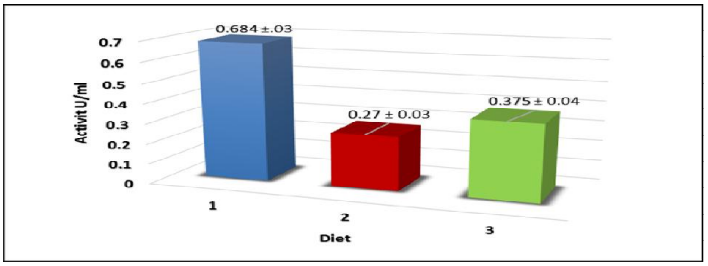

Figure 4. Lipase activity of common carp *C. carpio* fingerlings fed three different diets.

Discussion

Digestive enzymes play a significant role in the hydrolysis of proteins, lipids and carbohydrates to change them to ingested nutrients. These nutrients will be transported into the tissues and changed into material or energy for the growth and reproduction of fishes (Furne' *et al.*, 2005). However, digestion of ingested nutrients starts with effect of digestive enzyme in the stomach, and continues in the intestine by digestive enzymes secreted by the pancreas such as trypsin, chymotrypsin, amylase, and lipase (Cockson and Bourne, 1972; Moriarty, 1973; Fang and Chiou, 1989). Lazzari *et al.* (2010) reported that the response of digestive enzymes might be affected by the feeding period of the fishes. Previously, Kaushik *et al.* (1995) and López *et al.* (1999) stated that the changes in protein synthesis and enzyme activity in fishes can be detected after a long feeding period. However, it might be concluded from the previous and present work that 10% fish meal protein can be replaced by corn and rice without affecting intestinal proteases.

Dietary protein used (Table 1) in the present study was 23.68% indicating that there is a proteolytic enzyme activity in the gut of common carp fingerling. In contrast to this, trypsin was found to be unresponsive to dietary crude protein in early-weaned sea bass (Cahu and ZamboninoInfante, 1995). Later, Lopez-Lopez *et al.* (2005) did not find any correlation between protease activity and dietary crud protein and between protease activity and growth of juvenile Crayfish.

In general, fishes require higher protein levels with less carbohydrate in their feeds (Kikuchi, 1999). Digestive functions capable of hydrolyzing a larger variety of carbohydrate-containing lower amylase levels may be indicative of the limited potential of fish to utilize diets containing high carbohydrate levels (Lazzari *et al.*, 2010). Higher activities of amylase of fish fed with diets in experimental trail T3 as compared with T1 suggest that carbohydrates are easily digestible. Therefore, 100% replacement of fish meal is possible as tested in diet T3 when 10% corn and 20% rice were used as ingredient in the formulated diet. However, Fountoulaki *et al.* (2005) reported that amylase in gilthead sea bream is affected by dietary fat level. In fact, further research is required to establish whether growth performance and digestive enzyme physiology of carp fingerlings are affected by plant protein-supplemented diets in a long-term trial.

The ability of fish to utilize food normally correlates with the increase in growth of body weight, which reflexed the changes in the activity of digestive enzymes. Previous studies reported the ability of fish to utilize feed changes at different growth stages and stays relatively stable with changes in environmental temperature and pH (Jun-sheng *et al.*, 2006). The capability of fish to hydrolyze feed reduces with a decline in the environmental temperature. Similar results of tilapia protease related to the changes in the growth of cultured tilapia were found by Jun-sheng *et al.* (2006). The maximum activity of pH for tilapia protease ranged from 7.5 to 10.5 and the optimal temperature in fish gut was 55°C. The maximum amylase activity was at pH 6-7 to 7.5, and high amylase activity was recorded at 25-35°C. The activity of lipase was at pH 6.0-9.0 and at temperatures between 25 and 35°C (Ugwumba, 1993; Tocher and Sargent,

1984; Jun-sheng *et al*., 2006). Tongsiri *et al*. (2010) found that amylase activities of the stomach of giant Catfish (*Pangasianodon gigas*) were alkaline amylase and the optimal temperatures ranged from 25 to 50°C. The amylase activities in the intestine of *P. gigas* were neutral as well as alkaline and the optimal temperature ranged from 25 to 30°C.

Previous study reported that amylase activity of trout increases with higher consumption, temperature and salinity (Steffens, 1987). The acidic protease activities of the stomach of *P. gigas* showed the optimal temperatures ranged from 40 to 60°C and the alkaline protease showed the optimal temperatures ranged from 40 to 70°C (Tongsiri *et al*., 2010). However, amylase and lipase have different temperature requirements from that of protease (Jun-sheng *et al*., 2006; De la parra *et al*., 2007). However, water parameters and pH through the experiment were within the necessary ranges for the enzyme activities.

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دراسة تأثير علائق مختلفة البروتين والدهن على فعالية الانزيمات الهاضمة في إصبعيات الكارب الشائع (*Cyprinus carpio* L.) المرباة في أقفاص عائمة

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المستخلص - أجريت الدراسة الحالية لمعرفة نشاط فعالية الانزيمات الهاضمة لإصبعيات الكارب الشائع التي غذيت بأنواع علائق مختلفة من مصادر البروتين والمستخدمة للتربية ولفترة 150 يوماً. إذ تزايدت قيم نشاط فعالية انزيم البروتيز القاعدي في المعاملة الثالثة (البروتين 13.82% والدهن 4.76% والكاربوهيدرات 67.81%) وبمستوى معنوي ($P < 005$) في حين لوحظ أن أعلى مستوى نشاط لفعالية البروتيزات الحامضية كان في المعاملة الاولى (البروتين 23.68% والدهن 16.28% والكاربوهيدرات 43.68%) عند نفس مستوى المعنوية. بشكل عام ان فعالية البروتيزات الحامضية كانت أعلى من فعالية البروتيزات القاعدية خلال فترة الدراسة. وأدت الزيادة في مستوى الكاربوهيدرات بالعليقة الثالثة والخالية من البروتين الحيواني إلى ارتفاع نشاط الفا املیز إلى أعلى مستوى له، وظهر أن أعلى نشاط لفعالية انزيم اللايبيز كان في المعاملة الاولى.

الكلمات الدالة: الانزيمات الهاضمة، التغذية ، الكارب الشائع، مسحوق السمك، الاقفاص العائمة.