

Influence of pH on Survival, Growth and Activities of Digestive Enzymes of *Odontobutis obscurus*

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Abstract: The aim of our research is to explore the suitable range of pH for *Odontobutis obscurus*. Include the influence of pH on the survival, growth and activities of digestive enzymes of *Odontobutis obscurus*. The results showed that *Odontobutis obscurus* can survive well at 6.5~8.5 of pH. At 8.0 of pH, *Odontobutis obscurus* gained fastest, the average weight gain rate was 21.23%. The activity of protease, amylase and lipase of the *Odontobutis obscurus* increase with pH; while attained the maximum, activities of digestive enzymes decreased with pH. The optimum pH of protease is 8.5, amylase is 7.5-8.0, lipase is 7.5.

Keywords: Activities of digestive enzymes, growth, *Odontobutis obscurus*, survival

INTRODUCTION

Odontobutis obscurus, commonly known as tiger shark, belongs to Perciformes, Eleotridae and *Odontobutis*. It is fleshy, has less bone, delicious taste and rich nutrition. Every 100 g flesh, there is 18.39 g protein, 0.98 g fat, 0.42 g calcium and also phosphate, iron and vitamin etc (Wu *et al.*, 2002). It contains more amino acid essential for human beings and flavor amino acid than ordinary fish, has the functions of nourishing, strengthening tendons and bones, intestines and stomach, treating phlegm and retained fluid and haemorrhoids and has health care functions for pregnant women and delivery women (Sun, 2009). Feeding, reproduction, age, growth and other biological habits, farming techniques and genetic variation of *Odontobutis obscurus* have been reported At home and abroad (Hu *et al.*, 2004; Sakai *et al.*, 1996; Huang *et al.*, 2008; Tan, 1996; Wang *et al.*, 2005; Ye, 2009; Zhu *et al.*, 1999).

pH is an important indicator to express the strength of acidity and alkalinity in water, the variation of which has a directly or indirectly influence on the survival, growth and digestive enzyme activity of aquatic organism, especially fish. In this test, the influence of pH on the survival, growth and digestive enzyme activity of *Odontobutis obscurus* is researched to provide reference for the scientific research and production practice of artificial breeding, water quality management and disease control etc. of *Odontobutis obscurus*.

MATERIALS AND METHODS

Experimental materials: The *Odontobutis obscurus* used for the present test was purchased from the aquatic product market of Taizhou. After temporary rearing for one week in laboratory condition, the strong and uniformly sized *Odontobutis obscurus* were selected for test. The water used for the test was aerated running water, temperature was natural temperature $18\pm 2^{\circ}\text{C}$ and dissolved oxygen was kept at above 5 mg/L. The test was conducted in a 100 cm×80 cm×60 cm aquarium.

Experimental design:

The influence of pH on survival and growth of *Odontobutis obscurus*: 9 horizontal test groups 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 and a control group (treated pond water pH 7.8) were set for the pH of survival test, each treatment had 3 repetitions, the *Odontobutis obscurus* were fed with living freshwater shrimps according to their weight every day, the same amount was given in each horizontal group everyday, the residual bait and excrement were cleared regularly and the aquarium was aerated regularly, the dissolved oxygen was kept at above 5.0 mg/L, the test lasted for 40 d, before and after test, the wet weight of *Odontobutis obscurus* was measured to calculate the growth rate. pHs-2 acidimeter was used to determine the pH, the pH was adjusted once every morning with 1 mol/L HCL and 1 mol/L NaOH, so bait was given during test, the variation of pH rarely exceeded ± 0.2 of the design value.

The influence of pH on digestive enzyme of *Odontobutis obscurus*: Uniformly sized, healthy and active *Odontobutis obscurus* were selected to divide into 9 horizontal test groups randomly: 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0 with 10 in each group and 3 parallels set in each group, which were raised in a 100 cm×80 cm×60 cm aquariums. The dissolved oxygen was kept at 5.0 mg/L, temperature was 18±2°C, the aquarium was aerated continuously and one third to a half of the water was changed every day.

Sample treatment and enzyme fluid preparation: In the process of test, each group of *Odontobutis obscurus* were taken and the moisture on the surface was slightly absorbed with filter paper, the intestinal structure of *Odontobutis obscurus* was anatomized immediately, cleaned and then weighted and kept in a -20°C refrigerator. During analysis, the samples were added with 10-times frozen deionized water (4°C, pH 7.0) according to the weight, after glass homogenizer was used in low temperature, the samples were centrifuged for 20 min at 4000 r/min in low temperature and the liquid supernatant was taken to determine the enzymatic activity.

Determination of enzymatic activity: The protease activity was determined with Folin-phenol reagent method (Gui, 2004; Wang, 2005).

Determination of amylase activity: amylase detection kit (produced by Nanjing Jiancheng Bioengineering Research Institute) was applied and reacted in 96-pore elisa plate; microplate reader was used to determine the Optical Density (OD) value of each reactant in wavelength of 660 nm. The amylase activity is defined as: when acted with substrate for 30 min at 37°C, 100 mg hydrolyzed starch is defined as an enzymatic activity unit (U).

Determination of lipase activity: lipase detection kit (produced by Nanjing Jiancheng Bioengineering Research Institute) was applied and reacted in 96-pore elisa plate, microplate reader was used to determine the Optical Density (OD) value of each reactant in

wave length of 420 nm. The lipase activity is defined as: in the condition of 37°C, each gram of tissue protein acts with the substrate in the reaction system for 1 min and each consumption of 1 μmol substrate is an enzymatic activity Unit (U).

Calculation formula:

$$\text{Survival rate (\%)} = (\text{number of fish surviving} / \text{total number of fish}) \times 100\%$$

$$\text{Average Final Weight, (AFW)} = \text{TW} / n$$

$$\text{Weight Gain, (WG)} = \text{W2} - \text{W1}$$

$$\text{Weight Gain Rate, (WGR)} = (\text{W2} - \text{W1}) / \text{W1}$$

Where W1 and W2 are the preliminary weight and final weight (g) of *Odontobutis obscurus*, TW is the total weight (g) of *Odontobutis obscurus* in single aquarium and n is the number of *Odontobutis obscurus* used for experiment.

Data processing: The data obtained were analyzed and processed with Excel 2007 and SPSS17.0 software, p<0.05 was taken as the significant level.

RESULTS AND DISCUSSION

Influence of pH on survival of *Odontobutis obscurus*: The test lasted for 4 d (96 h), no bait was given during the test, the temperature variation was 18±2°C, pH variation was ±0.2 of the design value and the dissolved oxygen amount was more than 5.0 mg/L on average. After 4 days (96 h) test, the survival rate of *Odontobutis obscurus* is 100% when pH is 6.5-8.5, so is the survival rate of *Odontobutis obscurus* in the control group (pH 7.8); when pH is less than 6.0 and more than 8.5, *Odontobutis obscurus* dies in different degrees (Table 1).

Influence of pH on growth of *Odontobutis obscurus*: At completion of the test, when the test pH was 6.5-8.5, the survival of *Odontobutis obscurus* in each test pH was 100%, when the pH is 8.0, *Odontobutis obscurus* grow fastest, followed by pH 7.5. Compared with test group of other pH, the difference is significance

Table 1: Influences of different pH on survival rate of *Odontobutis obscurus*

pH	Number of fish used for test	Survival rate within 24 h (%)	Survival rate within 48 h (%)	Survival rate within 72 h (%)	Survival rate within 96 h (%)
5.0	30	73.33	50.00	46.67	40.00
5.5	30	100.00	93.33	83.33	70.00
6.0	30	100.00	100.00	100.00	96.67
6.5	30	100.00	100.00	100.00	100.00
7.0	30	100.00	100.00	100.00	100.00
7.5	30	100.00	100.00	100.00	100.00
8.0	30	100.00	100.00	100.00	100.00
8.5	30	100.00	100.00	100.00	100.00
9.0	30	100.00	100.00	83.33	50.00
7.8 (control group)	30	100.00	100.00	100.00	100.00

Table 2: Influences of different pH on growth of *Odontobutis obscurus*

pH	Preliminary weight (g)	Final weight (g)	Average weight gain (g)	Average weight gain rate (%)
6.5	69.36±0.63 ^a	74.64±0.61 ^a	5.28 ^a	7.61 ^a
7.0	70.96±0.39 ^a	76.28±0.28 ^a	5.32 ^a	7.49 ^a
7.5	70.17±0.42 ^a	79.99±0.95 ^b	9.82 ^c	13.99 ^c
8.0	69.71±0.68 ^a	84.51±1.06 ^c	14.80 ^d	21.23 ^d
8.5	69.45±0.73 ^a	77.38±0.87 ^b	7.93 ^b	11.42 ^b

The inter-group difference between average value of different superscripts in the same line is not significant ($p < 0.05$); the value is the average value of three groups of *Odontobutis obscurus*

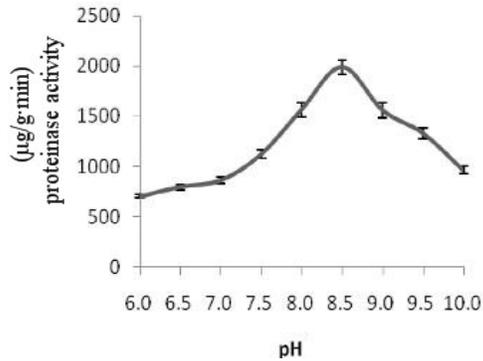


Fig. 1: Effects of different pH on activity of protease

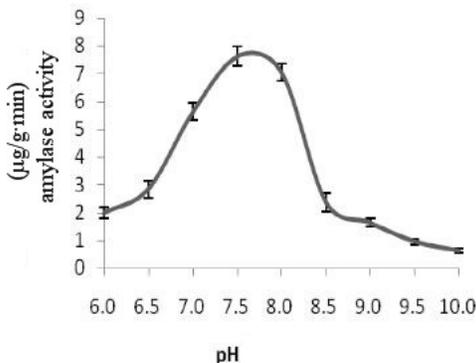


Fig. 2: Effects of different pH on activity of amylase

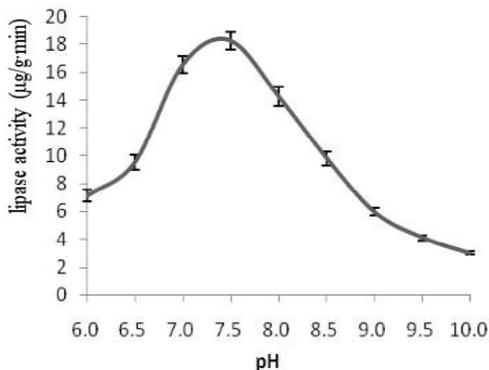


Fig. 3: Effects of different pH on activity of lipase

($p < 0.05$) and the slowest when pH is 6.5 and 7.0, when pH is 7.0, the average weight gain rate is slightly lower than that at pH of 6.5, but the difference is not significant ($p > 0.05$) (Table 2).

Influence of pH on digestive enzymatic activity of

Odontobutis obscurus: It can be seen from Fig. 1 that when pH is between 6.0 and 10.0, with the raising of pH, the protease activity presents a trend of first increasing then decrease, in which the activity is the highest when pH is 8.5 and relatively high when pH is between 8.0 and 9.0, therefore, the pH appropriate for *Odontobutis obscurus* should be about 8.5.

It can be seen from Fig. 2 that when pH is between 6.0 and 10.0, with the increasing of pH, the amylase activity presents a trend of first increasing and then decrease, the activity is the highest when pH is 7.5 and relatively high when between 7.0 and 8.0, therefore, the pH suitable for *Odontobutis obscurus* should be 7.5-8.0.

It can be seen from Fig. 3 that when pH is between 6.0 and 10.0, with the increasing of pH, lipase activity presents a trend of first increasing and then decrease and the activity is the highest when pH is 7.5 and relatively high when between 7.0 and 8.0, therefore, the pH appropriate for *Odontobutis obscurus* should be about 7.5.

Discussion:

Influence of pH on survival and growth of

Odontobutis obscurus: Fish have a certain enduring range to pH, in this range, fish can adjust themselves, when the pH in the water body exceeds the enduring range of fish, the fish may suffer from metabolic disorders and even death. According to result of the present test, the enduring range of *Odontobutis obscurus* to pH is 6.5-8.5, when pH is less than 6.5 and more than 8.5, *Odontobutis obscurus* may die in different degrees. The pH range appropriate for the growth of *Odontobutis obscurus* is 7.5-8.5 when pH is 8.0, the *Odontobutis obscurus* has the largest weight gain rate, followed by pH 7.5 and 8.5.

Influence of pH on digestive enzyme activity of

Odontobutis obscurus: When pH is between 6.0 and 10.0, the protease activity, amylase activity and lipase activity of *Odontobutis obscurus* all present a trend of first increasing and then decrease with the increasing of pH and there is an appropriate pH and different digestive enzymes are appropriate for different pH ranges. According to result of the present test and through comprehensive consideration of protease, amylase and lipase, the appropriate pH in the range of 7.5-8.0.

Under natural condition, pH plays an important role in the survival, ingestion and growth of *Odontobutis obscurus*. pH does not only directly influence the growth and digestive enzyme activity of *Odontobutis obscurus*, but also its metabolism. Therefore, in the artificial breeding of *Odontobutis obscurus*, it is required to pay attention to the regulation of pH in the water body, always stabilize pH within the range appropriate for the survival of *Odontobutis obscurus*, i.e., 7.5-8.5. In this range, *Odontobutis obscurus* grow the fastest has the lowest death rate and the economic benefit is the largest.

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