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Characterization of Amylase, Cellulase and Proteinase Enzyme in Stomach and Intestine of the Mekong Giant Catfish Fed with Various Diets Consisting of *Spirulina*

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Abstract: The amylase, cellulase and proteinase enzyme from the stomach and intestine of the Mekong Giant Catfish that had been fed with various diets consisting of *Spirulina*, were studied at pH 2-12 and at temperatures between 25-80ºC. This study found that; amylase activities of the stomach were alkaline amylase and the optimal temperatures to be 25 and 50ºC. Amylase activities of the intestine were neutral amylase as well as alkaline amylase and the optimal temperature range was at 25-30ºC. The cellulase activities were acidic cellulase and alkaline cellulase in both the stomach and the intestine. In the stomach, the acidic cellulase showed the optimal temperatures to be at 30, 40 and 50ºC, however alkaline cellulase were 30 and 50ºC. In the intestine, acidic cellulase showed the optimal temperatures to be at 25, 60 and 70ºC and the alkaline cellulase to be 80ºC. For proteinase activity, the stomach was acidic proteinase and alkaline proteinase, but the intestine was alkaline proteinase. The acidic proteinase activities of the stomach showed the optimal temperatures to be at 40, 50 and 60ºC and the alkaline proteinase activity proved to be at 40, 60 and 70ºC. The optimal conditions for amylase enzymes showed the higher specific activity in the intestine than in the stomach including the proteinase enzyme. At room temperature (25-30ºC), amylase and proteinase specific acitivity dominated in the intestine, while cellulase specific activity dominated in the stomach.

Key words: Amylase, cellulase, mekong giant catfish, proteinase

INTRODUCTION

The Mekong Giant Catfish, *Pangasianodon gigas* Chevey, is the world's largest freshwater catfish and an important fish that inhabits the Mekong Basin. It was listed as being critically endangered in the 2010 IUCN red list of threatened species (IUCN, 2010). Now the Mekong Giant Catfish is being commercially bred, for which the raiser must consider many factors, such as the specific requirements of investment and location because a vast and deep pond is needed, there is a high cost of feed, as well as a shortage of the fry and the necessary length of time needed for maturation (5-6 years). (Manosroii *et al*., 2004). Naturally, the Mekong Giant Catfish feed on plankton and algae, so to be cultivated in ponds, the environment must be adjusted to simulate nature. Nowadays, the pelleted feed has been added with some additives and/or supplements such as vitamins, minerals or *Spirulina*. *Spirulina* as a protein supplement source could improve growth, reduce of mortality, and improve overall elements of fish quality; such as the firmness of flesh and the brightness of skin color (Vonshak, 1997; Nandeesha *et al*., 2001).

The activity of enzyme, especially digestive enzyme, in fish was studied in much kind of fish. These studied could help to solve the nutritional problem and to know the limiting factor of protein, lipid and carbohydrate. The aim of this research was studied the characteristics of amylase, cellulase and proteinase enzyme from both the stomach and the intestine of the Mekong Giant Catfish fed with various diets consisting of *Spirulina*. The profile of temperature and pH were studied. This research could be a new knowledge on enzyme from the stomach and intestine of the Mekong Giant Catfish.

MATERIALS AND METHODS

Experimental place and time: This research was studied at the Faculty of Fisheries Technology and Aquatic Resource, Maejo University and Department of Biology,

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the Faculty of Science, Chiang Mai University, Chiang Mai, Thailand and the enzyme assays was studied for five months, 1 Aug.-30 Dec. 2009.

Experimental animal: Mekong Giant Catfish with an initial weight of 400±10 g and a total length of 20±5 cm were released in the pond. The different feeding combinations (4 formulas of isoenergy diets) were prepared as follows: Diet 1 feed containing 100% fish meal (T_1), Diet 2 feed-stuff supplemented with 5% dried *Spirulina* powder (T_2), Diet 3 feed-stuff supplemented with 10% dried *Spirulina* powder (T_3) and Diet 4 feed with 100% dried *Spirulina* powder (T_4).

Fish were fed two times each day at 3% of their body weight per day and the feeding rates were adjusted fornightly. After collection of the growth performance data, all fish were frozen and kept in -20ºC.

Enzyme study:

Enzyme extraction: Fish were washed and immediately frozen at -20ºC. The digestive systems of individual fish were removed using a glass plate maintained at 0ºC. The stomach was weighed, cut and the feed taken out. The feed was kept in 3% acetone. The intestine was weighed and length measured. The stomach and intestine were put in dry ice immediately and added into a centrifuge tube containing phosphate buffer pH 7 for homogenization. The homogenate was centrifuged at 10,000 x *g* at 4ºC for 10 min. and the supernatant was collected and stored at -80ºC. Three replicates were used for each sample. The method to measurement protein content was using the method described by Lowry *et al.* (1951).

Enzyme assays:

Amylase specific activity: Amylase activity was measured by using 1% (w/v) starch solution in pH 7 of phosphate buffer as substrate. The method was used the Areekijseree *et al.* (2004) modified method. Maltose was used for the preparation of standard curve. The amylase specific activity was defined as mmol of maltose produce per min per mg protein. The pH profile study was measured at room temperature and pH 2-12. The temperature profile study was measured at temperatures 25-80ºC in either the neutral or alkaline condition.

Cellulase specific activity: Cellulase activity was assayed by using 2% carboxymethyl cellulose (CMC) in phosphate buffer pH 7 as substrate. The method was used the Areekijseree *et al.* (2004) modified method. The standard curve was prepared by using maltose. The cellulase specific activity was defined as mmol of maltose produce per min per mg protein. The pH profile study was measured at room temperature and pH 2-12. The temperature profile study was measured at temperatures 25-80ºC in either the acidic or alkaline condition.

Proteinase specific activity: Total proteinase activity was measure by using 2% azocasein as substrate. The method was used the Areekijseree *et al.* (2004) modified method. Total proteinase specific activity was expressed as the number of proteinase units per mg of protein. One unit of proteinase activity was defined as the amount of enzyme giving an increase of per min per mg protein. The pH profile study was measured at room temperature and pH 2-12. The temperature profile study was measured at temperatures 25-80ºC in either the acidic or the alkaline condition.

Statistical analysis: The value of mean and standard error of mean were calculated. One-way ANOVA was used to test the effect of the treatment. All statistical analysis performed were done with SPSS version 11.5.

RESULTS AND DISCUSSION

The amylase specific activity: The amylase activity was measured in pH 2-12 and 25-80ºC; the profiles were similar in both the stomach and the intestine (Fig. 1-3). The amylase activities of the stomach showed the pH optima for hydrolysis of the substrate for each treatment, T_1 and T_4 were highest with the amylase activity at 8 and 12, but T_2 and T_3 were the highest amylase activity at 8 and 11. The amylase activities of the stomach were shown to be alkaline amylase. The amylase activities of the stomach showed the optimal temperatures at 25 and 50ºC (Fig. 2). The amylase activities of the intestine showed the pH optima for hydrolysis of the substrate for each treatment, T_1 displayed high activity at 6, 7, 11 and 12 and T_2 displayed high activity at 8, 11 and 12. T_3 and T_4 both displayed high activity at 7, 8, 11 and 12. The amylase activities of the intestine showed the optimal temperature to be at 25ºC, except T_2, which showed a high activity at 30ºC (Fig. 3). In the intestine, the neutral amylase specific activity was lower than the alkaline amylase specific activity and the optimal temperature range was found to be 25-30ºC.

The cellulase specific activity: The cellulase activity was studied in pH 2-12 and 25-80ºC; the profiles were similar in both the stomach and the intestine (Fig. 4). The cellulase activity of the stomach showed various pH optima for hydrolysis of the substrate, T_1 showed the optima pH at 5 and 10, T_2 and T_3 showed the optima pH at 6 and 10 and T_4 showed the optima pH at 3 and 8 (Fig. 4). The cellulase activity of the stomach showed the acidic cellulase (pH 3, 5 and 6) and alkaline cellulase (pH 8 and 10). The cellulase activities of the stomach showed the optimal temperature to be different in treatment , the acidic cellulase showed the optimal temperature at 40ºC (T_1), 50ºC (T_2,T_4) and 30ºC (T_3). All of the alkaline cellulase showed the optimal temperature at 50ºC, except

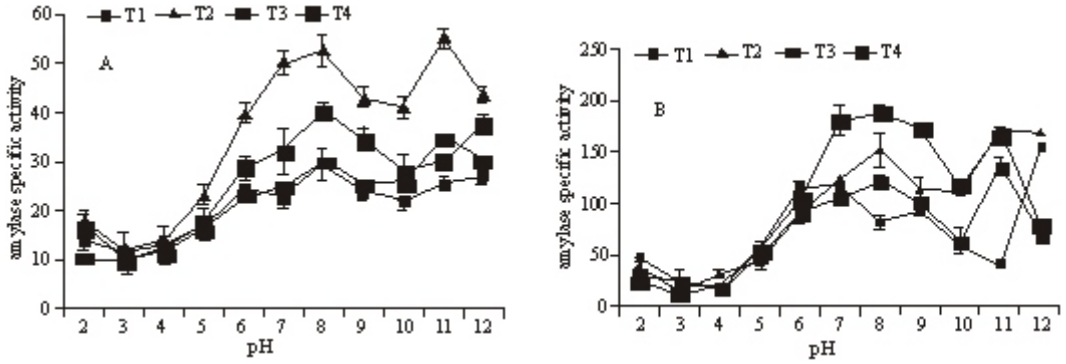


Fig. 1: The amylase specific activity in the stomach (A) and the intestine (B) extracts of the Mekong Giant Catfish fed with various diets supplemented with *Spirulina*

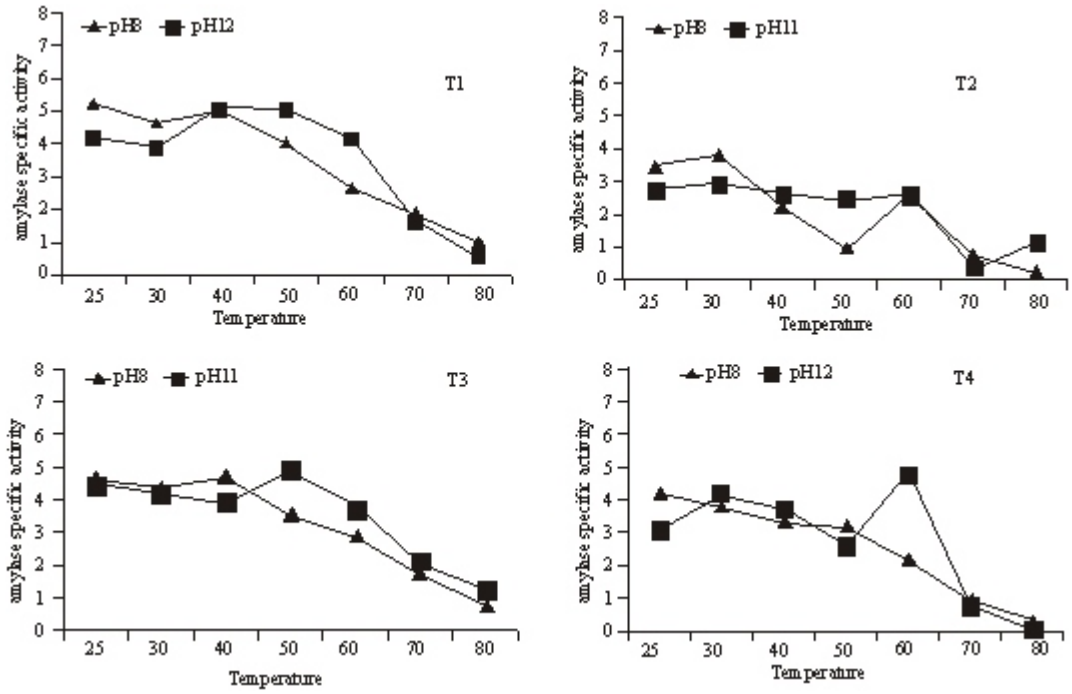


Fig. 2: The amylase specific activity in the stomach extracts of the Mekong Giant Catfish fed with various diets supplemented with *Spirulina*. The enzymatic reaction was performed at pH 8, 11 and 12 at 25-80ºC

T_4, which showed it to be at 30ºC (Fig. 5). In the intestine, the cellulase activity showed various pH optima for hydrolysis of the substrate, T_1 showed the optima pH at 4 and 11, T_2 and T_4 showed the optima pH at 4 and 10 and T_3 showed the pH at 3 and 11. These showed that the two groups of cellulase were acidic amylase (pH 3 and 4) and alkaline amylase (pH 10 and 11). The acidic cellulase in the intestine showed the optimal temperature at 25ºC (T_1), 60ºC (T_2), 70ºC (T_3) and 60ºC (T_4), all of the alkaline cellulase showed the optimal temperature at 80ºC (Fig. 6). Cellulase activity was found to be highest in fish fed on the cellulose incorporated diet, followed by those maintained on both the plant-protein based and reference diets, respectively. A diet-dependent variation in cellulase activity was apparent (Saha and Ray, 1998).

The proteinase activity: The proteinase activity was studied in pH 2-12 and 25-80ºC (Fig. 7-9). In the stomach, we found the acidic proteinase (pH 4) and alkaline proteinase (pH 11-12) but in the intestine, we found only alkaline proteinase (pH 10 and 12) (Fig. 7). The acidic proteinase activities of the stomach showed the optimal temperature to be different in treatment, T_1 showed high activity at 50ºC, T_2 and T_4 showed high

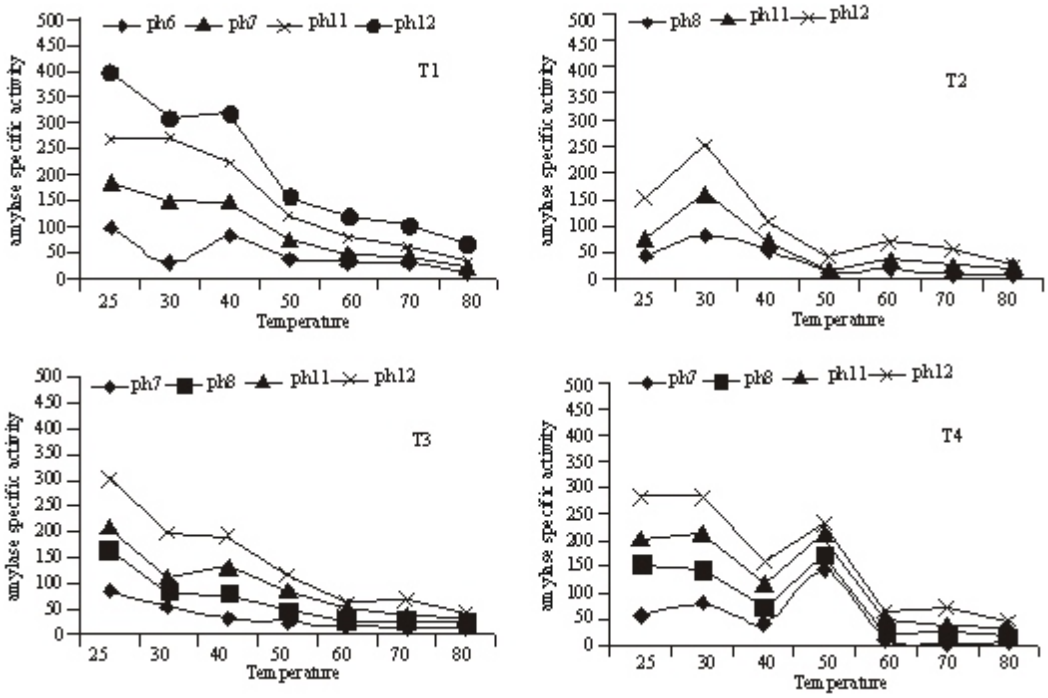


Fig. 3: The amylase specific activity in the intestine extracts of the Mekong Giant Catfish fed with various diets supplemented with *Spirulina*. The enzymatic reaction was performed at pH 6, 7, 8, 11 and 12 at 25-80ºC

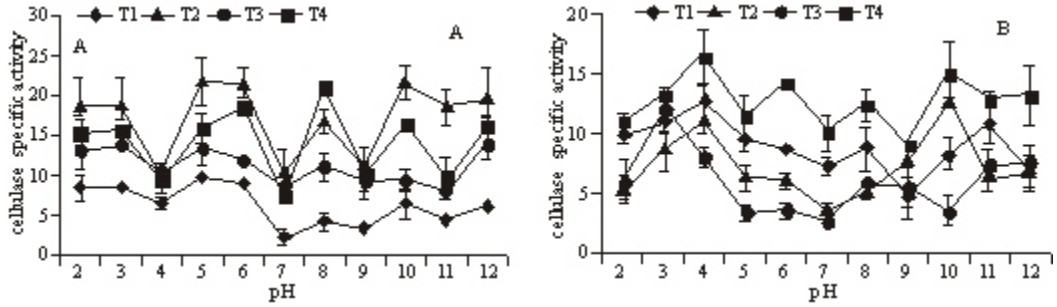
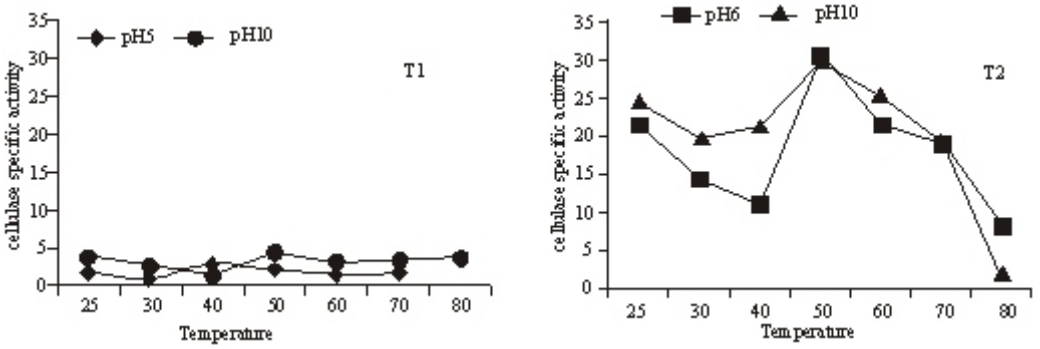


Fig. 4: The cellulase specific activity in the stomach (A) and the intestine (B) extracts of the Mekong Giant Catfish fed with various diets supplemented with *Spirulina*



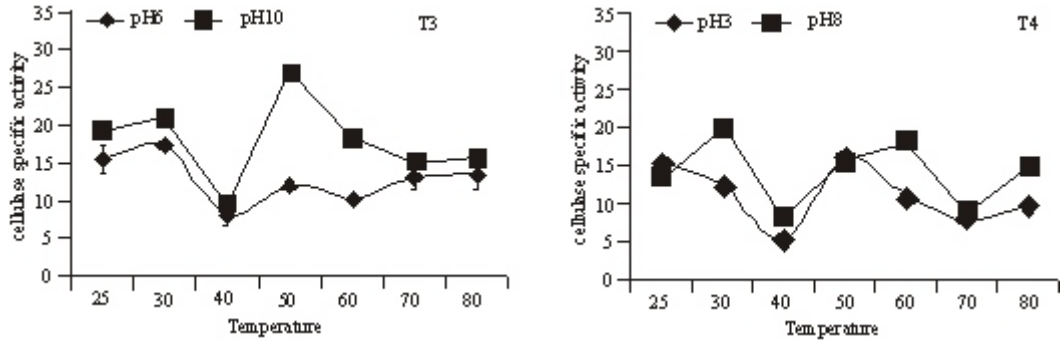


Fig. 5: The cellulase specific activity in the stomach extracts of the Mekong Giant Catfish fed with various diets supplemented with *Spirulina*. The enzymatic reaction was performed at pH 3, 5, 6, 8 and 10 at 25-80ºC

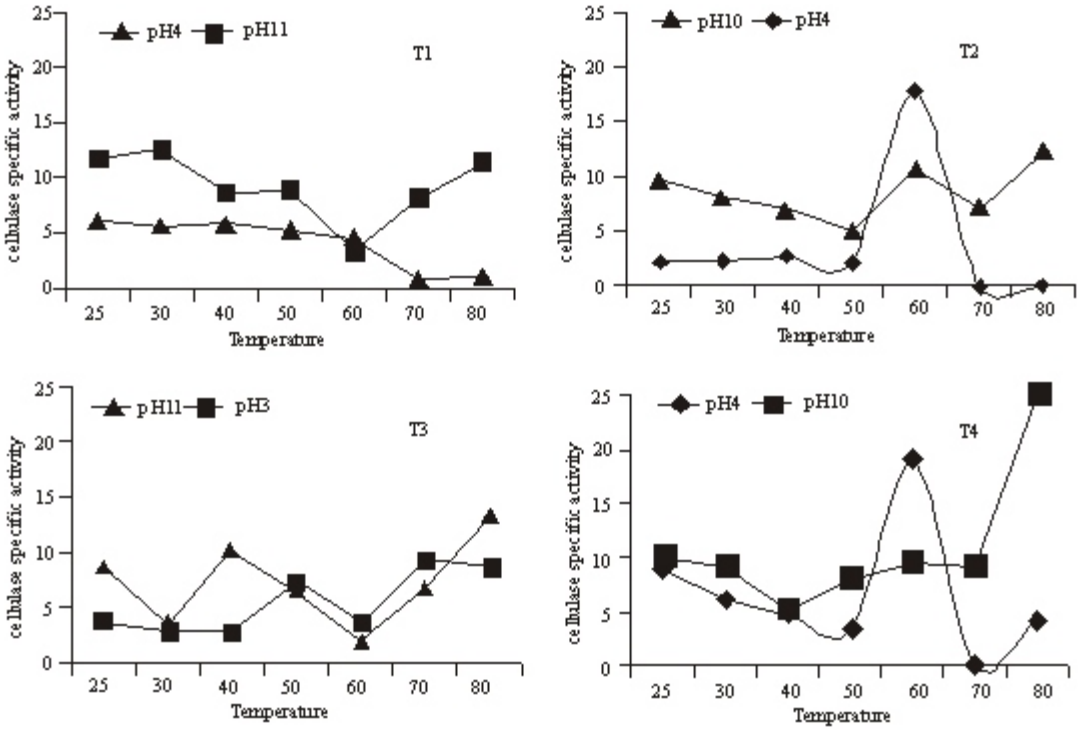


Fig. 6: The cellulase specific activity in the intestine extracts of the Mekong Giant Catfish fed with various diets supplemented with *Spirulina*. The enzymatic reaction was performed at pH 3, 4, 10 and 11 at 25-80ºC

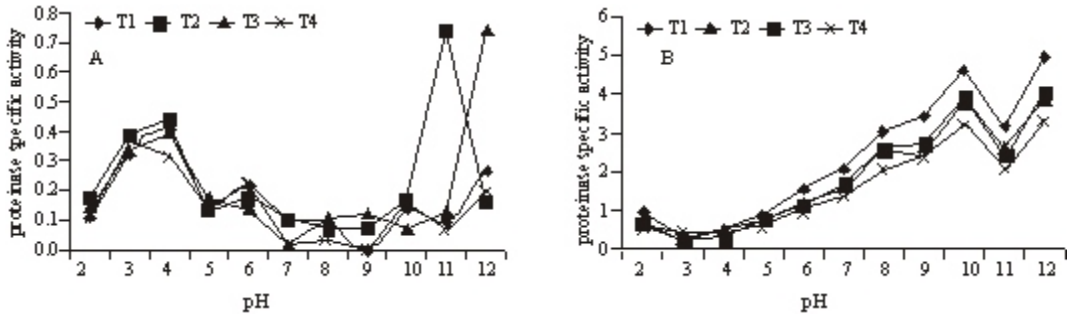


Fig. 7: The proteinase specific activity in the stomach (A) and the intestine (B) extracts of the Mekong Giant Catfish fed with various diets supplemented with *Spirulina*

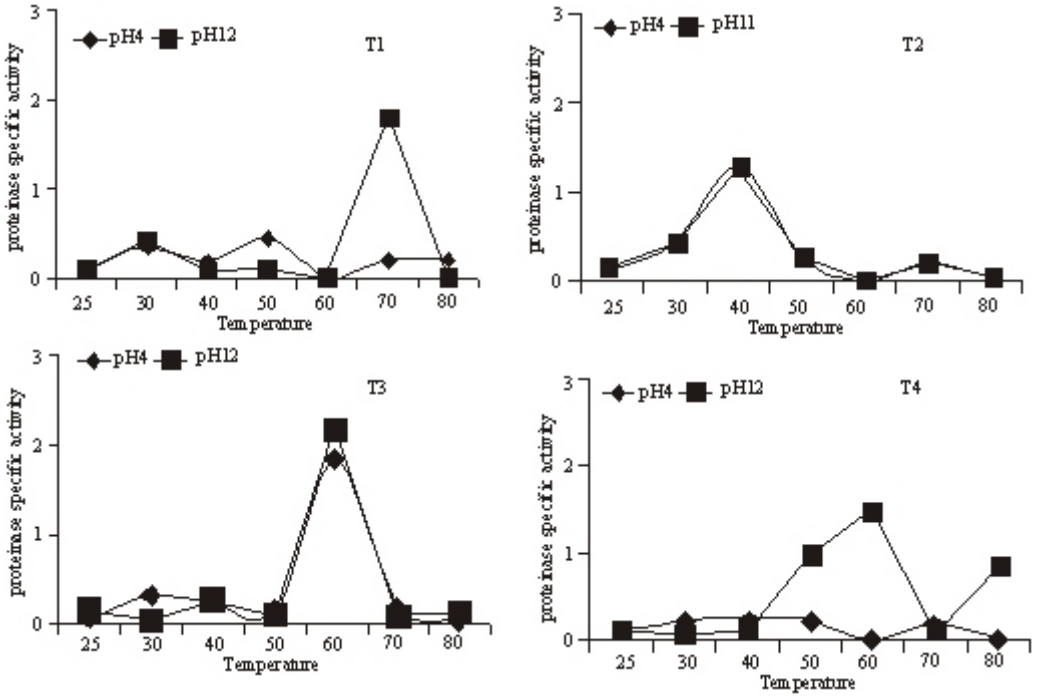


Fig. 8: The total proteinase specific activity in the stomach extracts of the Mekong Giant Catfish fed with various diets supplemented witb *Spirulina*. The enzymatic reaction was performed at pH 4, 11 and 12 at 25-80ºC

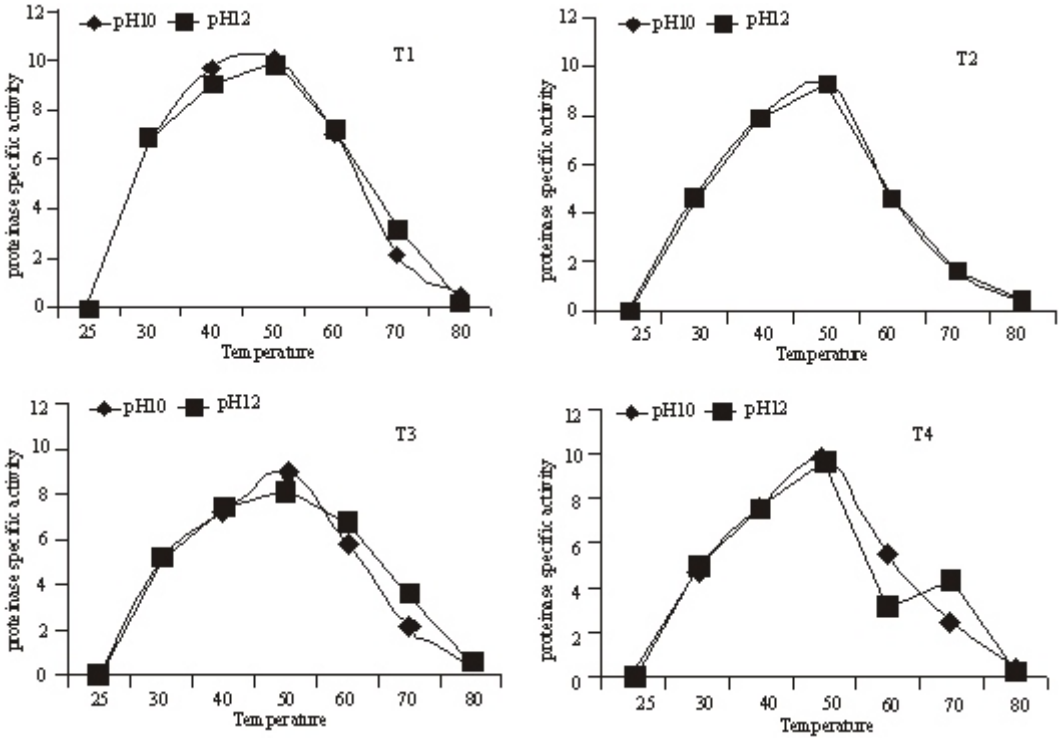


Fig. 9: The proteinase specific activity in the intestine extracts of the Mekong Giant Catfish fed with various diets supplemented with *Spirulina*. The enzymatic reaction was performed at pH 10 and 12 at 25-80ºC

activity at 40ºC and T_3 showed high activity at 60ºC but the alkaline proteinase activity showed the optima temperature at 40ºC (T_2), 60ºC (T_3 and T_4) and 70ºC (T_1) (Fig. 8). On the other hand, the alkaline proteinase activities of the intestine showed the optimal temperature at 50ºC in all treatments (Fig. 9). The proteases activities were high at pH from 8 to 10 in several fish species (Hidalgo *et al*., 1999). The most important of digestive enzyme was proteinase, it was used to recover protein from bones, hydrolyze blood protein, improve the quality of egg products and was also used for biomedical applications (Simpson, 2000).

CONCLUSION

The studied the characteristics of amylase, cellulase and proteinase enzyme from the stomach and intestine of the Mekong Giant Catfish fed with various *Spirulina* found that the amylase activities of the stomach to be alkaline amylase and showed the optimal temperature at 25 and 50ºC. The amylase activities of the intestine showed neutral amylase and alkaline amylase and revealed the optimal temperature range at 25-30ºC. The cellulase activities were acidic cellulase and alkaline cellulase in both the stomach and the intestine. In the stomach, the acidic cellulase showed the optimal temperatures to be at 30, 40 and 50ºC but all of the alkaline cellulase showed the optimal temperature to be at 50ºC, except T_4 which showed it to be at 30ºC. In the intestine, the acidic cellulase showed the optimal temperatures at 25, 60 and 70ºC, all of the alkaline cellulase showed the optimal temperature at 80ºC. For proteinase activity in the stomach, it was found to be acidic proteinase and alkaline proteinase but in the intestine, only alkaline proteinase was found. The acidic proteinase activities of the stomach showed the optimal temperature at 40, 50 and 60ºC but the alkaline proteinase activity showed the optima temperature at 40, 60 and 70ºC.

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