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RESEARCH ARTICLE

Ontogenic development of digestive enzyme activities in juvenile soft-shelled turtle (*Pelodiscus sinensis*) under cultured conditions

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Abstract The study was conducted to investigate the ontogenic development of main digestive enzymes (pepsin, trypsin, amylase and lipase) activities in juvenile soft-shelled turtle (*Pelodiscus sinensis*) (initial mean bodyweight 3.63 ± 0.27 g), in order to provide data on the digestive physiology of the juveniles during the first 30 days after hatching (DAH). The soft-shelled turtles were reared in an indoor rearing system, and fed with a formulated diet four times daily from 2 to 30 DAH. The results showed that the specific growth rate (SGR) of soft-shelled turtle ranged from 1.31 to 4.00%/d during the first 30 DAH. The specific activities of pepsin in stomachic segments, trypsin, amylase and lipase in intestinal segments first decreased slightly then increased to the maximum value ($P < 0.05$). The specific activities of these enzymes were first detected on 1 DAH, and the lowest values were observed from 4 to 6 DAH, while the highest values were found from 22 to 30 DAH. Results of the present study indicated that the activities of digestive enzymes in soft-shelled turtle developed during the development and were well correlated with growth.

Keywords soft-shelled turtle, *Pelodiscus sinensis*, juvenile, growth, enzyme activity

Introduction

Soft-shelled turtle *P. sinensis* lives in fresh water and is a highly valued cultured animal species in Asian countries including China, Japan, Korea, etc. (Yin et al., 2005). It has been also considered a product with medical benefits in Chinese tradition for a long time. Farming of soft-shelled turtle boomed after the success of its juvenile culture since the late 1990s. To our knowledge, the lack of optimal diets of the juveniles retarded the development of this species aquaculture. The study of digestive enzymes is an essential step toward understanding of the mechanism of digestion and the better knowledge of nutritional needs (Le Moullac et al., 1997). Moreover, the activity of the digestive enzyme could serve as an effective indicator of the digestive capacity and the development of larval and juvenile aquatic animal (Buddington et al., 1997; Suzer et al., 2006; Falcón-Hidalgo et al., 2011). It is therefore critical to study the physiology of juvenile including the ontogenic development of digestive enzymes activities during turtle juvenile from 1 DAH to 30 DAH. During the last two decades, an increasing number of studies were focused on the digestive physiology of aquatic animal larvae and juveniles (Cahu and Zambonino Infante, 2001). Ontogenic development of digestive enzymes has been well documented in frog and fish, such as anuran tadpole (Altig et al., 1975; Hourdry et al., 1996), European sea bass (Zambonino Infante and Cahu, 1994; Kamacı et al., 2010), Atlantic halibut (Gawlicka et al., 2000), Nile tilapia (Tengjaroenkul et al., 2001) and large yellow croaker (Ma et al., 2005). However, few studies on digestive enzymes activities were conducted in soft-shelled turtle juveniles (Tan, 1997). Moreover, no investigation was conducted on the ontogenetic development of digestive enzymes in juvenile soft-shelled turtle and the other reptile during the first 30 DAH.

The present study was designed to describe for the first time the ontogenic development of the main digestive enzymes (pepsin, trypsin, amylase and lipase) activities in soft-shelled turtle juveniles, to provide data on the digestive physiology of the juveniles during the first 30 DAH.

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Materials and methods

Juveniles rearing

The soft-shelled turtles (*Pelodiscus sinensisjaponicus*) juveniles were obtained from Hangzhou Wensli Biology Science and Technology Stock Co. Ltd. (Hangzhou, China). The juveniles were hatched under the same condition, and then reared in an indoor rearing system. Eight hundred juveniles with 3.63±0.27 g initial mean bodyweight were individually weighed. They were randomly distributed into four tanks (2 m × 1 m × 0.5 m, water volume: 460 L) and each with 200 individuals. The tanks were supplied with freshwater filtered through sand filter. During the rearing period, water depth was maintained at 0.2–0.3 m, with water temperature at 28–32°C, dissolved oxygen at 4.0–6.0 mg/L, pH at 7.5–8.5 and light intensity at 0–20 lx on the water surface. Approximately 20%–30% of the water volume was renewed daily. The bottom of the tank was cleaned by siphoning and the surface water was cleaned using a small net each morning.

The soft-shelled turtles were fed with a formulated diets manufactured by Hangzhou Wensli Biology Science and Technology Stock Co. Ltd. (Hangzhou, China), to apparent satiation four times daily (08:00, 12:00, 17:00 and 21:00) from 2 to 30 DAH. The diets were formulated with white fish meal, extruded full-fat soybean, beer yeast powder, pregelatinized starch, egg yolk powder, butylated hydroxytoluene, vitamin and mineral premix, and contained approximately 50.67% crude protein, 6.25% crude fat, 13.86% ash and 20.86 kJ/g gross energy.

Sampling and dissection

Eight healthy soft-shelled turtle juveniles were collected randomly from each tank before morning feeding on 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 22, 26 and 30 DAH, respectively. Each of the 32 individuals was weighed and dissected as described by Xiao et al. (2006), in order to separate stomachic and intestinal segments. Dissection was conducted under a microscope on a glass plate maintained at 0°C. All samples were collected and immediately stored at −20°C for further assays.

Enzymatic assays

Frozen samples were thawed, homogenized in ice-cold Glycine-HCl buffer (50 mmol/L, pH 2.0) for stomachic segments and Tris-HCl buffer (50 mmol/L, pH 8.0) for intestinal segments, then centrifuged at 8000 × g for 5 min and the supernatant was collected for enzymatic assays. The supernatants of stomachic segments were used for assay of pepsin activity, while those of intestinal segments were used for assay of trypsin, amylase and lipase, respectively.

Pepsin activity was measured using Folin-phenol reagent method (Lowry et al., 1951) as modified by Long et al. (1997), and the reaction was carried out at pH 2.0–2.5 and 37°C. Trypsin activity was assayed according to a modified Schwerdt method (Stellmach, 1992), and the reaction rate was measured by the increase absorbance of 0.003 per min at 253 nm, pH 7.5–8.0 and 37°C. Activities of amylase and lipase activities were measured using iodine-starch colorimetric method (Bernfeld, 1955) and olive oil emulsion method (Shihabi and Bishop, 1971), respectively.

Enzymatic activities were expressed as specific activity (U/mg protein). Protein was determined according to Bradford (1976) using bovine serum albumin (Sigma A-2153) as a standard.

Calculation and statistical analysis

Specific growth rate (SGR, %/d) = 100 (ln W_f − ln W_i)/D,

where W_f (g) and W_i (g) are the final and initial wet bodyweight of the soft-shelled turtle, respectively; D (d) is the experimental period in days.

Results were given as means±S.D. The software of the SPSS for Windows (version 13.0, USA) was used in data analysis. Duncan's multiple range test was used to detect the significant differences, followed by one-way analysis of variance (ANOVA). Effects with a probability at $P < 0.05$ were considered to be statistically significant.

Results

Growth performance

The curve of bodyweight showed a rapid growth during the period from 12 to 30 DAH (Fig. 1). The mean SGR were approximately 2.71 %/d and ranged from 1.31 to 4.00%/d during the first 30 DAH (Fig. 1). The SGR of the juveniles first increased from 2 to 4 DAH, then decreased from 5 to 10 DAH, next increased again after 12 DAH, and finally decreased slightly.

Mouth opening and yolk-sac

The mouth opening time of soft-shelled turtle occurred on 2 DAH after hatching at 28–32°C (water temperature). The yolk-sac of juveniles was small in body on 1 DAH, and then absorbed gradually, ultimately disappeared around 5 DAH.

Specific activities of digestive enzymes

The specific activities of pepsin in stomachic segments, trypsin, amylase and lipase in intestinal segments all changed significantly ($P < 0.05$) during the first 30 DAH (Figs. 2–5, respectively). The activities of each enzyme first decreased

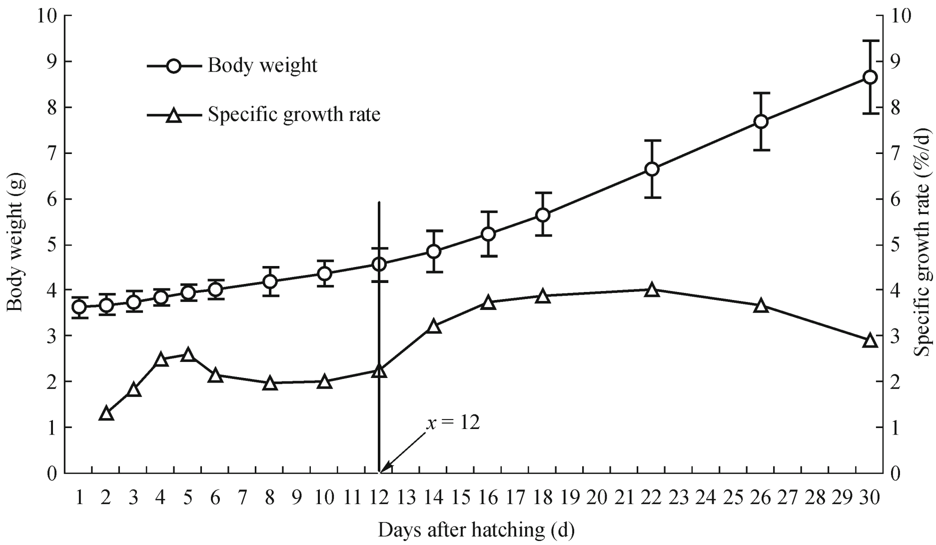

Figure 1 Bodyweight and specific growth rate of *P. sinensis* juvenile during the first 30 DAH. Means±S.D. (n = 32), specific growth rate (SGR) = 100 (ln final wet bodyweight – ln initial wet bodyweigh) / experimental days.

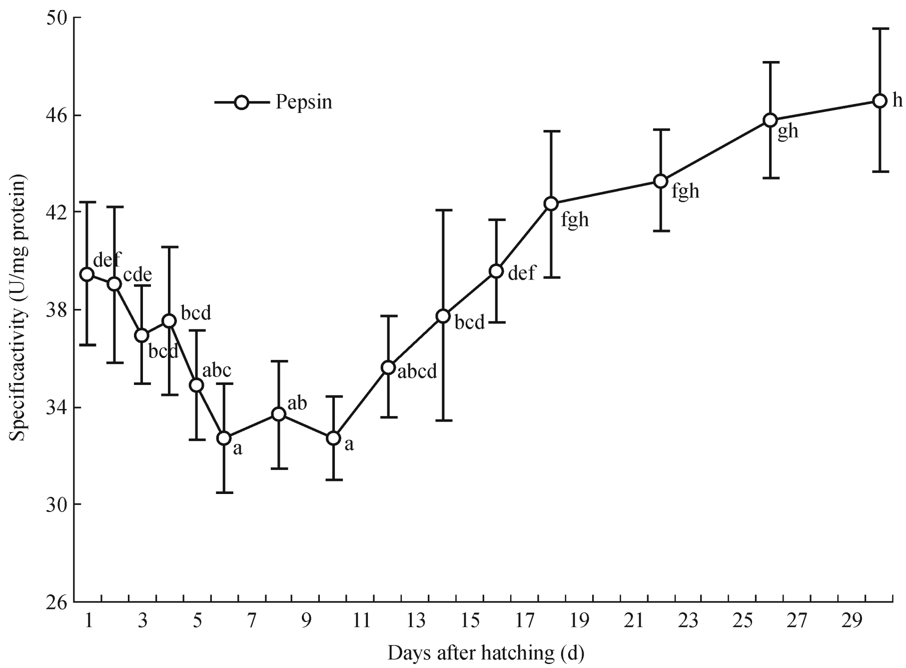

Figure 2 Specific activities of pepsin in stomachic segments of *P. sinensis* juvenile during the first 30 DAH. Means±S.D. (n = 4) with the same superscript letter are not significantly different determined by Duncan's test ($P > 0.05$).

and then increased gradually to the highest level. The specific activities of these enzymes were first detected on the 1st DAH, and the lowest values were observed from 4 to 10 DAH, while the highest values were found from 22 to 30 DAH.

The specific activity of pepsin in stomachic segments gradually decreased from 1 to 5 DAH, and remained the lowest from 6 to 10 DAH. Then, it abruptly increased from 10 to 26 DAH and reached the peaks (46.60±2.08 U/mg protein) at 30 DAH. The specific activity of trypsin in intestinal segments followed a pattern: slightly decreased from 1 to 4 DAH, and then sharply increased from 5 to 22 DAH, finally reached a plateau until 30 DAH. The highest specific activity of trypsin determined was 368.79±34.48 U/mg protein on 30 DAH, while the lowest was 186.68±10.02 U/mg protein on 4 DAH. The specific activity of amylase in intestinal segments slowly decreased from 1 to 5 DAH, and then remained the lowest value (0.65±0.05 U/mg protein) from 6 to 10 DAH.

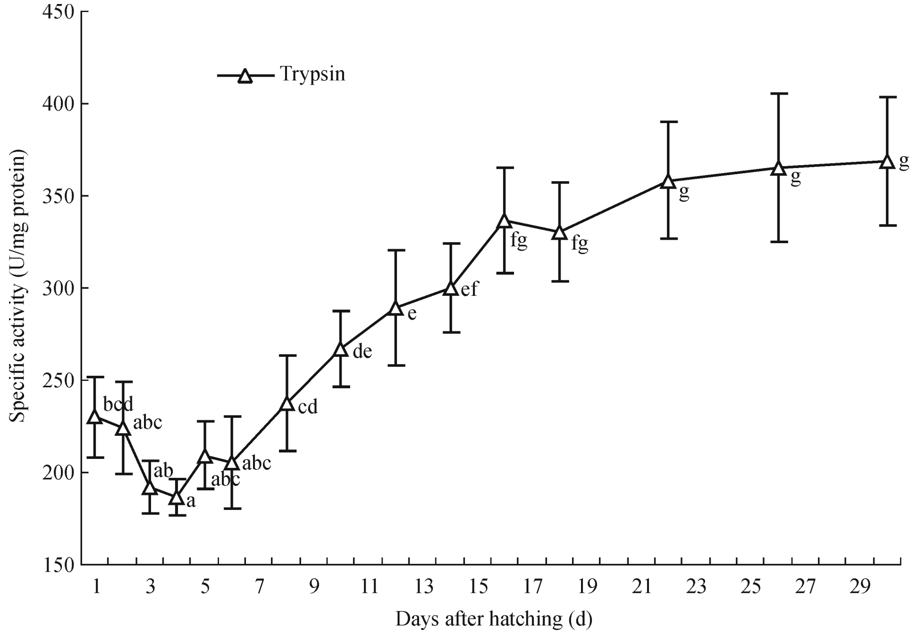

Figure 3 Specific activities of trypsin in intestinal segments of *P. sinensis* juvenile during the first 30 DAH. Means±S.D. ($n = 4$) with the same superscript letter are not significantly different determined by Duncan's test ($P > 0.05$).

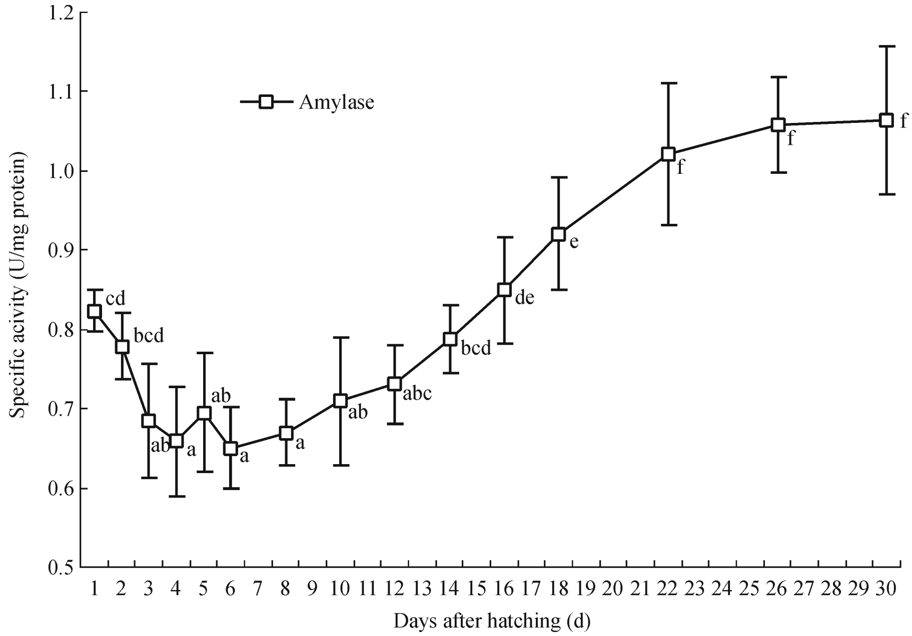

Figure 4 Specific activities of amylase in intestinal segments of *P. sinensis* juvenile during the first 30 DAH. Means±S.D. ($n = 4$) with the same superscript letter are not significantly different determined by Duncan's test ($P > 0.05$).

Then, it abruptly increased from 12 to 26 DAH, and maintained the highest value (1.06±0.09 U/mg protein) until 30 DAH. The specific activity of lipase in intestinal segments was first detected on 1 DAH and slightly increased to 2 DAH. Then, it slowly declined to the lowest value (7.19±1.19 U/mg protein) until 5 DAH and progressively increased until 22 DAH. Afterwards, it remained more or less constant (14.16±2.03 U/mg protein) until the end of the experiment.

Discussion

Activities of digestive enzymes in development

The mouth opening period represented a transitional period when the juveniles turned endogenous nutrients into exogenous food. In this study, the specific activities of the prime digestive enzymes in soft-shelled turtle juveniles first decreased then increased significantly during mouth opening

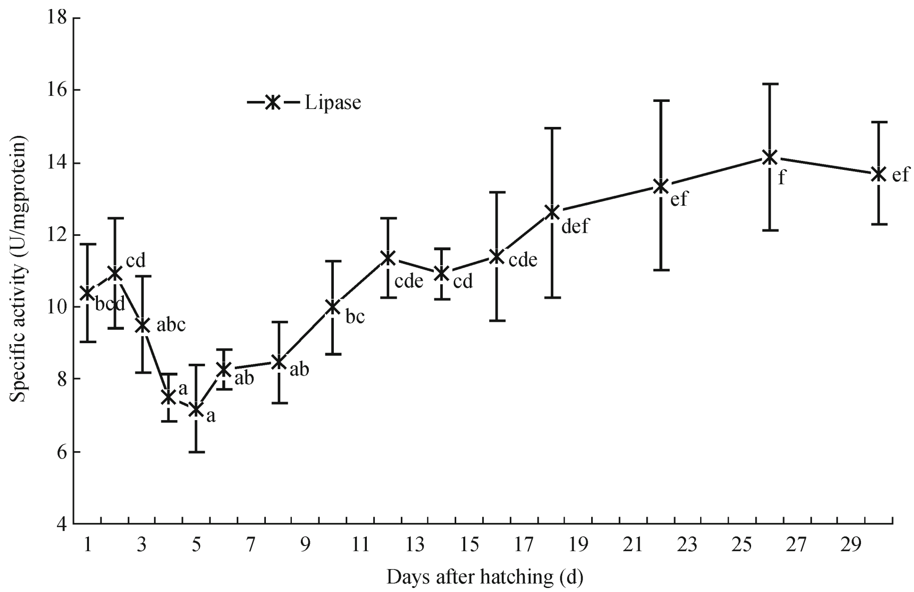

Figure 5 Specific activities of lipase in intestinal segments of *P. sinensis* juvenile during the first 30 DAH. Means±S.D. ($n = 4$) with the same superscript letter are not significantly different determined by Duncan's test ($P > 0.05$).

period from 2 to 12 DAH. This result was in agreement with the observation of Suzer et al. (2007), who reported that the development of trypsin, amylase and lipase activities in red porgy showed a similar pattern. Chen et al. (2006) also reported that activity of lipase in yellowtail kingfish larvae first decreased and then increased during this period. Similar changes in amylase activities were also found in larvae of anuran tadpole (Altig et al., 1975), large yellow croaker (Ma et al., 2005) and shorthead catfish (Li et al., 2008). The decrease in specific activities was due to the normal increase of tissue proteins in growing larvae (Zambonino Infante and Cahu, 2001; Li et al., 2008). This obvious increase in specific activities was possibly due to the changes of turning endogenous nutrients into exogenous food, showing a strengthening of enzyme synthesis and secretion during this period (Zambonino Infante and Cahu, 2001). The specific activities of digestive enzymes may provide information about the digestive capacity and the efficiency of species reared to use feeding components (Buddington et al., 1997). The digestive capacity in aquatic animal larvae and juveniles depended on the ontogenetic development of digestive system (Kuz'mina, 1996). In the present study, all measured digestive enzymes activities were at their lowest when the yolk was fully depleted, and it was necessary to incorporate highly digestible ingredients into feeds at this life stage. Therefore, under cultured condition, the soft-shelled turtles after 5 DAH should be fed with highly digestible diets due to the low value of digestive enzymes activities.

The specific activities of pepsin in stomachic segments, and trypsin, amylase and lipase in intestinal segments were first detected in soft-shelled turtle juveniles on 1 DAH, which was in agreement with the results of previous studies on the other aquatic species larvae including sea bass (Zambonino Infante and Cahu, 1994), Atlantic halibut (Gawlicka et al., 2000), Nile tilapia (Tengjaroenkul et al., 2001) and blue-spotted mudhopper (Wu et al., 2006). These high values in activities of digestive enzymes before first feeding were not induced by diets, but could be better explained as a result of programmed gene expression (Péres et al., 1998; Zambonino Infante and Cahu, 2001).

Though the general patterns of trypsin and amylase activity in soft-shelled turtle juveniles observed in this study was similar to that already described in the other aquatic species, there were some differences between juvenile soft-shelled turtle and fish in ontogenetic development of digestive enzymes. Activity of pepsin was first detected in soft-shelled turtle juveniles on 1 DAH, whereas those were rarely detected in tadpole and fish larvae on 1 DAH. (Altig et al., 1975; Zambonino Infante and Cahu, 2001). On the other hand, the specific activity of trypsin in soft-shelled turtle juveniles was first detected with a high value on 1 DAH and then significantly decreased, whereas those in anuran tadpole were first detected after 25 DAH (Altig et al., 1975). Most of fish larvae were first detected until 2 DAH or later and an initial sudden increase occurred from 3 to 30 DAH (Walford and Lam, 1993; Lazo et al., 2000; Zambonino Infante and Cahu, 2001; Suzer et al., 2007). Soft-shelled turtle is a kind of reptile without the larval period, and the ontogenetic development in soft-shelled turtles should be complete after hatching compared with fish, resulting in the differences in ontogenetic development of digestive enzymes. It is well known that pepsin activity usually becomes apparent concurrent with the formation of functional stomach, and the trypsin activity occurs in response to the secretion of pancreas. The development of stomach in soft-shelled turtle was nearly complete around 1 DAH (Su and Chen, 2004;

Xiao et al., 2006), whereas that of anuran tadpoles was nearly complete around 28 DAH (Altig et al., 1975), and that of most of fish was around 15 DAH (Walford and Lam, 1993). Moreover, gastric glands in soft-shelled turtle were observed on 1 DAH (Su and Chen, 2004; Xiao et al., 2006), whereas in frog and most of fish species with stomach, they were not observed on 1 DAH (Walford and Lam, 1993; Segner et al., 1994; Hourdry et al., 1996). For instance, the development of stomach in sea bass larvae was nearly complete around on 15 DAH, the appearance of gastric glands was around 25 DAH indicating the timing of stomach development, and pepsin activity could be first detected at this developmental stage (Walford and Lam, 1993).

Relationship between growth performance and specific activities of digestive enzymes

The growth of aquatic animal was related with changes of the digestive enzymes activities during juvenile development (Lee and Lawrence, 1985). In the present study, the similar trend was observed between specific growth rate and specific activities of digestive enzymes during juvenile development. There was a correlative relationship between growth and activities of digestive enzymes in soft-shelled turtle juveniles, which was similar to some previous findings on gilthead sea bream (Kolkovski et al., 1993), Indian white shrimp (Ziaei-Nejad et al., 2006) and shorthead catfish (Li et al., 2008). Nevertheless, several previous studies suggested that there was no obvious relationship between enzyme activity and growth in shrimp larvae (Chen and Lin, 1992; Brito et al., 2001). The difference on this relationship was probably due to different species, ontogenic development of digestive organ and nutrition condition.

The higher specific activities of digestive enzymes could, to some extent, explain the better growth for turtle. The increasing activities of digestive enzymes potentially help aquatic animal better digest and absorb diets, thereby indirectly promote the growth. In addition, the different species, life stages, health, diet composition, feeding habits and water temperature can also influence the growth performance in aquatic animals (Altig et al., 2007). Therefore, to some extent, the growth of soft-shelled turtle juveniles was decided by the activities of digestive enzymes.

In conclusion, the results from this study represent the first description of the ontogenic development of the main digestive enzymes in the soft-shelled turtle juveniles. In addition, these data indicated that the activities of digestive enzymes developed during soft-shelled turtle juvenile development and were well correlated with growth.

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