Characteristics of some digestive enzymes in sobaity, *Sparidentex hasta*

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Abstract:

BACKGROUND: Determination of digestive enzymes activity would provide critical information in the design of appropriate diet. Sobaity, *Sparidentex hasta* is found in the Persian Gulf and cultured in countries adjacent to it. **OBJECTIVES:** This study investigated proteolytic, amylase and lipase activities in the intestine of *Sparidentex hasta* captured from the Persian Gulf. **METHODS:** 60 immature *S. hasta* (mean weight: 520 ± 50g) were captured during summer and autumn of 2012 from Mussa Creek, North West of the Persian Gulf. After euthanization, fsh were dissected and the complete digestive tracts (from stomach to anus) were removed. The intestines were separated for each fsh and divided to 3 parts; proximal, mid portion and distal. After homogenization, supernatants were removed and enzymes activities were assayed chemically. **RESULTS:** The highest activity of protease (395.2 ± 32.6 mU mg-1 protein) was recorded in the proximal portion of the intestine compare to the 2 other parts ($p<0.05$) but amylase and lipase activities did not show a signifcant difference in 3 parts of the intestine ($p>0.05$). **CONCLUSIONS:** This study showed that the pattern of activity of digestive enzymes in *S. hasta* is consistent with the overall pattern of digestive enzymes activity in carnivorous fsh. These data can be used to design special diets for this species.

Introduction

The ability of fsh to metabolize a diet depends on the proper functioning of its digestive enzymes that mediate digestive pathways (Phillips, 1969). Measurement of proteases, carbohydrases and lipases activities could help to determine the digestive capacity and effciency of fsh in the digestion of feeding components (Buddington et al., 1997).

Digestion is a progressive process by which smaller size molecules such as amino acids, simple sugars and fatty acids are produced by the hydrolysis of much more complex compounds such as proteins, sugars or lipids, respectively (Caruso et al., 2009). In fsh, like other vertebrates, digestive enzymes play a crucial role in the hydrolysis of protein, carbohydrate and lipid to form small absorbable units. Nutrients resulting from digestion are absorbed in the intestine and transported to tissues by the circulatory system where they are converted into energy to supply biological demands (Furne et al., 2005). The characterization and quantifcation of digestive enzymes may lead to better understanding of digestive physiology, improve feeding regimes and developing proper diets in aquaculture. Therefore, gathering information about the main pancreatic digestive enzymes like protease,

amylase and lipase from each species is of importance as previously mentioned. There is a vast literature regarding the development and digestive capacities of different fsh species as regards their feeding habits (Ribeiro et al., 1999; Cuvier-Peres and Kestemont, 2002; Perez-Casanova et al., 2004). However, there is not enough information about the activity of these enzymes in *Sparidentex hasta* which is found in the Persian Gulf. This species has been introduced and cultured in Iran and other countries adjacent to the Persian Gulf. Considering that feeding costs represent up to 70% of the total production expenses in aquaculture, reducing feeding costs is of extreme importance and for this purpose obtaining practical knowledge of the digestive capacities of fsh is equally important (Thompson et al., 2005; Chaudhuri et al., 2012). Therefore the aim of this study is to improve current knowledge on the amount and distribution of digestive enzymes in the intestine of sobaity, *Sparidentex hasta*.

Materials and Methods

A total of 60 immature (30 in each season) *S. hasta* (mean weight: 520 ± 50g) were captured from Mussa Creek, Persian Gulf during the summer and autumn of 2012. Fish were euthanized with high doses of clove oil, dissected and the complete digestive tract (from esophagus to anus) was removed.

For enzyme analysis, the intestine was separated for each fsh and divided into 3 parts; proximal, mid portion and distal. Each part was washed with cold deionized water to remove as much mucus as possible and were then homogenized in cold sodium phosphate buffer (0.1 M, at pH 7.0, and 4 ºC) by a ratio of 1:9 (m/v) (Liu et al., 2008). The homogenate was centrifuged at 4 ºC at 10000 g for 30 min. The supernatant was then stored at -80 ºC (Operon, Korea) prior to analyses. The soluble protein content in the enzyme extract was measured by Lowry method (Lowry et al., 1951).

α-Amylase was determined by starch-hydrolysis method according to Robyt and Whelan (1968). The enzymatic reaction mixture consisted of 2% (w/v) starch solution (0.125 ml), 0.1 M citrate–phosphate buffer at pH 7.5 (0.125 ml) and a digestive extract (0.05 ml). The reaction mixture was incubated for 1 h at 37ºC. Absorbance was determined at 600 nm. Maltose was used as a standard and the activity unit of a-amylase was defned as the quantity of enzyme that produced 1mmol of maltose ml-1 min-1.

Lipase activity was determined by the evaluation of the degradation of triacylglycerols, diacylglycerols, and monoacylglycerols to free fatty acids following the method of Metin and Akpınar (2000). For the emulsion, a 1% solution of polyvinyl alcohol (PVA) in distilled water was used. Then 5 ml of 0.1 N HCl were added, heating to 75–85 ºC for 1 h, followed by cooling, fltering, and adjusting pH to 8.0 with 0.1 N NaOH. To an aliquot of the above solution, virgin olive oil was added to a substrate concentration of 0.1 M. The mixture was emulsifed for 5 min. The reaction mixture composed of a PVA solution-emulsifed substrate (1 ml), McIlvaine buffer at pH 8 (0.5 ml), and digestive extract (0.5 ml). The McIlvaine's buffer was prepared from 0.1 M citric acid and 0.2 M bisodium phosphate. The reaction mixture was incubated for 4 h at 37°Cafter which 3 ml of a 1:1 ethanol–acetone solution was added to stop the reaction and break the emulsion. A few drops of 1% phenolphthalein in ethanol were added to the reaction mixture and titrated with 0.01 M NaOH. For the blank tubes, the same procedure was followed but with boiled enzyme. One unit of lipase activity was defned as the hydrolysis of 1.0 microequivalent of fatty acids from triacylglycerols in 1 h at pH 7.7 and 37°C..

Total proteolytic activity was measured using the casein hydrolysis method by Walter (1984). The assay was conducted using a wide

range of pH values. The buffers used were 0.1 M KCl–HCl (pH 1.5), 0.2 M glycine–HCl (pH 3.0), 0.1 M citrate–0.2 M phosphate (pHs 4.0 and 7.0), 0.1 M Tris–HCl (pHs 8.5 and 9.0) and 0.1 M glycine–NaOH (pH 10.0), at 25°C. Enzyme reaction mixtures consisted of 1% (w/v). Casein in water (0.25 ml), buffer (0.25 ml) and enzyme sample (0.1 ml)were incubated for 1 h at 37ºC. The reaction was stopped by adding 0.6 ml of 8% w/v trichloroacetic acid. After holding for 1 h at 28ºC, samples were centrifuged at 1800g for 10 min and the absorbance of the supernatant recorded at 280 nm. Tyrosine was used as standard and one unit of enzyme activity was defined as the amount of enzyme needed to catalyze the formation of 1 mg of tyrosine per 1 min.

All the samples were assayed in triplicate. Data were analyzed by one-way ANOVA followed by Duncan's multiple comparisons test and presented as mean±SD. Multiple comparisons tests were only applied when a significant difference, $p<0.05$, was determined in the ANOVA analysis.. The SPSS 13.0 (Chicago, USA) software was used for data analyses.

Results

As seen in Figure 1, α-amylase activity did not show a significant difference along the different regions of the intestine assayed ($p>0.05$). It was 3.42±.54, 3.68±0.65 and 3.90±0.45 in proximal, middle and distal of intestine, respectively. lipase activity in. Figure 2 shows lipase activity in the proximal, middle and distal parts of *S. hasta* intestine to be 3.9±1.2, 3.78±0.82 and 2.75±0.61, respectively and these values did not show any significant ($p>0.05$). Total protease activity was 395.2 ± 32.6, 112.8 ± 23.6 and 125.6 ± 15.2 mU mg-1 protein in proximal, middle and distal of intestine, respectively (Fig. 3). It was highest in the proximal portion as compared to others ($p<0.05$).

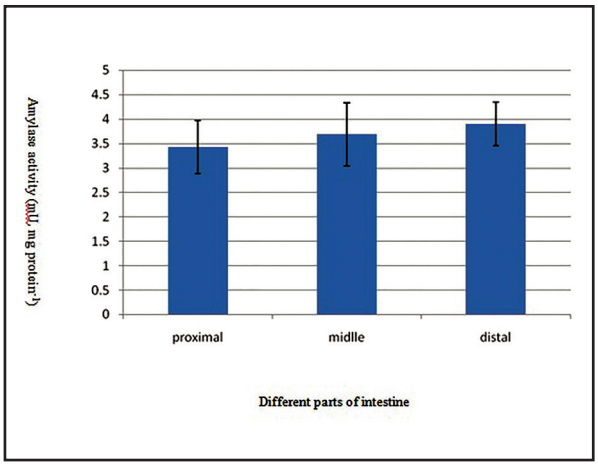

Figure 1. Specific activity of amylase in different parts of intestine in *S.hasta*. Data are showed as mean ± SD.

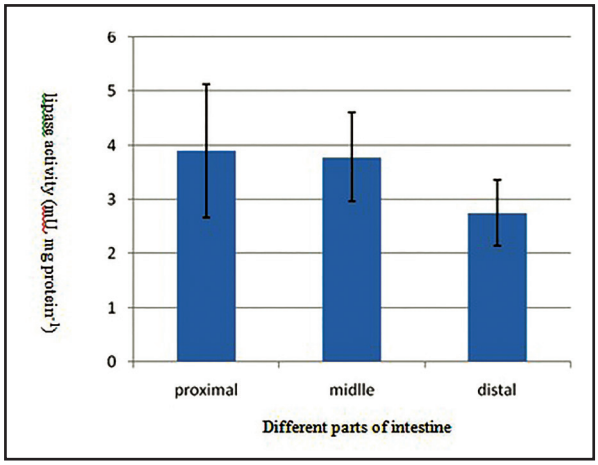

Figure 2. Specific activity of lipase in different parts of intestine in *S.hasta*. Data are showed as mean ± SD.

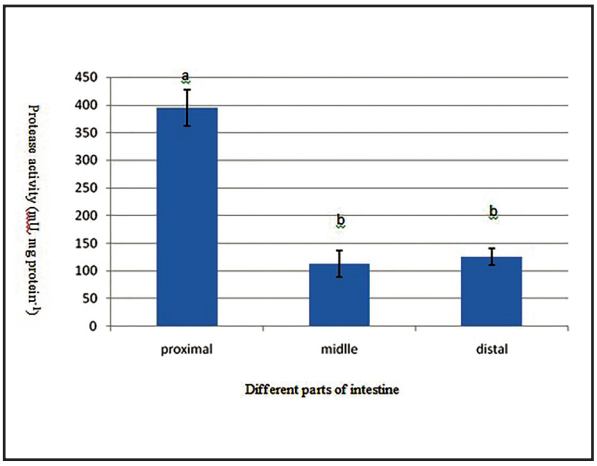

Figure 3. Specific activity of protease in different parts of intestine in *S.hasta*. Different letters showed statistically significant differences ($p<0.05$).

Discussion

In aquaculture, determination of digestive

physiology and responses to dietary composition could help in diet development and eventually in choosing the most appropriate ingredients for each species (Pérez-Jiménez et al., 2009). This study showed the activities of protease, amylase and lipase in different regions of the intestine in *S. hasta* captured from the Persian Gulf. Fishes like other vertebrates are grouped as carnivores, omnivores and herbivores on the basis of their food habit (Chakrabarti et al., 1995). *S. hasta* is characterized as a carnivorous fsh (Fishbase, 2008). Previous researches have demonstrated that no classifcation for protease was possible based on feeding behavior. (Furne et al. (2005) and Hidalgo et al. (1999) reported that rainbow trout and common carp had high protease activity levels whereas carnivorous fsh such as European eel and gilthead seabream had lower activities. Amylase activity in general, has been considered to be more dependent on nutritional habits rather than proteolytic activity (Ji et al., 2012). It is believed that amylase activity is higher in herbivorous and omnivorous fsh than in carnivorous fsh. Hidalgo et al. (1999) reported that irrespective of the food habit of the fsh, adaptations of the digestive system of different species exhibit close correlation with their diet than their microenvironment and taxonomic category. The activities of digestive enzymes show a relative functional specialization in the intestines of terrestrial vertebrates. Enzyme distribution and transport activities along the small intestine is related to the adaptation of its parts to various functional loads (e.g., changes in diet composition, velocity of chyme movement) (Ugolev and Iezuitova, 1985). In fshes, the activities of major digestive enzymes are present along the whole length of the digestive tract (Sklan et al., 2004). In this study only protease showed higher activity in the proximal portion compared to other parts of the intestine but in burbot (*Lota lota*), protease activity was similar in all parts of the intestine (Izvekova et al., 2013).

Our results showed that the digestive enzyme profle of *S. hasta* is well-suited to protein digestion. It is similar to other carnivorous species such as *Sparus aurata* (Deguara et al., 2003), *Pseudoplatystoma corruscans* (Lundstedt et al., 2004) and *Scleropages formosus* (Natalia et al., 2004) with a predominance of protease activity throughout the digestive tract. However, like omnivorous species such as Black Pacu, *Colossoma macropomum* (De Almeida et al., 2006; Corrêa et al., 2007) and Sharp snout seabream, *Diplodus puntazzo* (Tramati et al., 2005), *S. hasta* showed potential for carbohydrate digestion as demonstrated by the little amylase activity observed throughout its intestine. There is controversy on amylase activity in carnivorous fsh; so its role in these species is controversial (Chakrabarti et al., 1995; Hidalgo et al., 1999; Deguara et al., 2003; Natalia et al., 2004; Lundstedt et al., 2004; Fountoulaki et al., 2005). In some species such as *Notopterus notopterus* (Chakrabarti et al., 1995) no amylase activity have been reported in the digestive tract whereas in dentex (*Dentex dentex*) amylase activity was present throughout its digestive tract (Pérez-Jiménez et al., 2009). In the wild, carnivorous fsh can ingest high levels of lipids and consequently, lipase activity is present in their digestive tract (Chakrabarti et al., 1995). In *S. hasta*, enzyme activitywas highest in the proximal portion of the intestine. However, species with different feeding habits seem to present different lipase activity patterns. Therefore, herbivorous fsh such as *Oreochromis niloticus* showed limited lipase activity throughout its digestive tract (Tengjaroenkul et al., 2000) whileomnivorous fsh such as *Colossoma macropomum* (De Almeida et al., 2006) presented higher activity in the stomach than in the other parts of the digestive system (Pérez-Jiménez et al., 2009).

Our fndings showed that the assayed enzymes showed the same pattern as in other carnivorous species, however, decreased amylase

activity found in this study suggested that *S. hasta* could have the potential of using carbohydrates in its diet.

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