Ciência Rural, Santa Maria, v.47: 03, e20160113, 2017 http://dx.doi.org/10.1590/0103-8478cr20160113
ISSNe 1678-4596
BIOLOGY

Different feeding habits influence the activity of digestive enzymes in freshwater fish

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ABSTRACT: *The aim of this study was to verify the activity of some digestive enzymes in four fish species with different feeding habits. Knowledge of these enzymatic activities can help us to understand the species' digestive processes. The species chosen for this study were* ***Ctenopharyngodon idella*** *(herbivore),* ***Rhamdia quelen*** *(omnivore),* ***Leporinus obtusidens*** *(omnivore) and* ***Hoplias malabaricus*** *(carnivore). The digestive tract of these species was divided into four portions to estimate enzymatic activity: stomach, anterior, mid and posterior intestine.* ***Ctenopharyngodon idella*** *had the highest amylase and maltase activities in all portions of the gut, followed by* ***L. obtusidens****. The highest trypsin activity was observed in all gut portions of H. malabaricus, followed by the mid intestine of* ***L. obtusidens*** *and the anterior intestine of* ***C. idella****. The highest chymotrypsin activity was found in all portions of* ***C. idella*** *followed by the anterior intestines of* ***R. quelen****,* ***L. obtusidens*** *and* ***H. malabaricus****. In the stomach, acid protease activity was significantly lower in* ***R. quelen*** *and* ***L. obtusidens*** *compared to* ***H. malabaricus****.* ***Ctenopharyngodon idella*** *showed high activity of enzymes that hydrolyze carbohydrates, represented in this study by amylase and maltase and* ***H. malabaricus*** *showed higher protease activity and low amylase activity.*
Key words: *freshwater fishes, feeding habits, enzymatic activity, amylase, alkaline protease, maltase, trypsin.*

Diferentes hábitos alimentares influenciam a atividade das enzimas digestivas em peixes de água doce

RESUMO: *O objetivo deste estudo foi verificar a atividade de algumas enzimas digestivas em quatro espécies de peixes com hábitos alimentares diferentes. O conhecimento das atividades dessas enzimas pode nos ajudar a compreender os processos digestivos das espécies. As espécies escolhidas para este estudo foram* ***Ctenopharyngodon idella*** *(herbívoro),* ***Rhamdia quelen*** *(onívoro),* ***Leporinus obtusidens*** *(onívoro) e* ***Hoplias malabaricus*** *(carnívoro). O trato digestivo dessas espécies foi dividido em quatro partes para estimar a atividade enzimática: estômago, intestino anterior, médio e posterior.* ***Ctenopharyngodon idella*** *teve a maior atividade da amilase e maltase em todas as porções do intestino, seguido por* ***L. obtusidens****. A maior atividade da tripsina foi observada em todas as porções do intestino de* ***H. malabaricus****, seguido pelo intestino médio de* ***L. obtusidens*** *e o intestino anterior de* ***C. idella****. A maior atividade de quimotripsina foi encontrada em todas as partes do intestino do* ***C. idella****, seguida pelo intestino anterior de* ***R. quelen****,* ***L. obtusidens*** *e* ***H. malabaricus****. No estômago, a atividade de protease ácida foi significativamente menor em* ***R. quelen*** *e* ***L. obtusidens*** *comparado com* ***H. malabaricus****.* ***Ctenopharyngodon idella*** *mostrou alta atividade de enzimas que hidrolisam carboidratos, representadas neste estudo por amilase e maltase e* ***H. malabaricus*** *mostrou maior atividade de proteases e baixa atividade de amilase.*
Palavras-chave: *peixes de água doce, hábitos alimentares, atividade enzimática, amilase, protease alcalina, maltase, tripsina.*

INTRODUCTION

Fish usually exhibit high versatility in their feeding habits that is reflected in different anatomical and functional features. This allows fish to explore a wide range of food resources and maximize the use of available food in the environment (PERETTI & ADRIAN, 2008). The digestive potential of fish is highly variable, changing with species, age, size, food and feeding history, stage of maturity and temperature (GARCÍA-CARREÑO et al., 2002). The analysis of digestive enzymes provides information on fish nutritional physiology and on their ability to take advantage of the different nutritional fractions of the feed (TENGJAROENKUL et al., 2000; ODEDEYI & FAGBENRO, 2010; LAZZARI et al., 2010; 2015).

Ctenopharyngodon idella (grass carp) (Characiformes, ***Cyprinidae***), is an herbivorous fish species that feeds on aquatic plants (BILLARD & BERNI, 2004). The omnivorous ***Leporinus obtusidens*** (piava) (Characiformes, ***Anostomidae***) feed on plants, insects and fishes (REYNALTE-TATAJE & ZANIBONI-

Received 02.04.16 Approved 05.30.16 Returned by the author 12.06.16
CR-2016-0113.R1

FILHO, 2010; LAZZARI et al., 2015). ***Rhamdia quelen*** (silver catfish) (Siluriformes, ***Heptapteridae***) is an omnivorous fish with a tendency towards ichthyophagy, depending on food availability in the environment (BALDISSEROTTO et al., 2013). ***Hoplias malabaricus*** (traira) (Characiformes, ***Erythrinidae***) is a carnivorous fish species that, in the adult stage, ingests intact preys (MENIN & MIMURA, 1991).

Several studies of digestive enzymes in fish species with different feeding habits have been reported (KUZ´MINA & KUZ´MINA, 1990; CHAKRABARTI et al., 2006; LÓPEZ-VÁSQUEZ et al., 2009) and some showed that fish growth might be related to digestive enzyme capacity (CHAKRABARTI et al., 2006; FILIPPOV et al., 2013; LAZZARI et al., 2015). The aim of this study was to investigate protease and carbohydrase activities in four fish species raised in Brazil. The major propose is collected information concerning fish cultivated in Southern Brazil for improved in the future fish diets according to digestive enzymatic profile.

MATERIALS AND METHODS

Ten individuals of each species, ***C. idella*** (20.46±3.9g and 12.95±1.7cm), ***R. quelen*** (66.20±10.5g and 18.75±1.5cm) and ***L. obtusidens*** (69.80±11.7g and 25.41±0.8cm) with approximate age were obtained from the fish culture sector at Universidade Federal de Santa Maria. ***Hoplias malabaricus*** (60.30±10.9g and 16.64±1.6cm) was obtained from regional pond producer. Both species were fasted for 12h before euthanasia by section of spinal cord. Body weight and length were taken subsequently.

The fish were kept in 250L tanks with proper charge density for each species, and water quality parameters were monitored regularly: dissolved oxygen (5.8±0.6mg L^{-1}); temperature (23±0.4ºC, using an oxygen meter Y5512; YSI Inc., Yellow Springs, OH, USA); pH 7.5±0.3 (DMPH-2 pH meter, Digimed, São Paulo, SP, Brazil); total ammonia nitrogen levels (0.14±0.04mg L^{-1}) (EATON et al., 2005); un-ionized ammonia (NH_3) levels 0.007±0.001mg L^{-1} (COLT, 2002); alkalinity (42±2.7mg L^{-1} $CaCO_3$); nitrite (0.0033±0.003mg L^{-1}) (BOYD & TUCKER, 1992) and; water hardness (26.0±1.4mg L^{-1} $CaCO_3$) (EDTA titrimetric method).

The digestive tract was immediately removed after euthanasia and divided into four sections: stomach (except for the grass carp, which does not possess stomach), anterior (or pyloric ceca), mid and posterior intestines. Portions were placed into ice and then stored at -20ºC. Tissues were homogenized in buffer solution containing phosphate (10mM) and Tris (20mM), pH 7.0 using a Potter-Elvehijen homogenizer. The homogenates were centrifuged at 10.000*g* for 10min at 4ºC and the supernatant (crude extract) was used as an enzyme source for all assays.

The effect of different pH on the incubation medium was studied for protease alkaline and amylase activities. Total acid protease activity was measured in the stomach, using a non-specific substrate (casein 1.5%) according to HIDALGO et al. (1999).

Trypsin, chymotrypsin, amylase and maltase were determined in homogenates from the stomach and anterior, mid and posterior intestine. The experimental protocol was modified according to BERNFELD (1955). The starch hydrolyzed by the enzyme and glucose levels were determined according to PARK & JOHNSON (1949). The protein content of crude extracts was determined by the method of LOWRY et al. (1951), using bovine serum albumin as a standard. For more details, see the technical of study LAZZARI et al. (2010).

Differences between species were analyzed by one-way analysis of variance followed by the Duncan test (Statistica 5.0). Data were expressed as mean ± SEM, and differences were considered significant at a probability level of 95% ($P<0.05$).

RESULTS

Trypsin, chymotrypsin and maltase activities were observed in the stomach of ***R. quelen***, ***L. obtusidens***, and ***H. malabaricus***. The greatest trypsin activity was found in ***H. malabaricus*** following for ***L. obtusidens***. The low values for trypsin were recorded to ***R. quelen***. Chymotrypsin activity in the stomach was similar to ***R. quelen*** and ***H. malabaricus***, and ***L. obtusidens*** showed lower activity as compared to other fish species. Maltase activity was highest in ***L. obtusidens*** comparing to ***H. malabaricus*** and ***R. quelen*** that showed similar maltase activity in stomach (Table 1).

Ctenopharyngodon idella and ***L. obtusidens*** showed the highest amylase activity at pH 7.0 and ***H. malabaricus*** at pH 8.5 and pH 8.0 in the anterior and midintestine, respectively (Figure 1A and B). ***Rhamdia quelen*** showed highest activity at pH 7.0 in the middle intestine (Figure 1B). Higher amylase activity in the anterior and mid in ***C. idella*** (Figure 1A and B). In the stomach the highest amylase activity was observed at pH 7.0 for ***L. obtusidens*** and ***R. quelen*** and pH 8.0 for ***H. malabaricus*** (Figure 1C).

Table 1 - Trypsin, chymotrypsin and maltase activities in the stomach of the studied species data on enzyme digestive activities (n=10) are expressed as U mg protein^{-1} where U=1µmol of substrate hydrolyzed min^{-1}. Different superscript letters represent significant difference of digestive enzymes activity comparing different fish species (P<0.05).

Species	Trypsin	Chymotrypsin	Maltase
Rhamdia quelen	0.102 ± 0.027^{c}	114.6 ± 11.7^{a}	1.30 ± 0.36^{a}
Leporinus obtusidens	0.362 ± 0.031^{b}	71.86 ± 16.5^{b}	1.62 ± 0.29^{b}
Hoplias malabaricus	1.150 ± 0.11^{a}	113.5 ± 12.6^{a}	1.14 ± 0.19^{a}

Alkaline protease presented the highest activities at pH 8.0 or 8.5 for all species, but ***R. quelen*** and ***H. malabaricus*** also showed high activities at pH 9.0 and 10.0. Compared to the other species, ***C. idella*** continued to show high activity of alkaline protease in the middle portion of the intestine (Figure 2A and B). The highest acid protease activity in the stomach was at pH 2.5 for ***H. malabaricus*** and ***L. obtusidens*** and pH 2.0 for ***R. quelen*** (Figure 2C). In ***C. idella*** the mid intestine showed the highest activity for amylase and maltase when compared to anterior and posterior

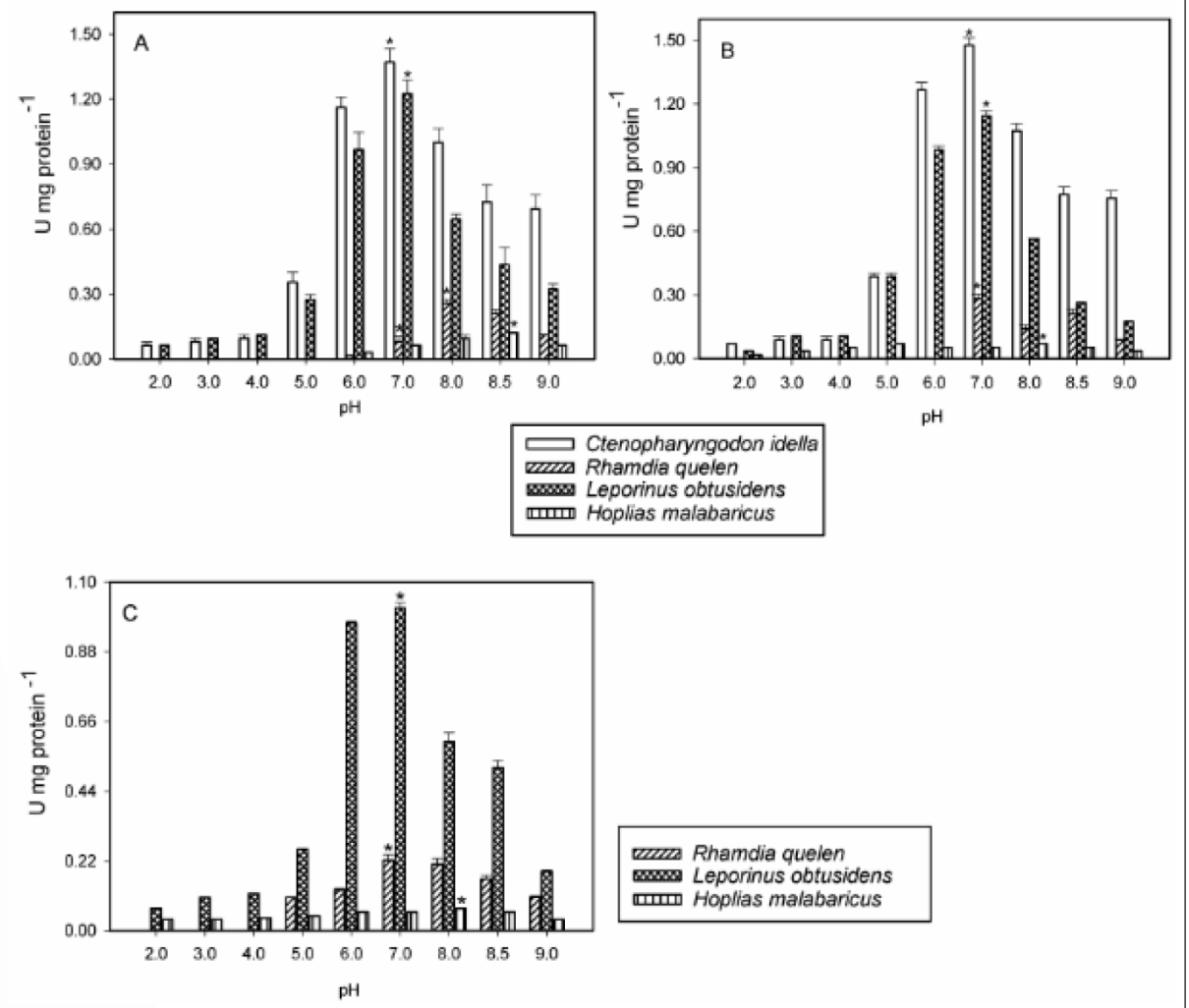

Figure 1 - Amylase activity at different pHs in the anterior (A) and min^{-1} (B) intestine and stomach (C) of the studied species. Activity is expressed as U mg protein^{-1}, where U=1 µmol glucose min mg protein^{-1}. Data represent mean ± SEM (n=10). *Represents the pH at which the enzyme has the highest activity.

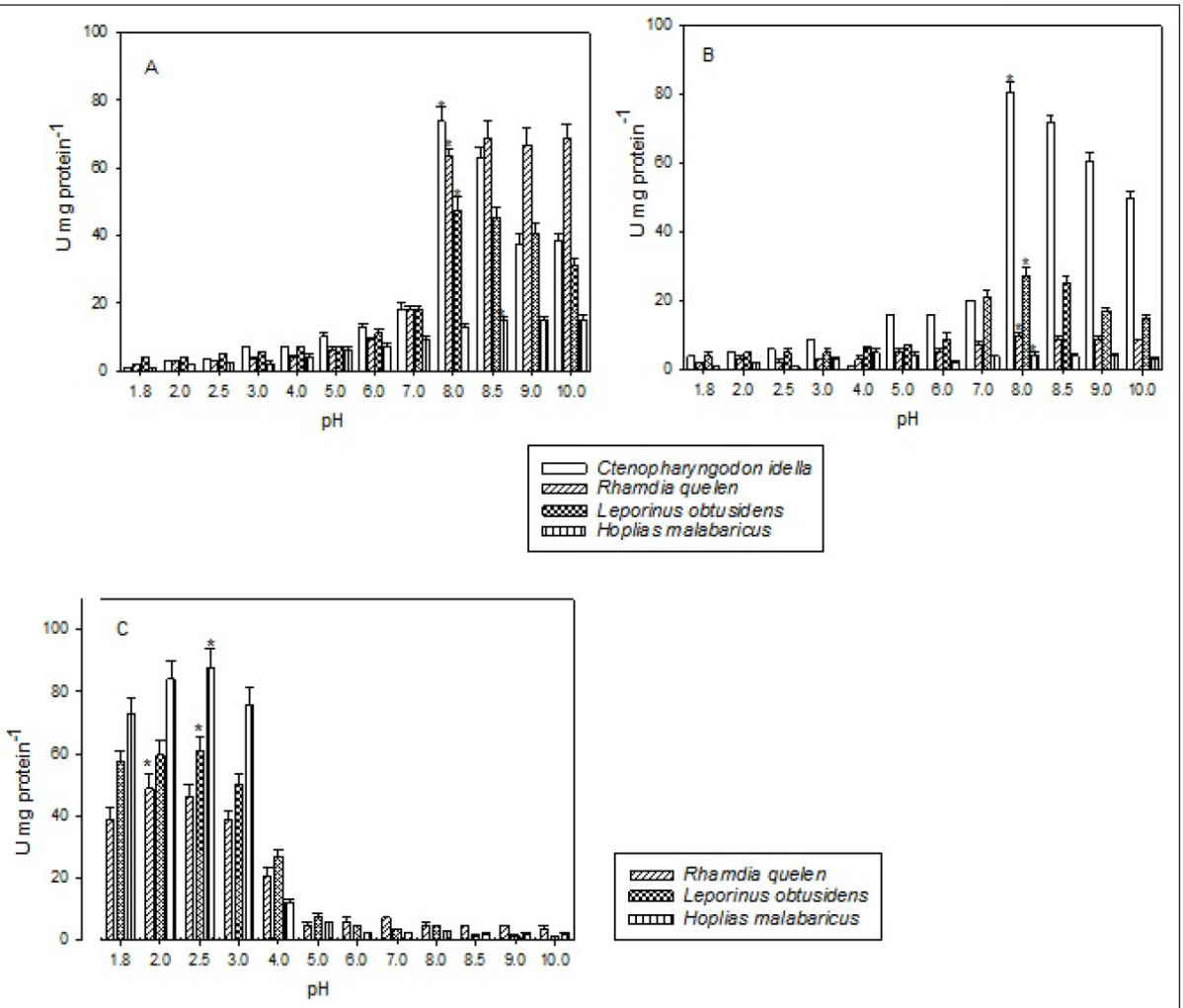

Figure 2 - Alkaline protease activity at different pHs in the anterior (A) and mid (B) intestine and acid protease activity in the stomach (C) of the studied species. Activity is expressed as U mg protein^{-1}, where U=1µg tyrosine min^{-1} mg protein^{-1}. Data represent mean ±SEM (n=10). *Represents the pH at which the enzyme has the highest activity.

segments. In ***R. quelen*** the highest activity of these enzymes was exhibited in the anterior intestine. In ***L. obtusidens*** the highest amylase activity was detected in the anterior intestine and maltase in the mid intestine, but ***H. malabaricus*** showed very low amylase activity in all intestine portions. On the other hand, ***H. malabaricus*** presented maltase activity in middle and posterior intestine portions similar to that obtained for ***R. quelen***, but lower than ***L. obtusidens*** and ***C. idella***. (Figure 3).

The highest trypsin activity was observed in all intestine portions of ***H. malabaricus***. ***Ctenopharyngodon idella*** exhibited the highest trypsin activity in the anterior intestine, ***L. obtusidens*** in the mid intestine and ***R. quelen*** in the posterior intestine (Figure 3C). ***Ctenopharyngodon idella*** showed the highest chymotrypsin activity at all intestine portions. ***Rhamdia quelen*** and ***H. malabaricus*** showed high activity of this enzyme in the anterior portion but for ***L. obtusidens*** the highest activity was observed in the posterior intestine (Figure 3D).

DISCUSSION

The knowledge of the feeding habits of different fish species associated with enzymes activities in the digestive tract is important to provide an appropriate diet for each species because digestive enzymes activity very reflects the changes in dietary (PERETTI & ANDRIAN, 2008; LAZZARI et al., 2010; 2015).

The carnivorous ***H. malabaricus*** showed lower amylase, maltase, alkaline protease and chymotrypsin activity compared to the other fish

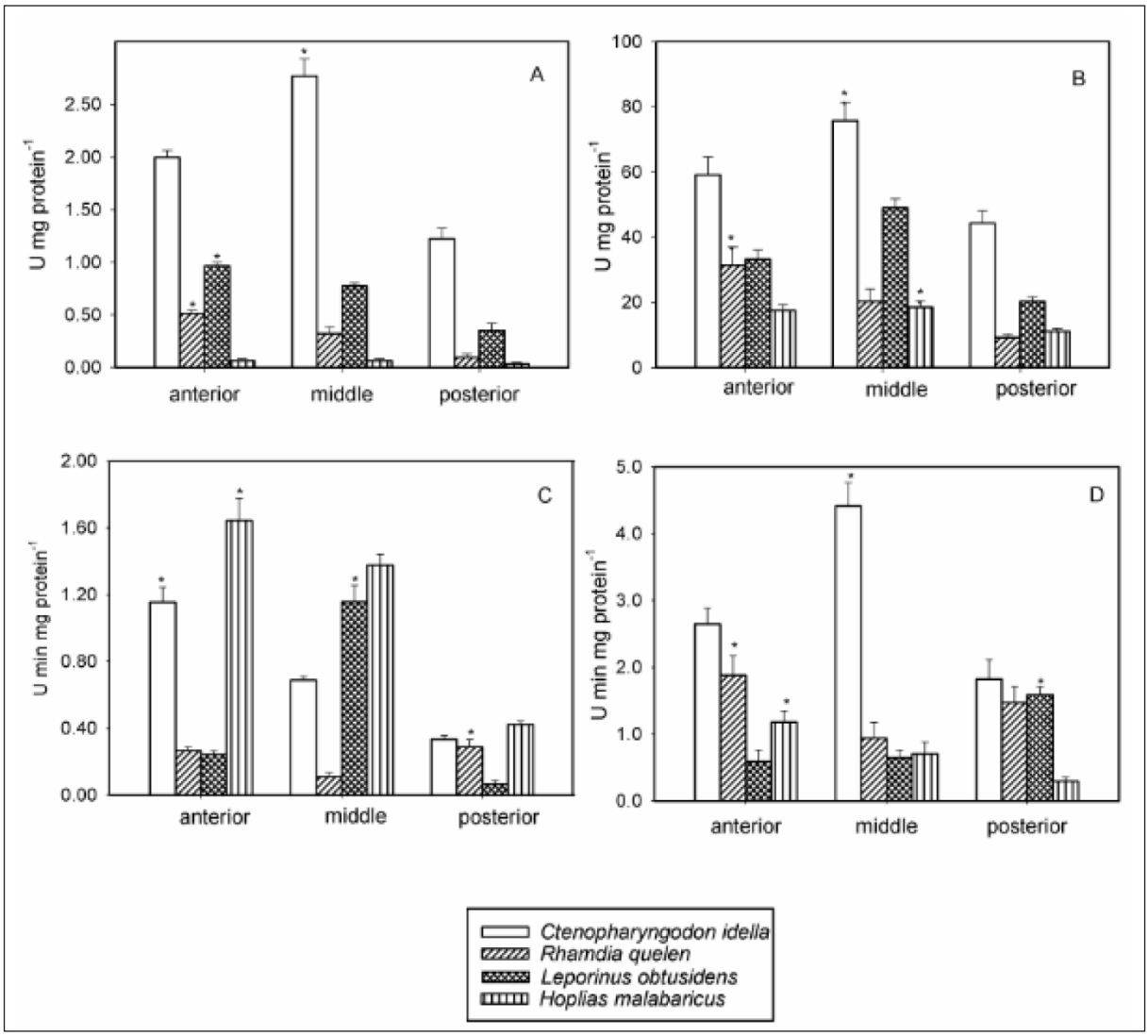

Figure 3 - Amylase (A) and maltase (B) activities in anterior, middle and posterior intestine of the studied species. Activity is expressed as U mg protein^{-1}, where U=1µmol glucose min^{-1} mg protein^{-1}. Trypsin (C) and chymotrypsin (D) activity in anterior, middle and posterior intestine of the studied species. Activity is expressed as U mg protein^{-1}, where U= g of substrate hydrolyzed (TAME or BTEE) min^{-1} mg protein^{-1}. Data represent mean ± SEM (n=10). *Represents the portion of the intestine where the highest activity is observed for each enzyme.

species studied. The main digestive enzymes of ***H. malabaricus*** are represented by acid protease in the stomach and trypsin in the intestine, but this species also showed some chymotrypsin and maltase activity in the stomach and all intestine portions. Other carnivorous species such as ***Oncorhynchus mykiss*** and ***Pseudoplatystoma corruscans*** also showed high protease activity in the stomach (HIDALGO et al., 1999; LUNDSTEDT et al., 2004). In the present study, amylase and maltase activities were found in ***H. malabaricus***, likely because they would be required to digest the glycogen present in animal tissues. Another carnivorous fish, the sea bass (***Lates calcarifer***), also presented amylase activity in the digestive tract (SABAPATHY & TÉO, 1992).

The highest total alkaline protease activity measured in the intestine was found at pH 8.0 and 8.5 for all fish species studied. However, some activity was also detected at pH 9.0 and 10.0 for all species, mainly in the anterior intestine. On the other hand, GARCÍA-CARREÑO et al. (2002) verified that the optimum pH for intestinal enzymes of ***Brycon orbignyanus*** was 10.0. The results observed at high alkaline pHs (9.0 and 10.0) are probably due to other alkaline proteases as carboxipeptidase-like, elastase-like or collagenase-like activities (HIDALGO et al., 1999).

The herbivore ***C. idella*** presented the highest amylase and maltase activities within the fish species studied, in accordance with CHAKRABARTI et al. (2006), who found high amylase activity in herbivorous fish. However, ***C. idella*** also presented alkaline protease, trypsin and chymotrypsin activities in the intestine. In agreement with the present study, KUZ′MINA & KUZ′MINA (1990) and CHAKRABARTI et al. (2006) also found high protease activity in non-carnivorous fish. In addition, the herbivorous ***Oreochromis niloticus*** (Nile tilapia) demonstrated higher carbohydrase activity than protease activity when compared to carnivorous and omnivorous fish (TENGJAROENKUL et al., 2000). Different authors have reported a close relationship between herbivorous feeding habits and higher amylase activity (HIDALGO et al., 1999; ODEDEYI & FAGBENRO, 2010).

In conclusion our study showed higher acid protease and trypsin activities in the carnivorous species studied, while higher amylase and maltase activities were found in the herbivorous species. Omnivorous species presented intermediate activity values. The results are within the expected range for each species and their feeding habits. The application of these feedings in the formulation of specific diets for each species using sources of low cost and good digestibility to fish.

ACKNOWLEDGEMENTS

The authors thank Dr. Everton Behr for his technical help. V.L. Loro and B. Baldisserotto received research fellowships from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq - Brazil).

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