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Enzyme activity in the Nile perch gut: Implications to Nile perch culture

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Nile perch (*Lates niloticus)* is a high-value freshwater fish of great social economic importance in Africa's great lakes region. High demand for this fish has caused its extensive harvest, resulting into a decline in the species stocks and consequently financial loss to the Nile perch industry. Several strategies have been suggested to reinstate the fish population, including the domestication of Nile perch, however, this will greatly depend on the ability of this fish to digest artificial diets, since these diets greatly determine the survival of fish in aquaculture systems. In this study gut enzymes were assayed using standard procedures. Results indicated presence of amylase and trypsin, which is an indicator that carbohydrates and proteins have to be included in the artificial diets that will be formulated for this species. The study further revealed a variation in enzyme activity in the juvenile stages of this fish, indicating that the most critical stage in the nutrition of the Nile perch is the juvenile stage. This variation reveals insights in the requirement for different diets formulations for the different juvenile developmental stages of Nile perch. These findings make Nile perch a potential aquaculture candidate, since carbohydrates and proteins contribute substantively to artificial diets in cultured fish.

Key words: Aquaculture, aquafeeds, enzyme activity, Nile perch.

INTRODUCTION

Artificial feeds are an important aquaculture input, and their efficacy depends on the ability of the cultured fish to digest and hence utilize the feeds for growth and development (Jauncey et al., 2007). It is for this reason that the ability of any aquaculture candidate to digest artificial diets is investigated before it is considered for culture (Kolkovski, 2001). Therefore, information about the characteristics of the digestive enzymes is important because it provides insights into the ability of a fish species to digest dietary components (proteins, carbohydrates, vitamins, minerals and lipids). This research is therefore vital to the artificial diet formulation and manufacturing process for any new aquaculture species (Britz, 1995), since it guides the selection of suitable ingredients to constitute the diets. Several suitable artificial diets have been developed following detailed studies of the functioning of the digestive systems in fish. For example, the formulation of appropriate diets in *Anaba testudineus*, followed an *in vitro* assessment of protein digestibility of different feed ingredients by the crude enzyme extracts obtained from the fish gut (Ali et al., 2009), and in *Labeo rohita*, where diets were formulated following an assessment of digestive enzymes of fingerlings fed on different diets

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(Debnath et al., 2007).

Due to the previously limited interest in Nile perch aquaculture, no published information is available in relation to the physiology of the digestive system of this fish in this respect. The development of artificial diets for carnivorous fish such as Nile perch therefore continues to be a challenge because of the lack of knowledge of the specificity and activity of digestive enzymes (D'Abramo, 2002; Oliveira and Cyrino, 2004). The ability to utilize artificial diets in other carnivorous fish has been widely studied and research reveals that digestion is greatly attributed to the presence of digestive enzymes that can efficiently hydrolyze the different components especially the proteins and carbohydrates which occur in the artificial diets (Caruso et al., 2009). Proteins contribute a large percentage of artificial diets in fish; where it serves as a major source of amino acids, constituting between 40 to 56% of the diets (Cerda et al., 1994; Garcia-Ortega et al., 2001; Razzaque et al., 2008; Mukherjee et al., 2011). Carbohydrates on the other hand, occur in diets as binders, additives and sources of energy and are therefore frequently utilized in artificial feed development (Ovie et al., 2005). Several studies have therefore focused on the proteolytic and amyolytic ability of the fish gut to utilize artificial diets (Walford and Lam, 1993; Garcia-Carreno et al., 2002; Matsumiya et al., 2006). This study used similar approaches toestablish the specific activity of amylases and trypsin in the gastro-intestinal tract of Nile perch, given that the activities of these enzymes in the Nile perch gut will determine its ability to efficiently utilize the formulated artificial diets. Several studies engaged in weaning candidate aquaculture species onto artificial diets begin with the larval stages (Herbert and Graham, 2003; Vega-Orellana et al., 2006; Cassiano et al., 2009). This approach was not possible with Nile perch in this study, because of the inability to obtain *L. niloticus* hatchlings during the period of this study. In the absence of fish weaned on to artificial diets, we commenced the study by testing the ability of wild Nile perch juveniles to digest ingredients that constitute artificial diets. Strategies towards the culture of Nile perch require information regarding its ability to digest artificial diets; this can be obtained by investigating the nature and abundance the gut enzymes.

The purpose of this study was to characterize amylase and trypsin-like activity present in Nile perch gut and to identify their specific location. The results from this study provide information to guide the formulation of artificial diets for the culture of Nile perch.

PROCEDURES

Sample collection

Wild Nile perch were collected live using beach seines from the northern side of Lake Victoria in Uganda near Kiggungu Fish landing site located at 32° 26′ 15″E, 00° 2′ 49″N . The fish were starved for twenty-four hours in a tank containing fresh water and continuously aerated with oxygen. Fish samples were divided into eight size classes namely; 1 - 5, 6 -10, 11- 15, 16 – 20, 21 - 25, 26 - 30, 31 – 35 and 36 - 40 cm of total length. This classification was adapted from Hopson (1972) and Hughes (1990) Nile perch length-growth models.

Preparation of enzyme extracts

Fish samples were individually measured and weighed and dissected on ice (German et al., 2010) to access the gut. The guts were sectioned into the oesophagus, stomach, liver, pyloric ceaca, anterior intestine, middle intestine and hind intestine (Féral, 1989; García-Carreńo et al., 2002). Each of the gut sections from the different samples were separately mixed with distilled water and homogenized. Extracts for enzyme assays were then placed in 2 ml microcentrifuge tubes and centrifuged at 10 rpm for 10 min and stored at - 40°C until needed.

Amylase activity

Amylase activity was measured using modified methods adapted from Tongsiri et al. (2010) and Xiong et al. (2011) by estimating the reducing sugar produced using dinitrosalicylic acid (DNS). The reaction mixture consisted of 400 µl of 4% (v/v) warmed soluble starch as substrate and citric acid-Na_2HPO_4 buffer (at pH 6 for the stomach extracts and pH 7 for the intestine, liver and oesophagus extracts) plus 100 µl of homogenate. All gut extracts were assayed at 20°C for 60 min. The enzyme assays were terminated by adding 150 µl of each reaction mixture to 300 µl of DNS and heating at 100°C for 5 min. The mixture was then cooled on ice for 5 min, 250 µl from each aliquot was transferred to a 96 well plate and absorbance was measured at 540 nm. A standard curve wave was developed with maltose (0.1 to 1.0 mg/ml), where one unit of enzyme was expressed as the mg of maltose produced per minute. Amylase specific activity was presented as amylase unit per mg of total protein.

Trypsin-like activity

Trypsin-like activity was measured using the Protease Fluorescent Detection Kit (Sigma PF0100) (Boitano et al., 2011; Fu et al., 2011). In this assay, 20 µl of Fluorescein Isothiocyanate (FITC) - labeled casein was mixed with 20 µl of incubation buffer (20 mM sodium phosphates with 150 mM sodium chloride, pH 7.6) and 10 µl of homogenate which were incubated at 37°C for 60 min. The blank sample constituted 20 µl of incubation buffer, 20 µl of FITC-Casein substrate and 10 µl of distilled water. To each aliquot of the reaction mixture, 150 µl of 0.6 M trichloroactic acid (TCA) solution was then added and incubated at 37°C in the dark for 30 min. The mixture was centrifuged for 10 min at 10,000 x g; 10 µl of the supernatant was then mixed with 1 ml of assay buffer, the fluorescence intensity with excitation wavelength at 485 nm and emission wavelength of 535 nm was recorded. Trypsin-like activity was equivalent to the amount of the reference protease (trypsin) that produced a significant amount of fluorescence above the value obtained with the blank sample, after 60 min at 37°C. One unit of protease was defined as the amount of trypsin (µg) detected per minute at 37°C, while trypsin specific activity was presented as the enzyme activity per total mg of protein.

Total protein

Protein was assayed using a modified Bradford protein assay (Bradford, 1976), using bovine serum albumin (BSA) as a standard.

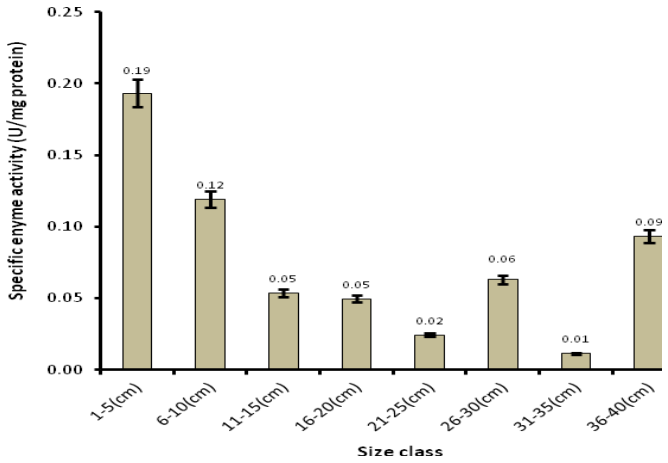

Figure 1. Amylase specific activity of gut extracts from Nile perch samples of different size classes.

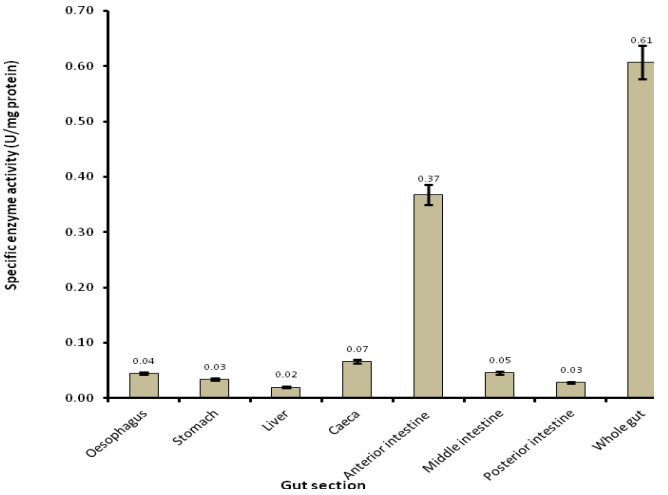

Figure 2. Amylase specific activity in the different gut sections of the Nile perch.

Bradford reagent (240 µl) was mixed to 10 µl of homogenate and incubated at ambient room temperature for 10 min; the absorbance was measured at 595 nm.

Statistical analysis

The comparisons of enzyme specific activity in the different size classes and gut regions were performed using the Kruskal-Wallis non-parametric test (SPSS version 16); and a probability level of $p<0.05$ was considered statistically significant.

RESULTS

Amylase specific activity

The quantitative results for the amylase assay of the different Nile perch gut extracts are presented in Figures 1 and 2. Results indicate a significant difference ($p<0.05$) of enzyme activity amongst the different size classes and gut regions. The specific activity decreased with increasing fish length. Gut extracts from the youngest fish (1 to 5 cm) showed higher amylase specific activity (0.19 U/mg protein) compared to the older fish (6 to 40 cm). Most amylase specific activity was observed in gut extracts from the anterior intestine (0.37 U/mg protein) compared to the other sections of the gut (Figure 2). The lowest activity was observed in the liver (0.02 U/mg protein), stomach (0.03 U/mg protein) and posterior intestine (0.03 U/mg protein) extracts.

Trypsin-like activity

Trypsin-like activity was observed in all gut extracts (Figures 3 and 4); but with a significant difference ($p<0.05$) between the different size classes and amongst the different gut regions. Activity decreased with increasing fish length. Activity of the gut extracts from the youngest fish (1 to 5 cm) was high (0.75 U/mg protein) compared to that in the older fish (6 to 40 cm) (Figure 3). Trypsin-like specific activity was highest in the anterior intestine (0.36 U/mg protein) and lowest in the oesophagus, liver and ceaca compared to the other sections of the gut (Figure 4).

DISCUSSION

Enzyme activity has been reported in the gut of several fish species (Al-Tameemi et al., 2010; Lazzari et al., 2010; Chaudhuri et al., 2012). Studies have revealed that herbivorous and omnivorous fishes tend to have high amylase activity in comparison to the carnivores, since the former need it to breakdown the polysaccharides that dominate their natural diets (Hidalgo et al., 1999). No wonder, the amylase activity levels observed in Nile perch in this study are much lower than those observed in some herbivorous and omnivorous fish (Table 1). However, different amylase specific activity levels have been reported in several carnivorous fish including *Pseudoplatystoma corruscans* and *Glyptosternum maculatum* (Table 1); and these vary from those observed in Nile perch in this study. This variance in results could have been caused by the difference in the analytical procedures used in the different studies or the difference in amylase quantities in the gastrointestinal tracts of different fishes.

The occurrence of amylase in predatory fish cannot be well explained, since very little or no carbohydrates are found in their diet. Some studies (Natalia et al., 2004) have, however, proposed that its role is in the digestion of glycogen (a source of energy in animals). In the case of Nile perch; amylase activity is observed in the oesophagus, liver, stomach, and in all intestinal extracts

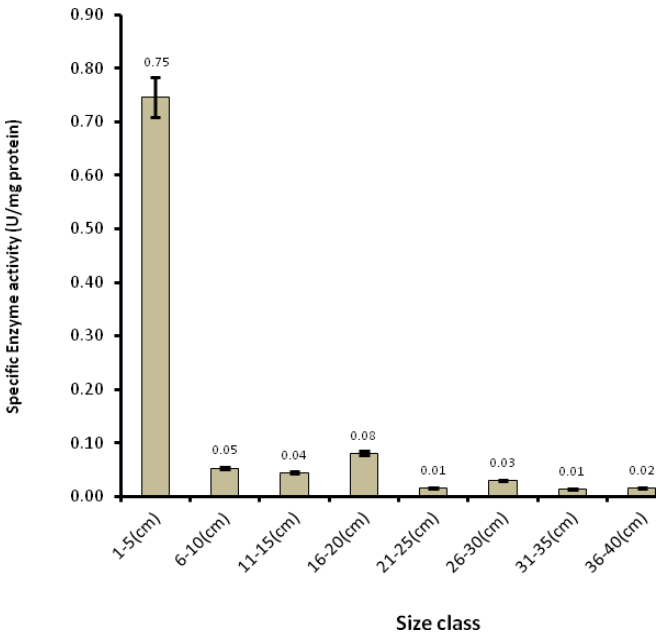

Figure 3. Trypsin-like specific activity of gut extracts of Nile perch samples of different size classes.

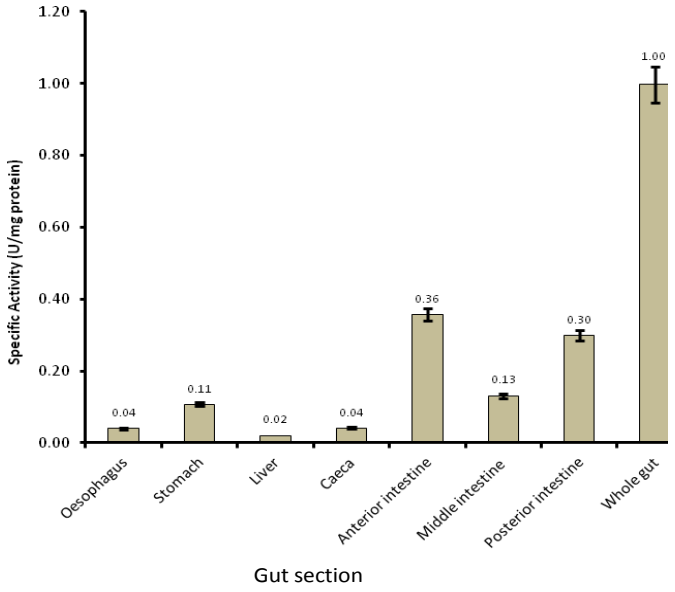

Figure 4. Trypsin-like specific activity in the different gut sections of the Nile perch.

(Figure 2). The amylase activity observed in the oesophagus (Figure 2) of Nile perch suggests that digestion of carbohydrates occurs in this region. The amylase activity observed in this region could be a mainfestation of saliva amylase in the bucal cavity, which is observed in some mammals that masticate their food (Meisler and Ting, 1993). However, the need for amylase in the bucal cavity and oeophagus of this fish might not be necessary given that mastication does not occur in Nile perch due to its non-flexible toungue (Namulawa et al., 2011). Amylase activity could instead be important to ease digestion in the subsequent regions of the gut. Activity of the same enzyme was also observed with the liver extracts from this fish (0.02 ± 0.002 U/mg protein); this amylase activity is lower than that recorded in extracts from other gut sections. The stomach extracts also showed activities levels lower (0.03 ± 0.002 U/mg protein) than those observed in the oesophagus (0.04 ± 0.005 U/mg protein); this observation is in agreement with reports from other carnivorous fish such as *Scleropages formosus* (Natalia et al., 2004), *Pseudoplatystoma corruscans* (Lundstedt et al., 2004), and in *Glyptosternum maculatum* (Xiong et al., 2011). The presence of amylase in the stomach of Nile perch and other carnivores can however not be explain, given that natural diet in Nile perch is rich in protein (Hopson, 1972).

The anterior intestine section extracts showed the highest amylase activity level (0.37 ± 0.005 U/mg protein) compared to that observed in the other gut section extracts. This level of activity in this fish was not expected, given that high amylase activity levels are synonymous with omnivorous and herbivours fishes and not carnivores. However, similar observations have been reported in other carnvorous fish species such as *Uranoscopus scaber* (Papoutsoglou and Lyndon, 2003), and *Glyptosternum maculatum* (Xiong et al., 2011).The presence of amylase in the anterior intestine suggests that starch digestion and glucose absorption mainly occur in this section of the gut and this gives prospects of artificial feed digestion in this fish. Further analysis also showed that amylase activity decreased in the hind intestine section extracts (0.03 U/mg protein). Similar observations have been reported in *Clarias gariepinus* by Uys and Hecht (1987), who described this phenomenon as the descending proximo-distal gradient (Kuz'mina, 1985). This could suggest that the hind intestine has limited participation in the chemical digestion of carbohydrates in Nile perch. Analyses from this study showed that the gut extracts of the youngest fish (1 to 5 cm) displayed the highest amylase activity (0.19 U/mg protein); compared to the gut extracts from the other size classes (Figure 2); as observed in *Trachinotus ovatus* (Youjun et al., 2011). Activity of this enzyme decreased with increasing fish length, suggesting a reduction in carbohydrase hydrolysis with increasing age. This observation is similar to that made in *Pagrus pagrus* (Suzer et al., 2007) and in *Anoplarchus purpurescens* (German et al., 2004) where their diets shift from small invertebrates to a carnivorous diet. The high carbohydrate activity levels observed in these fishes is probably due to the need for such enzymes to digest the tough exoskeleton in the small invertebrates that is constituted of complex carbohydrates (Kleinow, 1993;

Table 1. Comparative amylase activity of gut extracts of fish species of differing feeding behaviour.

Fish species	Gut section	Activity (U/mg protein)	Feeding behaviour	References
Pseudoplatystoma corruscans	Stomach	0.012±0.010 -0.18±0.016	Carnivorous	Lundstedt et al. (2004)
P. corruscans	Anterior Intestine	0.023±0.008 - 0.053±0.010	Carnivorous	Lundstedt et al. (2004)
P. corruscans	Middle intestine	0.013±0.005 - 0.022±0.016	Carnivorous	Lundstedt et al. (2004)
P. corruscans	Posterior intestine	0.003±0.002 - 0.029±0.019	Carnivorous	Lundstedt et al. (2004)
Glyptosternum maculatum	Stomach	0.0032±0.0007	Carnivorous	Xiong et al. (2011)
G. maculatum	Anterior Intestine	0.0062±0.0009	Carnivorous	Xiong et al. (2011)
G. maculatum	Middle intestine	0.0023±0.0005	Carnivorous	Xiong et al. (2011)
G. maculatum	Posterior intestine	0.0062±0.007	Carnivorous	Xiong et al. (2011)
Cyprinous carpio	Whole gut	72.73±8.46	Omnivorous	Hidalgo et al. (1999)
Carassius auratua	Whole gut	75.47±15.76	Omnivorous	Hidalgo et al. (1999)
Tinca tinca	Whole gut	19.37±2.67	Omnivorous	Hidalgo et al. (1999)
Sparus aurata	Whole gut	1.75±0.28	Carnivorous	Hidalgo et al. (1999)
Oncorhynchus mykiss	Whole gut	1.30±0.07	Carnivorous	Hidalgo et al. (1999)
Anguilla Anguilla	Whole gut	1.40±0.07	Carnivorous	Hidalgo et al. (1999)
Sparisoma cretense	Whole gut	35.9±2.21	Herbivorous	Papoustsoglou and Lyndon (2003)
Uranoscopus scaber	Whole gut	0.202±0.04	Carnivorous	Papoustsoglou and Lyndon (2003)
P. bogaraveo	Stomach	0.13±0.01	Omnivorous	Caruso et al. (2009)
P. bogaraveo	Pyloric ceca	0.273±0.03	Omnivorous	Caruso et al. (2009)
P. bogaraveo	Intestine	0.195±0.020	Omnivorous	Caruso et al. (2009)

Table 2. Comparative trypsin-like activity of gut extracts of fish species of differing feeding behaviour.

Fish species	Gut section	Activity (U/mg protein)	Feeding behaviour	References
P. corruscans	Stomach	2.70±0.45 - 4.20±0.60	Carnivorous	Lundstedt et al. (2004)
P. corruscans	Anterior Intestine	0.24±0.05 - 0.41±0.06	Carnivorous	Lundstedt et al. (2004)
P. corruscans	Middle intestine	0.22±0.04 - 0.75±0.14	Carnivorous	Lundstedt et al. (2004)
P. corruscans	Posterior intestine	0.19±0.05 - 0.53±0.22	Carnivorous	Lundstedt et al. (2004)
G. maculatum	Anterior Intestine	3.18±0.25	Carnivorous	Xiong et al. (2011)
G. maculatum	Middle intestine	1.52±0.23	Carnivorous	Xiong et al. (2011)
G. maculatum	Posterior intestine	1.76±0.21	Carnivorous	Xiong et al. (2011)
Cyprinous carpio	Whole gut	2.50±0.62	Omnivorous	Hidalgo et al. (1999)
Carassius auratua	Whole gut	2.01±0.11	Omnivorous	Hidalgo et al. (1999)
Tinca tinca	Whole gut	1.70±0.29	Omnivorous	Hidalgo et al. (1999)
Sparus aurata	Whole gut	0.81±0.24	Carnivorous	Hidalgo et al. (1999)
Oncorhynchus mykiss	Whole gut	3.44±0.45	Carnivorous	Hidalgo et al. (1999)
Anguilla anguilla	Whole gut	0.46±0.05	Carnivorous	Hidalgo et al. (1999)
Polyodon spathula	Whole gut	1.90±0.01	Omnivore	Ji et al. (2012)
Symphysodon aequifasciata	Intestine	0.79±0.05	Carnivorous	Chong et al. (2002)
S. aequifasciata	Stomach	0.09±0.03	Carnivorous	Chong et al. (2002)

Segers, 2008). The decrease in amylase activity observed in Nile perch in this study could similarly be caused by the dietary shift that occurs in this fish; from rotifers and small arthropods in the juvenile to a fish diet as Nile perch matures (Hopson, 1972).

This study shows trypsin-like activity in all the Nile perch gut extracts (Figure 3). Trypsin-like activity has been reported in the guts of several carnivorous fish (Table 2), since it contributes 40 to 50% in protein digestion that makes up much of their diet (Natalia et al., 2004). Studies have also reported protease activity in the guts of herbivorous fish (Table 2); which is attributed to the fact that herbivorous fish require this enzyme for the lysis of algal cell before amylase activities proceed (Benitez and Tiro, 1982; Zemk-White et al., 1999). Despite the presence of proteases in some herbivorous fish species, the ratio of proteases to amylases in the herbivores remains lower than that observed in their

carnivore counterparts (Natalia et al., 2004). Studies have also shown high protease activity in some omnivores such when compared to that observed in some carnivores (Table 2); suggesting that difference in enzyme activity cannot efficiently be used in fish classification (Kuz'mina and Kuz'mina, 1990; Hidalgo et al., 1999).

The trypsin-like activity was observed in the oesophagus (Figure 3) of Nile perch in this study and this may be important in the facilitation of digestion in the subsequent regions of the gut; since the food residence time in the oesophagus is too low to enable chemical digestion, given that Nile perch quickly swallows its prey (Hopson, 1972). On the other hand, trypsin-like activity in the liver in this study is relatively low (Figure 3), possibly due to the fact that this enzyme is largely inactive (trypsinogen) in the pancreas which is located in the liver of this fish. Yet the stomach extract trypsin-like activity (Figure 3) was a little higher, probably due to other digestive enzymes and the ambient acidic environment in this region suggesting that the initial trypsin-like activity occurs in this region before digestion progresses to the intestinal region.

Observations in this study showed highest trypsin-like activity levels in the anterior intestine extracts (0.368 ± 0.042 U/mg protein) compared to those observed in extracts from other sections of the gut. This corresponds with reports from *Symphysodon aequifasciata* (Chong et al., 2002), *Siganus canaliculatus* and *Lates calcarifer* (Sabapathy and Teo, 1993), *Engraulis encrasicholus* (Martinez and Seera, 1989), *Scophthalmus maximus* (Munilla-Moran and Stark, 1990), and *Leptosynapta galliennei* (Féral, 1989). This suggests that the anterior intestine is the primary site for the hydrolysis of proteins to amino acids prior to absorption (Uscanga et al., 2010) in other fish and in the Nile perch.

Further observations made from this study reveled higher trypsin-like activity in the gut extract from the youngest fish (1 to 5 cm; 0.045 U/mg protein) compared to that observed in gut extracts from fish of older size classes (Figure 3). Similarly, decrease in Trypsin-like activity as fish increases in length and age has been reported in the *Coregonus sp* (Lauff and Hofer, 1984). It is probable that the action of trypsin is very crucial during digestion in the younger fish, especially because their stomachs are not yet fully developed for pepsin activity as observed in *Pagrus pagrus* larvae (Suzer et al., 2007); and when the fish matures pepsin becomes more important in the breakdown of long-chain polypeptide chains in the acidic stomach (Xiong et al., 2011), than trypsin. The high trypsin-like activity observed in the juveniles at this stage in could therefore be important to maximize protein digestion efficiency since young fish have higher specific metabolic demands in comparison to older fish (Mehner, 1996). This observation is emphasized by the fact that protein is a critical requirement for the growth and development of the fish at this stage (Izquierdo, 2004). This is probably the reason why gut extracts from the mature fishes in this study exhibited lower trypsin-like activity compared to the younger ones.

Conclusion

This study demonstrates the presence of amylases and proteases in the gut of Nile perch fish. The existence of these enzymes implies that Nile perch has the ability to digest carbohydrates and proteins; this makes it able to digest the most important ingredients that make up artificial diets. In this way Nile perch qualifies as a possible aquaculture candidate with criteria that it is able to utilize aqua feeds. The high protease activity observed in the youngest juveniles suggests that protein is critical at this development stage; therefore the larval feed should contain substantive amounts of proteins, probably between 45 and 55% as observed in other carnivorous larvae (Kpogue et al., 2013). In the same light, the high amylase activity observed in the juveniles suggests that it is much easier to wean Nile perch juveniles onto artificial diets, because at this stage the gut can easily utilize the carbohydrates that form much of the formulated aquafeeds on the market.

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