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Full Length Research Paper

Formative changes in intestinal brush border chemical action in wild, adolescent Nile roost Lates niloticus (Linnaeus, 1758)

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The Nile perch, *Lates niloticus* is a carnivorous fish and a potential candidate for aquaculture. The relationship between fish size (total length) and the activity of three brush border enzymes: leucine aminopeptidase (LAP), γ-glutamyl transferase (γ-GT) and maltase was evaluated in six size groups (1 to 5, 6 to 10, 11 to 15, 16 to 20, 21 to 25 and 26 to 30 cm) of wild juvenile Nile perch. Enzymatic activity was evaluated in three intestinal sections: pyloric caeca, upper intestine and lower intestine. All the three enzymes were influenced by the fish size, intestinal section, and the interaction between the two factors ($p< 0.05$). The highest specific activity of LAP and maltase was observed in the upper intestine while that of γ-GT was highest in the lower intestine. The specific enzyme activities were significantly higher ($p< 0.05$) in the 11 to 15 cm and 16 to 20 cm size groups in all the tested enzymes. The results reveal that, the most critical stage in the nutrition of juvenile Nile perch occurs when the fish attains a total length of 11 to 20 cm. Dietary formulations for this species should hence be in cooperate substantive amount of proteins and carbohydrates at this stage so as to maximize weight gain. The presence of maltase is an indicator that carbohydrates cannot be precluded from formulated diets for this species.

Key words: Nile Perch, enzyme activity, aquaculture, brush border.

INTRODUCTION

The Nile perch, *Lates niloticus* is a carnivorous fish common to all major river basins in Africa including the Nile, Volta, Senegal, Chad and Congo. The species is also native to Lakes Albert and Turkana in Uganda and Kenya, respectively. *L. niloticus* was introduced to Lake Victoria in the late 1950s (Hughes, 1992) near Jinja, Uganda and then spreading to both Kenya and Tanzania. The population of the Nile perch grew over the years

to become the most economically important species of the East African freshwater fisheries (Ogutu-Ohwayo, 1993). Fish products are Uganda's second most important export for generating export revenue, providing employment and supporting food security in the region (Benkenstein, 2011). According to the Lake Victoria Fisheries Organisation (2008) the species supported the regional fish export industry valued at USD 250 million in 2007. However, the high international demand has led to a decline in biomass and catch per unit effort (Matsuishi et al., 2006). Consequently, decreased fish catches have been reported in most areas of the lake and fish factories are operating with inadequate raw material (LVFO, 2008; Muhoozi et al., 2005; Ogutu-Ohwayo and Kirema-Mukasa, 2005). In order to revive the fish industry, *L. niloticus* is currently being explored as a potential candidate for aquaculture at the Kajjansi Aquaculture Research and Development Center, Uganda. However, before culture of any candidate species its ability to digest artificial diets has to be investigated (Kolkovski, 2001). The Nile perch is a carnivorous fish feeding on haplochromine cichlids, shrimp, dagaa and juvenile Nile perch. Attempts to feed the Nile perch on artificial commercial diets designed for the catfish (omnivorous) and *Lates calcarifer* (carnivorous*)* have so far been unsuccessful since the fish would not consume the diet or it would pass out half-digested compared to its natural diet of live fish. In order to understand the nutritional requirements for this species, there is need to investigate its digestive physiology. Elucidation of digestive enzyme activity is key to understanding the nutritional physiology of fish. Of special interest are enzymes located in the brush border membrane that are responsible for the final stages of degradation and assimilation of nutrients (Klein et al., 1998). The characteristics of digestive enzymes provide insights into the ability of a given species to digest and utilize dietary components (Ali et al., 2009; Debnath et al., 2007).

Fish, like other animals, are thought to exhibit plasticity in production of digestive enzymes (Caviedes-Vidal et al., 2000). For instance, carnivorous fish have been shown to have higher proteolytic enzyme activities to digest high protein diets while herbivorous fish exhibit higher carbohydrase activities (Cahu et al., 1998; Hakim et al., 2007; Harpaz et al., 2005b; Harpaz and Uni, 1999; Hidalgo et al., 1999; Sabapathy and Teo, 1993). Nevertheless, Chakrabarti et al. (1995) studied the food habits and enzymatic activity of multiple freshwater fish species and found that adult fish produce almost all digestive enzymes, irrespective of diet. Similar results were reported by Harpaz and Uni (1999) in the carnivorous hybrid striped bass, omnivorous tilapia hybrids and the silver carp fed on natural food and commercial pellets containing 30% protein and 5% fat.

The intestinal section has been shown to influence the level of enzyme activity with variations across species. A higher proteolytic enzyme activity has been reported in the pyloric caeca of *L. calcarifer* (Harpaz et al., 2005a) indicating the role of this section in protein degradation and absorption (Horn, 2005). Furthermore the carnivorous species on high protein diets have been shown to exhibit a 3-fold increase in proteolytic enzyme activity compared to omnivorous species in the pyloric caeca. However studies by Tibaldi et al. (2006) in the European sea bass (*Dicentrarchus labrax*) found the highest activity of γ-GT, a protein degrading enzyme in the lower intestinal tract. Though the role of the pyloric caeca is still unclear, it has been suggested that this section takes part in absorption of carbohydrates and fats hence supplementing the role of the stomach and intestine (Hepher, 1988).

Digestive enzyme activity is also influenced by age and diet (Péres et al., 1998). The influence of diet and age on digestive enzyme activity can best be demonstrated in species that undergo an ontogenetic change in diet (German et al., 2004; Kuz'mina, 1996). Indeed, prickleback fishes (*Cebidichths violaceus* and *Xiphister mucosus)* from the central California coast increase their carbohydrase activities with an ontogenetic shift from carnivory to herbivory (German et al., 2004). Studies based on gut content analysis have shown that juvenile Nile perch undergo an ontogenetic shift in diet (Katunzi et al., 2006; Mkumbo and Ligtvoet, 1992) depending on food availability in the different habitants (Paterson and Chapman, 2009). Nile perch of <5 cm exclusively fed on zooplankton before shifting to midge larvae and shrimp. Above 10 cm the main prey for Nile perch in most habitants was reported to be juvenile Nile perch (< 5 cm) and dagaa (*Rastrineobola argentea*) (Katunzi et al., 2006). Therefore, the aim of this paper was to investigate if brush border enzyme activities would vary with this ontogenetic shift in diet.

Although, attempts to wean candidate aquaculture species onto artificial diets typically begins with larval stages (Creswell et al., 2010; Vega-Orellana et al., 2006), induced spawning of Nile perch has not yet been successful, hence nutritional studies can only be done on wild fish. We studied the activity of three brush border enzymes in three intestinal sections: pyloric caeca, upper intestine and lower intestine in six different size groups. Two proteolytic enzymes, leucine amino peptidase (LAP, EC 3.4.11.1), gamma glutamyl transferase (γ-GT, EC 2.3.2.2) and a carbohydrase, maltase (EC 3.2.1.20) were assayed. The activity of gut enzymes like trypsin and amylase in the wild juvenile Nile perch have been investigated (Namulawa et al., 2013). The following questions were specifically addressed: Are there differences in the enzymatic activities of LAP, γ-GT and maltase across the different fish size groups? How does the activity of these enzymes vary along the different intestinal sections? Is there a correlation between the activities of these enzymes along the different intestinal

sections and the different fish size groups? In order to answer these questions, juvenile *L. niloticus* at different stages of development were captured from the shores of Lake Victoria and their digestive enzyme activities at specific intestinal sites analyzed. Results from this study would provide useful information to guide the formulation of artificial diets for the Nile perch.

MATERIALS AND METHODS

Study design and sampling strategy

Wild juvenile Nile perch at different stages of development were collected from Kigungu landing site on the shores of Lake Victoria (GPS: 36" 43755 E, 0003036 N, and 1131 m above sea level). Fish with a total length ranging from 1 to 30 cm were considered juvenile (Katunzi et al., 2006). They were captured by beach seining and immediately transferred to oxygenated tanks (2 m × 2 m) containing lake water and taken to the laboratory where they were kept in the tank overnight so as to recover from handling stress. The fish were classified into six size groups of total length (1 to 5, 6 to 10, 11 to 15, 16 to 20, 21 to 25 and 26 to 30 cm) based on the observation by Katunzi et al. (2006) that wild juvenile *L. niloticus* undergo an ontogenetic dietary shift within the selected range of size groups.

Ten (10) fish from each group were randomly selected, sacrificed using a scalpel to sever the spine and their body weight taken. The abdomen was opened and the entire digestive tract removed. The intestine was dissected, adipose tissue carefully cleaned off and the total intestinal weight was measured using a weigh balance (Sartorius, Germany). The intestinal tract was then washed with distilled water in order to remove any residual prey debris and divided into the pyloric caeca, upper intestine and lower intestine. For fish between 1 to 10 cm total lengths, the whole gut was used because intestinal sectioning was not possible. The dissected sections were then washed with double distilled water and immediately stored in a -20°C freezer for evaluation on the following day.

Evaluation of brush border enzymes

Brush border enzyme activity evaluation was done following the method of Harpaz and Uni (1999). Prior to evaluation, stored sections were briefly thawed and eight milliliters of double distilled water added to 1 ml of tissue. Zirkonium glass beads (Muhlmeier, Germany) were added to each tube and the mixture homogenized using a mini-Bead Beater (Bio-Spec, U.S.A.) for 5 min. The Homogenate was then centrifuged for 5 min at a speed of 4,000 rpm using an Eppendorf Centrifuge (Eppendorf, Germany). The extract was then used to assay the specific activity of LAP, γ-GT and maltase. Specific activity was calculated as the amount of enzyme required to release 1 µmol of product per minute per milligram protein.

Leucine amino peptidase (EC 3.4.11.1) activity

The activity of leucine amino peptidase (LAP) was measured following the method of Harpaz and Uni (1999) using L-leucine-p-nitroanilide reagent (Randox, Ireland). The reagent (250 µl) was added to 15 µl of the homogenate in an ELISA plate and read at 1 min intervals for 15 min at 405 nm using a microplate reader (Tecan Sunrise, Switzerland). One unit of activity was determined as the quantity required to release 1 µmol L-leucine and p- nitro aniline per minute per milligram protein. Units of activity were determined as below

$$LAP\ (U/mgP) = ((\Delta A/min * TV) / (EF * SV)) * (D/P)$$

Where: ΔA/min: total increment per min, TV: 265 µl: The total reaction volume (homogenate + reagent), EF: 1.167=excitation coefficient of P-nitrophenoxide at 405 nm, SV: homogenate=15 µl, D: dilution factor, P: protein (mg) in the homogenate.

Gamma glutamyl transferase (EC 2.3.2.2) activity

The activity of γ-GT was measured following the method of Harpaz and Uni (1999) using a kit manufactured by Randox, Ireland. Twenty microliters of homogenate sample was placed on an ELISA plate followed by 200 µl of reagent. The plate was immediately read at 405 nm at 1 min intervals for 15 min using a microplate reader (Tecan Sunrise, Switzerland). One unit of activity was determined as the quantity required to release 1 µmol of 5-amino-2-nitrobenzoate per minute per milligram protein. Units of activity were as for LAP except for the EF coefficient, which is 9.5 for the 5-amino-2-nitrobenzoate produced in this reaction at 405 nm.

Maltase (EC 3.2.1.20) activity

The hydrolysis of maltose by maltase was assayed following the method of Harpaz and Uni (1999) following the equation below:

Twenty microliters of sample extract were mixed with 20 µl of substrate (0.056M maltose) in an ELISA plate. The plate was then incubated at 37^{O}C for 45 min for glucose to form. The reaction was stopped by putting the plate on ice for 4 min. The presence of glucose as an indicator of enzymatic activity was then measured using the glucose oxidase reagent (Thermo electron corporation, USA). After incubation, 2 µl were taken from the reaction mixture and placed in an ELISA plate. Glucose oxidase reagent (300 µl) was then added to all the wells and the plate incubated at 37°C for 10 min. After incubation, the plate was read using a microplate reader (Tecan Sunrise, Switzerland) at 500 nm and compared to a glucose standard (Thermo Scientific, USA).

Protein determination

Protein concentration in the sample extracts was determined using a kit (BIO-RAD, USA) so as to calculate the specific activity per milligram protein. The protein concentration was measured by putting 5 µl of sample extract in an ELISA plate and adding 5 µl of reagent A to all wells followed by 200 µl of reagent B and incubation at room temperature for 10 min. The OD was read at 750 nm using a microplate reader (Tecan Sunrise, Switzerland) and compared with a 2 mg/ml BSA standard (BIO-RAD, USA).

Statistical analysis

Statistical analysis of enzyme activity was done using GraphPad 5.0 statistical package. The effect of intestinal section, size group and their interaction on the activity of Leucine amino peptidase,

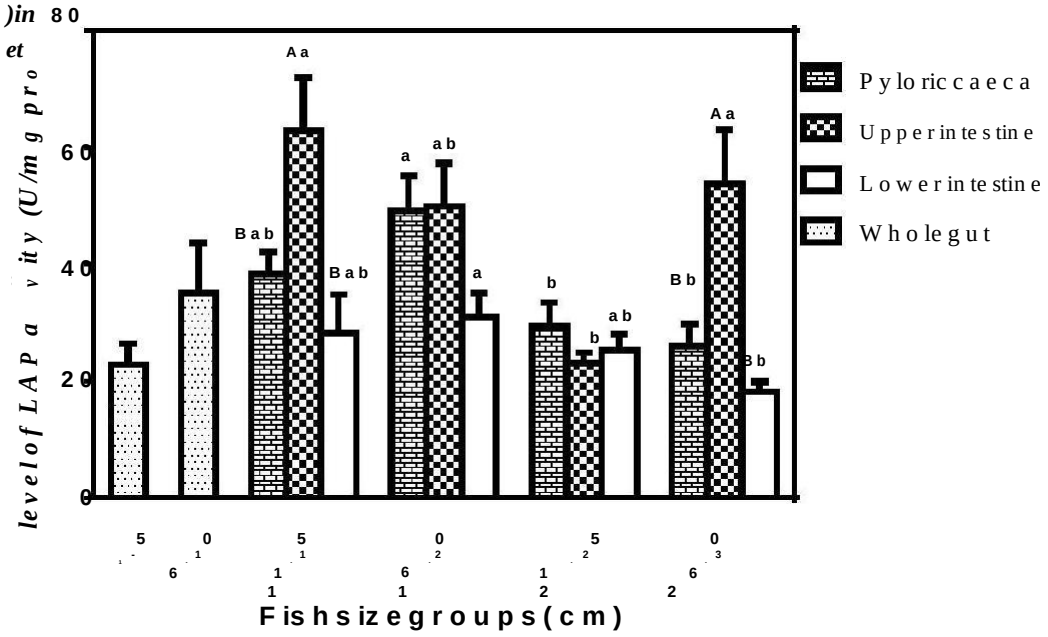

Figure 1. Specific activity of leucine amino peptidase measured in the different intestinal sections of six different fish size groups. Capital letters above the columns represent the significant differences ($p<0.05$) among intestinal sections in each size group. Small letters represent significant difference among the fish size groups within each segment (Size Groups 1 to 5 and 6 to 10 were excluded from the analysis).

Gamma glutamyl transferase and maltase was analyzed using a two-way ANOVA. The 1 to 5 and 5 to 10 cm size groups were not included in the ANOVA. Comparison between the enzymatic activity of the different intestinal segments and that between the different size groups was done using Tukey`s Multiple Comparison Test set at $P<0.05$. To determine the relationships between the activity of Leucine amino peptidase, Gamma glutamyl transferase and maltase across the different size groups, and correlation tests using Pearson correlation matrix set at a $P<0.05$ were done.

RESULTS

Leucine amino peptidase (LAP)

LAP specific activity was influenced by the intestinal section ($F_{2,\ 92}=14.38$, $p<0.0001$), the fish size ($F_{3,\ 92}=6.64$, $p<0.0004$) and the interaction between the two factors ($F_{6,\ 92}=3.37$, $p<0.0048$). Significantly higher activity was detected in the upper intestine of the 11-15 cm (62.83±9.154 U/mg protein) and 26-30cm (53.81±9.27 U/mg protein) groups as compared to the pyloric caeca and lower intestine. Comparison of LAP activity in the same intestinal segment among different groups revealed significantly higher activity ($p=0.005$) in the pyloric caeca of the 16 -20 cm group (49.15±6.0 U/mg protein) as compared to the 21-25 and 26-30 groups. In the upper intestine, a higher activity ($p=0.002$) was found in the 11-15 and 26-30 groups as compared to 21-25 group. In the lower intestine, a significantly higher activity ($p=0.03$) was found in the 16-20 group (31.014±4.02 U/mg protein) as compared to the 26-30 group (Figure 1).

Gamma glutamyl transferase (γ-GT)

The results show that the activity of γ-GT was influenced by the intestinal section ($F_{2,\ 88}=2.72$, $p<0.0247$), the fish size ($F_{3,\ 88}=8.25$, $p<0.0001$) and the interaction between the two factors ($F_{6,\ 88}=2.72$, $p< 0.018$). Comparing the activity of γ-GT in the same size group among different intestinal sections revealed a significantly higher activity in the lower intestine of fish in the 21 to 25 (814.35±152.08 U/mg protein) and 26 to 30 (703.87± 99.05 U/mg protein) cm groups as compared to the upper intestine. Comparison of the activity of γ-GT in the same intestinal tract among different groups revealed a higher activity ($p=0.01$) in the pyloric caeca of the 16 to 20 cm group (1145.68±241.5 U/mg protein) as compared to the 21 to 25 and 26 to 30 groups. In the upper section, a higher γ-GT activity ($p=0.001$) was found in the 11 to 15 group (1010.5±194 U/mg protein) as compared to the 21 to 25 and 26 to 30 groups. No significant difference was found in the activity of the enzymes in the lower intestine across the different size groups (Figure 2).

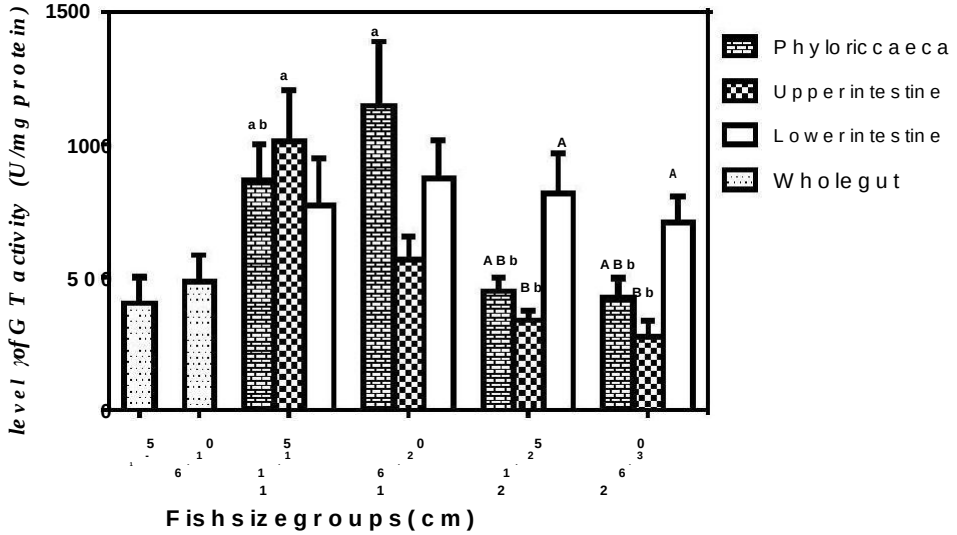

Figure 2. Specific activity of γ-glutamyl transferase measured in the different intestinal sections of six different fish size groups. Capital letters above the columns represent significant differences ($p<0.05$) among intestinal sections in each size group. Small letters represent significant difference among the fish size groups within each segment (Size groups 1 to 5 and 6 to 10 were excluded from the statistical analysis).

Maltase

The activity of maltase was influenced by the intestinal section ($F_{2, 103}=5.90$, $p<0.0038$), the fish size ($F_{3, 103}=21.23$, $p<0.0001$) and the interaction between the two factors ($F_{6, 103}=3.48$, $p< 0.0036$). Comparing the activity of maltase in the same fish size group among different intestinal sections revealed a significantly higher activity in the upper intestine of the 11 to 15 (112.32±11.08 U/mg protein) and 26 to 30 (66.32±12 U/mg protein) groups. Comparison of maltase activity in the same intestinal tract among different groups revealed a significantly higher activity ($p=0.002$) in the pyloric caeca of the 11 to 15 (65.23±3.89 U/mg protein) and 16 to 20 (56.61±8.20 U/mg protein) groups. In the upper intestine, a significantly higher activity ($p=0.01$) was found in the 11 to 15 (112.32±11.08 U/mg protein) and 26 to 30 (66.32±12 U/mg protein) groups. In the lower intestine, a higher activity ($p=0.04$) was also revealed in the 11 to 15 (73.7±15.51 U/mg protein) size group (Figure 3).

Correlations between enzyme activities

The highest correlation among enzyme activities was observed between LAP and maltase in all fish size groups, followed by that between γ-GT and maltase and lowest between LAP and γ-GT. The strongest correlations were revealed in the pyloric caeca of all size groups as compared to other intestinal segments (Table 1).

DISCUSSION

The ability of fish to utilize a given diet depends on the activity of its enzymes along the digestive tract (Tengjaroenkul et al., 2000). Digestive capacity of fish relates to the age and dietary composition (Lovett and Felder, 1989; Péres et al., 1998). Chakrabarti et al. (1995), while studying 11 adult fresh water teleost fish demonstrated that at any time, fish possess a panel of enzymes with activities varying according to feeding habit. Indeed, Harpaz and Uni (1999) reported similar findings while comparing carnivorous, omnivorous and herbivorous fish species. Similar results were observed in this study in that all tested enzymes were present in the different size groups of the wild juvenile *L. niloticus* but with activity variation relating to size and intestinal section. These findings will help reveal dietary needs for juvenile Nile perch.

Maltase is a disaccharide degrading enzyme required for the breakdown of maltose produced during the hydrolysis of starch and its highest activity was observed in the upper intestine. A high activity of maltase has also been reported in other carnivorous fish like the Asian sea bass (Harpaz et al., 2005b; Sabapathy and Teo, 1993) and in birds fed on a high protein, carbohydrate free diet

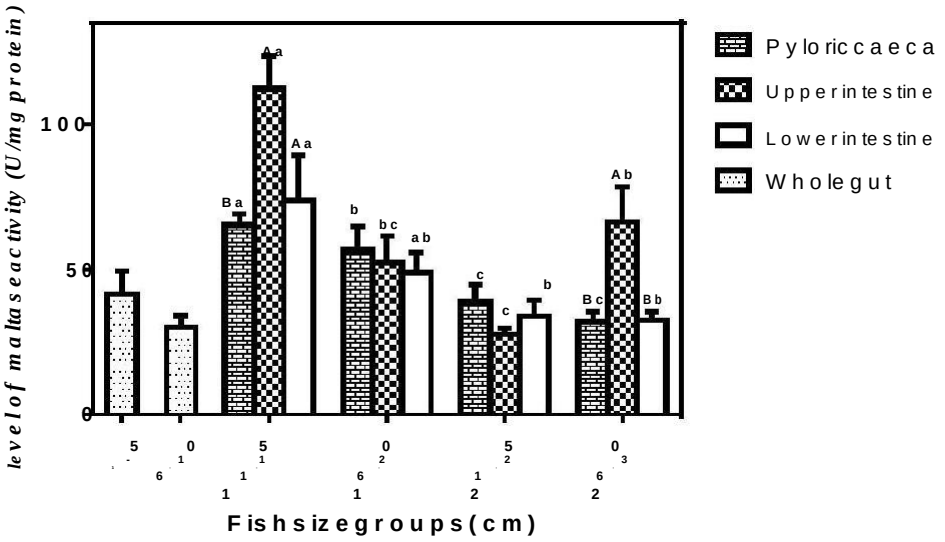

Figure 3. Specific activity of maltase measured in the different intestinal sections of six different fish size groups. Capital letters above the columns represent significant differences (p<0.05) among intestinal sections in each size group. Small letters represent significant differenc e among the fish size groups within each segment (Size groups 1 to 5 and 6 to 10 were excluded from the statistical analysis).

Table 1. Correlation between the specific enzyme activities of brush border enzymes among the different fish size groups and intestinal segments.

Size group (cm)	Intestinal segment	Lap/γ-GT	Lap/Maltase	γ-GT/Maltase
	Pc	0.362	0.978*	0.323
11-15	Upper	0.298	0.676*	0.610
	Lower	0.512	0.688*	0.862*
	Pc	0.831	0.914*	0.634
16-20	Upper	0.632	0.824*	0.654
	Lower	0.369	0.845*	0.778*
	Pc	0.790	0.920*	0.273
21-25	Upper	0.660	0.850*	0.570
	Lower	0.680	0.579	0.920*
	Pc	0.954*	0.935*	0.900*
26-30	Upper	0.234	0.342	0.965*
	Lower	0.321	0.314	0.600

The values presented are correlation coefficients (r). Figures with asterisk indicate significant values (p< 0.05). Pc is the pyloric caeca.

(Sabat et al., 1998). Presence of maltase and its strong correlation to other brush border enzymes in a carnivorous fish on a natural diet suggests that the activity of brush border digestive enzymes is regulated under the same gene control mechanism. Moreover there was a strong correlation in the activity of these enzymes especially in the pyloric caeca confirming that this region is an extension of the intestine with an additive role in

nutrient digestion and absorption (Horn, 2005). Similarly, Hakim et al. (2007) observed a linear correlation between mRNA expression for the different brush border enzymes and transporters in European sea bass (*Dicentrarchus labrax*). The presence of maltase in the Nile perch gives prospects for the design of artificial diets.

Our results revealed that intestinal segment had a significant effect on all assayed enzymes. As was shown previously (Hakim et al., 2007; Harpaz et al., 2005a; Tibaldi et al., 2006) the highest activity of LAP was revealed in the upper intestine. LAP is responsible for the digestion of the products of pancreatic proteases. The high activity of this enzyme in the upper intestine indicates importance of this intestinal section in protein digestion in the wild juvenile *L. niloticus*. The highest activity of γ-glutamyl transferase was revealed in the lower intestine. These results are in agreement with other studies in the hybrid striped bass (Harpaz and Uni, 1999), the Asian sea bass (Harpaz et al., 2005b) and the European sea bass (Tibaldi et al., 2006). The activity of γ-GT is indicative of active amino acid transportation across the intestinal surface (Semenza, 1986). A higher activity of this enzyme in the lower intestine indicates that absorption of amino acids is elevated with movement along the gut and might be an adaptation to efficiently absorb nutrients in order to compensate for the short intestinal tract in this species.

The results revealed a significant influence of the fish size on the activity of all the tested brush border enzymes. Several studies have shown that in the European sea bass (*D. labrax*) and Senegal sole (*Solea senegalensis*), juveniles have a higher enzymatic activity than adults (Infante and Cahu, 1994; Martinez et al., 1999; Ribeiro et al., 1999; Wang et al., 2006). Kuz'mina (1996) also observed an abrupt increase in the activity of proteolytic enzymes in carnivorous pike at an early age. Fish diet is one of the factors that influence the activity of digestive enzymes (Fernandez et al., 2001) especially in species that undergo ontogenetic shifts in diet (German et al., 2004). Different authors (Katunzi et al., 2006; Mkumbo and Ligtvoet, 1992; Ogutu-Ohwayo, 2004; Schofield and Chapman, 1999) have reported that *L. niloticus* undergo an ontogenetic dietary shift. Katunzi et al. (2006) observed that in most habitats, juvenile *L. niloticus* < 5 cm exclusively fed on zooplankton before shifting to midge larvae and shrimp. Above 10 cm the main prey for Nile perch in most habitats was reported to be juvenile Nile perch (< 5 cm) and *R. argentea*. In this study it was observed that the specific activity of both carbohydrase and proteolytic enzymes was significantly higher in the 11 to 15 and 16 to 20 cm groups, coinciding with the change to a higher protein diet. This high protein diet necessitates an increase in the activity of proteolytic enzymes in order to degrade and absorb these nutrients. Furthermore, maturation of the intestinal tract and the relative increase in intestinal length increase the total capacity of the intestine to digest and assimilate nutrients (Ferraris et al., 1986). The lower enzyme activity in Groups 1 to 5 and 6 to 10 cm relates to immaturity of the digestive tract as compared to the larger groups. This increase in enzyme activity with stage of development has been reported in other species (Kuz'mina, 1996; Kvåle et al., 2007; Zouiten et al., 2008).

Results from this study reveal the presence of proteases and carbohydrase enzymes in the gut of the Nile perch. Presence of these enzymes show that the Nile perch is capable of utilizing artificial diets. The results further reveal that juvenile Nile perch have the highest capacity to utilize dietary proteins and carbohydrates at a total length of 11 to 20 cm. This shows that proteins and carbohydrates are critical at this stage of development and hence artificial diets should contain substantive amounts at this critical stage. Moreover, this stage has been characterized by a shift to a higher protein diet in the wild further showing that it is possible to wean the juveniles at this stage since they are capable of utilizing carbohydrates that make up much of the ingredients in aquaculture feeds on the market. Presence of maltase is an indicator that carbohydrates cannot be precluded from formulated diets for this species and would hence form a cost effective venture to the aqua-culturist. Research to try and spawn Nile perch in captivity is ongoing. When this induced spawning becomes successful, it will be important to study the activity of digestive enzymes in the larval stages since this stage is critical in determining survival.

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