Effects of water depth on digestive enzymes activities of *Clarias gariepinus* (Burchell, 1822) fingerlings

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Abstract

The effects of water depth on the digestive enzymes activities of *Clarias gariepinus* was investigated. Ten concrete tanks (50x150x100 cm capacity) were used; each tank was filled with water at different experimental water depths (25, 50, 75,100 and 125 cm) under static culture system. The activity of protease, lipase and amylase from the pancreas of *Clarias gariepinus* was determined. Digestive protease, lipase and amylase showed optimum activities at 125 cm water depth with amylase activity showing greater variation across treatments compared to the other two digestive enzymes. Water depth positively influenced the release of these enzymes for feed digestion to occur with lipase being the most influenced by water depth. In conclusion water depth affects digestive enzymes activities in *Clarias gariepinus* fingerlings and it is recommended that 125 cm water depth should be used in culturing *Clarias gariepinus*.

Keywords: Amylase; *Clarias gariepinus*; lipase; protease; water depth.
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Introduction

Depth is usually determined for reasons related to construction costs, habitat preference of the primary cultured species, or for management of cohabiting organisms such as phytoplankton, benthos or rooted macrophytes (Carballo *et al* 2008). Fish farming is commonly practiced in ponds of about 1m depth, but there is a wide range of depths in use. Fish naturally tend to select the habitat that is most suitable for their physiological requirements. This behaviour is known as 'habitat selection' or 'enviroregulation'. Catfish species of the family Clariidae, particularly the African catfish *C. gariepinus* are becoming more important in African and Asian fish culture. *C. gariepinus,* however is a bottom dwelling fish spending a large proportion of time on the bottom of the culture tank (Fagbenro *et al* 2003). The study of digestive enzymes in fish has a wide range of potential interest, it is an essential step towards understanding the mechanism of digestion and how the organism adapts to changes in the nutritional environment (Sunde *et al* 2004). On the other hand, the assessment of the activity of digestive enzymes in cultured species may be helpful in the selection of feed ingredients. Recent investigations on digestive processes have focused on evaluating the ability of organisms to hydrolyze, absorb and assimilate the principal dietary nutrients, these processes can be initially examined by analyzing the activity of digestive enzymes (total proteinases, trypsin, chymotrypsin α-amylase and lipase) (Guzman *et al* 2005).

Fish go to the water surface to feed, after feeding they go down to the bottom of the water to break down the food into smaller particles (this process is known as digestion), during digestion some enzymes are being released (called digestive enzymes) for growth to take place. Digestive enzymes, such as lipase, protease and amylase are specific proteins that are produced in the gastrointestinal tract to break down food into digestible nutrients that can be readily absorbed and utilised by the body (Caruso *et al* 2009). The digestive enzymes in the pancreas break substances down into even smaller parts than the mouth and stomach. Digestive enzymes are inhibited by drugs, poisons and some harmful chemicals in the water which can alter the growth of fish. Although several comparative studies of the digestive enzymes in different fish species have been reported (Kuz'mina and Kuz'mina, 1990; Kuz'mina and Smirnova, 1992) there is a dearth of information on the quality and activities of digestive enzymes for our locally culturable fish species in Nigeria. This study was conducted to determine the effects of water depth on the digestive enzymes activities of *Clarias gariepinus* fingerlings.

Materials and methods

This study was carried out in outdoor concrete ponds located in the experimental garden of the Department of Biology, Faculty of Life Sciences, Ahmadu Bello University (ABU), Zaria. One hundred fingerlings of *C. gariepinus* produced by artificial spawning of domesticated broodstock of *C. gariepinus* was used in the experiment. They were obtained from the fish hatchery in the Department of Biology, ABU, Zaria and

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acclimatized for two weeks within the experimental area. Ten concrete ponds (50x150x100 cm capacity) were used; each pond was rain- fed though supplemented with dechlorinated tap water at different experimental water depths. One PVC pipe (1 inch diameter) each, cut to the desired length from experimental water depth, was fitted to each compartment to drain excess water. Water depth was measured using a metre rule and maintained at different experimental levels.

Fish were reared at five different water depths (25, 50, 75, 100 and 125 cm) under static culture system. The experiment lasted for 190 days (June 2nd 2016-December 2nd 2016). *Clarias* species were stocked into five experimental ponds at stocking rate of ten (10) fish per concrete ponds, each treatment was duplicated. Sampling of experimental fish was done biweekly within the experimental period. During fish sampling, weight of fish was measured using a sensitive weighing balance (Sartorius sensitive scale Model CP8201). While total length of fish was determined using a measuring board (FishBase, 2004). Feeding was done twice daily, morning (8.00am-9.00am) and evening (5.00 pm-6.00 pm), feeding was at 5% body weight of fish. Throughout the study, experimental fish fed on Coppens, a floating pelleted diet with 42% crude protein.

Water quality parameter

Temperature (°C) of the water was taken on field using HANNA Instrument model HI-98129.

Sample preparation for digestive enzymes activities

Ten fish per depth were used for the analysis. The fish were dissected a day after the final sampling and the pancreases were removed and rinsed with distilled water. A gram of the pancreas was weighed and homogenized (using ceramic mortar and pestle) with 5ml of ice cold phosphate buffer solution pH 8 (Furne *et al* 2008). The homogenate was centrifuged at 4°C at 2,000 rpm for 20 minutes; the supernatant was collected and kept in ice-cold condition in a plastic container for further analysis.

Amylase activity determination

Amylase assay was carried out using a modified procedure of McCue and Shetty (2004). A total of 250 µl of extract was placed in a tube and 250 µl of 0.02M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5 mg/ml) was added. The content of the tubes were pre-incubated at 25°C for 10 minutes after which 250 µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at timed intervals. The reaction was stopped by adding dinitrosalicylic acid (DNS) reagent and further incubated in boiling water for 5 minutes and cooled to room temperature. The content of each test tube was diluted with 5 ml of distilled water and the absorbance rate measured at 540 nm using a spectrophotometer. A standard curve was made and α-amylase activity was calculated using this formula:

$$\% \text{ inhibition} = \frac{(Ac - Ac)}{Ac} \times 100$$

Where: Ac = Absorbance of the control, Ae = Absorbance of the extract.

Protease activity determination

Protease activity was determined using a casein substrate by a modification of the Anson method (Keay and Wildi, 1970). One ml of the culture supernatant was mixed with 1 ml 0.05M phosphate buffer and 0.1M sodium hydroxide (pH 7.0 adjusted with phosphoric acid) containing 2% casein, and incubated for 10 minutes at 37°C. The reaction was stopped by adding 2 ml 0.4M Trichloroacetic acid. After 30 minutes stand at room temperature, the precipitate was removed by centrifugation and the optical density of the assays was measured at 660 nm. A standard curve was generated using solutions of 0-60 µg/ml tyrosine. One unit of protease activity was defined as the amount of enzyme required to liberate 1 µg/ml tyrosine under the experimental conditions used.

Lipase activity determination

Lipase activity was assayed as reported by Bülow and Mosbach (1987), with slight modifications. The substrate solution was 50 mM of p-NPB in ethanol and activity buffer was 50 mM Tris-HCl, pH 8.0, containing 4% ethanol. Lipase activity was assayed spectrophotometrically by measuring the rate of hydrolysis of p-NPB at 405 nm and 30°C in Ultra violet double beam spectrophotometer. The change in the absorbance at 405 nm and 30°C was read at 30 seconds intervals for a period of 5 minutes against blank. The reaction mixture composed of 293 µl activity buffer, 10 µl of enzyme solution and 60 µl of p-NPB. One unit of activity was defined as the amount of enzyme that catalyze the release of 1 µmol of p-nitrophenol (p-NP) per minutes under assay condition. The extinction coefficient of p-NP was taken as 11.500 M (Metin and Akpinar, 2000).

Data analysis

Data obtained were summarized using descriptive statistics. Means obtained with respect to digestive enzymes activities and water quality parameter were subjected to one-way ANOVA using SPSS Version 20, where significant difference was observed between the treatments, the means were separated using Duncan's Multiple Range Test (Duncan, 1955). Principal component analysis was used to determine the relationship between water depth and digestive enzymes activities. $p \leq 0.05$ was considered statistically significant.

Results

Digestive enzymes activities

The activities of digestive enzymes are presented in Table 1. In this study, digestive protease, lipase and amylase showed optimum activities at pond water depth of 125 cm. There was significant difference ($p \leq 0.05$) in digestive enzymes activities among all treatments. Amylase activity showed great variation across treatments compared to the other two digestive enzymes.

Water depth relationship with digestive enzymes activities

The association between water depth and digestive enzymes activities of *C. gariepinus* is presented in Figure 1. The activities of protease, lipase and amylase abruptly increase with increased water depth; this means that water depth positively influenced the release of these digestive enzymes during digestion for growth to occur, with lipase being the most positively influenced by water depth.

Water quality parameter of the pond water

The mean water quality parameter of the pond water is presented in Table 2. Temperature ranged between 25.50 and 26.07°C. There was significant difference ($p \leq 0.05$) in the water quality parameter among the treatments.

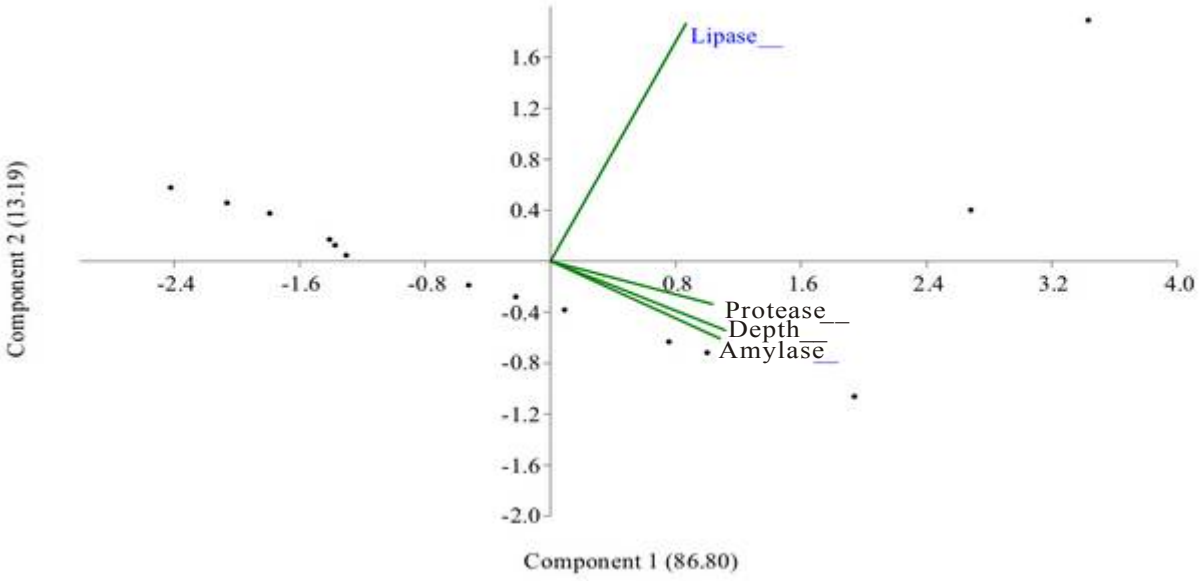

Figure 1. Principal component analysis biplot for digestive enzymes activities and water depth.

Table 1. Digestive enzymes activities of *C. gariepinus* cultured at various water depth.

	Water depth (cm)				
Digestive enzymes	25	50	75	100	125
Protease (U/mg)	0.17 ± 0.00^{bc}	0.16 ± 0.00^{c}	0.17 ± 0.00^{b}	0.18 ± 0.00^{a}	0.19 ± 0.00^{a}
Lipase (U/ml)	0.03 ± 0.00^{b}	0.04 ± 0.00^{b}	0.06 ± 0.00^{b}	0.09 ± 0.00^{b}	0.54 ± 0.24^{a}
α-Amylase (U/mg)	3.27 ± 0.19^{c}	4.56 ± 0.07^{d}	5.11 ± 0.12^{c}	5.76 ± 0.03^{b}	6.79 ± 0.10^{a}

Means with the same superscript along rows do not vary significantly (p>0.05).

Table 2. Mean water quality parameter of the experimental ponds.

	Water depth (cm)				
Parameter	25	50	75	100	125
Temperature (°C)	25.53 ± 0.21^{b}	25.50 ± 0.18^{b}	25.70 ± 0.14^{ab}	26.01 ± 0.11^{ab}	26.07 ± 0.03^{a}

Discussion

Fish subjected to lower water depth (25 cm) were prone to experience low metabolism, while greater water depth (125 cm) increased metabolic activities in fish. With good feeding and release of digestive enzymes in the system at desired water level, fish readily consume all that is fed and readily digest such resulting in high feed utilization that extend to fish growth (Adebola and Adetunji, 2015). Higher amylase activities were found in all treatments despite *C. gariepinus* consuming a high-protein, low-starch diet, this agrees with the findings of Hidalgo *et al* 1999 and Lundstedt *et al* 2004 who reported the presence of carbohydrases in many omnivorous fish. Fish species differ greatly in their ability to digest carbohydrates. Digestive functions capable of hydrolyzing a greater variety of carbohydrate-containing feed are more developed in omnivorous fish in contrast to carnivorous fish (De Almeida *et al* 2006). Lipase was the most positively influenced by water depth and this is probably linked to the fact that lipase being a type of enzyme known as hydrolase is responsible for catalyzing the hydrolysis of triglyceride into fatty acids and glycerol. It is referred to as hydrolase because the reaction it catalyzes is a hydrolysis reaction in which large food particles are broken down into smaller ones with the addition of water (McKee and McKee, 2003). Inspite of their omnivorous feeding habit, *C. gariepinus* require high amount of protein and fat in the diet similar to other carnivorous fish (Meyer and Fracalossi, 2004).

Water temperature is one of the most important physical factors affecting fish growth and production (Gaber *et al* 2012). The highest temperature value (26.07°C) recorded in this study was observed at 125 cm pond water depth, this indicates that as the water depth increases the temperature of the water also increases, this could be due to high heat capacity which enable water to retain heat due to higher volume as the fish agitate the water. Fish metabolic rate at greater depth (125cm) increased at higher temperature during digestion for growth to occur.

Conclusion

Digestive enzymes secreted by the pancreas of *C. gariepinus* at greater water depth (125 cm) are well adapted to protein (0.19 U/mg), lipid (0.54 U/ml) and carbohydrate (6.79 U/mg) digestion for efficient growth. This makes the fish able to digest the most important ingredients that make up artificial diet.

Recommendation

The release of enzyme quantity of substrate in diets should be carried out. However, further work should be carried out to evaluate the effects of water depth on other physiological processes.

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References

Adebola, O. A. and Adetunji, Q. A. 2015. Effects of water level on growth, nutrient utilization and survival of African Catfish, *Clarias gariepinus*. *International Journal of Agricultural Sciences and Natural Resources, 2(4):* 109-112.

Bülow, L. and Mosbach, K. 1987. The expression in *Escherichia coli* of a polymeric gene coding for an esterase mimic catalyzing the hydrolysis of p-nitrophenyl esters. *210(2):* 147-152.

Carballo, E., Eer, A., Schie, T. and Hilbrands, A. 2008. *Small scale freshwater fish farming*. Agromisa Foundation and CTA, Wagenningen, Book, 1:83

Caruso G., Denaro, M. G. and Genovese, L. 2009. Digestive enzymes in some teleost species of interest for Mediterranean Aquaculture.*The Open Fish Science Journal, 2:* 74-86.

De Almeida, L. C., Lundstedt, L. M. and Moraes, G. 2006. Digestive enzymes responses of Tambaqui (*Colossoma macropomum*) feed on different levels of protein and lipid. *Aquaculture Nutrition, 12:* 443-450.

Duncan, D. B. 1955. Multiple range and multiple (F) test. *Biometrics, 11:* 1-12.

Fagbenro, O. A., Adeparusi, E. and Fapohunda, O. 2003. Feed stuffs and dietary substitutions for farmed fish in Nigeria. *Proceedings of the National Workshop on Fish Feed Development and Feeding Practices in Aquaculture, (NWFFDFPA'03), National Freshwater Fisheries Research Institute*, pp. 60-65.

FishBase. Org. 2004. *Term: total length*, pp. 11-18.

Furne, M. M., García-Gallego, M.C., Hidalgo, A.E., Morales, A., Domezain, J. and Sanz, A. 2008. Effect of starvation and refeeding on digestive enzymes activities in Sturgeon (Acipensernaccarii) and Trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology, 149:* 420-425.

Gaber, M. M., Omar, E. A., Abdel-Rahim, M., Nour, A. M., Zaki, M.A. and Srour, T. M. 2012. Effects of stocking density and water exchange rates on growth performance of Tiger Shrimp, *Penaeus semisulcatus* cultured in earthen ponds. *Journal of Aquaculture and Research Development, 3(6):* 1-5.

Guzman, M. L., Rossi, R. M., Lilliana, K., Derick, R. P., Dianna, S. H., Xiaojie, L. and Craig, T. J. 2005. The sesquiterpene lactones parthenolide induces apoptosis of human acute myelogenous leukemia stem and progenitor cells. *Blood, 105(11):* 4163-4169.

Hidalgo, M. C., Urea, E. and Sanz, A. 1999. Comparative study of digestive enzymes in fish with different nutritional habits. Proteolytic and amylase activities. *Aquaculture. 170:* 267-283.

Keay, L. and Wildi, B. S. 1970. Proteases of the genus Bacillus II. Alkaline proteases. *Biotechnology. Bioengineering, 12:* 213-249.

Kuz'mina, V. V. and Kuz'mina, Y. G. 1990. Level of total proteolytic activity in some species of fish from the Volga barin. *Journal of Ichthyology, 30:* 25-35.

Kuz'mina, V. V. and Smirnova, Y. G. 1992. Distribution of alkaline phosphatase activity along the length of the

intestine of freshwater teleosts. *Journal of Ichthyology, 32:* 1-9.

Lundstedt, L. M. Melo, J. F. B. and Moraes, G. 2004. Digestive enzymes and metabolic profile of *Pseudoplatystoma corruscans* (Teleostei: Siluriformes) in response to diet composition. *Comparative Biochemistry and Physiology, 137B:* 331-339.

McCue, P. and Shetty, K. 2004. Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase in vitro. *Asia Pacific Journal of Clinical Nutrition, 13:* 101-106.

McKee, T. and McKee, J. R. 2003. *Biochemistry*, *3rd Edition.* Boston: McGraw-Hill.

Metin, K. and Akpýnar, M. A. 2000. *Oncorhynchus mykiss* Walbaum, 1792 karaciðer lipaz enziminin bazý kinetik özellikleri. In Turkish. *Turkish Journal of Biology, 24:* 489-502.

Meyer, G. and Fracalossi, D. M. 2004. Protein requirement of Jundiá fingerlings, Rhamdia quelen, at two dietary energy concentrations. *Aquaculture, 240:* 331-343.

Sunde, J. Eiane, S. A., Rustad, A., Jensen, H. B., Nygard, E., Venturini, G. and Rungruangsak-Torrissen, K. 2004. Effect of fish feed processing conditions on digestive protease activities, free amino acid pools, feed conversion efficiency and growth in Atlantic salmon (Salmo salar L.). *Aquaculture Nutrition, 10:* 261-277.

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