International Journal of Innovative Studies in Aquatic Biology and Fisheries
Volume 5, Issue 3, 2019, PP 16-20
ISSN 2454-7662 (Print) & ISSN 2454-7670 (Online)
DOI: http://dx.doi.org/10.20431/2454-7670.0503003
www.arcjournals.org

Enzymes Activities in Two Sizes of Clarias. Gariepinus Exposed to on Farm Stress

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Abstract: *The enzyme activities in the plasma of C. gariepinus juveniles and adults subjected to handling stress in the laboratory was carried out. Blood samples were collected from the fish and analyzed with standard methods. Results obtained indicate that Alkaline phosphatase (ALP) and Alanine transaminase (ALT), and Aspartate transaminase (AST) were significantly elevated (P<0.05) in all the handling procedures under consideration. These alterations were more pronounced in the fish exposed to starvation and sorting. An indication that fish exposed to these handling activities were more stressed than fish in procedures.*

Keywords: *Catfish, Enzymes, Aquaculture, Stress, Immune system.*

1. INTRODUCTION

The health issues have become a very important aspect of cultured animals, more importantly in farmed fish (Kestin *et al.;* 1995; Hastein, 2004). Stress in farmed fish is a very vital aspect to the health and productivity of fish because it affects their growth and can also lead to abnormal behaviour and depression in their immune system (Wedemeyer *et al*., 1996, Ashley, 2007). Wedemeyer *et al*. (1996) opined that environmental stress is inevitable in the life of fish, particularly those in intensive culture. Cultured fish are exposed to management practices that can lead to stress (Gabriel and Akinrotimi, 2011). Aquaculture operations such as handling, transportation or confinement have been reported to have some degrees of stress on fish (Akinrotimi et al., 2011). Some studies have been carried out to examine the response of fish to some stressors in aquaculture practices (Barton, 2000; Acerete *et al.*, 2005). In nature, normally fish are exposed to natural things that can lead to stress but more stress are been caused to fish in artificial conditions such as aquaculture or in the laboratory. According to Davis *et al*., (2002), human activities is one factor that have led to an increased pollution of our natural water bodies and is one category of things that causes stress in the environment. Fishes respond to this stress with all levels of organization (Hur *et al*.; 2007), from the cells to the individual organism (Barton and Iwama, 1991) and also extend to the general population (Barton, 2002).

Enzymes are biochemical macromolecules that control the metabolic process of organisms, thus a slight variation in enzyme activities would affect the organism metabolic integrity (Roy, 2002). They are indispensable for signal transduction and cell regulation, often via kinases and phosphates. The activities of alkaline phosphatase, alanine aminotransferase, aspartate and aminotransferase, are useful marker enzymes of damage to the system of the fish (Akanji *et al.*, 1993; Akinrotimi *et al*., 2013). Maintenance of internal homeostasis through biochemical processes in the Kreb's cycle may be reflected by variation in the levels of the enzymes AST, ALT, ALP in the plasma of the fish, triggered by cellular damage in the functional organs such as liver, heart, gill, muscles and kidney as they are generally found in the tissues of these organs (Gabriel *et al*., 2010). Both serum AST and ALT activities in the cell of an organism are raised when infections affects cell integrity (Gabriel and George, 2005).

Moreover, evaluation of blood biochemistry was considered as a useful tool for the diagnosis of diseases and assessing the physiological status of fish (Gabriel *et al*., 2011). This is because stress response in fish is characterized by biochemical and physiological changes which may be manifested in changes induced by culture systems (Tiwari and Singh, 2004; Akinrotimi *et al*., 2012). The disruption of the biochemical and physiological integrity is assessable by the changes in the enzyme activities in

plasma of the fish (Van Der Oost *et al.*, 2003). Hence, the present study was carried out to evaluate the effects of different handling aquaculture procedures on the plasma enzymes of African Catfish *C. gariepinus* which is widely cultured in different parts of Nigeria.

2. MATERIALS AND METHODS

2.1. Experimental Process

This study was carried out at the African Regional Aquaculture Centre (ARAC) Aluu, Port Harcourt, Rivers State. A total of 120 *C.gariepinus* comprising 60 adult size (mean total length 32.81cm ± 3.01SD; mean weight 972.42g ± 30.01SD) and 60 juvenile size (mean length 15.02±4.77; mean weight 309.01±11.44) were procured from African Regional Aquaculture Centre (ARAC) Aluu, Port Harcourt, Rivers State, Nigeria. They were held in circular plastic aquaria in the hatchery unit before they were been subjected to the selected on-farm-procedures (treatments). All the experimental procedures were carried out in the hatchery unit the same day.

The fish were subjected to some common selected handling procedures in aquaculture that result in stress in fish, namely: Sorting; Overcrowding; Starvation. Blood samples were collected from the kidney of the *C.gariepinus* by puncturing it using a hypodermic needle. Blood samples were collected from three fish at rest state before and after exposure to these on-farm stresses.

2.2. Determination of Alkaline Phosphatase (ALP)

The concentration of alkaline phosphatase in plasma was determined spectrophotometrically using RANDOX diagnostic kit on RX Monza analyzer made by Randox laboratories limited, United Kingdom (model RX MONZA AP 542). This method was carried out according to Rec *et al*., (1972) and Englehardt, (1970). Three cuvettes marked Macro, Semi micro and Micro was arranged in a rack. 0.05ml of plasma sample was pipetted into Macro cuvette, 0.02ml sample was pipetted into semi-micro and 0.01 ml sample was pipetted into micro cuvette. 3.00ml reagents were pipetted into macro cuvette, 1.00ml of the reagents were pipetted into semi-micro cuvette and 0.50ml of the reagents was pipetted into the micro cuvette. The solution was mixed and the initial absorbance was read at Hg 405 nm at a temperature of 37^0C. It was read again after 1, 2, and 3 minutes. (Timer was set to run simultaneously).

ALP concentration was calculated using the following formular:

$$U/l = \frac{2760 \times \text{Absorbance 405 nm}}{\text{Minute}}$$

2.3. Determination of Aspartate Aminotransferase (AST)

The concentration of Aspartate aminotransferase in plasma was determined spectrophotometrically using RANDOX diagnostic kit on RX Monza analyzer made by Randox laboratories limited, United Kingdom (Model RX MONZA AS 101). This method was carried out according to Reitman *et al*., (1975) and Schmidt *et al*., (1963). AST was measured by monitoring the concentration of Oxaloacetate hydrazone formed with 2,4dinitrophenylhydrazine.

Two test tubes were labeled blank (B) and sample (S). In the reagent blank test tube was pipetted 0.5ml of buffer (reagent 1) followed by 0.1ml distilled water, while the sample test tube labeled (S) was pipetted 0.1ml plasma sample and 0.5ml of buffer. The mixture was incubated for exactly 30 minutes at 37^0C. Later 0.5 ml of 2,4-dinitrophenylhydrazine (reagent 2) was pipetted into the two test tubes, mixed and allowed to stand for exactly 20minutes at 25^0C. Later, 5.0 ml of Sodium hydroxide was pipetted into the two test tubes. The solution was mixed and the absorbence of the sample was read at 546 nm against the reagent blank after 5 minutes. The activity was extrapolated from the standard curve.

2.4. Determination of Alanine Aminotransferase (ALT)

Alanine amino transferase was measured spectrophotometrically using Randox diagnostic kit (Model AL100) according to the method of Reitman et al., (1957) and Schmidt et al., (1963). This was done by monitoring the concentration of Pyruvate hydrazone formed with 2,4 dinitrophenylhydrazine. Two test tubes were labeled Reagent blank (B) and Sample (S). 0.5ml of 100 mmol/l phosphate buffers and 0.5 ml of 200 mmol of L- alanine and 0.1 ml of distilled water was pipetted into reagent blank test tube. 0.1 ml of plasma sample, 0.5 ml of 100 mmol/l phosphate buffers and 0.5ml of 200 mmol of L-alanine was pipetted into test tube (S). The solution in the three tubes was mixed, incubated for exactly 30 minutes at 370C. Later, 0.5ml of 2.0mmol/l 2,4-dinitrophenylhydrazine was pipetted into reagent blank tube and sample test tubes. The solution was mixed, incubated for exactly 20 minutes at 250C. Lastly, 5.0 ml of Sodium hydroxide was pipetted into reagent test tube and sample test tube. The solution was mixed and

the sample absorbance was read at 578 nm against the reagent after 5 minutes. The activity was extrapolated from the standard curve.

2.5. Statistical Analysis

The data were collated and analyzed using one-way analysis of variance, (ANOVA) at 5% level of significance. Post-hoc comparison of significance of variance results gotten from ANOVA was done using DMRT (Duncan Multiple Range Test) tests. These analyses were carried out based on a computer programme SPSS 10.0.

3. RESULTS

The enzyme activities in the plasma of *C. gariepinus* juveniles exposed to different on farm procedures is presented in Table 1. The highest enzyme activities were observed in fish exposed to sorting, and closely followed by the one exposed to starvation. However, AST and ALT were within the same range in overcrowding and control values. The values of ALP, ALT and AST in adult sizes of *C.gariepinus* subjected to handling stress were elevated in all the farming procedures compared to the control values with the highest values of 488.02±42.02, 32.02±1.33 and 59.66±6.51U/l for ALP, ALT and AST respectively (Table 2).

Table1. *Enzymes in Plasma Juvenile Sizes of C. gariepinus Exposed to Some Handling Procedures (Mean ± SD)*

Handling Procedures	Enzymes (U/I)		
	ALP	ALT	AST
Control	238.89±34.42^{a}	11.33±2.08^{a}	23.33±2.08^{a}
Starvation	400.64±31.48^{c}	13.33±1.34^{a}	43.33±4.04^{b}
Overcrowding	383.40±45.60^{b}	15.09±1.34^{a}	32.66±4.04^{b}
Sorting	409.40±45.60^{c}	25.89±1.09^{b}	43.66±4.61^{b}

Means within the Same Column with Different Superscripts Are Significantly Different (P > 0.05)

Table2: *Enzymes in Plasma Adult Sizes of C. gariepinus Exposed to Some Handling Procedures (Mean ± SD)*

Handling Procedures	Enzymes (U/I)		
	ALP	ALT	AST
Control	308.77±41.092^{a}	14.02±2.018^{a}	28.92±2.21^{a}
Starvation	456.91±30.32^{b}	16.44±1.37^{a}	53.77±3.79^{b}
Overcrowding	403.21±33.01^{b}	18.88±1.92^{a}	42.66±5.67^{b}
Sorting	488.02±42.02^{c}	32.02±1.33^{b}	59.66±6.51^{b}

Means within the Same Column with Different Superscripts Are Significantly Different (P > 0.05)

4. DISCUSSION

In this study, the enzymes under consideration are very vital and are useful biomarker that reveals intricate cellular damage long before revealing the structural damage by some standard techniques (Mgbenka *et al*., 2005). The activities of the enzymes (alkaline phosphatase, alanine aminotransferase, aspartate and aminotransferase), considered in this study, are useful marker in revealing damaged liver and kidney (Akanji *et al.*, 1993). These biochemical changes try to maintain equilibrium in the presence of stress. A shift in the activities of enzymes from the control is also used as a relevant stress indicator. A major biochemical response to the effect of stress in fishes is the elevation of a number of enzymes (Akinrotimi et al., 2007). In this study exposure of *C. gariepinus* to different on farm procedures generally resulted in elevation of these enzymes when compared to the control values. In this study AST activity was elevated in the muscles probably to enable the fish cope with the energy demand during stress condition. Similar findings, suggest that this energy demand could be satisfied through amino acid. ALP activity and ALT increased in the plasma of exposed fish. Alanine transaminase (ALT, Aspartate transaminase (AST) and Alkaline phosphate (ALP) are important enzymes giving indication for liver function through controlling transferring amino group function of alpha amino acids to alpha keto acids. Large amount of ALT and AST are released into animal blood, mostly during liver cell damage (Thomas *et al.*, 1999).

Moreover, stressful aquaculture practices have been reported to affect enzyme profiles in fish (Akinrotimi *et al*., 2012). Nte *et al*. (2018) observed that increase in ALT, ALP and AST activities in

fish stressed by low DO and high stocking density. They reported that elevation of tese enzymes may reflect the use of excess hydrocarbons from amino acids to supply energetic demands. The rise in ALT and AST in stressed fish may indicate use of dietary amino-acids for growth as well as compensatory for energy demand as a response to the stressor. These enzymes have no other known functions in the serum other than to provide information about hepatic state and disorders. These disorders could be as a result of injury or liver disease. The injury could be caused by reactive metabolites, resulting from xenobiotic metabolism in the liver (Akinrotimi *et al*., 2018). Also, the observed elevated levels of ALP may indicate an increase in the rate of phosphorylation and transport of molecules across the cell membrane, which may result to increased detoxification effects of the kidney and thus a possible stress on the kidney membrane that could cause cell injury (Luskova et al., 2002). The increases could also result in a shift in biosynthesis, mixed-function oxidase and energy metabolism pathways (George *et al*., 2017).

5. CONCLUSION

This study revealed that common on-farm procedures such as the ones studied in this study can lead to stress in fishes as a whole as evident in the elevation of these enzymes. Hence, this should be minimized as much as possible to reduce the effect of these procedures on the fish and this can be achieved through a better farm management practises.

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Citation: *Momoh M. Y, et.al, "*Enzymes Activities in Two Sizes of Clarias. Gariepinus Exposed to on Farm Stress*". International Journal of Innovative Studies in Aquatic Biology and Fisheries, 5(3), pp.16-20. http://dx.doi.org/10.20431/2454-7670.0503003*