OXIDATIVE STRESS BIOMARKERS AND LIVER FUNCTION PROFILE OF AFRICAN CATFISH JUVENILES TRANSPORTED BY OPENED SYSTEM

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Abstract:

This study assessed the effect of transportation on oxidative stress biomarkers (malondialdehyde (MDA), glutathione peroxidase (GPX) and protein (PRO)) and liver function profile (aspartate transaminase (AST) and alanine transaminase (ALT)) of African catfish juveniles transported for six hours in plastic jerry cans. The tested durations were 1hr, 3 hrs, and 6 hrs (three replicates each) and blood sample was collected immediately after transportation (day 0), three (day 3) and six (day 6) recovery days. MDA showed a significant decrease ($P < 0.05$) across the recovery periods with the highest and lowest MDA mean values observed in fish on Day 0 and day 6 respectively. However, the MDA mean values increased with increase transport duration. A significant increase ($P<0.05$) of GPX and PRO mean values were observed across the recovery periods and the transport durations as compared to the control group. The mean values of AST and ALT for both control group and transported fish decreased as the recovery day progressed. Each transport duration showed significant increase ($P<0.05$) among the recovery days with day 0 having the highest values while the lowest values were observed on day 6. From the result of this study, it is concluded that transportation may be considered a strong stressor to catfish juveniles and thus affect the health status of the fish.

Keywords: Catfish, Stress, Open system, Transport, Oxidative stress biomarkers, Liver function profile

Introduction

Stress is typically referred to as the general physiological response of an organism to threatening situations and challenges in the environment (Schreck and Tort, 2016). Stressful farming conditions can translate into reduced fish health and performance, and thus compromise fish welfare (Segner *et al.*, 2012). Handling and transport are some of the most common causes of acute and /or chronic stress in fish (Meinelt *et al.*, 2008) resulting to immune-suppression, physical injury or even death (Crosby *et al.*, 2006). Duration of stress influences the stress impact so fish should be moved as quickly and as efficiently as possible to minimize their stress (Noga, 2000).

Open system of transportation consists of water filled containers in which the basic requirements for survival are supplied continuously from outside sources. The simplest of these are small tanks, plastic containers, cans, buckets, bowls, boxes, calabashes, clay pots, trucks, vans, and many others. It is suitable for movement of fish within the farm for short distances and for periods not longer than 2 hour except for catfishes which can endure 5 hours (N.A.E.R.L.S., 2001). During transport, fish are exposed to multitude of stressors such as density changes, handling stress, water movement, noise, vibrations, and poor water conditions. Exposure to such stressors simultaneously or in rapid succession may induce severe physiological stress (Koolhaas *et al.*, 2011). Numerous chemical additives/anaesthetic (Velisek *et al.*, 2006), probiotics (Mohapatra *et al.*, 2013), non-iodized salt and palm oil (Idowu *et al.*, 2016) and oxygen (Orina *et al.*, 2014) are added to the transport water to alleviate several problems associated with transporting fish, the reduction in these stress factors during transportation lowers mortality and improves appearance (Crosby *et al.*, 2006).

Malondialdehyde (MDA) is the product of lipid peroxidation formed by lipoperoxidation reaction of oxygen free radicals, phospholipids in biofilm and lipoperoxidation of macro molecules such as side chains of polyunsaturated fatty acids and nucleic acids associated with enzymes and membrane receptors (Uner *et al.*, 2001). It is an important index to evaluate the degree of oxidative damage in cells (Mozhdeganloo *et al.*, 2015). glutathione peroxidase (GPX) could convert hydroperoxides into hydroxyl compounds with GSH as substrate and its main detoxification function is to terminate the propagation of the radical chain, thus protecting membranes from oxidative damage (Maharajan *et al.*, 2018). Increased GPx activity is associated with a compensatory response of SOD activities (Lee *et al.*, 2017a).

Changes in liver function profile are important toxicity indices (biomarkers) and have been used to assess the biochemical and physiological health of vital tissues and organs in fishes (Gabriel and George, 2005). Enzymes assays such as AST and ALT, are parts of standard laboratory tests to detect abnormalities in animals (Gabriel *et al.*, 2010; Ayalogu *et al.*, 2001; Simonato *et al.*, 2008) and their increase in the plasma indicates tissue injury or organ dysfunction (Shalaby and Abbassa, 2009). Changes in the activity of these enzymes resulting from toxicant or contaminant effects in various organs of fish have been reported (Mgbenka *et al.*, 2005; Ribeiro *et al.*, 2006; Ruas *et al.*, 2008). The change in serum protein content is a physiological response that use as indicators of fish health (Tabmasebi-Kohyam *et al.*, 2012). Negative impacts of

transport stress on serum total protein (TP) detected in several of fish species e.g., common carp, *Cyprinus carpio* L. (Dobikova *et al.*, 2009) and rohu, *Labeo rohita* (Pakbira *et al.*, 2015). Reduced serum TP may be result of modifying rates of protein synthesis in liver under stress condition. The liver is the chief organ in the synthesis and export of serum proteins (Wright and Anderson, 2001).

African catfish (*Clarias gariepinus*) is of great commercial importance because it is the most common fresh water fish widely consumed in Nigeria (Olaifa *et al.*, 2004). It is therefore a good model to study responses to handling and transportation stress.

Materials and Methods

Study Area

The acclimatization and recovery period were carried out at the Department of Marine Sciences, University of Lagos while the oxidative stress assay and liver function profile were carried out at Nigerian institute of medical research (NIMR), Yaba, Lagos state. The fish were transported within the school premises, University of Lagos Akoka, Lagos, Nigeria.

Weather Condition

The transportation was carried out at 11.00 am to 5.00 pm during the rainy season (September, 2021). The Weather conditions at the day of transportation were as follows; no rainfall, cloudy and ambient temperature 26^{o}C

Source of Fish and Acclimatization

Clarias gariepinus juveniles were obtained from a commercial fish farm in Lagos state, Nigeria. Fish were acclimatized for three days in concrete tanks filled with fresh water from tap. During this period, fish were fed to satiation with a commercial floating feed twice a day (9.00 am and 16.00 pm). The feeding of fish was stopped 24 h before transportation process.

Fish Transportation

Prior to transportation, the fish were exposed to handling and then divided into two groups; the control group (containing 45 fish distributed into three plastic tanks with a density of 15 fish per tank) which was not transported (stationed at the aquaculture unit), and the treatment group (containing 360 fish distributed into plastic kegs with a density of 40 fish per keg) which were exposed to transportation stress. The fish in the treatment group were distributed into 10 liter plastic kegs which were slightly cut opened at the top then filled with 7.5 liter of fresh water. The kegs were labeled according to the duration of transport as T1, T3, and T6 then were moved into a vehicle (Honda Odessy). The transportation was carried out in triplicate and upon return the transported fish were placed in labeled plastic tanks for recovery also in triplicate to the same density as the control group.

Sample Collection

Blood samples for transported fish and the control group were taken via the caudal vein and collected in sample bottles (lithium heparin bottles) using 2ml syringes at successive time intervals immediately after transportation (day 0), three days after transportation (day 3) and six days after transportation (day 6) to determine the oxidative stress parameters (Total protein, Glutathione peroxidase, and Malondialdehyde) and liver function profile (Aspartate transaminase and Alanine transaminase). Samples for each treatment group (T1, T3, and T6) and the control group were taken in triplicate.

Determination of Oxidative Stress Biomarkers

The assay for MDA was done by the method of Wright *et al.* (2003) with some modifications. The reaction mixture in a total volume of 2.1 ml contained 0.1 ml sample, 2.0 ml of (TCA 15%, TBA 0.37% and 0.25N HCl in ratio 1:1:1). All the test tubes were placed in a boiling water bath for a period of 15 minute. The tubes were allowed to cool and then centrifuged at 2500×g for 10 minute. The amount of malondialdehyde (MDA) formed in each of the samples was assessed by measuring the optical density of the supernatant at 535 nm. The results were expressed as the nmol MDA formed/gram tissue by using a molar extinction coefficient of 1.56×10^{5} M^{-1} cm^{-1}. With the help of this formula

$$\text{Lpo}= \frac{\text{Vol.of assay x O.D x } 10^{9}}{1.56 \text{ x} 10^{5} \text{ x } 10^{3} \text{ gm tissue}}$$

GPx was determined by the method of Ellman, (1959). Glutathione peroxidase (GPx) activity was measured by the addition of 0.2ml of 0.4M phosphate buffer pH 7.0, 0.1ml of 10mM sodium azide, 0.2ml of plasma, 0.2ml of glutathione salt (GSH) and 0.1ml of 0.2mM H_20_2. The mixture was incubated at 37^{o}C for 10mins. The reaction was arrested by 0.4ml of 10% TCA, and centrifuged. The supernatant was assayed for glutathione content by using Ellman's reagent.

Liver Function Profile Determination

AST and ALT were estimated by the Randox Laboratories Kit procedures. In this method, Aspartate transaminase (AST) catalyses the transfer of the amino group from aspartate to oxoglutarate. Aspartate is converted to oxaloacetic acid (OAA) which subsequently decarboxylated into pyruvate. Alanine transaminase (ALT) catalyses the transfer of the amino group from alanine to oxoglutarate. Alanine is converted directly to pyruvate. The pyruvate produced by the transamination reactions reacts with the chromogen 2,4 dinitrophenylhydrazine (DNPH) to give a brown coloured hydrazone whose intensity reflects the concentration of the oxoacids present. These reactions were carried out at alkaline PH and the brown coloured measured at 546 nm in spectrophotometer. The total plasma proteins were measured by using the standard Biuret method as described by Lawrence, (1986) 1.0ml of biuret reagent (test) and 1.0ml of blank reagent (reagent blank) was pipette into each sample test tubes, 0.02ml (20µl) of each sample to the test and 20µl water to the blank were added, a standard test tube was also set up for each batch and contains 20 µl of standard protein 1.0ml of biuret reagent, it was mixed and allowed to stand at room temperature for 30 minutes at 37^{o}C, the instrument was zeroed with the reagent blank solution and absorbance of the test and the standard were measured at 546 nm wavelength.

Data Analyisis

The oxidative stress biomarkers and liver function profile were subjected to one-way analysis of variance using SPSS version 23.0 and results were presented with means ± SD of three replicates. The Duncan multiple range test (DMRT), a post hoc test, was employed to investigate further significant changes across variables over days, with $P \leq 0.05$ considered statistically significant.

Results and Discussion

Results

Oxidative Stress Biomarkers of Fish Transported by Opened System

There was significant decrease across the recovery periods with the highest and lowest MDA mean values observed in fish immediately after transportation (Day 0) and on sixth recovery day (day 6) respectively. However, the MDA mean values increased with increase transport duration. A significant increase ($P \leq 0.05$) in mean values of GPx was observed across the recovery periods and the transport durations as compared to the control group (**Table 1**). The mean values of Protein shows a significant ($P \leq 0.05$) increase across the recovery periods for each of the transport durations as compared with the control group (**Table 2**).

The mean values of AST and ALT for both control group and transported fish decreased as the recovery day progressed. Each transport duration showed significant ($P<0.05$) increase among the recovery days with day 0 having the highest values while the lowest values were observed on day 6 (**Table 3**).

Table 1: Effect of stress transportation on Malondialdehyde (MDA) and Glutathione peroxidase (GPx) on transported fish

	Days	Control	1hr	3hrs	6hrs
MDA	0	$7.471^{A} \pm 0.350$	$11.503^{B} \pm 0.505$	$13.483^{B} \pm 0.507$	$16.695^{B} \pm 2.410$
	3	$9.154^{A} \pm 0.389$	$9.781^{AB} \pm 0.873$	$11.710^{A} \pm 0.387$	$15.275^{B} \pm 1.516$
	6	$8.705^{A} \pm 1.050$	$8.818^{A} \pm 0.039$	$8.952^{A} \pm 0.194$	$12.855^{B} \pm 1.048$
GPx	0	$7.476^{A} \pm 0.325$	$7.789^{A} \pm 1.055$	$7.838^{A} \pm 0.054$	$9.288^{A} \pm 0.240$
	3	$10.025^{B} \pm 0.175$	$10.542^{B} \pm 1.156$	$10.743^{B} \pm 0.376$	$11.240^{B} \pm 0.612$
	6	$10.475^{B} \pm 0.136$	$10.619^{B} \pm 0.570$	$11.777^{B} \pm 0.366$	$13.224^{B} \pm 0.908$

Values with different superscript in each column are statistically significant ($P < 0.05$).

Table 2: Effect of stress transportation on Protein levels on transported fish in opened system over recovery days

Days	Control	1hr	3hrs	6hrs
0	$36.467^{A} \pm 1.685$	$36.230^{A} \pm 1.022$	$39.230^{B} \pm 2.891$	$39.873^{B} \pm 0.425$
3	$38.100^{A} \pm 0.561$	$38.320^{A} \pm 0.283$	$39.897^{B} \pm 2.091$	$40.375^{B} \pm 0.546$
6	$41.290^{B} \pm 0.260$	$41.325^{B} \pm 0.696$	$43.055^{B} \pm 0.052$	$43.255^{B} \pm 0.118$

Values with different superscript in each column are statistically significant ($P < 0.05$).

Table 3: Effect of stress transportation on Aspartate transaminase (AST) and Alanine transaminase (ALT) on transported fish

	Day	Control	1hr	3hrs	6hrs
AST	0	$26.655^{A} \pm 2.110$	$67.185^{B} \pm 0.257$	$63.445^{B} \pm 1.689$	$92.190^{B} \pm 3.645$
	3	$22.675^{A} \pm 0.857$	$22.855^{A} \pm 0.569$	$34.360^{A} \pm 0.947$	$35.710^{A} \pm 2.142$
	6	$20.130^{A} \pm 1.207$	$22.045^{A} \pm 0.453$	$26.123^{A} \pm 0.538$	$34.017^{A} \pm 0.761$
ALT	0	$20.265^{B} \pm 1.296$	$27.253^{B} \pm 2.006$	$28.613^{B} \pm 0.910$	$29.390^{B} \pm 0.491$
	3	$8.467^{A} \pm 0.820$	$11.080^{A} \pm 0.323$	$11.680^{A} \pm 0.092$	$13.527^{A} \pm 0.300$
	6	$5.120^{A} \pm 0.629$	$5.507^{A} \pm 0.232$	$6.520^{A} \pm 0.139$	$7.413^{A} \pm 0.192$

Values with different superscript in each column are statistically significantly ($P < 0.05$).

Discussion

In the present study, a significant decrease in MDA activity was observed in both transported *C. gariepinus* juveniles and the control groups with significant increase in the recovery days. However, the mean values of MDA for transport durations increased with increase duration. The increased MDA values observed as the transport durations increase suggests that, the antioxidant defense system of *C. gariepinus* juveniles was activated to some extent by transportation but was insignificant to eliminate the large amount of ROS produced. The increased MDA observed in this study corroborates the report of Refaey and Li, (2018) which indicated rise in MDA content in transported Channel catfish. In another study, similar observations were reported in Zebrafish, large yellow croaker, and rainbow trout exposed to methyl-mercury (Mozhdeganloo *et al.*, 2015; Wu *et al.*, 2018; Strungaru *et al.*, 2018). The decreased MDA observed in this study is in agreement with the report of Vinagre *et al.* (2012) which observed low values of MDA in juvenile Seabass exposed to optimum temperature of 28°c. Also in the gills, liver and kidney of *C.*

punctatus exposed to effluents, a decrease in MDA was observed (Ahmad and Alib, 2013).

The present study observed significant increase in GPX level with increase in the recovery days and transport duration in transported *C.gariepinus* juveniles and the control group. Also, the GPX activities of transported fish on the third and sixth recovery days were not significantly different from that of the control group. The increased GPx activity is associated with a compensatory response of SOD activities (Lee *et al.*, 2017a) and this suggests a participation of the molecules in the detoxification of H_20_2 produced in the transported fish. This is in agreement with the observation by Tian *et al*. (2016) in which the exposure of yellow catfish to transport stress led to increase in the activities of GPx.

The mean values of Protein for both control group and transported fish increased with increase in the recovery days and transport duration. However, the protein levels of some transported fish were not significantly different from the control group. The increase could be attributed to the protein synthesis for utilization to meet high energy demand. The increase in the protein levels observed in this study suggests a recovery of *C. gariepinus* juveniles from stress caused by transport stressors. This observation agrees well with an increased in serum protein in *Channa punctatus* exposed to heavy metal loaded waste water and river polluted by thermal power plant effluent (Javid *et al.*, 2017; Javid and Usmani, 2015) also in the muscle of *O. niloticus* increase in total protein was reported (El-serafy *et al.*, 2013). Similarly, increase in total protein was also reported in air breathing catfish by Palanisamy *et al.* (2011). This is in contrast with other studies that showed decrease in serum total protein observed after transportation of several fish species (Dobsikova *et al.*, 2009; Pakhira *et al.*, 2015). Similar decrease in serum protein with increase in concentration of ivermectin in *C. gariepinus* was reported by Ogueji *et al.* (2020).

The mean values of AST for both control group and transported fish decreased across the recovery days but a significant increase was observed immediately after transportation (Day 0) across the transport durations. However, there were no significant differences among control group, third and sixth recovery days. The increase in AST suggest the impairment of normal liver function and this is in agreement with the report of Refaey and Li, (2018) in which the outcomes obtained on AST in Channel catfish exposed to transport stress were elevated. Similarly Saravanan *et al.* (2012) and Ogueji *et al.* (2017a) reported an increased AST in *C. gariepinus* juveniles exposed to Ibuprofen drug. The decrease in the activities of AST observed in the present study could be attributed to inhibition of the enzyme or a reduction in the rate of synthesis of the enzyme in the liver. This is in agreement with the observation of Mousa and Khattab, (2003) which revealed inhibition of AST activity in the liver of catfish after intoxication with dietary ochratoxin.

The mean values of ALT for the control group and transported fish decreased across the recovery days but increased with increase duration. However, the values for transported fish in transport durations showed no significant difference with the control group. The decrease in the activities of ALT observed in the present study suggests a recovery from impaired liver caused by transport stress and it could be attributed to inhibition of the enzyme or a reduction in the rate of synthesis of the enzyme in the liver. This is in agreement with the observation of Dobsikova *et al*. (2006) in which ALT significantly decreased after transportation of Common carp. The increase in the ALT activity observed in the present study could be attributed to the diversion of alpha-amino acid in the tricarboxylic acid (TCA) cycle as keto-acids to augment energy production and also suggest the impairment of normal liver function. This is in agreement with the increased observation in ALT in African catfish (Adesina *et al.*, 2017; Amin and Hashim, 2012; Ogueji *et al.*, 2017a; Saravanan *et al.*, 2012).

Conclusion

The result of this study clearly indicated that the transported *C. gariepinus* juveniles were stressed as a result of transportation requiring a recovery period of at least six days. However, some of the transported fish exhibited strong antioxidant defense system against transportation-induced oxidative stress in African catfish juveniles transported by opened system. This study therefore, postulates that, transportation is a significant stressor to African catfish juveniles.

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Conflict of Interest

The authors declare no conflict of interest.

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