Journal of Stress Physiology & Biochemistry, Vol. 19, No. 3, 2023, pp. 58-71 ISSN 1997-0838

ORIGINAL ARTICLE

Evaluation of transportation stress-induced changes in serum biochemistry of African catfish (*Clarias gariepinus*) transported in palm oil-treated water

Oscar Dahenji Kolndadacha [1], Raphael John Ogbe [2]*,
Elizabeth Buba [1], and Anastasia Aleji [1]

[1] *Department of Animal Health and Production, Federal University of Agriculture, Makurdi, Benue State, Nigeria.*

[2] *Department of Veterinary Physiology and Biochemistry, Federal University of Agriculture, P. M. B. 2373, Makurdi, Benue state, Nigeria.*

*E-Mail:raphael.ogbe@uam.edu.ng ORCID iD: 0000-0002-1512-9275

Received February 24, 2023

Background: A study was conducted to evaluate the serum biochemical and oxidative stress parameters of catfish (*Clarias gariepinus*) obtained from homestead ponds in Makurdi metropolis, Nigeria, in order to establish changes due to stress from transportation, following the pretreatment of transporting water with palm oil and aqua anti-stress. Serum samples were obtained from the blood collected from the fish a day before, just before loading the fish, immediately after journey, and 3 days post-transportation. The samples were used to analyze for some oxidative stress markers like malon dialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and other biochemical parameters such as; alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total cholesterol (TC) and triglyceride (TRG).

Results: There was a significant ($p<0.05$) increase in MDA levels of low dose palm oil-treated water (PA) group compared with the control (C) just before journey, while there was no significant ($p>0.05$) difference in MDA levels of PA group compared with the control after the journey. However, there was no significant ($p>0.05$) difference in the MDA levels of aqua anti-stress agent-treated water (A) group compared with the control, before the journey and after the journey. There was a significantly ($P<0.05$) elevated activities of ALT, AST and ALP in palm oil-pretreated water (PA and PB) groups when compared with the control, just before the journey and immediately after the journey. However, there was no significant ($p>0.05$) difference in the ALT, AST and ALP activities of aqua anti-stress agent-treated water (A) group when compared with the control, before the journey and after the journey. There was no significant ($p>0.05$) difference in the TC and TRG levels of A, PA and PB groups when compared with the control group, before the journey and after the journey. The ALT, AST and ALP activities of the fish transported in water treated with palm oil were markedly reduced at the end of the journey when compared with the levels just before the journey.

Conclusion: The findings of this study have shown that both aqua anti-stress agent and palm oil may improve the biochemical parameters of African catfish, which enhanced their responses to stress during the eight hour journey but the conventional anti-stress agent was more effective than palm oil.

Key words: Anti-stress, Catfish, Hepatotoxicity, Oxidative stress, Palm oil, Transportation

The persistent high demand for animal protein by the increasing human population, which is easily obtainable from fish, have made catfish to be raised and marketed outside the places where they are produced, which makes it a necessity to transport fish all over the world (Perez *et al*., 2002). *Clarias gariepinus* is one of the fish species that is cultured commonly in Africa, because of the advantage of its hardness which can withstand handling and stress (Eyo and Ezechie, 2004).The fish has been recognized to possess high growth rate, voracious feeding habit, tolerance to harsh aquatic condition and have the ability to manage low dissolved oxygen (DO) and utilizes both the DO and atmospheric oxygen (Okechi, 2004), right from the stage the fish could possess functionally developed accessory of the respiratory organ.

Transportation has been considered as one of the main causes of stress (Adenkola, 2008) which affects the production of fish adversely. Transportation of live fish is an inevitable phase in fish production, which has been recognized as a critical part (Adeyemo *et al*., 2009) leading to a stressful condition that may cause great loss. Fish may be moved at any life stage to cover short or long distance involving few to many hours of time. Proper handling and transportation is important to the success of a producer. Mishandling or stressing the fish during transit may result in mass mortality upon arrival or stress-related conditions shortly after delivery (Johnson, 1993). The goal of transporting fish is to move as many fish or weight of fish in as little water as possible, since the whole water cannot be moved, and to arrive the destination safely, with active and healthy fish. Activities like capture, sampling, handling and transportation from one locality or region to another are normally employed in intensive culture (Krieger-Azzolini *et al*., 1989; Narnaware *et al*., 1994) in aquaculture. The weight of fish that can be safely transported depends on many factors such as fish size, type of tanks, type of aeration, the duration of the journey, and water temperature (Johnson, 1993).

Fish transportation which involves counting, grouping, loading and unloading, shaking of vehicle, especially when traveling along rough road before arriving final destination, may affect the health or welfare of the fish (Refaey and Li, 2018). Stoskopf (1993) reported that any procedure is stressful to fish, and they exhibit most, if not all of the physiological and biochemical responses to stressors which are seen in mammals. Stress responses are generally considered adaptive in natural situation and total lack of stress would be an impossible goal. Metabolic disturbances, enzymatic dysfunction and many other malfunctions in fish may occur as a result of physiological changes caused by handling (Kurovskaya and Osadchaya, 1993) and/or transportation. Adenkola *et al*. (2009) reported that physical and psychic exertion occur during transportation of food producing animals, which disrupt their homeostasis and metabolism. As a result of the exertion, road transportation stress increases the activities of enzymes and hormones.

Handling and transportation of fish may cause physiological changes, which may lead to adverse events in the fish (Kurovskaya and Osadchaya, 1993). Living creatures have an antioxidant defense system which can neutralize the harmful effects of reactive oxygen species (ROS) including hydroxyl radicals, superoxide anion radicals, and hydrogen peroxide. The antioxidant enzymes are found in almost all tissues of vertebrates, and their activities are especially high in the liver, a major organ responsible for the transformation of ROS (Refaey and Li, 2018). The ROS are generated during normal metabolism and the amounts are well-controlled under normal physiological conditions (Ogbe *et al*., 2019). When undesirable elements enter the body, they undergo redox cycling and generate ROS. The body will then produce more antioxidant enzymes to get rid of the undesired ROS. This response is called induction of antioxidant enzymes (Eden, 2002). When the generation of ROS overwhelms the antioxidant defense (AD) system, damages to lipids and proteins occur, and this condition is referred to as oxidative stress (Refaey and Li, 2018). Oxidative stress, a pathological process relating to overproduction of ROS in tissues, is one important general mechanism of toxicity in many mammals (Momoh *et al*., 2019). Many organisms including fish have evolved mechanisms to counteract the impact of ROS. These include various antioxidant defense enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione S-transferase (GST) (Uzoma *et al*., 2015). Antioxidants play the role of attenuating the adverse environmental stress and stress-induced tissue damage. Malon dialdehyde (MDA) is one of the most common by-products of lipid peroxidation during oxidative stress (Adenkola *et al*., 2009). Generation of ROS is inevitable for aerobic organisms and in healthy cells it occurs at a controlled rate. Under oxidative stress, ROS production is dramatically increased, resulting in subsequent degradation of membrane lipids, proteins and nucleic acids. Thus, serum biochemical parameters may be useful tools to evaluate the health and/or stress condition of the fishes (Narnaware *et al*., 1994; Refaey and Li, 2018). Therefore, this study was designed to investigate the changes in biomarkers of oxidative stress and liver injury in catfish after transportation on road for 8 hours in transporting water, pretreated with local anti-stress (palm oil) and conventional anti-stress agents.

MATERIALS AND METHODS

Experimental fish and management:

A total of 180 adult African catfish (*Clarias gariepinus,* Burchell 1822) with mean weight (550 ± 18 g) and mean length (25 ± 4 cm) were bought and harvested with seine net measuring 10 x 3 meters, from a homestead fish pond in Makurdi metropolis, Benue State, Nigeria. The fish were acclimatized for 7 days (Davis, 1990; Oriakpono *et al.*, 2012) in 5 x 5 x 1 earthen pond, during which they were fed *ad libitum* at every time of feeding, 2 times a day with commercial fish pelleted feed (Coppens^{R} Int'l Helmond, Holland). Mortality during the period of acclimatization was 0% before the journey. The fish were fasted 24 hrs before the day and throughout the journey (NAERLS, 2001), to reduce the risk of regurgitation of food that may lodge in the gills of the fish, and fecal contamination of the water used for the journey.

Experimental Design

One hundred and eighty fish were distributed randomly into twelve 25-liter Jeri-cans containing 15 fish each in 15 liters of water at the stocking rate of approximately 2 kg/L of water (Falaye *et al*., 2012). These twelve Jeri-cans represent 3 treatments and control in triplicate, consisting of 45 fish per group.

Group 1 (Control): No anti-stress agent was added to the transporting water.

Group 2: A conventional anti-stress agent (Aqua anti-stress®) was added to the fish transporting water at a concentration of 1 g/L of water according to the manufacturer's instruction.

Group 3: A palm oil was added to the transporting water of this group, at a lower concentration of 1.5 ml/L (22.5 ml) to serve as an anti-stress agent.

Group 4: The transporting water of this group was treated with higher concentration of palm oil at 2.5 ml/L (37.5 ml), so as to act as an anti-stress agent.

All other conditions in the 3 treatments remain the same with that of the control. The fish were then transported for a period of 8 hours by road.

Blood Collection and Processing

Blood samples were collected as described by Schmitt *et al*. (2007) four times during this research, which include: 24 hours before, just before, immediately after the journey, and 3 day post- transportation. The fish was held by an assistant in a vertical position with the ventral part of the fish facing the person collecting the blood. The blood samples were collected from each fish one after another with sterile 5 ml syringe and 21 G needle. The needle was introduced into the ventral mid-line between the anal opening and the beginning of the anal fin to access the caudal vein beneath the vertebral column according to Schmitt *et al*. (2007). After successful access, 2 - 3 ml of blood was withdrawn from each fish and dispensed into non-heparinized sample bottles for the serum biochemical analysis. The blood was centrifuged at 4000 rpm for 10 minutes to obtain the serum which was decanted into sterilized cryo-vials, to determine the serum biochemical parameters (Ogbe *et al*., 2022).

Physicochemical analyses

Water quality parameters such as dissolved oxygen (DO), pH and Temperature (T^{o}), total dissolved solids and electrical conductivity of the transporting water were determined during the journey, at every 2 hours of the journey using digital multi aqua-parameter kit.

Biochemical analyses

The biochemical assays of serum markers of liver injury in fish such as ALT, AST, ALP, TC, and TRG were conducted, by following the procedures in the manual supplied by the reagent kits manufacturer, TECO Diagnostics Ltd, USA. The serum markers of oxidative stress and antioxidant status of fish such as MDA, SOD, CAT, and GPx were estimated, according to the procedures in the manual supplied by the reagent kits manufacturer, Randox Laboratories Ltd, UK.

Statistical analyses

Data were presented as mean ± standard error of mean. The data collected were analyzed by the one-way analysis of variance (ANOVA) using Graph Pad Prism statistical package version 5.03. The differences between the mean values were determined using Duncan's multiple range tests at 95% confidence interval ($p<0.05$). A total of forty five (45) fish per group were used, therefore (n) = 45.

RESULTS

Physicochemical parameters of the catfish transporting water

The trends of physicochemical parameters of the transporting water are presented in figure 1 (A-E). The fluctuations in the parameters observed were within the desirable range for catfish production. However, the pH of the control group was found to be highest at the 0, 4 and 6 hours into the journey, while the group added aqua anti-stress agent (A) was the second highest at 0 and 6th hours, but decreased to the lowest value at the 4th hour. All the other treatments maintained similar levels of pH (Figure 1A). There was a steady increase in the temperature of transporting water as the journey progressed but not far from each other, and the values were within the acceptable ranges (Table 1). The temperature of control group have higher values than the treatment groups throughout the journey except at 2 H where the group added aqua anti-stress agent recorded the highest. At the end of transportation (8th H), the control group has the highest temperature, while the treatment groups (A, PA, and PB) showed similar degrees of temperature (Figure 1B).

The dissolved oxygen (DO) in the transporting water at 0 H in the control group has the highest value compared to the two groups which palm oil was added to the transporting water at low and high concentrations, while the group added aqua anti-stress agent (A) had the lowest value at the beginning of the journey. There was generally a steady decrease as the journey progressed, reaching down to 1 mg/L at the end of the journey for all the groups. The transporting water which has no additive (control) had the highest dissolved oxygen at the take-off point (initial level, 0 H). The transporting water containing palm oil recorded a uniform level of DO throughout the journey, as it dropped uniformly from 4 mg/L to 1 mg/L for all the treatment groups at the end of the journey (Figure 1C). The total dissolved solids (TDS) increased steadily from 0 H to the highest level at the end of the 8th H of transportation. At 4 and 6th H, the groups added low concentration of palm oil (PA) and high concentration of palm oil (PB) maintained higher levels of TDS, while at 8th H the PA has TDS levels close to that of control (Figure 1D).

Electrical conductivity (EC) of transporting water used in the treatment groups was unstable throughout the journey. The electrical conductivity maintained a similar trend of steady increase as observed with the levels of TDS. The PA recorded the highest level of EC throughout the journey, although at the 4th and 6th H, PA and PB values of EC were the same (Figure 1E). However, all these changes in the physicochemical properties of water were not statistically significant ($p>0.05$), and remained within acceptable ranges (Table 1). There was no mortality recorded throughout the experiment, despite the fluctuations in these parameters, and the stress of transportation.

Changes in the markers of oxidative stress in catfish transported in water by road

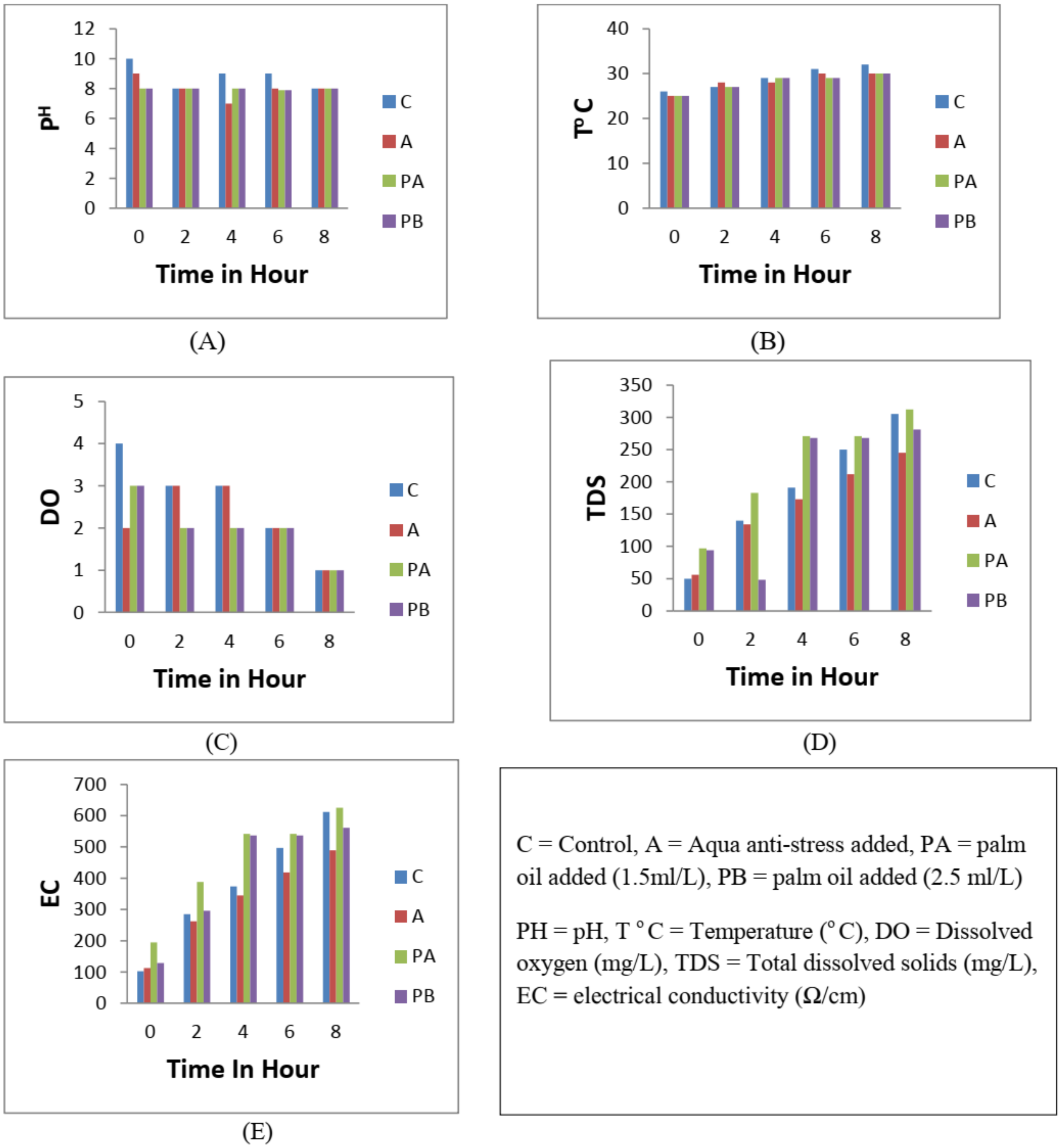
The changes in serum biochemical parameters or markers of oxidative stress in catfish transported in water are presented in Table 2. There was a significant ($p<0.05$) increase in MDA levels of the fish transported in PA-treated group compared with the control just before journey, while there was no significant (p>0.05) difference in MDA levels of this fish group when compared with the control immediately after journey and 3 days post-transportation. The levels of MDA in the fish transported in PA-treated water just before and immediately after the journey recorded the same values (1.48±0.04 nmol/mg p) which decreased to 1.35±0.16 nmol/mg p three days post-transportation. The group which was treated with high concentration of palm oil (PB) has higher values (1.53±0.06 nmol/mg p) compared to that of those in the control (1.37±0.08 nmol/mg p) three days post-transportation, though there was no significant (p>0.05) difference in the MDA levels of the fish in PB group compared with that of those in C group just before the journey, immediately after journey and 3 days post-transportation. The group treated with aqua anti-stress agent (A) maintained the lowest values throughout the period of transportation, and there was no significant (p>0.05) difference in the MDA levels of the fish in this group and those of the control (C) before and after the journey.

There was no significant (p>0.05) difference in SOD activities of the fish in groups A, PA and PB before the journey when compared with the control but the SOD activities of the fish in groups PA and PB were significantly (p<0.05) reduced when compared with the control immediately after the journey. There was no significant (p>0.05) difference in the SOD activities of the fish in A and PA groups compared with the C group 3 days post-transportation but the SOD activity (1.82±0.19 U/mg p) of the fish in group PB was significantly (p<0.05) lower than that of the control (2.27±0.04 U/mg p). There was no significant (p>0.05) difference in CAT activity of the fish in A group while there was a significant (p<0.05) decrease in the CAT activities of the fish in PA and PB groups when compared with the C group a day before the journey. There was no significant (p>0.05) difference in the CAT activities of groups A, PA and PB when compared with the control (C) just before the journey. Immediately after the journey, the CAT activities of the fish transported in water treated with PA (47.0±1.0 U/mg p) and PB (48.3±1.9 U/mg p) were significantly (p<0.05) reduced when compared with that of the C (54.2±1.2 U/mg p) but the CAT activity of the fish in A group (51.5±0.8 U/mg p) was not significantly (p>0.05) different from the C group. There was no significant (p>0.05) difference in the CAT activities of the fish in A, PA and PB groups when compared with C group 3 days post-transportation.

There was a significant (p<0.05) decrease in the GPx activities of the catfish transported in water treated with PA and PB while there was no significant (p>0.05) difference in the GPx activities of the catfish in A group when compared with the C group a day before the journey. The GPx activities of the fish in A, PA and PB groups did not significantly (p>0.05) differ when compared with C group just before the journey. Immediately after the journey, the GPx levels of the fish in PA (45.3±1.2 U/mg p) and PB (44.5±0.3 U/mg p) groups were significantly (p<0.05) reduced compared to the C group (49.8±1.1 U/mg p) but there was no significant (p>0.05) difference in GPx activities of the fish in A group (50.3±1.4 U/mg p) when compared with that of the fish in C group. Furthermore, there was no significant (p>0.05) difference in the GPx activities of the fish in A, PA and PB compared with those in the C group 3 days post-transportation.

Changes in the markers of liver injury in catfish transported in water by road

The changes in biomarkers of hepatic injury in catfish transported in water was reported in Table 3. The ALT activities of the catfish in PB (67.83±2.8 U/L) was significantly (p ≤ 0.05) higher, followed by the values of those in PA (56.67±3.19 U/L) compared with that of those in the control group (41.00±1.00 U/L) a day before the journey. There was a significant (p<0.05) increase in the ALT and AST activities of the catfish in the PA and PB groups compared with C just before the journey, immediately after the journey and 3 days post-transportation while the ALT and AST activities of those in the A group have no significant (p>0.05) difference with that of the C group throughout the same period. The AST and ALT activities of the catfish in A group recorded the lowest values in the treatment groups, which are significantly (p ≤ 0.05) lower than that of those in PA and PB groups, just before the journey, immediately after and 3 days after the journey but not significantly different (p ≥ 0.05) from the control group.



(A) (B) (C) (D) (E)

C = Control, A = Aqua anti-stress added, PA = palm oil added (1.5ml/L), PB = palm oil added (2.5 ml/L)

PH = pH, T °C = Temperature (° C), DO = Dissolved oxygen (mg/L), TDS = Total dissolved solids (mg/L), EC = electrical conductivity (Ω/cm)

Figure 1. Physicochemical properties of water used for transporting fish throughout the journey

Table 1. Desirable ranges of physicochemical properties of water used for African catfish production

Parameter	Acceptable range	Reference
pH	5.5 - 10.0	Stone and Thormford (2003)
Temperature (°C)	20 - 32	Keremah *et al*. (2014)
Dissolved oxygen (mg/L)	1 - 5 (saturation)	Fafioye *et al*. (2005)
Total dissolved solids (mg/L)	500	Keremah *et al*. (2014)
Electrical conductivity (Ω/cm)	20 - 1500	Keremah *et al*. (2014)

Table 2. Changes in the markers of oxidative stress in catfish transported in water by road

Parameters	Treatment groups	A day before journey	Just before the journey	Immediately after journey	3 days after the journey
MDA (nmol/mg p)	C	1.05 ± 0.09^{b}	1.18 ± 0.09^{b}	1.30 ± 0.06^{a}	1.37 ± 0.08^{a}
	A	1.04 ± 0.06^{b}	1.12 ± 0.04^{b}	1.27 ± 0.02^{a}	1.30 ± 0.05^{a}
	PA	1.3 ± 80.02^{a}	1.48 ± 0.04^{a}	1.48 ± 0.04^{a}	1.35 ± 0.16^{a}
	PB	1.30 ± 0.03^{a}	1.23 ± 0.04^{b}	1.43 ± 0.15^{a}	1.53 ± 0.06^{a}
SOD (U/mg p)	C	2.40 ± 0.08^{a}	2.33 ± 0.04^{a}	2.22 ± 0.07^{a}	2.27 ± 0.04^{a}
	A	2.00 ± 0.38^{a}	2.38 ± 0.04^{a}	2.28 ± 0.03^{a}	2.27 ± 0.09^{a}
	PA	2.02 ± 0.04^{a}	1.98 ± 0.16^{a}	1.92 ± 0.02^{b}	2.27 ± 0.02^{a}
	PB	2.07 ± 0.08^{a}	2.00 ± 0.36^{a}	1.82 ± 0.09^{b}	1.82 ± 0.19^{b}
CAT (U/mg p)	C	52.17 ± 0.88^{a}	50.17 ± 0.44^{ab}	54.17 ± 1.17^{a}	52.33 ± 1.20^{a}
	A	53.00 ± 0.58^{a}	51.83 ± 0.88^{a}	51.50 ± 0.76^{ab}	53.00 ± 0.24^{a}
	PA	45.58 ± 0.60^{b}	47.67 ± 1.36^{b}	47.00 ± 1.04^{c}	49.50 ± 2.08^{a}
	PB	48.33 ± 1.89^{b}	49.67 ± 1.67^{ab}	48.33 ± 1.92^{bc}	49.50 ± 2.08^{a}
GPx (U/mg p)	C	46.83 ± 1.59^{a}	48.33 ± 0.60^{a}	49.83 ± 1.09^{a}	47.83 ± 1.20^{a}
	A	50.00 ± 1.26^{a}	45.83 ± 3.00^{a}	50.33 ± 1.36^{a}	48.50 ± 0.29^{a}
	PA	44.33 ± 0.88^{b}	44.17 ± 1.88^{a}	45.33 ± 1.17^{b}	47.67 ± 1.64^{a}
	PB	43.50 ± 1.15^{b}	45.96 ± 0.58^{a}	44.50 ± 0.29^{b}	45.00 ± 2.02^{a}

Values are means ± SEM, n = 45; Different alphabet superscript in a column indicates significant difference ($p<0.05$); MDA: Malon dialdehyde, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase, A: Group aqua anti-stress agent was added, PA: Group palm oil was added (low concentration, 1.5ml/L), PB: Group palm oil was added (high concentration, 2.5ml/L), C: control.

Table 3. Changes in biomarkers of hepatic injury in catfish transported in water by road

Parameters	Treatment groups	A day before journey	Just before the journey	Immediately after journey	3 days after journey
ALT (U/L)	C	41.00 ± 1.00^{c}	48.17 ± 1.45^{c}	51.17 ± 1.88^{c}	44.83 ± 0.44^{b}
	A	46.00 ± 0.29^{c}	46.50 ± 1.73^{c}	54.17 ± 2.33^{c}	43.33 ± 2.09^{b}
	PA	56.67 ± 3.19^{b}	80.67 ± 1.45^{b}	76.50 ± 0.29^{a}	68.17 ± 3.66^{a}
	PB	67.83 ± 2.80^{a}	87.67 ± 2.63^{a}	63.67 ± 3.18^{b}	73.50 ± 5.00^{a}
AST (U/L)	C	38.83 ± 0.62^{b}	46.50 ± 1.00^{b}	47.33 ± 0.93^{c}	43.17 ± 1.69^{b}
	A	43.67 ± 1.42^{b}	42.33 ± 1.01^{b}	49.50 ± 2.18^{c}	41.00 ± 1.26^{b}
	PA	59.83 ± 6.62^{a}	76.33 ± 0.88^{a}	73.00 ± 0.29^{a}	63.33 ± 3.42^{a}
	PB	63.53 ± 2.80^{a}	78.00 ± 4.80^{a}	61.83 ± 2.68^{b}	70.00 ± 4.54^{b}
ALP (U/L)	C	79.67 ± 3.28^{b}	82.83 ± 0.93^{b}	73.33 ± 1.30^{b}	79.67 ± 1.92^{b}
	A	77.00 ± 1.60^{b}	80.83 ± 2.52^{b}	75.33 ± 0.73^{b}	73.50 ± 1.00^{b}
	PA	107.67 ± 8.08^{a}	125.17 ± 2.62^{a}	99.17 ± 12.92^{a}	116.67 ± 6.66^{a}
	PB	116.50 ± 3.25^{a}	125.50 ± 3.25^{a}	119.17 ± 2.62^{a}	117.50 ± 4.90^{a}
TC (mmol/L)	C	2.18 ± 0.09^{a}	1.10 ± 0.13^{a}	2.33 ± 0.02^{a}	2.35 ± 0.06^{a}
	A	2.30 ± 0.03^{a}	0.93 ± 0.03^{a}	2.27 ± 0.08^{c}	2.35 ± 0.08^{a}
	PA	2.37 ± 0.02^{a}	1.08 ± 0.06^{a}	2.55 ± 0.08^{a}	2.30 ± 0.10^{a}
	PB	2.35 ± 0.09^{a}	0.95 ± 0.10^{a}	2.42 ± 0.09^{ab}	2.45 ± 0.10^{a}
TRG (mmol/L)	C	1.57 ± 0.22^{a}	2.33 ± 0.09^{a}	0.78 ± 0.08^{a}	1.00 ± 0.10^{a}
	A	0.93 ± 0.04^{a}	2.18 ± 0.09^{a}	0.87 ± 0.10^{a}	1.47 ± 0.38^{a}
	PA	2.28 ± 0.12^{a}	1.98 ± 0.38^{a}	0.88 ± 0.09^{a}	0.92 ± 0.12^{a}
	PB	1.35 ± 0.10^{a}	2.28 ± 0.12^{a}	1.03 ± 0.10^{a}	1.12 ± 0.12^{a}

Values are means ± SEM, n = 45; Different superscript alphabet in a column indicates significant difference ($p<0.05$). ALT: Alanine transaminase (U/L), AST: Aspartate transaminase (U/L), ALP: Alkaline phosphatase (U/L), TC: Total cholesterol (mmol/L), TRG: Triglyceride, C: Control (Nothing was added to water), A: Group aqua anti-stress agent was added to water, PA: Group palm oil was added to their water (low concentration, 1.5ml/L), PB: Group palm oil was added to their water (high concentration, 2.5ml/L).

The ALP activities of the catfish in PA and PB groups were significantly ($p<0.05$) higher than that of those in the C group just before the journey, immediately after the journey, as well as 3 days post-transportation while the ALP activity of the catfish in A group was not significantly ($p>0.05$) different from the C group throughout the period of the experiment i.e. before and after transportation. There was no significant ($p>0.05$) difference between the ALP activities of the catfish in the group water was pretreated with low concentration of palm oil (PA) and high concentration of palm oil (PB). There was no significant ($p>0.05$) difference in the TC levels of the catfish in A, PA and PB a day before journey, just before the journey and 3 days post-transportation when compared with that of those in the C group. However, immediately after the journey, the level of TC in the catfish transported in water treated with A group was significantly ($p<0.05$) reduced when compared with C while the TC levels of PA and PB were not significantly ($p>0.05$) different from the C group. Furthermore, there was no significant ($p>0.05$) difference in the TRG levels of catfish transported in water treated with A, PA and PB groups when compared with the C group throughout the period of the experiment. i.e. a day before the journey, just before the journey, immediately after the journey, and 3 days after the journey.

DISCUSSION

The physicochemical properties of the transporting water evaluated for all the treatments (A, PA and PB) and control are within the acceptable ranges required for the survival of African catfish, especially as the fish has a wide tolerance for temperature, low DO, salinity etc., according to Keremah *et al*. (2014) and Fafioye *et al.* (2005). The changing pattern of physicochemical properties of the transporting water recorded in this study are in agreement with Falaye *et al*. (2012), as well as with the study by Crosby *et al.* (2006) who reported that, as the duration of transportation increases, the temperature and pH increases, and the dissolved oxygen content of the water decreases. The no significant difference in all the physicochemical properties of the transporting water may indicate that all the additives did not have adverse effect on the water and the fish survival. These findings are in agreement with the study by Abalaka (2013) who reported that the physicochemical parameters of water from earthen ponds taken from Tiga dam were not different from concrete ponds. The zero mortality observed in this experiment may be attributed to the fact that the catfish has high tolerance to changes in physicochemical properties of water (Ozmen *et al*., 2006). The catfish in the treated water containing palm oil had a better activity (calmness) than the control and the group added aqua anti-stress agent, thus this conducive environment may have contributed to the physical appearance of the fish. This is in agreement with Falaye *et al.* (2012) who reported that the physical appearance of the fish transported in palm oil-treated water was better because the palm oil maintains the water pH and fish appearance, resulting in high survival of the fish.

The determination of serum biochemical indices is a valuable approach for ascertaining the health status of farm animals because these biomarkers will provide reliable information on metabolic disorders, and the oxidative stress status of the animals (Bahmani *et al.,* 2001). The antioxidant defense mechanism to counteract the impact of ROS is found in many mammalian species including aquatic animals such as fish. In aquatic animals, the generation of reactive oxygen species (ROS) activates stress pathway in tissues and the subsequent changes in antioxidant enzymes activities (Refaey and Li, 2018). The antioxidant profiles of animals have been used for long as biomarkers of oxidative stress (Owumi and Dim, 2019). Stress may cause chemical changes in mucus, blood or plasma which decrease the effectiveness of the fish under such conditions. Most stressful situations cause a quick increase in lipid peroxidation, leading to induced oxidative stress (Famurewa *et al*., 2022). Substances such as conventional anti-stress agent, non-iodide salt, anesthetic agents, and palm oil have been used to ameliorate stress, by maintaining the physical appearance of catfish, according to a report by Falaye *et al*. (2012). Palm oil possesses phytonutrients such as carotenoids, sterols, vitamin E and some water soluble antioxidants, giving it an edge over other oils in reducing the risk of a variety of disease processes. Thus, the use of proper concentration of palm oil for the treatment of water for transporting adult catfish will go a long way in reducing fish loss during transportation, due to reduced effect of oxidative stress, and the prevention of toxicity of the liver (Eden, 2002).

The levels of MDA, CAT and SOD are often monitored in aquatic and terrestrial animals to determine the extent of stress (Esiegbe *et al*., 2013). The malon dialdehyde (MDA) is used as an indicator of cellular ROS and a sign of lipid peroxidation, resulting in cellular injury. Thus, it is regarded as the most commonly used lipid marker of oxidative stress. The incomplete reduction of oxygen forms ROS, which includes superoxide anion radical ($O_2^{.-}$), hydrogen peroxide (H_2O_2) and the hydroxyl ion radical ($OH^{.-}$). The MDA is a product of lipid peroxidation, so an elevation of MDA level in animal tissues is an indication of increased lipid peroxidation and oxidative stress (Ogbe *et al*., 2022). The rise in MDA levels of the fish transported in water treated with low concentration palm oil, just before the journey, in the current study, may be due to lipid peroxidation and oxidative stress caused by ROS, as a result of elevated oxidation by molecular oxygen (O_2), which may be attributed to an increased cellular oxidation of food molecules in the fish. The no significant difference in MDA levels in the fish transported in low concentration palm oil-treated water and the control or reference medium after the journey, may be attributed to the capacity of palm oil to reduce lipid peroxidation and oxidative stress at an appropriate concentration in water during the transportation. Furthermore, the MDA levels of the fish transported in low concentration palm oil-treated water remain relatively stable throughout the 8 hours journey but the MDA levels of the fish transported in water treated with high concentration palm oil, the control water, and aqua anti-stress agent-treated water were increased during the same period, which may indicate that the low concentration of palm oil used provided appropriate amount of phytonutrients required by the fish for the mitigation of lipid peroxidation and oxidative stress. This finding is in agreement with Ogbe *et al*. (2019) who earlier reported that certain phytochemicals have protective effect against oxidative stress-induced hepatic tissue damage in animals, and Eden (2002) who showed that palm oil contains β-carotene and vitamin E, which are potent antioxidants that scavenge ROS in the tissues of animals.

The cellular concentration of ROS depends on the production and/or removal of the cellular antioxidants. Three important antioxidant enzymes necessary for healthy life of all oxygen-metabolizing cells are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (Owumi and Dim, 2019). Low level of ROS is necessary in regulating several key physiological mechanisms in fish, but elevated level is injurious to cells, thus SOD as an antioxidant enzyme plays an important role of scavenging these harmful oxidative biomolecules, so as to prevent their accumulation in the animal tissues. The SOD catalyzes the reduction of superoxide anion radical to form hydrogen peroxide and oxygen (Vasudevan and Sreekumari, 2007). The significant reduction in SOD activity of the fish transported in high and low concentrations palm oil-treated water immediately after the journey, may indicate the depletion of this antioxidant enzyme, which may be attributed to an increased utilization of the enzyme in the antioxidant defense system of the fish. However, the significant reduction of SOD activity of only the fish transported in high concentration palm oil-treated water, 3 days after the journey, may indicate that the accumulation of ROS, with the resultant oxidative stress was greater in the fish transported in this water than the low concentration palm oil-treated water. This may be attributed to the presence of required amounts of phytonutrients in the low concentration palm oil, which enhanced the antioxidant defense system of the fish and protected the antioxidant enzymes during the transportation. This is in accord with Ogbe *et al*. (2019) who reported that antioxidant defense status of animals was enhanced by phytochemicals, and Eden (2002) who reported that palm oil contains phytonutrients such as β-carotene and vitamin E which act as antioxidants.

The CAT activity is considered as an important and reliable biomarker of oxidative stress and antioxidant status of animal tissues. The metabolic role of CAT is to break down hydrogen peroxide in the tissues of animals into oxygen and water, which prevent the formation of carbon dioxide bubbles in the blood, as well as using hydrogen peroxide to break down potentially harmful toxins in the body such as alcohol, phenol, and formaldehyde into harmless or less harmful molecules (Papagiannis *et al*., 2004). The significantly reduced

CAT activities in the fish transported in palm oil-treated water (A and PB) immediately after the journey, may suggest that there was loss of this antioxidant enzyme activity, which may be attributed to the accumulation of ROS and the consequent oxidative stress in the fish. However, the no significant difference in the CAT activities of fish transported in palm oil-treated water and the control or aqua anti-stress agent 3 days after the journey, may indicate that the fish had stabilized and there was no further oxidative stress in the fish. This may be attributed to the presence of phytonutrients in the palm oil which acted as antioxidants that scavenged the excess ROS, thereby complementing the action of antioxidant enzyme, prevented its depletion in the cellular antioxidant defense system of the fish, and protected them against oxidative damage by these harmful oxidants. This finding is in agreement with Falaye *et al*. (2012) who reported better survival and good physical appearance of the fish transported in water treated with palm oil. This finding also agreed with Ogbe *et al*. (2020) and Famurewa *et al*. (2022) who reported that plant products contain phytochemicals which act as antioxidants that can protect animal tissues against oxidative damage by the ROS. The no significant difference in the CAT activities of fish transported in water treated with aqua anti-stress agent and the control before the journey, immediately after the journey, and 3 days post-transportation may suggest that it is an effective anti-oxidative stress agent for the treatment of water for transporting fish.

The GPx is regarded as an important component of the cellular antioxidant defense system against oxidative stress involved in detoxification and conjugation of xenobiotics, and in the protection of animal tissues against per-oxidative damage (Famurewa *et al*., 2022). GPx is considered to play an important role in protecting cells against membrane damage due to lipid peroxidation, by the conversion of hydrogen peroxide to oxygen and water (Owumi and Dim, 2019). The significant decrease in GPx activity of the fish transported in water pretreated with palm oil (PA and PB), immediately after the journey, may indicate loss of this enzyme activity which may be attributed to oxidative damage of the antioxidant enzyme, as a result of excess ROS, and the resultant oxidative stress during transportation of the fish. The decreased activity of GPx in this study agreed with Momoh *et al*. (2019) who reported reduced GPx activity immediately after journey. This may be due to protein structure modification leading to change in its function, and the accumulation of peroxides may also be responsible. The no significant difference in the GPx activities of fish transported in water pretreated with palm oil (PA and PB), and the control or aqua anti-stress agent-treated water, 3 days post-transportation, may suggest that the fish had stabilized after the stress of the journey and there was no further accumulation of ROS or oxidative stress. This may be attributed to the presence of phytonutrients in the palm oil which acted as antioxidants to scavenge the excess ROS, thereby protecting the antioxidant enzyme from further oxidative damage, and promoting the rapid synthesis of this enzyme required in the antioxidant defense system of fish. The finding of this study is in agreement with Ogbe *et al*. (2020) who reported that phytochemicals have antioxidant activities, thus they may scavenge the harmful ROS, and Eden (2002) who reported the presence of potent antioxidants; β-carotene and vitamin E in palm oil, which can prevent oxidative damage of animal tissues.

The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are enzymes regarded as markers of liver injury, for the assessment of function and integrity of the heart and liver including plasma membrane and endoplasmic reticulum (Achilike and Anyanwu, 2019). They are also responsible for rearranging building blocks of proteins (Okoye *et al*., 2016; Nwamba *et al*., 2020). The ALP is a marker of cholestasis or biliary duct obstruction of the liver (Vasudevan and Sreekumari, 2007). The marked increases in ALT and AST activities of catfish transported in the palm oil-treated water (PA and PB) pre-transportation (a day before journey) and 3 days after the journey, may suggest that there was oxidative damage of cell membrane, resulting in the leakage of these membrane-bound enzymes, and liver tissue injury in the animals. This is because serum enzymes are cytoplasmic in nature and are only released into blood circulation after cellular damage by substances which have adverse effects on the tissues (Gomaa, 2018; Ogbe *et al*., 2022). In addition, the treatment of transporting water with palm oil altered the environmental conditions of the fish, which required their quick adaptation, and may have contributed to the sudden increase in the liver enzymes. The significant increase in the serum ALP activities of this water before and after the journey may indicate cholestasis, which may be attributed to increased production of toxicants, as a result of the effect of handling stress and alteration in environmental conditions before the journey, and the effect of transportation stress on the fish at the end of the journey. The no significant difference between the serum enzymes (ALT, AST and ALP) activities of the fish transported in water treated with aqua anti-stress agent and that of the control, before the journey may indicate that the reference agent is more effective in protecting the liver tissues of fish against oxidative damage due to handling stress, and initially created a more conducive environment for the fish than the palm oil.

However, the markedly reduced enzymes (ALT, AST and ALP activities of the fish transported in palm oil-treated water immediately after the journey may indicate that the palm oil was more effective than aqua anti-stress agent in protecting the liver tissues of fish against oxidative damage due to the stress of transportation, and that the fish had adapted well to the palm oil-treated water. Therefore, the reduced enzymes activities in the fish transported in palm oil-treated water after the journey, when compared with the activities before the journey, may be attributed to the beneficial effect of the palm oil in maintaining the integrity of the liver cells, which made the enzymes activities quickly returned close to the initial levels, despite the transportation stress. Though, it appears the treatment of transporting water with palm oil contributed to the elevation of enzymes activities of fish when compared with the control pre-transportation, but it has beneficial effect on the fish in protecting their liver tissues against transportation stress, as seen in the enzymes activities of the fish post-transportation. Previous studies by Ogbe *et al*. (2019) and Famurewa *et al*. (2022) showed that certain chemicals in plants have health benefits, and act by protecting hepatic tissues against oxidative injury in animals. However, the generally lower activities of these enzymes in the aqua anti-stress agent-treated water close to the control in all the stages of the study, may indicate a better performance of the conventional anti-stress agent in maintaining physiology of the fish, and protecting the fish liver tissues from oxidative damage induced by stress in the animals. The perturbation in the maintenance of internal homeostasis through biochemical processes, may be reflected by the changes in these enzymes (AST, ALT, and ALP) in the plasma of the fish, which might be triggered by cellular damage in the functional organs such as liver, heart, gills, muscles and kidneys, as they are generally found in the tissues of these organs (Nwamba *et al*., 2020) but the ALT is a more specific marker of liver dysfunction than the AST or ALP (Owumi and Dim, 2019). The activities of these liver marker enzymes in the brood stock African catfish reported by Okoye *et al*. (2016) have lower values of ALT and ALP but higher values of AST compared to our study, which may be due to differences in environmental conditions and the stress of transportation. Though, the enzymes activities in the present study are close to the normal values for catfish (Okoye *et al*., 2016; Refaey and Li, 2018).

Cholesterol is a lipid used by the body for many functions including building of cell membrane and making important hormones. The right levels of total cholesterol play a vital role in maintaining cell membrane and the synthesis of hormones. The triglycerides are used to provide energy in animals and synthesize other important lipid molecules (Vasudevan and Sreekumari, 2007). The high total cholesterol levels have been found to be associated with stress responses in fish and the extent of the responses is dependent on the intensity of the stress (Refaey and Li, 2018). The no significant difference between the levels of TC and TRG of fish transported in palm oil-treated water and the control or aqua anti-stress agent-treated water, before the journey, immediately after the journey, and 3 days post-transportation, may suggest that the oxidative stress resulting from transportation stress did not have demonstrable adverse effect on the metabolism of cholesterol and triglycerides in fish. This may be attributed to the fact that short duration transportation stress does not have adverse effect on the metabolism of lipids in fish, hence there was no significant alteration in the total cholesterol and triglycerides levels in this study. The reduced TRG levels and elevated TC levels in all the transported catfish, immediately after the journey, may be attributed to the utilization of TRG in lipid metabolism, as it was incorporated into chylomicron for the synthesis of cholesterol-rich very low density lipoprotein (VLDL) in the fish (Vasudevan and Sreekumari, 2007). The elevation of cholesterol levels in the catfish transported in palm oil-treated water immediately after the journey compared with before the journey, was more than the fish in the control and aqua anti-stress-treated water. Previous report by Refaey and Li (2018) showed that transportation stress induces hypercholesterolaemia in fish, while Eden (2002) showed that oxidation of palm oil generates toxicants and elevates cholesterol levels in animal tissues. Therefore, the elevated cholesterol levels observed after transportation in our study, may be attributed to transportation stress and the oxidation of palm oil added to the transporting water.

This study has shown that handling and transportation of catfish in water by road for short duration of 8 hours may cause stress-induced generation of ROS in the animal tissues, as demonstrated by markedly reduced antioxidant status in the fish transported in palm oil-treated water. However, the treatment of fish transporting water with palm oil has health benefit or a beneficial effect in protecting the animal liver tissues against hepatocellular injury during the period of transportation, as demonstrated by markedly reduced liver marker enzymes activities in fish after the journey. This protective effect of palm oil against oxidative damage of the liver tissues of fish may be attributed to the presence of phytonutrients which may act as antioxidants.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

REFERENCES

Abalaka E.S. (2013) Evaluation of the haematology and biochemistry of *Clarias gariepinus* as biomarkers of environmental pollution in Tiga dam, Nigeria. *Braz. Arch. Biol. Technol.*, 56(3), 371-376.

Achilike N.M. and Anyanwu P.E. (2019) Enzymes activities in juveniles and adults of *Clarias gariepinus* reared in earthen ponds and concrete tanks. *Int. J. Fish. Aqua. Stud*., 7(2), 258-262.

Adenkola A.Y. and Ayo J.O. (2009) The effect of road transportation on erythrocyte osmotic fragility in pig administered ascorbic acid during the harmattan season in Zaria, Nigeria. *J. Cell Anim. Biol*., 3(1), 004-008

Adenkola A.Y., Ayo J.O., Sackey A.K.B. and Minka N.S. (2008) Ameliorative effect of ascorbic acid on rectal temperature of pigs transported by road for eight hours during the harmattan season. *Proc. 13th Annual Conference of Animal Science Association of Nig.* pp. 177-181.

Adeyemo O.K., Naigaga I. and Alli A.R. (2009) Effects of handling and transportation on haematology of African catfish (*Clarias gariepinus*). *J. Fish. Sci. Comm.*, 3(4), 333-341.

Bahmani M., Kazemi R. and Donskaya P. (2001) A comparative study of some hematological features in young reared sturgeons (*Acipenser persicus* and *Huso huso*). *Fish Physiol. Biochem*., 24, 135-140.

Crosby, T. C., Hill, J. E., Martinez, C. V., Watson, C. A., & Yanong, R. P. (2006). On-Farm Transport of Ornamental Fish: FA-119/FA119, 11/2006. EDIS, *2006(33)*.

Davis T. J. (1990) Brood stock and hatchery production. Southern Regional Aquaculture Center (SRAC) Publication No. 323.

Eden D.O. (2002) Palm oil: biochemical, physiological, nutritional, hematological, and toxicological aspects: a review. *Plant Foods Hum. Nutr*. 57(3-4), 319-341.

Esiegbe F.J., Doherty F.V., Sogbanmu T.O. and Otitoloju A.A. (2013) Histopathology alterations and lipid peroxidation as biomarkers of hydrocarbon-induced stress in the earthworm, *Eudrilus eugeniae*. *Environ. Monit. Assessm*., 185 (3), 2189-2196.

Eyo J.E. and Ezechie C.V. (2004) The effects of rubber (*Havea brasiliensis*) seed meal based diets on diets acceptability and growth performance of *Heterobranchus bidorsalis* (male) x *Clarias gariepinus* (female) hybrid. *J. Susten. Trop. Aquacult. Res.*, 10, 20-5.

Fafioye O.O. Olurin K.B. and Sowunmi A.A. (2005) Studies on the physicochemical parameters of Omi water body of Ago-Iwoye, Nigeria. *Afr. J. Biotechnol.*, 4(9), 1022-2024.

Falaye E., Omoike A., Folorunso L.A. and Bello O.S. (2012) The effects of palm oil on the physical appearance of *Clarias gariepinus* during transportation. *Int. J. Plant Anim. Environ. Sci.,* 2(4), 82-89.

Famurewa A.C., Maduagwuna E.K., Aloke C., Azubuike-Osu S.O. and Narayanankutty A. (2022) Virgin coconut oil ameliorates arsenic hepatorenal toxicity and NO-mediated inflammation through suppression of oxidative stress in rats. *Thai J. Pharmaceut. Sci.*, 46(2), 167-172.

Gomaa S. (2018) Adverse effects induced by diclofenac, ibuprofen, and paracetamol toxicity on immunological and biochemical parameters in Swiss albino mice. *J. Basic Appl. Zool*., 79:5-14.

Johnson S.K. (1993). Transport of live fish. *Aquacult. Mag.*, 5(6), 20–4.

Keremah R., Davies O.A., and Abezi I.D. (2014) Physico-Chemical analysis of fish pond water in freshwater areas of Bayelsa State, Nigeria. *Greener J. Biol. Sci*., 4(2), 033-038.

Krieger-Azzolini M.H., Delattre E., Carolsfeld J., Ceccarelli P.C. and Menezes F.V. (1989) A time-course study of physiological indicators of handling stress in the tropical fish (*Piaractus mesopotamicus*). *Braz. J. Med. Biol. Res*., 22, 1019-1022.

Kurosvskaya L.N. and Osadchaya S.Y. (1993) The influence of *Ichthyophthirus multifilis* on under-yearling carp, *Cyprinus carpio*. *J. Ichthyol*., 33, 81-92.

Momoh M.Y., Deekae S.N. and Gabriel U.U. (2019) Effect of handling stress on selected antioxidants in two sizes of African catfish (*Clarias gariepinus*). *Int. J. Innov. Stud. Aquat. Biol. Fish*., 5 (3), 21-27.

NAERLS (2001) Transporting Fish for culture. Extension bulletin number 151, Fisheries series no 6. Pp 1-21 published by National agricultural research liaison services (NAERLS), A.B.U. Zaria.

Narnaware Y.K., Baker I.B. and Tomlinson M.G. (1994) The effects of various stresses, corticosteroids and adrenergic agents on phagocytosis in the rainbow trout *Oncorhynchus mykiss*. *Fish Physiol. Biochem*., 13, 31-40.

Nwamba H.O., Nwani C.D., Njom V.S., Ogamba E.E. (2020) Toxic effect of ladosulfan-pesticides on biochemical indices of Clarias gariepinus (Burchell 1822) juveniles. *J. Mar. Sci. Res. Ocean*., 3(3), 113-116.

Ogbe R. J., Luka C. D. and Adoga G. I. (2019) Effect of aqueous ethanol extract of *Dialium guineense* leaf on diclofenac-induced oxidative stress and hepatorenal injuries in Wistar rats. *Comp. Clin. Pathol.*, *28*, 241-248.

Ogbe R. J., Luka C. D. and Adoga G. I. (2020) Comparative study of the effects of *Cassia spectabilis* and *Newbouldia laevis* leaf extracts on diclofenac-induced hepatorenal oxidative damage in rats. *Clin. Phytosci.*, *6*, 28.

Ogbe R.J., Luka C.D. and Adoga G.I. (2022) Influence of hydroethanolic extract of *Cassia spectabilis* leaves on diclofenac-induced oxidative stress and hepatorenal damage in Wistar rats. *J. Basic Appl. Zool.*, 83, 13.

Okechi J.K. (2004) Profitability assessment: A case study of African catfish (*Clarias gariepinus*) in the Lake Victoria basin, Kenya. Final project. *Fisheries Training Programme*. Iceland: The United Nations University; p. 12.

Okoye C.N., Dan-Jumbo S.O., Agina O.A., Eze U.U. and Udoumoh A.F. (2016) Reference intervals for the serum biochemistry and lipid profile of male broodstock African catfish (*Clarias gariepinus:* Burchell, 1822) at varied ages. *Not. Sci. Biol.*, 8(4), 437-443.

Oriakpono O., Hart A. and Ekanem W. (2012) Acute haematological response of a cichlid fish *Sarotherodon melanotheron* exposed to crude oil. *J. Toxicol. Environ. Health Sci*., 4(9), 151-155.

Owumi S.E. and Dim U.J. (2019) Biochemical alterations in diclofenac-treated rats: Effect of selenium on oxidative stress, inflammation, and hematological changes. *Toxicol. Res. Appl*., 3, 1-10.

Ozmen M., Gungordu A., Kucukbay F.Z. and Gular R.E. (2006) Monitoring the effects of water pollution in *Cyprinus carpio* in Karakara lake Turkey. *Ecotoxicology,* 15, 167-169

Papagiannis I., Kagalou I., Leonardos J., Petridis D. and Kalfakakou V. (2004) Copper and Zinc in four freshwater fish species from Lake Pamvotis (Greece). *Environ. Int.*, 30, 357-362.

Perez M.P., Palacio J., Santolaria M.P., Acena M.C., Chacon G., Gascon M., Calvo J.H., Zaragoza P., Beltran J.A. and Gracia-Belenguer S. (2002) Effect of transport time on welfare and meat quality in pigs. *Meat Sci*., 61, 425-433.

Refaey M.M. and Li D. (2018) Transport stress changes blood biochemistry, antioxidant defense system, and hepatic HSPs mRNA expressions of channel catfish *Ictalurus punctatus. Front. Physiol*., 9, 1628.

Schmitt C.J., Brumbaugh W.G. and May T.W. (2007) Accumulation of metals in fish from lead-zinc mining areas of southeastern Missouri, USA. *Ecotoxicol. Environ. Safety*, 67, 14-30.

Stone N. M. and Thormford H. K. (2003) Understanding your fish pond water analysis report. University of Arkansas cooperative extension printing series. pp. 1-4.

Stoskopf, M.K., (1993) Clinical pathology. In Stoskopf, M.K. (ed.), Fish Medicine. WB Saunders Publishers, Philadelphia, USA, pp. 113-131.

Uzoma C.E., Obinna C.L. and Nnamndi A.H. (2015) Cellular biomarker responses of bagrid catfish, *Chrysichthys nigrodigitatus* in a contaminated coastal ecosystem. *Afr. J. Biotechnol*., 14(25), 21 14 - 2123, https://doi.org/10.5897/AJB2014.14265.

Vasudevan D.M. and Sreekumari S. (2007) Textbook of biochemistry for medical students (5th ed.). Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, India, pp. 148-357.