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Effects of Stocking Density and Palm Oil Additive on Water Quality and Blood Parameters of the African Catfish, *Clarias gariepinus* During Transportation

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ABSTRACT

Stocking density is an important factor to be considered while subjecting fish seeds to transportation, since it disposes fish to stress especially under crowded conditions. Hence, palm oil has been documented as a natural product used as an anti-stress during fish transportation. Therefore, this study was designed to investigate the effects of stocking density during transportation on water quality of the transportation media and hematological parameters of the African catfish, *Clarias gariepinus*. Two hundred and forty fish samples of average weight 600±0.5g were randomly allotted at ten fish per treatment and replicated thrice with palm oil added to the water at two levels (0.5 and 1.5ml) and at varying water concentrations (1.0, 1.5 and 2.0 liters per fish). The fish were transported over a distance of 150km for a duration of 180 minutes. Water quality parameters of the transportation media, hematology, and serum biochemistry were determined according to standard procedures. Data obtained were analyzed using the analysis of variance (ANOVA), and means were separated using Duncan multiple range test. The results demonstrated that both palm oil concentration and transportation density significantly ($P<0.05$) influenced the serum and hematological parameters. The control group exhibited the highest values ($P<0.05$), with these values increasing with higher stocking densities. Furthermore, the duration of transit had a significant ($P<0.05$) impact on the blood parameters, with values increasing over time. Water quality parameters followed a similar trend, with the control group (0ml palm oil) showing the highest values, which increased with higher densities. However, the best performance for all measured parameters was observed with 1.5ml palm oil inclusion and 2.0L per fish stocking density. These findings contribute to the ongoing scientific discussion on optimizing transportation conditions for aquaculture species, promoting sustainable practices in the industry.

INTRODUCTION

The African catfish (*Clarias gariepinus*) is a major fish species cultured in sub-Saharan Africa. It is more resistant because of its bimodal breathing **(Mbanga *et al.*, 2018)**. Nevertheless, catfish are susceptible to physiological stress especially transportation and handling, which are perceived as a bottleneck in their production

(Sandrine *et al*., 2021). Stress in animals is caused by stressors which can originate from within an individual (endogenous) or from the environment (exogenous) **(Kumar *et al*., 2022)**. According to **Kannan *et al*. (2023)**, exposure of animals to new situations such as transportation stresses them and causes them to react by eliciting certain physiological and behavioral functions to cope with the situation. **Manuel *et al.* (2014)** reported that the transportation of fish among many stressors in aquaculture remained poorly studied.

Live fish are transported in a water medium on land, making it essential to assess the quality of the water as it can contribute to stress. Understanding these factors is crucial for studying the physiological responses of fish while maintaining homeostatic balance. **Ahn *et al.* (2019)** reported that the optimal ranges of water quality parameters such as dissolved oxygen, pH, water temperature, carbon dioxide, and ammonia vary depending on the type of fish, which in turn causes physiological changes in the fish. **Faudzi *et al*. (2021)** stated that the water quality of the holding water fluctuates during transportation, thereby putting the fish in transit under stress. This happens as a result of metabolic disruption brought on by subpar conditions during transit, which raises the death rate.

Fish transportation density is also an important factor that contributes to the exhibition of some physiological responses in fish during transportation. **Faudzi *et al.* (2021)** hypothesized that excessive stocking density may trigger a stress reaction in fish through metabolic pathways brought on by overpopulation and a decline in water quality. This stress response would then result in detrimental changes to fish's biochemistry and physiology. It can therefore be said that there is a relationship between fish density, holding water quality and transportation as factors that contribute to stress in fish, thereby causing a change in the physiology of the fish.

However, to ameliorate the transportation stress in fish, various chemical additives have been used to transport live fish with few exceptions. There is an opinion that anaesthetics are beneficial for calming excitable fish that might injure themselves in transit. The addition of natural sedatives such as essential oils or osmoregulatory salts viz. sodium or calcium chloride in the transported media have been reported **(Carneiro *et al*., 2019; Souza *et al.*, 2019; Vanderzwalmen *et al.*, 2019)**.

Palm oil generated from the West Africa's local palm oil tree *Elaeis guineensis* of West Africa, has long been renowned for its flexibility as cooking oil. Palm oil contains beneficial components such as saturated and monounsaturated fatty acids, organic antioxidants like carotenes and vitamin E (which act by scavenging free radicals and reactive oxygen species (ROS) that are generated during stress conditions) and has a high oil yield, making it a suitable material for maintaining water quality, thereby protecting cells from oxidative damage and lipid peroxidation **(Shahid *et al*., 2018; Samsi & Asthutiirundu, 2022)**.

The antioxidant activity of palm oil components helps stabilize cellular membranes, preventing oxidative damage to lipids, proteins, and DNA, as well as regulating cellular signalling pathways involved in stress responses **(Amri *et al.*, 2021)**.

However, palm oil could have potential toxic effects when introduced into water; there could be oxidative stress and lipid peroxidation resulting from exposure to palm oil **(Yu *et al.*, 2020)**. The oxidation of oils, including palm oil, has been linked to adverse impacts on development, immunological response, antioxidant capacity, and intestinal microbiota composition in the sea urchins and yellow catfish **(Li *et al*., 2022; Liu *et al.*, 2022)**. Additionally, studies have demonstrated that oxidized fish oil can worsen liver diseases and intestinal dysbiosis in animal models, indicating potential harmful effects on fish health **(Feng *et al*., 2020)**.

Therefore, this study was designed to investigate how the blood parameters of adult *C. gariepinus* and water quality were affected by transportation stress when palm oil was added as a water additive at different levels of stocking densities. The outcome of this study will contribute to previous studies' findings and will validate the effect of stocking density and the ameliorating effect of palm oil as a water additive during the transportation of *C. gariepinus*.

MATERIALS AND METHODS

1. Sample collection

A total of two hundred and forty live *C. gariepinus* of average weight 600 ± 0.5g were procured from a reputable fish farm in Oluponna, Osun State, Nigeria. The fish were acclimatized in a concrete tank on the Teaching and Research Farm, Bowen University, Iwo, Nigeria.

2. Experimental design

The experiment employed a partially nested design with two factors: transportation density and palm oil inclusion. Three levels of fish stocking density during transportation were used: 2.0, 1.5, and 1.0L/ fish. Additionally, two levels of palm oil inclusion were used: 0.5 and 1.5ml per tank. The variation in water volume per fish during transportation was based on recommendations from previous studies, which suggest that this fish species should be transported at a density of 500 grams per liter, within a temperature range of 20-30°C **(Piper *et al*., 1982; Berka 1986)**. Fish were randomly assigned to replicates within each density and palm oil combination. Each replicate contained 10 fish. The transportation used for the trip was a Toyota Hiace bus. The car has a 4 x 1.2m internal ground area and a metal ceiling with foam and rug material cushions. The metal floor of the car was padded with foam and covered with a rug. The windshields on vehicles are made of glass, positioned on both sides, and include a sliding mechanism to allow for ventilation. Appropriate ventilation was made possible by the windows, which were placed opposite to one another and 800cm from the interior floor. Each window was 50 by 35cm. Fish were carefully put into transportation containers so that the conditions on the bus for each treatment and replication were the same **(Buckham *et al.*, 2008; Minka & Ayo, 2011)**. The car covered 150km in three hours at an average speed of 50km/ h on a mixture of rocky and unpaved roads.

3. Limitations of the design

It is important to acknowledge a limitation in the originally planned 3 x 3 factorial design. Data for the 0ml palm oil inclusion level were missing at densities of 1.5 and 1.0L/ fish. This restricted a complete evaluation of the independent effects of density and palm oil on the fish. A control group was established using the 0ml palm oil inclusion level at a density of 2.0L/ fish. This group served as the reference point for comparison at this specific density. The remaining fish were assigned to treatment groups defined by the combination of transportation stocking density (2.0, 1.5, or 1.0L/ fish) and palm oil inclusion level (0.5 or 1.5ml).

4. Meteorological data

Frequent measurements of the relative humidity (RH) and ambient temperature (AT) were obtained using a wet-and dry-bulb thermometer, both inside and outside the vehicle (Brannan, England). In the car, the average temperature and relative humidity were 38.8^0C and 63.4%, respectively, whereas outside the car, they were 37.50^0C and 54.7%, respectively. Throughout the trip, the average wind speed was 37km/ h. The temperature humidity index (THI) was determined using the formula:

$$THi = 0.8 \times T + RH \times (T - 14.4) + 46.4$$ **(Scope *et al.*, 2002)**

Where, T = ambient or dry-bulb temperature in °C

RH = relative humidity expressed as a proportion, that is, 75% humidity is expressed as 0.75

$$THi_{Outside\ the\ vehicle} = 0.8 \times 37.5 + 0.547 \times (37.5 - 14.4) + 46.4$$

$$= 89.04$$

$$THi_{Inside\ the\ vehicle} = 0.8 \times 38.8 + 0.634 \times (38.8 - 14.4) + 46.4$$

$$= 92.91$$

5. Blood sample collection

Before loading, during, and right after the trip (post-transportation), a blood sample was taken. Using a 2ml disposable heparinized syringe treated with EDTA as an anticoagulant, 2.5ml of blood was drawn from the heart puncture. As per **Schalm *et al.***

(1975) and **Oladele *et al.* (2001)**, haematology and serum biochemical analyses were performed on all blood samples. Bottles containing an anticoagulant called ethylene diethyl trichloro acetate (EDTA) were used to collect samples intended for investigation.

The measurements of hematological parameters included total white blood counts (WBC), packed cell volume (PCV), red blood counts (RBC), and hemoglobin concentration (Hb). WBC was determined using a hemocytometer, PCV was measured by the microhematocrit method, RBC was counted using an automated blood cell counter, and Hb concentration was assessed using a hemoglobinometer. The following serum metabolites were also measured: cortisol, creatinine, aspartate aminotransferase (AST), and alanine aminotransferase (ALT). Cortisol levels were determined by enzyme-linked immunosorbent assay (ELISA), creatinine was measured using a colorimetric assay, and AST and ALT levels were determined using spectrophotometric methods.

6. Water sample collection

Water samples were also collected to check for the water quality (pH, nitrite, ammonia, dissolved oxygen, nitrate). All parameters listed, except for dissolved oxygen, were tested using a PONDLAB-200 test kit. Dissolved oxygen was measured in the laboratory using the DO meter.

7. Data analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS) application, version 20. At $P<0.05$, the differences were revealed by the Duncan multiple range test.

RESULTS

Table (1) shows the interaction effect of palm oil additive and stocking density on the hematological parameters of *C. gariepinus*. For fish treated with 0.5 ml of palm oil additive and stocked at varying densities, the results for packed cell volume (PCV), platelet count (PLT), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) indicated no significant differences ($P > 0.05$) among the stocking densities of 2.0, 1.5, and 1.0L. However, the results for red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), and total count (TC) showed significant differences ($P < 0.05$) among these stocking densities.

For fish treated with 1.5ml of palm oil additive and stocked at varying densities, PCV, RBC, PLT, MCV, and MCH also showed no significant differences ($P> 0.05$) among the stocking densities (2.0, 1.5, and 1.0L). In contrast, Hb, WBC, and TC exhibited significant differences ($P< 0.05$) among the different stocking densities.

Table 1. Interaction effect palm oil additive and transportation density on haematological parameters of the transported African catfish

Parameter		Treatment					
	(Control)	Palm oil additive (ml)					
	0.0ml	0.5			1.5		
		Fish stocking density					
	T_1 2.0L	**T_2 2.0L**	**T_3 1.5L**	**T_4 1.0L**	**T_5 2.0L**	**T_6 1.5L**	**T_7 1.0L**
PCV (m%/ml)	**40.01±0.92^{a}**	**36.80±0.59^{ab}**	**38.08±0.72^{a}**	**38.93±0.91^{a}**	**34.80±0.59^{ab}**	**36.08±0.72^{a}**	**36.93±0.91^{a}**
Hb (g/dl)	**25.10±0.56^{a}**	**17.93±0.23^{c}**	**20.73±0.45^{ab}**	**23.45±0.56^{a}**	**15.93±0.23^{c}**	**17.73±0.45^{ab}**	**21.45±0.56^{a}**
RBC (m/mm^3)	**16.13±0.77^{a}**	**13.15±0.14^{b}**	**14.25±1.08^{a}**	**15.53±0.78^{a}**	**10.15±0.14^{ab}**	**11.25±1.08^{a}**	**11.53±0.78^{a}**
WBC (k/µl)	**10.02±0.56^{a}**	**4.50±0.14^{c}**	**6.07±0.43^{b}**	**8.62±0.55^{a}**	**3.20±0.14^{c}**	**4.77±0.43^{b}**	**5.62±0.55^{a}**
PLT (10^3/µL)	**3.07 x10^2±1.27^{a}**	**2.03 x10^2±1.66^{a}**	**2.06 x10^2±1.74^{a}**	**2.14 x10^2±1.27^{a}**	**2.13 x10^2±1.66^{a}**	**2.18 x10^2±3.74^{a}**	**2.24 x10^2±3.27^{a}**
MCV (µm^3/10^{-15}Fl)	**2.70±0.23^{a}**	**2.45±0.22^{a}**	**2.27±0.41^{a}**	**2.60±0.23^{a}**	**2.30±0.22^{a}**	**2.47±0.41^{a}**	**2.50±0.23^{a}**
MCH (10^{-12}g)	**4.11±0.20^{ab}**	**6.80±0.14^{a}**	**5.73±0.49^{a}**	**4.12±0.20^{ab}**	**4.10±0.24^{a}**	**4.73±0.29^{ab}**	**5.72±0.30^{a}**
TC (g)	**3.60 x10^2±6.09^{a}**	**1.80 x10^2±2.93^{c}**	**2.30x10^2±7.45^{ab}**	**3.40 x10^2±6.09^{a}**	**1.72 x10^2±2.93^{c}**	**2.40x10^2±7.45^{ab}**	**3.32 x10^2±6.09^{a}**

Values along the same row with different superscripts are significantly different ($P<0.05$)

PCV- Packed Cell Volume; **Hb**- Hemoglobin; **RBC**- Red Blood Cells; **WBC**- White Blood Cells; **PLT**- Platelets; **MCV**- Mean Corpuscular Volume; **MCH**- Mean Corpuscular Hemoglobin; **TC**- Total Count.

Table (2) displays the hematological parameters of *C. gariepinus* subjected to different transportation distance. The results revealed that hematological parameters were significantly affected ($P<0.05$) by transportation duration, which varied significantly ($P<0.05$) across the three transportation periods, except for the total count (TC), which was not significant ($P>0.05$) across the three transportation period (0km, 75km, and 150km) for the transported fish.

Table 2. Effect of transportation distance on hematological parameters of *C. gariepinus* transported in media with palm oil additive

	Transportation distance (period)		
Parameters	**Initial (0km)**	**Middle (75km)**	**Final (150km)**
PCV (m%/ml)	35.5 ± 0.85^{c}	37.40 ± 0.76^{ab}	38.46 ± 0.76^{a}
HB (g/dl)	15.14 ± 0.97^{bc}	17.93 ± 1.05^{b}	25.83 ± 1.31^{a}
RBC (m/mm^3)	16.35 ± 0.52^{bc}	17.31 ± 0.71^{ab}	19.34 ± 1.11^{a}
WBC (k/µl)	4.55 ± 0.59^{c}	6.89 ± 0.75^{ab}	8.46 ± 0.87^{a}
PLT (10^3/µL)	$2.01\ x10^{2}\pm8.93^{c}$	$2.48\ x10^{2}\pm10.56^{ab}$	$3.53\ x10^{2}\pm11.38^{a}$
MCV (µm^3/10^{-15}Fl)	3.70 ± 0.35^{c}	4.29 ± 0.46^{ab}	5.56 ± 0.49^{a}
MCH (10^{-12}g)	3.45 ± 0.48^{c}	5.05 ± 0.59^{ab}	7.73 ± 0.69^{a}
TC (g)	$2.01\ x10^{2}\pm9.50^{a}$	$2.14\ x10^{2}\pm9.85^{a}$	$2.29\ x10^{2}\pm12.07^{a}$

^{abc} values along the same row with different superscripts are significantly different ($P<0.05$)
PCV- Packed Cell Volume; **Hb**- Haemoglobin; **RBC**- Red Blood Cells; **WBC**- White Blood Cells; **PLT**- Platelets; **MCV**- Mean Corpuscular Volume; **MCH**- Mean Corpuscular Haemoglobin; **TC**- Total Count.

The interactive effect of palm oil additive and transportation stocking density on serum biochemical parameters of *C. gariepinus* is presented in Table (3). For the fish transported under 0.5ml of palm oil additive and stocked at varying densities, the results showed that measured ALT, AST, and cortisol levels were significantly ($P<0.05$) different across different densities. Creatinine was not significantly different ($P>0.05$), but values increased with stocking densities. In addition, the fish transported under 1.5ml of palm oil additive followed the same trend as those on 0.5ml of palm oil additive for all measured serum parameters. Compared to other treatments, fish in the control group which did not include palm oil had the highest values for the parameters that were tested. Significant differences ($P>0.05$) were not observed between the control group and the high density (1.0L) fish.

Table 3. Effect of palm oil additive and fish stocking density on serum biochemical parameters of transported *C.gariepinus*

Parameter		Treatments					
	(Control) 0.0ml	Palm oil additive 0.5ml			1.5ml		
	Fish stocking density						
	T_1 2.0L	**T_2 2.0L**	**T_3 1.5L**	**T_4 1.0L**	**T_5 2.0L**	**T_6 1.5L**	**T_7 1.0L**
ALT (U/L)	$\mathbf{12.07\pm0.45^{a}}$	$\mathbf{4.05\pm0.20^{c}}$	$\mathbf{9.68\pm0.33^{b}}$	$\mathbf{11.57\pm0.45^{a}}$	$\mathbf{3.01\pm0.10^{c}}$	$\mathbf{7.25\pm0.31^{b}}$	$\mathbf{9.17\pm0.35^{a}}$
AST (IU/L)	$\mathbf{13.08\pm0.40^{a}}$	$\mathbf{4.10\pm0.19^{c}}$	$\mathbf{10.83\pm0.39^{b}}$	$\mathbf{12.48\pm0.37^{a}}$	$\mathbf{3.0\pm0.11^{c}}$	$\mathbf{8.21\pm0.25^{b}}$	$\mathbf{10.16\pm0.27^{a}}$
CREATININE (nmol/L)	$\mathbf{0.18\pm0.08^{a}}$	$\mathbf{0.02\pm0.00^{a}}$	$\mathbf{0.12\pm0.06^{a}}$	$\mathbf{0.16\pm0.08^{a}}$	$\mathbf{0.01\pm0.00^{a}}$	$\mathbf{0.08\pm0.05^{a}}$	$\mathbf{0.11\pm0.06^{a}}$
CORTISOL (nmol/L)	$\mathbf{1.51\pm9.06^{a}}$	$\mathbf{0.72\pm0.47^{c}}$	$\mathbf{1.00\pm8.61^{b}}$	$\mathbf{1.11\pm10.96^{a}}$	$\mathbf{0.50\pm0.27^{c}}$	$\mathbf{0.70\pm5.11^{b}}$	$\mathbf{0.91\pm8.06^{a}}$

^{abc}Values along the same row with different superscripts are significantly different ($P<0.05$)
ALT- alanine aminotransferase; AST- aspartate aminotransferase.

Effects of transportation period on measured serum biochemical parameters of transported fish are denoted in Table (4). Measured parameters were significantly ($P<0.05$) different across the transportation distance. All values increased significantly ($P<0.05$) with the duration of fish transportation irrespective of the density and concentration of palm oil additives.

Table 4. Effect of transportation period on serum biochemical parameter of *C.gariepinus* transported in media with palm oil additive

	Transportation distance (period)		
Parameter	**Initial (0km)**	**Middle (75km)**	**Final (150km)**
ALT (U/L)	8.16 ± 1.01^{b}	8.85 ± 1.13^{ab}	9.61 ± 1.15^{a}
AST (IU/L)	9.08 ± 1.21^{b}	9.99 ± 1.38^{ab}	10.24 ± 1.24^{a}
CREATININE (nmol/L)	0.03 ± 0.00^{b}	0.11 ± 0.05^{ab}	0.19 ± 0.64^{a}
CORTISOL (nmol/L)	0.93 ± 1.32^{c}	1.41 ± 7.34^{b}	2.01 ± 9.20^{a}

^{abc}Values along the same row with different superscripts are significantly different ($P<0.05$)

ALT- alanine aminotransferase; **AST**- aspartate aminotransferase.

Table (5) shows the effect of palm oil additive and stocking density on water quality parameters, dissolved oxygen, pH, ammonia, and temperature. The fish transported at varying densities (2.0, 1.5 and 1.0L) with 0.5ml of palm oil added to the water showed significant ($P<0.05$) differences in all the water quality parameters assessed, except in the case of pH where there was no significant ($P>0.05$) difference between the treatments.

Table 5. Effect of palm oil additive and fish stocking density on water quality parameters of transported fish

Parameter	**Treatments**						
		Palm oil additive					
		0.5 (ml)			**1.5 (ml)**		
		DENSITY					
	T_1(Control)	**T_2 2.0L**	**$T_3$1.5L**	**$T_4$1.0L**	**T_5 2.0L**	**$T_6$1.5L**	**$T_7$1.0L**
Nitrate	17.25 ± 1.13^{a}	8.10 ± 0.20^{c}	12.07 ± 0.45^{b}	17.30 ± 0.31^{a}	7.80 ± 0.00^{c}	11.67 ± 7.60^{b}	16.73 ± 1.63^{a}
Nitrite	7.05 ± 1.68^{a}	0.89 ± 0.19^{c}	3.97 ± 1.67^{b}	5.18 ± 1.68^{a}	0.48 ± 0.19^{c}	2.11 ± 0.37^{b}	3.05 ± 0.42^{a}
Ammonia	10.15 ± 1.01^{a}	1.14 ± 0.05^{c}	3.17 ± 1.70^{b}	8.33 ± 1.11^{a}	0.32 ± 0.05^{c}	1.07 ± 1.70^{b}	3.53 ± 1.11^{a}
pH	7.00 ± 0.00^{a}	7.00 ± 0.00^{a}	7.00 ± 0.00^{a}	7.00 ± 0.00^{a}	7.00 ± 0.00^{a}	7.00 ± 0.00^{a}	7.00 ± 0.00^{a}
DO (mg/L)	2.1 ± 0.50^{c}	3.6 ± 0.40^{a}	2.8 ± 1.20^{b}	2.5 ± 0.50^{c}	4.0 ± 0.40^{a}	3.1 ± 1.20^{b}	2.7 ± 0.50^{c}

^{c} Values along the same row with different superscripts are significantly different ($P<0.05$)

Fish transported at varying densities (2.0, 1.5, and 1.0L) with 1.5ml of palm oil added to the water showed significant ($P<0.05$) differences in all the water quality parameters examined except in the case of pH, where there was no significant ($P>0.05$) difference between the treatments. The water quality of fish in the control (T_1) was the highest and similar ($P<0.05$) to those on T_4 and T_7 stocking densities. The results of transportation duration on the measurement of water quality parameters are presented in Table (6). Measured parameters increased significantly ($P<0.05$) across the three durations, except for pH which was not significant ($P>0.05$). The pH values increased with transportation duration.

Table 6. Effect of the transportation distance on water quality parameters of the African catfish transported in media with palm oil additive (1.5ml)

	Transportation distance (period)		
Parameter	**Initial (0km)**	**Middle (75km)**	**Final (150km)**
Nitrate	0.00 ± 0.00^a	7.54 ± 1.65^b	7.54 ± 1.65^b
Nitrite	0.00 ± 0.00^a	0.18 ± 0.4^b	0.18 ± 0.4^b
Ammonia	0.00 ± 0.00^a	1.43 ± 0.05^a	1.43 ± 0.05^a
pH	7.00 ± 0.00^a	8.50 ± 0.00^a	8.80 ± 0.00^a
DO (mg/L)	4.2 ± 0.40^a	3.4 ± 1.20^b	2.6 ± 0.50^c

^{abc}Values along the same row with different superscripts are significantly different ($P<0.05$)

DISCUSSION

Packed cell volume (PCV) haematocrit, also known as haematocrit, was characterized as the amount of red blood cells present in the blood as a whole **(Chidozie *et al*., 2020)**. **Dinesh *et al.* (2021)** described PCV as the proportion of erythrocytes in the blood which is also a well-known stress indicator in fish. It was stated by **Radoslav *et al.* (2013)** that the oxygen demand of organisms can be determined using the alterations in PCV levels. In a study by **Fazio *et al.* (2015)**, upon examining the effects of acute handling stress on blood parameters in cultured sea bream, it was observed that stressed fish exhibited higher levels of PCV, hemoglobin, and RBC compared to non-stressed fish. It was therefore reported that the contraction of the spleen must have caused this increase in values to release blood into the circulatory system (**Ruane *et al*., 2000)**. The mechanism observed in the spleen was described as such to improve the capacity of the blood to transport oxygen under the energy-demanding condition.

Olopade *et al*. (2022) reported that the level of PCV was low for non-stressed fish, but higher for stressed ones. However, the level of haemoglobin in the study of **Olopade *et al.* (2022)** was higher in non-stressed fish compared to stressed ones and this was stated to have been caused by the structural damage to the erythrocytes' membrane, thereby causing haemolysis and haemoglobin production impairment. It was also noted by **Ayoola *et al.* (2020)** and **Yanuhar *et al.* (2021)** that a high level of PCV or increase in the level of RBC can be attributed to transportation stress. In this study, the levels of PCV, Hb, and RBC increased with higher stocking densities (2.0 to 1.0L), indicating greater stress in fish stocked at 1.0L compared to those at 2.0 and 1.5L with 0.5ml of palm oil. Similarly, with 1.5ml of palm oil, the levels of PCV, Hb, and RBC also rose with increasing transportation density, following the same trend as observed with 0.5ml, but with higher overall levels compared to 1.5ml, indicating that the addition of palm oil at 1.5ml was more effective in reducing the oxygen demand of the fish compared to those exposed to the addition of 0.5ml of palm oil.

The WBCs defend the body of the fish against foreign matters that can cause disease. Therefore, an increase in the production of WBCs in the body of a fish is an indication of the presence of a stressor that the immune system of the fish is trying to resist **(Yanuhar *et al.* 2021, Ayoola *et al*., 2023)**. In this study, the decreased level of WBC with a decrease in transportation stocking density (1.0 to 2.0L) shows that the fish stocked within 1.0L were more stressed compared to 1.5 and 2.0L for 0.5ml addition of palm oil. With 1.5ml of palm oil, the level of WBC decreased as stocking density increased (1.0 to 2.0L). This trend mirrored the results observed with 0.5ml of palm oil, though higher WBC levels were recorded with the 0.5ml treatment. This indicates that fish transported at varying densities with 0.5ml of palm oil experienced greater stress compared to those with 1.5ml of palm oil. It also revealed that adding palm oil at 1.5ml was more effective in moderating the amount of WBC produced by the fish compared to those exposed to 0.5ml addition of palm oil.

The MCV and MCH are two out of the three red blood cell indices that respectively measure the volume (average size of erythrocytes) and weight of haemoglobin (amount of haemoglobin per RBC) **(Dinesh *et al.*, 2021)**. Toxic stress in fish was observed by **Kavitha *et al.* (2021)** as cited by **Dinesh *et al.* (2021)** and it was attributed to a decrease in MCHC leading to an increase in MCV and MCH values. The values of the MCV exhibited a consistent pattern across different stocking densities. This consistency may be attributed to haemolysis and impaired haemoglobin production, as noted by **Yanuhar *et al.* (2021)**.

The analysis of MCV and MCH in relation to transportation density and palm oil inclusion reveals significant insights into the stress levels experienced by the *C gariepinus*. Table (1) indicates that the highest transportation density of 1.0L resulted in the greatest MCV value (2.60 $\mu m^3/10^{-15}$ Fl), while the lower density of 2.0L yielded the lowest MCV (2.45 $\mu m^3/10^{-15}$ Fl). This trend suggests that higher density transportation may lead to increased red blood cell (RBC) indices, potentially reflecting elevated stress levels. Notably, the inclusion of palm oil at 1.5ml appeared to mitigate this effect, as indicated by a decrease in MCV with increasing transportation density. Specifically, at 1.0L density, the MCV was higher (2.60 $\mu m^3/10^{-15}$ Fl) with 0.5ml of palm oil compared to 1.5 ml, which resulted in a lower MCV (2.50 $\mu m^3/10^{-15}$ Fl). This suggests that the higher concentration of palm oil may be more effective in reducing MCV, thereby alleviating stress in the fish. Regarding MCH, a similar pattern was observed. The results demonstrated a decrease in MCH with increasing transportation density, indicating that fish transported in denser conditions (2.0L) experienced greater stress than those in less dense conditions (1.0L). Interestingly, when palm oil was added at 1.5ml, MCH levels increased with decreasing transportation stocking density, paralleling the trends observed with 0.5ml palm oil. However, the overall levels of MCH were greater under the 0.5ml treatment, suggesting that fish subjected to lower palm oil concentrations experienced higher stress levels compared to those with 1.5ml. These findings underscore the importance of both stocking density and palm oil inclusion in managing stress levels in *C. gariepinus* during transportation. The results indicate that optimizing these parameters can enhance the welfare of the fish, ultimately contributing to better survival rates and overall health in aquaculture practices.

This shows that 1.5ml addition of palm oil was more effective than 0.5ml in lowering the amount of MCH produced in the fishes thereby reducing stress in the fishes. In this study, transported fish are subjected to elevated stress conditions with an increase in transportation. During transportation stress, fish may experience oxidative stress due to increased production of reactive oxygen species (ROS). The ameliorating effect of palm oil on the aforementioned measured parameters may be attributed to the antioxidants present in palm oil which helps to scavenge reactive oxygen species (ROS) and prevent oxidative damage to cellular components. This helps maintain cellular homeostasis and reduces stress-induced damage.

The body uses the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) to analyze how well the liver and heart are functioning **(Achilike & Anyanwu, 2019)**. In normal circumstances, they are low and mostly stable, but when the body experiences tissue injury and organ malfunction, a significant amount of AST and ALT is released into the plasma **(Paulino *et al.*, 2017; Dong *et al.*, 2022)**. In this study, the AST and ALT which increased significantly ($P<0.05$) with the interactive effect of palm oil concentration and increase in stocking could be explained by oxidative damage to the cell membrane, which led to the leakage of these membrane-bound enzymes and liver damage as a result of the transportation media's toxicity **(Gomaa, 2018; Ogbe *et al.*, 2022)**. The inclusion of 1.5ml palm oil as an antistress agent, revealed that the level of AST and ALT decrease with a decrease in transportation density (1.0 to 2.0L), which corresponds with the pattern of the result in 0.5ml, but higher values of AST and ALT were recorded under 0.5ml treatment compared to that of 1.5ml, and this shows that the fish transported at varying densities under 0.5ml addition of palm oil were more stressed compared to those under 1.5ml. The reduced measured ALT and AST enzymes of the fish transported in 1.5ml palm oil treated water may indicate that the palm oil was more effective at such quantity in protecting fish liver cells from oxidative damage brought on by transportation stress, it corroborated **(Ogbe *et al.*, 2019; Famurewa *et al.*,**

2022; Kolndadacha *et al*., 2023). Palm oil contains tocotrienols, tocopherols, and carotenoids, which have antioxidant properties. It is suggested that these compounds diffuse into the fish through the gills to reduce oxidative stress, capable of causing cellular damage. Antioxidants assist lessen oxidative stress and eliminate free radicals, thereby mitigating stress-induced damage on the blood cells and tissues.

A nitrogenous metabolic byproduct of muscle catabolism is creatinine. Renal filtration is the body's practically exclusive method of eliminating creatinine, hence measuring creatinine typically yields an accurate estimate of renal filtration efficiency. Blood levels of creatinine can fluctuate due to physiological events that affect the kidneys' capacity to filter, including dehydration, stress, and illnesses, among others. In this study, values of creatinine are not significantly different ($P>0.05$) across the treatments but increased with the interaction effect of palm oil concentration and increase in transportation density. The inclusion of palm oil as an antistress at 1.5ml was better than 0.5ml. However, measured creatinine value increases with journey duration. All measured creatinine values are within the normal range for healthy fish **(McDonald & Milligan, 1992)**.

The significant ($P<0.05$) effect of treatments and period on the cortisol level of transported fish can be attributed to stress. Most fish emit cortisol as their primary glucocorticoid during stress responses, and this hormone has been used to study a variety of stressors including organic contaminants. Most fish emit cortisol as their primary glucocorticoid during stress responses, and this hormone has been used to study a variety of stressors including organic contaminants **(Odhiambo *et al*., 2020; Lemos *et al*., 2023)**. The glucocorticoid hormone released by steroidogenic cells in the inter-renal region is called cortisol. When an organism experiences stress, the hypothalamus-pituitary-inter-renal (HPI) axis is activated, releasing this hormone **(Grosell, 2021; Schumann *et al*., 2022)**. It was evident from this study that the inclusion of palm oil as an anti-stress with an increased concentration of 1.5ml, as compared to 0.5ml in transporting water, reduced cortisol levels ($P<0.05$). Palm oil's fatty acid composition, particularly its high content of monounsaturated and saturated fats, possibly have been caused by cortisol's regulating impact. According to research, the hypothalamic-pituitary-adrenal (HPA) axis, which controls the body's stress response, may be influenced by specific fatty acids, such as omega-3s and omega-6s **(Famurewa *et al*., 2022; Kolndadacha *et al*., 2023)**.

The production of nitrate (NO_3) results from the oxidation of nitrite to form ammonia and nitrate by an autotrophic aerobic Nitrobacter bacterium **(Verma *et al*., 2022)**. This process of formation is called nitrification. It was further stated that the nitrate levels stay around 50-100mg/ L. **Caesar *et al*. (2021**) also stated that the ideal level of nitrate in water is less than 100mg/ L. Nitrate is the primary form of nitrogen in water which can be obtained from the nitrogen cycle and the process of nitrification by autotrophic bacteria. The nitrate level increased significantly ($P<0.05$) with an increase in stocking density (2.0-1.0L) of the fish from 7.80 to 16.73mg/ L, respectively, for the addition of palm oil at 1.5ml. The same pattern was observed in the results of the fish under 0.5ml treatment of palm oil, revealing a significant ($P<0.05$) increase in nitrate level from 8.10 to 17.30mg/ L with an increase in transportation density from 2.0-1.0L. Nevertheless, the values observed under the palm oil addition of 0.5ml were higher for each transportation density compared to that of 1.5ml addition. This is a pointer that more ammonia and nitrite were produced under 0.5ml treatment compared to 1.5ml treatment of palm oil. This also indicates that the 1.5ml addition of palm oil to the varying transportation densities was more effective in reducing the concentration of nitrate compared to 0.5ml, although nitrate encourages algae growth which is not being considered in this study. Though the nitrate level increased with an increase in transportation density, the recorded nitrate levels were lower than the range- of 50-100mg/ L, and this simply implies that the nitrate levels were not toxic to the fishes (**Caeser *et al*., 2021; Verma *et al.*, 2022)**.

Nitrite (NO_2) is the product obtained from the oxidation of ammonia (NH_3) and ammonium ions (NH_4^+) **(Philminaq, 2018)**. **Verma *et al.* (2022)** stated that it has the capability of changing haemoglobin to methemoglobin in the blood, and this changes the color of the blood and gills to brown, thereby hindering respiration. **Akongyuure and Alhassan (2021)** stated that nitrite serves as a step in the process that converts ammonium to nitrate. According to **Melard (1999)**, as cited by **Sanou *et al*. (2022)**, 0.1mg/ L is the recommended limit for freshwater aquaculture. In this present study, it can be observed from Table (5) that the nitrite concentrations increased with an increase in transportation densities for both 0.5 and 1.5ml palm oil addition. The concentrations under 1.5ml addition of palm oil increased from 0.48 to 3.05mg/ L, while that of 1.5ml addition of palm oil increased from 0.89 to 5.18mg/ L. The concentrations recorded in this study are higher than the recommended limit (0.1mg/ L) stated by **Melard (1999)**, and this indicates that the environment was toxic for the fish, thereby causing them stress. It was also observed that the nitrite concentration values for 0.5ml are higher than those under 1.5ml, and this revealed that the fish under the treatment of 0.5ml treatment of palm oil addition were more stressed. This is an indicator that the 1.5ml addition of palm oil to the varying transportation densities was more effective in reducing the concentration of nitrite produced compared to 0.5ml and the control (0ml).

Boyd (1990) stated that the standard for total ammonia content in water is 0.4-3.1mg/ L. **Ariffin *et al.* (2019)** stated that ammonia in water is produced by decomposing urea, feces and organic matter. In this present study, the ammonia concentrations increased with an increase in transportation stocking densities for both 0.5 and 1.5ml palm oil addition treatments. The concentrations of ammonia under the 0.5ml addition of palm oil increased from 1.14 to 8.33mg/ L, while those under the 1.5ml addition increased from 0.32 to 3.53mg/ L. It was observed that the ammonia concentration values for the 0.5ml treatment were higher than those for the 1.5ml treatment, indicating that fish receiving the 1.5ml palm oil addition experienced more stress. **Verma *et al.* (2022)** reported that ammonia levels greater than 0.1mg/ L can damage gills, harm mucus-producing membranes, and lead to less severe consequences, such as poor feed conversion and decreased disease resistance. According to records from the Ministry of Agricultural Animal Industry and Fisheries (MAAIF), as published by **Tumwesigye *et al.* (2022)**, the maximum ammonia concentration limit for aquatic species is 0.1mg/ L. It was also noted that an acceptable concentration of un-ionized ammonia (<0.05mg/ L) is considered safe for tropical fish species. However, in this study, the ammonia concentrations for both the 0.5 ml and 1.5 ml additions of palm oil at varying transportation densities exceeded these recommended limits **(Boyd, 1999; Verma *et al.*, 2022; Tumwesigye *et al.*, 2022)**. This indicates that the transportation media were highly toxic and potentially lethal to the fish.

Upon comparing the effects of the two palm oil treatments, the 1.5ml addition was more effective in limiting ammonia production compared to the 0.5ml addition and the control.

The World Health Organization **(WHO, 2011)**, as cited in the study of **Omer (2019)**, stated that the safe pH range for drinking water, domestic use, and living organisms is between 6.5 and 8.5. **Sanou *et al.* (2022)** reported that the ideal pH for biological activity ranges from 7 to 8.5. **Boyd (1982)**, as cited in **Tumwesigye *et al*. (2022)**, indicated that a pH range of 6-9 is acceptable for most fish. In this study, the pH values of the varying transportation densities for both palm oil treatments (0.5 and 1.5ml) were neutral, recorded at 7.00. This pH value is within the acceptable range for aquatic species and promotes biological productivity. It can be deduced that the varying transportation densities and the palm oil additions had no significant ($P > 0.05$) impact on pH, which is a key water quality parameter.

Sanou *et al*. (2022) stated that dissolved oxygen is a crucial water quality characteristic that is not harmful to fish even in high concentrations but can be lethal at very low levels. **Rachmawati *et al.* (2015)** and **Caesar *et al.* (2021)** noted that catfish thrive in water with an oxygen concentration of at least 3mg/ L. The dissolved oxygen concentrations under the 0.5ml addition of palm oil decreased from 3.6 to 2.5mg/ L, while those under the 1.5ml addition fluctuated from 2.7 to 4.0mg/ L. As transportation density increased, the dissolved oxygen concentration decreased; however, the values from the 1.5ml palm oil addition are higher than those of the 0.5ml addition. This indicates that the 1.5ml addition of palm oil was more effective in conserving dissolved oxygen levels in the transportation media.

CONCLUSION

The research focused on the effects of transportation stress on the physiological responses of *Clarias gariepinus*. Transporting this fish species at different densities significantly contributed to stress, while the duration of transportation also affected the physiological status of the fish. Based on these findings, natural anti-stress agents, such as palm oil at varying inclusion levels, can help mitigate the effects of transportation stress due to their ability to reduce oxidative stress in fish. The oil contains α-tocopherol, a powerful antioxidant that can improve redox balance.

The inclusion of palm oil at 1.5ml in varying water densities for transporting fish provided a greater ameliorating effect against transportation stress compared to the 0.5ml inclusion. Therefore, it is essential to consider transportation density to reduce stress, while using palm oil as an anti-stress agent against oxidative stress is recommended. Further research is needed to explore the potency and optimal concentration of this anti-stress component.

STATEMENTS & DECLARATIONS

Limitations

This analysis cannot account for the independent effect of density due to the missing control data points at other densities.

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Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Mathew O. Ayoola, Isaac A. Abioye and Emmanuel B. Adeleye. The first draft of the manuscript was written by Mathew O. Ayoola. Faith Ayoade, Ajibola Ajiboye and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declares that there is no conflict of interests regarding the publication of this article

Data availability statement

The document includes all the data and findings that were employed in writing this article.

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