



Ameliorative Effects of *Elaeis guineensis* Oil on Water Quality, Hematological and Biochemical Parameters of *Clarias gariepinus* under Transport Condition

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ABSTRACT

To ascertain the effects of *Elaeis guineensis* oil on the haematological and water quality parameters of *Clarias gariepinus* to road transportation, experiments were conducted. Forty-two clinically healthy adult *Clarias gariepinus*, weighing an average of 450.46 ± 26.06 g and measuring an average total length of 38.23 ± 4.46 cm were utilized for the experiment and split into two groups. *Elaeis guineensis* oil was given to group I every day for one month and on the day of transportation, but not to group II. After transportation, the group I's packed cell volume and erythrocyte counts were significantly elevated ($P < 0.05$). In comparison to group II, the group I's total leucocyte count, neutrophil count, and stress index (neutrophil/lymphocyte ratio) were all considerably lower ($P < 0.05$). Following transportation, group II had significantly higher levels ($P < 0.05$) of nitrite, nitrate, and ammonia than group I. However, the dissolved oxygen level was significantly elevated in group I than in group II. As a result, it was determined that palm oil improved the various *Clarias gariepinus* haematological and water quality indicators. As a result, it can be beneficial to the fish to reduce the stress of transportation consequently mitigating the effect of stress leading to disease susceptibility and mortality of the fish.

Keywords: *Clarias gariepinus*, Haematological parameters, Palm oil, transport stress, Water quality.

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INTRODUCTION

Several aquaculture processes require moving fish between facilities or refilling rivers, lakes, or ponds with fish from a hatchery. Transportation is known to stress fish and trigger a variety of physiological reactions, including elevated blood glucose levels, as well as the release of catecholamines and corticosteroids (Pottinger, 2017; de Fátima Pereira de Faria *et al.*, 2021).

Studies on the impact of stress on fish's immune systems have mainly examined hematological indicators and systemic immune responses (Lima-Cabello *et al.*, 2014). Additionally, several research has demonstrated how stress increases illness vulnerability in fish due to immunological suppression (Ibrahim *et al.*, 2020; Campbell *et al.*, 2021). The creation of a lot of skin and gill mucus is one of the most obvious ways that fish react to stress (Jerez-Cepa and Ruiz-Jarabo 2021; Jia *et al.*, 2022). Fish mucosal barriers are crucial stress sensors, according to gross

observations of stressed fish (Bolner *et al.*, 2014; Bouyoucos *et al.*, 2021).

The oil palm tree (*Elaeis guineensis*), which is a member of the Palmae family, produces its fruits with fresh mesocarp, which is where palm oil is extracted. Triacylglycerols (TAG) are the main component of palm oil, while carotenoids, tocopherols, tocotrienols, phytosterols, phospholipids, glycolipids, terpenic and aliphatic hydrocarbons make up around 1% of the crude palm oil's minor components (Ng *et al.*, 2007). In addition to preserving the stability and quality of palm oil, some minor components, particularly the carotenoids, tocopherols, and tocotrienols, also have important biological properties, such as antioxidation, anti-cancer, anti-inflammatory, atherosclerosis-controlling, and cholesterol-lowering effects (Olurin *et al.*, 2004; Ochang *et al.*, 2007).

Numerous investigations of palm oil and its minor components have been performed. In addition to its antioxidant action and protection against a number

of diseases, the beta-carotene in palm oil may be crucial for addressing vitamin A insufficiency (Ayisi *et al.*, 2019; Absalome *et al.*, 2020; Yan *et al.*, 2021). The aim of this study was to evaluate the effectiveness of palm oil on the aquatic environment quality and hematological parameters of *Clarias gariepinus* subjected to transport stress

MATERIALS AND METHODS

Study Area

The experiment was carried out as a field study in Ilorin, Kwara State, located in the transitional zone within the forest and the guinea savannah regions of Nigeria (Lat 8° 08' 49.20" N, Log 4° 43' 12.00" E). The total annual rainfall ranges from 800 to 1200 mm in the NW and 1000–1500 mm in SE.

Fish Sample

A total of 42 healthy adult *Clarias gariepinus* with an average weight of 450.46 ± 23.06 g and an average length of 38.23 ± 4.46 cm was used for the experiment. The fish had no clinical manifestation of disease and were acquired from a commercial catfish farm. On arrival, the fish were released into the plastic holding facility with water supplied in a flow-through system initially and topped up. The fish were acclimatized for 2 weeks before the experiment and were fed with a commercial pelleted feed once per day. The commercial diet contains 34% crude protein and 3.5% crude fat. The fish samples were divided into two groups. Group, I (PPF) had their feed supplemented with palm oil at the rate of 2ml/L of water every day for one month while group II (OPF) which served as the control, was not supplemented with palm oil.

Evaluation of the Water Quality Parameters

Parameters of water quality were measured before and after the fish transportation. Before and after the transport process, measurements of temperature, dissolved oxygen (DO), pH, nitrate, nitrite, and ammonia of water were evaluated and recorded, respectively. In situ measurements of the water's temperature, pH, and dissolved oxygen were made using portable dissolved oxygen meters (HI 9146) and a Combo pH/EC/TDS/Temperature Hanna meter (HI98129). While samples of the water were taken from the holding facility before and after transportation for ammonia, nitrate, and nitrite concentrations and were measured spectrophotometrically by the 2005 American Public Health Association (APHA) guidelines.

Experimental Design

Transportation of *Clarias gariepinus*

Before being transported, the fish were starved for 24 hours and then handled, graded, and netted. Two built black 50-litre open-cut portable containers with dimensions of 310 mm in width, 400 mm in length, and

575 mm in height were used to transport the fish. Group, I fish samples were placed in one tank with palm oil added to the water, while the group II fish samples were placed in a tank without palm oil added. The two groups were then subjected to a 100 km trip on a tarred plain road between 06 00 h and 09 00 h. Blood samples were collected from representative fish samples from each group (I and II) before transportation and post-transportation.

Haematological parameters

Blood samples were obtained from the caudal vein of the fish using a 22-gauge needle and a sterile disposable plastic syringe in vacuum containers coated with the anticoagulant sodium heparin (1 %). The samples were placed in a Coleman box containing ice packs and transported to the laboratory for analysis. Erythrocytes were diluted with Grower's solution before being measured using a Neubauer hemocytometer (Voigt, 2000). After dilution with Dacie's solution, the white blood cells were counted using a Neubauer hemocytometer (Dacie and Lewis, 2001). The cyano-haemoglobin technique was used to calculate the haemoglobin (g /dL) content. Hematocrit levels were calculated using the microhematocrit technique (McMullin *et al.*, 2005). The neutrophil/lymphocyte ratio (stress index) was also calculated. Using a total protein kit, plasma protein was calculated by the Biuret method using a dye reagent (Qualigens Fine Chemicals, Mumbai, India). The plasma glucose was determined using the GOD-POD-based kit procured from Diatek, Kolkata, India.

Analyses of Data

Data generated from the experiment were expressed as mean \pm SEM and analyzed using the student's *t*-test to compare the two groups. Values of $P < 0.05$ were considered significant. Data generated were analyzed using GraphPad Prism (Version 5.3).

RESULTS

Table 1 shows the water quality parameters of the water before and after transportation. After transportation, the dissolved oxygen level measured in the water containing Group I was significantly elevated ($P < 0.05$) than the level measured in Group II. After transportation, there was a significant decline ($P < 0.05$) in the ammonia concentration in the water containing Group I in contrast with the concentration of the Group II.

The nitrate and nitrite levels in the water of Group I declined significantly ($P > 0.05$) than those found in Group II (Table 1). There was no significant difference in the values for temperature and pH in both groups I and groups II. The haematological parameters of *Clarias gariepinus* before and after transportation are shown in Table 2. In comparison to Group II,

Group I's post-transport packed cell volume was considerably higher ($P < 0.05$) than that of Group II. Group I's erythrocyte counts were higher than those recorded in Group II. When compared to the value obtained in Group II the mean corpuscular volume

obtained in Group, I was considerably higher ($P < 0.05$). When compared to the value obtained in Group II, the reported hemoglobin concentration in Group I was higher ($P < 0.05$) (Table 2).

Table 1: Water Quality Parameters of *Clarias gariepinus* subjected to Road Transportation

Water Quality Parameters		Group I (Mean \pm SEM)	Group II (Mean \pm SEM)
Water Temperature ($^{\circ}$ C)	Pre-Transportation	27.55 \pm 2.10	26.65 \pm 1.40
	Post-Transportation	27.65 \pm 1.70	29.50 \pm 1.80
Dissolved Oxygen (mg/mL)	Pre-Transportation	5.04 \pm 1.22	5.45 \pm 0.78
	Post-Transportation	4.76 \pm 1.58 ^a	3.11 \pm 0.07 ^b
pH	Pre-Transportation	6.52 \pm 0.76	6.94 \pm 0.54
Ammonia (Mg/mL)	Pre-Transportation	0.02 \pm 0.012	0.03 \pm 0.016
Nitrate (mg/mL)	Pre-Transportation	20.67 \pm 2.89	20.23 \pm 3.56
	Post-Transportation	21.53 \pm 1.18 ^a	25.47 \pm 5.06 ^b
Nitrite (mg/mL)	Pre-Transportation	0.03 \pm 0.02	0.03 \pm 0.03
	Post-Transportation	0.05 \pm 0.02 ^a	0.08 \pm 0.07 ^b

^{a,b} Means for the same row having different superscript letters are significantly ($P < 0.05$) different

Table 2: Erythrocyte Parameters of *Clarias gariepinus* subjected to Road Transportation

Erythrocyte Parameters	Time	Group I (Mean \pm SEM)	Group II (Mean \pm SEM)
Packed Cell Volume (%)	Pre-Transportation	26.86 \pm 5.11	20.12 \pm 1.43
	Post-Transportation	27.75 \pm 4.65 ^a	21.66 \pm 0.98 ^b
Erythrocyte Count ($\times 10^6$ mm ⁻³)	Pre-Transportation	2.94 \pm 0.26	1.75 \pm 0.78
	Post-Transportation	2.97 \pm 2.98 ^a	2.01 \pm 0.55 ^b
Mean Corpuscular Volume (fl)	Pre-Transportation	87.11 \pm 8.76	74.32 \pm 4.54
	Post-Transportation	87.43 \pm 7.98 ^a	83.56 \pm 3.83 ^b
Haemoglobin Concentration (g/100mL)	Pre-Transportation	12.43 \pm 2.55	11.23 \pm 1.97
	Post-Transportation	12.65 \pm 3.76 ^a	9.34 \pm 1.05 ^b
Mean Corpuscular Hb Conc. (g%)	Pre-Transportation	25.83 \pm 6.76	20.33 \pm 1.45
	Post-Transportation	26.44 \pm 4.61	20.78 \pm 0.87
Mean Corpuscular Haemoglobin (pg)	Pre-Transportation	31.45 \pm 7.29	24.23 \pm 1.56
	Post-Transportation	33.59 \pm 6.58	24.47 \pm 3.38

^{a,b} Means for the same row having different superscript letters are significantly ($P < 0.05$) different

Table 3 shows the leucocyte indices of *Clarias gariepinus* before and after transportation. Leucocyte counts in the Group I and Group II were different; the Group I'S result was lower. When compared to the result obtained in the Group II, the heterophils count in Group I was lower ($P > 0.05$). In comparison to the value found in Group I, the stress index found in Group II was higher.

Table 3: Leucocyte parameters of *Clarias gariepinus* subjected to Road Transportation

Leucocyte Parameters	Time	Group I	Group II
Leucocyte Count ($\times 10^3 \text{ mm}^{-3}$)	Pre-Transportation	1.74 \pm 0.02	1.85 \pm 0.28
	Post-Transportation	1.87 \pm 0.38 ^a	2.91 \pm 0.89 ^b
Heterophil Count ($\times 10^3 \text{ mm}^{-3}$)	Pre-Transportation	1.11 \pm 0.86	1.32 \pm 0.54
	Post-Transportation	1.03 \pm 0.18 ^a	1.76 \pm 0.87 ^b
Lymphocyte Count ($\times 10^3 \text{ mm}^{-3}$)	Pre-Transportation	1.43 \pm 0.57	1.36 \pm 0.77
	Post-Transportation	1.55 \pm 0.14	1.38 \pm 0.85
Monocyte Count ($\times 10^3 \text{ mm}^{-3}$)	Pre-Transportation	0.73 \pm 0.06	0.33 \pm 0.02
	Post-Transportation	0.44 \pm 0.11	0.78 \pm 0.37
Stress Index	Pre-Transportation	0.25 \pm 0.09	0.63 \pm 0.06
	Post-Transportation	0.59 \pm 0.18 ^a	1.47 \pm 0.92 ^b

^{a,b} Means for the same row having different superscript letters are significantly different at ($P < 0.05$)

When compared to the value obtained in Group II, the total protein recorded in the Group I group was significantly elevated ($P < 0.05$) (Table 4). Following the transportation of *Clarias gariepinus*, the blood glucose level in the group I was greater than the value obtained in the group II (Table 4).

Table 4: Biochemical Parameters of *Clarias gariepinus* subjected to Road Transportation

Biochemical Parameters	Time	Group I	Group II
		(Mean \pm SEM)	(Mean \pm SEM)
Total Protein (g/L)	Pre-Transportation	68.76 \pm 8.91	65.72 \pm 7.63
	Post-Transportation	64.65 \pm 9.85 ^a	59.56 \pm 3.38 ^b
Blood Glucose ($\mu\text{Mol/L}$)	Pre-Transportation	2.94 \pm 3.42	2.45 \pm 0.68
	Post-Transportation	2.05 \pm 2.88 ^a	1.07 \pm 0.21 ^b

^{a,b} Means for the same row having different superscript letters are significantly ($P < 0.05$) different

DISCUSSION

The changes in the water quality parameters recorded in this study were similar to the findings of **Vanderzwalmen et al., (2021)** and **Huiyan et al., (2022)**, where the transportation of fish was observed to alter the water quality parameters. A previous study reported that the ammonia nitrogen content in aquatic water should be less than 0.02 mg/L to minimize physiological stress and ammonia accumulation in fish blood (**Sinha et al., 2015**). In this study, compared to Group I, the concentration of ammonia nitrogen increased significantly during transportation in Group II indicating a palm-oil-modulatory effect. Fish mostly excrete ammonia, which, at high concentrations, impairs metabolism, changes development, and even results in mortality (**Golombieski et al., 2013; Yichao et al., 2022**).

According to **Pottinger (2017)**, ammonia, nitrate, and nitrite may have an impact on how the stress axis in fish functions. As a result, a sudden change in ammonia nitrogen during transportation can be lethal therefore a measure such as the administration of an anti-stress agent must be adopted to ameliorate

the effect of stress of transportation. According to this study, Group II's water's dissolved oxygen (DO) level significantly dropped during travel. A decrease in DO concentration in the Group II indicated that the journey was extremely stressful.

Transport-related stress frequently results in an increase in breathing rate, which in turn increases carbon dioxide excretion and dissolved oxygen consumption in the transport tanks, having a detrimental effect on pH and dissolved oxygen levels. In addition, as was already indicated, the increased outflow of nitrogenous wastes increased the amount of ammonia in the fish's aqueous medium, which is one of the primary causes of stress. Additionally, handling, confinement, and declining water quality all contribute to the stress that fish experience during transportation (**EFSA, 2004; Manuel et al., 2014**).

When evaluating the impact of transportation stress on fish health, packed cell volume (PCV) and erythrocyte count are important factors to take into account (**Sampaio et al., 2016; Seibel et al., 2021**). They help determine how well the blood can carry

oxygen as well. Due to Group I's higher erythrocyte count and PCV levels, it is suggested that palm oil modulated the erythrocyte indices of group I. This shows even more that the group handled the stress of transportation better than Group II.

The stress of transportation may have had an impact on the fish's immunological and defense systems since the transported fish had greater leucocyte counts thereafter. Group II in this study had higher leucocyte counts, which suggested that transportation stress had a more noticeable effect on the group. Additionally, given that palm oil has a considerable antioxidant effect under stressful circumstances; it suggests that palm oil had a modulatory and ameliorative effect in Group I.

CONCLUSION

Palm oil supplementation before transport ameliorates the deleterious effect of transportation stress in *Clarias gariepinus* thereby reducing the impact of stress on fish health, disease susceptibility, and mortality.

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Conflicts of interest

The authors declared no competing interests.

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