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Ameliorative Effects of Ascorbic Acid on Haematological Indices of Adult *Clarias Gariepinus* Following a 100 km Transportation

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ABSTRACT

Experiments were performed to determine the effects of ascorbic acid on haematological of *Clarias gariepinus* and water quality parameters to road transportation. A total of 40 apparently healthy adult *Clarias gariepinus* of an average weight of 450.46 ± 23.06 g and an average length of 38. 23 ± 4.46 cm were used for the experiment and were divided into two groups. Group I (AAF) was administered ascorbic acid daily for one month and on the day of transportation while group II (NAF) was not administered. After transportation of the erythrocyte counts and packed cell volume was significantly higher in group I ($P < 0.05$). Total leucocyte count, neutrophil count, and neutrophil/lymphocyte ratio of group I were significantly lower ($P < 0.05$) compared to group II. The concentrations of nitrite, nitrate, and ammonia were significantly higher ($P < 0.05$) in group II compared to group I post-transportation. The dissolved oxygen content of group I was however higher ($P < 0.05$) compared to group II. It was therefore concluded that ascorbic acid modulated some haematological parameters of *Clarias gariepinus* and water quality parameters. Thus, may be beneficial to the fish in ameliorating the stress of transportation.

Keywords: Erythrocyte indices; Fish; Leucocyte indices; Transportation stress; Vitamin C

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INTRODUCTION

The transport of live fish is a typical practice in aquaculture and may cause stress to fish due to overcrowding, deterioration in water quality arising from changes in ammonia, pH, and dissolved oxygen during transport can cause varying degrees of stress response and physiological changes leading to high mortality (Hong *et al.*, 2019; Serafini *et al.*, 2019). According to previous studies, physiological changes in fish can damage both specific and nonspecific immunity, resulting in a high prevalence of diseases (Wendelaar Bonga, 1997; Si *et al.*, 2019). Stress also promotes the production of metabolites in the blood such as corticosteroids and catecholamines, as well as the generation of free radicals in fish (Park*et al.*, 2016; Stara *et al.*, 2018).

Various environmental stress factors, such as air exposure, handling, physical disturbance, and variations in water quality parameters are present during the transport process and the early stages of aquatic culture after transportation (Manuel *et al.*, 2014; Abdel-Tawwab *et al.*, 2019). These elements have an effect on the body's natural physiological state of aquatic animals, triggering stress and immunological responses (Jerez-Cepa and Ruiz-Jarabo, 2021).

Nervous, immunological, and hormonal systems are among the internal physiological mechanisms involved in adapting to a stressor in fish (Sampaio and Freire, 2016). However, there is a metabolic cost involved with this adaptation, which involves diverting energy from normal metabolic operations to stress-response functions (Harmon, 2009). Primary, secondary, and tertiary stress responses are commonly used to classify these reactions. The release of hormones into the circulatory system is the initial response, which subsequently triggers secondary responses such as increased heart rate, gill blood flow, and metabolic rate, as well as decreased plasma chloride, sodium, and potassium levels (Portz *et al.*, 2006; Cook *et al.*, 2011).

Increases in erythrocyte count accompanied the considerable increases in hemoglobin concentration, possibly to boost blood oxygen capacity and provide tissues with more oxygen during hypoxic circumstances leading to changes in blood protein levels are physiological reactions that are used as a fish health indicators (Tahmasebi-Kohyani *et al.*, 2012; Sampaio and Freire, 2016). Several fish species, including common carp (Dobšikova *et al.*, 2009) and *Clarias gariepinus* (Manuel *et al.*, 2014), showed negative effects of transport stress on serum total protein (Pakhira *et al.*, 2015). Both internal variables like sex, age, size, and health as well as external ones including seasonal dynamics, water temperature, environmental quality, nutrition, and

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stress can affect the fish's haematological characteristics (Tacchi *et al*., 2015; Abdel-Tawwab *et al.,* 2019). Because neutrophils and lymphocytes are affected by stress in opposing directions, researchers frequently take this ratio into account when assessing the stress response (Davis *et al*., 2008).

Most animals can produce enough ascorbic acid to maintain normal functions of life, but fishes are unable to do so because they lack the enzyme L-gluconolactone oxidase which produces vitamin C from glucose (Smirnoff, 2018).As a result, ascorbic acid is necessary for the reason stated above and it should be taken in feed (Bowzer *et al*., 2014). The blood of fish is an important tissue of the body associated with its vital activities and an indicator of stress (Shahjahan *et al*., 2022). Transportation of fish may result in the outbreak of diseases and mortality due to stress therefore this study aims to evaluate the effect of ascorbic acid on the haematological response of *Clarias gariepinus* due to transportation 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 40 apparently healthy adult *Clarias gariepinus* of an average weight of 450.46 ± 23.06 g and an average length of 38. 23 ± 4.46 cm were 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 (AAF) had their feed supplemented with Vitamin C at the rate of 400 mg/kg every day for a period of one month (Ibrahim *et al*., 2020) while group II (NAF) which served as the control, their feed was not supplemented with vitamin C.

Measurement of Water Quality

Water quality parameters were determined before and after the transport of the fish. Measurement of temperature, dissolved oxygen (DO), pH, nitrate, nitrite and ammonia of water respectively were examined and recorded before and after the transport process directly. Water temperature and pH were measured using Combo

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pH/EC/TDS/ Temperature Hanna meter (HI98129) and dissolved oxygen was determined using a portable dissolved oxygen meter (HI 9146), they were determined in situ. For ammonia, nitrate and nitrite water samples were collected from the holding facility before and after transport, and were immediately saved at −20 °C until analysis and were determined spectrophotometrically in according to the methods described by American Public Health Association (APHA) (2005).

Transportation of *Clarias gariepinus*

The fish were starved for 24 hoursbefore the transport process and were subjected to netting, handling, and grading. The fish was transported using two constructed black 50litre open-cut portable container measuring 310 mm in width, 400 mm in length, and 575mm in height. The group I fish samples were put in one tank with ascorbic acid added to the water, while the group II fish samples were put in the other tank without ascorbic acid and transported for 100 km on a tarred plain road between 06 00 h to 09 00 h. The 100 km journey took three hours and involved the fish samples. Blood samples were taken from representative fish samples from each group before transportation and following transport.

Haematological Analysis

Blood samples were obtained from the caudal vein of the fish using a 22-gauge needle and a sterile disposable pl astic syringe into 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). Using a total protein kit, plasma protein was calculated (Biuret method using dye reagent, Qualigens Fine Chemicals, Mumbai, India). Using a readily available kit, plasma glucose was calculated (GOD-POD-based kit for estimation of blood glucose procured from Diatek, Kolkata, India 2.10).

DATA ANALYSIS

Data generated from the study was expressed as mean ± SEM and analysed using the student's *t*-test to compare between the two groups. Values of $P < 0.05$ were considered significant. Data generated from this study were analysed using GraphPad Prism (Version 5.3).

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RESULTS

The water quality parameters of the water before and after transportation is shown in table 1. The dissolved oxygen value of recorded in the water holding the AAF group was significantly higher ($P < 0.05$) than the value obtained in the NAF group post transportation. The concentration of ammonia in the water holding the AAF group obtained post-transportation was significantly lower ($P < 0.05$) than the concentration of obtained in the NAF group post-transportation.

Table 1. Water Quality Parameters of *Clarias gariepinus* Subjected to Road Transportation

Water Quality Parameters		AAF (Mean ± SEM)	NAF (Mean ± SEM)
Water Temperature (°C)	Pre-Transportation	27.70 ± 1.10	27.65 ± 1.40
	Post-Transportation	29.65 ± 1.70	29.50 ± 1.80
Dissolved Oxygen (mg/ml)	Pre-Transportation	5.04 ± 1.22	5.45 ± 0.78
	Post-Transportation	4.50 ± 1.48[a]	3.01 ± 0.05[b]
pH	Pre-Transportation	6.72 ± 0.76	6.84 ± 0.54
	Post-Transportation	7.13 ± 0.38	7.86 ± 1.23
Ammonia (Mg/ml)	Pre-Transportation	0.02 ± 0.012	0.03 ± 0.016
	Post-Transportation	0.03 ± 0.02[a]	0.17± 0.8[b]
Nitrate (mg/ml)	Pre-Transportation	20.45 ± 2.89	20.23 ± 3.56
	Post-Transportation	20.59 ± 1.18[a]	23.47 ± 5.06[b]
Nitrite (mg/ml)	Pre-Transportation	0.03 ± 0.02	0.03 ± 0.03
	Post-Transportation	0.04 ± 0.02[a]	0.09 ± 0.07[b]

[a,b] Means for the same column having different superscript letters are significantly ($P < 0.05$) different. Key: AAF = Administered with ascorbic acid; NAF = Not Administered with ascorbic acid.

The concentration of nitrate in the water holding the AAF group was lower ($P < 0.05$) than the value obtained in the NAF group. The concentration of nitrite in the water holding the AAF group was lower ($P < 0.05$) than the value obtained in the NAF group. There was no significant difference in the values obtained for temperature between the group.

Table 2 shows the erythrocyte indices of *Clarias gariepinus* before and after

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transportation. The packed cell volume obtained post-transport in the AAF group was significantly higher ($P < 0.05$) than the obtained in the NAF group post-transportation. The erythrocyte counts in the AAF group were higher than the value obtained in the NAF group.The mean corpuscular volume obtained in the AAF was significantly higher ($P < 0.05$) than the value obtained in the NAF group. The recorded haemoglobin concentration in the AAF group was higher ($P < 0.05$) than the value obtained in the NAF group.

Table 2. Erythrocyte Parameter of *Clarias gariepinus* subjected to Road Transportation

Erythrocyte Parameters	Time	AAF (Mean ± SEM)	NAF (Mean ± SEM)
Packed Cell Volume (%)	Pre-Transportation	26.76 ± 5.11	20.12 ± 1.43
	Post-Transportation	27.65 ± 4.65[a]	21.46 ± 0.88[b]
Erythrocyte Count (x10^6 mm^{-3})	Pre-Transportation	2.94 ± 0.22	1.85 ± 0.78
	Post-Transportation	2.97 ± 2.98[a]	2.01 ± 0.55[b]
Mean Corpuscular Volume (fl)	Pre-Transportation	88.11 ± 8.76	78.32 ± 4.54
	Post-Transportation	89.43 ± 7.98[a]	81.56 ± 3.23[b]
Haemoglobin Concentration(g/100ml)	Pre-Transportation	12.43 ± 2.55	11.23 ± 1.97
	Post-Transportation	12.65 ± 3.76[a]	9.34 ± 1.05[b]
Mean Corpuscular Haemoglobin Concentration (g%)	Pre-Transportation	25.33 ± 6.76	20.33 ± 1.45
	Post-Transportation	26.44 ± 4.11[a]	20.78 ± 0.87[b]
Mean Corpuscular Haemoglobin (pg)	Pre-Transportation	31.45 ± 7.89	24.23 ± 1.56
	Post-Transportation	33.59 ± 6.88 [a]	26.47 ± 3.06 [b]

[a,b] Means for the same column having different superscript letters are significantly ($P < 0.05$) different Key: AAF = Administered with ascorbic acid; NAF = Not Administered ascorbic acid

The Leucocyte indices of *Clarias gariepinus* before and after transportation is shown in table 3. The leucocyte count obtained in the AAF group was lower than the value obtained in the NAF group. The

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neutrophil count obtained in the AAF group was lower (P < 0.05) than the value obtained in the NAF group. The neutrophil/lymphocyte ratio obtained in the NAF group was higher than the value obtained in the AAF group.

Table 3. Leucocyte parameters of *Clarias gariepinus* to Road Transportation

Leucocyte Parameters	Time	AAF (Mean ± SEM)	NAF (Mean ± SEM)
Leucocyte Count ($x10^3$ mm^{-3})	Pre-Transportation	1.74 ± 0.02	1.85 ± 0.28
	Post-Transportation	1.87 ± 0.18[a]	2.91 ± 0.79[b]
Neutrophil Count ($x10^3$ mm^{-3})	Pre-Transportation	1.11 ± 0.56	1.32 ± 0.54
	Post-Transportation	1.03 ± 0.08[a]	1.76 ± 0.67[b]
Lymphocyte Count ($x10^3$ mm^{-3})	Pre-Transportation	1.43 ± 0.55	1.33 ± 0.47
	Post-Transportation	1.55 ± 0.16	1.34 ± 0.85
Monocyte Count ($x10^3$ mm^{-3})	Pre-Transportation	0.73 ± 0.06	0.33 ± 0.02
	Post-Transportation	0.44 ± 0.11	0.78 ± 0.37
Neutrophil/ Lymphocyte Ratio	Pre-Transportation	0.25 ± 0.09	0.63 ± 0.06
	Post-Transportation	0.59 ± 0.18[a]	1.47 ± 0.93[b]

[a,b] Means for the same column having different superscript letters are significantly (P < 0.05) different. Key: AAF = Administered with ascorbic acid; NAF = Not Administered with ascorbic acid.

The total protein obtained in the AAF group was higher (P < 0.05) than the value obtained in the NAF group (table 4). The blood glucose level obtained in the AAF group was higher than the value obtained in the NAF group post-transportation of *Clarias gariepinus* (Table 4).

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

Biochemical Parameters	Time	AAF (Mean ± SEM)	NAF (Mean ± SEM)
Total Protein (g/L)	Pre-Transportation	66.76 ± 8.11	65.12 ± 7.43
	Post-Transportation	64.65 ± 9.65[a]	59.46 ± 3.88[b]
Blood Glucose (µMol/L)	Pre-Transportation	2.94 ± 3.22	2.85 ± 0.78
	Post-Transportation	2.09 ± 2.98[a]	1.07 ± 0.15[b]

[a,b] Means for the same column having different superscript letters are significantly ($P < 0.05$) different. Key: AAF = Administered with ascorbic acid; NAF = Not Administered with ascorbic acid.

DISCUSSION

Fish transportation results in severe changes to water quality parameters. The production of ammonia is one of the main causes of the deterioration of water. According to a previous study (Sinha *et al.,* 2015), the ammonia nitrogen level in aquatic water should be less than 0.02 mg/L for fish aquaculture because it might cause physiological stress and ammonia deposition in the blood in fish (Sinha *et al.,* 2015). During transportation in this study, the ammonia concentration increased significantly in the NAF group compared to the AAF group suggesting an ameliorative effect by ascorbic acid. Fish excrete predominantly ammonia (Golombieski *et al.,* 2013), which, at high concentrations, affects metabolism, changes development, and can even cause mortality (Cavero *et al.,* 2004; Bolner *et al.,* 2014). The possibility that ammonia, nitrate, and nitrite impact the function of the stress axis in fish was recently reported by Pottinger (2017). According to a recent report, an increase in ammonia levels may increase mortality by causing the reaction that turns NH_3 into NH^{4+} and slightly increases the alkalinity of the water (Yichao *et al.,* 2022). Therefore, a sudden change in ammonia nitrogen during transit can result in death. In this study, it was observed during the transportation, the dissolved oxygen (DO) concentration was significantly reduced in the NAF group. The reduction in the DO concentration in the NAF group is indicative of severe stress during the transportation.

The transport process in this study contributed to the worsening of water quality especially in the NAF group by lowering the pH, increasing the concentration of ammonia, and lowering the DO level. Both the respiration rate and the excretion of

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nitrogenous waste increased due to the high stocking density and increased fish motor activity during the transit process (Gatica *et al.*, 2008; Jia *et al.*, 2022). Transportation stress usually results in an increased respiration rate which led to increased consumption of dissolved oxygen and increased emission of carbon dioxide in the transport tanks, which had an adverse effect on the levels of dissolved oxygen and pH. Furthermore, the increased excretion of nitrogenous wastes as discussed above raised the concentration of ammonia in the water medium, which is one of the primary factors that trigger stress. When being transported, fish are frequently subjected to stressors such as handling, confinement, and declining water quality (EFSA, 2004; Manuel *et al.*, 2014). The transport usually causes an increase in water temperature due to increased metabolic processes occasioned by the stress of transportation as observed in this study. It was noticed in this study that the temperature of water in the AAF group was lower than in the NAF group suggesting an ameliorative effect of ascorbic acid.

Blood parameters are crucial in determining the physiological and functional status of fish exposed to transportation stress since hematological assessment is a pathophysiological reflection of the entire body When analyzing the impacts of the stress of transportation on fish health, packed cell volume (PCV) and erythrocyte count are crucial (Shahjahan*et al.*, 2022). They also help assess the blood's ability to deliver oxygen. Higher erythrocyte counts and PCV values in the AAF group suggest a modulatory role by ascorbic acid in the group and further indicate that the group tolerated the transportation stress better than the NAF group.

The increased higher leucocyte counts in the transported fish post-transportation indicate that the stress of transportation stimulated the fish's immune system and defense mechanisms. In the current study, fish in the NAF group had significantly higher leucocyte counts, indicating a greater impact of transportation stress. It further suggests a modulatory and ameliorative role by ascorbic acid. Ascorbic acid has been reported to play an important role as a potent antioxidant during stressful conditions.

The lower values of Hb, RBC, PCV, MCH, MCHC, and plasma glucose recorded in the NAF group when fish are exposed to the stress of transportation suggest decreased ability to transport oxygen in the group (Aguirre-Guzman *et al.*, 2016). However, supplementation of ascorbic acid in the AAF group ameliorated the deleterious effect of the stress as the values of Hb, RBC, PCV, MCH, and MCHC were higher in the group.

It was therefore concluded that supplementation of ascorbic acid to *Clarias gariepinus* before transportation

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will be beneficial in ameliorating the effects of stress of transportation.

APPROVAL OF ETHICAL COMMISSION

This research project was approved and endorsed by the Faculty of Veterinary Medicine's ethical review committee of the University of Ilorin, with approval reference number: UERC/FVM/2022/042.

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