Original Article

Melatonin Modulates Haematological and Water Quality Parameters Following a 100 Km Transportation of *Clarias gariepinus* by Road

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How to Cite This Article Adah, AS., Adah, DA., Nwonuma, CO., Oyekunle, T., & Olaosebikan, B. (2023). Melatonin Modulates Haematological and Water Quality Parameters Following a 100 Km Transportation of Clarias gariepinus by Road. *Iranian Journal of Veterinary Medicine, 17*(3):199-208. http://dx.doi.org/10.32598/ijvm.17.3.1005310

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ABSTRACT

Background: The transportation of fish is a common practice in aquaculture. However, the transportation of fish results in significant stress that can cause mortality and disease outbreaks due to compromised immune status. To ameliorate the effect of this stress, it is advocated that a suitable antioxidant be supplemented to fish before subjecting to transportation.

Objectives: The present experiment was performed to evaluate the effect of melatonin on haematological parameters and water quality indices of *Clarias gariepinus* post-transportation.

Methods: Forty adult *C. gariepinus* fishes, weighing an average of 450.46 g and measuring an average of 38.23 cm and 4.46 cm were used for the experiment. They were divided into two groups. Group I (MMF) was supplemented with melatonin in their feed every day for one month while group II (OMF) was not administered melatonin. The subjects were fed a commercial pelleted diet once a day throughout the experiment. They were maintained in a tank made of plastic, and water was originally provided through a flow-through system. Haematological and water quality parameters were determined before and after transportation.

Results: After the transportation process, erythrocyte count and packed cell volume of the group I was significantly higher (P<0.05). In comparison to group II, the total leucocyte count, neutrophil count, and neutrophil/lymphocyte ratio of group I were all significantly lower (P<0.05). After transit, group II had considerably higher quantities of nitrite, nitrate, and ammonia (P<0.05) than group I. Following transportation, group II had significantly higher nitrite, nitrate, and ammonia (P<0.05) than group I. However, in group I, dissolved oxygen concentration was greater (P<0.05) than that of group II.

Conclusion: Accordingly, it was concluded that melatonin affected various haematological and water quality variables in *C. gariepinus* and will ameliorate the stress of transportation.

Keywords: Catfish, Haematology, Melatonin, Transportation, Water properties

Article info:
Received: 24 Sep 2022
Accepted: 14 Dec 2022
Publish: 01 July 2023

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1. Introduction

In aquaculture, live fish transportation is a common practice. Fishes experience stress during transportation among other reasons because of high stocking density and deteriorating water qualities (Hong et al., 2019; Serafini et al., 2019). Furthermore, several physiological changes happen as a result of the stress. Studies have shown that physiological modifications in fish can impair both specific and nonspecific immunity, leading to a high incidence of diseases (Wendelaar-Bonga, 1997; Si et al., 2019; Ghorbani et al., 2021). On the other hand, stress encourages the synthesis of blood metabolites, including corticosteroids and catecholamines as well as the increased levels of oxidative stress markers in fish (Park et al., 2016; Stara et al., 2018; Shamohamadi et al., 2021).

There are a variety of natural stressors present during transportation, including air exposure, handling, physical disturbance, temperature change, salinity fluctuations, and high level of dissolved ammonia (Manuel et al., 2014; Abdel-Tawwab et al., 2019; Rahmati et al., 2022). These factors affect the physiological condition of the body of aquatic animals causing stress and immunological reactions (Jerez-Cepa & Ruiz-Jarabo, 2021).

Nervous, immunological, hormonal, and haematological systems are among the internal physiological mechanisms involved in adapting to a stressor (Sampaio & Freire., 2016). However, there is a metabolic cost involved in this adaptation, which involves diverting energy from normal metabolic operations to stress-response functions (Chabot et al., 2016). Primary, secondary, and tertiary stress responses are commonly used to classify these reactions (Tacchi et al., 2015; Abdel-Tawwab et al., 2019). The release of hormones into the circulatory system is the initial response, which subsequently triggers secondary responses, such as increased heart rate, increased gill blood flow, and increased metabolic rate, as well as decreased plasma glucose, chloride, sodium, and potassium levels (Campbell et al., 2021; Cook et al., 2011).

There is usually a significant increase in hemoglobin concentration, which is accompanied by increases in erythrocyte count, potentially increasing blood oxygen capacity and supplying tissues with more oxygen under hypoxic conditions following stressful situations (Bowzer et al., 2014). These changes in haematological indicators are associated with low levels of dissolved oxygen (DO). Physiological responses, such as changes in blood protein levels also occur and are employed as a fish health indicator (Tahmasebi-Kohyani et al., 2012). Several fish species, including rohu, Labeo rohita, and common carp have been reported to experience adverse changes in serum total protein due to stress of transportation (Dobšikova et al., 2009; Bowzer et al., 2014; Pakhira et al., 2015). The fish haematological parameters can be altered by factors, such as changes in water temperature and stress (Tacchi et al., 2015; Abdel-Tawwab et al., 2019). Researchers usually consider the ratio of neutrophils to lymphocytes in evaluating stress response because neutrophils and lymphocytes are altered by stress in opposite directions (Davis et al., 2008; Forget et al., 2017).

Melatonin not only acts as a highly effective antioxidant but also as a direct scavenger of free radicals (Tan et al., 2000; Maitra & Hassan, 2016, Nisembaum et al., 2021). Melatonin protects cells from DNA damage against peroxynitrite (Tan, 2000). In the macrophage cell line (J774A.1), melatonin reduced lipid peroxidation levels and also enhanced free radical detoxification (Tain et al., 2010). It also enhances the effects of other antioxidants, such as melatonin and glutathione, thereby stabilizing the erythrocyte membrane and improving the erythrocyte indices (Tan et al., 2002; Sadowska-Bartosz, 2014).

In vertebrates, the pineal gland and the retina both secrete the hormone melatonin, which is made from the amino acid tryptophan (Saha et al., 2019). The majority of the hormone are created during the photoperiod's dark phase, which is characterized by strong daily rhythms for melatonin (Nisembaum et al., 2015; Galano et al., 2018). Melatonin fluctuations in fish indicate its role in circadian rhythms and regulation of a variety of physiological and behavioural processes (Lima-Cabello et al., 2014; Ngasainao & Lukram, 2016).

This study was done to evaluate the effect of melatonin on haematological and selected water quality parameters due to the stress of transportation in *Clarias gariepinus*.

2. 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 healthy adult *C. gariepinus* with 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 samples had no clinical manifestation of disease and were acquired from a commercial catfish farm. On arrival, they were released into the plastic holding facility with water supplied in a flow-through system initially and topped up. They were acclimatized for two weeks before the experiment and fed with a commercial pelleted feed once a day. The commercial diet contains 34% crude protein and 3.5% crude fat. The samples were divided into two groups. Group I (MMF: Administered with melatonin) had their feed supplemented with melatonin at the rate of 2 mg/mL of water every day for one month (Ngasainao & Lukram, 2016) while group II (OMF: Not administered with melatonin) which was served as the control, was not supplemented with melatonin.

Evaluation of the water quality parameters

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

Transportation of *C. 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. While the group II was were transported 100 km on a tarred plain road between 06 00 h and 09 00 h, the group I placed in one tank with melatonin added to the water, and the other tank was left empty. The fish samples were transported 100 km in three hours. Blood samples were taken from representative fish samples from each group before and after transportation.

Haematological analysis

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 & Swist, 2000). After dilution with Dacie's solution, the white blood cells were counted using a Neubauer hemocytometer (Dacie & 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 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

The data were expressed as mean±SEM and analyzed using the student's t-test to compare the two groups. A $P<0.05$ was considered significant. Data generated were analyzed using GraphPad Prism, Version 5.3.

3. Results

The water quality parameters before and after transportation are shown in Table 1. The DO value of 4.66±1.58 mg/mL recorded in the water holding the MMF group was significantly higher ($P<0.05$) than the value of 3.01±0.05 mg/mL obtained in the OMF group post-transportation. The concentration of ammonia in the water holding the MMF group obtained post-transportation (0.03±0.02 mg/mL) was significantly lower ($P<0.05$) than the concentration obtained in the OMF group post-transportation (0.17±0.8 mg/mL).

The concentration of nitrate in the water holding the MMF group (20.59±1.18 mg/mL) was lower ($P<0.05$) than the value of 23.47±5.06 mg/mL obtained for the OMF group. The concentration of nitrite in the water holding the MMF group (0.04±0.02 mg/mL) was lower ($P<0.05$) than the value of 0.09±0.07 mg/mL obtained for the OMF group. There was no significant difference in the values obtained for temperature between the group.

Table 1. Water quality parameters of *C. gariepinus* subjected to road transportation

Water Quality Parameters	Time	Mean±SEM	
		MMF	OMF
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	4.66±1.58[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 different (P<0.05).

Abbreviation: MMF: Administered with melatonin; OMF: Not administered with melatonin; SEM: Standard error of the mean

Table 2. Erythrocyte parameters of *C. gariepinus* subjected to road transportation

Erythrocyte Parameters	Time	Mean±SEM	
		MMF	OMF
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 ($\times 10^6$ 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/100 mL)	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	20.78±0.87
Mean corpuscular haemoglobin (pg)	Pre-transportation	31.45±7.89	24.23±1.56
	Post-transportation	33.59±6.88	24.47±3.06

[a, b]Means for the same column having different superscript letters are significantly different (P<0.05).

Abbreviations: MMF: Administered with melatonin; OMF: Not administered with melatonin; SEM: Standard error of the mean

Table 3. Leucocyte parameters of *C. gariepinus* subjected to Road Transportation

Leucocyte Parameters	Time	Mean±SEM	
		MMF	OMF
Leucocyte count ($\times10^3$ 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 ($\times10^3$ 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 ($\times10^3$ mm^{-3})	Pre-transportation	1.43±0.55	1.33±0.47
	Post-transportation	1.55±0.16	1.34±0.85
Monocyte count ($\times10^3$ 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 different (P<0.05).

Abbreviations: MMF: Administered with melatonin; OMF: Not administered with melatonin; SEM: Standard error of the mean.

Table 2 shows the erythrocyte indices of *C. gariepinus* before and after transportation. The packed cell volume obtained post-transport (27.65±4.65%) in the MMF group was significantly higher (P<0.05) than (21.46±0.88%) the OMF group post-transportation. The erythrocyte counts of 2.97±2.98×10^6 mm^{-3} in the MMF group were higher than (2.01±0.55×10^6 mm^{-3}) the OMF group. The mean corpuscular volume of 89.43±7.98 fl obtained in the MMF was significantly higher (P<0.05) than the value of 81.56±3.23 fl obtained for the OMF group. The haemoglobin concentration (12.65±3.76 g/100 mL) recorded in the MMF group was higher (P<0.05) than (9.34±1.05 g/100 mL) the OMF group.

The leucocyte indices of *C. gariepinus* before and after transportation are shown in Table 3. The leucocyte counts of 1.87±0.18×10^3/mm^3 obtained in the MMF group were lower than the value of 2.91±0.79×10^3/ mm^3 obtained in the OMF group. The neutrophil counts of 1.03±0.08×10^3/mm^3 obtained in the MMF group was lower (P<0.05) than the value of 1.76±0.67×10^3/mm^3 obtained in the OMF group. The neutrophil/lymphocyte ratio obtained in the OMF group (1.47±0.93) was higher than the value of 0.59±0.18 obtained in the MMF group.

The total protein content obtained in the MMF group (64.65±9.65 g/L) was higher (P<0.05) than the value obtained in the OMF group (59.46±3.88 g/L) (Table 4). The blood glucose levels obtained in the MMF group (2.09±2.98

Table 4. Biochemical parameters of *C. gariepinus* subjected to road transportation

Biochemical Parameters	Time	Mean±SEM	
		MMF	OMF
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 different (P<0.05).

Abbreviations: MMF: Administered with melatonin; OMF: Not administered with melatonin; SEM: Standard error of the mean

µMol/L) were higher than the value obtained in the OMF group (1.07±0.15 µMol/L) post-transportation (Table 4).

4. Discussion

Water quality parameters significantly alter as a result of fish transportation. One of the key reasons why water quality declines are the formation of ammonia nitrogen. To prevent physiological stress and ammonia deposition in fish blood, earlier research recommended that the ammonia nitrogen level in water be less than 0.02 mg/L.(Sinha et al., 2015). In this study, the concentration of ammonia nitrogen rose considerably during transportation in the OMF group compared to the MMF group, indicating an ameliorative effect of melatonin. According to Golombieski et al. (2013), fish predominantly excretes ammonia, which, at high amounts, disrupts metabolism, alters growth, and even causes death (Bouyoucos et al., 2021; Bolner et al., 2014). Pottinger (2017) assessed the potential influence of ammonia, nitrate, and nitrite on the operation of the stress axis in fish. A rise in ammonia levels may result in a process that converts NH_3 into NH_4^+ and slightly raises the water alkalinity, which may increase mortality (Ren et al., 2022). Thus, a rapid shift in ammonia nitrogen while transportation can be fatal. This study found that the DO content of the water considerably decreased in the OMF group during transit. Reduced DO content in the OMF group is a sign of significant stress during transportation.

By lowering pH, raising ammonia concentration, and decreasing DO level, the transport mechanism in this study led to the degradation of water quality, notably in the OMF group. Due to the high stocking density and increased fish motor activity during transportation, the respiration rate and excretion of nitrogenous waste both increased (Gatica et al., 2008). Stress induced by transportation often causes a rise in breathing rate, which increased DO consumption and carbon dioxide excreation in the transport tanks, which had a negative impact on the levels of DO and pH. Additionally, as mentioned earlier, the increased excretion of nitrogenous wastes increased the ammonia content in the aqueous medium of the fish, which is one of the main causes of stress.

Additionally, fish are usually stressed during transportation due to factors, like handling, confinement, and deteriorating water quality (EFSA, 2004; Manuel et al., 2014). As observed in this study, the stress of transportation typically produces an increase in metabolic activities, which increases the temperature of the water. In this study, we observed that the water temperature in the MMF group was lower than in the OMF group, indicating that melatonin played a modulatory role in the group.

Haematological evaluation is a physiological mirror of the complete organism. Blood parameters are essential in identifying the physiological and functional state of fish subjected to transportation stress. Packed cell volume (PCV) and erythrocyte count are critical variables to consider when assessing the effects of transportation stress on fish health. They also aid in determining how well the blood can transport oxygen. Melatonin played a modulatory function in the MMF group because this group had higher erythrocyte count and PCV values. This further suggests that this group was able to withstand the transportation stress better than the OMF group. This is because of the antioxidant and anti-inflammatory properties of melatonin, which help stabilize the membranes of erythrocytes during stress.

Following transportation, the transported fish had higher leucocyte counts, which suggests that the stress of transportation had an effect on the fish's immune system and defensive systems. The OMF group in this study had significantly higher leucocyte counts, indicating a more pronounced effect of transportation stress in the group. Additionally, it implies that melatonin played a modulatory and ameliorative effect in the MMF group because melatonin has a significant antioxidant effect under stressful conditions.

Following the stress of transportation, lower values of Hb, RBC, PCV, MCH, MCHC, and plasma glucose were observed in the OMF group. These findings suggest that the OMF group had a decreased capacity to transport oxygen. Melatonin supplementation in the MMF group, however, mitigated the negative effects of the stress as the group's Hb, RBC, PCV, MCH, and MCHC levels were higher. During stressful conditions, erythrocytes are mobilized from the spleen into the peripheral circulation explaining why pre-transportation erythrocyte parameters were lower compared to the post-transportation values. It was, therefore, concluded that supplementing *C. gariepinus* with melatonin before transportation helps to ameliorate the negative effects of the stress of transportation.

Ethical Considerations

Compliance with ethical guidelines

All procedures were performed based on the ethical approval of the Ethical Committee of the University of Ilorin.

Funding

The paper was extracted from the research project of Taiwo Oyekunle in the Department of Veterinary Physiology and Biochemistry, University of Ilorin.

Authors' contributions

Conceptualization and supervision: Adakole Sylvanus Adah and Deborah Arimie Adah; Methodology: Charlse Obiora Nwonuma and Taiwo Oyekunle; Data collection: Taiwo Oyekunle and Boluwaji Olaosebikan; Data analysis: Adakole Sylvanus Adah; Funding acquisition and resources, investigation, writing the original draft, review & editing: All authors.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors wish to appreciate all the technical staff that helped in the laboratory analyses.

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