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

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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 result in 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 them to transportation

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

Methods: 40 adult *Clarias gariepinus* fish, 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 Fish 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 group I's total leucocyte

count, neutrophil count, and neutrophil/lymphocyte ratio 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, the group I's 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 *Clarias gariepinus* and will ameliorate the stress of transportation.

Keywords: *Clarias gariepinus*, haematological parameters, melatonin, transportation stress, water quality parameters

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 happened 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 and these include 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 have an impact on the physiological condition of the body of aquatic animals causing stress and immunological reactions (Jerez-Cepa and Ruiz-Jarabo, 2021).

Nervous, immunological, hormonal, and haematological systems are among the internal physiological mechanisms involved in adapting to a stressor (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 (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 and Hassan, 2016, Nisembaum *et al*. 2021**)**. Melatonin protects cells from DNA damage against peroxynitrite (Tan, 2000). In the macrophage cell line (J774A.1), melatonin was observed to reduce lipid peroxidation levels and also enhance free radicals' 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; Acharyya *et al*. 2021).

The majority of the hormone is 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 imply that it plays a role in circadian rhythms and that it regulates a variety of physiological and behavioural processes (**Lima**-Cabello *et al* 2014; Ngasainao, and Lukram, 2016**).**

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

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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 *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 (MMF) had their feed supplemented with melatonin at the rate of 2 mg/ml of water every day for one month (Ngasainao and Lukram, 2016) while group II (OMF) which 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, 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). Nitrate and nitrite concentrations in water samples were measured

spectrophotometrically by the 2005 American Public Health Association (APHA) guidelines for ammonia, and samples of the water were taken from the holding facility before and after transportation.

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

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, 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 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 ± 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

The water quality parameters of the water before and after transportation are shown in table 1. The dissolved oxygen 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 of 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 in 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 in the OMF 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 transportation. The packed cell volume obtained post-transport (27.65 ± 4.65 %) in the MMF group was significantly higher ($P < 0.05$) than the 21.46 ± 0.88 % obtained in the OMF group post-transportation. The erythrocyte counts of 2.97 ± 2.98 $x10^6$ mm^{-3} in the MMF group were higher than the value of 2.01 ± 0.55 $x10^6$ mm^{-3} obtained in 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 in the OMF group. The 12.65 ± 3.76 g/100ml recorded as the haemoglobin concentration in the MMF group was higher ($P < 0.05$) than the 9.34 ± 1.05 g/100ml obtained in the OMF group.

The Leucocyte indices of *Clarias gariepinus* before and after transportation are shown in table 3. The leucocyte counts of 1.87 ± 0.18 $x10^3$/ mm^3 obtained in the MMF group were lower than the value of 2.91 ± 0.79 $x10^3$/ mm^3 obtained in the OMF group. The neutrophil count of 1.03 ± 0.08 $x10^3$/ mm^3 obtained in the MMF group is lower ($P < 0.05$) than the 1.76 ± 0.67 $x10^3$/ 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 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 level obtained in the MMF group (2.09 ± 2.98 µMol/L) was higher than the value obtained in the OMF group (1.07 ± 0.15 µMol/L) post-transportation of *Clarias gariepinus* (table 4).

Discussion

Water quality parameters are significantly altered 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 aquatic 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 excrete ammonia, which, at high amounts, disrupts metabolism, alters growth, and even causes death (Bouyoucos *et al*., 2021; Bolner. *et al*., 2014). Pottinger (2017) reported on 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's alkalinity, which may increase mortality, according to a recent study (Yichao *et al*. 2022). Because of this, a rapid shift in ammonia nitrogen while in transportation can be fatal. This study found that the dissolved oxygen (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 dissolved oxygen consumption and increased carbon dioxide excreation in the transport tanks, which had a negative impact on the levels of dissolved oxygen 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 because the group had higher erythrocyte count and PCV values. This further suggests that the 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 to 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 group had a decreased capacity to transport oxygen. Melatonin supplementation in the MMF group, however, mitigated the negative effects of the stress since the group's Hb, RBC, PCV, MCH, and MCHC levels were higher in the group. 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 *Clarias gariepinus* with melatonin before transportation helps to ameliorate the negative effects of the stress of transportation.

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Ethical Statement

All procedures were performed based on the ethical approval of the ethical committee of the University of Ilorin, Nigeria.

Conflict of interest

The authors declare that there are no conflicts of interest

References

Abdel-Tawwab, M., Monier, M.N. Hoseinifar, S. H and Faggio, C. (2019). Fish response to hypoxia stress: growth, physiological, and immunological biomarkers. *Fish Physiology and Biochemistry*, *45*(3): 997–1013. https://doi.org/10.1007/s10695-019-00614-9. PMID:30715663

Aguirre-Guzman, G., V. Carvajal-de-la-Fuente, M. Neri-Coronado, J. Loredo-Ostiand J. L. Rábago-Castro. (2016). Hematological and clinical chemistry changes induced by acute stress during handling and capture of catfish (*Ictalurus punctatus). Revista MVZ Córdoba; 21*:5345–5354 https://doi.org/10.21897/rmvz.601

American Public Health Association (APHA). (2005). *Standard Methods for the Examination of Water and Wastewater* (21st ed). Washington DC: American Public Health Association. 1220 p

Bolner, K. C. S., Copatti, C. E. Rosso, F. L.Loro, V.L and Baldisserotto, B. (2014). Water pH and metabolic parameters in Silver catfish (*Rhamdia quelen*). *Biochemical Systematics and Ecology*; 56:202-208. DOI: 10.1016/j.bse.2014.06.006

Bouyoucos, I. A., Schoen, A. N., Wahl, R. C., & Anderson, W. G. (2021). Ancient fishes and the functional evolution of the corticosteroid stress response in vertebrates. *Comparative biochemistry and physiology. Part A, Molecular & Integrative Physiology*, *260*, 111024. https://doi.org/10.1016/j.cbpa.2021.111024. PMID: 34237466

Bowzer, J., Bergman, A. and Trushenski, J. (2014). Growth performance of largemouth bass-fed fish meal derived from Asian carp. *North American Journal of Aquaculture; 76*(3):185–9. DOI: 10.1080/15222055.2014.893473

Campbell, J. H., Dixon, B., & Whitehouse, L. M. (2021). The intersection of stress, sex and immunity in fishes. *Immunogenetics*, *73*(1), 111–129. https://doi.org/10.1007/s00251-020-01194-2. PMID: 33426582

Chabot, D., McKenzie, D. J., and Craig, J. F. (2016). Metabolic rate in fishes: definitions, methods, and significance for conservation physiology. *Journal of Fish Biology, 88*(1), 1–9. doi:10.1111/jfb.12873. PMID: 26768969

Cook, K.V., McConnachie, S.H. Gilmour, K.M. Hinch, S.G and Cooke, S.J. (2011). Fitness and behavioral correlates of pre-stress and stress-induced plasma cortisol titers in pink salmon (*Oncorhynchus gorbuscha*) upon arrival at spawning grounds. *Hormone and Behaviour; 60*: 489–497. https://doi.org/10.1016/j.yhbeh.2011.07.017. PMID: 21839080

Dacie, J.V. and Lewis, S.M (2001). *Practical Haematology*. 9th Edition, Churchill Livingstone, London, 633.

Davis, A.K., Maney, D.L and Maerz, J.C. (2008). The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. *Functional Ecology, 22*: 760-772. https://doi.org/10.1111/j.1365-2435.2008.01467.x

Dobšikova R, Svobodova, Z., Blahova, J., Modra, H. and Velišek, J. (2009). The effect of transport on biochemical and haematological indices of common carp (*Cyprinus carpio* L.). *Czech Journal of Animal Science*. *54*:510–518. https://doi.org/10.17221/52/2009-CJAS

EFSA. (2004). Opinion of the scientific panel for animal health and welfare on a request from the commission related to the welfare of animals during transport. *European Food and Safety Authority Journal*. *44*: 1–36. https://doi.org/10.2903/j.efsa.2004.44

Forget, P., Khalifa, C., Defour, J. P., Latinne, D., Van Pel, M. C., and De Kock, M. (2017). What is the normal value of the neutrophil-to-lymphocyte ratio? *BMC Research Notes*, *10*(1), 12. https://doi.org/10.1186/s13104-016-2335-5. PMID: 28057051

Galano, A., Tan, D.X. and Reiter, R.J. (2018) Melatonin: a versatile protector against oxidative DNA damage. *Molecules 23*(3):530. https://doi.org/10.3390/molecules23030530. PMID: 29495460

Gatica, M. C., Monti, G., Gallo, C., Knowles, T.G. and Warriss, P.D. (2008). Effects of well-boat transportation on the muscle pH and onset of rigor mortis in Atlantic salmon. *Veterinary Records; 163*:111–116. https://doi.org/10.1136/vr.163.4.11

Gevi, F., D'Alessandro, A., Rinalducci, S., & Zolla, L. (2012). Alterations of red blood cell metabolome during cold liquid storage of erythrocyte concentrates in CPD-SAGM. *Journal of Proteomics*, *76*:168–180. https://doi.org/10.1016/j.jprot.2012.03.012. PMID: 22465715. PMID: 18660520

Ghorbani, A., Peighambari, S M. and Razmyar, J. (2021). Molecular and in vivo Characterization of an Iranian Infectious Bursal Disease Virus Containing a Mixed Virus Population. *Iranian Journal of Veterinary Medicine*, 15(2), 155-167. Doi:10.22059/IJVM.2020.305989.1005108

Golombieski, J. I., Koakoski, G., Becker, A.J., Almeida, A. P. G. Toni, C., Finamor, I.A. and Baldisserotto, B. (2013). Nitrogenous and phosphorus excretions in juvenile silver catfish (*Rhamdia quelen*) exposed to different water hardness, humic acid, and pH levels. *Fish Physiology and Biochemistry; 39*(4): 837-849. https://doi.org/10.1007/s10695-012-9744-8. PMID: 23135153

Hong, J., Chen, X..Liu, S. Fu, Z., Han, M., Wang, Y. Zhifeng, Z. and Gu, Z. (2019). *Impact of fish density on water quality and physiological response of golden pompano (Trachinotus ovatus)* fingerlings during transportation. *Aquaculture; 507*: 260-265.

Ibrahim, R. E., Ahmed, S. A. A., Amer, S. A., Al-Gabri, N. A., Ahmed, A. I., Abdel-Warith, A.-W. A., Metwally, A. E. (2020). Influence of vitamin C feed supplementation on the growth, antioxidant activity, immune status, tissue histomorphology, and disease resistance in Nile tilapia, *Oreochromis niloticus. Aquaculture Reports, 18: 100545*. doi:10.1016/j.aqrep.2020.100

Jerez-Cepa, I and Ruiz-Jarabo, I. (2021). Physiology: An Important Tool to Assess the Welfare of Aquatic Animals. *Biology*; *10*: 61. https://doi.org/10.3390/ biology1001006. PMID: 33467525

Jia, R., Wang, L., Hou, Y., Feng, W., Li, B., & Zhu, J. (2022). Effects of Stocking Density on the Growth Performance, Physiological Parameters, Redox Status and Lipid Metabolism of *Micropterus salmoides* in Integrated Rice-Fish Farming Systems. *Antioxidants (Basel, Switzerland)*, *11*(7), 1215. https://doi.org/10.3390/antiox11071215. PMID: 35883706

Lima-Cabello, E., Díaz-Casado, M. E., Guerrero, J. A., Otalora, B. B., Escames, G., López, L. C., Reiter, R. J., & Acuña-Castroviejo, D. (2014). A review of the melatonin functions in zebrafish physiology. *Journal of Pineal Research*, *57*(1), 1–9. https://doi.org/10.1111/jpi.12149. PMID: 24920150

Maitra, S. K., & Hasan, K. N. (2016). The Role of Melatonin as a Hormone and an Antioxidant in the Control of Fish Reproduction. *Frontiers in Endocrinology*, *7*, 38. https://doi.org/10.3389/fendo.2016.00038. PMID: 27199895

Manuel, R., Boerrigter, J. Roques, J. van der Heul, J., van den Bos, R., Flik, G. and van de Vis, H. (2014). Stress in African catfish (*Clarias gariepinus*) following overland transportation. *Fish Physiology and Biochemistry 40*(1): 33–44. https://doi.org/10.1007/s10695-013-9821-7. PMID: 23801364

McMullin, M.F., Bareford, D., Campbell, P. Green, A.R. Harrison, C. Hunt, B., Oscier, D., Polkey, M.I., Reilly, J.T. Rosenthal, E., Ryan, K., Pearson, T.C. and Wilkins, B. (2005). General Haematology Task Force of the British Committee for Standards in Haematology. Guidelines for the diagnosis, investigation, and management of polycythemia/erythrocytosis. *British Journal of Haematology;130*(2):174-95. https://doi.org/10.1111/j.1365-2141.2005.05535.x. PMID: 16029446

Ngasainao, M., and Lukram, I. (2016). A review on melatonin and its prospects in fish aquaculture. *Journal of Zoological Sciences. 4*, 34–41.

Nisembaum, L. G., Martin, P., Lecomte, F., & Falcón, J. (2021). Melatonin and osmoregulation in fish: A focus on Atlantic salmon Salmo salar smoltification. *Journal of Neuroendocrinology*, *33*(3), e12955. https://doi.org/10.1111/jne.12955. PMID: 33769643

Nisembaum, L., Besseau, L., Paulin, C., Charpantier, A., Martin, P., Magnanou, E., et al. (2015). In the heat of the night: thermo-TRPV channels in the salmonid pineal photoreceptors and modulation of melatonin secretion. *Endocrinology 156,* 4629–4638. doi: 10.1210/en.2015-1684. PMID: 26389691

Pakhira, C., T. Nagesh, S., Abraham, T.J., Dash, G. and Behera, S. (2015). Stress responses in rohu, *Labeo rohita* transported at different densities. *Aquaculture. Reports; 2*:39–45. https://doi.org/ 10.1016/j.aqrep.2015.06.002

Park, J. Y., Han, K. H., Cho, J. K., Kim, K. M., Son, M. H., Park, J. M., & Kang, H. W. (2016). Survival Rate and Hematological Responses with Temperature Changes of Red Spotted Grouper, *Epinephelus akaara* in South Korea. *Development and Reproduction, 20*(2), 103–112.

https://doi.org/10.12717/DR.2016.20.2.103**https://doi.org/10.12717/DR.2016.20.2.103.** PMID: 27660825

Pottinger, T. G. (2017). Modulation of the stress response in wild fish is associated with variation in dissolved nitrate and nitrite. *Environmental Pollution; 225*:550-558. **https://doi.org/10.1016/j.envpol.2017.03.021.** PMID: 28318786

Rahmati, M., Morovvati, H. and Abdi, R. (2022). Histomorphometric Analysis of Skin and Stress Indices of Nile Tilapia (*Oreochromis niloticus*) Exposed to Different Concentrations of Ammonia. *Iranian Journal of Veterinary Medicine*, 16(3), 288-297. DOI: 10.22059/IJVM.2022.337549.1005230

Sadowska-Bartosz, I. and Bartosz, G. (2014). Effect of antioxidants supplementation on ageing and longevity. *Biomed Research International* 2014:404680. **https://doi.org/10.1155/2014/404680.** PMID: 24783202

Saha, S., Singh, K. M., & Gupta, B. (2019). Melatonin synthesis and clock gene regulation in the pineal organ of teleost fish compared to mammals: Similarities and differences. *General and Comparative Endocrinology*, *279*, 27–34. https://doi.org/10.1016/j.ygcen.2018.07.010. PMID: 30026020

Sampaio, F. D. F. and Freire, C.A. (2016). An overview of stress physiology of fish transport: changes in water quality as a function of transport duration. *Fish and Fisheries; 17*(4):1055–1072. https://doi.org/10.1111/faf.12158

Serafini, S., Souza, C.F., Baldissera, M.D., Baldisserotto, B. and Silva, A.S. (2019). Fish exposed to eprinomectin show hepatic oxidative stress and impairment in enzymes of the phosphor transfer network. *Aquacultur;e 508*:199–205. https://doi.org/ 10.1016/j.aquaculture.2019.04.081

Shahjahan, M., Islam, M.J. Hossain, M. T., Mishu, M.A., Hasan, J. and Brown, C. (2022). Blood biomarkers as diagnostic tools: An overview of climate-driven stress responses in fish (Review). *Science of the Total Environment;843*: 156910, **https://doi.org/10.1016/j.scitotenv.2022.156910.** PMID: 35753474

Shamohamadi, M., Pooyanmehr, M., Maleki, A. and Haghnazari, L. (2021). Biochemical Modulatory and Protective Effects of the Hydroalcoholic Extract of Scrophularia striata on the Hepatotoxicity of Silver Nanoparticles in the Rat Model. *Iranian Journal of Veterinary Medicine*, 15(3), 311-324. Doi: 10.22059/IJVM.2021.308066.1005119

Si, L. J., Pan, L.Q., Zhang, X., Wang, H.D. and Wei, C. (2019). Evidence that dopamine is involved in neuroendocrine regulation, gill intracellular signaling pathways and ion regulation in *Litopenaeus vannamei*. *Journal of Experimental Biology; 222*(15): https:// doi.org/10.1242/jeb.204073. PMID: 31266778

Sinha, A. K., Rasoloniriana, R. Dasan, A.F., Pipralia, N. Blust, R. and De Boeck, G. (2015). Interactive effect of high environmental ammonia and nutritional status on ecophysiological performance of European sea bass (*Dicentrarchus labrax*) acclimated to reduced seawater salinities. *Aquatic Toxicology*; *160*:39–56. **https://doi.org/10.1016/j.aquatox.2015.01.005**. PMID: 25625520

Stara, A., Kouba, A. and Velisek, J. (2018). Biochemical and histological effects of subchronic exposure to atrazine in crayfish Cherax destructor. *Chemico-Biological Interactions; 291*:95–102. **https://doi.org/10.1016/j.cbi.2018.06.012.** PMID: 29908168

Tacchi, L., Lowrey, L., Musharrafieh, R., Crossey, K., Larragoite, E.T. and Salinas, I. (2015). Effects of transportation stress and addition of salt to transport water on the skin mucosal homeostasis of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*; *435*:120–127. PMID: 25705060

Tahmasebi-Kohyani, A., Keyvanshokooh, S., Nematollahi, A., Mahmoudi, N. and Pasha-Zanoosi, H. (2012). Effects of dietary nucleotides supplementation on rainbow trout (*Oncorhynchus mykiss*) performance and acute stress response. *Fish Physiology and Biochemistry; 38*:431–440. **https://doi.org/10.1007/s1069 5-011-9524-x.** PMID: 21671024

Tain, Y.L., Kao, Y.H., Hsieh, C.S., Chen, C.C., Sheen, J.M., Lin, I.C. and Huang, L.T. (2010) Melatonin blocks oxidative stress-induced increased asymmetric dimethylarginine. *Free Radicals Biology and Medicine;* 49(6):1088–1098. PMID: 20600827

Tan, D.X., Manchester, L.C., Reiter, R.J., Qi, W.B., Karbownik, M. and Calvo, J.R. (2000) Significance of melatonin in antioxidative defense system: reactions and products. *Biological signals and Receptors;* 9:137–159. PMID: 10899700

Tan, D.X., Reiter, R.J., Manchester, L.C., Yan, M.T., El-Sawi, M., Sainz, R.M., Mayo, J.C., Kohen, R., Allegra, M. and Hardeland, R. (2002) Chemical and physical properties and potential mechanisms: melatonin as a broad-spectrum antioxidant and free radical scavenger. *Current Topics in Medicinal Chemistry;* 2:181–197. PMID: 11899100

Voigt, L. (2000). *Heamtology: Techniques and concept for Veterinary Technician*s. Iowa State University Press Ames, p139

Wendelaar-Bonga, S. E. (1997). The stress response in fish. *Physiological Reviews;* 77(3):591–625. **https://doi.org/10.1152/physrev.1997.77.3.591.** PMID: 9234959

Yichao, R., Xianhui, M., Yu, Y., Bing, L., Yangen, Z. and Chunyan, Z. (2022). Effects of transportation stress on antioxidation, immunity capacity and hypoxia tolerance of rainbow trout (*Oncorhynchus mykiss*), *Aquaculture Reports;* 22: 100940. https://doi.org/ 10.1016/j.aqrep.2021.100940.

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

Water Quality Parameters		MMF (Mean ± SEM)	OMF (Mean ± SEM)
Water Temperature	Pre-Transportation	27.70 ± 1.10	27.65 ± 1.40

(ºC)			
	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 ($P < 0.05$) different

Key

MMF = Administered with melatonin

OMF = Not Administered with melatonin

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

Erythrocyte Parameters	Time	MMF (Mean ± SEM)	OMF (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	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 ($P < 0.05$) different

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Key

MMF = Administered with melatonin

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

Leucocyte Parameters	Time	MMF	OMF
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	Pre-Transportation	0.73 ± 0.06	0.33 ± 0.02

($x10^3$ mm^{-3})			
	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

MMF = Administered with melatonin

OMF = Not Administered with melatonin

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

Biochemical Parameters	Time	MMF (Mean ± SEM)	OMF (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

MMF = Administered with melatonin

OMF = Not Administered with melatonin