Journal homepage: http://fens.usv.ro/index.php/FENS
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XXI, Issue 3 - 2022, pag. 230 - 236

FOOD AND ENVIRONMENT SAFETY

HAEMATOLOGICAL PROFILE OF CATFISH (*CLARIAS GARIEPINUS* BURCHELL, 1822) FOLLOWING THE STRESS ASSOCIATED WITH TRANSPORTATION AND HANDLING IN THE MARKET

***Olaniyi Alaba OLOPADE[1], Henry Eyina DIENYE[1], Kingsley Kenechukwu AGBO[1]**
[1]*Faculty of Agriculture, University of Port Harcourt, Choba, East-West Road, PMB 5323, Port Harcourt, Rivers State, Nigeria,* olaniyi.olopade@uniport.edu.ng
*Corresponding author
Received 7th April 2022, accepted 29th September 2022

Abstract: *This study aimed to investigate the haematological profile of Clarias gariepinus after stress from transportation and handling in the market. C. gariepinus mixed sexes and siblings reared in the same fish tank were subjected to transportation and market stressors associated with transportation and handling in the market. Ten fish were collected at random at successive time intervals at the fish farm (A), another immediately after arriving at the Choba market (B), and then after 3 hours (C), 6 hours (D), and 9 hours (E) at the same location. The results revealed that red blood cell (RBC) levels were higher in the non-stressed group (Group A) than in the test populations (Groups B-E).The differences in values were not statistically significant (p<0.05). Haemoglobin levels were consistent with the RBC. The non-stressed group had the highest value of 14.87±0.81 g/dL. Mean values of the platelets ranged from 195.00±12.50 ×10 /L to 242.33±12.99 ×10 /L for all the samples, and this parameter showed a significant difference. The mean packed cell volume (PCV) values differed significantly between different population groups. There was no statistically significant difference (p<0.05) in neutrophils, lymphocytes, or monocytes values, but eosinophils showed a significant difference (p <0.05) across all treatments. The glucose mean values did not differ significantly across the populations, with the lowest value of 24.3±52.71g/dL recorded for the control relative to the test populations. It can be concluded that acute stress alters blood parameters in fish, and all the haematological parameters determined in the present study are important stress markers.*

Keywords: *Blood, glucose, stressors, haematological parameters, aquaculture.*

1. Introduction

In Nigeria catfish accounts for roughly 64% of aquaculture fish production [1], with production put at 189,562 tonnes in 2017 [2]. Increased production of Clarias gariepinus, one of the Clarrid species, was supported by consumer and farmer acceptance of the fish due to beneficial characteristics such as fast growth and high market demand. [3]. At current levels of production, there is a need for permanent exchange relationships to exist between producers and consumers. The availability of fish to consumers at the right time and place necessitates an efficient marketing system.

As such, the marketing system must develop well to provide the necessary services to ensure the machineries for enhancing marketing efficiency are put in place. There is a consumer perception that live fish are healthier than fresh or frozen products, and many consumers perceive that live fish are superior to frozen in terms of taste, freshness, and nutritional value. As a result, catfish are frequently distributed and sold live from farm gates to

DOI: https://doi.org/10.4316/fens.2022.022

designated fish markets in the majority of cities and towns across the country, where the fish are sold to restaurants, processors, and final consumers. [4].

During transport and marketing, fish are exposed to a multitude of stressors such as loading density, handling stress, water movement, noise, thirst, hunger, malnutrition, vibrations, poor water conditions, pain, injury, discomfort, disease, restriction of normal behaviors, fear, and suffering [5].

Exposure to such stressors simultaneously or in rapid succession may induce severe physiological stress [5]. Fish struggle during harvest has been shown to cause rapid decreases in post-mortem muscle pH, affecting fillet quality through loss of fillet firmness and increased fillet gaping. [6]. Therefore, since the transport of live fish to markets some miles away is stressful for fish, it deserves special attention. Knowledge on stress in fish following transport and handling in the market is important to delineate the health status and quality of fish and provide a future understanding of stress impacts that could modify the physiological state of the fish.

It is well known that haematological and serum biochemical changes are termed as pre-requisite to determine the health status of fish [7]. Among haematological indices, blood cells are one of the best indicators of body health [8]. Furthermore, blood glucose is a marker of the metabolic rate of the stress response [9]. The majority of past research on stress physiology in fish during the last few decades has focused on laboratory experiments, which enforce scientific control and a highly controlled setting in a laboratory. This study was designed to determine the physiological response of *C. gariepinus* to acute stress of transportation and handling in the field to reflect real-life conditions.

2. Matherials and methods

Adult catfish (*C. gariepinus*) in excellent health status, (mean weight 973 ± 3.34 g and mean length 32 ± 00.3 cm mixed sexes) were purchased from a commercial fish farm in Ahoada and Choba market, Rivers State, Nigeria. They were siblings reared in the same fish tank. At the farm, the catfish are starved prior to transportation to the market a distant of about 47.8km**.** Food is being withheld for 24 h. The experiment was carried out in the rainy season (June 2019). The following experimental protocol was used: fish were divided into six groups (Groups A, B, C, D, E and F), Fish of Group A were not subjected to stress (control group), while those of Group B were subjected to acute handling and transportation stress. Group C, D, E and F were subjected to transportation stress and acute handling in the market. At the time of sampling, ten fish were collected at random at a successive time intervals at the fish farm (A), another immediately after arriving at the Choba market (B) and then 3 hours (C), 6 hours (D), 9 hours (E) at the same location, to determine the effects of stress associated with transportation and handling in the market on the haematology of fish. Blood samples were collected using 5ml syringe, and transferred to test tubes after each collection and immediately put on melting ice. The experiment was conducted in triplicates for each category. Samples were stored on ice for immediate analysis in the Laboratory.

Laboratory Experiment

Blood collection and Processing

Blood was drawn from the posterior caudal vein using needle and syringes according to [10] and 2ml was decanted in EDTA bottles for the assessment of haematological parameters, while another

2ml was decanted into heparinized bottles for the BLL determination. Whole blood (50µl) was stained for enumeration of red and white blood cells [11]. Blood smears were air-dried for five minutes, fixed in absolute methanol, and stained for 60 seconds in Giemsa stain.

Haematological Analyses

Red blood cells (RBC) and white blood cells (WBC) were counted in a Neubauer chamber; packed cell volume (PCV) by the microhematocrit technique; and haemoglobin level (Hb) by the cyanomethemoglobin method. Haematological indices were computed using standard formulae while glucose was measured in the laboratory using an electronic blood glucose meter.

Data Analysis

Statistical analysis of the data was performed using a Statistical Package for the Social Sciences (SPSS v16.0). A one-way ANOVA was applied to understand the difference in haematological parameters between and among groups of fish, and significance was set at 0.05

3. Results and discussion

The red blood Parameters Profile of *C. gariepinus* exposed to handling, transportation, and market stressors is summarized in Table 1. The red blood cell (RBC) levels of the stressed groups were generally lower than those of the non-stressed group (Group A). The highest value of 24.17±17.97 (×10^12) was recorded for the non-stressed group (Group A), while the values recorded for stressed groups ranged from 4.73±0.12 (×10^12) to 5.90±0.21 (×10^12). The variations in values, however, were not statistically significant ($p<0.05$).

Stress caused by capture, handling, and sampling procedures was observed to cause intraspecies haematological value variation [12]. The haemoglobin mean values ranged between 10.13±0.30 g/dL and 14.87±0.81 g/dL with the non-stressed (Group A) having the highest (14.87±0.81 g/dL) relative to the test populations (Groups B-E). The mean platelet values for all samples were extremely high, as indicated in table 1, ranging between 195.00±12.50 ×10 /L recorded for non-stressed and 242.33±12.99 ×10 /L (Group E), and this parameter showed a significant difference. Haemoglobin values were consistent with red blood cell. The highest value of 14.87±0.81 g/dL was recorded for the non-stressed group. These changes were attributed to a direct or feedback response to structural damage to the RBC membrane, which resulted in haemolysis and impaired haemoglobin synthesis, as well as stress-related RBC release from the spleen [13,15]. The mean packed cell volume (PCV) values decreased sharply from the non-stressed group (44.67±2.40 %) to 40.00±1.15 % (Group B) and then decreased gradually to 30.33±0.88 % (Group E). HATTINGH & VAN PLETZEN [14] reported a decrease in the fish blood haemoglobin concentration and PCV. This is consistent with the findings of [15] studies, which show that the park cell value is lower in the non-stressed group compared to the stressed group.

As follows, four different types of white blood cells were identified in the peripheral blood of *C. gariepinus*: lymphocytes, neutrophils, eosinophils, and monocytes (Figures 1, 2, 3, 4, and 5). There were no statistically significant ($p<0.05$) in the values of neutrophils, lymphocytes, and monocytes.

Table 1.
Red Blood Parameters Profile of *C. gariepinus* exposed to handling transportation and market stressors

Parameters	Initial stage GroupA	landing site Group B	3 hours after landing Group C	6 hours after landing Group D	9 hours after landing Group E
Hgb (g/dL)	14.87±0.81 [a]	13.33±0.38 [ab]	12.23±0.79 [b]	11.33±0.69 [bc]	10.13±0.30 [c]
RBC (×10^12)	24.17±17.97	5.90±0.21	5.50±0.36	4.97±0.26	4.73±0.12
Plat.(×10^9)	195.00±12.50 [b]	194.00±15.95 [b]	217.00±10.69 [ab]	230.00±8.14 [ab]	242.33±12.99 [a]
PVC (%)	44.67±2.40 [a]	40.00±1.15 [ab]	36.67±2.40 [b]	34.00±2.08 [bc]	30.33±0.88 [c]

PCV: packed cell volume, Hgb: haemoglobin, RBC: red blood cell, Plat: Platelet
Mean in same row with different superscripts are significantly different ($p<0.05$).

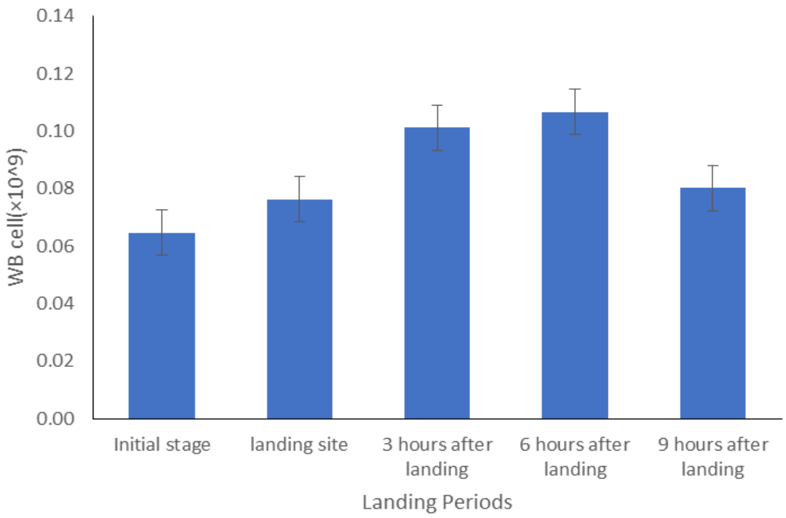

Fig. 1. White blood cell of *C. gariepinus* exposed to handling transportation and market stressors

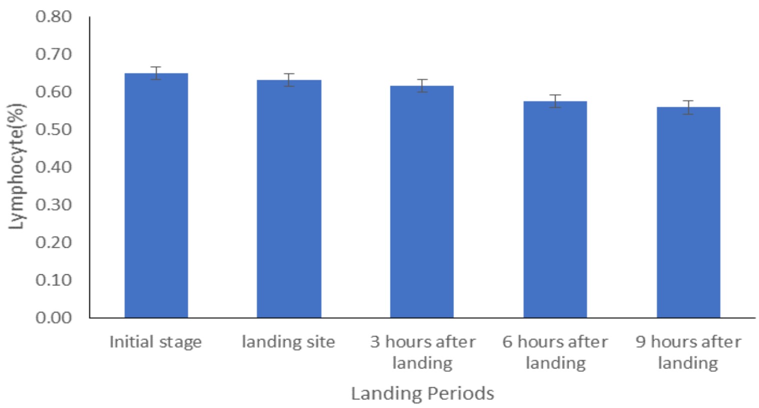

Fig. 2: Lymphocyte of *C. gariepinus* exposed to handling transportation and market stressors

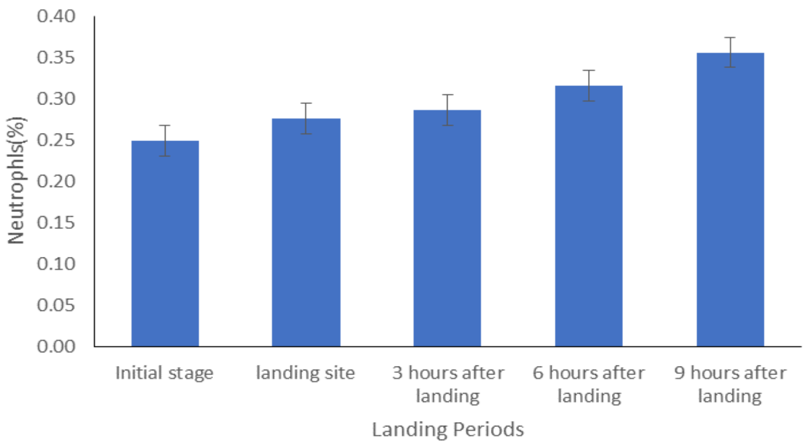

Fig. 3. Neutrophils of *C. gariepinus* exposed to handling transportation and market stressors

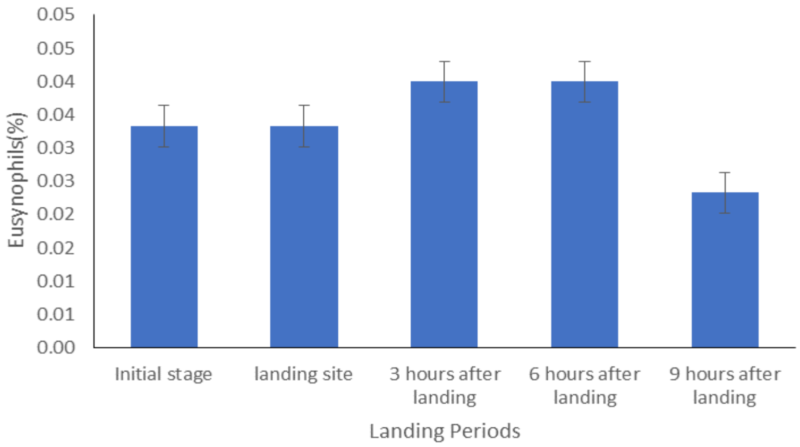

Fig. 4: Eusynophils of *C. gariepinus* exposed to handling transportation and market stressors

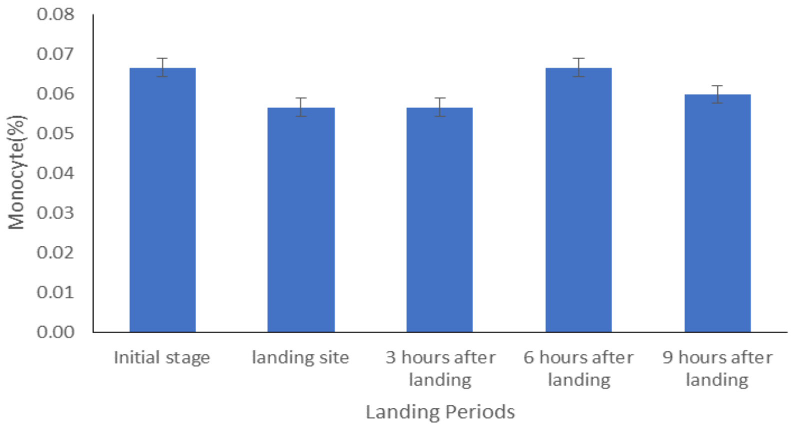

Fig.5: Monocyte of *C. gariepinus* exposed to handling transportation and market stressors

The numbers of eosinophils were significantly ($p < 0.05$) in all the groups.

The mean values of white blood cells and neutrophils followed the same pattern, with the non-stressed group having the lowest values compared to the stressed groups, while the mean value of lymphocytes decreased gradually from the non-stressed group to the stressed groups. From the present studies, the mean values of white blood cells and neutrophils in the non-stressed population had the lowest values compared to the test population. This is in agreement with the report of [16] that changes in the composition of circulating white blood cells are more reliable indicators of chronic crowding stress. Stress or glucocorticoid treatment specifically causes increases in neutrophil numbers (neutrophil) and decreases in lymphocyte numbers (lymphopenia or lymphocytopenia) [17]. The non-stressed group had the highest mean values in terms of lymphocytes and then decreased gradually in test populations (Groups B–E). Leucocyte profiles have recently been considered significant in assessing stress [18], and the reduction in the number of lymphocytes is generally considered a stress response [19]. The percentages of eosinophilis and monocytes in the contents of the white blood cells differed widely among the populations, with eosinophilis showing significantly lower values ($p < 0.05$). An absolute monocytes high can also be a response to stress, chronic infections or autoimmune disorders, while too much tension and anxiety can lead to higher eosinophilic inflammation. Eosinophils numbers decreased below the reference range, or eosinopenia, may occur as part of a stress response but is a fairly nonspecific response.

The blood glucose values of C. gariepinus in this study is presented in Table 2. There were differences in the values blood glucose ($p>0.05$). The glucose values ranged from 24.3±52.71g/dL to 41.75±3.2 g/dL with the lowest value recorded in the non-stressed group (Group A) and the highest value of 41.75±3.2 g/dL recorded in the stressed groups (Group E). VIJAYAN *et al.* [20] reported that increased or decreased glucose levels are considered a signs of stress. According to SEIBEL *et al.* [21] stress-induced change in muscle activity accelerate the conversion of glucose to lactate (or alternatively, to pyruvate) by anaerobic glycolysis.

Table 2

Plasma Glucose Values of *C. gariepinus* exposed to handling transportation and market stressor

Parameter	Initial stage Group A	landing site Group B	3 hours after landing Group C	6 hours after landing Group D	9 hours after landing Group E
Glucose (g/dL)	24.3±52.71	32.00±3.85	36.91±3.91	38.32±0.37	41.75±3.22

Mean in same row with different superscripts are significantly different (p<0.05)

4. Conclusion

The results obtained during the examination of the haematological profile of *C. gariepinus* after stress from transportation and handling in the market reveal that blood biochemical parameters in fish change with acute stress. As a result, proper training in handling fish during transportation and marketing is required because these activities cause stress responses in fish, which have an impact not only on the welfare of the animals but also on the quality of the fish products.

5. Acknowledgments

Authors would like to thank staffs of the Department of Fisheries, University of Port Harcourt for their help at the lab as well as in the field.

6. References

[1]. FAO. World Aquaculture Performance Indicators (WAPI): Information, knowledge and capacity for Blue Growth. Rome. FAO (2018). URL: http://www.fao.org/documents/card/en/c/I9622EN

[2]. FAO. Fishery and Aquaculture Country Profiles: The Federal Republic of Nigeria. Fisheries and Aquaculture Department. Rome FAO (2017). URL: http://www.fao.org/fishery/facp/NGA/en

[3]. DAUDA A. B., NATRAH I., KARIM M., KAMARUDIN M. S. & BICHI A. H., African Catfish Aquaculture in Malaysia and Nigeria: Status, Trends and Prospects. *Fish Aqua Journal*, 9,237, 2018. doi:10.4172/2150-3508.1000237.

[4]. IGONI-EGWEKE Q. N., Analysis of value addition in commercial catfish (*Clarias gariepinus and Heterobranchus* spp.) production in Rivers State Nigeria (Doctoral dissertation). Federal University of Technology, Owerri, 2018.

[5]. http://futospace.futo.edu.ng/xmlui/bitstream/handle/123456789/1894/IGONI.pdf?sequence=1&isAllowed=y. [Google Scholar].

[6]. MCEWEN B. S. & WINGFIELD J. C., The concept of allostasis in biology and biomedicine. *Horm Behav*., 43:2–15, 2003.

[7]. BERG T., ERIKSON U. & NORDTVEDT T. Rigor Mortis Assessment of Atlantic Salmon (*Salmo Salar*) and Effects of Stress. J. Food Sci. 62, 439–446, 1997.

[8]. OKOMODA V.T., KOH I.C.C. HASSAN A. AMORNSAKUN T. & SHAHREZA M.S. Hematological parameters of pure and reciprocal crosses of *Pangasianodon hypophthalmus* (Sauvage, 1878) and *Clarias gariepinus* (Burchell, 1822). *Comparative Clinical Pathology* 27:549–554, 2018. DOI: 10.1007/s00580-017-2623-z.

[9]. AHMED I., SHEIKH Z.A. Comparative study of hematological parameters of snow trout *Schizopyge plagiostomus* and *Schizopyge niger* inhabiting two different habitats. European Zoological Journal 12-19, 2020. doi.org/10.1080/24750263.2019.1705647

[10]. DAVIS K. B. Management of physiological stress in finfish aquaculture. *N Am J Aquacult* 68(2):116-121, 2006.

[11]. SCHMITT C. J., BLAZER V. S., DETHLOFF G. M., TILLITT D. E., GROSS T .S., BRYANT JR. W. L., DEWEESE L. R., SMITH S. B., GOEDE R. W., BARTISH T. M. & KUBIAK T. J., Biomonitoring of Environmental Status and Trends (BEST) Program: Field Procedures for Assessing the Exposure of Fish to Environmental Contaminants. Information and Technology Report USGS/BRD-1999-0007. U.S. Geological Survey, Biological Resources Division, Columbia, 68 pp, 1999.

[12]. SHAW A.F., A direct method for counting the Leucocytes, thrombocytes and erythrocytes of bird's blood, *Journal of Pathology Bacteria*, 33, 833-835, 1930.

[13]. BAUCK G. R. & BALL R. C., Influence of capture methods on blood characteristics and mortality in the rainbow trout (*Salmo gairdneri*), Translantic American Fisheries Society, 95(2), 170-176, 1966.

[14]. SHAH S. L., Haematological parameters iu tench, *Tinca tinca* after short term exposure to lead. *Journal of applied toxicology*, 26 (3), 223-228, 2006.

[15]. HATTINGH J. & VAN PLETZEN A. J. J., The influence of capture and transportation on some blood parameters of fresh water fish. Comp. *Biochem. Phys*., 4TA, 607-609, 1974.

[16]. GABRIEL U. U., AKINROTIMI O. A. & ESEIMOKUMO F., Haematological responses of wild Nile Tilapia *Oreochromis niloticus* after acclimatation to captivity. *Jordan Journal Biol Sci.*, 4,225-30, 2011.

[17]. PICKERING A. D. & POTTINGER T. G., Crowding causes prolonged leucopenia in Salmonid fish, despite interrenal acclimation, *Journal of Fish Biology*, 30: 701–712, 1987.

[18]. DAVIS A. K., MANEY D. L. & MAERZ J. C., The use of leukocyte profiles to measure stress in vertebrates; a review for ecologists. *Functional Ecology* 22, 760-772, 2008.

[19]. CHEN W. H., SUN L.T., TSAI C. L., SONG Y. L. & CHANG C. F., "Cold-stress induced the Modulation of Catecholamines, Cortisol, Immunoglobulin M, and Leukocyte Phagocytosis in Tilapia." *General and Comparative Endocrinology* 126: 90100, 2002.

[20]. PICKERING A. D. & POTTINGER T. G., Cortisol can increase the susceptibility of brown trout, *Salmo trutta L*., to disease without reducing the white blood cell count, *Journal of Fish Biology*, 27, 611-619, 1985.

[21]. VIJAYAN M. M, BALLANTYNE J. S. & LEATHERLAND J. F., High stocking density alters the energy metabolism of brook charr, *Salvelinus fontinalis*. *Aquaculture*, 88:371–81, 1990. doi: 10.1016/0044-8486(90)90 162-G.

[22]. SEIBEL H, BAßMANN B. & REBL A., Blood Will Tell: What Hematological Analyses Can Reveal About Fish Welfare. Front. Vet. Sci. 8, 616955, 2021. doi: 10.3389/fvets.2021.616955