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Blood Indices and Chemical Characteristics of *Clarias gariepinus* (Burchell 1822) Collected from Three Different Environments

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Authors' contributions

This work was carried out in collaboration among all authors. Author SHAH made the draft of the initial manuscript and reviewed and revised the draft and literature review. Authors AYH, AAB, RMA, FYM, HSE and NMM collected samples and did laboratory work and data analysis. Authors MYMA and MEKS did a final review. All authors read and approved the final manuscript.

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ABSTRACT

Hematological analysis of teleost fish blood offers valuable insights into the physiological adaptations of these organisms to alterations in their external milieu. Additionally, hematological parameters are well-established for their clinical utility in the prognosis and diagnosis of various disease states in fish. The results of this study on blood indices and chemical characteristics of 108 samples of *Clarias gariepinus* collected during March - April 2021, indicate that, there are no significant differences in red blood cell (RBC), hemoglobin (Hb), packed cell volume (PCV) and mean corpuscular volume (MCV), white blood cell (WBC), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH), the levels of neutrophils (N), lymphocytes (L), monocytes (M) and eosinophils (E), the levels of glucose values, protein levels and calcium level (ca) between Jebel Awalia reservoir of the Dam in the White Nile south of Khartoum state and El-Salait culture pond which considered as natural and culture water and there are significant differences in Abu-Adam sewage ponds in all these parameters.

Keywords: Clarias; external environments; physiological response.

1. INTRODUCTION

Global fish production has experienced a substantial rise over the past six decades, reaching approximately 179 million metric tons valued at $401 billion in 2018; Correspondingly, per capita fish consumption worldwide has increased from 9.0 kg in 1961 to 20.5 kg in 2018; Aquaculture now accounts for 46 % of total fish production and 62% of total market value [1]. Driven by escalating demand for high-quality protein, declining wild fish catches, and technological advancements in aquaculture, global aquaculture output is anticipated to double by 2050 [2].

The African catfish, *Clarias gariepinus* (Burchell 1822) (synonymous with *C. lazera*), has long been recognized as one of the most suitable species for aquaculture development in Africa [3]. Since the 1970s, this species has been considered highly promising for fish farming in Africa due to its rapid growth rate, resilience to handling and stress, and widespread popularity across many African countries. The large African catfish species of interest for aquaculture belong to the subgenus *Clarias*; Earlier taxonomic studies [4,5] identified five distinct species within this subgenus, distinguished by morphological characteristics such as vomerine tooth shape, vomerine to premaxillary tooth band ratio, and gill rakers number; These five species are: *C. anguillaris*, *C. senegalensis*, *C. lazera*, *C. mossambicus*, and *C. gariepinus*.

Environmental stressors, both natural and anthropogenic, can induce cellular alterations that disrupt the physiology of organ systems in fish [6,7,8]. Numerous environmental and physiological factors are known to influence fish hematology; environmental pollution, especially in industrialized areas, is a major concern. Wastewater containing heavy metals from manufacturing processes and abattoirs often contaminates aquatic ecosystems [7]; Exposure to such polluted waters poses challenges, including the risk of extinction, for fish and other organisms, including humans [6-8].

Fish can accumulate toxic compounds in their tissues due to their position at the top of the aquatic food chain; These pollutants may be essential at low concentrations but can have adverse health effects at higher levels, overriding their importance [8].

The complete blood cell count (CBC) is a valuable diagnostic and monitoring tool in fish health management. It can be used to assess the impact of nutritional, water quality, and disease factors, as well as evaluate response to therapeutic interventions [9]. Traditionally, fish blood parameters have been manually evaluated using a Neubauer or Burker hemocytometer [9]. Seasonal variations were observed in key serum constituents of fish; During summer, serum protein and glucose were elevated, while cholesterol and urea were depressed, the inverse relationship occurred in other seasons. Multiple hematological, biochemical, and immunological parameters, including enzyme activities, exhibited significant correlations with fish health status across seasonal changes [10]; Seasonal fluctuations primarily modulate the physiological state of the fish species.

This investigation aims to compile and update the available knowledge on the hematology and select chemical characteristics of the African catfish, *Clarias gariepinus*, with a particular emphasis on how different aquatic environmental conditions influence these parameters.

2. MATERIALS AND METHODS

2.1 Collection of Blood Samples

During March to April 2021, 108 samples of *C. gariepinus* (average body weigh 53 g) were collected from three locations (Jebel Awalia reservoir El-Salait culture pond and Abu-Adam sewage ponds 36 specimens each); The fishes were sacrificed, blood samples were collected by puncturing the posterior caudal fin of the fish using a clean, sharp knife; The samples were collected in bottles containing the anticoagulant ethylenediamine tetra acetate (EDTA) [11].

2.2 Hematological Analysis

Two ml of blood sample were transferred to heparinized bottles for blood parameter analysis. The packed cell volume (PCV/hematocrit) was determined using the microhematocrit method described by [12]; Blood samples were diluted with 0.85% NaCl solution (1:5 ratio) prior to analysis. Hemoglobin (Hb) concentration was measured using the cyanmethemoglobin method [13]. Blood cells were counted within 5 of the 25 chambers of a Neubauer hemocytometer, and the mean value was calculated according to [14]. Differential cell counts were determined using the procedure mentioned in [15]. The counting was performed under an Olympus Vanox Research Microscope (Mag 60), Model 230485. The values of hematological indices were calculated using the method described in [16].

Mean cell volume (MCV)

Values of MCV were calculated using the formula:

$$MCV = \frac{PCV \times 10}{RBC}$$

Mean cell Hemoglobin (MCH)

Values of MCH were calculated using the formula:

$$MCH = \frac{Hb\ (g/dl)10}{RBC}$$

Mean cell Hemoglobin concentration (MCHC)

Values of MCHC were calculated using the formula:

$$MCHC = \frac{Hb\ (g/dl) \times 100}{PCV\ \%}$$

Blood plasma was obtained by centrifuging the samples at 1500 rpm for 15 minutes; The plasma from each fish was pooled and stored in plastic screw-top tubes at -18°C until analysis. Total plasma protein, plasma glucose, and Ca++ concentrations were measured using the Biuret reaction method as described by [17] (Clonital, Carvico, Italy) with a Shimadzu UV-1200 UV spectrophotometer.

2.3 Statistical Analysis

The data were statistically analyzed using one-way analysis of variance (ANOVA), and the results are presented as mean ± standard deviation. Statistical significance was set at $p < 0.05$. All analyses were performed using SPSS software (version 13.0).

3. RESULTS AND DISCUSSION

The result shown in Table 1 revealed no significant difference in red blood cell (RBC), Hemoglobin (Hb), packed cell volume (PCV) and mean corpuscular volume (MCV), white blood cell (WBC), mean corpuscular Hemoglobin concentration (MCHC), and mean corpuscular Hemoglobin (MCH), the levels of neutrophils, lymphocytes, monocytes and eosinophils, the levels of glucose values, protein levels and calcium level (Ca) between Jebel Awalia reservoir of the Dam in the White Nile south of Khartoum state and El-Salait culture pond; The same result obtained by [18 and 19] In a study comparing the blood parameters and chemical analysis of *C. gariepinus* collected from the White Nile and Blue Nile, the results revealed no significant difference (p>0.05) between the two river systems for all parameters examined, except for hemoglobin (Hb) concentration.

The current study classified the following hematological parameters for Clarias fish: PCV (20 – 15 ± 4.7), Hb (9 – 95 ±2.8), RBCs (2.67 ± 0.95), WBCs (19.23 ± 6.63), hemoglobin value (36.25 ± 13.85), and PCV range (76.49 ± 24.5); The chemical analysis the following mean values for Clarias fish: protein (157.85 ± 19.35), Glucose (91.75 ± 28.35), Urea (45.45 ± 23.4) as shown in Table 2.

The results showed a significant increase (p<0.05) in RBC, Hb, and WBC counts in fish from the Abu Adam sewage pond. Interestingly, the WBC count exhibited a marked increase at the lowest treated concentration (0.15 mg/L), followed by the highest concentration (0.74 mg/L); This response could be an adaptive mechanism by the fish to combat the effects of the pollutant [20]. Specifically, the rise in WBC count may be viewed as the fish's efforts to adapt and resist the detrimental effects of the pollutant, in order to maintain a healthy physiological state [21]; The current findings differ from the previous research conducted by [22] on the effects of wastewater exposure on *C. lazera*; Whereas [22] reported a decrease in RBC counts in the treated fish compared to the control group, the present study observed a significant increase (p<0.05) in RBC, Hb (HGB), and WBC counts in fish from the Abu Adam sewage pond. Conversely, [22] found the mean values of Hb (HGB) and hematocrit (HCT) were significantly decreased (p<0.05) in the treated group relative to the control; In contrast, the current study did not observe such a decrease in these parameters.

Additionally, [22] noted an increase in the mean values of mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) in the treated group, although the changes were not statistically significant (p>0.05). Similarly, an increase in WBC, MCV, and MCH was reported, which are considered indicators of a functional immune system response [23]; However, the present study did not find the same pattern of changes in these hematological indices.

The current findings are in agreement with the results reported by [24], their study on the exposure of *C. gariepinus* to atrazine and metolachlor indicated an increase in WBC, neutrophils, monocytes, MCH, and MCHC in both male and female fish [24].

Similarly, [25] found that infected fish had higher WBC content compared to uninfected fish. In contrast, [26] reported different results in their study on the effects of abattoir effluents on *C. gariepinus* juveniles; They observed the RBC level of the control group ($2.37 \pm 0.04 \times 10^{12}$ L^{-1}) was statistically (p<0.05) higher than those of the treatment groups ($1.24–1.86 \pm 0.04 \times 10^{12}$ L^{-1}) [26].

The decrease in RBC count in the treatment groups was possibly due to hemolysis, i.e., the breakdown of red blood cells by the effluents as a result of their toxicity [26,27]. This indicates erythrocyte damage or reduction in red cell glutathione, leading to an increase in free radicals and cell death [27].

The current study on the effects of wastewater from the Abu Adam sewage pond on *C. gariepinus* found that packed cell volume (PCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH) were significantly reduced (p<0.05) in the treated fish compared to the natural and culture water; These findings are similar to the results reported by [19], who evaluated the toxic effects of two sublethal concentrations of the pesticide carbofuran on hematological and blood-biochemical parameters in *C. gariepinus*; They observed a significant (p<0.05) decrease in red blood cells (RBCs) count, hemoglobin (Hb) concentration, hematocrit (Hct), and MCHC levels in the treated fish. In contrast, MCV and MCH levels were increased. Additionally, [19] found that white blood cells (WBCs) count, neutrophils, eosinophils, basophils, and monocytes were significantly increased (p<0.05), while lymphocytes were decreased.

Furthermore, [28] investigated the changes in morphological, hematological, and biochemical indices in *C. gariepinus* juveniles exposed to (CLO); After acute exposure, the 96 h LC50 value of CLO was 38.79 mg L^{-1}; Fish were then exposed to sublethal concentrations of 7.76, 3.89, 1.94, and 0.00 mg L^{-1} (control) of CLO for 21 days, followed by a 7-day recovery period. The results revealed concentration- and time-dependent significant decreases in RBC, Hb, PCV, and MCV, accompanied by significant increases in WBC, MCHC, and MCH in the exposed groups compared to the control.

The current study found significant differences in the levels of neutrophils, lymphocytes, monocytes, and eosinophils between the Jebel Awalia reservoir of the White Nile, the El-Selait culture pond, and the Abu Adam sewage pond.

Similarly, [28] reported a mixed trend in the levels of these white blood cell (WBC) differentials in *C. gariepinus* juveniles exposed to (CLO). Furthermore, [29] observed a significant reduction (p<0.05) in total WBC counts and the differential WBC counts in common carp (*Cyprinus carpio*) after exposure to zinc in the water, compared to the control group. These findings suggest that environmental pollutants, such as pesticides, sewage effluents, and heavy metals, can have significant impacts on the hematological parameters, including the WBC differentials, in various fish species.

The current study on the Abu Adam sewage pond found a significant decrease in calcium levels and significant increases in glucose and protein levels ($p<0.05$) in the water samples. [28] reported that in *C. gariepinus* juveniles exposed to (CLO), glucose values significantly increased, while protein levels were reduced ($p<0.05$) throughout the 21-day exposure and the 7-day recovery period.

The present research suggests that CLO may have potential toxic effects on non-target organisms, especially fish, and should be monitored in the aquatic ecosystem. Furthermore, [30] investigated the effect of copper on the blood chemistry of *C. gariepinus*; The fish were exposed to sublethal concentrations of copper for 96 hours in a continuous-flow experimental system. The results showed that the concentration of copper in the river exerts a physiological effect on *C. gariepinus*, manifesting in changes in blood chemistry. Pathological conditions, such as *erythrocytopenia* (decreased red blood cells), *leukocytosis* (increased white blood cells), *hyperglycemia* (elevated glucose), and *hyperproteinemia* (elevated protein), were observed.

These findings highlight the potential impacts of various environmental pollutants, including pesticides and heavy metals, on the physiology and blood parameters of fish species, which are important indicators of ecosystem health.

According to [31], various aquaculture studies have measured several key hematological and biochemical parameters, including red blood cells, white blood cells, hemoglobin, hematocrit, and total protein. However, the author noted that these parameters do not always exhibit the same trend across different experimental fish studies.

As a result, [31] stated that it is difficult to draw a firm conclusion about which specific parameter should be considered the most reliable or informative indicator when assessing the physiological status of fish in aquaculture settings.

This observation highlights the complexity and variability of fish hematology and biochemistry, which can be influenced by numerous factors, such as species, age, environmental conditions, and the nature of the stressors or contaminants present. The lack of a consistent pattern across studies underscores the importance of considering multiple parameters and the need for further research to better understand the relationships between various blood and biochemical markers in different fish species and aquatic environments.

Table 1. Blood parameters of *C. gariepinus* collected from three different areas

Area / parameter	Jebel Awalia (reservoir)	El-Selait culture (pond)	Abu Adam (sewage pond)
Weight (g)	50.00±1.02	52.80^{ab}±5.50	57.40^{a}±5.60
Length (cm)	62.50^{a}±2.45	32.20^{c}±2.29	40.90^{b}±2.52
TWBCs* $_{10}{}^{3}$	51.90±5.90	47.80±6.00	54.40±6.12
TRBCs*$_{10}{}^{6}$	2.10^{bc}±0.40	3.20^{b}±0.40	4.70^{a}±0.40
Hb (g/dl)	1.60^{b}±90	1.40^{bc}±0.90	5.40^{a}±0.90
PCV%	29.60^{a}±1.58	28.20^{ab}±1.60	21.80^{c}±1.65
MCV/cl	89.70^{a}±6.89	89.00^{ab}±6.94	63.40^{c}±7.06
MCH/pg	30.50^{ab}±1.51	30.60^{a}±1.54	7.30^{c}±1.57
N%	18.70±2.43	12.50±1.54	16.30±2.48
E%	3.80^{a}±0.40	2.40^{c}±0.40	2.60^{abc}±0.60
L%	53.20^{c}±3.38	65.40^{ab}±3.88	66.50^{a}±3.92
M%	13.20±1.39	14.10±1.40	12.20±1.43

Table 2. Some biochemical analysis of *C. gariepinus* collected from three different areas

Area / parameter	Jebel Awalia reservoir	El-Selait culture pond	Abu Adam sewage pond
Calcium	3.80^{bc}±1.58	15.80^{a}±1.59	2.50^{c}±1.62
Protein	3.50^{c}±1.08	7.40±1.09	9.70±1.11
Glucose	72.50±20.62	74.50^{bc}±20.77	148.10^{a}±21.21

4. CONCLUSION

It is concluded that Jebel Awalia reservoir of the Dam in the White Nile south of Khartoum state and El-Salait culture pond exhibit similar hematological and biochemical profiles in terms of red blood cell (RBC), hemoglobin (Hb), packed cell volume (PCV) and mean corpuscular volume (MCV), white blood cell (WBC), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH), the levels of neutrophils (N), lymphocytes (L), monocytes (M) and eosinophils (E), the levels of glucose values, protein levels and calcium level (ca) between which considered as natural and culture water. On the other hand, there are significant differences in Abu-Adam sewage ponds in all these parameters.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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