Blood indices and chemical characteristics of *Clarias gariepinus* (Burchell 1822) collected from three different environments

Abstract

Hematological evaluation of fish blood provides valuable facts concerning the physiological response of fish to changes in the external environment. Furthermore, hematological variables are well known for their clinical value in prognosis and diagnosis. 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.

Key word: *Clarias*, external environments, physiological response.

Introduction

According to the Food and Agriculture Organization [1], world fish production has increased dramatically during the past 60 years, to around 179 million tons in 2018 with a value of $401 billion; Global fish consumption also increased from 9.0 kg per capita in 1961 to 20.5 kg in 2018. Aquaculture production presents 46% of the total production and 62% of the total sale value. Due to the increasing demand for high-quality protein, reduction of wild fish catch, and advancement in fish farming technologies, global aquaculture production is expected to double by 2050 [2].

It was soon recognized that the African catfish *Clarias gariepinus* (Burchell 1822) was one of the most suitable species for aquaculture in Africa [3] and since the seventies it has been considered to hold great promise for fish farming in Africa; the African catfish having a high growth rate, being very resistant to handling and stress, and being very well appreciated in a wide number of African countries; The large African species which are of interest for aquaculture belong to the subgenus *Clarias*. In earlier systematic studies on the large African catfish species [4] as well as [5] recognized five species within this subgenus; both authors used morphological criteria such as form of vomerine teeth, ratio of vomerine to premaxillary teeth band and the number of gills rakers, the five species were: · *C. anguillarus*, · *C. senegalensis*, · *C. lazera*, *C. mossambicus* and *C. gariepinus*

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Environmental stressors, both natural and humanly induced, could cause changes in cellular function which alter the physiology of organ systems in fish; many environmental and physiological factors are known to influence fish hematology; Environmental problems are water pollution especially in places with many industrial activities taking place as said by [6], waste water containing heavy metals are produced by many manufacturing processes and abattoirs find their way into the aquatic environments [7], fishes and other organisms with humans inclusive in their habitats come across many challenges including extinction as a result of exposure to such polluted water; Fishes could accumulate some toxic compounds in their body because they are at the tip of sea food chain [8]; These pollutants can be essential at a very low concentration and at higher levels have the ability to override their importance there by resulting in many adverse health.

In the present paper an attempt is made to compile and update available knowledge on haematology and some chemical characteristics concerning the of *C. gariepinus* with particular emphasis on different water environmental conditions.

Materials and methods

Collection of blood samples:

During March to April 2021, 108 samples of *C. gariepinus* 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 using a clean sharp knife in bottles containing ethylenediamine tetra acetate (EDTA) as anticoagulant [9].

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Haematological analysis

Two ml of blood sample was decanted in heparinized bottles for determination of blood parameters; The microhematocrit method described by [10] was used to determine the packed cell volume (PCV/ hematocrit), blood samples were diluted with 0.85% NaCl solution i.e. 1 (blood): 5 (NaCl solution), haemoglobin (Hb concentration was determined by cyanmethemoglobin method [11]. Blood cells were counted within 5 of 25 chambers of a Neubauer haemocytometer and the mean value was calculated according to [12], differential cell count were determined by procedure mentioned [13]; The counting was done under an Olympus Vanox Research Microscope (Mag 60), Model 230485. The values of haematological indices were calculated [14].

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 at 1500 round/sec for 15 min and stored in plastic screw top tubes at -18 ^{o}C until analyses; the plasma obtained from each of fish was pooled, total plasma protein, plasma glucose and Ca^{++} were measured by the Biuret reaction as described by [15] (Clonital, Carvico, Italy) with a Schimatzu UV-1200 UV spectrophotometer.

Statistical analysis

data were statistically analyzed by means of analysis of variance (ANOVA) using the mean ± standard deviation; significance was set at $p < 0.05$; All analysis was performed using SPSS software (version 13.0).

Result and discussion

The result shown in Table I 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 [16] and [17] in study conducted to compare of blood parameters and chemical analysis of *Clarias gariepinus* collected from the White Nile and Blue Nile, the results obtained revealed no significant different (p<0,05) between the White Nile River and Blue Nile River in all parameters examined except haemoglobin concentration (Hb).

In addition to that the current study classified that mean value of compact blood PCV in Clarias fish was (20 – 15 ± 4.7), haemoglobin Hb (9 – 95 ±2.8), RBCs (2.67 ± 0.95), WBCs (19.23 ± 6.63), value of hemoglobin (36.25 ± 13.85), and range value of the cell PCV (76.49 ± 24.5); In case of chemical analysis, the result revealed that the mean value of protein in Clarias fish was varies between (157.85 ± 19.35), Glucose (91.75 ± 28.35), Urea (45.45 ± 23.4) as shown in table II.

There was significant increase in red blood cell (RBC), haemoglobin (Hb), white blood cell (WBC) ($p < 0.05$) in Abu Adam sewage pond; The

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WBC showed a marked increase in the least treated concentration (0.15 mg/L) followed by the highest concentration (0.74 mg/L); this could be due to attempts made by the fish to fight against the effects of the pollutant which could have led to its increment in order to ameliorate healthy state of fish; [18] stated that a rise in WBC count is

viewed as adaptation of organisms and their efforts to combat invaders from cells. [19] suggests that sharp increase in exposed blood cells above that of the control group could be resistance to prevalent unwanted change and adaptability to the environment; Also result differ from the finding of [20] who conducted research on effects of wastewater on *Clarias lazera* and found a decrease in the RBC counts of the treated fishes; Also found mean values of HGB and HCT has significantly decreased when compared with control ($p < 0.05$). Mean values of MCV and MCH has increased when compared with control but not significantly significant ($p > 0.05$). Similarly, an increase was also observed in the WBC, MCV and MCH which are all indicators of a functional immune system [21]. Also, the result in agreement with [22]. The result obtained in their study indicated that the exposure of *C. gariepinus* to different concentrations of atrazine and metolachlor indicated that there was increase in WBC, neutrophils, monocytes, MCH, MCHC in both male and female *C. gariepinus*. The same result obtained by [23] who mentioned that the infected fishes had higher content of white blood cell (WBC) than the uninfected; While [24] found different result in study determining the effects abattoir effluents on *Clarias gariepinus* juveniles under study involved the determination of physicochemical parameters of the water and the hematological parameters of *Clarias gariepinus* juveniles. The RBC level of the control group ($2.37 \pm 0.04 \times 10^{12}$ L^{-1}) was observed to be statistically ($p < 0.05$) different from those of the treatment groups of *C. gariepinus* juveniles ($1.24–1.86 \pm 0.04 \times 10^{12}$ L^{-1}). The decrease in the RBC count is possibly as a result of hemolysis i.e. the breakdown of the red blood cells by the effluents as a result of their toxicity. This indicates erythrocyte damage or reduction in red cell glutathione leading to increase free radical which causes cell death [25].

Also packed cell volume (PCV), mean corpuscular Hemoglobin concentration (MCHC), and mean corpuscular Hemoglobin (MCH) were reduced ($p < 0.05$) in Abu Adam sewage pond semi similar result was obtained on study aimed to evaluate the toxic effects of two sublethal concentrations of carbofuran pesticide (0.16 and 0.49mg/L, for 35 days) on hematological and blood biochemical parameters of catfish, *Clarias gariepinus*, by [17]. The results revealed a significant ($p<0.05$) decrease in red blood cells (RBCs) count, Hemoglobin (Hb) concentration, hematocrit (Hct) and the mean corpuscular hemoglobin concentration (MCHC) levels, but the mean corpuscular volume (MCV) and the mean corpuscular hemoglobin (MCH) levels were increased. White blood cells (WBCs) count, neutrophils, eosinophils, basophils and monocytes were increased significantly ($p<0.05$), while the lymphocytes were decreased. Also, study done by [26] evaluated the changes in

morphological, hematological and biochemical indices in *Clarias gariepinus* juveniles exposed to CLO; After the acute exposure, the 96 h LC_{50} value of CLO determined by probit analysis was 38.79 mgl^{-1}. Based on this value, fish were exposed to sublethal concentrations of 7.76, 3.89, 1.94 and 0.00 mgl^{-1} (control) of CLO for 21 days and were allowed to recover for 7 days. The result revealed no significant effect on the hepatosomatic index and condition factor of the exposed fish. There were concentration and time-dependent significant decreases in red blood cell (RBC), Hemoglobin (Hb), packed cell volume (PCV) and mean corpuscular volume (MCV) with significant increase in the white blood cell (WBC), mean corpuscular Hemoglobin concentration (MCHC), and mean corpuscular Hemoglobin (MCH) in the exposed group when compared with the control.

Also, there were significant difference in the levels of neutrophils, lymphocytes, monocytes and eosinophils, between Jebel Awalia reservoir of the Dam in the White Nile south of Khartoum state and El-Selait culture pond and Abu Adam sewage pond. In study done by [26] on *Clarias gariepinus* juveniles exposed to CLO. indicated that mixed trend was observed in the levels of neutrophils, lymphocytes, monocytes and eosinophils; Also [27] mention that there had been a significant reduction in values of WBC counts and the differential WBC counts have decreased in common carp *Cyprinus carpio* after exposure to zinc in water when compared with controls ($p < 0.05$).

In the case of chemical analysis, the study investigated significant decrease in calcium and significant increase in glucose and protein level in Abu Adam sewage pond ($p < 0.05$). In study done by [26] on *Clarias gariepinus* juveniles exposed to CLO. indicated that 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 indicated that CLO may have potential toxic effect on non-target organisms especially fish and, therefore, should be monitored in the aquatic ecosystem. [28] conducted study on effect of copper on the blood chemistry of *Clarias gariepinus* which exposed to sublethal concentrations of copper for 96 hr in a continuous-flow experimental system; The results proved that the concentration of copper in the river exerts a physiological effect on *Clarias gariepinus* which manifests in changed blood chemistry. Pathological conditions, such as *erythrocytopenia*, *leucocytosis*, *hyperglycemia*, and *hyperprotonemia*, are evident.

[29] stated that various aquaculture studies have measured the following components: red blood cells, white blood cells, hemoglobin, hematocrit, and total protein; However, because these parameters do not always follow the same trend across experimental fish, it is difficult to draw a firm conclusion about which parameter should be considered.

Table I: blood parameter of *C. gariepinus* collected from three different areas

Area / parameter	Jebel Awlia (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 II: some biochemical analysis of *C. gariepinus* collected from three different areas

Area / parameter	Jebel Awlia 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

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