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Influence of Different Water Environments on the Differential Leukocyte Counts in Nile Tilapia

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ABSTRACT

Aquaculture production has become increasingly important for ensuring food security, supported by the expanding variety of cultivated species. This study evaluated the effects of different water environment characteristics on the differential leukocyte counts of the Nile tilapia (*Oreochromis niloticus*), a key farmed fish species in Africa. Fish from four different water sources were compared with healthy individuals from the natural water of the Nile River. A total of 64 Nile tilapia, each weighing 80–100 grams, were evenly divided into four groups and maintained at temperatures ranging from 25.00± 2.5°C to 30.00 ± 2.5°C. The groups included fish from experimental tanks (ET), ponds managed by the General Administration of Fisheries Ponds (GAFP), the Fisheries Research Center Ponds (FRCP), and the White Nile River (WNR, control). Significant differences in water quality parameters, particularly NH_4, NH_3, NO_3, and NO_2 concentrations, were observed among the water sources. GAFP and ET waters showed higher concentrations of these compounds (NH_4, NH_3, NO_3, and NO_2) compared to FRCP and WNR. Differential leukocyte counts varied significantly across the groups. The ET group showed the highest eosinophil percentage (9.68 ± 0.44%), while the GAFP group exhibited the highest percentages of lymphocytes (46.40 ± 0.13%), monocytes (15.43 ± 0.14%), and neutrophils (18.31 ± 0.16%) compared to WNR. In contrast, the FRCP group recorded the highest platelet percentage (32.34 ± 0.49%), while the ET group had the lowest (13.65 ± 0.15%). Additionally, the ET group recorded the highest overall white blood cell count (191.46 ± 0.61 × 10^3). A strong positive correlation was found between the blood profiles of Nile tilapia and the water environment parameters. This study highlighted significant differences in water quality among experimental groups, with FRCP and WNR showing lower parameters. In addition, examining white blood cells in fish is crucial for biological monitoring of surface water pollution.

Keywords: Differential leukocyte count, Nile tilapia, Water quality deterioration

INTRODUCTION

Marine organisms are an important food source for humans, as highlighted by the Food and Agriculture Organization of the United Nations (Tacon, 2019).

Nile tilapia (*Oreochromis niloticus*) farming has expanded rapidly in recent years, paralleling the global expansion of aquaculture. However, fish farming is often linked to the intensification of fish culture and the nutritional requirements (Lal, 2009; Moffitt and Cajas-Cano, 2014), which may have an impact on the well-being and, eventually, productivity, making it challenging to distinguish between overt disease and suboptimal development or reduced health status (Tacon, 2019).

Due to its extraordinary sensitivity to environmental changes, blood is a pathophysiological reflector of the body and a useful instrument for researching the impacts of toxicants (Ali and Ansari, 2023). Morphologically, seven types of cells have been identified in the blood of Nile Tilapia, including erythrocytes, thrombocytes, neutrophils, eosinophils, basophils, lymphocytes, and monocytes (Ueda et al., 2001; Handin et al., 2003).

The most important aspect of a fish farmer's skill set is the ability to recognize performance degradation during developmental stages and take corrective measures. Fish welfare can be negatively impacted by water quality degradation because it can introduce ineffective or toxic compounds into the fish (Naigaga and Kaiser, 2006; Srivastava and Reddy, 2020; Ibrahim and ElSaye, 2023). Successful fish farming relies on two critical factors, water source and water quality. Historically, the success of aquaculture intensification was largely determined by the engineer's ability to manage water and the nutritionist's ability to formulate cost-effective, nutritionally adequate diets (Shepherd et al., 2004).

In Sudan, aquaculture remains in its early stages, with most fish farming conducted by government or state organizations using low-tech methods. The small-scale sector dominates, with limited involvement from foreign entities

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or organizations. *Tilapia spp*. is the most fascinating and widely used fish in fish farms. However, issues such as excessive reproduction and its associated challenges have hindered broader adoption. Various methods have been attempted to prevent overpopulation and stunted growth, including separating parents from offspring at hatching, mono-sex culture, introducing predators alongside tilapia, and selective breeding for larger fish. However, none have proven successful (Miller and Atanda, 2011). The current study addresses one of the critical issues in aquaculture, the accumulation of decomposed food and nitrogenous waste products from cultured fish. Excessive stocking levels in aquaculture systems can result in elevated levels of the formation of hazardous ammonia and a decline in water quality. As such, it is a significant factor that should be taken into account when evaluating fish culture. Hematology and other diagnostic tools such as enzyme activities can help identify stressors and diseases that impact fish performance (Fazio, 2019; Chew and Gibson- Kueh, 2023). These tools also assess fish health in response to changes in nutrition, water quality, and disease resulting from treatment, as reported by Fazio (2019). Over the last 20 years, there has been a rapid development of knowledge of the immunology of fish, particularly at the biochemical and molecular level, in terms of the sophisticated intercellular interactions that accompany any immune response (Sheldon et al., 2014).

The primary objectives of this study were to identify suitable water quality characteristics for aquaculture systems by comparing water from tanks, ponds, and the White Nile River, establish a method for assessing potential environmental impacts on cultured *Oreochromis niloticus*, and determine reference values for leukocyte differential counts in cultured Nile Tilapia.

MATERIALS AND METHODS

Ethical approval

Blood sample collection from the fish in this study was conducted in accordance with the National Animal Health Monitoring System (NAHMS) guidelines (USDA, 2022).

Experimental design

A Completely Randomized Design (CRD) was used to evaluate the effects of water quality parameters from four different water sources on the blood indices, specifically leukocyte differential counts, of Nile tilapia (*Oreochromis niloticus*). The studied fish were randomly collected from various water sources, regardless of their age or sex. The experiment was conducted in six glass tanks (100 cm x 35 cm x 50 cm), each equipped with an aerator positioned in the center. Two additional tanks, each fitted with two aerators, were provided to replace fish sacrificed for analysis during the study period. The experimental fish, with an average weight of 80–100 g and a total length of 19.0-22.7 cm, were collected using gillnets from the White Nile River in the El Shaggara area, located 10 km south of Khartoum (Sudan). A total of 16 fish were stocked in each tank and fed a commercial fish meal at 5% of their biomass per day for six months. The water sources included the General Administration Ponds (GAFP), Fisheries Research Centre Ponds (FRCP), and the White Nile River (WNR), which served as the control site.

Species

Nile tilapia (*Oreochromis niloticus*, [Cichlidae, Teleostei], Linnaeus, 1758) was selected for this study due to its widespread use in Sudanese fish farming and its consistent availability (Mahdi, 1972).

Blood collection and white blood cell differential count

The wedge smear technique is the most convenient and widely used method for preparing peripheral blood smears. For the present study, six fish were randomly selected from each water source to create peripheral blood smears for investigation. No anesthesia was used during blood collection. A small volume of whole blood (2.0 ml) was drawn from the caudal peduncle vein using a sterile 2.5 mL disposable syringe. The blood was transferred into a 0.5 mL mini-plastic tube containing EDTA (1.26 mg/0.6 mL) as an anticoagulant. Peripheral blood smears were promptly prepared and analyzed for differential cell counts using the wedge smear method (Dacie and Lewis, 2017). The differential counts of neutrophils, monocytes, lymphocytes, eosinophils, basophils, and total white blood cells are presented in Table 2.

Water quality parameters

Colorimetric analysis was performed to assess water quality parameters, including ionized ammonia (NH_4^+), non-ionized ammonia (NH_3), nitrate (NO_3^-), and nitrite (NO_2^-), utilizing the Lovibond test system (2000). This widely used quantitative water analysis technique measures the intensity of color produced by a chemical reaction to determine the concentration of specific substances and identify the chemicals present in the sample.

pH determination

The pH levels of water samples from the surveyed locations were measured using an electronic digital paper pH meter (Stick Meter, 2001).

Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS, version 20). A two-way Analysis of Variance (ANOVA), as described by Stern (1986), was utilized to assess the effects of water quality parameters (NH_4, NH_3, NO_3, and NO_2) on the white blood cell (WBC) counts of fish samples collected from various water sources. This method effectively evaluates interactions and differences between multiple independent variables, such as water quality parameters and fish from different water sources, on the dependent variable (WBC counts). Additionally, Pearson's correlation coefficient was applied to determine linear relationships between the physio-chemical parameters of water quality and the WBC counts across the study groups.

RESULTS

Water quality characteristics

The water quality parameters of the WNR (control) and the water sources from FRCP, GAFP, and ET during the survey period are summarized in Table 1 and illustrated in Figures 1, 2, and 3a. Ionized ammonia (NH_4) concentrations were the highest in GAFP (8.36 ± 0.05) and ET water (6.18 ± 0.04), whereas the lowest levels were recorded in FRCP (0.57 ± 0.06) and WNR (0.15 ± 0.02). The pH of GAFP water (9.00 ± 0.85) was significantly higher than that of FRCP (8.85 ± 0.37), WNR (7.34 ± 1.35), and ET water (7.21 ± 0.17). Additionally, the WNR (control) exhibited the highest recorded temperature (30 ± 0.00), showing a significant difference compared to the other sources ($p > 0.05$).

White blood cell count

The white blood cell counts for Nile tilapia in ET, GFAP, WNR, and FRCP groups are presented in Tables 2 and 3, as well as Figures 3b and 4. The water from the experimental tanks (ET) exhibited a significant positive correlation with the differential white blood cell count in the fish, compared to those from the White Nile River ($p < 0.05$, control). The total mean WBC count increased progressively from 113.26±0.24 ×10³ in the FRCP group to 191.46±0.61 ×10³ in the ET group, alongside an increase in neutrophils (from 10 ± 0.11% to 18.31 ± 0.16%) and lymphocytes (from 27.43 ± 0.12% to 46.40 ± 0.13%).

Table 1. Quality parameters for water samples procured from various water environments in Sudan

Treatment / Water Environment	Samples size	Ionized ammonia (µg/dL)	Unionized ammonia (µg/dL)	Nitrate (µg/dL)	Nitrite (µg/dL)	hydrogen ions level	Temperature (° C)
WNR	16	$0.15^{c}±0.02$	$0.01^{c}±0.00$	$0.10^{b}±0.01$	$0.03^{b}±0.01$	$7.34^{b}±1.35$	$30.00^{a}±2.50$
FRCP	16	$0.57^{c}±0.06$	$0.08^{c}±0.01$	$0.10^{b}±0.00$	$0.03^{b}±0.00$	$8.85^{a}±0.37$	$29.44^{a}±0.93$
GAFP	16	$8.36^{a}±0.05$	$0.42^{a}±0.005$	$0.10^{b}±0.00$	$0.03^{b}±0.00$	$9.00^{a}±0.00$	$28.37^{a}±3.95$
ET	16	$6.18^{b}±0.04$	$0.31^{b}±0.02$	$0.65^{a}±0.07$	$0.35^{a}±0.04$	$7.21^{b}±0.17$	$25.45^{b}±4.32$
Sig.		**	**	**	**	**	**

[a,b] Superscript letters in the identical column demonstrate a significant difference ($p < 0.05$). Data are expressed as averages ± standard deviation. WNR: White Nile River, FRCP: Fisheries Research Centre Pond, GAFP: General Administration of Fisheries Pond, ET: Experimental Tanks, Sig: Significance.

Table 2. Blood profiles of Nile Tilapia (*Oreochromis niloticus*) as influenced by the various water environmental sources in Sudan

Deferential count / Water Source	Eosinophils (%)	Basophils (%)	Platelets (%)	Neutrophils (%)	Monocyte (%)	Lymphocytes (%)	WBC (X 103)	Sig.
White Nile River (control)	$1.11^{c}±0.2$	$3.62^{c}±0.02$	$29.63^{a}±0.52$	$10.87^{c}±0.04$	$6.22^{c}±0.34$	$27.43^{c}±0.12$	$116.96^{b}±0.10$	
Fisheries Research Centre Pond	$1.17^{c}±0.04$	$3.24^{c}±0.05$	$32.34^{b}±0.49$	$10.37^{a}±0.11$	$5.75^{c}±0.06$	$30.25^{c}±0.08$	$113.63^{b}±0.20$	
General administration of fisheries pond	$5.68^{b}±0.18$	$9.46^{b}±0.017$	$14.27^{c}±0.15$	$18.31^{c}±0.16$	$15.43^{a}±0.14$	$46.40^{a}±0.13$	$113^{b}.26±0.24$	**
Experimental tank	$9.68^{a}±0.44$	$10.82^{a}±0.12$	$13.65^{c}±0.15$	$15.50^{c}±0.19$	$7.69^{b}±0.14$	$45.57^{b}±0.30$	$191^{a}.46±0.61$	

Sample sizes are average ± Standard Deviation (SD) [a, b, c] in the same column with different superscript letters show significant ($p < 0.05$) differences. **: Significant ($p < 0.05$). WBC: White blood cells

Table 3. Correlations between blood profile of Nile tilapia (*Oreochromis niloticus*) and different water environment parameters (NH4, NH3, NO3, and NO2)

White blood cells	Lymphocytes	Eosinophils	Basophils	Thrombocytes	Neutrophils	Monocytes	NH4	NH3	NO3	NO2
Lymphocytes	.519** .									
Eosinophils	.837**	.894**								
Basophils	.667**	.970**	.956**							
Thrombocytes	-.569**	-.995**	-.918**	-.980**						
Neutrophils	.280	.945**	.747**	.899**	-.930**					
Monocytes	-.183	.725**	.374	.607**	-.688**	.887**				
NH4	.369	.978**	.807**	.930**	-.965**	.977**	.835**			
NH3	.366	.976**	.801**	.911**	-.958**	.955**	.816**	.986**		
NO3	.923**	.670**	.882**	.776**	-.700**	.467*	.040	.542**	.559**	
NO2	.807**	.807**	.927**	.864**	-.837**	.667**	.297	.692**	.710**	.871**

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed). Where NH4: Unionized ammonia, NH3: Ionized ammonia, NO3: Nitrates, NO2: Nitrites and Number of Samples: 24. A correlation of - 0.96 and -0.95 are a strong negative correlation, whereas a correlation of -0.70 is stronger than +0.10.

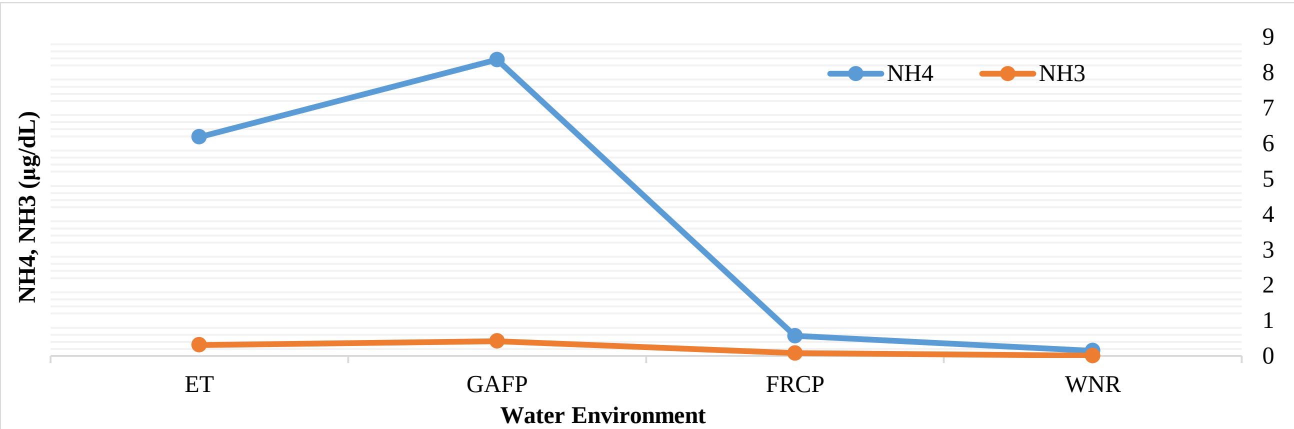


Figure 1. Ionized ammonia (NH4) and Unionized ammonia (NH3) concentrations (µg/dL) appeared in various water environments. Where ET is the experimental Tank, GAFP is General Administration Fisheries Ponds, FRCP is Fisheries Research Centre Ponds, and WNR is White Nile River (water sources).

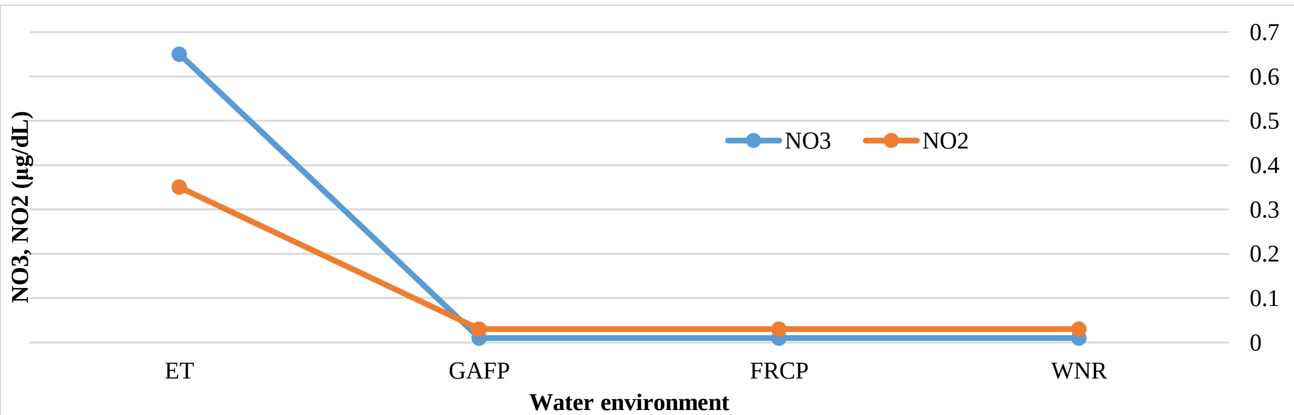
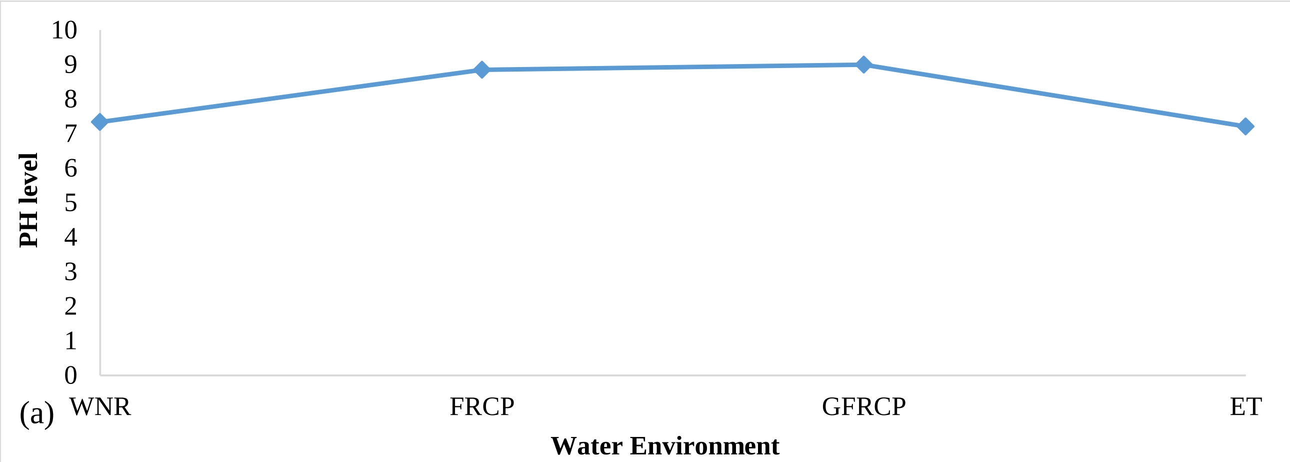


Figure 2. Nitrates (NO3) and Nerites (NO2) concentration (µg/dL) appeared in various water environments. Where ET is the experimental Tank, GAFP is General Administration Fisheries Ponds, FRCP is Fisheries Research Centre Ponds, and WNR is White Nile River (water sources).



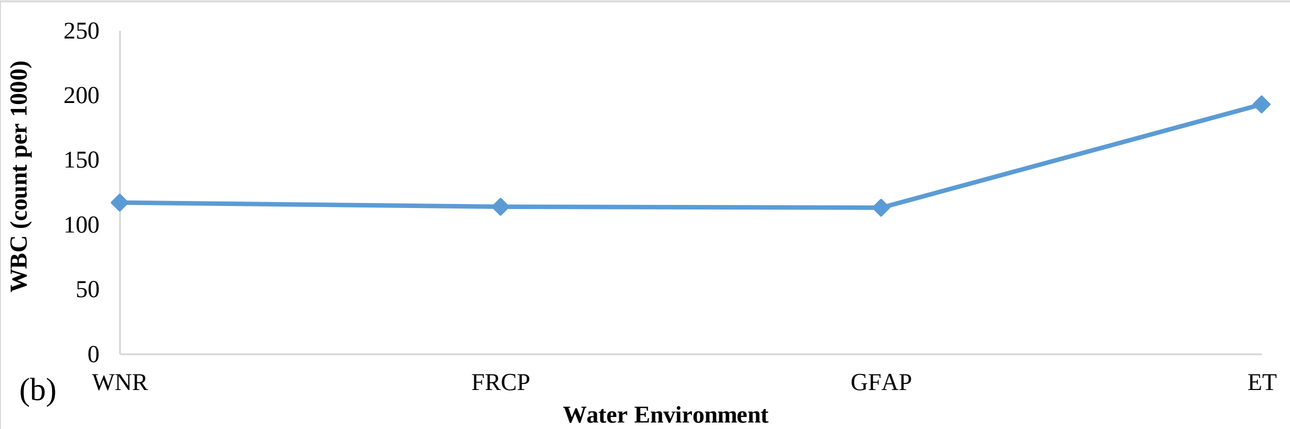


Figure 3. Hydrogen ion concentration (pH) levels as appeared at various water environment sources (**a**). White blood cell (WBC) count of Nile Tilapia (*Oreochromis niloticus*) per 1000 as appeared in various water environments (**b**). Where ET is the experimental Tank, GAFP is General Administration Fisheries Ponds, FRCP is Fisheries Research Centre Ponds, and WNR is White Nile River (water sources).

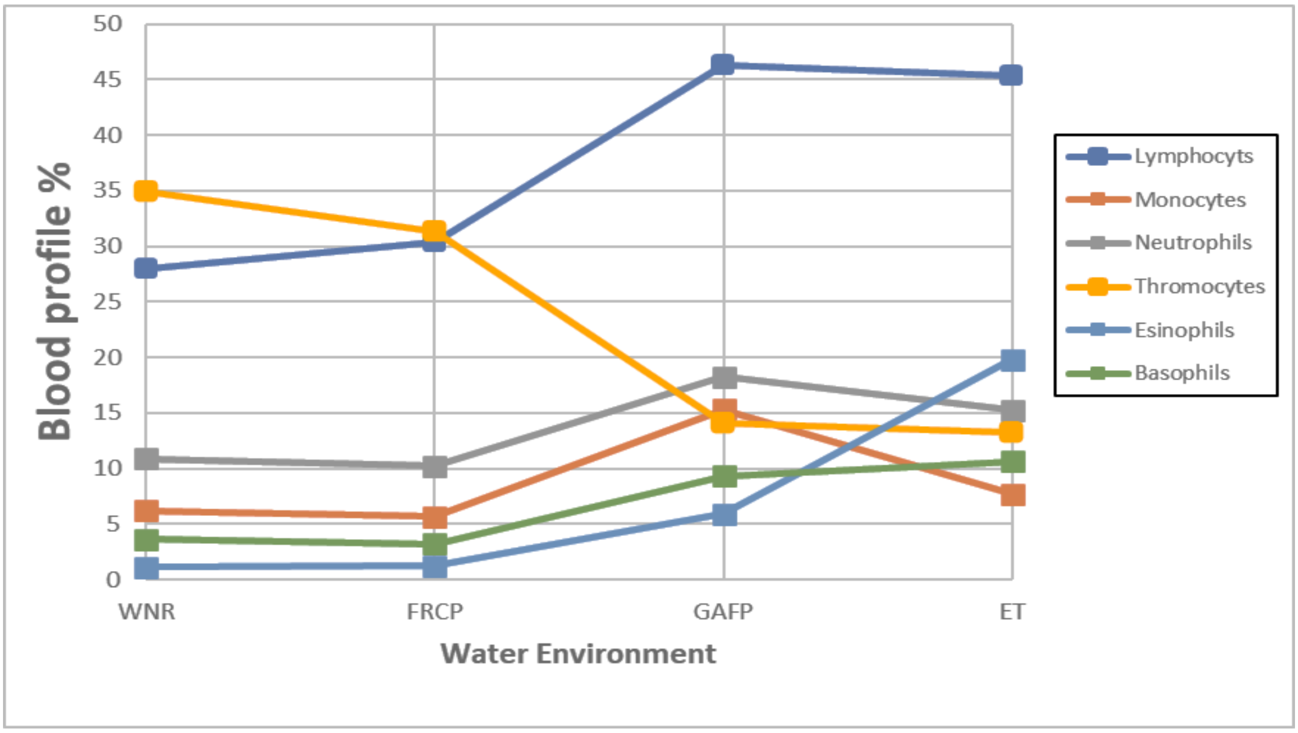


Figure 4. White blood profile (%) of Nile Tilapia (*Oreochromis niloticus*) influenced by different water environment parameters. Where ET is the experimental Tank, GAFP is General Administration Fisheries Ponds, FRCP is Fisheries Research Centre Ponds, and WNR is White Nile River (water sources).

DISCUSSION

The findings of this study revealed significant variations in the white blood cell (WBC) counts of Nile tilapia (*Oreochromis niloticus*) from different water sources. In comparison to the control, the fish from the ET had the highest eosinophils percentage and total leukocyte count. In comparison to the control fish in WNR, the GAFP fish demonstrated the highest levels of lymphocytes, monocytes, and neutrophils. Conversely, fish from the FRCP showed the highest platelet count. Significant changes in the percentage of individual leukocyte types are influenced by stress factors, particularly various forms of pollution (NH_4, NH_3, NO_3, and NO_2), including extreme pH levels (Modrá et al., 1998). This observation aligns with and supports the findings of the study by Modrá et al. (1998) and Lutnicka et al. (2016).

In general, the results of this study are confirmed by Lutnicka et al. (2016), Ueda et al. (2001), and Ibrahim and ElSayed (2023). Additionally, Ibrahim and ElSayed (2023) demonstrated that alterations in water quality variables significantly affect the blood profile of fish, as evidenced by the results of their examination. The measured water quality parameters for the White Nile River (WNR, control) and FRCP, as shown in Table 1, were within acceptable limits for Nile tilapia culture (Ibrahim and ElSayed, 2023; Aljadeff and Morlandt, 2024). Even so, the ET and GAFP water contained the highest concentration of water quality parameters. The water quality parameters measured in the WNR (control) and FRCP, specifically NH_4, NH_3, NO_3, NO_2, pH, and temperature fell within the acceptable limits for Nile tilapia culture, as reported by Ibrahim and ElSayed (2023). Research by Annas et al. (2023) indicates that ammonia can decrease dietary intake in fish at concentrations as low as 0.08 mg/L, with extended exposure to 0.2 mg/L causing serious harm.

In this study, the highest mean ammonia concentrations were observed in water samples from GAFP, ET, FRCP, and WNR, respectively. While nitrate (NO_3) is relatively non-toxic to tilapia, prolonged exposure can weaken the immune system and cause mortality (Hulata, 2001). Conversely, nitrite (NO_2) is highly toxic, impairing physiological functions and reducing hemoglobin’s oxygen transport capacity, leading to growth retardation (El-Sayed, 2006).

Leukocytosis is the phenomenon characterized by an elevated white blood cell (WBC) count in a blood profile (Nussey et al., 1995; Lutnicka et al., 2016). This increase in leukocyte counts reflects the body's natural defense against foreign invaders, such as water pollutants, which can disrupt regular physiological processes in fish. The precise WBC count slightly increases in response to water environment pollutants. These data essentially demonstrate the impact of water pollution and indicate that essential physiological and immunological functions in fish may eventually be compromised after prolonged exposure to the deteriorated water quality of studied water sources.

Significant increases in percentage in neutrophils (neutrophilia), monocytes (monocytophilia), and eosinophils (eosinophilia) are indicative of changes in various water sources, as are significant increases in lymphocytes (lymphocytosis, Nwani et al., 2014; Saravanan et al., 2017). This conclusion is supported by the observation that more *Oreochromis mossambicus* leucocytes are produced at both temperatures, likely as a defense mechanism against potential pollution-related water deterioration (Nussey et al., 1995; Lutnicka et al., 2016).

The impact of contaminated water on the white blood parameters of the fish under study revealed a reduction in platelets and an increase in leukocytes, as observed in the ET water. This indicates that chemical contaminants in the water acted as antigens, triggering the proliferation of defense cells in the fish species (Svoboda et al., 2001; Venkateswara, 2006). The GAFP fish showed the highest monocyte percentage, while all fish from the WNR (control), ET, and FRCP groups remained within the normal monocyte range. Monocytosis in GAFP suggests an elevated monocyte count, which is sporadically linked to persistent water deterioration, with no clinical monocytopenia (Nussey et al., 1995; Svoboda et al., 2001; Venkateswara, 2006).

The highest percentage of neutrophils was observed in fish from GAFP. This finding aligns with studies like that of Pichhode et al. (2020), which reported increased neutrophil counts in Tilapia (*Oreochromis mossambicus*). Neutrophilia, or elevated neutrophil levels, can be triggered by factors such as stress, excitement, physical activity, and deteriorating water quality (Nussey et al., 1995; Mazon et al., 2002; Tavares- Dias and Moraes, 2007).

The lowest percentage of eosinophils and basophils were found in fish from the WNR and FRCP groups. These values resemble those reported by Henry and Kishimba (2006) and Bolade and Ndidi (2021). Basophils are extremely uncommon in all livestock species and rare in the blood of dogs and cats. According to Hrubec et al. (2000), basophils are observed only sporadically or in low numbers when present and are rarely found consistently in the blood. Eosinopenia, which is defined as a decreased eosinophil count, may be normal or result from stress or deteriorating water quality. Generally, the variation in WBC differential counts in this study may be linked to declining water quality in GAFP and ET water sources. In contrast, acute toxicity studies by Youssef et al. (2023) corroborated the findings of this study, reporting increased blood leukocyte counts linked to adverse effects observed during the study period due to deteriorating water quality.

Stress caused by high concentrations of water quality deterioration parameters, such as NH4, NH3, NO3, and NO2, leads to increased leukocyte levels, particularly neutrophils characterized by lymphocytosis and neutrophilia (Bolade and Ndidi, 2021). In this investigation, leukocyte counts considerably fluctuated to values above the control (WNR) and FRCP fish, likely due to abnormal concentrations of water quality parameters (NH_4, NH_3, NO_3, and NO_2) in GAFP and ET. Similar findings have been reported by Briggs and Bain (2017).

CONCLUSION

In conclusion, this study sheds light on the challenges affecting the growth of Tilapia (*Oreochromis niloticus*) in fish pond culture in Sudan. Poor water quality can adversely impact fish health by causing toxicosis or introducing harmful agents. Such deterioration significantly hampers fish productivity and compromises health, ultimately leading to the onset of recognizable diseases. Moreover, the study underscores the potential of blood parameter values as reliable indicators for evaluating fish health.

Further research is needed to determine whether the elevated WBC levels are caused by the deterioration of the water source or by immune stimulation from other factors, such as heavy metals.

DECLARATIONS

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Availability of data and materials

The original data supporting this study are included in the article and are available upon reasonable request to the corresponding author.

Ethical considerations

The authors of the current study declare that this study is an original study that was authored by contributions of all authors and submitted for the first time to the present journal.

Authors' contributions

Prof. Hassan Sulieman contributed to the study design and experiment scheduling, while Talaat Hassan conducted the data analysis. All authors reviewed the analyzed data and approved the final manuscript draft.

Competing of interests

The authors declare that there is no competing interest.

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