

The Effect of Salinity Variation on the Blood Profile and Physiological Adaptation of Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

Nile tilapia (*Oreochromis niloticus*) is a major freshwater aquaculture species with potential for culture in brackish environments, but increasing salinity may impose osmotic stress and alter physiological performance. This study evaluated the effects of salinity on the blood profile, survival, and physiological adaptation of Nile tilapia. A completely randomized design was used with five salinity treatments (0, 5, 10, 15, and 20 ppt), each with three replicates. Hematocrit, hemoglobin concentration, erythrocyte count, leukocyte count, blood glucose concentration, survival rate, and water quality were assessed. Increasing salinity significantly affected the hematological and physiological responses of Nile tilapia. Erythrocyte counts and hemoglobin concentrations decreased as salinity increased, whereas leukocyte counts, hematocrit values, and blood glucose concentrations increased. Survival tended to decline with increasing salinity, with the lowest value (73.33%) recorded at 20 ppt. These responses indicate increasing osmoregulatory and metabolic stress at higher salinity levels. Under the conditions tested, salinity levels of ≤ 10 ppt provided a more favorable range for maintaining physiological stability and survival of Nile tilapia.

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is one of the most important freshwater fish species cultured in Indonesia. This commodity is highly valued due to its distinctive taste, high nutritional value, and strong export potential. From an aquaculture perspective, Nile tilapia offers several advantages, including a relatively fast growth rate, broad environmental tolerance, adaptability to various culture systems (such as ponds, recirculating aquaculture systems (RAS), and aquaponics), and generally high survival rates. In addition, its relatively large body size produces white flesh that is highly preferred by consumers (Magbanua & Ragaza, 2022 in Novodvorski *et al.*, 2024).

In practice, freshwater Nile tilapia can also be cultured in brackish and marine waters through a gradual acclimation process. Tilapia that successfully adapt to high-salinity environments are commonly referred to as saline tilapia. However, the salinity of the culture medium directly affects the osmotic pressure of the fish, thereby influencing growth performance. Energy that would otherwise be allocated for growth is diverted to maintain

osmotic balance. Furthermore, gonadal development is inhibited under high-salinity conditions, resulting in reduced reproductive performance. Therefore, freshwater Nile tilapia cultured at salinity levels of ≥ 5 ppt must undergo gradual acclimation. Without proper acclimation, the fish may experience severe stress or even mortality (Aminin *et al.*, 2024).

Although Nile tilapia production in Indonesia has continued to increase, current production remains insufficient to meet both domestic and international market demand. One of the major limiting factors is water salinity. Salinity is defined as the total concentration of dissolved ions in water. Excessively high, low, or highly fluctuating salinity levels may cause mortality in Nile tilapia because they disrupt the osmotic balance between the fish and its surrounding environment, particularly when salinity exceeds the species' physiological tolerance limits (Takvam *et al.*, 2021). Under such conditions, fish must expend additional energy to maintain osmotic homeostasis through osmoregulatory mechanisms.

Osmoregulation is a fundamental physiological process that maintains the balance of body fluids by regulating water and ion exchange to preserve optimal osmotic pressure. This process primarily involves the kidneys, gills, skin, and buccal membranes. Disruption of osmotic balance may cause cells to undergo lysis due to excessive water uptake or crenation due to excessive water loss, both of which can ultimately lead to mortality (Aminin *et al.*, 2024).

Each fish species possesses different osmoregulatory capabilities depending on its natural habitat. Freshwater fish are hyperosmotic relative to their environment and maintain ion and water balance through active transport mechanisms in the gills, kidneys, and intestines (Fridman *et al.*, 2020). In contrast, marine fish drink seawater and excrete excess ions through the gills as well as through concentrated urine and feces. Hormonal regulation, particularly by prolactin, together with genetic regulation, plays a crucial role in salinity tolerance. Studies on *Oreochromis* have demonstrated genotypic variation and differential gene expression that enable certain strains of Nile tilapia to adapt to brackish and high-salinity environments following gradual acclimation (Yamaguchi *et al.*, 2018; Yu *et al.*, 2022; Sarker *et al.*, 2023). Therefore, proper adaptation and acclimation are essential when transferring or culturing freshwater Nile tilapia in brackish or marine environments.

Therefore, this study aimed to evaluate the effects of different salinity levels (0, 5, 10, 15, and 20 ppt) on the blood profile and physiological adaptation of Nile tilapia (*Oreochromis niloticus*). Specifically, erythrocyte count, leukocyte count, hematocrit, hemoglobin, blood glucose concentration, and survival rate were assessed to characterize the physiological response of Nile tilapia to increasing osmotic stress.

METHODS

Time and Place

This study was conducted over a period of 14 days, commencing in March 2026. The rearing of Nile tilapia (*Oreochromis niloticus*) was carried out at the Fish Production and Reproduction Laboratory, Faculty of Agriculture, University of Mataram.

Tools and Materials

The equipment used in this study included culture containers, hand nets, syringes, aerators, siphon hoses, a digital balance, label paper, stationery, a pH meter, a refractometer, a digital camera, a thermometer, pipettes, a centrifuge, microhematocrit capillary tubes, Critoseal, a Sahli hemoglobin meter, a hematocrit reader, and a hemocytometer.

The materials used in this study consisted of Nile tilapia (*Oreochromis niloticus*), commercial fish feed, freshwater, salt, distilled water (aquades), 0.1 N hydrochloric acid (HCl), Hayem's solution, Turk's solution, blood glucose test strips, 10% EDTA solution, and a microscope.

Research Design

This study employed an experimental research method. According to Sugiyono (2022), experimental research is a method used to determine the effect of a particular treatment under controlled conditions. In this study, the experimental method was applied to evaluate the effects of different salinity levels on the blood profile and physiological adaptation of Nile tilapia (*Oreochromis niloticus*). The experiment was arranged in a Completely Randomized Design (CRD) consisting of five treatments with three replicates each, adapted from Royan *et al.* (2014). The treatments were as follows:

- P1 : 0 ppt salinity (control)
- P2 : 5 ppt salinity
- P3 : 10 ppt salinity
- P4 : 15 ppt salinity
- P5 : 20 ppt salinity

Research Procedures

• Preparation of Experimental Fish

Total of 150 Nile tilapia (*Oreochromis niloticus*) were selected as the experimental fish. Prior to the experiment, the fish were acclimated in holding tanks for 14 days. During the acclimation period, the fish were fed ad libitum twice daily, in the morning and afternoon, to maintain good health and ensure their readiness for the experiment.

• Preparation of Salinity Treatments

The salinity treatments were prepared by dissolving non-iodized salt in freshwater to obtain the desired salinity levels. The amount of salt required was calculated from the mass-concentration relationship for salinity, in which 1 ppt is approximately equivalent to 1 g of dissolved salt per liter of water at low concentrations (Boyd, 2015), using the following formula:

$$\text{Salt (g)} = \text{Water volume (L)} \times \text{Target salinity (ppt)}$$

The calculated amount of salt was first dissolved in approximately 20% of the total water volume and then mixed thoroughly with the remaining water in the aquarium while stirring continuously. After the solution was completely homogenized, the salinity was measured using a refractometer. A few drops of the prepared water were placed on the refractometer prism, and the salinity reading was recorded once the measurement had stabilized.

• Preparation of the Rearing Media

Total of 15 experimental units were prepared according to the number of treatments and replicates. Each culture container was filled with water adjusted to the designated salinity levels of 0, 5, 10, 15, and 20 ppt, corresponding to the experimental design. To maintain optimal water quality, uneaten feed and fish waste were removed daily by siphoning. Nile tilapia were then randomly stocked into each experimental unit at a density of 10 fish per container. Each treatment was replicated three times, resulting in a total of 15 experimental units. The fish were reared for seven days, during which the assigned salinity levels were maintained throughout the experimental period.

• Data Collection

Data were collected through daily observations of the fish blood profile. The measured parameters included hematocrit, hemoglobin, erythrocyte count, leukocyte count, and blood

glucose concentration. Blood samples were collected using a 0.3 mL syringe that had been rinsed with 10% EDTA solution to prevent blood coagulation. Hematocrit was determined using microhematocrit capillary tubes sealed with Critoseal and centrifuged at 3,500 rpm for 15 minutes. Hemoglobin concentration was measured using the Sahli method with 0.1 N hydrochloric acid (HCl) and a Sahli hemoglobin meter. Erythrocyte counts were determined following the method of Blaxhall & Daisley (1973) using Hayem's solution, while leukocyte counts were determined using Turk's solution. Both parameters were analyzed with a hemocytometer under a light microscope. Blood glucose concentration was measured using a digital glucometer with disposable test strips. In addition to blood profile analysis, fish survival rate (SR) was determined by comparing the number of fish at the beginning and the end of the experiment. Water quality parameters, including temperature, pH, dissolved oxygen (DO), and salinity, were also monitored throughout the study to ensure that the culture conditions remained within the desired range.

Research Parameters

• Survival Rate

The survival rate represents the percentage of Nile tilapia that remain alive throughout the culture period, from the beginning of stocking until the end of the experiment. Survival rate is influenced by both biotic and abiotic factors that affect fish viability during the rearing period. The survival rate was calculated using the formula proposed by Hidayat *et al.* (2013) :

$$SR = \frac{N_t}{N_0} \times 100\%$$

Where:

SR = Survival rate (%)

N₀ = Number of fish alive at the beginning of the stocking period

N_t = Number of fish alive at the end of the rearing period

• Blood Profile

The blood profile of Nile tilapia was evaluated to determine the physiological responses of the fish to different salinity treatments. The blood parameters observed, based on the methods described by Royan *et al.* (2014), included the following:

a. Hematocrit

Hematocrit was determined using the microhematocrit method described by Anderson (1993). Microhematocrit capillary tubes were filled with blood samples and sealed with Critoseal. The tubes were centrifuged at 3,500 rpm for 15 minutes. The hematocrit value was then calculated as the percentage of packed red blood cell volume relative to the total blood volume using the following formula:

$$H = \frac{\text{Volume of packed red blood cells}}{\text{Total blood volume}} \times 100\%$$

b. Hemoglobin

Hemoglobin concentration was determined using the Sahli method as described by Wedemeyer & Yasutake (1977). A blood sample was reacted with 0.1 N hydrochloric acid (HCl) and diluted until its color matched the standard color scale of the Sahli hemoglobin meter, after which the hemoglobin concentration was read directly from the scale and expressed in grams per deciliter (g/dL).

c. Erythrocyte Count

The erythrocyte count was determined using the method of Blaxhall & Daisley (1973) in Royan *et al.* (2014) with Hayem's solution as the diluent. The diluted blood sample was loaded

into a hemocytometer and examined under a light microscope. The number of erythrocytes was calculated using the following formula:

$$\text{RBC} = \frac{N \times DF}{A \times D}$$

Where:

N = Number of cells counted

DF = Dilution factor

A = Area of the counting chamber (counting grid)

D = Depth of the counting chamber

d. Leukocyte Count

The leukocyte count was determined using the method of Blaxhall & Daisley (1973) in Royan *et al.* (2014) with Turk's solution as the diluent. Similar to the erythrocyte count, the diluted blood sample was loaded into a hemocytometer and examined under a light microscope. The number of leukocytes was calculated using the following formula:

$$\text{WBC} = \frac{N \times DF}{A \times D}$$

Where:

N = Number of cells counted

DF = Dilution factor

A = Area of the counting chamber (counting grid)

D = Depth of the counting chamber

e. Blood Glucose

Blood glucose concentration was measured using a digital glucometer with disposable test strips following the procedure used for Nile tilapia by Royan *et al.* (2014). The blood sample was applied directly to the test strip, and the glucose concentration was displayed on the glucometer screen and recorded in milligrams per deciliter (mg/dL).

• Water Quality

Water quality parameters, including dissolved oxygen (DO), salinity, and pH, were measured daily throughout the 14-day rearing period. DO was measured using a DO meter, salinity using a refractometer, and pH using a calibrated pH meter. All measurements were performed directly in the rearing units at approximately the same time each day, and the results were recorded in mg L⁻¹, ‰, and pH units, respectively.

Data Analysis

The data obtained during the study were subjected to statistical analysis. Data on blood profile parameters (hematocrit, hemoglobin concentration, erythrocyte count, leukocyte count, and blood glucose concentration) and survival rate (SR) were analyzed using one-way Analysis of Variance (ANOVA) at a 5% significance level. When significant differences among treatments were detected, Duncan's Multiple Range Test (DMRT) was performed as a post hoc test to compare treatment means. Water quality parameters, including temperature, pH, dissolved oxygen (DO), and salinity, were analyzed descriptively.

RESULTS

Survival Rate

The results showed that the highest survival rate was recorded in treatment P2, with a survival rate of 96%, whereas the lowest survival rate was observed in treatment P5, at 73%. These findings indicate that lower salinity levels were associated with higher survival of Nile

tilapia. One-way Analysis of Variance (ANOVA) at a significance level of $p < 0.05$ revealed a significant effect of salinity on the survival rate of Nile tilapia. This finding indicates that survival rates differed significantly among the salinity treatments, demonstrating that salinity variation influenced the survival performance of Nile tilapia under the experimental conditions.

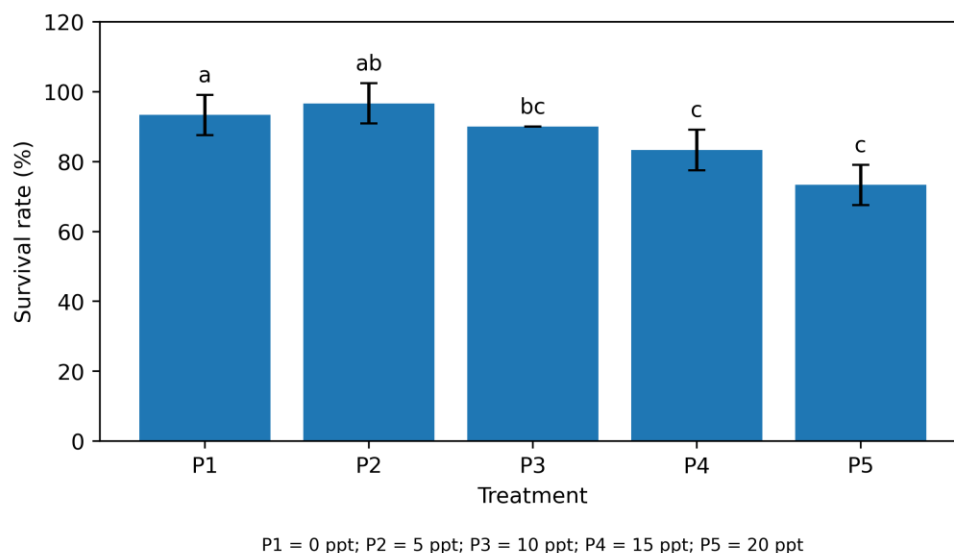


Figure 1. Survival Rate

Erythrocyte Count

The results showed that the highest erythrocyte count was observed in treatment P1, with a value of 9.01, whereas the lowest value was recorded in treatment P5, at 1.38. These findings indicate that increasing salinity resulted in a reduction in the erythrocyte count of Nile tilapia. Statistical analysis revealed that salinity had a significant effect on erythrocyte count. Duncan's Multiple Range Test (DMRT) showed that treatment P1 differed significantly from treatments P2, P3, P4, and P5. Treatment P2 did not differ significantly from P3 but differed significantly from P4 and P5.

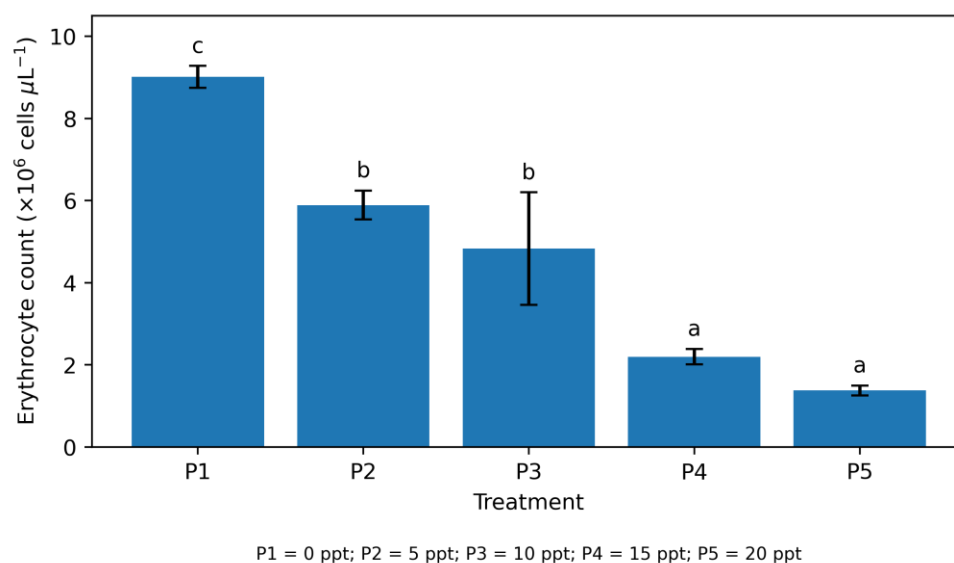


Figure 2. Erythrocyte Count

Leukocyte Count

The results showed that the highest leukocyte count was recorded in treatment P5, whereas the lowest count was observed in treatment P1. These findings indicate that increasing salinity influenced the leukocyte count of Nile tilapia. Statistical analysis revealed that salinity had a significant effect on leukocyte count. Duncan's Multiple Range Test (DMRT) showed that treatment P1 did not differ significantly from treatments P2 and P3 but differed significantly from P4 ($p < 0.05$). However, no significant difference was observed between treatments P1 and P5. Likewise, treatment P4 differed significantly from P1 ($p < 0.05$) but did not differ significantly from P5.

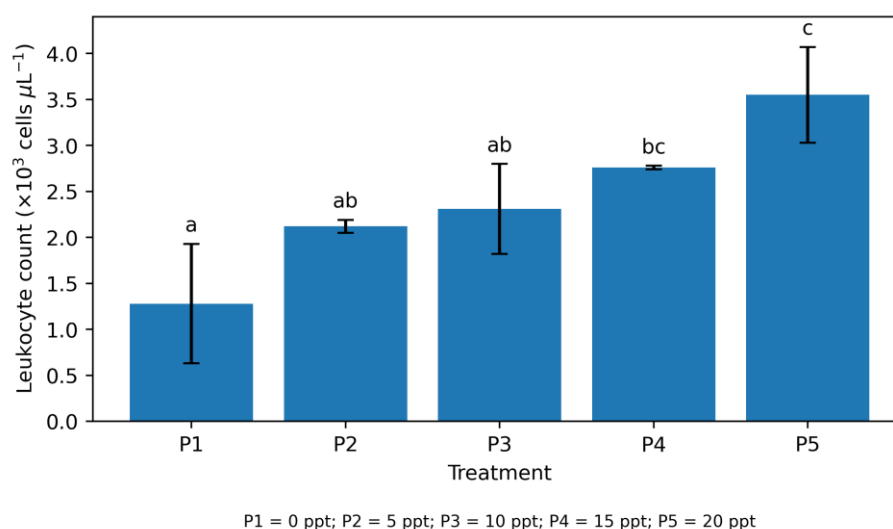


Figure 3. Leukocyte Count

Hematocrit

The results showed that the highest hematocrit value was recorded in treatment P4 (9.39%), whereas the lowest value was observed in treatment P1 (4.03%). These findings indicate that salinity influenced the hematocrit values of Nile tilapia. Statistical analysis revealed that salinity had a significant effect on hematocrit. Duncan's Multiple Range Test (DMRT) showed that treatment P1 differed significantly from treatments P2, P4, and P5 ($p < 0.05$). In contrast, treatment P3 did not differ significantly from P1, nor did it differ significantly from treatments P2, P4, and P5.

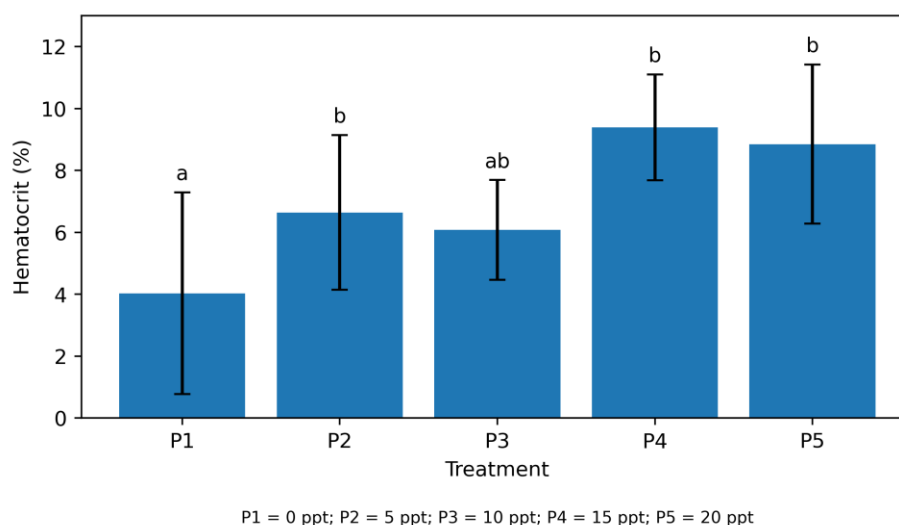


Figure 4. Hematocrit

Hemoglobin

The results showed that the highest hemoglobin concentration was recorded in treatment P1 (8.50 g/dL), whereas the lowest value was observed in treatment P5. These findings indicate that increasing salinity affected the hemoglobin concentration of Nile tilapia. Statistical analysis revealed that salinity had a significant effect on hemoglobin concentration. Duncan's Multiple Range Test (DMRT) showed that treatments P1 and P2 did not differ significantly from each other. Likewise, treatments P3 and P4 were not significantly different from one another but differed significantly from P1 and P2. In addition, treatment P5 differed significantly from all other treatments.

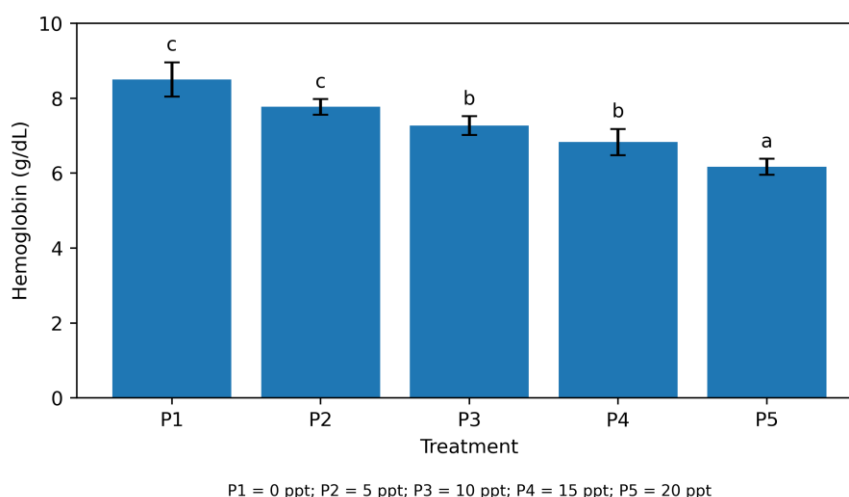


Figure 5. Hemoglobin

Blood Glucose

The results showed that the highest blood glucose concentration was recorded in treatment P5 (119.3 mg/dL), whereas the lowest value was observed in treatment P1 (63.33 mg/dL). These findings indicate that increasing salinity elevated the blood glucose concentration of Nile tilapia. Statistical analysis revealed that salinity had a significant effect on blood glucose concentration. Duncan's Multiple Range Test (DMRT) showed that treatment P1 differed significantly from treatments P2, P4, and P5. Treatment P3 did not differ significantly from treatments P2 and P4. In contrast, treatment P5 differed significantly from all other treatments (P1, P2, P3, and P4).

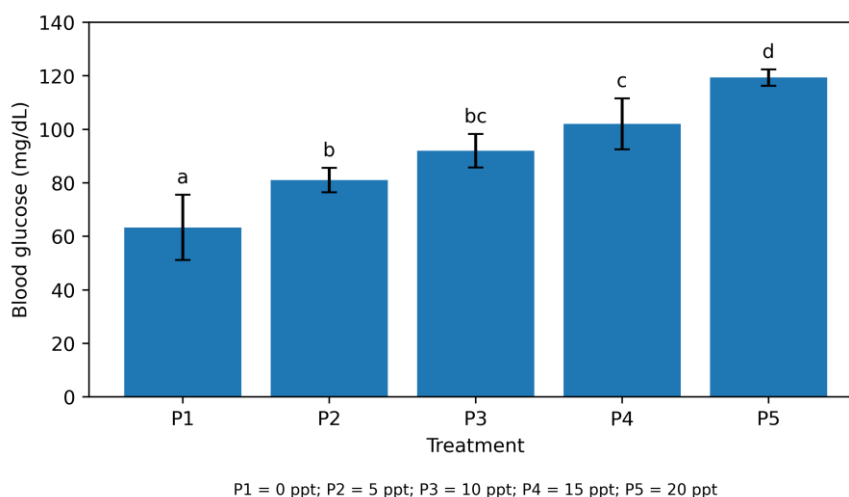


Figure 6. Blood Glucose

Water Quality

The water quality parameters measured in this study included temperature, pH and dissolved oxygen (DO). The results of the water quality measurements are presented in Table 1.

Table 1. Water Quality

Parameters	Observed Range	Optimal Range
Temperature (°C)	26 - 36	27 – 30 (Safsafubun <i>et al.</i> , 2023)
DO (mg/L)	6	>5 (Safsafubun <i>et al.</i> , 2023)
pH	6 - 10	6,5 - 8,5 (Safsafubun <i>et al.</i> , 2023)

DISCUSSION

Survival Rate

Survival rate is one of the most important biological indicators for evaluating the tolerance limits and physiological adaptation of Nile tilapia (*Oreochromis niloticus*) to different salinity levels. Based on the experimental results, survival rate tended to decline with increasing salinity, although the statistical analysis indicated that the differences among treatments were not significant. The highest survival rate (100%) was recorded in the control (0 ppt) and 5 ppt treatments, followed by a gradual decline at 10 and 15 ppt, with the lowest survival observed at the highest salinity level (20 ppt).

The reduction in survival at higher salinity levels (particularly 15–20 ppt) was consistent with the deterioration of hematological parameters observed in this study. Under hyperosmotic conditions, fish experience a substantial increase in metabolic energy demand because a greater proportion of energy is allocated to ion transport mechanisms required for osmoregulation. This energy trade-off reduces the energy available for growth, tissue maintenance, and immune defense. Furthermore, chronic hyperglycemia accompanied by marked reductions in erythrocyte count and hemoglobin concentration may impair oxygen transport to tissues, resulting in tissue hypoxia, immunosuppression, and, under prolonged stress, dysfunction of vital organs that ultimately increases mortality. According to Dawood *et al.* (2020), exposure to excessively high salinity may damage the integrity of the gill epithelium, induce tissue necrosis, and disrupt acid–base regulation in euryhaline fish. When salinity exceeds the physiological tolerance of Nile tilapia, the cumulative osmotic stress surpasses the fish's adaptive capacity, leading to reduced survival. Similarly, Rebouças *et al.* (2018) reported that mortality in Nile tilapia exposed to acute saline conditions is primarily associated with circulatory failure and cellular dehydration caused by excessive osmotic stress.

The variation in survival rate among treatments demonstrates that salinity is an important environmental factor influencing the successful adaptation of Nile tilapia. At salinity levels within the species' tolerance range, fish are able to acclimate effectively and maintain normal physiological functions, thereby sustaining high survival rates. In contrast, salinity levels exceeding the adaptive capacity of the fish impose a greater osmoregulatory burden, induce prolonged physiological stress, and increase the risk of mortality. These findings support those of Royan *et al.* (2014), who reported that successful physiological adaptation to saline environments is a key determinant of survival in Nile tilapia.

Erythrocyte Count

The results of this study demonstrated that the erythrocyte count of Nile tilapia (*Oreochromis niloticus*) decreased significantly with increasing salinity of the culture medium.

In the control treatment (0 ppt), the mean erythrocyte count was the highest, reaching $9.01 \pm 0.27 \times 10^6$ cells/ μL . This value declined markedly at 5 ppt ($5.89 \pm 0.35 \times 10^6$ cells/ μL) and 10 ppt ($4.83 \pm 1.37 \times 10^6$ cells/ μL), reaching its lowest value at 20 ppt ($1.38 \pm 0.12 \times 10^6$ cells/ μL), which was not significantly different from the 15 ppt treatment ($2.20 \pm 0.19 \times 10^6$ cells/ μL).

The decline in erythrocyte count can be attributed to the hyperosmotic conditions of the rearing environment, which promote the movement of intracellular water from erythrocytes into the surrounding medium through exosmosis. As a consequence, red blood cells undergo shrinkage (crenation) and become more susceptible to premature structural damage and hemolysis. In addition, severe osmotic stress stimulates the production of reactive oxygen species (ROS), leading to lipid peroxidation of erythrocyte membranes and accelerating erythrocyte destruction. According to Kestin *et al.* (2020), acute osmoregulatory failure under saline conditions may suppress the activity of hematopoietic organs, particularly the anterior kidney and spleen, thereby reducing erythropoiesis and resulting in a significant decline in the number of circulating mature erythrocytes.

The present findings are consistent with those reported by Royan *et al.* (2014), who demonstrated that changes in salinity alter the blood profile of Nile tilapia as a physiological response to osmotic stress. At elevated salinity levels, disruption of osmoregulatory balance induces changes in erythrocyte count as part of the physiological mechanism to maintain oxygen transport. However, when osmotic stress persists beyond the adaptive capacity of the fish, the rate of erythrocyte production is no longer sufficient to compensate for the increased destruction of red blood cells, resulting in a progressive decline in erythrocyte count.

Leukocyte Count

The leukocyte count increased significantly with increasing salinity of the culture medium. The lowest leukocyte count was observed in the control treatment (0 ppt), with a mean value of $1.28 \pm 0.65 \times 10^3$ cells/ μL . The count increased progressively at 5 ppt ($2.12 \pm 0.07 \times 10^3$ cells/ μL), 10 ppt ($2.31 \pm 0.49 \times 10^3$ cells/ μL), 15 ppt ($2.76 \pm 0.02 \times 10^3$ cells/ μL), and reached the highest value at 20 ppt ($3.55 \pm 0.52 \times 10^3$ cells/ μL). This increase in leukocyte count represents an innate immune response of Nile tilapia to environmental stress caused by elevated salinity. Exposure to hyperosmotic conditions activates the hypothalamic–pituitary–interrenal (HPI) axis, stimulating the release of cortisol into the bloodstream. Acute elevations in cortisol promote the mobilization of leukocytes from lymphoid tissues into the circulation, preparing the fish to respond to cellular damage induced by osmotic stress. According to Tort (2021), the increase in leukocyte count is primarily associated with the proliferation and recruitment of neutrophils and monocytes, which actively remove damaged cells and cellular debris through phagocytosis following osmotic injury.

The elevated leukocyte count observed in this study indicates that changes in salinity activated the immune system as part of the physiological response to environmental stress. Leukocytes are key components of the innate immune system and play essential roles in recognizing, phagocytosing, and eliminating foreign particles as well as damaged cells. As salinity-induced osmotic stress increased, the immune response intensified, as reflected by the higher leukocyte counts, to help maintain physiological homeostasis. However, once the fish successfully acclimated to the new environmental conditions, the stress response gradually subsided, allowing physiological functions and hematological parameters to stabilize. Therefore, leukocyte count can be considered a reliable hematological indicator of environmental stress and immune activation in Nile tilapia exposed to salinity changes, consistent with the findings of Royan *et al.* (2014).

Hematocrit

A contrasting response was observed in hematocrit values compared with erythrocyte count and hemoglobin concentration. While erythrocyte count and hemoglobin decreased with increasing salinity, hematocrit values exhibited a significant upward trend. The lowest hematocrit value was recorded in the control treatment (0 ppt), at $4.03 \pm 3.26\%$, and increased to $6.64 \pm 2.50\%$ at 5 ppt and $6.08 \pm 1.61\%$ at 10 ppt. The highest hematocrit value was observed at 15 ppt ($9.39 \pm 1.71\%$), followed by a slight decrease at 20 ppt ($8.85 \pm 2.57\%$).

The increase in hematocrit despite the decline in erythrocyte count may be attributed to physiological adjustments induced by osmotic stress. Exposure to hyperosmotic conditions can alter erythrocyte volume through osmoregulatory mechanisms, while simultaneous plasma water loss results in hemoconcentration. Consequently, although the total number of circulating erythrocytes decreases, the proportion of packed cell volume relative to total blood volume may increase. Ndong *et al.* (2015) reported that elevated hematocrit values accompanied by reduced erythrocyte counts are indicative of acute extracellular dehydration in fish exposed to osmotic shock. In addition, osmotic stress may temporarily alter erythrocyte cell volume through ion transport processes involved in maintaining intracellular osmotic balance.

The increase in hematocrit observed in the present study indicates that elevated salinity altered the physiological characteristics of the blood as a consequence of osmotic imbalance. Because hematocrit represents the proportion of erythrocyte volume relative to total blood volume, it is influenced not only by erythrocyte abundance but also by changes in plasma volume. Under osmotic stress, dehydration and plasma volume reduction can increase hematocrit values even when erythrocyte numbers decline. As the fish gradually acclimate to the saline environment, physiological homeostasis may be restored, resulting in stabilization of hematocrit values. However, when salinity exceeds the adaptive capacity of the fish or osmotic stress persists for an extended period, hematocrit alterations remain evident as part of the physiological response to environmental stress, consistent with the findings of Royan *et al.* (2014).

Hemoglobin

The hemoglobin (Hb) concentration of Nile tilapia decreased progressively with increasing salinity, exhibiting a clear linear decline across the treatments. The highest hemoglobin concentration was recorded in the control treatment (0 ppt), with a mean value of 8.50 ± 0.46 g/dL, followed by 7.77 ± 0.21 g/dL at 5 ppt, 7.27 ± 0.25 g/dL at 10 ppt, 6.83 ± 0.35 g/dL at 15 ppt, and the lowest value of 6.17 ± 0.21 g/dL at 20 ppt. This progressive decline indicates that increasing salinity adversely affected the oxygen-carrying capacity of the blood.

Physiologically, hemoglobin is contained within erythrocytes; therefore, the reduction in erythrocyte count caused by osmotic stress directly contributes to a decrease in hemoglobin concentration. Exposure to elevated salinity imposes osmotic stress that may damage erythrocyte membranes, shorten erythrocyte lifespan, and increase erythrocyte destruction, thereby reducing the total hemoglobin available for oxygen transport. In addition, osmotic stress activates the Hypothalamic–Pituitary–Interrenal (HPI) axis, resulting in increased cortisol secretion. Prolonged elevation of cortisol has been reported to interfere with normal hematopoiesis and may impair hemoglobin synthesis, further reducing the oxygen-carrying capacity of the blood. According to Fazio *et al.* (2018), diminished hemoglobin concentration decreases oxygen delivery to metabolically active tissues, potentially leading to tissue hypoxia and compensatory increases in respiratory activity to satisfy the oxygen demand of the fish under hyperosmotic conditions.

The present findings indicate that changes in salinity induced physiological disturbances in Nile tilapia, as reflected by alterations in hemoglobin concentration. Because hemoglobin is closely associated with erythrocyte abundance, a reduction in erythrocyte count is generally accompanied by a decline in hemoglobin concentration. During successful acclimation, hematological parameters may gradually recover as physiological homeostasis is restored. However, when salinity exceeds the adaptive capacity of the fish, hemoglobin concentration continues to decline, reducing the efficiency of oxygen transport to body tissues. This impairment may disrupt metabolic processes and compromise the overall physiological performance of the fish, consistent with the findings of Royan *et al.* (2014).

Blood Glucose

Blood glucose concentration exhibited a consistent and significant increase with increasing salinity. The lowest glucose concentration was recorded in the control treatment (0 ppt), with a mean value of 63.33 ± 12.22 mg/dL. Blood glucose increased progressively to 81.00 ± 4.58 mg/dL at 5 ppt, 92.00 ± 6.24 mg/dL at 10 ppt, 102.00 ± 9.54 mg/dL at 15 ppt, and reached the highest concentration of 119.33 ± 3.06 mg/dL at 20 ppt. This progressive increase indicates that elevated salinity induced a marked physiological stress response in Nile tilapia.

Hyperglycemia is widely recognized as one of the primary physiological indicators of stress in fish. Exposure to hyperosmotic environments increases the energetic demands of osmoregulation, particularly through enhanced activity of ion transport systems such as the Na^+/K^+ -ATPase pump in the gill epithelium, which actively maintains ionic and osmotic balance. To satisfy these increased energy requirements, activation of the hypothalamic–pituitary–interrenal (HPI) axis stimulates the release of cortisol and catecholamines, promoting glycogenolysis and gluconeogenesis in the liver. These metabolic processes increase circulating glucose levels, providing readily available energy to support osmoregulatory functions. Bonga (2016) reported that elevated blood glucose under saline conditions reflects an energy trade-off, in which metabolic energy that would normally support growth and other physiological processes is redirected toward maintaining osmotic homeostasis and survival.

The elevated blood glucose concentrations observed in this study demonstrate that increasing salinity triggered a substantial metabolic response in Nile tilapia. The rise in blood glucose indicates the mobilization of energy reserves required to maintain internal homeostasis under osmotic stress. During successful acclimation, the physiological stress response gradually diminishes, allowing blood glucose levels to return toward baseline values. However, persistent exposure to salinity beyond the adaptive capacity of the fish may sustain elevated glucose concentrations, reflecting prolonged physiological stress. Therefore, blood glucose concentration can be considered a sensitive indicator of stress and metabolic adaptation in Nile tilapia exposed to changes in environmental salinity, consistent with the findings of Royan *et al.* (2014).

CONCLUSION

Based on the statistical analysis and discussion of the effects of different salinity levels on the blood profile and physiological adaptation of Nile tilapia (*Oreochromis niloticus*), it can be concluded that increasing salinity significantly affected the blood profile and physiological responses of the fish. As salinity increased, erythrocyte count and hemoglobin concentration decreased, whereas leukocyte count, blood glucose concentration, and hematocrit values increased as physiological responses to osmotic stress. In addition, survival rate tended to

decline with increasing salinity, with the lowest survival rate of 73.33% recorded at the highest salinity treatment. Overall, elevated salinity imposed physiological stress that adversely affected the health and adaptive capacity of Nile tilapia. Therefore, salinity levels of ≤ 10 ppt are recommended for the culture of Nile tilapia to maintain optimal physiological performance and survival.

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