Assessment of food quality and antibiotic residue toxicity in *Florida red* tilapia *Oreochromis sp* exposed to erythromycin

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ABSTRACT

Erythromycin (ERY) is widely used as an antibiotic, leading to its pervasive presence in the environment and potential risks to non-target species. This study investigated the under sub-lethal effects of ERY on Florida red tilapia *(Oreochromis sp.)* by examining changes in their hematological and biochemical parameters. Over 28 days, fish were exposed to a range of ERY concentrations (0.3, 1.7, 10, 60, and 300 µg/L), with the control group at 0.00 µg/L.

This study demonstrated that exposure to low environmental concentrations and legally permissible aquaculture doses of antibiotics (ERY) (under sub-lethal concentration) can result in blood effects in *Oreochromis sp*. An exploratory principal component analysis (PCA) was conducted to simplify the data and highlight the most influential factors. The first two principal components accounted for 80.9 % of the variance, indicating strong dataset representation. Subsequent analysis of variance (ANOVA) revealed significant dose-dependent variations in the hematological and biochemical profiles, linking specific bioassays (Aq1 through Aq5) to particular changes in the fish's physiology. These findings underscore the importance of monitoring antibiotic levels in aquatic environments to safeguard the health of aquatic organisms.

Keywords: Erythromycin, hematology, *Oreochromis sp.*, pathologies, toxicology.

1. Introduction

Given the challenges presented by increasing urbanization and industrial expansion, environmental pollution has become an increasingly urgent global concern, posing a threat to the integrity of ecosystems and human health (Nasroallahi *et al.,* 2022). Furthermore, agricultural runoff, a multitude of pollutants saturates our air, water, and soil, inflicting adverse effects on biodiversity and ecological stability (Iftikhar & Hashmi, 2021; Nasroallahi *et al.,* 2022). Among the complex and multifaceted issues of environmental contamination, pharmaceutical pollutants have emerged as a critical concern due to their pervasive presence in aquatic ecosystems and their potentially harmful effects. This study aims to highlight the urgent need to address pharmaceutical pollution within the broader context of environmental degradation. Pharmaceuticals are complex, carbon-based compounds with diverse physicochemical and therapeutic properties, which complicate their behavior and impact once released into the environment (Iftikhar & Hashmi, 2021). By examining the intricate relationships between pharmaceutical contaminants and other environmental challenges, this research seeks to emphasize the growing importance of tackling pharmaceutical pollution as an integral part of efforts to mitigate overall environmental damage.

These pharmaceutical compounds (ATBS) ultimately become part of the environment via various point sources such as manufacturing facilities, hospitals, agricultural and land runoff, household use, and improper disposal (Rosner *et al.,* 2024). Antibiotics are biologically active compounds that may interact with specific biological systems or generically act all over the body depending on their chemical properties (Zheng *et al.,* 2021). Among all pharmaceutical classes, antibiotics have the highest consumption rate (Castillo-Zacarias *et al.,* 2021).

Owing to the high demand for food security, the aquaculture sector has grown very rapidly worldwide. In the recent decade, antibiotics have attracted significant public attention as a prevalent pollutant and their potential risk to human health and the ecological environment. Antibiotics play a key role in inhibiting infectious diseases in the aquaculture industry. For the last decade, it has been calculated that 200,000 tons of antibiotics have been used worldwide (Tu *et al.,* 2024). Due to their

high consumption, increasing detection of pharmaceuticals has been found largely around the world, including North America, China, the UK, and Europe (González-González *et al.*, 2022). Among the total antimicrobials employed, sulfur drugs occupy 6%. Among them, macrolide groups are widely used as veterinary antibiotics (Zhang *et al*., 2023).

Compared to other antibiotics, Erythromycin (ERY) has been identified as an antibiotic of particular concern for the aquatic compartment due to its consumption, discharge, persistence, and toxic properties (Rodrigues *et al*., 2016). Some studies have shown that ERY can cause deleterious effects on non-target organisms; among aquatic organisms, it seems that cyanobacteria are the most sensitive to the direct toxicity of ERY (Li *et al*., 2020). Until recently, antibiotics were not considered a significant ecological risk to fish species (Ispir *et al*., 2023).

However, scarce data exist on sub-lethal effects (e.g., genotoxicity, hematology, and oxidative stress) resulting from the biochemical action of antibiotics (Gabriel *et al.*, 2022). Therefore, this study aims to provide novel insights into the direct physiological and behavioral responses of tilapia to erythromycin exposure to legal aquaculture doses of (Ery) elucidating the potential risks posed by pharmaceutical pollutants in aquatic ecosystems (Gill *et al.*, 1991; Hasimuna *et al.*, 2021).

The primary objective of this research is to assess the effects of ERY on the hematological parameters of *Oreochromis sp.* Specifically, the main contributions of this study are:

- To evaluate the effect of legal aquaculture doses of Erythromycin (ERY) on hematological and biochemical parameters in Florida red tilapia *(Oreochromis sp.)* exposed to varying concentrations over 28 days.
- To identify key patterns and significant differences in the distribution of physiological profiles, elucidating potential associations between specific bioassays and distinct parameters.
- To contribute to the broader understanding of the physiological responses of *Florida red* tilapia to ERY exposure, providing insights into the environmental risks associated with this commonly used antibiotic in aquatic ecosystems.

2. Materials and methods

2.1. Chemicals and Acute exposure

For each assay, a stock solution was obtained by dissolving ERY (Sigma Aldrich - CAS: 114-07-8) in saltwater. Test solutions were prepared by successive dilution of the stock solutions (acute exposure - 29 mg/L and chronic exposure - 1 mg/L), immediately before the beginning of the assay and at each renewal of the exposure medium, as described by (Rodrigues *et al.*, 2016). The Bradford test reagent was purchased from Bio-Rad UK. (Perveen *et al.*, 2019). (Table 1).

2.2 Test organisms

Florida red tilapias *(Oreochromis sp.)* is a farmed fish that plays a major role in the economics of Mediterranean countries and, according to the Federation of European Aquaculture Producers, it is the most farmed species in the Mediterranean region, with an estimated annual production of 129,000 tons. *Florida red* tilapia *(Oreochromis sp.)* has a total weight (Wt) which varies (19≤Wt (g)≤89) with a length varies (10.5 ≤Lt(cm)≤16.4) was investigated using a static bioassay system for 28 days. The sub-lethal concentrations used were (0.3, 1.7, 10, 60, and 300 µg/L) and 0.00(control) mg.L-1 (Table I). The conditions for keeping aquatic organisms were completely favorable, maintaining the density of fish stocking and the conditions of the hydrochemical regime. All work was carried out in strict compliance with the provisions of the framework of the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, 1986); International Principles of the Declaration of Principles of Tolerance (28th session of UNESCO, 1995), the Rules of Conducting Studies using Experimental Animals, according to the Order of the Ministry of Healthcare of Ukraine No. 281 as of 1 November 2000, The Measures of Further Improvement of Organizational Forms of Study using Experimental Animals and according to the Law of Ukraine On the Protection of Animals from Abuse (No.3447-IV as of 02/21/2006, Kyiv). The research and procedures on the breeding of *Nile tilapia* were carried out in accordance with the guidelines of the Ethics Committee on Animal Experimentation

(CETEA) Decree No. 2013-118 of February 1, 2013 relating to the protection of animals used for scientists. from the Pasteur Institute of Algeria.

2.3 Water quality control

For test validation purposes, and according to both adopted OECD guidelines (1992, 2000), water quality parameters (pH, temperature (T°), dissolved oxygen (DO), and electrical conductivity (E.C)) during the exposure assays were monitored every 48 h (Table 2). During exposures, no mortality was recorded in the control group complying with the OECD guidelines (1992, 2000) requirements: mortality < 10%.

2.4 Biochemical analysis

Biochemical analysis, including glucose (GLU), triglyceride (TG), and cholesterol (TCHO), was selected for the present study of fish blood samples. The analysis was conducted according to the method stated by Perveen *et al.* (2019) to assess the toxic impacts of antibiotics. For the biochemical analysis, blood samples were withdrawn using a syringe and collected in tubes with gel activators for the preparation of blood serum.

To produce serum, the samples were centrifuged at 4,000 rpm for 10–20 minutes and then analyzed using an AMP Picco II Chemistry analyzer. Before the commencement of hematological analysis, blood samples were centrifuged on Platform shaker LABCON-SPo-MP3 for 10–15 min at 300 rpm to avoid formation of any clots.

2.5 Hematological parameters

To determine the effect of applied doses on hematological parameters of exposed fish blood samples, a test was carried out for 28 days of exposure duration and weekly blood samples were collected Sample preparation and analysis was done according to methodology stated by Perveen *et al.* (2019). The blood was collected through cardiac puncture from the caudal vein below the dorsal fins using a 5 mL heparinized syringe. The blood was collected in sterile purple topped EDTA vials containing anticoagulant. After collecting blood, the vials were gently shaken by hand to dissolve anticoagulant agent properly. Finally, red blood cells (RBCs), white blood cells (WBCs),

hemoglobin (Hb) count, lymphocyte (LYM) and platelets (PLT) were measured using Sysmex blood analyzer XP-100. using the reference keys of Hoffman (2000).

2.6 Residue analysis

Residue analysis is an essential method in science, aimed at evaluating the remains or traces left by a process or reaction. This analysis helps detect undesirable substances, contaminants, or impurities in a sample, providing crucial information for product quality and safety. Several methods of residue analysis are used in the literature, but we will place particular emphasis on the UV-visible spectrophotometer method due to its simplicity, sensitivity, speed, relatively low instrument cost, precision and selectivity. Considering these advantages, this method can be used for routine quality control of drug in fish samples.

This method relies on measuring the absorption of light by chemical components in the ultraviolet and visible range. Molecules present in a sample absorb light at specific wavelengths, enabling the identification and quantification of substances present (Zhou *et al.,* 2015).

In our study, the residues of erythromycin were quantified in the flesh of the bioassays for the five concentrations at variable wavelength set at 215 nm according to ml of each sample was measured in duplicate to obtain the average absorbance of the sample and the reference standard. The concentrations of erythromycin residues in the samples were calculated from the linear equation obtained from a calibration curve. Furthermore, according to the results reported in the literature, the maximum wavelength value of the UV-Visible spectrophotometer has been set at 215 nm for Erythromycin. Finally, in residue analysis, ensuring the substance against its known concentrations.

This approach allows for the establishment of a linear relationship between concentration and absorbance, facilitating precise quantification of residue levels in samples, and emphasizing the adherence to stringent quality standards required in residue analysis.

2.7 Statistical analysis

Firstly, a descriptive analysis of the population was conducted: qualitative variables were described with their frequencies and percentages, and quantitative variables with their means and standard

deviations or median and quartiles. Significant difference was defined by using the criterion, $p < 0.05$ as significance level. To address the main objectives of this study, an in-depth statistical analysis was conducted to assess the under sub-lethal effects of erythromycin antibiotics on the hematological parameters of *Florida red* tilapia *"Oreochromis sp."* The statistical analysis primarily focused on two crucial aspects: Principal Component Analysis (PCA) and Analysis of Variance (ANOVA).

PCA was used as a pivotal tool for a descriptive exploration of the data. Initially, emphasis was placed on visualizing the correlation matrix. This preliminary step highlighted the relationships between in this process involves verifying the linearity of the various accuracy and reliability of quantitative measurements is paramount. One essential step analytical method through adherence to Beer's law. Specifically, for compounds like erythromycin, it is imperative to construct a calibration curve by plotting the absorbance of various hematological variables, providing an initial understanding of underlying data structures.

This preliminary step highlighted the relationships between various hematological variables, providing an initial understanding of underlying data structures. For quantitative measurements, verifying the linearity, accuracy, and reliability is paramount. One essential step in this process is the validation of the analytical method through adherence to Beer's law. Specifically, for compounds like erythromycin, it is imperative to construct a calibration curve by plotting the absorbance of standard solutions against their known concentrations. This step ensures the linearity of the response and the validity of the method within the working range of concentrations.

The objectives of PCA were twofold:

Descriptive - Exploratory: Data visualization through simple graphs allowed for an initial exploration of trends and patterns in hematological data. This facilitated the visual identification of any clustering or emerging trends.

Synthesis - Summary of Large Tables: PCA results were utilized to summarize large individual × variable tables, thus simplifying the complexity of the data. This synthesis provided a concise yet informative representation of relationships between individuals and variables.

Following PCA, a One-Factor Analysis of Variance (ANOVA) was applied to the first two factorial axes. This approach statistically assessed significant differences between erythromycin concentration groups.

The major benefit of combining PCA with ANOVA lies in the reduction of data dimensionality.

PCA had previously extracted the most significant information from the initial data, allowing for a more efficient ANOVA. This reduced dimensional approach facilitated the interpretation of ANOVA results and strengthened the reliability of conclusions drawn from the analysis.

3. Results and Discussion

The examination of hematological responses in *Florida red* tilapia exposed to low concentrations of Erythromycin antibiotics has yielded a wealth of insights, shedding light on the intricate interplay between environmental conditions and physiological outcomes. This section presents a comprehensive analysis structured around three key aspects. Firstly, a meticulous exploration of the physicochemical parameters within the experimental tank sets the stage, establishing the baseline environmental context crucial for interpreting hematological variations. Following this, a detailed descriptive analysis delves into the central tendencies and variations of hematological data, offering a holistic view of the observed effects. Subsequently, we venture into the realm of statistical analyses, unraveling complex relationships through correlation matrices, Principal Component Analysis (PCA), and Analysis of Variance (ANOVA). This trifecta of analyses provides a nuanced understanding of the hematological responses, adding depth to our exploration and paving the way for insightful discussions on the impact of Erythromycin on *Florida red* tilapia.

3.1. Physicochemical parameters of the experimental tank

In this sub-section, the physicochemical parameters of the experimental tank are examined to establish the foundational environmental context necessary for the interpretation of the observed

hematological responses. Understanding the environmental conditions is paramount to comprehend the nuances of the hematological responses observed in *Florida red* tilapia under the influence of the lowest concentrations of Erythromycin antibiotics. By scrutinizing these parameters, we aim to establish a baseline for the experimental setting, providing context for the subsequent hematological assessments. The water quality of experimental tanks was determined by investigating different parameters as prescribed by OECD.

The pH levels varied within the range of (6.89 ± 0.34), with the highest recorded pH observed in the test tank containing the highest concentration of Erythromycin (300 µg/l), while the lowest pH value was recorded in the control tank (0 mg/l).

The dissolved oxygen (DO) content exhibited a decreasing trend with increasing Erythromycin concentration, ranging from 7.3 to 13.2 mg/l, whereas the control tank recorded a DO value of 9.2 mg/l. Furthermore, the temperature remained constant across all tanks, registering at 22.68°C, including the control tank. Nonetheless, minor variations were observed. A one-way ANOVA was conducted for pH, temperature (T°), DO, and electrical conductivity (EC), revealing a non-significant difference ($p > 0.05$). These findings align with the guidelines outlined in the OECD Guideline (2000) for Testing Chemicals 203, ensuring compliance with fish acute toxicity test standards. The summarized results of water quality parameters are presented in (Table 2).

3.1.2. Correlation Matrix, PCA, and ANOVA Results

This sub-section ventures into the intricate realm of statistical analyses, unraveling the relationships between variables through correlation matrices, Principal Component Analysis (PCA), and Analysis of Variance (ANOVA). These multivariate approaches are instrumental in discerning underlying patterns, reducing data dimensionality, and assessing significant differences between antibiotic concentration groups.

3.1.3. Correlation Matrix Analysis

The correlation table (Table 3) reveals significant associations involving the variable WBC and several other variables. Particularly, very strong relationships were observed with the variables

RBC, Hb, and PLT, showcasing remarkably high correlation coefficients of 0.952, 0.934, and 0.892, respectively. These findings suggest a strong dependence between these variables, indicating that fluctuations in GB are closely linked to variations in RBC, Hb, and PLT.

In contrast, the variable WBC exhibits a moderate correlation with the variable GLU, illustrated by a correlation coefficient of 0.543. This less pronounced Assessment of Food Quality and Antibiotic Residue Toxicity relationship suggests a relatively weaker dependence between WBC and GLU, highlighting the diversity of connections within the dataset.

In addition to these correlations, further insights are gained by examining specific pairs of variables. The relationships between (RBC and Hb) and (RBC and PLT) were of very good quality, with correlation coefficients of 0.957 and 0.942, respectively, and positive signs indicating increasing trends. Similarly, the relationships between (LYM and Hb) and (LYM and PLT) are of good quality, with correlation coefficients of -0.621 and -0.699, respectively, both showing negative signs, suggesting decreasing trends.

Furthermore, the relationship between (TG and TCHO) has a very strong correlation with a coefficient of -0.753, again indicating a negative correlation, implying a decreasing nature of this association. These additional insights contribute to a more comprehensive understanding of the interdependencies within the dataset, highlighting both strong and moderate correlations and their directional trends.

3.1.4. Principal Component Analysis (PCA)

After gaining valuable insights from the correlation analysis, we turn our attention to Principal Component Analysis (PCA) to offers a holistic approach to understanding the overall structure and variance in our multidimensional data.

The variables with high correlations may contribute significantly to certain principal components, reflecting their joint influence on the overall variation in the dataset. On the other hand, variables with weaker or negligible correlations may have a more limited impact on specific principal components.

3.1.5 Total variance of the data

The table (4) indicates the proportion of variance in the original variables accounted for by each principal component, helping to understand their contributions to the overall variability in the dataset. In Table 4, the first principal component (Factorial Axis 1) accounts for 52.467 % of the total variance, while the second principal component (Factorial Axis 2) accounts for 28.467 %. Together, the factorial plane defined by axes 1 and 2 captures 80.934% of the overall variance. This high percentage indicates that our interpretations and results derived from this analysis are highly reliable and allow us to draw meaningful conclusions from these principal components. The dominance of Factorial Axis 1 suggests that it represents the primary dimension of variability in the data, likely linked to the most influential variables in the dataset. The significant contribution of Factorial Axis 2 further complements this, capturing secondary but meaningful variability. Together, these components provide a compressed yet comprehensive view of the dataset, ensuring key patterns and relationships are preserved and facilitating accurate interpretations.

3.1.6 Study of the measured variables

For a more interpretable and meaningful representation of the data, the Matrix of components after rotations is determined and shown in (Table 4).

In (Table 5), we can observe the contributions of each variable on the factorial axis. We notice that the set of variables (WBC, RBC, Hb, and PLT) contributes to axis 1, while the set of variables (CHOL, TG) contributes to axis 2. The most significant influence (or contribution) on the results comes from the variable Hb, followed by the variable PLT.

Moreover, in (Figure 1), it is noticeable that the variables (RBC, PLT, Hb, WBC) exhibit concurrent trends, demonstrating mutual increases or decreases in tandem, while showing an opposite relationship with LYM. Additionally, there is an opposite relationship observed between the variables TCHO and TG confirming the results of (Table 4).

In summary, variables such as white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), and platelets (PLT) significantly influence the first component, while cholesterol (CHOL) and triglycerides (TG) are more relevant to the second component.

This means that changes in these blood parameters can greatly affect the overall patterns we observe in our study.

3.1.7 Analysis of individuals (Doses)

The hierarchical tree analysis dendrogram in (Figure 2), based on the average distance between classes, aims to establish homogeneity among doses. Observing the tree in Figure 2, it becomes apparent that doses (Aq4, Aq3, Aq5, Aq2) exhibit homogeneity and have a similar effect on the measurable parameters. Among these doses, Aqtest stands out as the most influential, while Aq2 has the least pronounced effect. The hierarchical tree analysis provides valuable insights into the relative impact of different doses on the studied parameters, guiding our understanding of the experimental outcomes.

Moreover, the scatterplot (Figure 3) shows the parameter groupings:

LYM Grouping (Group 1):Aq4, Aq3, Aq2, and Aq5 are grouped together according to their similarity through LYM parameter. RBC, PLT, Hb, and WBC Grouping (Group 2):Aq1 and Aqtest share similarities according to these parameters and were grouped together. Moreover, it is seen that Group 1 and Group 2 share similarities according to TCHO and TG.

This analysis reveals distinct patterns and associations among the doses, providing insights into how certain parameters contribute to the observed variations. The identified groupings help in understanding the relationships and effects of different doses on the measured parameters. All the previous interpretations are entirely meaningful with a rate of 80 %.

In summary, this analysis reveals distinct patterns and associations among the doses, providing insights into how certain parameters contribute to the observed variations. Doses Aq4, Aq3, Aq5, and Aq2 are closely related, suggesting they have similar effects on the measured outcomes.

Among all doses tested, Aqtest was found to have the most substantial impact on results, while Aq2 showed a lesser effect.

The identified groupings help in understanding the relationships and effects of different doses on the measured parameters. All the previous interpretations are entirely meaningful with a rate of 80 %.

3.1.8 ANOVA Results

A comprehensive analysis is currently being conducted to assess the influence of the dose factor on Hb and PLT parameters. Before applying the one-way ANOVA test, it was imperative to ensure that the prerequisites for its use were met. Two crucial conditions were the normality of measurable quantitative variables and homogeneity of variance. These conditions were verified using the Shapiro-Wilk test for normality and the Levene test for variance homogeneity. Focusing specifically on the influence of the dose factor on the variance of the Hb and PLT parameters, the analyses revealed significant findings. Regarding the PLT parameter, the Dose factor demonstrated a statistically significant influence on its variance, at a significance level of 100%.

This suggests that the various doses applied have a significant impact on the observed variation in platelet levels see (Table 6) ANOVA, Signification column for V2PLT). Furthermore, examining the Hb parameter, the Dose factor also showed a significant influence on its variance, at a significance level of 97.8%. This finding indicates that the different dose levels applied have a notable effect on the variation in hemoglobin levels see (Table 5) ANOVA, Signification column for (V1Hb).

Consequently, it is reasonable to conclude that the specific modalities (Aq1, Aq2, Aqtest) of the Dose factor (or Aquarium) do not have a uniform effect on the Hb and PLT parameters. These results underscore the importance of considering the different dose levels in interpreting the observed variations in the measured blood parameters. This discussion reinforces the validity of the conclusions drawn from the one-way ANOVA analysis and provides significant insights into how the Dose factor influences the studied blood parameters. So, the one-way ANOVA

results provide critical insights into how different doses affect hemoglobin (Hb) and platelet (PLT) levels:

1. **Significance of Dose Factor**: The significant p-values (0.022 for Hb and <0.001 for PLT) indicate that different doses have a statistically significant impact on these parameters. This suggests that varying dosages can lead to markedly different outcomes in blood health indicators, reinforcing the importance of dose selection in experimental designs.
2. **Implications of Variability**: The findings imply that not all doses exert uniform effects; rather, specific doses (Aq1 and Aq6) show pronounced impacts compared to intermediate doses (Aq2, Aq3, Aq4, Aq5). This variability emphasizes the need for careful consideration when interpreting results related to dose-response relationships.

In addition to the one-way ANOVA analysis, pairwise comparisons were conducted using the Tukey test to further elucidate the specific influences of each dose level on the parameters PLT and Hb. This approach allows for a detailed examination of the interplay between different dose levels and their effects on the measured blood parameters. The pairwise comparisons revealed that, for doses Aq2, Aq3, Aq4, and Aq5, there was a consistent and similar impact on both the PLT and Hb parameters see (Table 7) and (Table 8), test Tukey for V1Hb and V2PLT respectively). These doses exhibited comparable effects, indicating that their influence on blood parameters is consistent across the two parameters under consideration. Contrastingly, the doses Aq1 and Aq6 demonstrated the most significant effects on both PLT and Hb parameters. The pairwise comparisons suggest that these doses exhibit a distinct influence compared to the intermediate doses in (Aq2, Aq3, Aq4, and Aq5). Specifically, doses in Aq1 and Aq6 show cased the most pronounced effects, signifying their superior impact on the observed variations in both PLT and Hb levels.

3.1.9 Residue analysis results

First, the linearity of the method was verified at five points within the calibration range, spanning concentrations from 0.3 to 300 µg/L of erythromycin. The results are presented in (Table 7) and (Figure 4).

According to these, the data perfectly adheres to Beer's law within this concentration range. Specifically, a linear correlation was observed between the absorbance of erythromycin and its concentration at the maximum wavelength, yielding a correlation coefficient greater than 0.999 and a linear equation of $y = 0.0100x + 0.2659$, where y represents absorbance values and x represents concentration values in µg/L. The protocol's validation was further confirmed through a recovery experiment, which determined the percentage recovery (yield, ρ).

Three replicates of erythromycin-free fish spiked with different concentrations of standard erythromycin were subjected to extraction procedures and measured by our method. As seen in (Table 8) and (Figure 5), the results obtained by the proposed method are consistent with the examined quantities and fall within the limits allowed by the AFSSA for the quality of animal-origin food products. Recovery rates within the range of 09 to 140% were obtained, indicating that the developed method is suitable for the determination of erythromycin in fish samples without any matrix interference.

Discussion

In the present study, valuable insights into the complex interplay between environmental conditions and physiological outcomes were provided by the examination of hematological responses in Red Tilapia exposed to five distinct nominal concentrations of erythromycin antibiotics. This comprehensive analysis encompasses three critical aspects: the scrutiny of physicochemical parameters in the experimental tank, a detailed descriptive analysis of hematological data, and the unraveling of complex relationships through correlation matrices, Principal Component Analysis (PCA), and Analysis of Variance (ANOVA). The obtained results were compared with existing literature to provide a comprehensive understanding of the potential effects of ERY on fish health. The discussion focused on key hematological parameters, including Hb, RBC, WBC, PLT, and LYM.

First, the physicochemical parameters examined within the experimental tank included pH, temperature, dissolved oxygen (DO), and electrical conductivity (E.C). The consistent temperature

across all tanks, along with non-significant differences in pH, DO, and E.C, ensured compliance with fish acute toxicity test standards. The established baseline provides context for interpreting subsequent hematological assessments. Biochemical parameters (glucose, cholesterol, and triglycerides) across different aquariums and Erythromycin concentrations. The diversity in parameter responses emphasizes the nuanced impact of Erythromycin on various aspects of *Red Tilapia* physiology.

- The study revealed a significant decrease in Hemoglobin (Hb) levels in fish treated with high doses of ERY (Aq5, Aq4, Aq1, and Aq2) compared to Aq3 and control.

These findings align with previous research by Omar (2023), indicating that changes in Hb levels can reflect the oxygen content in the blood. The observed hypochromic microcytic anemia, similar to findings in other studies, emphasizes the sensitivity of Hb as an indicator of environmental stress.

- Variations in Red Blood Cell (RBC) numbers were recorded, with the highest values observed in Aq5 individuals. Similar alterations in RBCs were found in other fish species exposed to antibiotics. The ellipsoidal structure of their RBCs, sensitivity to environmental changes, and the potential effects erythromycin on membrane fragility were discussed. The observed changes may be a compensatory response to antibiotic toxicity.

Alterations in White Blood Cell (WBC) levels were indicative of stress and tissue damage. A significant decrease in WBC levels was observed in fish treated with high doses of ERY (Aq5, Aq4, Aq1, and Aq2). The immune system's crucial role in maintaining physiological processes and responding to pathogens was highlighted. The decrease in blood leukocytes and potential correlations with antibiotic-induced immune responses were discussed. Platelet (PLT) levels exhibited a significant decrease in fish treated with high doses of ERY (Aq5).

The discussion included insights into thrombocytopenia and its potential causes, such as drug-induced immune reactions. The variations in PLT levels and their implications on fish health were underscored. Triglyceride (TG) and cholesterol levels showed fluctuations, with TG levels peaking in Aq2. The potential reasons behind the observed changes, including energy mobilization under

stress, lipid metabolism disturbances, and the lipophilic nature of macrolides affecting cell membranes, were discussed. Liver dysfunction and the inhibition of enzymes converting cholesterol to bile acid were considered as factors contributing to hypercholesterolemia. Second, the subsequent exploration involved complex statistical analyses, starting with a correlation matrix. Significant associations, particularly with variables like RBC, Hb, and PLT, revealed intricate interdependencies. The PCA results provided a holistic understanding of data variability, with a total variance of 80.93 %, indicating the accuracy of interpretations. The outcomes revealed significant associations, particularly emphasizing the variables Hb and PLT, which were identified as having the most substantial influence on the results. Specifically, the variable Hb emerged as the most influential, followed closely by PLT. This hierarchy of influence underscores the pivotal role that these hematological parameters play in capturing the nuanced responses of *Florida Red Tilapia* to low concentrations of Erythromycin antibiotics.

The application of one-way ANOVA to assess the influence of the Dose factor on hematological parameters (Hb and PLT) required verification of normality and variance homogeneity, which were confirmed through appropriate tests. The ANOVA analyses indicated a significant influence of the Dose factor on both PLT and Hb parameters. Pairwise comparisons, employing the Tukey test, revealed consistent effects for doses Aq2, Aq3, Aq4, and Aq5, while doses Aq1 and Aqtest exhibited more pronounced impacts on both parameters.

The findings underscore the intricate relationship between Erythromycin exposure and hematological responses in Red Tilapia. The consistent effects of intermediate doses on blood parameters suggest a dose-dependent impact. Doses Aq1 and Aqtest and out as having the most significant effects, emphasizing the need for a nuanced consideration of different doses in interpreting hematological variations.

These results contribute to the understanding of under sublethal effects of Erythromycin on fish health, highlighting the importance of considering environmental conditions and dose-specific

responses. Hematological parameters are indicators of the water balance, nutritional status and general health of fish (Park *et al*., 2008; Shahjahan *et al.,* 2022).

Therefore, hematological variables have been used as indicators of the health status of fish species in order to detect physiological changes due to stress, such as exposure to pollutants, hypoxia, transport, anesthesia and acclimatization (Park *et al.,* 2008).

The ideal dose should be determined by considering the overall health of the species and minimizing adverse effects on hematological parameters. However, even if the residue analysis results confirm the effectiveness and reliability of the method used for quantifying erythromycin levels in fish samples, trace quantities of erythromycin residues were still detected in the flesh of bioassays, as indicated by the recovery rates within the range of 09 to 140 %. This finding suggests that while the developed method is effective, there may still be residual contamination present in fish samples. Such residual traces of erythromycin could potentially pose risks to consumer health and highlight the need for further investigation into alternative antibacterial treatments. The natural compounds have demonstrated antibacterial properties and may offer safer alternatives to synthetic antibiotics like erythromycin (Sabbobeh *et al.*, 2019; Li *et al.*, 2021).

Conclusions

In conclusion, hematological variables serve as crucial indicators of fish health, reflecting physiological changes induced by various stressors, including exposure to pollutants, hypoxia, transport, anesthesia, and acclimatization. The determination of an ideal dosage should prioritize the overall health of the species while minimizing adverse effects on hematological parameters.

The results of the present study demonstrate significant alterations in respiratory burst activity, hematological profile, and biochemical parameters of *Oreochromis sp.* following chronic exposure to erythromycin at nominal concentrations. These findings provide baseline data on the potential effects of antibiotics on non-target organisms, particularly fish under prolonged exposure. Additionally, the biomarker approach employed in this study may serve to evaluate the risks associated with antibiotics in aquatic environments.

Further exploration into molecular toxicity mechanisms could enhance our understanding of erythromycin's mode of action on organisms. The nuanced effects of different dose levels on blood parameters underscore the importance of identifying doses with similar effects and recognizing superior effects from specific doses.

This nuanced understanding of the dose-response relationship is essential for refining dosage recommendations or interventions aimed at optimizing blood parameter levels in relevant medical or research settings.

Since residual erythromycin contamination remains a concern despite the effectiveness of current quantification methods, exploring antibacterial treatments derived from natural sources presents a promising avenue for addressing residual erythromycin contamination. Natural compounds like garlic, algae, tea tree oil, propolis, or grapefruit seed extract have demonstrated antibacterial properties and may offer safer alternatives to synthetic antibiotics like erythromycin.

Embracing these natural solutions not only mitigates the risks associated with antibiotic residues but also aligns with the growing demand for sustainable and eco-friendly practices in aquaculture and food production. Therefore, future research efforts should prioritize the evaluation of these natural antibacterial treatments to promote safer and more environmentally friendly practices in the aquaculture sector.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval

The author declares that this study complies with research and publication ethics. The research and procedures on the breeding of Nile tilapia were carried out in accordance with the guidelines of the Ethics Committee on Animal Experimentation (CETEA) Decree No. 2013-118 of February 1, 2013 relating to the protection of animals used for scientists, issued by the Pasteur Institute of Algeria. Specific ethical challenges were addressed to ensure humane treatment and minimize suffering.

Tilapia were acclimated to laboratory conditions for two weeks prior to the study, with water quality parameters such as temperature, oxygen levels, and pH maintained within species-specific optimal ranges to reduce stress. Humane euthanasia was conducted using MS-222 (Tricaine methanesulfonate) in accordance with the American Veterinary Medical Association (AVMA) guidelines, which provide standards for minimizing pain and distress in animals. The principle of reduction was upheld by using the minimum number of fish necessary to achieve statistically valid results. Predefined humane endpoints, such as the euthanasia of fish exhibiting severe distress (e.g., loss of equilibrium or respiratory failure), were established to prevent undue suffering. All protocols were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) or an equivalent ethical review board, ensuring compliance with ethical standards throughout the study. Regular consultations with the committee further addressed challenges, such as dose determination and monitoring criteria, reinforcing ethical rigor.

Informed consent

Not available.

Data availability statement

There was no data used in the present study.

Funding organizations

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Author contributions

Sabrine Boucetta: conceptualization, writing - review and editing; Olena Honcharova: review and editing; Soumia Kharfouchi: acquisition, writing and statistical formulation; Adel Chala: using Spss software writing - review and editing; Khawla Aouation: editing and statistics graphs and Erkan Can: supervision, writing, review, editing.

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Figure	Title
01	Component diagram in the space after rotation
02	Hierarchical tree using average distance (between classes). Resized class combination distance.
03	Component diagram in the space after rotation.
04	Water-based detector calibration curves (erythromycin)
05	Graphical representation of the results of the ERY dosage study in the flesh of bioassays.

Table	Title
01	*The experimental aquaria with five legal doses of "Ery" including the control*
02	*Physiochemical parameters of the experimental aquarium*
03	Table of correlations between the measured quantitative variables
04	Table of explained variances
05	Matrix of components after rotations
06	One factor ANOVA
07	Overall results of Erythromycin (ERY) residues in the flesh of Tilapia bioassays
08	Experimental results of erythromycin levels in the flesh of bioassays treated with ERY

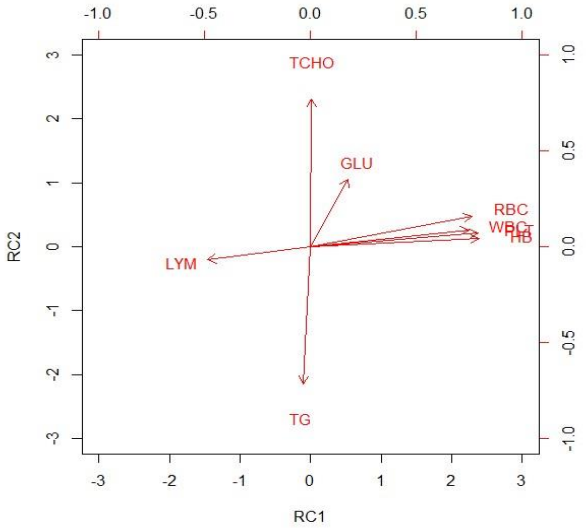

Figure 1. Component diagram in the space after rotation.

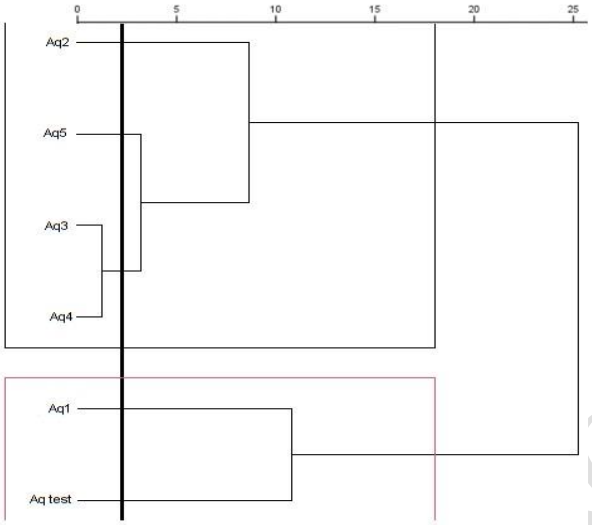

Figure 2. Hierarchical tree using average distance (between classes). Resized class combination distance

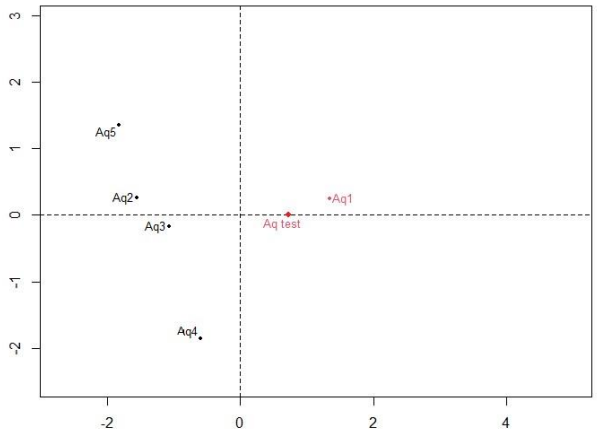

Figure 3. Component diagram in the space after rotation

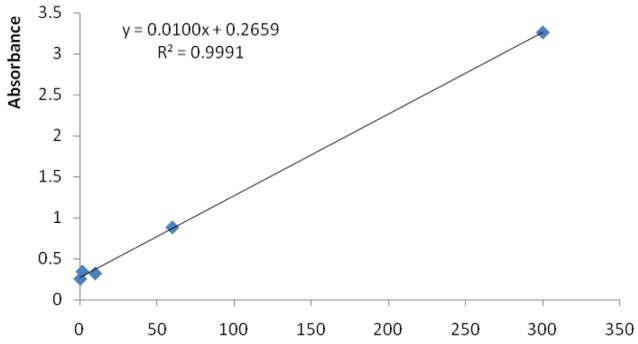

Figure 4. Water-based detector calibration curves (erythromycin)

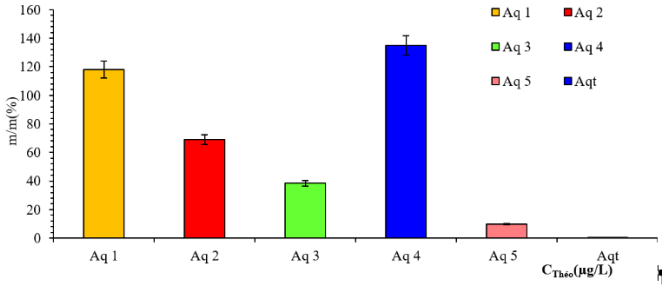

Figure 5. Graphical representation of the results of the ERY dosage study in the flesh of bioassays.

Table 1. *The experimental aquaria with five legal doses of "Ery" including the control*

Aquaria	Aq1	Aq2	Aq3	Aq4	Aq5	Aqtest
ERY doses (µg/l)	0.3	1.7	10	60	300	Control

Table 2. *Physiochemical parameters of the experimental aquarium*

Experimental aquarium	Parameters			
	Mean values (Minimum-maximum)			
	Temperature (C°)	pH	DO (mg/l)	E.C (µS)
	22.68 ±1.12 (20-25.1)	6.89 ± 0.34 (6.28-7.29)	9.99 ± 2.03 (7.3-13.2)	294 ± 7.8 (286-301)
OECD guidelines	20–24	6 – 8.5	80% of air saturation	10–250

Table 3. Table of correlations between the measured quantitative variables

		WBC	RBC	Hb	PLT	CHOL	GLU	TG	LYM
Correlation	WBC	1,000	**0,952**	**0,934**	**0,892**	0,028	**0,543**	-0,056	-0,325
	RBC	0,952	1,000	**0,957**	**0,942**	0,192	0,369	-0,174	-0,466
	Hb	0,934	0,957	1,000	**0,994**	-0,006	0,232	-0,134	**-0,621**
	PLT	0,892	0,942	0,994	1,000	0,034	0,154	-0,194	**-0,699**
	CHOL	0,028	0,192	-0,006	0,034	1,000	0,298	**-0,753**	-0,061
	GLU	0,543	0,369	0,232	0,154	0,298	1,000	-0,141	0,422
	TG	-0,056	-0,174	-0,134	-0,194	-0,753	-0,141	1,000	0,353
	LYM	-0,325	-0,466	-0,621	-0,699	-0,061	0,422	0,353	1,000

Table 4. Table of explained variances

Explained total variance						
	Initial proper values			Sum of squares of factors retained for rotation		
Component	Total	% variance	% cumulative	Total	% variance	% cumulative
1	5,328	53,283	53,283	5,247	52,467	52,467
2	2,765	27,651	80,934	2,847	28,467	**80,934**
3	1,559	15,590	96,523			
4	0,249	2,493	99,016			

Table 5. Matrix of components after rotations

Parameters	Component	
	1	2
WBC	0,954	
GR1	0,966	0,126
Hb	**0,997**	
PLT	**0,986**	
CHOL		**0,937**
GLU	0,291	0,241
TG	-0,117	**-0,929**
LYM	-0,582	-0,178

Table 6. One factor ANOVA

		Sum of squares	ddl	Mean of squares	F	Sign
V1Hb	Inter-groups	80.365	5	16.073	4.033	**0.022**
	Intra-groupes	47.826	12	3.986		
	Total	128.191	17			
V2PLT	Inter-groups	152550.421	5	30510.084	126.885	**0.000**
	Intra-groups	2885.453	12	240.454		
	Total	155435.875	17			

Table 7. Overall results of Erythromycin (ERY) residues in the flesh of Tilapia bioassays

	Erythromycin Concentration (µg/L)
Aq 1	6.982
Aq 2	50.369
Aq 3	1.595
Aq 4	327.934
Aq 5	255.803
Aqtest	0.293

Table 8. Experimental results of erythromycin levels in the flesh of bioassays treated with ERY

$C_{Théo}$(µg/L)	300	60	10	1.7	0.3	0.2
Abs	-	0.204	-	-	0.069	0.084
$C_{Théo}$(µg/L)	-	0.041	-	-	0.00011	$1.08.10^{-4}$
%m/m	-	69%	-	-	38.4	54.1 9%
Abs	0.017	-	0.37	0.458	-	-
$C_{Théo}$(µg/L)	29.653	-	111.803	2.297	-	-
%m/m	9.8 8%	-	18.037	135.110 3%	-	-