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Original Article

Effects of *Streptococcus agalactiae* infection and oral florfenicol administration on the hemato-biochemistry, erythrocyte morphology and histopathology of *Oreochromis niloticus*

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Abstract
Streptococcosis is considered one of the most important diseases affecting farmed tilapia, causing severe economic losses. Antimicrobial therapy is the principal control measure applied during outbreaks. This research assessed the efficacy of florfenicol (FFC) when fed at 15 mg kg biomass^{-1} day^{-1} for 10 days against *Streptococcus agalactiae* infection in *Oreochromis niloticus* in terms of survival, changes in hemato-biochemistry, erythro-morphology, and histoarchitecture of the vital organs. *Streptococcus agalactiae* was moderately virulent to *O. niloticus* with a lethal dose (LD_{50}) of 1.26×10^8 cells fish^{-1} and sensitive to FFC with a minimal inhibitory concentration of 0.78 µg mL^{-1}. It caused systemic infection in tilapia. FFC therapy reduced bacterium-induced mortality and physiological stress. The histopathological findings indicated significant improvement in the kidney and liver tissues of fish. The erythrocyte morphological alterations noted in the challenged fish were irregularly shaped, elongated, crenated, and teardrop cells, hypochromic erythrocytes, ragged cytoplasmic membranes, hypertrophied nuclei, eccentric nuclei, and vacuolation, all of which were mitigated with FFC therapy. Eventually, FFC therapy improved wound healing, normalized plasma biochemistry, and aided recovery from bacterial infection. This study revealed that the therapeutic dose of FFC was effective against *S. agalactiae* infection in *O. niloticus* and lessened the physiological stress.

Keywords: antibiotic medication; blood cells; clinical biochemistry; histological aberrations; Nile tilapia; Streptococcosis

1 | INTRODUCTION

Aquaculture has drawn attention as a result of its rapid growth over other animal-producing sectors (Assefa and Abunna 2018). Aquaculture provides a growing share of the total number of fish available for human consumption and is expected to rise from 57% in the base period (2022) to 61% by 2032 (FAO 2023). The Food and Agriculture Organization (FAO) has predicted that tilapia production in India will increase by 26% between 2018 and 2030, which is 6.8% and 11.5% faster than the projected growth rates for Asia and the world, respectively (FAO 2023). The largest producer of tilapia is China (1,241,410 t), followed by Indonesia (11,72,633 t), Egypt (954,154 t), Brazil (343,596 t), and Thailand (205,971 t) (FAO 2022). Widely consumed in numerous Asian countries, tilapia serves as a crucial dietary protein source (Engle *et al.* 2023). Tilapias are preferred over carp because of their firm, white fleshes and lack of intermuscular bones and they are hardy, fast-growing, and able to tolerate a wide range of environmental conditions, including the high stocking densities normally used during their culture (El-Sayed 2019). Diseases in aquatic organisms severely constrain the expansion and development of sustainable aquaculture. In tilapia farming, the majority of bacterial infections such as motile *Aeromonas* septicemia, *Pseudomonas* septicemia, hemorrhagic septicemia, streptococcosis, columnaris, franciselliosis, edwardsiellosis, and piscirickettsiosis are significant problems, accounting for 80% of fish mortality (El-Sayed 2019; Haenen *et al.* 2023). One of the most significant diseases in tilapia culture worldwide, particularly in indoor systems, is caused by bacteria in the genus *Streptococcus* (Haenen *et al.* 2023). The first confirmed streptococcal infection in cultured fish was reported in 1958 in rainbow trout *Oncorhynchus mykiss* cultured in Japan (Agnew and Barnes 2007). The two primary species of *Streptococcus* affecting tilapia are *S. agalactiae* and *S. iniae*. It is a multifactorial disease in fish, depending on host variety, age, immune status, type of pathogen, and environmental conditions (Haenen *et al.* 2023). The reported cases of *S. agalactiae* infection, which included wild-type and cultured tilapia, are widespread (Amal *et al.* 2013; Haenen *et al.* 2023). Antimicrobial drugs are often the main goal of farmers to control disease and stop massive economic losses (Bondad-Reantaso *et al.* 2012). When used properly, antimicrobials are among the most effective measures available for controlling outbreaks.

Florfenicol (FFC) is a synthetic, broad-spectrum, fluorinated analog of thiamphenicol with the same mechanism of action as chloramphenicol and has often been recommended as a therapeutic agent to control several diseases in intensive aquaculture (USFDA 2023). It was among the most commonly used antibacterial drugs in aquaculture to treat diseases of both warm and cold-water fish (Assane *et al.* 2019). Oxytetracycline (OTC), sulfadiazine and FFC are used in 11 of the 15 main-producing countries (Chen *et al.* 2020). In *O. niloticus*, FFC exhibits fast absorption with maximum serum concentration after 12 h upon oral administration and broad distribution in the host tissues. Drug metabolisation is temperature-dependent, and faster in high water temperatures (Feng and Jia 2009). Since the use of several antibiotics such as chloramphenicol, furazolidone, and nitrofurans have been banned (Bondad-Reantaso *et al.* 2012), FFC has been promoted as an alternative drug for use in aquaculture (Chen *et al.* 2020). Although Indian aquaculture uses a variety of drugs to prevent and control bacterial and other diseases (Patil *et al.* 2022), FFC application in Indian aquaculture is uncommon due to a lack of scientific data and regulation. The current study, therefore, aimed to determine whether the antibiotic FFC when fed orally at 15 mg kg biomass^{-1} day^{-1} for 10 consecutive days could lower the mortality in *S. agalactiae*-challenged *O. niloticus* and improve the physiological status in terms of plasma biochemistry, hematology, erythrocyte morphology, and histoarchitecture of the kidney and liver.

2 | METHODOLOGY

2.1 Experimental fish and bacterial strain and its pathogenicity

Nile tilapia *Oreochromis niloticus* juveniles (n = 400) in healthy and active conditions were obtained from a farm in Sonarpur, South 24 Parganas district, West Bengal, India and acclimatized for 15 days under optimal conditions in 500-L circular tanks containing nonchlorinated borewell-water and continuous aeration (Bardhan *et al.* 2022a). A glycerol stock *Streptococcus agalactiae* LCR1 (NCBI accession number OP752129) was obtained from the State Referral Laboratory for Aquatic Animal Health, Tamil Nadu Dr J Jayalalithaa Fisheries University, Madhavaram, India and revived to determine the lethal dose (LD_{50}) through intramuscular (IM) challenge following Patel *et al.* (2024) at an estimated dose of approximately 2.30×10^{8} – 2.30×10^{5} cells fish^{-1}, in duplicate, with 10 fish tank^{-1}. The LD_{50} was determined from the fish mortality data (Reed and Muench 1938).

2.2 In-vivo therapeutic effectiveness of florfenicol

Florfenicol is an approved antibiotic for aquaculture use at 10–15 mg kg biomass^{-1} day^{-1} for 10 days (USFDA 2023). The medicated and control diets used to feed the fish at 2% body weight (BW) were prepared following Bardhan *et al.* (2022a). Twenty-five *O. niloticus* (17.67 ± 1.27 g) juveniles were housed in nine polypropylene tanks (L58 × H45 × B45 cm), covered with nylon netting and conditioned for 3 days. After conditioning, the fish were assigned to three groups, in triplicate. Group 1 was the unchallenged and control feed-fed, i.e., saline-injected and considered as the negative control. Group 2 fish were challenged with *S. agalactiae* LCR1 at a concentration of 1.32×10^{7} cells fish^{-1} and fed FFC at 15 mg kg biomass^{-1}

day^{-1} for 10 consecutive days (FFC treatment) and group 3 fish were challenged with *S. agalactiae* LCR1 at a concentration of 1.32×10^7 cells fish^{-1} and fed control feed, i.e., untreated and considered the positive control. Before the bacterial challenge, the fish were anaesthetized with clove oil at 40 µL L^{-1} water. Group 1 received 0.1 mL of sterile saline intramuscularly. Groups 2 and 3 were given a sublethal dose of *S. agalactiae* LCR1 at 1.32×10^7 cells fish^{-1} at the base of the dorsal fin intramuscularly (Sa-challenged). The fish were returned to their respective tanks after the challenge. The injection was followed by feeding the fish with or without medication at 2% BW thrice daily in equal ratios. The control feed was offered to groups 1 (negative control) and 3 (untreated positive control). Group 2 was given FFC feed for 10 days at 15 mg kg biomass^{-1} day^{-1} (FFC treatment), then switched to a control diet until the study ended. After 75 min of each feeding, unconsumed feeds were carefully collected, air-dried, pooled tank-wise, and weighed daily. Behavioural changes, mortality, feed intake, wound healing, and external infections were documented daily. During the experimental period, half the water was exchanged biweekly to maintain the water quality parameters within optimal ranges of pH (7.53 – 7.85), dissolved oxygen (5.49 – 5.54 mg L^{-1}), temperature (24.30 – 31.40°C), ammonia (0.02 – 0.03 mg L^{-1}), nitrite (0.16 – 0.21 mg L^{-1}), and nitrate (0.27 – 0.34 mg L^{-1}).

2.3 Collection of blood and plasma

Two fish from each tank were carefully moved to plastic buckets with the same water temperature on day 0, day post injection (DPI) 1, 10, 17 and 24, and anaesthetized. Blood was drawn through a caudal puncture using 2-mL sterile syringes (Roberts 2012). Blood samples were placed in 2 mL Eppendorf tubes, which were washed with 5% ethylene diamine tetra acetic acid to avoid clotting. The blood was centrifuged at 4500 rpm for 15 min, to collect the plasma, which was transferred to new Eppendorf tubes, and stored at –20°C for analysis.

2.4 Hemato-biochemistry

The plasma biomarkers used to measure physiological status were glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine, calcium and chloride. The specific test kits and methods used for each biomarker were as follows: Glucose kit from Diasys Diagnostic Systems, Germany by GOD FS 10 (Schmidt *et al.* 1961); ALT and AST kits or ERBA SGPT and SGOT kits from Erba Diagnostics Ltd., Mannheim, Germany by IFCC and Kinetic methods (Wolf *et al.* 1972); ALP kit from Erba Diagnostics Ltd., Mannheim, Germany by FS test (Tietz 1994); Creatinine kit from Span Diagnostics Ltd., India by modified Jaffe's Reaction, Initial rate assay (Junge *et al.* 2004). The calcium content was determined with a calcium test kit AS FS from Diasys Diagnostic Systems, Germany (Michaylova and Ilkova 1971). The chloride content was determined using the chloride test kit from Coral Clinical Systems, India (Schoenfeld *et al.* 1964). All the parameters were measured via a Photometer (model: 5010 v5 +, Robert Riele KG, Berlin) according to the commercial kit protocol.

2.5 Erythrocyte morphology

The total erythrocyte counts (TECs) were measured in Hayem's solution at 1:200 and the total leukocyte counts (TLCs) in Turke's solution at 1:20. Both counts were performed with a Neubauer hemocytometer. The thrombocyte counts (TCs) were measured using Rees-Ecker dilution fluid. The cell count was performed with a 40× lens under an Olympus BX51 microscope. For erythrocyte morphology, a qualitative assessment was performed by using an oil immersion lens (100×) to observe blood smears, which were prepared by Giemsa staining (Bardhan *et al.* 2022b). Cell visualization was carried out using an Olympus BX51 microscope with a 16 MP SCOLUX camera, connected to the computer. Using previous descriptions, cell and nucleus shape changes were observed to acquire descriptive data (Bojarski *et al.* 2018; Bardhan *et al.* 2022b).

2.6 Histopathology

On 0, 1, 10, 17, and 24 DPI, kidney and liver samples were taken from control, FFC-treated, and untreated fish, in triplicates. After blood collection, the fish were euthanized with clove oil (100 µL L^{-1}) and carefully dissected. The kidney and liver tissue samples were kept in Bouin's solution for 24 hours. After conventional treatments, paraffin wax was applied to embed the samples. Sections 5 µm thick were double stained with hematoxylin and eosin (Roberts 2012). ToupTek ToupView (version ×64, 4.11) software was used to capture and process photomicrographs. The histological alterations were evaluated using a six-point ordinal scale, following the methodology described by Bowker *et al.* (2013).

2.7 Discoloration, wound progression and healing

The 30-day study involved digitally imaging wounds after IM injection, scoring tissue damage using a scale of 0–6 developed and modified by Bernet *et al.* (1999), and categorizing wound progression, healing, and discoloration into mild, moderate, and severe disease based on pathological importance. The intensity of damage was qualitatively categorized as 0: no damage/discoloration with no pathological importance, 0.5: very mild damage/discoloration with no pathological importance, 1: very mild damage/ discoloration with minimal pathological importance, 2: mild damage/discoloration with minimal pathological importance, 4: moderate damage/discoloration with moderate pathological importance, 6: severe damage/discoloration with marked

pathological importance.

2.8 Data analyses

The results are expressed as means ± standard deviations. The mortality, biomass, feed intake, hematology, and plasma biochemistry data were analyzed via one-way ANOVA. The Tukey post-hoc test was used to confirm treatment and period differences by mean comparison. Friedman ANOVA for related samples and Mann-Whitney U test for independent samples were used for the analysis of the qualitative scores of wound progression, healing, and discoloration. The nonparametric Kruskal-Wallis test with pairwise comparisons was used to examine the histopathological scores. All the statistical analyses were conducted via IBM-SPSS (version 22.0) at an α level of significance of 0.05.

3 | RESULTS

3.1 Efficacy of florfenicol against *Streptococcus agalactiae* LCR1

Streptococcus agalactiae LCR1 was moderately pathogenic to *O. niloticus*, with an LD_{50} of 1.26×10^{8} cells $fish^{-1}$. In the efficacy trial, mortality in the FFC-treated group was significantly lower (16%) than in the untreated (32%) group. The challenged fish were lethargic, hanging and lying at the bottom of the tank, whereas the control showed typical behaviour. Feed intake was markedly lower in the untreated group compared with the treated and control groups. Despite the initial nominal feed intake, the treated group became more aggressive over time and ultimately resulted in significantly higher biomass ($p < 0.05$) than those of the control and untreated groups.

3.2 Hemato-biochemistry

The plasma glucose levels of the challenged groups increased and peaked at DPI 1. A reduction in glucose was observed starting at DPI 17, but it did not return to normal. The levels of glucose were still high compared with those on day 0. The untreated group had higher glucose levels than the treated group on DPI 24 (Figure 1A). The plasma creatinine level of the treated group peaked at DPI 1, followed by a reduction on and after DPI 10. The untreated group followed the same trend. However, the creatinine levels of both groups did not fully recover at DPI 24 (Figure 1B). The plasma ALT, AST, and ALP of the challenged groups increased significantly at DPI 1 compared to the control group. The levels of these parameters were reduced in the treated and untreated groups but did not reach a normal state at DPI 24 (Figures 1C–E). The plasma calcium and chloride concentrations were reduced at DPI 1 in the treated and untreated groups. Although the levels increased with time, normalcy was not achieved at DPI 24 in both groups. Compared with the treated group, the untreated group had lower calcium and chloride levels. Significant differences were detected among the groups and the DPI (Figures 1F, 1G). The total erythrocyte, leukocyte, and thrombocyte counts were assessed, and the values were compared with those of the control group (Figures 2A–C). The challenged groups had significant reductions in TECs at DPI 1. A gradual but significant decrease in TECs was observed in both groups at DPI 10 ($p < 0.05$). However, after DPI 24, the number of TECs significantly increased (Figure 2A). The challenged groups showed a significant increase in TLCs and TCs from DPI 1 to 10 ($p < 0.05$). At DPI 24, TLCs (Figure 2B) and TCs (Figure 2C) were significantly reduced but did not reach normal levels.

3.3 Erythrocyte morphology

The erythrocytes of the treated and untreated groups displayed size and shape variations compared with those of the control, and the morphological changes observed in the DPIs are depicted in detail in Figures 3A-H. The erythrocytes presented the greatest degree of alterations in challenged *O. niloticus* at DPI 1. The erythrocytes of the treated group were irregularly shaped, elongated, crenated, teardrop shaped, and hypochromic erythrocytes, ragged cytoplasmic membranes, hypertrophied nuclei, eccentric nuclei, and vacuolation. Over time, the number of morphological alterations in the treated group decreased after DPI 17 compared with those of the control and untreated groups. The treated group had almost normal erythrocytes at DPI 24 with mildly crenated and teardrop cells. The highest intensity of erythrocytic damage was observed in the untreated group at DPI 1 and 10. The untreated group showed slower recovery at DPI 17 than in the treated group. However, the erythrocyte aberrations were only mild, with the persistence of crenated, teardrop, and elongated cells on DPI 24.

3.4 Histopathology

Qualitative histopathological scores based on alterations in the kidney tissues of Sa-challenged *O. niloticus* with or without FFC treatment compared to the control (Figures 4A–I) are presented in Table 1. A normal renal tubule structure with a well-organized glomerulus was observed in the kidney tissues of control *O. niloticus* (Figure 4A). Kidney tissues of the treated group on DPI 1 exhibited hemocyte infiltration, glomerulopathy, inflammation, nephropathy and nephrocalcinosis (Figure 4B). The intensities of all the histopathological alterations were significantly lower ($p < 0.05$) on and after 10 DPI in the treated group (Figures 4D, 4F). The recovery of the renal tissues at DPI 24 was noted in the treated group, although nephropathy indicated degeneration of the renal epithelium, vacuolation within the renal tubules, a widened lumen and slight inflammation persisted (Figure 4H). In contrast, the untreated group presented significantly greater degrees ($p < 0.05$) of alterations compared with those in the treated group, such as nephropathy, hemo-

cyte infiltration, glomerulopathy, inflammation and nephrocalcinosis occurred at DPI 1 (Figure 4C). The untreated group presented a slower recovery than did the treated group at DPI 10 (Figures 4E, 4G). A decrease in kidney tissue aberrations was noted on DPI 24 despite the minimal persistence of nephropathy and glomerulopathy (Figure 4I). The bacterial challenge resulted in significant alterations in the liver tissues of *O. niloticus*. The treated group exhibited cytoplasmic degeneration, cellular hypertrophy, cytoplasmic vacuolation, glycogen-type vacuolation, and necrotized areas at DPI 1 (Figure 5A). All histopathological changes showed a significant decrease ($p < 0.05$) after DPI 17 (Figures 5D, 5F, 5H) showing gradual recovery of the liver tissues. However, trivial cytoplasmic and glycogen-type vacuolation persisted at DPI 24. In contrast, the untreated group presented significantly greater degrees ($p < 0.05$) of alterations. Increased glycogen-type vacuolation and cellular hypertrophy were observed at DPI 10 and DPI 17, respectively and the subsequent recovery was slower (Figures 5E, 5G). However, at DPI 24, a reduction in aberrations was documented, with mild persistence of cytoplasmic degeneration and vacuolation (Figure 5I).

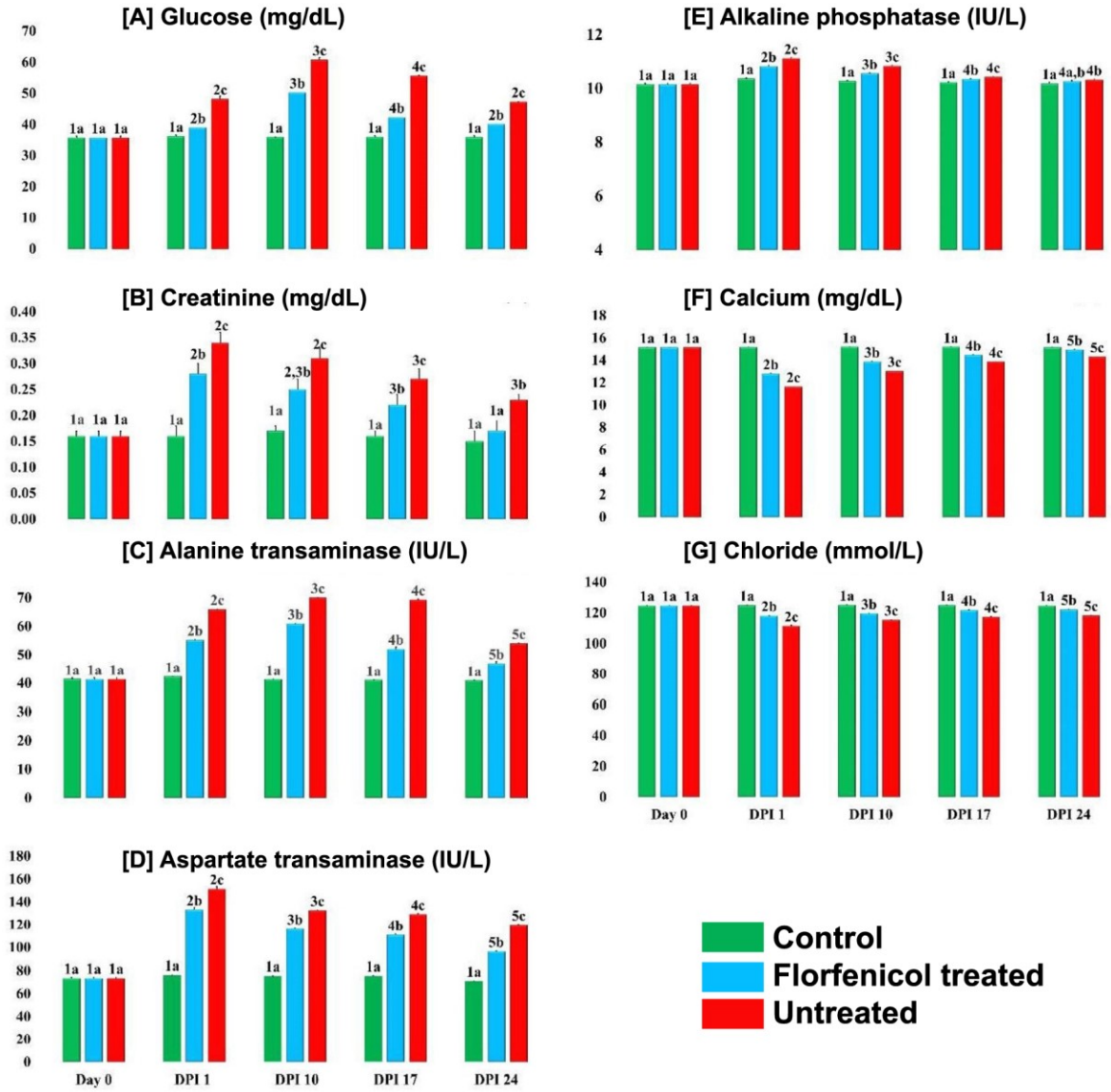

FIGURE 1 Effect of *Streptococcus agalactiae* LCR1 intramuscular challenge at 1.32×10^7 cells fish^{-1} and florfenicol-treatment at 15 mg kg biomass^{-1} day^{-1} for 10 consecutive days on the plasma [A] glucose, [B] creatinine, [C] alanine transaminase, [D] aspartate transaminase, [E] alkaline phosphatase, [F] calcium, and [G] chloride levels at different time points in *Oreochromis niloticus* juveniles in comparison with those in the untreated and control groups. a–c: Bars sharing a common alphabet for a particular time point differed insignificantly ($p > 0.05$). 1–4: Bars sharing a common numeral for a particular treatment differed insignificantly ($p > 0.05$).

TABLE 1 Qualitative assessment of major histopathological changes in the kidney and liver tissues at different time points in *Oreochromis niloticus* juveniles intramuscularly challenged with *Streptococcus agalactiae* LCR1 at 1.32×10^7 cells fish^{-1} and fed florfenicol at 15 mg kg biomass^{-1} day^{-1} for 10 consecutive days in comparison with normal architecture.

Histopathological changes	Qualitative assessment on an ordinal scale*							
	DPI 1		DPI 10		DPI 17		DPI 24	
	FFC treated	Untreated	FFC treated	Untreated	FFC treated	Untreated	FFC treated	Untreated
Kidney								
Hemocyte infiltration	0.35 ± 0.09^{1a}	0.72 ± 0.08^{2A}	0.50 ± 0.10^{1b}	0.91 ± 0.11^{2B}	0.32 ± 0.08^{1a}	0.65 ± 0.08^{2A}	0.19 ± 0.07^{1c}	0.38 ± 0.15^{1C}
Glomerulopathy	0.47 ± 0.13^{1a}	0.56 ± 0.17^{1A}	0.65 ± 0.20^{1b}	0.73 ± 0.12^{1B}	0.48 ± 0.12^{1a}	0.59 ± 0.08^{1A}	0.31 ± 0.09^{1a}	0.38 ± 0.08^{1C}
Inflammation	1.11 ± 0.12^{1a}	1.20 ± 0.09^{1A}	1.24 ± 0.07^{1b}	1.42 ± 0.10^{2B}	1.01 ± 0.11^{1a}	1.12 ± 0.09^{1A}	0.65 ± 0.10^{1c}	0.84 ± 0.16^{1C}
Nephropathy	1.11 ± 0.10^{1a}	1.25 ± 0.07^{2A}	1.44 ± 0.15^{1b}	1.50 ± 0.04^{1B}	1.25 ± 0.11^{1a}	1.35 ± 0.08^{1A}	0.83 ± 0.10^{1c}	0.92 ± 0.07^{1C}
Nephrocalcinosis	0.29 ± 0.12^{1a}	0.38 ± 0.12^{1A}	0.43 ± 0.14^{1b}	0.51 ± 0.11^{1B}	0.24 ± 0.07^{1a}	0.36 ± 0.09^{1A}	0.18 ± 0.06^{1a}	0.22 ± 0.04^{1C}
Liver								
Glycogen-type vacuolation	0.20 ± 0.04^{1a}	0.25 ± 0.06^{1A}	0.34 ± 0.05^{1b}	0.76 ± 0.12^{2B}	$0.30\pm0.30^{1a,b}$	0.53 ± 0.12^{2C}	$0.23\pm0.06^{1a,b}$	0.31 ± 0.08^{1A}
Cytoplasmic vacuolation	0.38 ± 0.09^{1a}	0.93 ± 0.15^{2A}	1.04 ± 0.07^{1b}	1.19 ± 0.07^{2B}	0.54 ± 0.14^{1a}	0.81 ± 0.20^{2A}	0.24 ± 0.04^{1c}	0.29 ± 0.10^{1C}
Cytoplasmic degeneration	0.33 ± 0.08^{1a}	0.59 ± 0.17^{2A}	0.70 ± 0.16^{1b}	1.02 ± 0.08^{2B}	0.54 ± 0.54^{1b}	0.71 ± 0.15^{1A}	0.21 ± 0.07^{1c}	0.35 ± 0.16^{1C}
Cellular hypertrophy	1.22 ± 0.08^{1a}	1.34 ± 0.07^{1A}	1.55 ± 0.06^{1b}	1.65 ± 0.08^{1B}	1.04 ± 0.08^{1c}	1.14 ± 0.08^{1C}	0.45 ± 0.28^{1d}	0.65 ± 0.15^{1D}
Necrosis	0.24 ± 0.04^{1a}	0.29 ± 0.04^{1A}	0.31 ± 0.08^{1b}	0.37 ± 0.06^{1B}	0.23 ± 0.08^{1a}	0.27 ± 0.08^{1A}	0.15 ± 0.06^{1c}	0.22 ± 0.06^{1A}

*Qualitative assessment ordinal scale: 0 = no change; 1 = normal with <5% of tissues affected; 2 = mild with 5–15% of tissues affected; 3 = moderate with 15–25% of tissues affected; 4 = marked with 25–50% of tissues affected and 5 = severe with >50% of tissues affected. The qualitative assessment was based on six observations (mean ± standard deviation) for each organ of the respective group. No changes were noted in the control group. 1–2: Values sharing identical numerical superscripts for a particular row differed insignificantly among the treatment groups for a particular day ($p > 0.05$). a–d; A–D: values sharing identical alphabetical superscripts within a row differed insignificantly for a particular treatment group ($p > 0.05$). DPI: Day post injection. Glomerulopathy includes changes such as dilated Bowman's space (DBS) and fragmented glomerulus (FG); Nephropathy includes changes such as degeneration of renal tubular epithelium (DRE), degeneration of renal tubule (DRT), vacuolation within the renal tubules (VRT), necrotized areas (N) and widened lumens (W).

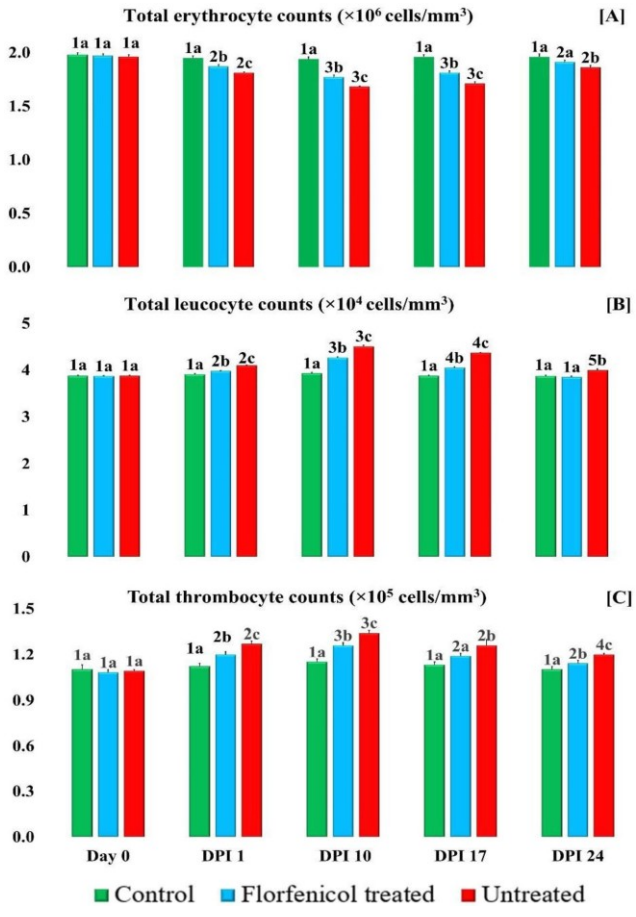

FIGURE 2 Effect of *Streptococcus agalactiae* LCR1 intramuscular challenge at 1.32×10^7 cells fish^{-1} and florfenicol-treatment at 15 mg kg biomass^{-1} day^{-1} for 10 consecutive days on the counts of [A] total erythrocytes, [B] leucocytes, and [C] thrombocytes at different time points in *Oreochromis niloticus* juveniles in comparison with those in the untreated and control groups. a–c: Bars sharing a common alphabet for a particular time point differed insignificantly ($p > 0.05$). 1–4: Bars sharing a common numeral for a particular treatment differed insignificantly ($p > 0.05$).

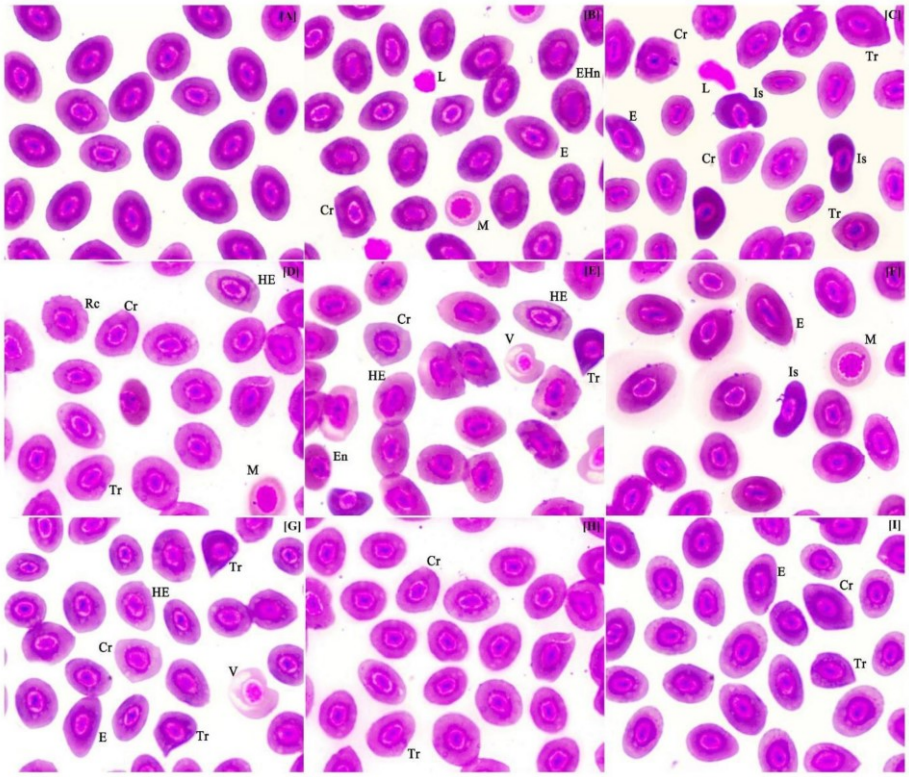

FIGURE 3 Effect of *Streptococcus agalactiae* LCR1 intramuscular challenge at 1.32×10^{7} cells fish^{-1} and florfenicol-treatment at 15 mg kg biomass^{-1} day^{-1} for 10 consecutive days on the erythrocytic morphological alterations in *Oreochromis niloticus* juveniles at different time points in comparison with untreated and control groups. [A] Control, [B] day post injection (DPI) 1 treated and [C] untreated, [D] DPI 10 treated and [E] untreated, [F] DPI 17 treated and [G] untreated and [H] DPI 24 treated and [I] untreated groups. Cr: Crenated cell; E: Elongated cell; En: Eccentric nucleus; Ehn: Hypertrophied nucleus; HE: Hypochromic erythrocyte; Is: Irregularly shaped cell; Rc: Ragged cytoplasmic membrane; Tr: Tear-drop cell; V: Vacuolation; L: Lymphocyte; M: Monocyte; ×1000 Giemsa-staining.

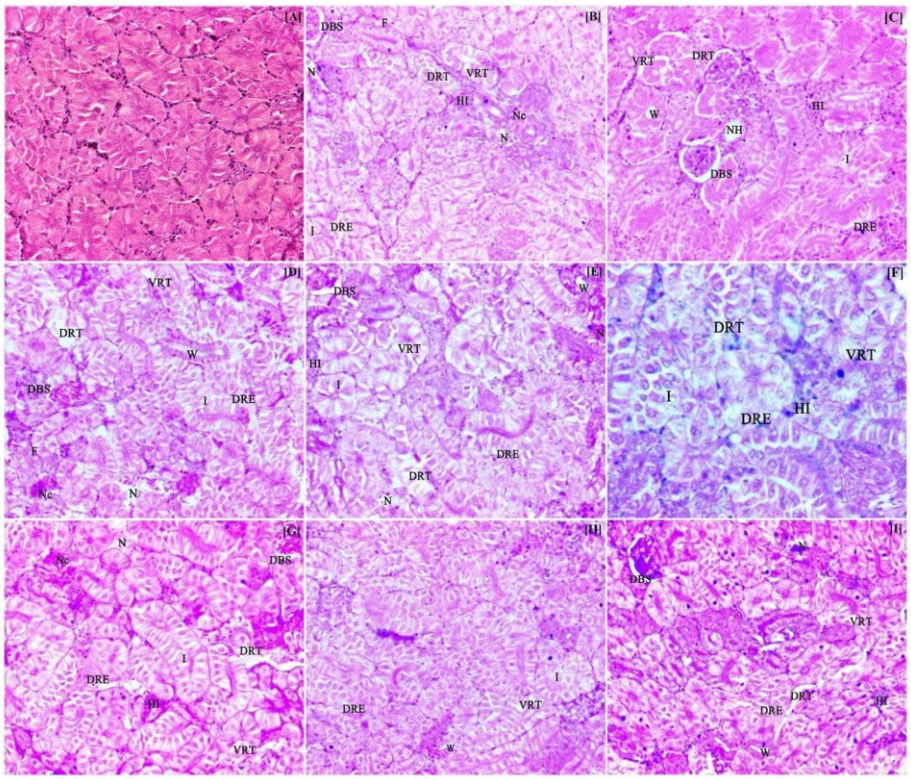

FIGURE 4 Effect of *Streptococcus agalactiae* LCR1 intramuscular challenge at 1.32×10^{7} cells fish^{-1} and florfenicol-treatment at 15 mg kg biomass^{-1} day^{-1} for 10 consecutive days on the kidney histoarchitecture of *Oreochromis niloticus* juveniles at different time points. A] Control, [B] Day post injection (DPI) 1 treated and [C] untreated, [D] DPI 10 treated and [E] untreated, [F] DPI 17 treated and [G] untreated, and [H] DPI 24 treated and [I] untreated. DRE: degeneration of renal tubular epithelium; DRT: degeneration of renal tubules; I: inflamed renal tubules; VRT: vacuolation within the renal tubules; W: widened lumen; DBS: dilated Bowman's space; F: fibrosis; HI: hemocyte infiltration; N: necrotized areas; Nc: nephrocalcinosis and NH: necrotized hematopoietic area; ×200 H&E staining.

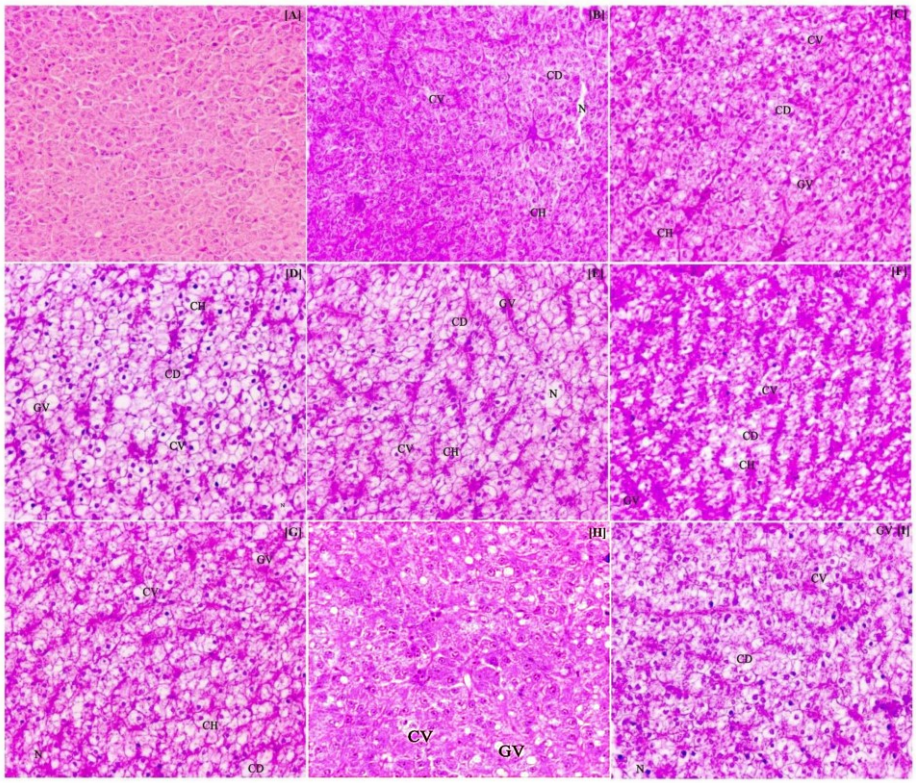

FIGURE 5 Effect of *Streptococcus agalactiae* LCR1 intramuscular challenge at 1.32×10^{7} cells $fish^{-1}$ and florfenicol-treatment at 15 mg kg $biomass^{-1}$ day^{-1} for 10 consecutive days on the liver histoarchitecture of *Oreochromis niloticus* juveniles at different time points. [A] Control, [B] Day post-injection (DPI) 1 treated and [C] untreated, [D] DPI 10 treated and [E] untreated, [F] DPI 17 treated and [G] untreated, and [H] DPI 24 treated and [I] untreated. CD: cytoplasmic degeneration; CH: cellular hypertrophy; CV: cytoplasmic vacuolation; GV: glycogen-type vacuolation; and N: necrotized areas; ×200 H&E staining.

3.5 Discoloration, wound progression and healing

Wound formation commenced on and from DPI 1 for both groups. The open subepithelial wounds became obvious after 72 hours of challenge with signs of tail rot, subconjunctival hemorrhage, exophthalmia, protrusion of scales and skin discolouration. The qualitative scores peaked at DPI 6 for the treated and untreated groups. The intensity of the wounds in the treated and untreated groups significantly decreased from DPI 7 to DPI 8 and DPI 7 to DPI 12, respectively, and eventually returned to normal. Discolouration began on and from DPI 1 in challenged groups. Between DPI 6 and DPI 8, the highest levels of discolouration were recorded in both groups. The degree of discolouration significantly decreased from DPI 7 to DPI 12 and DPI 9 to DPI 14 before returning to normal in the treated and untreated groups, respectively. Within 6 days of FFC therapy, mild reddening and inflammation subsided with the formation of a black scar at the ulcerated site. All mild wounds closed within 8 days of therapy, and by DPI 10, the black scar had disappeared, with dermal fibrous tissue regrowth and skin development in the ulcerated scar region. The epidermis gradually thinned with the development of new scales in the underlying tissues, and almost returned to normal at DPI 12. Between DPI 13 and DPI 30, normal skin architecture was restored. Wound healing was greater in the treated group than in the untreated group (Figure S1; Table 2).

4 | DISCUSSION

The results of the pathogenicity test indicated that *S. agalactiae* was moderately pathogenic because of its ability to cause septicemia and meningoencephalitis in challenged *O. niloticus*. The gross pathological abnormalities observed in diseased fish were similar to those reported by Adikesavalu *et al.* (2017), who confirmed that *S. agalactiae* infection is characterized by septicemia and meningoencephalitis. The challenged fish presented obvious signs of infection on the skin and eyes post-challenge. The FFC administration resulted in significantly lower mortalities than those in the untreated group, suggesting the potential of FFC therapy in controlling *S. agalactiae* infection and consistent with the findings of a previous study on the effectiveness of FFC against *S. iniae* in *M. chrysops* × *M. saxatilis* (Darwish 2007). However, the mortality rates observed in the treated group of the present study were higher (16%) than those reported in the Darwish (2007) study (13%). Although the two studies are not directly comparable, adult and subadult fish are reportedly more susceptible to streptococcosis than are younger life stages (Agnew and Barnes 2007). Mortalities persisted until DPI 18 and DPI 20 in treated and untreated groups, respectively. These findings indicated reinfection post treatment. Such recurrent infection was also documented previously in the treatment of *S. agalactiae* infection with OTC in *O. niloticus* (Faria *et al.* 2013). Compared to a previous study (Faria *et al.* 2013), the present study showed promising results during the treatment period, indicating a significant reduction in mortality with FFC.

As the fish became anorexic, their feeding activity gradually declined during the early stages of infection,

and the medicated feed initially appeared ineffective. The feed intake in the treated group increased significantly beginning at DPI 7 and then gradually returned to normal over time. At the end of the experiment, the feed intake increased to 99.80-100% in all the groups. With the lack of bacterial recovery and the increased efficacy, the FFC dose of 15 mg kg biomass^{-1} day^{-1} can, thus, be considered for the treatment of *O. niloticus* with *S. agalactiae* infection. The FFC-fed challenged group presented greater biomass, i.e., a 1.73-fold increase compared with that of the untreated group at DPI 24 concomitant with increased feed consumption, indicating the growth-promoting effects of FFC in *O. niloticus* similar to those of Reda *et al.* (2013). The scientific data concerning the influence of FFC on the growth of different fish species are conflicting. For example, FFC administration in hybrid striped bass did not alter growth (Straus *et al.* 2012), while a significant dose-dependent reduction in the fish biomass occurred during the antibiotic administration, including FFC has been reported (Limbu *et al.* 2018; Bardhan *et al.* 2022a, 2022b).

TABLE 2 Qualitative scores of the changes in discolouration, wound progression and healing in *Streptococcus agalactiae*-challenged and florfenicol (FFC)-fed *Oreochromis niloticus* juveniles at 15 mg kg biomass^{-1} day^{-1} for 10 consecutive days during the treatment regime in comparison with the negative control.

Treatment days	Discolouration		Wound progression and healing	
	FFC treated	Untreated	FFC treated	Untreated
Day 0	0.00^{1a}	0.00^{1a}	0.00^{1a}	0.00^{1a}
DPI 2	0.13^{1b}	0.57^{2b}	1.08^{1b}	1.18^{1b}
DPI 4	0.83^{1c}	1.37^{2c}	1.17^{1b}	1.64^{2c}
DPI 6	1.39^{1d}	1.76^{2c}	1.36^{1c}	2.47^{2d}
DPI 8	1.11^{1e}	2.04^{2d}	0.73^{1d}	1.63^{2c}
DPI 10	0.41^{1f}	1.51^{2c}	0.00^{1a}	1.00^{2b}
DPI 12	0.17^{1b}	0.80^{2e}	0.00^{1a}	0.50^{2e}
DPI 14	0.00^{1a}	0.21^{2f}	0.00^{1a}	0.00^{1a}
DPI 16-30	0.00^{1a}	0.00^{1a}	0.00^{1a}	0.00^{1a}

DPI: Day post injection; Control: Unchallenged and fed control feed (negative control). Florfenicol (FFC) treatment: Fish were challenged with *S. agalactiae* LCR1 at a concentration of 1.32×10^7 cells fish^{-1} and fed FFC at 15 mg kg biomass^{-1} day^{-1} for 10 consecutive days. Untreated: Fish were challenged with *S. agalactiae* LCR1 at a concentration of 1.32×10^7 cells fish^{-1} and fed control feed (positive control). DPIs 1–10: FFC treatment period. a–f: Values sharing identical alphabetical superscripts within a specific column differed insignificantly ($p > 0.01$). 1–2: Values sharing identical numerical superscripts within a row differed insignificantly for a specific parameter ($p > 0.01$). The even sampling days data only were furnished in the table.

The bacterial challenge caused stress, as indicated by the increase in secondary stress response indicator, i,e., glucose levels. The degree of glucose increments at DPI 1 and DPI 10 in the treated group was 1.09- and 1.28-fold greater, respectively. Similarly, elevated glucose in Sa-challenged *Oreochromis* spp. was reported previously (Alsaid *et al.* 2014; Wardani *et al.* 2021). The untreated group recorded higher glucose levels than the FFC-treated group, which provided conclusive indications of impaired hepatic glucose metabolism caused by a pathogenic bacterial infection. The glucose had decreased significantly at DPI 24 but was significantly greater than the initial levels recorded on day 0. The concentrations of inorganic ions in plasma play a role as physiological variables for the maintenance of total osmolarity and ionic balance (Bardhan *et al.* 2022a). Within 24 h of the challenge, both groups presented significant reductions in calcium and chloride contents corroborating the findings noted in *S. iniae*-infected *O. niloticus* (Bowser *et al.* 1998; Chen *et al.* 2004) and *Streptococcus* spp. infected salmonids (Barham *et al.* 1980; Bowser 1993). Although increments in calcium and chloride levels were observed in both groups, at DPI 24, their levels in the treated group reached near normal. In the untreated group, the recovery of these ions was low at DPI 24, indicating the persistence of *S. agalactiae*-induced stress.

The plasma creatinine level increased significantly at DPI 1 in the challenged groups, which indicated kidney impairment and was in agreement with Oda *et al.* (2016), who reported a high creatinine in *S. iniae*-infected *O. niloticus*. However, at DPI 10, the treated group presented lower creatinine compared with the untreated group, indicating the efficacy of FFC in protecting the kidney from the destructive effects of *S. agalactiae* infection. The creatinine level in the treated group was reduced by almost one-fold at DPI 10 and 0.8-fold at DPI 24, indicating the anti-*S. agalactiae* activity of FFC in *O. niloticus*. Similarly, a significant decrease in creatinine in *S. iniae*-infected *O. niloticus* upon FFC therapy has been documented (Aboyadak e*t al.* 2016). Within 24 h of the chal-

lenge, a significant increase in plasma ALT and AST was observed in both groups compared with the unchallenged control group, thus supporting earlier studies on *S. iniae*-infected *O. niloticus* (Aboyadak *et al.* 2016; Oda *et al.* 2016), and *S. agalactiae*-infected *Oreochromis* spp. (Abuseliana *et al.* 2010; Alsaid *et al.* 2014). The treated group showed a significant decrease in ALT and AST after DPI 10, indicating the positive influence of the FFC on enhancing functions and protecting liver cells. The treated group demonstrated approximately 1.14- and 1.13-fold reduction in ALT and AST, respectively compared with those in the untreated group at DPI 10, possibly attributed to the protective effect of FFC, its bactericidal and certain hepatoprotective properties. This enabled the fish to recover from hepatic damage and prevent infection. Although the ALP levels increased, there was a significant difference between the treated and untreated groups at DPI 1, indicating the positive effects of FFC medication on the inflammatory response of fish. A comparable result by Chen *et al.* (2011) reported a significant increase in ALP at 12 h post infection with *S. iniae*. The treated group demonstrated an approximately 1.02-fold reduction in ALP compared with that of the untreated group at DPI 10. The results indicated the positive effects of FFC in alleviating Sa-challenge in the liver and boosting the adaptive responses of fish to bacterial infection. The findings on significantly higher levels of ALP at DPI 24 compared with those on day 0 indicated persistent liver inflammation in both groups. However, the decrease in ALP activity upon cessation of FFC treatment was significant and prominent.

The impact of the Sa-challenge was evident in various hematological parameters of *O. niloticus*. The TECs in the challenged groups were significantly reduced at DPI 1, indicating the immediate impact of the bacterial challenge on fish. Similarly, a substantial decrease in the TECs of *O. niloticus* upon *S. iniae* challenge (Martins *et al.* 2011) and *O. mykiss* during *Aeromonas* and *Streptococcus* coinfection (Barham *et al.* 1980), and *Oreochromis* sp. fed essential oils of *Mentha piperita* (Vo *et al.* 2022) and lemongrass essential oils of *Cymbopogon citratus* after Sa-challenge (Thuong *et al.* 2022) have been documented. Additionally, a gradual yet significant decline in TECs was observed in both groups at DPI 10. Over time, a substantial increase in TECs was noted in both groups, indicating the recovery of the fish. However, normalcy was not noted on DPI 24, suggesting prolonged stress caused by bacterial infection. The TLCs play a vital role in the immune response of fish, particularly for inflammation. A considerable increase in TLCs was observed in the challenged groups at DPI 1, possibly the result of stimulation caused by the entry of pathogens. The results corroborated the increase in TLCs following *S. agalactiae* infection in *Oreochromis* spp. (Alsaid *et al.* 2014; Vo *et al.* 2022). There was an increase in TLCs at DPI 10, which then decreased at DPI 24. These results demonstrated that fish stressed by the bacterial infection have their nonspecific immune system activated, similar to the earlier studies by Martins *et al.* (2009). Significant increases in TCs were observed on DPI 1 for the treated and untreated groups, similar to the results of Vo *et al.* (2022) in *Oreochromis* sp. after *S. agalactiae* infection. Subsequently, at DPI 10, the treated group experienced a decrease in TCs, indicating that FFC played a role in reducing hemolysis caused by the bacterial destruction of TECs and its capacity to stimulate the immune system. On and from DPI 17, both groups experienced a significant decrease in TCs, indicating fish recovery, but the levels did not return to normalcy. The results of fast wound healing in treated fish highlighted the importance of high-value thrombocytes overpowering Sa-challenge and mitigating infection.

Fish erythrocytes are believed to play an important role in immune system participation (Thuong *et al.* 2022). The observed alterations in erythrocyte morphology indicated stress responses of the fish to Sa-challenge as well as FFC therapy. Compared with the control group, the erythrocytes of the treated group at DPI 1 recorded elongated, and crenated cells and hypertrophied nuclei. These changes were comparable to those reported by Vo *et al.* (2022) for *Oreochromis* sp. against *S. agalactiae* infection. At DPI 10, the treated group exhibited similar changes as was observed at DPI 1 which persisted until DPI 17. These alterations were reduced after DPI 17 in the treated group. In contrast, the untreated group presented the greatest degree of erythrocytic damage, such as irregularly shaped, elongated, teardrop-shaped, and crenated cells at DPI 1. The decrease in TECs is an indication of an increase in immature erythrocytes in circulation due to the stress response of *O. niloticus* against the Sa-challenge in the untreated group. Similarly, the number of immature erythrocytes increased and gradually replaced erythrocytes because of the influence of bacteria (Clauss *et al.* 2008). The erythrocyte aberrations were only mild, with the persistence of crenated, teardrop, and elongated cells at DPI 24. The increase or decrease in hematological parameters is often uneven, which helps the fish body find ways to cope with and adapt to environmental conditions (Esmaeili *et al.* 2021). They can inhibit bacterial action in the body to reduce the hemolysis of erythrocytes, as was observed in the challenge experiment with *S. agalactiae*. During and after infection, FFC at 15 mg kg biomass^{-1} day^{-1} helped stimulate the improvement of erythrocytes and their anomalies.

The pathological changes observed in the kidneys of untreated *O. niloticus* suggested nephropathy with severe tissue-level alterations. Several earlier reports revealed that *S. agalactiae* caused systemic infection in tilapia (Amal and Zamri-Saad 2011; Adikesavalu *et al.* 2017; Mishra *et al.* 2018). Histopathological lesions observed on DPI 1 in the kidney tissues of Sa-challenged fish corroborated previously documented kidney alterations in *S. aga-*

lactiae-infected *O. niloticus* (Adikesavalu *et al.* 2017), *O. mossambicus* (Abraham *et al.* 2019), and *Oreochromis* spp. (Alsaid *et al.* 2013). At DPI 10, the challenged groups presented a significant increase in the intensity of histopathological alterations, possibly due to bacterial invasion, release of exotoxins and renal dysfunction. Similar outcomes were documented in *Sciaenops ocellatus* against *S. iniae* (Eldar *et al.* 1999). During the 10 days of FFC treatment, the kidney tissue-level anomalies in the Sa-challenged *O. niloticus* resulted in improved and enhanced architecture and organization of nephritic tubules compared with the untreated group. These findings indicated that FFC therapy can result in enhanced functionality of kidneys carrying infectious agents. The results supported the observations of Sherif *et al.* (2022), who recorded almost similar creatinine levels, and the histopathological alterations of kidney tissues of Sa-infected and FFC-treated groups. At DPI 24, the maximum recovery of renal tissues was noted in the treated group compared with the untreated group, which exhibited a significant decrease in plasma creatinine, supporting the beneficial impact of FFC therapy. The liver is seldom used in pathological studies of *S. agalactiae* infection in tilapia, even though the bacteria could induce significant pathological damage (Isiaku *et al.* 2017). Hepatic tissues of the Sa-challenged groups at DPI 1 along with the damaged liver cells and the release of large quantities of enzymes such as ALT and AST into the blood, indicated liver damage similar to that caused by *S. iniae* infection (Chen *et al.* 2004), and *S. agalactiae* infection (Abuseliana *et al.* 2010; Alsaid *et al.* 2013) in *Oreochromis* spp. The abnormal changes in the treated group gradually decreased after 17 DPI but were faster than those in the untreated group. These results indicated that the treated fish were able to mount adaptive responses to overcome the hepatopathic effect of *S. agalactiae* and protect the liver tissues. These histopathological findings suggested marked improvements in the liver tissues of *S. agalactiae*-infected and FFC-treated *O. niloticus*. The accumulation of FFC residues in fish tissues (Bardhan *et al.* 2022a) might have reduced the potency and virulence of *S. agalactiae*. Similarly, the treatment with FFC and *Spirulina platensis* significantly impacted the liver and renal tissues, as the values of liver enzymes and creatinine demonstrated tissue deterioration as a result of oxidative stress (Abu-Zahra *et al.* 2024).

The present study further assessed the rates of wound progression and healing in Sa-challenged and FFC-treated *O. niloticus*. Minor tissue damage and subepithelial wounds were obvious after 72 hours of challenge. Tissue reddening, inflammation and formation of a membrane over the wound at DPI 7 indicated the initial protective reactions of the fish to ward off the bacterial challenge in the treated group. Afterwards, there was a notable decrease in wound progression in the treated (DPIs 7 – 8) and untreated (DPIs 7 – 12) groups. Subsequently, the damage was reverted to normal. Compared with the untreated group, the wounds of FFC-fed groups healed faster. The melanin within the dermis of *O. niloticus* indicated mature healing of the ulcer at the site of injection. The areas surrounding the wounds became very dark at 9 DPI in the treated group possibly because of the increased number of melanocytes, suggesting increased melanocyte activity after injury. The disappearance of the black scar, the onset of dermal fibrous tissue regrowth and the development of skin at the ulcerated scar region were observed at DPI 10, indicating regeneration of the muscle tissue in the treated group, thus, supporting the observations of Agius and Robert (2003). The repair of dermal and muscle structures took a much shorter time than the repair of the epidermis because no deeper wounds were observed. The epidermis gradually thinned with the development of new scales at the site of injection during the recovery period (DPI 12), which was an indication of faster tissue regrowth than that in the untreated group. Similarly, Bereiter-Hahn and Zylberberg (1993) and Ohira *et al.* (2007) observed complete regrowth of tissue and new scales within a few weeks of time-course changes in calcium and phosphorus during the scale regeneration. The restoration of normal skin architecture was achieved after DPI 13 in both groups. The results confirmed that the degree of wound healing promoted by FFC therapy was more prominent during the treatment period.

5 | CONCLUSIONS

Streptococcus agalactiae infection has a major impact on aquaculture, and its control measures involve the use of antibiotics. FFC is an approved drug of choice for treating *Streptococcus* in fish. The present study demonstrated the potential and effectiveness of FFC therapy against *S. agalactiae* infection in *O. niloticus*. FFC treatment at 15 mg kg biomass^{-1} day^{-1} for 10 successive days by the oral route improved the survival, plasma biochemistry, hematology, erythro-morphology, and histoarchitecture of the vital organs, indicating the positive impact of FFC on enhancing the functions and physiological status of the infected fish. The observations on the efficient therapy against *S. agalactiae* infection suggested that FFC can be safely recommended for commercial use in *O. niloticus* farming and other aquaculture activities. However, vaccines, natural and eco-friendly products, and immunostimulants as probable adjuvants to FFC in *O. niloticus* diets may help increase disease resistance and reduce antibiotic usage in aquaculture and the spread of antibiotic resistance. Nevertheless, the results of the present study provided valuable scientific data on the efficacy of FFC against *S. agalactiae* infection to tropical aquaculturists, policymakers, and regulatory authorities. The reported findings on the morphological alterations in the erythrocytes of *O. niloticus* in response to the Sa-

challenge marked a stepping stone for subsequent analyses in future research.

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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.

AUTHORS' CONTRIBUTION

Joshi Sharon, Arya Sen, Ratnapriya Das, Priyanka Sinha: Formal analysis, data generation, statistical analyses, data interpretation, writing-original draft; Thangapalam Jawahar Abraham: Conceptualization, experimental design, data curation, resources, project administration, writing - review and editing of the manuscript; Satyanarayana Boda: Statistical analyses; Arumugam Uma: Resources, project administration; Prasanna Kumar Patil: Resources, project administration and funding acquisition.

DATA AVAILABILITY STATEMENT

The data supporting this study's findings are available on request from the corresponding author. The data are not publicly available due to privacy restrictions.

REFERENCES

Aboyadak IM, Ali NG, Abdel-Aziz MM, Gado MS, El-Shazly KA (2016) Role of some antibacterial drugs in control *Streptococcus iniae* infection in *Oreochromis niloticus*. Asian Journal of Pharmaceutical and Clinical Research 1(5): 555–573.

Abraham TJ, Namdeo MS, Adikesavalu H, Banerjee S (2019) Pathogenicity and pathology of *Streptococcus agalactiae* in challenged Mozambique tilapia *Oreochromis mossambicus* (Peters 1852) juveniles. Aquatic Research 2(4): 182–190.

Abuseliana A, Hassan D, Saleha AA, Siti KB, Milud A (2010) *Streptococcus agalactiae* is the etiological agent of mass mortality in farmed red tilapia (*Oreochromis* sp.). Journal of Animal and Veterinary Advances 9(20): 2640–2646.

Abu-Zahra NI, Elseify MM, Atia AA, Al-sokary ET (2024). Impacts of florfenicol on immunity, antioxidant activity, and histopathology of *Oreochromis niloticus*: a potential protective effect of dietary *Spirulina platensis*. Veterinary Research Communication 48: 125–138.

Adikesavalu H, Banerjee S, Patra A, Abraham TJ (2017) Meningoencephalitis in farmed monosex Nile tilapia (L.) caused by *Streptococcus agalactiae*. Fisheries and Aquatic Life 25(3): 187–200.

Agius C, Roberts RJ (2003) Melano-macrophage centers and their role in fish pathology. Journal of Fish Diseases 26(9): 499–509.

Agnew W, Barnes AC (2007) *Streptococcus iniae* an aquatic pathogen of global veterinary significance and a challenging candidate for reliable vaccination. Veterinary Microbiology 122(1–2): 1–15.

Alsaid M, Abuseliana AF, Daud HH, Mustapha NM, Bejo SK, ... Hamdan RH (2014) Hematological, biochemical and clinical signs changes following experimental infection of *Streptococcus agalactiae* in red hybrid tilapia (*Oreochromis* sp.). Aquacultura Indonesiana 4(9): 289–295.

Alsaid M, Daud HHM, Mustapha NM, Bejo SK, Abdelhadi YM, ... Hamdan RH (2013) Pathological findings of experimental *Streptococcus agalactiae* infection in red hybrid tilapia (*Oreochromis* sp.) (pp. 70–73). In: Proceedings of the International Conference on Chemical, Agricultural and Medical Sciences. CAMS-2013, International Institute of Chemical, Biological & Environmental Engineering, Kuala Lumpur, Malaysia.

Amal MNA, Zamri-Saad M (2011) Streptococcosis in tilapia (*Oreochromis niloticus*): a review. Pertanika Journal of Tropical Agricultural Science 34(2): 195–206.

Amal MNA, Zamri-Saad M, Siti-Zahrah A, Zulkafli AR (2013) Transmission of *Streptococcus agalactiae* from a hatchery into a newly established red hybrid tilapia, *Oreochromis niloticus* (L.) × *Oreochromis mossambicus* (Peters), farm. Journal of Fish Diseases 36(8): 735–739.

Assane IM, Gozi KS, Valladão GMR, Pilarski F (2019) Combination of antimicrobials as an approach to reduce their application in aquaculture: emphasis on the use of thiamphenicol/florfenicol against *Aeromonas hydrophila*. Aquaculture 507: 238–245.

Assefa A, Abunna F (2018) Maintenance of fish health in aquaculture: review of epidemiological approaches for prevention and control of infectious disease of fish. Veterinary Medicine International 2018: 5432497.

Bardhan A, Abraham TJ, Singha J, Saha S, Sarker S, Patil PK (2022b) The effects of extended feeding of florfenicol-coated medicated diets on the safety, serum biomarkers and blood cells morphology of Nile tilapia *Oreochromis niloticus* (L.). Environmental Science and Pollution Research 29: 39914–39927.

Barham WT, Smit GL, Schoonbee HJ (1980) The hematological assessment of bacterial infection in rainbow trout, *Salmo gairdneri* Richardson. Journal of Fish

Biology 17(3): 275–281.
Bereiter-Hahn J, Zylberberg L (1993) Regeneration of teleost fish scale. Comparative Biochemistry and Physiology 105A: 625–641.
Bernet D, Schmidt H, Meier W, Burkhardt-Holm P, Wahli T (1999) Histopathology in fish: proposal for a protocol to assess aquatic pollution. Journal of Fish Diseases 22(1): 25–34.
Bojarski B, Lutnicka H, Swadźba-Karbowy M, Makulska J, Jakubiak M, ... Witeska M (2018) Effects of herbicides pendimethalin and ethofumesate on common carp (*Cyprinus carpio*) erythrocyte morphology. Folia Biologica (Kraków) 66(3): 143–149.
Bondad-Reantaso MG, Arthur JR, Subasinghe RP (Eds) (2012) Improving biosecurity through prudent and responsible use of veterinary medicines in aquatic food production. FAO Fisheries and Aquaculture Technical Paper. No. 547. Food and Agriculture Organization of the United Nation, Rome. 207 pp.
Bowker JD, Carty D, Bowman MP (2013) The safety of Aquaflor (50% florfenicol) administered in feed to fingerling yellow perch. North American Journal of Aquaculture 75(4): 517–523.
Bowser PR (1993) Clinical pathology of salmonid fishes (pp. 327–332.). In: Stoskopf M (Ed) Fish medicine, W.B. Saunders, Philadelphia, Pennsylvania.
Bowser PR, Wooster GA, Getchell RG, Timmons MB (1998) *Streptococcus iniae* infection of tilapia *Oreochromis niloticus* in a recirculation production facility. Journal of the World Aquaculture Society 29(3): 335–339.
Chen CY, Wooster GA, Bowser PR (2004) Comparative blood chemistry and histopathology of tilapia infected with *Vibrio vulnificus* or *Streptococcus iniae* or exposed to carbon tetrachloride, gentamicin or copper sulfate. Aquaculture 239(1–4): 421–443.
Chen D, Wang K, Geng Y, Wang J, Huang L, Li J (2011) *Streptococcus iniae* isolated from channel catfish (*Ictalurus punctatus)* in China. Israeli Journal of Aquaculture-Bamidgeh 63(1): 593–600.
Chen J, Sun R, Pan C, Sun Y, Mai B, Li QX (2020) Antibiotics and food safety in aquaculture. Journal of Agricultural and Food Chemistry 68(43): 11908–11919.
Clauss TM, Dove AD, Arnold JE (2008) Hematologic disorders of fish. Veterinary Clinics of North America: Exotic Animal Practice 11(3): 445–462.
Darwish AM (2007) Laboratory efficacy of florfenicol against *Streptococcus iniae* infection in sunshine bass. Journal of Aquatic Animal Health 19(1): 1–7.
Eldar A, Perl S, Frelier PF, Bercovier H (1999) Red drum *Sciaenops ocellatus* mortalities associated with *Streptococcus iniae* infection. Diseases of Aquatic Organisms 36(2): 121–127.
El-Sayed AFM (2019) Tilapia culture, 2nd edition. Academic Press, London.
Engle CR, van Senten J, Clark C, Boldt N (2023) Has the regulatory compliance burden reduced the competitiveness of the US tilapia industry? Fishes 8(3): 151.
Esmaeili M (2021) Blood performance: a new formula for fish growth and health. Biology 10(12): 1236.
FAO (2022) The state of world fisheries and aquaculture 2022, towards blue transformation. Food and Agriculture Organization of the United Nation, Rome.
FAO (2023) OECD-FAO Agricultural outlook 2023-2032. OECD Publishing, Paris.
Faria FC, Leal CAG, Carvalho-Castro GA, Leite RC, Figueiredo HCP (2013) Carrier state induced by oxytetracycline therapy against streptococcosis in Nile tilapia, *Oreochromis niloticus* (L.). Journal of Fish Diseases 37(9): 853–857.
Feng JB, Jia XP (2009) Single dose pharmacokinetic study of florfenicol in tilapia (*Oreochromis niloticus* × *O. aureus*) held in freshwater at 22°C. Aquaculture 289(1–2): 129–133.
Haenen OLM, Dong HT, Hoai TD, Crumlish M, Karunasagar I, ... Bondad-Reantaso MG (2023) Bacterial diseases of tilapia, their zoonotic potential and risk of antimicrobial resistance. Reviews in Aquaculture 15: 154–185.
Isiaku AI, Sabri MY, Ina-Salwany MY, JHassan MD, Tanko PN, Bello MB (2017) Biofilm is associated with chronic streptococcal meningoencephalitis in fish. Microbial Pathogenesis 102: 59–68.
Junge W, Wilke B, Halabi A, Klein G (2004) Determination of reference intervals for serum creatinine, creatinine excretion and creatinine clearance with an enzymatic and a modified Jaffe method. International Journal of Clinical Chemistry 344(1–2): 137–148.
Limbu SM, Zhou L, Sun SX, Zhang ML, Du ZY (2018) Chronic exposure to low environmental concentrations and legal aquaculture doses of antibiotics cause systemic adverse effects in Nile tilapia and provoke differential human health risk. Environment International 115: 205-219.
Martins ML, Shoemaker CA, Xu D, Klesius PH (2011) Effect of parasitism on vaccine efficacy against *Streptococcus iniae* in Nile tilapia. Aquaculture 314(1–4): 18–23.
Martins ML, Vieira FN, Jeronimo GT, Mourino JL, Dotta G, ... Pereira G (2009) Leukocyte response and phagocytic activity in Nile tilapia experimentally infected with *Enterococcus* sp. Fish Physiology and Biochemistry 35: 219–222.
Michaylova V, Ilkova P (1971) Photometric determination of micro amounts of calcium with arsenazo III. Analytica Chimica Acta 53(1): 194–198.
Mishra A, Nam GH, Gim JA, Lee HE, Jo A, Kim HS (2018) Current challenges of *Streptococcus* infection and effective molecular, cellular, and environmental control methods in aquaculture. Molecules and Cells

41(6): 495–505.
Oda SS, Tohamy HG, Massoud RG (2016) Pathological alterations in Nile tilapia experimentally infected with *Streptococcus iniae* and *Candida albicans*. Turkish Journal of Fisheries and Aquatic Sciences 16(4): 779–788.
Ohira Y, Shimizu M, Ura K, Takagi Y (2007) Scale regeneration and calcification in goldfish *Carassius auratus*: quantitative and morphological processes. Fisheries Science 73(1): 46–54.
Patel JB, Abraham TJ, Bardhan A, Das R, Sen A, ... Patil PK (2024) Efficacy of oxolinic acid against *Aeromonas hydrophila* infection in Nile tilapia *Oreochromis niloticus* juveniles. Aquaculture Studies 24(3): AQUAST1653.
Patil PK, Mishra SS, Pradhan PK, Manna SK, Abraham TJ, ... Jena J (2022) Usage pattern of chemicals, biologicals and veterinary medicinal products in Indian aquaculture. Reviews in Aquaculture 14(4): 2038–2063.
Reda RM, Ibrahim RE, Ahmed EG, El-Bouhy ZM (2013) Effect of oxytetracycline and florfenicol as growth promoters on the health status of cultured *Oreochromis niloticus*. Egyptian Journal of Aquatic Research 39(4): 241–248.
Reed LJ, Muench H (1938) A simple method of estimating fifty per cent endpoints. American Journal of Epidemiology 27(3): 493–497.
Roberts RJ (2012) Fish Pathology, 4th edition. John Wiley and Sons, USA.
Schmidt H, Goetz K, Hallmann I, Holt CV (1961) The course of resynthesis of blood glucose. Biochemical Journal 334: 534–544.
Schoenfeld RG, Lewellan CJ (1964) A colorimetric method for determination of serum chloride. Clinical Chemistry 10(6): 533–539.
Sherif AH, Abdellatif JI, Elsiefy MM, Gouda MY, Mahmoud AE (2022) Occurrence of infectious *Streptococcus agalactiae* in the farmed Nile tilapia. Egyptian Journal Aquatic Biology Fisheries 26(3): 403–432.
Straus DL, Bowker JD, Bowman MP, Carty D, Mitchell AJ, Farmer BD (2012) Safety of aquaflor-medicated feed to sunshine bass. North American Journal of Aquaculture 74(1): 1–7.
Thuong HN, Tran TLN, Le TNT, Vo VT (2022) Hematological parameters of red tilapia (*Oreochromis* sp.) fed lemongrass essential oil (*Cymbopogon citratus*) after challenge with *Streptococcus agalactiae*. Israeli Journal of Aquaculture 55(3): 1123–1130.
Tietz NW (1994) Accuracy in clinical chemistry--does anybody care? Clinical Chemistry 40(6): 859–861.
USFDA (2023) Approved aquaculture drugs. U.S. Food and Drug Administration. https://www.fda.gov/animal-veterinary/aquaculture/approved-aquaculture-drugs. Accessed on 13 October 2023.
Vo VT, Tran TH, Nguyen TTT, Truong VT, Pham CT, ... Thuong HNT (2022) Hematological parameters of red tilapia (*Oreochromis* sp.) fed essential oils of *Mentha piperita* after challenge with *Streptococcus agalactiae*. Pakistan Journal of Zoology 2022: 1–8.
Wardani WW, Alimuddin A, Junior MZ, Setiawati M, Nuryati S, Suprayudi MA (2021) Growth performance, fillet quality and immunity after challenge with *Streptococcus agalactiae* of red tilapia (*Oreochromis* sp.) fed diet containing cysteamine and creatine. Aquaculture Research 52(9): 4237–4248.
Wolf PL, Williams D, Coplon N, Coulson AS (1972) Low aspartate transaminase activity in serum of patients undergoing chronic hemodialysis. Clinical Chemistry 18(6): 567–568.

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