Aquaculture Studies, 24(3), AQUAST1653
http://doi.org/10.4194/AQUAST1653

RESEARCH PAPER

Efficacy of Oxolinic Acid Against *Aeromonas hydrophila* Infection in Nile tilapia *Oreochromis niloticus* Juveniles

Jaykumar Bhagubhai Patel[1], Thangapalam Jawahar Abraham[1,*], Avishek Bardhan[1], Ratnapriya Das[1], Arya Sen[1], Satyanarayana Boda[2], Prasanna Kumar Patil[3]

[1]Department of Aquatic Animal Health, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Chakgaria, Kolkata 700094, West Bengal, India
[2]School of Fisheries and Aquaculture Sciences, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia.
[3]Aquatic Animal Health and Environment Division, ICAR-Central Institute of Brackishwater Aquaculture, Raja Annamalai Puram, Chennai 600028, Tamil Nadu, India

How to Cite

Patel, J.B., Abraham, T.J., Bardhan, A., Das, R., Sen, A., Boda, S., Patil, P.K. (2024). Efficacy of Oxolinic Acid Against *Aeromonas hydrophila* Infection in Nile tilapia *Oreochromis niloticus* Juveniles. *Aquaculture Studies*, *24(3), AQUAST1653*. http://doi.org/10.4194/AQUAST1653

Article History
Received 17 August 2023
Accepted 21 September 2023
First Online 30 October 2023

Corresponding Author
Tel: +919433368328
E-mail: abrahamtj1@gmail.com

Keywords
Antibiotics
Aquaculture
Fish health
Histopathology
Plasma biochemistry

Abstract

Oxolinic acid (OA), a broad-spectrum quinolone antibiotic that plays a crucial role in human medicine, is often used in aquaculture as a second-line treatment at 12 mg/kg biomass/day continuously for seven days. The objective of this study was to assess its effectiveness against *Aeromonas hydrophila* infection in Nile tilapia *Oreochromis niloticus* in terms of progression and healing of wounds, clinical biochemistry and histopathological changes upon challenge and treatment. To evaluate the efficacy, the juveniles were intramuscularly challenged with *A. hydrophila* at a sublethal dose of 1.93×10^6 cells/fish, followed by the administration of OA-supplemented feed at 2% body weight daily for seven days. OA treatment yielded better consequences in terms of increased feed intake and biomass and decreased plasma biochemical parameters, histopathological changes and improved recovery from *A. hydrophila* infection compared to the untreated group. Wounds of the OA-treated group healed at a faster rate, with complete healing within 12 days post-injection, while the wounds of the untreated group took longer to heal. Though the efficacy of OA against *A. hydrophila* infection in tilapia was established in this study, it is important to exercise caution regarding its application in aquaculture due to its classification as a critically important medicine for humans.

Introduction

Tilapias are commercially cultivated in >140 countries, making up approximately 11.20% of the global share in 2020. It holds the second position, following carps, in terms of sales and volume in international trade within the fish farming industry (FAO, 2022). Interestingly, despite their origins, 98% of tilapia production comes from countries outside their native habitat in Africa. The Nile tilapia *Oreochromis niloticus* is the most significant variety of farmed tilapia, accounting for 80% of the total tilapia production in 2020 (FAO, 2022), primarily due to its adaptability and resilience. Approximately 177 kg of tilapia were produced every second, with *O. niloticus,* the third most farmed fish species globally, reaching a production rate of 4514 thousand tonnes, equivalent to 143.15 kg per second in 2020 (FAO, 2022). Therefore, tilapias are often likened to aquatic chicken due to their resemblance to chicken as farmed animals (Maclean, 1984). Since their introduction to India in 1952 (NFDB, 2015) and recognizing the successful tilapia farming ventures, the

Indian government identified tilapia farming as a key sector in aquaculture (Menaga & Fitzsimmons, 2017). In recent times, aquaculture has faced significant challenges in contributing to food security, nutrition and poverty reduction due to various disease outbreaks (both exotic and endemic) and biosecurity issues (FAO, 2022). Infectious diseases pose a constant threat, leading to substantial losses in fish stocks and negatively impacting the livelihoods of farmers. Among the major bacterial diseases affecting tilapia aquaculture, motile *Aeromonas* septicemia (MAS), *Pseudomonas* septicemia, haemorrhagic septicemia, streptococcosis, columnaris, francisellosis, edwardsiellosis and piscirickettsiosis are the most important. These diseases have been responsible for more than 80% of mortality and significant production losses (El-Sayed, 2019; Haenen *et al.*, 2023).

In tilapia farming, MAS is commonly associated with various bacterial species, with *Aeromonas hydrophila* being the most prevalent (Julinta *et al.*, 2017; Nicholson *et al.*, 2020; Gewaily *et al.*, 2021). In the Indian context, tilapia farming has encountered various bacterial infections, specifically, the prevalence of MAS and streptococcal infections has risen due to the intensification (Adikesavalu *et al.*, 2017; Raj *et al.*, 2019). Oxolinic acid (OA), a broad-spectrum quinolone antibiotic, is currently not approved for use in fish production in the United States due to concerns about the potential selection of antibiotic-resistant bacteria that could pose a threat to human health (USFDA, 2022). However, it is recommended and utilized in several European countries as well as Japan for the prevention of bacterial infections in aquatic species such as trout, salmon, tilapia and shrimp (EMEA, 2005; Quesada *et al.*, 2013; Lulijwa *et al.*, 2020). While the effectiveness and safety of OA on temperate fish have been established, there is a lack of studies on tropical fish species. Therefore, the purpose of this study was to assess the effectiveness of OA in *O. niloticus* against *A. hydrophila* infection, focusing on plasma biomarkers, histopathological changes in the kidney, liver and spleen, as well as wound progression and healing.

Materials and Methods

Experimental Fish

The healthy and active Nile tilapia *Oreochromis niloticus* juveniles were obtained from Sonarpur, South 24 Parganas district, West Bengal. The fish were transported to the laboratory, disinfected and acclimated for 15 days in 500-L circular tanks with continuous aeration. Throughout the study, chlorine-free aerated bore-well water was used.

Bacterial strain and its pathogenicity

Aeromonas hydrophila AAH-28 strain used in this study was from the collections of the Department of Aquatic Animal Health, West Bengal University of Animal and Fishery Sciences, Kolkata. The revival of the glycerol stock of this strain, culture maintenance and pathogenicity testing by intramuscular injection were described in Julinta *et al.* (2017). The Reed & Muench (1938) method was followed for the lethal dose (LD_{50}) determination.

Efficacy of Oral Oxolinic Acid Therapy Against *Aeromonas Hydrophila* Infection

The recommended dose and dosage of oxolinic acid (OA) is 12 mg/kg biomass/day for 7 consecutive days (EMEA, 2005). The medicated feed containing OA (Sigma, India) and control feeds were prepared for feeding *O. niloticus* juveniles at 2% body weight (BW) as per our earlier reports (Julinta *et al.*, 2017; Bardhan *et al.*, 2022). The experiment was conducted using plastic tanks with dimensions of L58 × H45 × B45 cm and juveniles of size 8.39±0.67 cm and 15.20±0.72 g. Before use, the tanks were thoroughly cleaned, dried, filled with clean water to a volume of 80 L and conditioned for three days. After the conditioning, the nine tanks were stocked with 25 fish/tank, sourced from the acclimatized stocks, properly labelled, and covered with nylon netting for adequate protection. The fish were fed a control feed daily at 2% BW before the challenge. Approximately 50% of the water was exchanged regularly, and any leftover feed and faecal matter were removed daily. Throughout the experiment, regular measurements of physicochemical parameters were taken to ensure optimal conditions.

The experimental fish were divided into three groups in triplicates. Group 1 served as the unchallenged control. Group 2 was challenged and OA feed-fed (treated). Group 3 was challenged and fed a control feed (untreated). After three days of acclimatization, the fish of groups 2 and 3 were intramuscularly injected at the base of the dorsal fin with 0.1 ml of *A. hydrophila* cell suspension to achieve a sub-lethal dose of 1.93×10^6 cells/fish. The fish of group 1 were intramuscularly injected with 0.1 ml saline and served as unchallenged control. Following the injection, the fish were transferred to their respective tanks. All fish groups were provided with the appropriate feeds at 2% BW thrice daily. The treatment period for group 2 lasted for 7 days post-injection (DPI), during which period OA feed was offered. Following the treatment period, fish were switched to the control feed. The fish of groups 1 and 2 were fed a control feed. Any unconsumed feed in the tank was removed daily, air-dried and weighed accurately. Daily observations include mortality, external signs of infections, feed consumption and behavioural changes in the fish.

Wound Progression and Healing

The wounds at the site of injection were captured daily using a digital camera throughout the experimental

period. The extent of tissue damage was assessed using a scoring system ranging from 0 to 6, as outlined in the scale proposed by Bernet *et al.* (1999). The scoring was based on the degree and extent of damage observed. The progression and healing of the wounds were qualitatively assessed on an ordinal scale as follows: 0: no damage or undamaged with no pathological significance, 0.5: very mild damage with little or no pathological importance, 1: very mild damage with minimal pathological importance, 2: mild damage with minimal pathological importance, 4: moderate damage with moderate pathological importance and 6: severe damage with marked pathological importance. Intermediate values were also taken into consideration for the assessment.

Collection of Blood and Plasma

Two fish were arbitrarily removed from each tank and blood was collected by caudal puncture using 2 mL sterile syringes (Roberts, 2012). The blood was transferred immediately to 1.5 mL Eppendorf tubes that had been rinsed with a 5% ethylene diamine tetra acetic acid (EDTA) anticoagulant. The plasma was obtained by centrifuging at 4500 rpm for 15 min, carefully transferred to Eppendorf tubes and stored at -20°C to facilitate further analysis.

Plasma Biochemistry

Plasma glucose was measured using the glucose GOD FS kit through the glucose dehydrogenase photometric method (Schmidt *et al.*, 1961). The plasma calcium was determined photometrically using the calcium AS FS kit following the arsenazo III method (Michaylova & Ilkova, 1971). The ferric (III) perchlorate method was used to quantify plasma chloride photometrically using the chloride 21 FS kit (Young *et al.*, 1975). The plasma creatinine was determined using the creatinine test kit following the modified Jaffe's reaction and the initial rate assay method (Young *et al.*, 1975). The plasma alanine transaminase (ALT) and aspartate transaminase (AST) were measured using the ALT and AST kits through the modified UV (IFCC) and kinetic assay methods (Wolf *et al.*, 1972). The plasma alkaline phosphatase (ALP) was determined by the ALP FS kit following the kinetic photometric test method (Tietz, 1994). All the kits used for measuring plasma parameters are manufactured by Erba Diagnostics Ltd., Mannheim, Germany.

Histopathology

The kidney and liver samples of *A. hydrophila* AAH-28 challenged and OA-treated, untreated and control *O. niloticus* were fixed in Bouin's solution for 24 h. The fixed samples were subjected to conventional processing and embedded in paraffin wax. Thin sections of about 5 µm size were prepared and stained with eosin and haematoxylin (Roberts, 2012). The sections were scanned and photomicrographed in 200× magnification in a trinocular research microscope (Olympus, Japan, Model: BX51) to assess the abnormalities.

Statistical Analyses

The data were expressed as mean ± standard deviation. Feeding behaviour scores were analyzed using the non-parametric Kruskal Wallis test with pair-wise comparisons. The data relating to mortalities, biomass and plasma biomarkers were analyzed by one-way ANOVA and the significance of differences among the treatments and days was confirmed by the Tukey posthoc test for comparing means. The qualitative scores of wound progression and healing within and among the challenge groups were analyzed using related samples Friedman ANOVA for dependent samples and the Mann-Whitney U test for independent samples. All statistical analyses were performed using the Statistical Package for Social Sciences (IBM-SPSS), version 22.0, with a significance level set at $P<0.05$.

Results

Pathogenicity of *Aeromonas hydrophila* AAH-28 on *Oreochromis niloticus*

The clinical and behavioural signs and mortalities were recorded daily for up to 15 DPI. The challenged *O. niloticus* exhibited initial signs of lethargy, abnormal behaviour, wandering around the corners, resting at the bottom and vertical swimming. Inflammation and haemorrhages were observed at the site of injection. Within 72 h of injection, *A. hydrophila* at 1.03×10^8 cells/fish caused 80% mortalities. About 50%, 10% and 5%, mortalities were observed in fish injected with 10^7, 10^6 and 10^5 cells/fish, respectively. The LD_{50} value for *A. hydrophila* AAH-28 strain was estimated to be 1.93×10^7 cells/fish.

Efficacy of oxolinic Acid Against *Aeromonas hydrophila* AAH-28

The challenged *O. niloticus* juveniles were weak, lethargic, hanging and/or lying at the bottom of the tank. The feed intake was 100% in the control group, which reduced significantly to 87.54±0.80% and 80.25±0.36% in the OA-treated and untreated groups, respectively until 21 DPI. Only minor but insignificant moralities in the range of 4.00±4.00 - 5.33±4.62% were recorded in both challenge groups. The biomass recorded in the control, OA-treated and untreated groups were 193.90±7.43 g, 173.83±1.26g and 164.67±5.86 g, respectively, which differed significantly ($P<0.05$). However, the biomass of the OA-treated and untreated groups differed insignificantly ($P>0.05$) on DPI 21.

Wound Progression and Healing

The challenged fish exhibited abnormal movement, haemorrhages with loss of scales at the injected site, skin peeling with a haemorrhagic lesion, pale gills, haemorrhagic opercular region, tail rot and darkening of the body colour. Within 24 h, tissue reddening, inflammation and skin peeling at the site of injection became evident, along with the appearance of open sub-epithelial wounds. However, with the administration of OA therapy, the reddening and inflammation gradually diminished and a black scar formed in the ulcerated area. In the next three days, the areas surrounding the wound became very dark. On day 5 of OA therapy, all examined wounds were closed with the development of new skin and scales. By 7 DPI, the black scar disappeared and dermal fibrous tissue regrowth was evident, along with the formation of new skin at the ulcerated scar region. On 14 DPI, the black scars disappeared completely at the injection site. Notably, the wounds of the OA-treated group healed faster compared to the untreated group (Figure S1, S2; Table 1).

Plasma Biomarkers

The plasma glucose levels of the challenged groups increased significantly from 40.00±2.00 mg/dL ($P<0.05$) and peaked on DPI 1. It reduced significantly ($P<0.05$) thereafter and reached near normal level on 21 DPI, but the levels were still high compared to day 0. The untreated group had higher glucose compared to the OA-treated group on DPI 21 (Figure 1A). The calcium levels reduced insignificantly ($P>0.05$) on DPI 1 in both OA-treated and untreated groups. Later, its level increased and reached near normal level on DPI 21 (Figure 1B). The plasma chloride was also reduced in both groups on DPI 1, which increased later till DPI 21. Yet, the levels were still lower than on day 0 (Figure 1C). The plasma creatinine of the OA-treated group reached a peak on DPI 1, followed by a reduction with time till DPI 21. A similar trend with significantly higher levels ($P>0.05$) on DPI 21 was noted in the untreated group. However, the creatinine levels of both groups did not recoup fully (Figure 2A). An increment in plasma ALP was observed on DPI 1 in both groups from 11.33±2.08 IU/L. The ALP of the untreated group was significantly higher than the treated group on all days of observation ($P<0.05$). Though the ALP levels of both groups reduced significantly ($P<0.05$) on DPI 21, the levels were still higher compared to day 0 (Figure 2B). A significant increase in plasma AST ($P<0.05$) was observed in challenged groups on DPI 1, after which their levels were reduced. The AST levels of both groups returned to near normal on DPI 21 (Figure 2C). On DPI 1, the plasma ALT levels of challenged groups were significantly higher ($P<0.05$), which reduced significantly with time. However, the levels did not reach a normal state on DPI 21 (Figure 2D).

Histopathological Changes in the Kidney and Liver Tissues

Table 2 presents the qualitative histopathological scoring of the alterations in the kidney and liver tissues of *A. hydrophila*-challenged *O. niloticus* with or without OA treatment in comparison to the control (Figure 3, 4). The kidney tissues of control tilapia showed a normal histo-structural organization of the renal tubules with a well-organized glomerulus (Figure 3A). On DPI 1, the kidney tissues of the OA-treated group exhibited degeneration of renal tubules (3.49±0.13), hydropic swelling (3.23±0.16), haemocyte infiltration (1.82±0.26), necrotized area (1.43±0.18) and wide lumen (1.15±0.23) (Figure 3B). Subsequently, significant reductions ($P<0.05$) were observed in all histopathological alterations of the kidney structure on DPI 7 and 14 in the treated group (Figure 3D, F). On DPI 21, recovery of the renal tissues was noted in the treated group, although mild degeneration of renal tubules (1.25±0.10), hydropic swelling (1.20±0.13) and haemocyte infiltration (0.94±0.16) persisted (Figure 3H). In contrast, the untreated group documented significantly higher degrees ($P<0.05$) of alterations compared to the treated

Table 1. The rate of wound progression and healing in *Aeromonas hydrophila*-challenged *Oreochromis niloticus* juveniles fed oxolinic acid (OA) at 12 mg/kg biomass/day for 7 consecutive days during the treatment regime in comparison with control

Treatment days	Wound progression and healing score upon intramuscular challenge	
	OA treated*	Untreated**
Day 0	0.00±0.00	0.00±0.00
1 DPI	$3.05±0.21^{a3}$	$4.80±0.21^{b3}$
2 DPI	$2.45±0.33^{a23}$	$3.85±0.38^{b3}$
3 DPI	$1.50±0.40^{a123}$	$1.95±0.37^{a23}$
4 DPI	$1.05±0.11^{a123}$	$1.45±0.11^{b123}$
5 DPI	$0.75±0.18^{a12}$	$1.20±0.11^{b123}$
6 DPI	$0.50±0.25^{a1}$	$0.95±0.21^{b123}$
7 DPI	$0.25±0.00^{a1}$	$0.70±0.11^{b123}$
8 DPI	0.00±0.00	$0.50±0.00^{12}$
9 DPI	0.00±0.00	$0.25±0.18^{1}$
10 DPI	0.00±0.00	$0.15±0.14^{1}$
11-14 DPI	0.00±0.00	0.00±0.00

*: *Aeromonas hydrophila* challenged and OA treated; **: *Aeromonas hydrophila* challenged and untreated. DPI: day post-injection

group, such as degeneration of renal tubules (4.07±0.05), hydropic swelling (3.63±0.10), haemocyte infiltration (1.30±0.26), necrotized area (1.60±0.14) and wide lumen (1.63±0.25) on DPI 1 (Figure 3C). The untreated group showed slower recovery on DPI 7 and 14 compared to the treated group (Figure 3E, G). However, on DPI 21, a reduction in kidney tissue aberrations was documented, with the mild persistence of degeneration of renal tubules (2.22±0.21), hydropic swelling (2.03±0.10) and necrotized area (0.80±0.25) (Figure 3I).

Aeromonas hydrophila-challenge caused significant alterations in the hepatic tissues of *O. niloticus*. On DPI 1, the OA-treated group exhibited cytoplasmic vacuolation (3.96±0.22), lipid vacuolation (4.47±0.13) and degeneration of hepatocytes (3.05±0.15) compared to the control (Figure 4A, B). Subsequently, significant reductions ($P<0.05$) were observed in all histopathological alterations in the treated group (Figure. 4D, F, H). By DPI 21, the treated group showed near normal cytoplasmic vacuolation (1.47±0.10), lipid vacuolation (0.47±0.10) and degeneration of hepatocytes (1.18±0.25) (Figure 4H). In the untreated group, cytoplasmic vacuolation (4.12±0.09), lipid vacuolation (4.67±0.05) and degeneration of hepatocytes (3.55±0.10) were observed on DPI 1 (Figure 4C). This group exhibited slow recovery compared to other groups. By DPI 21, the changes like cytoplasmic vacuolation (2.97±0.16) and degeneration of hepatocytes (2.30±0.26) were mild to moderate, while the lipid vacuolation (0.93±0.10) was almost normal (Figure 4E, G, I).

Discussion

Aeromonas hydrophila is known for its consistent association with pathogenicity in various food fish species worldwide and is responsible for multiple infections (Austin & Austin, 2016; Sherif & Kassab, 2023). The determination of the LD_{50} value of the study organism before the experimental challenge is advantageous for ensuring successful experiments and inducing clinical signs and symptoms (Rey *et al.*, 2009). In the present study, when *A. hydrophila* AAH-28 was injected intramuscularly into healthy tilapia, inflammation and haemorrhages were observed at the

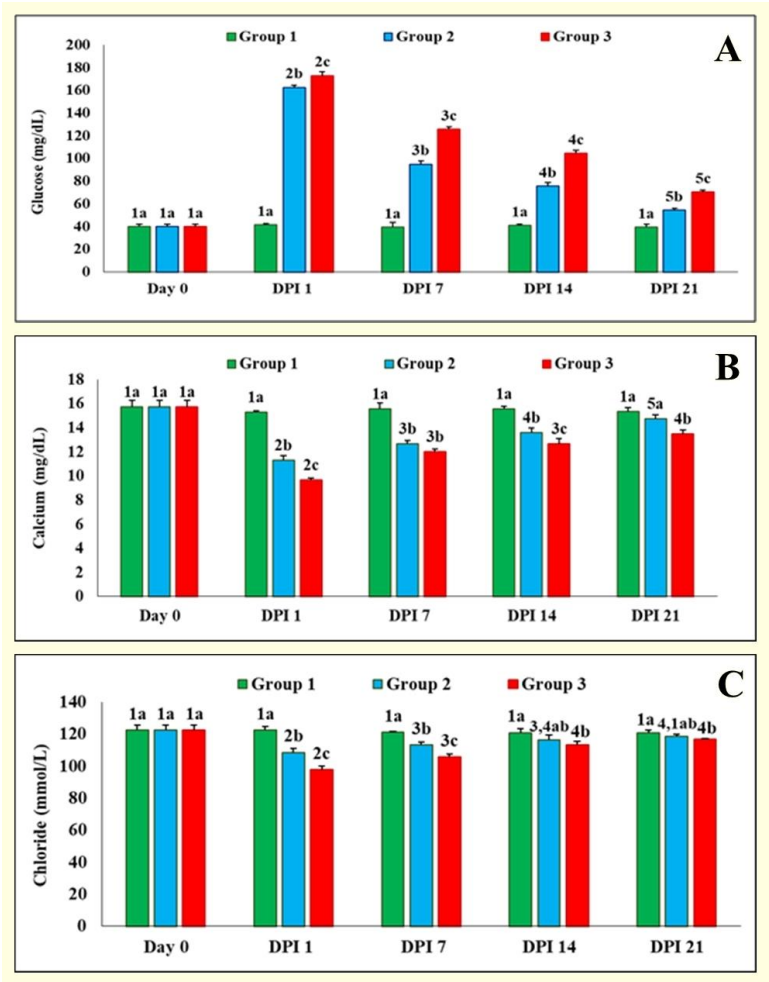


Figure 1. Plasma [A] glucose, [B] calcium and [C] chloride levels of *Aeromonas hydrophila* challenged *Oreochromis niloticus.* Group 1: Unchallenged control; Group 2: Challenged and oxolinic acid (OA) fed at the therapeutic dose of 12 mg/kg biomass/day for 7 consecutive days; Group 3: Challenged and untreated. a-c: Bars sharing a common alphabet for a particular day differed insignificantly ($P>0.05$). 1-5: Bars sharing a common numeral for a particular treatment differed insignificantly ($P>0.05$). DPI: day post-injection.

injection site and caused increased mortalities (80%) at higher doses. The fish that received lower doses remained active with no serious abnormalities or mortalities, except for the primary inflammatory response at the injection site. The LD_{50} value of 1.93×10^7 cells/fish indicated that *A. hydrophila* AAH-28 is moderately pathogenic to tilapia as per the criteria of Mittal *et al.* (1980). In the efficacy trial, *O. niloticus* juveniles were first challenged with *A. hydrophila* at a sub-lethal dose of 1.93×10^6 cells/fish through intramuscular injection. During the post-challenge period, only insignificant mortalities were noted at the sub-lethal dose in OA-treated and untreated groups. These mortalities could be attributed to factors such as

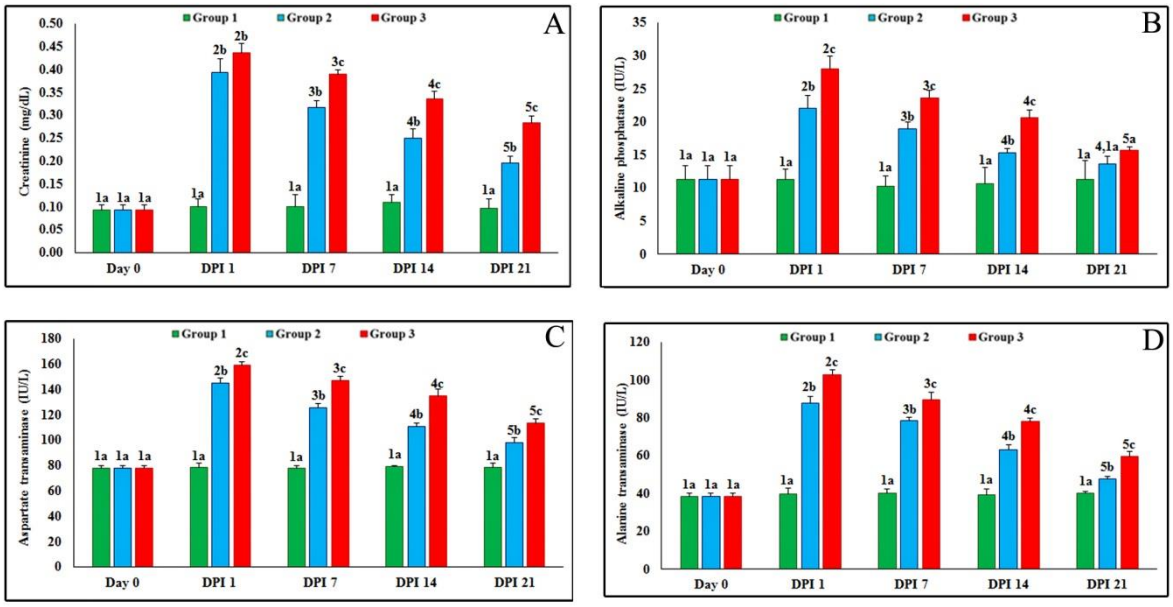


Figure 2. Plasma [A] creatinine, [B] alkaline phosphatase [C] aspartate transaminase and [D] alanine transaminase levels of *Aeromonas hydrophila* challenged *Oreochromis niloticus*. Group 1: Unchallenged control; Group 2: Challenged and oxolinic acid (OA) fed at the therapeutic dose of 12 mg/kg biomass/day for 7 consecutive days; Group 3: Challenged and untreated. a-c: Bars sharing a common alphabet for a particular day differed insignificantly (P>0.05). 1-5: Bars sharing a common numeral for a particular treatment differed insignificantly (P>0.05). DPI: day post-injection.

Table 2. Qualitative assessment* of the major histopathological changes in *Aeromonas hydrophila*-challenged *Oreochromis niloticus* juveniles fed oxolinic acid (OA) at 12 mg/kg biomass/day for 7 consecutive days in comparison with control

Histopathological changes	DPI 1		DPI 7		DPI 14		DPI 21	
	OA-treated*	Untreated**	OA-treated	Untreated	OA-treated	Untreated	OA-treated	Untreated
Kidney								
Degeneration of renal tubules	3.49 ± 0.13^{1a}	4.07 ± 0.05^{2a}	2.57 ± 0.28^{1b}	3.57 ± 0.11^{2b}	1.57 ± 0.15^{1c}	3.06 ± 0.20^{2c}	1.25 ± 0.10^{1d}	2.22 ± 0.21^{2d}
Hydropic swelling	3.23 ± 0.16^{1a}	3.63 ± 0.10^{2a}	2.75 ± 0.23^{1b}	3.32 ± 0.10^{2b}	1.60 ± 0.25^{1c}	2.86 ± 0.12^{2c}	1.20 ± 0.13^{1d}	2.03 ± 0.10^{2d}
Haemocyte infiltration	1.82 ± 0.26^{1a}	1.30 ± 0.26^{2a}	1.58 ± 0.24^{1b}	1.22 ± 0.21^{2a}	1.27 ± 0.10^{1c}	0.80 ± 0.18^{2b}	0.94 ± 0.16^{1d}	0.50 ± 0.21^{2c}
Necrotized area	1.43 ± 0.18^{1a}	1.60 ± 0.14^{2a}	1.32 ± 0.12^{1a}	1.39 ± 0.12^{1b}	0.95 ± 0.22^{1b}	1.18 ± 0.12^{2c}	0.47 ± 0.10^{1c}	0.80 ± 0.25^{2d}
Wide lumen	1.15 ± 0.23^{1a}	1.63 ± 0.25^{2a}	0.95 ± 0.12^{1b}	1.22 ± 0.21^{2b}	0.50 ± 0.17^{1c}	0.73 ± 0.24^{2c}	0.33 ± 0.10^{1c}	0.50 ± 0.21^{2c}
Liver								
Cytoplasmic vacuolation	3.96 ± 0.22^{1a}	4.12 ± 0.09^{2a}	3.46 ± 0.13^{1b}	3.65 ± 0.10^{2b}	1.97 ± 0.20^{1c}	3.37 ± 0.07^{2c}	1.47 ± 0.10^{1d}	2.97 ± 0.16^{2d}
Degeneration of hepatocytes	3.05 ± 0.15^{1a}	3.55 ± 0.10^{2a}	2.45 ± 0.36^{1b}	3.02 ± 0.17^{2b}	1.57 ± 0.12^{1c}	2.73 ± 0.18^{2c}	1.18 ± 0.25^{1d}	2.30 ± 0.26^{2d}
Lipid vacuolation	4.47 ± 0.13^{1a}	4.67 ± 0.05^{2a}	0.90 ± 0.21^{1b}	4.01 ± 0.11^{2b}	0.50 ± 0.11^{1c}	1.03 ± 0.14^{2c}	0.47 ± 0.10^{1c}	0.93 ± 0.10^{2c}

*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. 1-2: Values sharing a common numerical superscript within a row for a particular histopathological change for a particular day differed insignificantly (P>0.05). a-d: Values sharing a common alphabetical superscript for a particular row among the days (DPI) for a particular treatment differed insignificantly (P>0.05). DPI: day post-injection. *: *Aeromonas hydrophila* challenged and OA treated; **: *Aeromonas hydrophila* challenged and untreated.

physical handling during injection, injection stress, environmental factors, challenge doses and fish immunity. In a similar study by Yardimci & Aydin (2011), during the intramuscular challenge with *A. hydrophila* in *O. niloticus,* infections were noticed only in skin and muscles, whereas intraperitoneal injection established infections in visceral organs like the liver and kidney.

The challenged fish reduced the feed intake by about 12-15% and the feeding rate was at its lowest during the disease progression period. However, it gradually increased towards normal levels by the end. The OA-treated group exhibited higher feed intake compared to the untreated group, indicating a positive response to OA treatment against *A. hydrophila* infection. Though the biomass of all fish groups increased significantly with time, the challenged fish groups had lower biomass increments compared to the control. Nevertheless, on 21 DPI, the OA-treated group documented higher biomass than the untreated group, which correlated with their feeding behaviour. Decreased appetite and growth retardation are often early signs of stress and disease in fish (Julinta *et al.*,

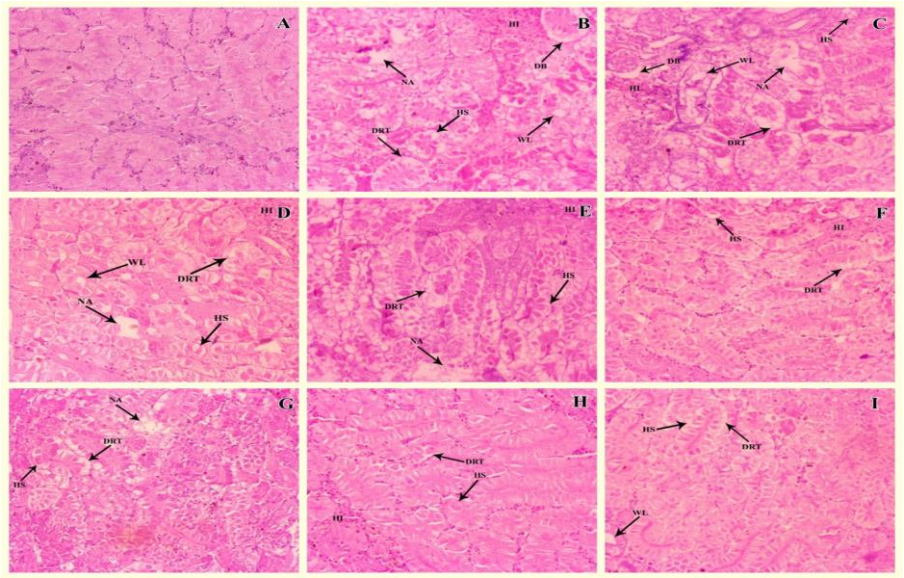


Figure 3. Histoarchitecture of the kidney tissues of *Aeromonas hydrophila* challenged *Oreochromis niloticus.* [A] Control, [B] Day 1 OA treated, [C] Day 1 untreated, [D] Day 7 OA treated, [E] Day 7 untreated, [F] Day 14 OA treated, [G] Day 14 untreated, [H] Day 21 OA treated, [I] Day 21 untreated. HS: hydropic swelling; HI: haemocyte infiltration; DB: dilated Bowman's space; WL: wide lumen; NA: necrotized areas and DRT: degeneration of renal tubule. H&E staining ×200. OA therapeutic dose was 12 mg/kg biomass/day for 7 consecutive days.

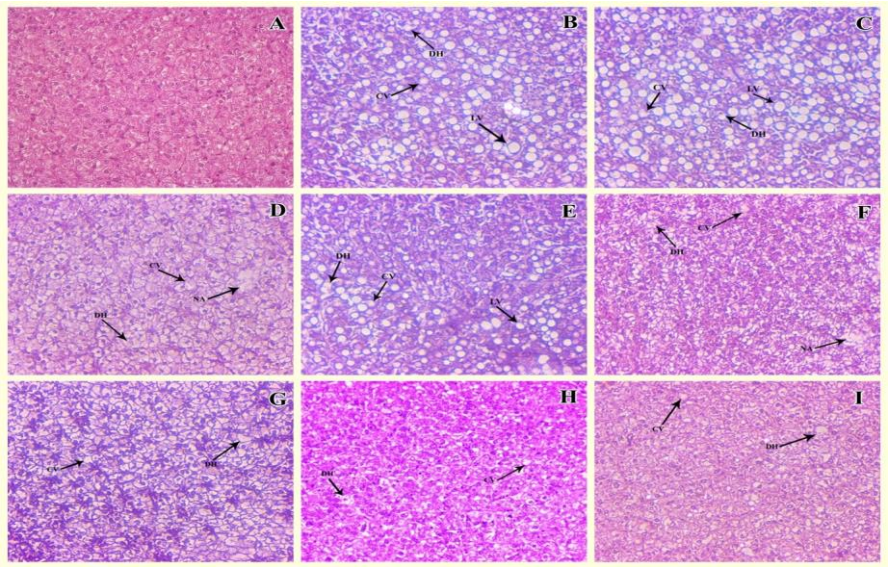


Figure 4. Histoarchitecture of the liver tissues of *A. hydrophila* challenged *O. niloticus.* [A] Control, [B] Day 1 OA treated, [C] Day 1 untreated, [D] Day 7 OA treated, [E] Day 7 untreated, [F] Day 14 OA treated, [G] Day 14 untreated, [H] Day 21 OA treated, [I] Day 21 untreated. DH: degeneration of hepatocytes; CV: cytoplasmic vacuolation; LV: lipid vacuolation and NA: necrotized areas. H&E staining ×200. OA therapeutic dose was 12 mg/kg biomass/day for 7 consecutive days.

2017). This present work proved that medicated feed is considered an effective and convenient method to treat fish. However, it is crucial to administer them promptly since sick fish tend to stop feeding, and early intervention can improve their recovery (Kelly, 2013).

Wound healing assessment was done through a selection of digital images, which gave illustrations of the healing process at different periods. The challenged fish were weak and lethargic initially. Tissue reddening, inflammation and skin peeling at the site of injection and open subepithelial wounds started to become obvious within 24 h of the challenge. During the OA therapy, reddening and inflammation subsided with the formation of a black scar in the ulcerated area. The reddening and inflammation subsided relatively faster in the OA-treated group compared to the untreated group. The areas surrounding the wound become very dark on 3 DPI may be due to an increased number of melanocytes, thus, suggesting an increased melanocyte activity after the injury (Guerra *et al.,* 2008; Julinta *et al*., 2017; Roy *et al.,* 2019). The black scar disappearance, the onset of dermal fibrous tissue regrowth and the formation of new skin at the ulcerated scar region were seen on 5 DPI, indicating regeneration of the muscle tissue. All wounds examined were closed with the disappearance of the black scar, the onset of dermal fibrous tissue regrowth and the formation of new skin at the ulcerated scar region on 7 DPI. The depression at the injection site during the recovery period of 9 DPI indicated that the tissue regrowth had not reached steady-state levels. On 12 DPI, the complete disappearance of the depression at the site of injection was noticed. Likewise, Roy *et al.* (2019) observed, within a few weeks, the complete disappearance of the black scar with mild depression at the site of injection with a new scale having the size and characteristics of a mature scale completely re-grown. The wounds of the OA-treated group healed faster than the untreated group. Though the rate of wound healing was initially faster in OA-treated fish, the wounds were healed completely within 14 DPI even in untreated fish. In some other studies of *A. hydrophila*-induced and *A. caviae*-induced wounds in *O. niloticus* by intramuscular challenge, the epidermis became normal by 26-30 DPI with oxytetracycline treatment (Julinta *et al*., 2017; Roy *et al.,* 2019). According to them, the main effect was generally seen on fibrous tissue, including the repair of damaged dermal fibres, revascularisation and the re-establishment of normal dermal and muscle structure. The results, thus, demonstrated that the degree of wound healing was promoted by OA medicated feed, which was more prominent during the treatment period.

Blood biochemical characteristics provide valuable insights into an organism's internal condition. In the present study, after intramuscular injection, a significant increase in stress indicator plasma glucose was observed compared to the control. The rise in glucose levels on DPI 1 for the challenged groups suggested that *A. hydrophila* infection may induce stress in fish like Pakhira (2013) and Dong et al. (2017). From 7 DPI onwards, the plasma glucose of the OA-treated group significantly decreased compared to the untreated group, implying that OA treatment contained the *A. hydrophila* infectivity, reduced the impact and alleviated the stressful condition. Until 21 DPI, the untreated group exhibited significantly higher glucose than the OA-treated group, indicating that the *A. hydrophila* infection might cause prolonged stress in fish. Changes in inorganic ions, such as calcium and chloride, can have an impact on an organism's physiological processes and osmoregulation (Ramesh *et al.*, 2021). Within 24 h, the challenged groups showed a significant reduction in plasma calcium and chloride levels, thus, causing ionic imbalance. Earlier studies also reported similar findings, where the levels of inorganic ions significantly decreased in *A. hydrophila*-challenged *C. carpio* (Harikrishnan *et al.,* 2003; Wu et al., 2021). Since the kidney plays a role in maintaining ionic balance, the observed decrease in calcium and chloride levels during bacterial infection suggested potential dysfunction of this excretory organ (Wei *et al.*, 2016). From DPI 7 to 21, both groups exhibited an increase in plasma calcium and chloride levels, indicating recovery from the *A. hydrophila* infection. Nevertheless, the OA-treated group showed a rapid increase in plasma calcium and chloride compared to the untreated group, suggesting that OA can significantly mitigate bacterial infection and facilitate improved recovery of the vital organs of fish. By DPI 21, the plasma calcium and chloride levels of the OA-treated group approached normal levels, although they remained slightly lower than on day 0. On the other hand, the untreated group showed a slower recovery of ionic balance on DPI 21, indicating the persistence of *A. hydrophila* infection-induced stress in fish.

Both challenged groups showed a significant increase in plasma creatinine on DPI 1 compared to the control and corroborated the study of Pakhira (2013) and El-Barbary (2017) in *A. hydrophila*-infected fish. The elevated plasma creatinine indicated kidney damage and impaired renal function. Interestingly, both groups differed significantly from each other immediately after intramuscular injection and on 14 DPI, with lower creatinine levels in the OA-treated group compared to the untreated group. The plasma creatinine decreased in both groups on DPI 21 but remained higher than on day 0. However, the reduction in plasma creatinine was more pronounced in the OA-treated group compared to the untreated group. These results highlight the therapeutic effect of OA, which provided resistance against bacterial infection in *O. niloticus*. Similarly, quinolone treatment demonstrated a reduction in kidney damage and decreased blood creatinine levels in *O. niloticus* injected with *A. hydrophila* (Hal & El-Barbary, 2021).

According to Van der Oost *et al.* (2003), changes in liver enzymes ALT and AST serve as essential indicators of liver tissue damage caused by stress and play a

significant role in assessing liver metabolism. The ALP is known to function as an immune factor that protects against stress and infection. Within 24 h, both challenged groups exhibited a significant increase in plasma ALT and AST compared to the control. However, the increment was relatively lower in the OA-treated group. Similar observations were made in *Streptococcus agalactiae*-infected *Oreochromis* sp. (Alsaid *et al.*, 2014) and *F. columnare*-challenged *C. carpio* (Tripathi *et al.*, 2005) and naturally infected fish (Racicot *et al.*, 2006), indicating hepatic injury leading to dysfunction and disturbance in enzymatic activities. Although the ALT and AST in the OA-treated group approached near-normal on 21 DPI, they were still higher than on day 0. In contrast, the untreated group showed significantly higher ALT and AST on 21 DPI. Alike, Ibrahim *et al.* (2020) noted a significant reduction in ALT and AST with doxycycline treatment in *A. hydrophila*-challenged *Clarias gariepinus*. The significant reduction in plasma ALT and AST in the OA-treated group indicated the bactericidal and certain hepato-protective properties of medicated feed, enabling the fish to recover from liver damage and thwart *A. hydrophila* infection. In the present study, upon intramuscular injection, both groups showed a significant increase in plasma ALP on DPI 1 compared to the control, which corroborated the findings in *A. hydrophila*-challenged *Ctenopharyngodon idella* (Wang & Li, 2017) and *L. rohita* by immersion (Manoj *et al.*, 2010). The increased ALP during the post-challenge period may be considered an adaptive response to mitigate the effects of *A. hydrophila* infection. The results of this study also implied that the increase in ALP is associated with liver tissue necrosis. By 21 DPI, plasma ALP was significantly reduced in both groups and the reduction was more pronounced in the OA-treated group. It implied the beneficial effects of therapeutic OA in mitigating *A. hydrophila* infection in the liver and enhancing the fish's adaptive responses.

Histology of the control fish kidney tissues revealed a regular and organized structure, including a well-defined glomerulus. However, on DPI 1, significant pathological changes were observed in the kidney tissues of the *A. hydrophila*-challenged groups. The observed changes indicated severe nephropathy with notable cellular and tissue level alterations. The untreated group exhibited more severe changes compared to the OA-treated group. The results indicated the pathogenic potential of *A. hydrophila* and its ability to cause systemic infection even at the sublethal dose. Hydropic swelling of the nephritic tubular epithelial cells indicated severe cellular oedema. The dilated Bowman's space suggested impaired glomerular filtration, leading to the inadequate removal of excess waste and fluids from the kidney. The results confirmed the similar findings reported earlier due to *A. hydrophila* infection (Skjolstrup *et al.*, 2000; Paul *et al.*, 2015). On DPI 14 and 21, the OA-treated group showed a reduction in glomerulopathy and improvement in the structural organization of renal tubules, possibly attributed to the inhibitory effects of OA on bacterial activity. It has been reported that antibiotics can limit the growth of bacterial populations and their production of toxins and by-products (Schlomann *et al.*, 2019) and the observed results supported their findings. The observed decrease in plasma creatinine from DPI 7 to 21 also indicated an improvement in kidney function in the OA-treated group similar to Hal & El-barbary (2021) in *A. hydrophila*-injected *O. niloticus*. In contrast, the untreated group showed minimal improvement, exhibiting marked degeneration of renal tubules, moderate to marked hydropic swelling, and mild necrotized areas on DPI 7. Subsequently, this group exhibited slower recovery from DPI 14 onwards. On DPI 21, a reduction in kidney tissue aberrations was observed, yet the changes were still evident. These observations suggested the beneficial effect of dietary OA on the kidney tissues against *A. hydrophila* infection.

The liver tissues of the control fish exhibited a normal structure and systematic arrangement of hepatocytes. The challenged groups showed severe pathological alterations in the liver tissues on DPI 1 concomitant with the significant increase in plasma ALT and AST. The observed histopathological changes of the present study were consistent with previous findings in different fish species infected with *Aeromonas* (Noor El Deen *et al.*, 2014; Paul *et al.*, 2015). The lipid vacuolation and necrosis of the liver are believed to be associated with toxins and extracellular products, such as hemolysin, protease, and elastase produced by *Aeromonas* (Yardimci & Aydin, 2011). These results are in line with the observations of Reda *et al.* (2013), who noted severe fatty changes and vacuolations in the hepatocytes of *O. niloticus* infected with *Aeromonas*. Shahjahan *et al.* (2020) also reported massive diffuse necrosis in the liver tissues of fish challenged with *Aeromonas*, characterized by vacuolation and atrophy. Importantly, significant reductions in the histopathological alterations of liver tissues and plasma ALT and AST levels were observed in the OA-treated groups on and from DPI 7. This implied that OA enabled the fish to recover from liver damage induced by the *A. hydrophila* challenge. In contrast, the untreated group showed severe pathological effects in the liver with higher levels of plasma ALT and AST on DPI 7. Although some recovery in the liver tissue was observed on DPI 14 and 21, the recovery was still lower than the degree of recovery observed in the OA-treated group.

Conclusion

Managing the health of aquatic organisms often involves the strategic and effective use of chemotherapeutics. In this context, the current study demonstrated the potential effect of oxolinic acid against *A. hydrophila* infections in fish. The intramuscular *A. hydrophila* challenge resulted in various histopathological changes in the vital organs and altered the clinical biochemistry, indicating possible

nephrotoxicity and hepatotoxicity. The OA treatment demonstrated its efficacy against *A. hydrophila* infection in *O. niloticus* juveniles, effectively stabilizing the clinical biochemical parameters and aiding in recovery from the infection. The wound healing was faster in the OA-treated group with complete wound closure within 12 DPI. Additionally, oral OA therapy improved the feed intake and biomass of *O. niloticus* compared to the untreated group. Nevertheless, it is important to note that quinolones including OA are categorized as critically important antimicrobials for humans and are considered highly important antimicrobial agents for veterinary animals (OIE, 2020; WHO, 2021). As per Office International des Epizooties (OIE) guidelines, critically important medicines should be used as a second-line treatment in food-producing animals only when no other alternatives are available (OIE, 2020). Though OA was efficacious against *A. hydrophila* infection, it should, therefore, be used as a second-line treatment choice, adhering to OIE recommendations.

Ethical Statement

The current investigations were performed in compliance with the guidelines for experimentation on fishes set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The experimental protocols were approved by the ICAR, Government of India, New Delhi, under the All-India Network Project on Fish Health (No. CIBA/AINP-FH/2015-16 dated 16.7.2015).

Funding Information

The work was supported by the Indian Council of Agricultural Research, Government of India, New Delhi under the All-India Network Project on Fish Health (Grant F. No. CIBA/AINP-FH/2015-16 dated 02.06.2015).

Author Contribution

Jaykumar Bhagubhai Patel, Avishek Bardhan, Ratnapriya Das**,** Arya Sen: Execution of wet laboratory experiments, laboratory investigations, formal analysis, generation of data, statistical analyses and drafting the manuscript. Satyanarayana Boda: Statistical analyses and interpretation of data; Thangapalam Jawahar Abraham: Conceptualization, experimental design, data interpretation, writing - review and editing the manuscript. Prasanna Kumar Patil: Resources, project administration and funding acquisition.

Conflict of Interest

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

Acknowledgements

The authors thank the Vice-Chancellor, West Bengal University of Animal and Fishery Sciences, Kolkata, for providing the necessary facilities for carrying out this work.

References

Adikesavalu, H., Banerjee, S., Patra, A., & Abraham, T.J. (2017). Meningoencephalitis in farmed monosex Nile tilapia (*Oreochromis niloticus* L.) caused by *Streptococcus agalactiae*. *Fisheries and Aquatic Life*. 25(3), 187-200. https://doi.org/10.1515/aopf-2017-0018.

Alsaid, M., Abuseliana, A.F., Daud, H.H., Mustapha, N.M., Bejo, S.K., Abdelhadi, Y.M., & Hamdan, R.H. (2014). Haematological, biochemical and clinical signs changes following experimental infection of *Streptococcus agalactiae* in red hybrid tilapia (*Oreochromis* sp.). *Aquaculture Indonesiana*. 15(2), 289-295.

Austin, B., & Austin, D.A. (2016). *Bacterial Fish Pathogens: Disease of Farmed and Wild Fish*. Springer International Publishing, Switzerland.

Bardhan, A., Abraham, T.J., Singha, J., Saha, S., Sarker, S., & Patil, P.K. (2022). 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(26), 39914-39927. https://doi.org/10.1007/s11356-021-18418-x.

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. https://doi.org/10.1046/j.1365-2761.1999.00134.x.

Dong, H.T., Techatanakitarnan, C., Jindakittikul, P., Thaiprayoon, A., Taengphu, S., Charoensapsri, W., Khunrae, P., Rattanarojpong, T., & Senapin, S. (2017). *Aeromonas jandaei* and *Aeromonas veronii* caused disease and mortality in Nile tilapia, *Oreochromis niloticus* (L.). *Journal of Fish Diseases*. 40(10), 1395-1403. https://doi.org/10.1111/jfd.12617.

El-Barbary, M.I. (2017). Serum biochemical and histopathological changes associated with *Aeromonas hydrophila* isolated from *Oreochromis niloticus* and *Sparus aurata* with multiple antibiotic resistance index. *Journal of Biological Sciences*. 17(5), 222-234. https://doi.org/10.3923/jbs.2017.222.234.

El-Sayed, A.F.M. (2019). *Tilapia Culture*. CABI Publication, Wallingford.

EMEA. (2005). *Committee for medicinal products for veterinary use: oxolinic acid (Extension to all food-producing species). European Medicines Evaluation Agency*. https://www.ema.europa.eu/en/documents/committee-report/committee-medicinal-products-veterinary-use-cvmp-monthly-report-application-procedures-guidelines_en-4.pdf (accessed 24 March 2022).

FAO. (2022). Towards Blue Transformation. *The State of World Fisheries and Aquaculture 2022*. FAO, Rome. https://doi.org/10.4060/cc0461en.

Gewaily, M.S., Shu kry, M., Abdel-Kader, M.F., Alkafafy, M., Farrag, F.A., Moustafa, E. M., Doan, H.V., Abd-Elghany, M.F., Abdelhamid, A.F., Eltanahy, A., & Dawood, M.A. (2021). Dietary *Lactobacillus plantarum* relieves Nile

tilapia (*Oreochromis niloticus*) juvenile from oxidative stress, immunosuppression, and inflammation induced by deltamethrin and *Aeromonas hydrophila*. *Frontiers in Marine Science*. 8, 621558. https://doi.org/10.3389/fmars.2021.621558.

Guerra, R.R., Santos, N.P., Cecarelli, P., Silva, J.R.M.C., & Hernandez-Blazquez, F.J. (2008). Healing of skin wounds in the African catfish *Clarias gariepinus*. *Journal of Fish Biology*. 73(3), 572-583. https://doi.org/10.1111/j.1095-8649.2008.01950.x.

Haenen, O.L.M., Dong, H.T., Hoai, T.D., Crumlish, M., Karunasagar, I., Barkham, T., Chen, S.L., Zadoks, R., Kiermeier, A., Wang, B., Gamarro, E.G., Takeuchi, M., Azmai, M.N.A., Fouz, B., Pakingking. Jr. P., Wei, Z.W., & Bondad-Reantaso, M.G. (2023). Bacterial diseases of tilapia, their zoonotic potential and risk of antimicrobial resistance. *Reviews in Aquaculture*. 15, 154–185. https://doi.org/10.1111/raq.12743.

Hal, A.M., & El-Barbary, M.I. (2021). Therapeutic effect of Nigella sativa oil and ciprofloxacin against bacterial infection based on interleukin 1β expression and kidney histopathological alterations in *Oreochromis niloticus*. *Aquaculture Research*. 52(6), 2772-2782. https://doi.org/10.1111/are.15129.

Harikrishnan, R., Rani, M. N., & Balasundaram, C. (2003). Hematological and biochemical parameters in common carp, *Cyprinus carpio*, following herbal treatment for *Aeromonas hydrophila* infection. *Aquaculture*. 221(1-4), 41-50. https://doi.org/10.1016/S0044-8486(03)00023-1.

Ibrahim, H.A., Eleiwa, N.Z., Galal, A.A., El-Ekiaby, W., & Abd El-Rahman, E.S. (2020). Antibacterial activity of doxycycline against *Aeromonas hydrophila* in experimentally challenged African catfish (*Clarias gariepinus*). *Zagazig Veterinary Journal*. 48(1), 46-56. https://doi.org/10.21608/zvjz.2019.15561.1071.

Julinta, R.B., Abraham, T.J., Roy, A., Singha, J., Dash, G., Nagesh, T.S., & Patil, P.K. (2017). Histopathology and wound healing in oxytetracycline treated *Oreochromis niloticus* (L.) against *Aeromonas hydrophila* intramuscular challenge. *Journal of Aquaculture Research & Development*. 8, 488. https://doi.org/10.4172/2155-9546.1000488.

Kelly, A.M. (2013). *Medicated Feed for Food Fish, Southern Regional Aquaculture Center (SRAC)*. SRAC Publication No. 473. https://srac.tamu.edu/index.cfm/event/getFactSheet/whichfactsheet/120/.

Lulijwa, R., Rupia, E.J., & Alfaro, A.C. (2020). Antibiotic use in aquaculture, policies and regulation, health and environmental risks: a review of the top 15 major producers. *Reviews in Aquaculture*. 12(2), 640-663. https://doi.org/10.1111/raq.12344.

Maclean, J.L. (1984). Tilapia: the aquatic chicken. *ICLARM Newsletter*. 7 (1), 17.

Manoj, C.K., Nair, C.M., Patel, M.B., & Salin, K.R. (2010). Haemato-biochemical and histopathological changes in *Labeo rohita* infected with *Aeromonas hydrophila* by immersion challenge. *Fishery Technology*. 47(2), 151-160.

Menaga, M., & Fitzsimmons, K. (2017). Growth of the tilapia industry in India. *World Aquaculture*. 48(3), 49-52.

Michaylova, V., & Ilkova, P. (1971). Photometric determination of micro amounts of calcium with arsenazo III. *Analytica Chimica Acta*. 53(1), 194-198. https://doi.org/10.1016/S0003-2670(01)80088-X.

Mittal, K.R., Lalonde, G., Leblanc, D., Olivier, G., & Lallier, R. (1980). *Aeromonas hydrophila* in rainbow trout: relation between virulence and surface characteristics. *Canadian Journal of Microbiology*. 26(12), 1501-1503. https://doi.org/10.1139/m80-248.

NFDB. (2015). *Guidelines for Responsible Farming of Tilapia in India*. National Fisheries Development Board. Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture and Farmers Welfare, Government of India, Hyderabad, India.

Nicholson, P., Mon-on, N., Jaemwimol, P., Tattiyapong, P., & Surachetpong, W. (2020). Coinfection of tilapia lake virus and *Aeromonas hydrophila* synergistically increased mortality and worsened the disease severity in tilapia (*Oreochromis* spp.). *Aquaculture*. 520, 734746. https://doi.org/10.1016/j.aquaculture.2019.734746.

Noor El Deen, A.E., Dorgham, S.M., Hassan, A.H.M., & Hakim, A.S. (2014). Studies on *Aeromonas hydrophila* in cultured *Oreochromis niloticus* at Kafr El Sheikh Governorate, Egypt with reference to histopathological alterations in some vital organs. *World Journal of Fish and Marine Sciences*. 6(3), 233-240. https://doi.org/10.5829/idosi.wjfms.2014.06.03.83136.

OIE. (2020). *Guidelines and Resolutions on Antimicrobial Resistance and the Use of Antimicrobial Agents*. World Organisation for Animal Health. https://www.oie.int/fileadmin/Home/eng/MediaCenter/docs/pdf/PortailAMR/book-AMR-ANG-FNL-LR. pdf.

Pakhira, C. (2013). *Effect of acute physical and biological stressors on the haematological and biochemical responses in rohu, Labeo rohita (Hamilton, 1822)*. Master's thesis, West Bengal University of Animal and Fishery Sciences, Kolkata.

Paul, P., Adikesavalu, H., Banerjee, S., & Abraham, T.J. (2015). Antibiotic-resistant motile aeromonads induced septicemia in Philippine catfish *Clarias batrachus* (Linnaeus, 1758) fingerlings. *Croatian Journal of Fisheries*. 73(4), 170-175. https://doi.org/10.14798/73.4.844.

Quesada, S.P., Paschoal, J.A.R., & Reyes, F.G.R. (2013). Considerations on the aquaculture development and on the use of veterinary drugs: special issue for fluoroquinolones—A review. *Journal of Food Science*. 78(9), 1321-1333. https://doi.org/10.1111/1750-3841.12222.

Racicot, J. G., Guadet, M., & leray, C. (2006). Blood and liver enzymes in rainbow trout with emphasis on their diagnostic use and a case of *Aeromonas* infection. *Journal of Fish Biology*. 7(6), 825-835. https://doi.org/10.1111/j.1095-8649.1975.tb04653.x.

Raj, N.S., Swaminathan, T.R., Dharmaratnam, A., Raja, S.A., Ramraj, D. and Lal, K.K. (2019). *Aeromonas veronii* caused bilateral exophthalmia and mass mortality in cultured Nile tilapia, *Oreochromis niloticus* (L.) in India. *Aquaculture*. 512, 734278. https://doi.org/10.1016/j.aquaculture.2019.734278.

Ramesh, M., Sujitha, M., Anila, P.A., Ren, Z., & Poopal, R.K. (2021). Responses of *Cirrhinus mrigala* to second generation fluoroquinolone (ciprofloxacin) toxicity: Assessment of antioxidants, tissue morphology, and inorganic ions. *Environmental Toxicology*. 36(5), 887-902. https://doi.org/10.1002/tox.23091.

Reda, R.M, Ibrahim, R.E., Ahmed, E.G., & El-Bouhy, Z.M. (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. https://doi.org/10.1016/j.ejar.2013.12.001.

Reed, L. J., & Muench, H. (1938). A simple method of estimating fifty per cent endpoints. *American Journal of Epidemiology*. 27(3), 493-497. https://doi.org/10.1093/oxfordjournals.aje.a118408.

Rey, A., Verjan, N., Ferguson, H.W., & Iregui, C. (2009). Pathogenesis of *Aeromonas hydrophila* strain KJ99 infection and its extracellular products in two species of fish. *Veterinary Record*. 164: 493-499. https://doi.org/10.1136/vr.164.16.493.

Roberts, R.J. (2012). *Fish Pathology*. John Wiley and Sons, USA.

Roy, A., Abraham, T.J., Namdeo, M.S., Singha, J., Julinta, R.B., & Boda, S. (2019). Effects of oral oxytetracycline-therapy on wound progression and healing following *Aeromonas caviae* infection in Nile tilapia (*Oreochromis niloticus* L.). *Brazilian Archives of Biology and Technology*. 62(1), e19180766. https://doi.org/10.1590/1678-4324-2019180766.

Schlomann, B.H., Wiles, T.J., Wall, E.S., Guillemin, K., & Parthasarathy, R. (2019). Sublethal antibiotics collapse gut bacterial populations by enhancing aggregation and expulsion. *Proceedings of the National Academy of Sciences*. 116(43), 21392-21400. https://doi.org/10.1073/pnas.1907567116.

Schmidt, H., Goetz, K., Hallmann, I., & Holt, C.V. (1961). The course of re-synthesis of blood glucose. *Biochemical Journal*. 334, 534-544.

Shahjahan, M., Khatun, M., Mun, M.M., Islam, S.M., Uddin, M., Badruzzaman, M., & Khan, S. (2020). Nuclear and cellular abnormalities of erythrocytes in response to thermal stress in common carp *Cyprinus carpio*. *Frontiers in Physiology*. 11, 543. https://doi.org/10.3389/fphys.2020.00543.

Sherif, A.H., & Kassab, A.S. (2023). Multidrug-resistant *Aeromonas* bacteria prevalence in Nile tilapia broodstock. *BMC Microbiology*. *23, 80.* https://doi.org/10.1186/s12866-023-02827-8

Skjolstrup, J., McLean, E., Nielsen, P.H., & Frier, J.O. (2000). The influence of dietary oxolinic acid on fluidised bed biofilter performance in a recirculation system for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. 183(3-4), 255-268. https://doi.org/10.1016/S0044-8486(99)00298-7.

Tietz, N.W. (1994). Accuracy in clinical chemistry-does anybody care?. *Clinical Chemistry*. 40(6), 859-861. https://doi.org/10.1093/clinchem/40.6.859.

Tripathi, N.K., Latimer, K.S., Gregory, C.R., Ritchie, B.W., Wooley, R.E., & Walker, R.L. (2005). Development and evaluation of an experimental model of cutaneous columnaris disease in koi *Cyprinus carpio*. *Journal of Veterinary Diagnostic Investigation*. 17, 45-55. https://doi.org/10.1177/104063870501700109.

USFDA. (2022). *Approved aquaculture drugs*. U.S. Food and Drug Administration. https://www.fda.gov/animal-veterinary/aquaculture/approved-aquaculture-drugs (accessed 24 March 2022).

Van der Oost, R., Beyer, J., & Vermeulen, N.P. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental Toxicology and Pharmacology*. 13(2), 57-149. https://doi.org/10.1016/S1382-6689(02)00126-6.

Wang, R.Q., & Li, J.L. (2017). Biological parameters, immune enzymes, and histological alterations in the livers of grass carp infected with *Aeromonas hydrophila*. *Fish and Shellfish Immunology*. 70, 121-128. https://doi.org/10.1016/j.fsi.2017.08.039.

Wei, K., Yin, Z., & Xie, Y. (2016). Roles of the kidney in the formation, remodeling and repair of bone. *Journal of Nephrology*. 29(3), 349-357. https://doi.org/10.1007/s40620-016-0284-7.

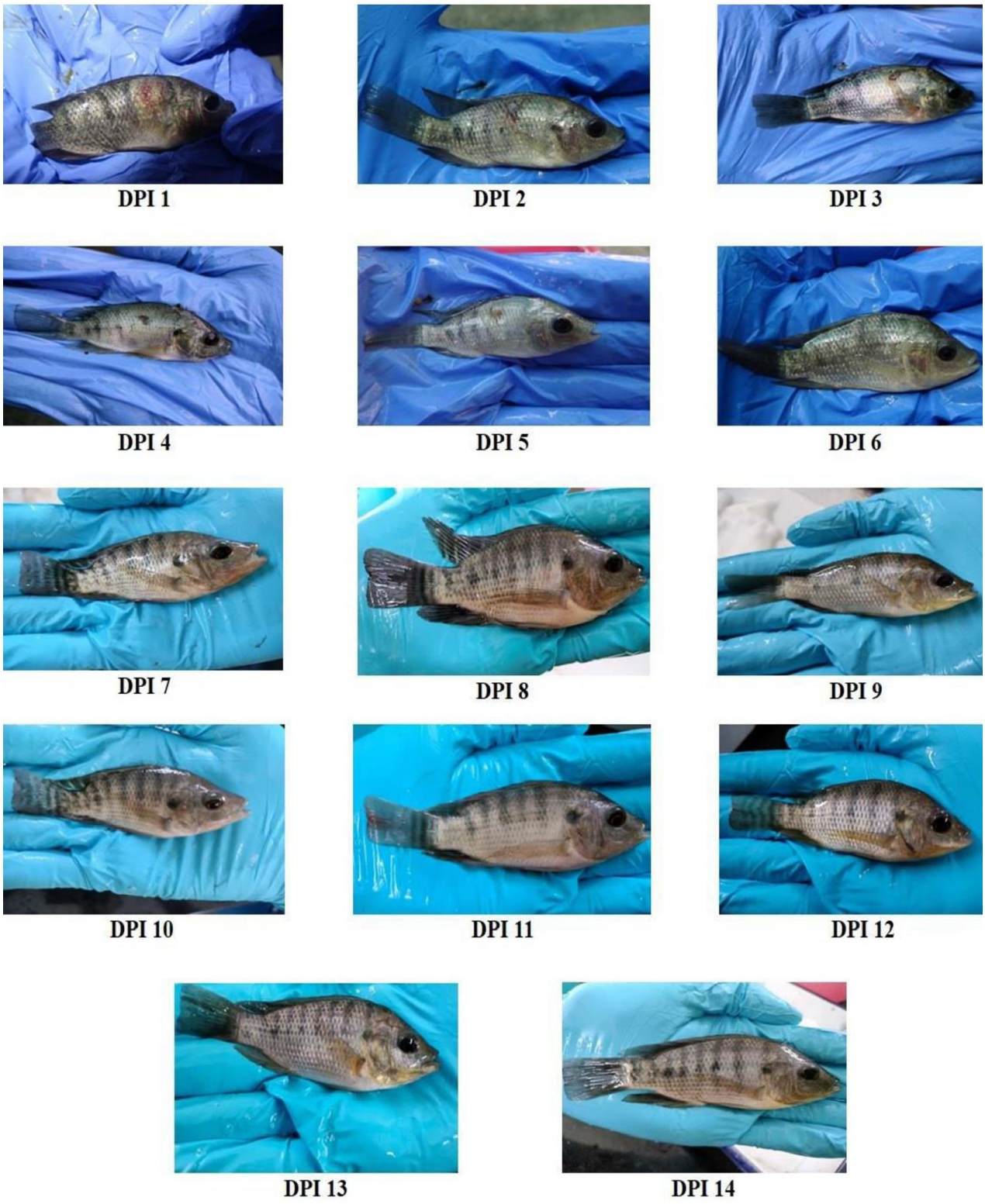
WHO. (2021). *Critically Important Antimicrobials for Human Medicine*. World Health Organization. https://apps.who.int/iris/bitstream/handle/10665/312266/ 9789241515528-eng.pdf.

Wolf, P.L., Williams, D., Coplon, N., & Coulson, A.S. (1972). Low aspartate transaminase activity in serum of patients undergoing chronic hemodialysis. *Clinical Chemistry*. 18(6), 567-568. https://doi.org/10.1093/clinchem/18.6.567.

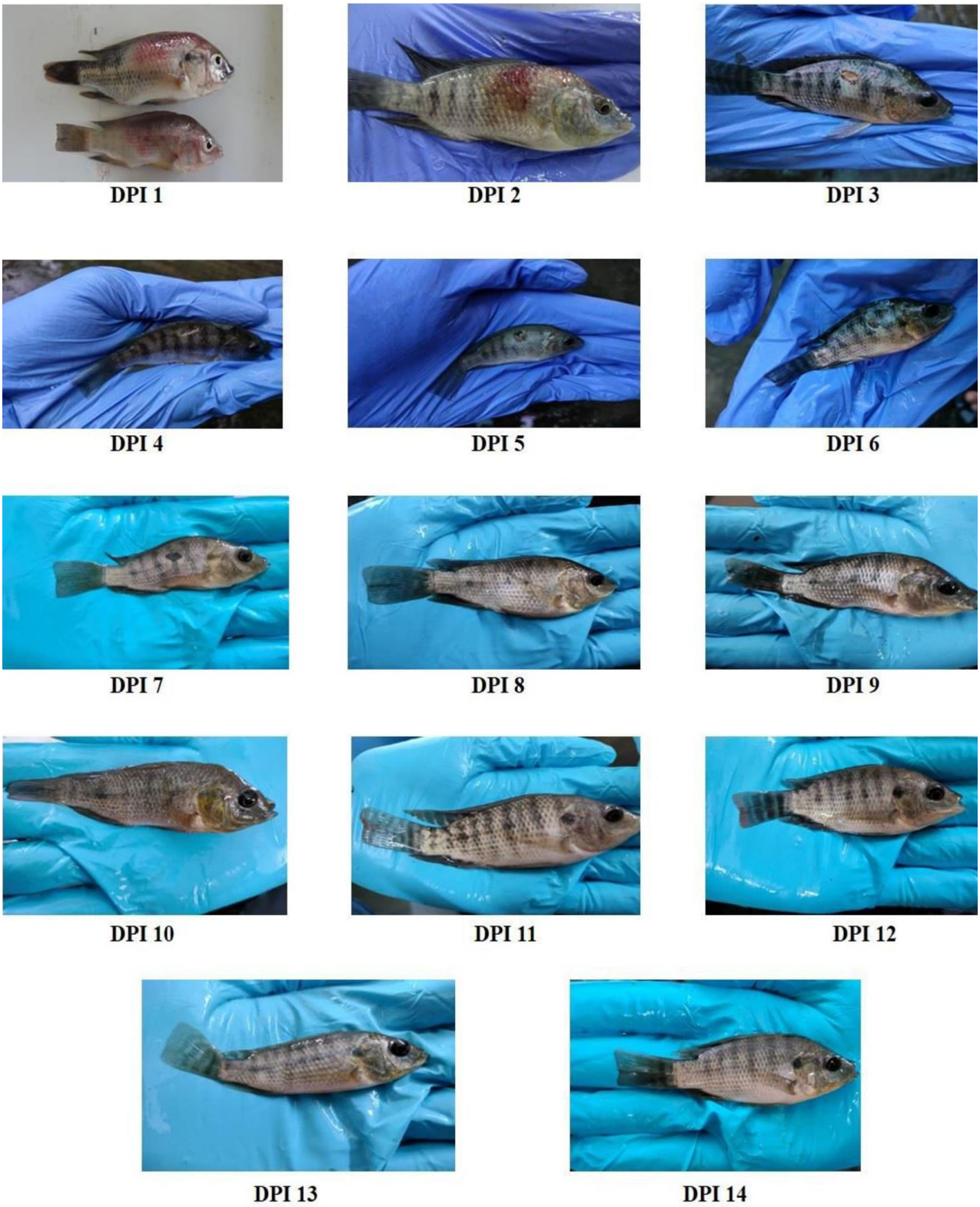
Wu, D., Fan, Z., Li, J., Zhang, Y., Wang, C. A., Xu, Q., & Wang, L. (2021). Evaluation of alpha-ketoglutarate supplementation on the improvement of intestinal antioxidant capacity and immune response in Songpu mirror carp (*Cyprinus carpio*) after infection with *Aeromonas hydrophila*. *Frontiers in Immunology*. 12, 690234. https://doi.org/10.3389/fimmu.2021.690234.

Yardimci, B., & Aydin, Y. (2011). Pathological findings of experimental *Aeromonas hydrophila* infection in Nile tilapia (*Oreochromis niloticus*). *Veterinary Journal of Ankara University*. 58, 47-54. https://doi.org/10.1501/Vetfak_0000002448.

Young, D.S., Pestaner, L.C., & Gibberman, V.A.L. (1975). Effects of drugs on clinical laboratory tests. *Clinical Chemistry*, 21(5), 1-432.



Supplementary Figure 1. The wound progression, healing, and the gross and clinical changes observed in *Aeromonas hydrophila*-challenged and oxolinic feed-fed *Oreochromis niloticus* juveniles for 7 consecutive days. DPI: Day post-injection.



Supplementary Figure 2. The wound progression, healing, and the gross and clinical changes observed in *Aeromonas hydrophila*-challenged and control-feed-fed *Oreochromis niloticus* juveniles. DPI: day post-injection.