



Enhancing Growth and Disease Resistance in North African Catfish Fingerlings: The Role of Aeration

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ABSTRACT

The research explores the impacts of aeration on the growth and well-being of North African catfish, *Clarias gariepinus*, raised in concrete tanks. Fish of mean weight 1.96 ± 0.22 g of three experimental groups in triplicates (30 fish per replicate group) were set to include, 0 h daily aeration group (control group), 12 h overnight aeration (i.e. 1800 h to 0600 h) group and a 24 h continues aeration (daily aeration) group, within a mean temperature of $28^\circ\text{C} \pm 0.21^\circ\text{C}$ fed a commercial diet for 8 weeks. Data on Weight Gain Rate percent (WGR%), Specific Growth Ratepercent (SGR%), Feed Conversion Ratio (FCR), Dissolved Oxygen (DO), and haematological parameters, Cumulative Mortality (CM), and Relative Percent Survival (RPS) to *Aeromonas hydrophila* infection were measured. Results from the study showed that 24 h aeration is best in improving WGR% and SGR% while both 12 h and 24 h of aeration are better in improving FCR compared to the 0 h aeration ($P < 0.05$). The water DO level was 8.24 ± 0.16 mg/l for the 24 h aeration groups throughout the 8 weeks of experimentation while significantly lower Mean Capsular Haemoglobin (MCH) and Mean Capsular Haemoglobin Concentration (MCHC) levels were found in 12 h and 24 h aeration groups compared to the 0 h aeration group ($P < 0.05$). The CM after *A. hydrophila* challenge test showed similarity ($P > 0.05$) in the 12 (12.22 ± 2.22 %) and 24 h (6.67 ± 2.22 %) groups in comparison to the 0 h (25.56 ± 1.92 %) group. The RPS was significantly higher in the 24 h group. The results suggest that aerating culture tank systems for 24 hours daily is an economically safe measure and could improve the growth and well-being of *C. gariepinus* as well as increase profits up to ~45 %.

Keywords: Aeration, Growth, *Clarias gariepinus*, Dissolved oxygen, *Aeromonas hydrophila*

IMPACT STATEMENT

Implementing 24 h daily aeration practices can significantly enhance the growth, elevate dissolved oxygen levels, and boost the resistance to *Aeromonas hydrophila* infection in North African Catfish *Clarias gariepinus* fingerlings, promoting sustainable and healthy catfish farming.

INTRODUCTION

The North African Catfish *Clarias gariepinus*, a native species of Africa is widely cultured, but 91% of its production comes from Sub-Saharan Africa, with Nigeria being the leading producer. In Ghana, Catfishes (*Clarias* sp. and *Heterobranchus* sp.) are the second largely cultured fish accounting for about 20 percent of

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aquaculture production. *Clarias gariepinus*' widespread cultivation is attributed to its wide acceptability, high tolerance to poor culture conditions, prolificity, and good feed conversion rate [1]. Despite its admirable cultural characteristics, the primary obstacles faced in the breeding, upkeep and extensive cultivation of *C. gariepinus* revolve around bacterial infections [2]. *Aeromonas* spp. are common bacterial pathogens that attack *C. gariepinus* [3]. In many parts of the world, data shows a downward trend in the supply of fish from capture fisheries and an increase in the demand for fish and fishery products especially in Ghana [4]. This has necessitated the adoption of intensive culture methods to increase fish production in culture systems [5]. To this end, the increase in intensive practices such as increased stocking densities and feed inputs has resulted in a surge in food production; however, critical issues such as increased exposure of fish to disease outbreaks and Dissolved Oxygen (DO) deficiency have emerged [6,7]. Ponds, concrete, fiberglass, and plastic tanks are the culture systems commonly used in the production of *C. gariepinus* globally. As many farmers believe *C. gariepinus* has high tolerance for low do, majority of the production cycle is done without aeration. With recent rise in demand of *C. gariepinus*, high stocking densities are employed to maximize production and profits resulting in hypoxia stress, exacerbation of disease occurrence and high mortality rates [8,9].

Disease management in fish culture depends on factors such as good feed and water management. Good water quality management, particularly managing the DO levels is essential for the survival and growth of fish [10]. To mitigate the effects of bacterial diseases in fish culture systems some reports have shown that many fish farmers and fish research scientists have resorted to the use of antibiotics, probiotics, prebiotics and medicinal plants with reports of varying degrees of success [11]. To attenuate DO deficiencies, fish cultivators have used aeration to increase DO levels in culture media [12,13]. Although aeration is a good way to solve the DO deficiency in culture systems, there is little research correlating the effects of aeration on fish growth, health, survival, and susceptibility to pathogens especially in *C. gariepinus*. In this study, the effects of aeration on growth performance, haematology, and *Aeromonas hydrophila* resistance in *C. gariepinus* fingerlings, were investigated. The comparative economic feasibility was also evaluated.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the Department of Aquaculture and Fisheries Sciences (DAFS) fish farm, Faculty of Biosciences, University for Development Studies, Nyankpala Campus, Ghana. The geographic coordinates of the experimental site are 9°24'50.1 N and 0°58'45.3"W.

Experimental fish and design

Two hundred and seventy fingerlings of the *C. gariepinus* of

mean weight 1.83 ± 0.18 g were purchased from a private fish farmer stationed in the Golinga community in the Tolon District of Northern Region of Ghana. The fish were transported to the fish farm of DAFS and placed in 9 circular concrete experimental tanks (90 cm in diameter x 65 cm in depth) containing static water to acclimatize to the experimental conditions within a week. Each tank containing 30 fingerlings were fed at 2% of their body weight twice daily at 0800 h and 1500 h with Ranaan Commercial Feed (Ranaan feed; protein, 42 %; ash, 10.0 %; moisture, 9.0 %; fiber, 3.0 %; vitamin A, 900 IU/kg; and vitamin C, 300 mg/kg). Three treatments including a 0 h aeration (control), 12 h aeration and a 24 h aeration in triplicate groups were randomly assigned to the stocked tanks. An aerator (BOYU air compressor of power 35 W, air flow max 30 L/min and pressure 0.03 MPa) was used to aerate the 12 h group from 6 pm to 6 am and the 24 h group continuously. All experimental groups were continually fed with the same commercial feed as before and observed for 8 weeks by monitoring growth and water quality.

Growth monitoring

The Initial Weight (IW), Final Weight (FW), Weight Gain Rate Percent (WGR%), and feed conversion ratio (FCR) were measured as have been described by Saheli et al. as [14]:

Weight Gain Ration (WGR, %)= $100 \times [(final\ weight\ (g)-initial\ weight\ (g))/initial\ weight\ (g)]$

Specific Growth Rate (SGR, %/day)= $100 \times [Ln\ final\ weight\ (g)-Ln\ initial\ weight\ (g)]/days$

Feed Conversion Ratio (FCR)=Dry feed intake (g)/wet weight gain (g) Protein

Monitoring of dissolved oxygen

Dissolved oxygen was monitored twice daily between 0600 h to 0700 h and between 1600 h to 1700 h with a multiparameter water meter (Bante, 900). The water level in the culture tanks was maintained by topping it up when necessary.

Blood sample collection

After 8 weeks of experimental feeding, blood samples from test fish were collected as previously described [15]. Briefly, whole blood was collected from the caudal fin region of the fish with a 1 ml syringe into EDTA tubes, stored on ice and transported to the laboratory for determination of haematological parameters including White Blood Cells (WBC), Red Blood Cells (RBC), Haemoglobin (HGB), Haematocrits (HCT), Mean Corpuscular Volume (MCH), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC). All blood tests were carried out in an Urit-5250 analyser using standard protocols at the Tamale Teaching Hospital, Haematology Laboratory Tamale, Ghana.

Artificial challenge test

A 10 day experiment was carried out prior to the challenge test to determine the LD50 of *A. hydrophila*. An LD50 of 1.0×10^8 CFU/ml was arrived at and used subsequently in the challenge trial. Eight weeks after the aeration trial, 25 fingerlings per replicate group of *C. gariepinus* from the respective treatments were infected with *A. hydrophila* prepared as previously described [16]. All test groups were injected intraperitoneally with 0.2 ml of the bacterial suspension and mortalities were recorded over 14 days. Dead fish were examined for clinical signs and bacteria were re-isolated to confirm the cause of death. Cumulative Mortality percent (CM %) and Relative Percent Survival (RPS) were computed as previously described as [17]: $CM\% = \frac{\text{Total mortality in each treatment after challenge}}{\text{Total number of fish challenged for same treatment}} \times 100$. $RPS = 1 - (\frac{\% \text{ of mortality in the treated group}}{\% \text{ of mortality in the control group}}) \times 100$

Statistical analysis

Data on initial and final weights, weight gain rate percent, specific growth rate percent, feed conversion ratio, haematological parameters and cumulative mortality of all test groups were subjected to one-way Analysis Of Variance (ANOVA). A repeated measures ANOVA was used to analyse bi-weekly weight measurements and dissolved oxygen levels of test fish. Turkey post hoc test was used to analyse differences in means and the results presented as means \pm standard error.

RESULTS

Effect of aeration on growth performance of *C. gariepinus*

As shown in Table 1, the experimentally tested fish in all treatments started with a similar IW ($P > 0.05$). After 8 weeks of experimentation, the FW and SGR% were in the order 24 h > 12 h > 0 h aeration with significant differences between 24 h and 0 h groups ($P < 0.05$). The WGR% showed similarities among all test groups ($P > 0.05$). It was observed that aeration significantly lowered the FCR of *C. gariepinus* in both 12 h and 24 h aeration treatments compared to those reared in 0 h aeration treatment.

Biweekly mean weight measurements for the various fish groups are shown in Figure 1. The fish group with 24 h aeration progressively demonstrated higher mean weight from week 2 throughout week 8 in comparison to the other fish groups. The fish group with 12 h aeration grew steadily in biweekly mean weight but began to rise above the 0 h aeration group after week 6 of experimentation. A repeated measures ANOVA with Mauchly's test of sphericity was used. The biweekly mean weight measurements served as dependent variables and treatments (0 h, 12 h, and 24 h) served as independent variables. The results revealed a significant difference within variables, ($F(4) = 279.29$, $p = 0.001$) with significant interaction between biweekly mean weight measurements and treatments ($F(2) = 6.17$, $p = 0.03$). However, no significant difference $F(2) = 4.98$, $p = 0.053$ was observed between treatments.

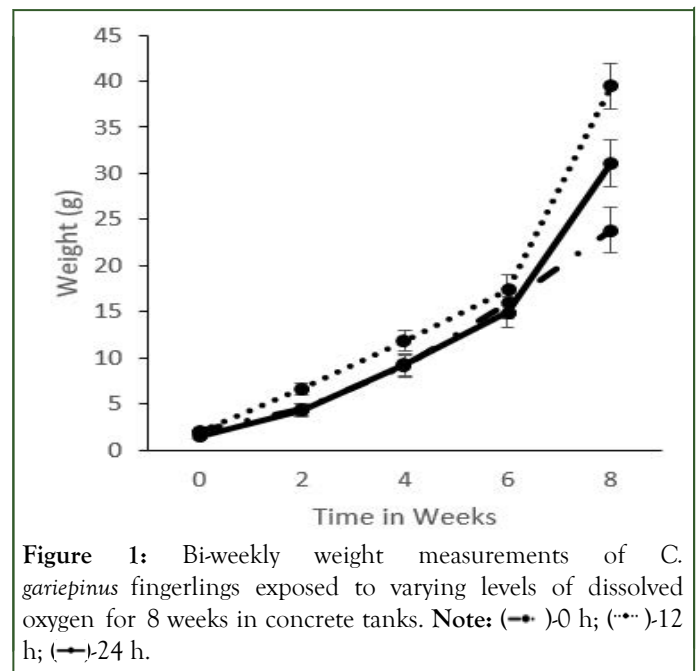


Figure 1: Bi-weekly weight measurements of *C. gariepinus* fingerlings exposed to varying levels of dissolved oxygen for 8 weeks in concrete tanks. Note: (—●—) 0 h; (·····●·····) 12 h; (---●---) 24 h.

Effect of aeration on dissolved oxygen level in *C. gariepinus*

The mean values of DO recorded over eight weeks in the respective groups are shown in Figure 2. A repeated measures ANOVA with Mauchly's sphericity test within and between subjects was run with time (weeks) of exposure to DO conditions as the dependent variable and treatments (i.e. 0 h, 12 h and 24 h) as independent variables. The analysis showed a significant difference within subjects, $F(4) = 16.17$, $p = 0.001$ but with no effect on interaction between time and treatment, $F(8) = 1.35$, $p = 0.27$. Between subject test, showed significant differences $F(2) = 269.57$, $p = 0.001$. Further analysis with Turkey post-hoc tests showed that treatments were in the order 24 h (8.24 ± 0.16 mg/l) > 12 h (5.82 ± 0.16 mg/l) > 0 h group (2.98 ± 0.16 mg/l). Generally, it was observed that the DO levels appeared to have continuously declined from the start (week 0) to the end (week 8) of the experiment in all tested groups in the ranges, 10.82 mg/L-5.78 mg/L, 8.08 mg/L-4.16 mg/L, 4.04 mg/L-2.28 mg/L for 24 h, 12 h and 0 h aeration respectively.

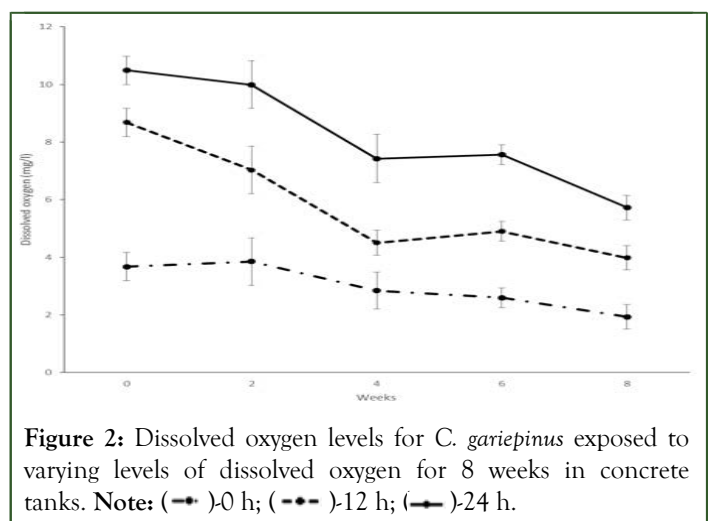


Figure 2: Dissolved oxygen levels for *C. gariepinus* exposed to varying levels of dissolved oxygen for 8 weeks in concrete tanks. Note: (—●—) 0 h; (---●---) 12 h; (·····●·····) 24 h.

Effect of aeration on the health of *C. gariepinus*

The effects of aeration on haematological parameters on test fish are shown in Table 2. Although slightly higher values of WBC, RBC, HGB, and HCT were noticed in the aerated groups compared to the non-aerated groups, no significant changes were observed. It was observed that the 0 h group demonstrated lowered MCV relative to the 24 h group. Aeration for 12 h and 24 h resulted in lowered MCH and MCHC in comparison to the 0 h (i.e. non-aerated).

Effect of aeration on disease resistance of *C. gariepinus*

As demonstrated in Figure 3, the cumulative mortality was similar among the aerated groups but significantly lower compared to the non-aerated group. In relation to the control, the relative per cent survival was significantly lower in the 12 h group compared to the 24 h with RPS of 52.91 ± 4.23 % and 74.60 ± 5.72 % respectively.

Economic feasibility of using aerators

Using Ghana as a case study, it will cost 0.966 cedis (1.15 cedis per kilowatt hour tariff) to run a 35 W aerator for a day (24 h) thus a total of 54.12 cedis to run the aerator for 8 weeks. At the current observed growth rate, it is 20.2% and 45.4% more profitable to culture *C. gariepinus* under 12 h and 24 h aeration

respectively than 0 h aeration under normal circumstance without disease outbreak (Table 1).

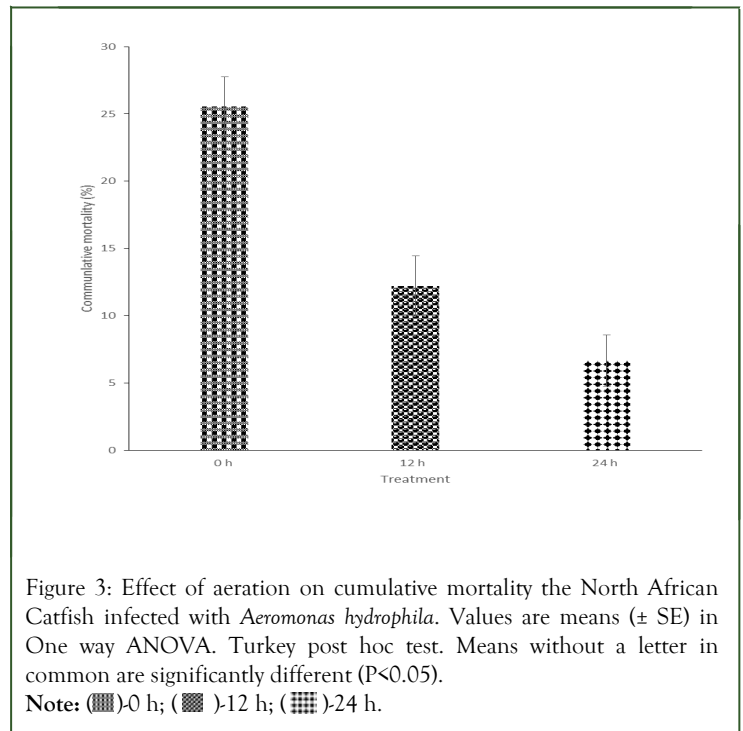


Figure 3: Effect of aeration on cumulative mortality the North African Catfish infected with *Aeromonas hydrophila*. Values are means (\pm SE) in One way ANOVA. Turkey post hoc test. Means without a letter in common are significantly different ($P < 0.05$). Note: (▨)-0 h; (■)-12 h; (▩)-24 h.

Measured parameter	Treatments			P-value
	0 h	12 h	24 h	
IW (g)	2.04 \pm 0.02	2.10 \pm 1.22	1.96 \pm 0.22	0.21
FW (g)	23.68 \pm 3.76 ^y	31.12 \pm 1.22 ^{zy}	39.44 \pm 1.91 ^z	0.01
WGR (%)	1071.90 \pm 190.23	2187.00 \pm 433.77	1942.94 \pm 161.23	0.08
SGR (%)	3.14 \pm 0.15 ^y	3.43 \pm 0.04 ^{zy}	3.66 \pm 0.05 ^z	0.00
FCR	2.57 \pm 0.09 ^z	2.07 \pm 0.10 ^y	2.06 \pm 0.10 ^y	0.02

Table 1: Growth performance of *C. gariepinus* with and without aeration after 8 weeks. Treatment designation are as follows: 0 h – fish group with no aeration serving as control group, 12 h-fish group with aeration from 1800 h-0600 h and 24 h-fish group with continues aeration for 2400 h. Note: IW: Initial weight; FW: Final weight; WGR: Weight Gain Rate Percent; SGR: Specific Growth Rate; FCR: Feed Conversion Ratio. ^{x,y,z}: values are means (\pm SE) in one way ANOVA(Analysis of Variance), Turkey post hoc test; Means without a letter in common are significantly different ($P < 0.05$); P: Probability.

Haematological parameters	Experimental Groups			P-value
	0 h	12 h	24 h	
WBC ($10^9/L$)	84.82 \pm 3.62	91.9 \pm 1.74	100.68 \pm 7.13	0.11
RBC ($10^{12}/L$)	1.672 \pm 0.08	1.93 \pm 0.07	2.38 \pm 0.35	0.12
HGB (g/dl)	8.44 \pm 0.37	8.80 \pm 0.44	10.92 \pm 1.72	0.26
HCT (%)	20.56 \pm 0.96	26.23 \pm 1.34	31.55 \pm 5.50	0.13
MCV (fL)	123.08 \pm 1.22 ^{yx}	135.88 \pm 4.00 ^z	131.02 \pm 3.01 ^{zy}	0.04

MCH (pg)	50.62 ± 1.50 ^z	45.57 ± 0.98 ^y	45.72 ± 0.47 ^y	0.01
MCHC (g/dL)	41.2 ± 1.36 ^z	33.67 ± 0.67 ^y	35.00 ± 0.73 ^y	0.00

Table 2: Treatment designation are as follows: 0 h-fish group with no aeration serving as control group, 12 h-fish group with aeration from 1800 h-0600 h and 24 h-fish group with continues aeration for 2400 h. **Note:** WBC: White Blood Cells; RBC: Red Blood Cells; HGB: Haemoglobin; HCT: Haematocrits; MCV: Mean Capsula Volume; MCH: Mean Corpuscular Hemoglobin and MCHC: Mean Corpuscular Hemoglobin Concentration; x,y,z: values are means (±SE) in one way ANOVA(Analysis of Variance), Turkey post hoc test; Means without a letter in common are significantly different (P<0.05); P: Probability.

DISCUSSION

Aeration is an effective means to increase DO levels in culture systems [18]. This was found to be true in the present study as aeration resulted in significantly enhanced DO in the 24 h group in comparison to those in the 12 h and 0 h groups. With daily aeration (24 h), the DO levels could be optimum thus 6 mg/L at the present stocking density as the fish grew from 1.83±0.18 g to about 31.21 ± 4.55 g of mean weight [19]. It is worth noting that DO levels in all tested groups decreased as the experiment progressed from the start to finish. This probably was because the supply of DO by the aerator was constant but with increasing demand as the fish grew bigger in size as the experiment progressed. This assertion is supported by the fact that, as fish grow bigger in size DO requirement for physiological activities increases [20]. Also, the findings suggest that DO levels in cultured systems need to be constantly monitored and supplemented, perhaps using higher aerator capacities to enhance the DO to meet the requirement of fish as they grow in size.

It was revealed in the experiment that supply of DO does not only enhance the water quality but also improve growth and health of fish. For example, daily aeration (24 hr) of tank water demonstrated significant improvement in %WGR and %SGR in *C. gariepinus* (P<0.05). Daily 12 h (1800-0600) aeration of pond water showed a slight improvement in %WGR and %SGR compared to 0 h (none-aerated tanks) (P>0.05). The findings in this study suggest that daily tank aeration (24 h) can improve the growth of *C. gariepinus* significantly and could increase profits by 45%. In terms of feed utilization thus a measure of the FCR, it was observed to be lower in both 24 h and 12 h aeration compared to 0 h aeration suggesting that the FCR of the fingerlings of *C. gariepinus* could be enhanced with aeration than without aeration. Similar results in *Oreochromis niloticus* have been reported elsewhere where aeration was found to improve growth and feed utilization in fish [6].

Haematological parameters namely RBC, HB, HCT, MCV, MCH and MCHC serve as oxygen transport vehicles in organisms. It has been found that culture conditions optimal levels of physicochemical parameters for example 6 mg/l of do not alter the levels of these oxygen transport vehicles [21]. Slightly contrary to the report by Abalaka SE et al, we found that an optimal and nearly optimal supply of DO in the 24 h and 12 h groups respectively by aeration did not alter RBC, HGB, and HCT significantly but lowered significantly the levels of MCH and MCHC in relation to the 0 h group. Significant lowering of some of these haematological parameters may be due to chance.

Despite the slight variation in levels in the treatment groups and the control group, it is worth noting that the levels recorded for these oxygen carrying blood parameters were similar to the normal haematological reference values reported by Sowunmi A et al, for *C. gariepinus* [22].

Normoxic conditions could support maximum growth and feed conversion efficiency, whereas prolonged hyperoxic and hypoxic conditions has the potential to induce stress, and increase disease susceptibility and mortality in fish [23-25]. A study of the haematological profile of fish can indicate the physiological status and health of the fish in response to different environmental stressors [26]. According to Momph P et al, DO levels below 6 mg/l in itself are not lethal to *C. gariepinus*, however, it promotes the proliferation of bacterial infection [18]. In this study, we found that aeration of *C. gariepinus* culture systems tend to increase resistance to bacterial infection as was shown in the bacteria challenge experiment with lower mortalities in the 24 h and 12 h groups compared to the 0 h group. Although no statistical increments were recorded in WBC, RBC, HGB and HCT among all groups under study, the increased resistance to *A. hydrophila* infection can be explained by the slight enhancement of WBC levels which primarily are involved in cellular immunity and oxygen delivery apparatus (RBC, HB, and HCT) [24]. Aeration can increase the profitability of culturing *C. gariepinus*, by 42.6% and 83.8 % for 12 h and 24 h of aeration per day, respectively, compared to no aeration when there is a risk of disease infection, such as *A. hydrophila* [27,28]. The observation in this study is in support of the work by Apriani et al, who reported an increase in infection of *A. hydrophila* in *C. gariepinus* in culture conditions with very low DO levels [29].

CONCLUSION

In conclusion, 24 h of daily aeration of culture systems showed significant improvement in the growth performance (i.e., weight gain, weight gain percent, specific growth rate percent) and feed utilization (i.e., feed conversion ratio) of *C. gariepinus* fingerlings as well as serve as a buffer to maintain an optimal supply of dissolved oxygen to the culture system. Daily aeration of these culture tanks increased the levels of haematological parameters and also resulted in increased levels of immune cells all of which culminated in increased resistance to *A. hydrophila* infection in *C. gariepinus* fingerlings. Therefore, daily aeration (24 h) can be a potential mechanism to enhance growth performance, health, and disease resistance in *C. gariepinus* fingerlings, and could reduce the use of other prophylactics which could be harmful or

expensive in *C. gariepinus* aquaculture. Future studies in varying water quality conditions common to *C. gariepinus* may be necessary to gain more knowledge and skills that could contribute to improving its' propagation.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest in this article. The authors also hereby declare that no funds, grants, or other support were received during the preparation of this manuscript.

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DATA AVAILABILITY STATEMENT

Data were created and analyzed in this research and will be made available upon request

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