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Full Length Research Article

Impact of Effective microorganisms (EM) and water-change frequency on growth and gut microbiota of *Heteroclarias*

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Abstract

Effective microorganisms (EM) have been proven as a natural remedy for enhancement of growth in livestock and fisheries. The lack of beneficial microbial population in pond water and the fish gut, particularly associated with water change frequency can adversely affect overall fish production. Thus this 60-day experimental study investigated the effects of water-change frequency and Effective Microorganisms (EM) on the growth performance and gut microbiota of *Heteroclarias* fingerlings. The experiment employed a completely randomized design with three treatments: A (5-day water change without EM), B (5-day water change with EM), and C (10-day water change with EM). Each treatment consisted of triplicate groups of fingerlings (initial mean weight = 4.50 g) at ten fish per 30 L plastic tank. Growth parameters including feed conversion ratio (FCR) and gut microbial diversity were evaluated. Treatment C achieved the highest mean weight gain (11.4 ± 0.95 g; $p < 0.05$) and the most efficient feed conversion ratio (FCR = 1.19 ± 0.04; $p < 0.05$), compared to treatments A and B. Although no significant differences ($p > 0.05$) were recorded in specific growth rate (SGR) or feed intake among treatments, EM application markedly enhanced gut microbial composition. The dominance of beneficial bacterial species such as *Bacillus subtilis* and *Bacillus licheniformis* in Treatment C, contrast with pathogenic taxa including *Enterococcus faecalis* observed in other groups. EM

inoculation with reduced water-change frequency supports microbial proliferation and low FCR. Integrating EM with a 10-day water-change interval improve growth and water-use efficiency in *Heteroclarias.*

Keywords: Effective Microorganisms (EM), Feed Conversion Ratio, Gut Microbiota, *Heteroclarias*, Sustainable Aquaculture, Water-Change Frequency

Introduction

Aquaculture remains one of the fastest-growing sectors of global food production, contributing substantially to nutritional security, economic development, and employment (Food and Agriculture Organisation (FAO), 2022). However, sustainable intensification of aquaculture systems requires balancing high productivity with ecological integrity and animal welfare. Among the major challenges constraining aquaculture efficiency are feed costs, water quality deterioration, and microbial imbalance within the rearing environment, all of which collectively influence fish health, growth, and feed utilization efficiency (Clavelle *et al*., 2019; Irabor *et al*., 2022).

Feed remains the single largest input cost in aquaculture, accounting for approximately 60–70% of total production expenditure (Omitoyin *et al.,* 2020*;* Khan *et al*., 2021). Consequently, enhancing feed conversion ratio (FCR) and optimizing nutrient assimilation have become central goals in modern aquaculture research. However, feed efficiency is intricately linked to water quality, the physiological condition of the fish, and the microbial environment of the culture system (Ebeniro *et al*., 2016; Irabor *et al*., 2021). Poor water quality, improper feeding regimes, and environmental stressors such as overcrowding, low dissolved oxygen, and excessive organic load can compromise fish health, leading to suppressed growth and increased susceptibility to diseases (Mzula *et al*., 2021).

Frequent water changes are traditionally employed to maintain water quality, yet this practice can be labour-intensive, costly, and stressful to cultured species. Each water-change operation disrupts the microbial equilibrium and alters physico-chemical parameters such as pH, conductivity, ammonia, and nitrate levels, potentially impairing fish growth and immunity Giatsis *et al.*, 2015; Ramos *et al*., 2017). Moreover, in developing area like Ishiagu, Ebonyi State, Nigeria, where access to clean and sufficient water is limited, frequent water replacement is impractical and unsustainable. This has necessitated the exploration of alternative eco-friendly strategies to manage water quality and microbial health within aquaculture systems.

Historically, synthetic additives and therapeutic agents such as antibiotics and chemical disinfectants have been used to control disease outbreaks and enhance growth in aquaculture (Yang *et al*., 2018). However, these interventions pose significant environmental and public health concerns, including antibiotic resistance, residue accumulation in fish tissue, and effluent contamination of aquatic ecosystems (Sylvain and Derome, 2017; Mzula *et al*., 2021). The need for safer and more sustainable alternatives has driven global interest in Effective Microorganisms (EM) based aquaculture management (Oyediran *et al.,* 2025).

Effective Microorganisms (EM) represent a consortium of beneficial microbes typically including *Lactobacillus* sp., *Bacillus* sp. and

Saccharomyces sp. that collectively promote biological equilibrium within aquatic systems (Dawood and Koshio, 2022; Ringo *et al*., 2023). These microbial consortia function by suppressing pathogenic bacteria, enhancing digestive enzyme activity, decomposing organic waste, and stabilizing water parameters (Balogun *et al*., 2016; Ebeniro *et al*., 2016; Yang *et al*., 2017). Furthermore, EM improves nutrient availability by promoting microbial mineralization processes, while simultaneously enhancing immune response and disease resistance in cultured fish (Sylvain and Derome, 2017; Ramos *et al*., 2017).

The hybrid African catfish (*Heteroclarias***),** a cross between *Clarias gariepinus* and *Heterobranchus bidorsalis*, has become a preferred species in African aquaculture due to its hardiness, fast growth, and tolerance to fluctuating environmental conditions (Irabor *et al*., 2021). However, despite these advantages, *Heteroclarias* production is often constrained by relatively high Feed Conversion Ratio (FCR)**,** particularly at the juvenile and nursery stages. Poor FCR not only increases production costs but also leads to longer culture period (Ebeniro *et al.,* 2016)

Recent research has highlighted the critical role of gut microbiota in mediating feed efficiency, metabolism, and immune function in fish (Zhang *et al*., 2021; Parata *et al*; 2023). A well-balanced gut microbial community facilitates nutrient absorption, modulates immune response, and contributes to disease resistance (Xiong *et al*., 2021). Conversely, disruption of the microbiome through frequent water changes, antibiotics, or poor feed management can lead to dysbiosis and reduced growth performance (Wei *et al*., 2017). Hence, the integration of EM and optimized water-change intervals represents a promising strategy for promoting both microbial stability and system efficiency in aquaculture.

Despite growing global evidence of EM's benefits, limited data exist in Nigeria regarding their application in *Heteroclaria*s production, particularly concerning their effects on gut microbial ecology under varying water-change regimes. This knowledge gap hinders the adoption of microbial-based management practices in small- and medium-scale aquaculture operations.

Therefore, this study investigates the combined effects of Effective Microorganisms (EM) application and water-change frequency on the growth performance and gut microbiota composition of *Heteroclarias* fingerlings. Specifically, it aims to assess how EM and water-change intervals influence growth parameters such as weight gain, specific growth rate, and feed conversion ratio evaluate the diversity and composition of the gut microbiota. The significance of the study is to determine optimal management practices for sustainable *Heteroclarias* aquaculture in a limited-water environment.

Materials and methods

Study Area

This research was conducted at the fish production unit of the Federal College of Agriculture, Ishiagu, Ebonyi State, Nigeria. Ishiagu lies within the derived savannah agroecological zone of southeastern Nigeria, located approximately between latitude 5°56′ N and longitude 7°33′ E, at an altitude of about 178 meters above sea level (Ogeh and Ogah, 2020). The area experiences a humid tropical climate with a mean annual rainfall ranging between 1,500–1,800 mm and mean daily temperatures between 26°C and 32°C (NIMET, 2023). Fish were reared in 50-litre plastic containers filled with 30 litres of water.

Experimental Design

A Completely Randomized Design (CRD) was employed with three treatments in triplicate. Ten juveniles (4.50 g each) were stocked per container. Fish were sourced from Ecodawn Fish Farmers and Consultants, Ibadan, Nigeria. The treatments were: A (5-day water change without EM), B (5-day water change with 10 ml EM), and C (10-day water change with 10ml EM). Fish were fed commercial feed at 5% body weight, twice daily for 60 days.

Fish Sampling and Measurement

Initial weights and lengths were recorded at beginning of the experiment, while further records were taken at 5-day interval. Five fish per replicate were collected randomly and their measurements taken. Lengths were measured with a 0.1 cm graduated meter rule, and weigh with a digital balance (Model SF-400, accuracy 0.01 g).

Growth and Survival Determination

The following parameters were determined from the fish samples using the formulae.

Weight gain = final weight – initial weight

$$\text{Specific growth rate} = \frac{\ln(\text{Final mean weight of fish}) - \ln(\text{Initial mean weight of fish})}{\text{No of days}} \times 100$$

$$\text{Survival Rate} = \frac{\text{Number of fish harvested}}{\text{No of fish stocked}} \times 100$$

$$\text{Feed Conversion Ratio} = \frac{Total\ Weight\ of\ feed\ fed\ to\ fish}{\text{Final mean body weight} - \text{Initial mean body weight}}$$

Determination of gut microbiota of the sampled fish

One fish from each container was used. A mid-central cut exposed the intestine, which was dissected longitudinally (Emikpe *et al*., 2011). One gram of gut content was mixed in 10 ml distilled water. Eosin Methylene Blue agar (EMBA), Nutrient Agar (NA), TCBS and Cetrimide Agar (CTA) were prepared according to manufacturer's specification and these were used for the cultivation of the micooganisms (Cheesbrough, 2000). The identities of the isolates were confirmed using standard identification manuals (Buchanan and Gibbons, 2000). Further. Bacterial load was measured using standard methods as described by Ogunbanwo *et al*. (2019). Microorganisms that were not identified by the colonial and microscopic characteristics were further subjected to biochemical tests described by Beishir (1987) and Cheesbrough (2000).

Gram Staining

Bacteria were gram-stained according to the standard of Cheesbrough (2000) to determine their type. Crystal violet and safranin were used, and slides were examined under a microscope. Gram-negative bacteria appeared pink/red, and gram-positive retained violet/blue.

Data Analysis

Data were analyzed using ANOVA in SPSS version 20. Duncan's multiple range test determined differences between means (significance at $p < 0.05$). Results were reported as means ± standard error of mean.

Results

Growth Performance

The results of the growth performance of *Heteroclarias* with EM-treated water are presented in Table 1. The obtained results showed a significant ($P<0.05$) weight gain (15.90± 0.12) g in the fish treated with EM at a 10-day water change when compared to the fish treated with EM at a 5-day water change and the control without EM. There were also significant higher values of length gain (7.7± 0.02) cm and lower FCR (1.19±0.04) in the fish treated with EM at the 10-day water change in relation to the other two treatments. However, the feed intake, specific growth rate and percentage

survival showed no significant variation (P>0.05) in all the treatments.

Gut Microbiota Diversity

Table 2 reveals high diversity of beneficial bacterial in Treatment C, with beneficial bacteria such as *Bacillus subtilis* and *Bacillus licheniformis*. Treatment A with a relative highest total count of bacteria (5.9×10^9), had predominantly pathogenic bacteria. The gut microbiota in treatment A was dominated by potentially pathogenic bacteria, including *Enterococcus faecalis* and *Pseudomonas aeruginosa* with minimal representation of beneficial probiotics species. The presence of *Salmonella* species in Treatment A suggests a less stable gut microbial environment, likely associated with poorer microbial regulation in the absence EM inclusion.

Treatment B showed a partial improvement in gut microbiota composition. Beneficial bacterial such as *Bacillus subtilis* and *Bacillus cereus* were detected, indicating that EM application promoted probiotics colonization. However, pathogenic species, *Pseudomonas aeruginosa* was still present, suggesting that frequent water changed may have disrupted microbial stability despite EM addition. Treatment C exhibited the highest microbial diversity and the most favourable gut profile. The dominance of *Bacillus* species in treatment C is indicative of effective probiotics colonization, enhanced competitive exclusion pathogenic bacterial and improved balanced within the fish gut

Table 1. Growth performance of *Heteroclarias* with EM treated water

Parameters	Treatments		
	A (Control with no EM)	B (5-day Water change with EM)	C (10-day Water change with EM)
W_1, (g)	4.50	4.50	4.50
W_2, (g)	14.22± 0.19[a]	14.28± 0.15[a]	15.90± 0.12[a]
WG, (g)	9.70± 1.05[a]	9.78± 1.02[a]	11.4± 0.95[b]
L_1, (cm)	7.40± 0.10[a]	7.50± 0.14[a]	7.40± 0.16[a]
L_2, (cm)	12.90± 1.22[a]	13.30± 1.32[a]	15.10± 1.05[b]
LG, (cm)	5.5± 0.05[a]	5.8± 0.02[a]	7.7± 0.02[b]
SGR, (% day^{-1})	1.91± 0.07[a]	1.92± 0.05[a]	2.10± 0.03[a]
FI, (g $fish^{-1}$)	13.3± 1.20[a]	13.4± 1.55[a]	13.6± 1.42[a]
FCR	1.37± 0.03[b]	1.37± 0.02	1.19± 0.04[a]
% Mortality	90[a]	80[a]	90[a]

Values are means ± S.EM. of triplicate groups of three plastic containers. Mean values with different superscript alphabets across each row are significantly different. Initial mean weight = W1, final mean weight =W2, mean Weight gain = (WG), initial mean length = L1, final mean length = L2 mean length gain = LG, specific growth rate (SGR), Feed intake (FI), feed conversion ratio (FCR). Values with the same superscript (across rows) are not significantly different (p>0.05), while those with different superscripts are significantly different (p<0.05)

Table 2. Diversity and count of microbiota in the gut of *Hetroclarias*

Treatment	Microbiota	Total count
A (Control with no EM)	*Bacillus subtilis* *Bacillus cereus* *Enterococcus faecalis* *Pseudomonas aeruginosa*	5.9×10^9
B (5-day Water change with EM)	*Bacillus subtilis* *Bacillus cereus*	1.06×10^{10}

C (10-day Water change with EM)	*Bacillus licheniformis* *Bacillus subtilis,* *Bacillus cereus* *Bacillus licheniformis*	1.34×10^{10}

Discussion

The present study evaluated the influence of Effective Microorganisms (EM) and varying water-change frequencies on the growth performance and gut microbiota composition of *Heteroclarias* fingerlings. The findings revealed that while EM application did not significantly affect specific growth rate (SGR) and feed intake (FI)**,** a marked improvement was observed in the feed conversion ratio (FCR) and weight gain among fish subjected to a 10-day water-change interval (Treatment C). Ebeniro *et al*. (2016) documented a similar observation on *Clarias gariepinus***.**

The non-significant variation in SGR and FI across treatments indicates that the impact of EM was not directly associated with feed consumption rate but rather with feed utilization efficiency. Similar trends were observed by Okomoda *et al*. (2021), who reported that low or stagnant SGR values in confined aquaculture systems could result from restricted space and physiological stress**,** limiting feed conversion despite stable intake levels. Likewise, Abdel *et al*. (2022) attributed variations in SGR to feed nutritional quality, a variable not assessed in the current experiment but one that could have interacted with microbial treatments to influence overall growth outcomes.

The superior FCR observed in Treatment C suggests that prolonged water-change intervals combined with EM supplementation may stabilize the rearing environment and reduce handling-induced stress. This stable environment allows for enhanced microbial colonization within the gut and rearing water, improving nutrient assimilation and reducing metabolic energy losses due to stress. As Ramos *et al*. 2017) noted, probiotics and EM agents promote intestinal morphological improvements**,** enhancing absorptive efficiency and fostering a healthier gut lining. Reduced disturbance from less frequent water changes likely fostered a more balanced microbial ecosystem, allowing beneficial bacteria to thrive while minimizing pathogen colonization.

Microbial profiling revealed a clear distinction between treatments. Fish in Treatment C harboured beneficial bacteria such as *Bacillus subtilis* and *Bacillus licheniformis*, while those in Treatments A and B exhibited a higher prevalence of pathogenic species, including *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Salmonella* spp. The dominance of *Bacillus* species in Treatment C underscores EM's capacity to enhance gut microbial symbiosis and competitive exclusion of pathogens**,** a phenomenon consistent with findings by Triantaphyllopoulos *et al*. (2020). According to Wei *et al*. (2017), beneficial and pathogenic bacteria naturally coexist in fish guts; however, an equilibrium that favours probiotic species like *Bacillus* is crucial for optimal digestion, enzyme secretion, and immune modulation.

The enhanced microbial diversity in the EM-treated, less frequently changed system (Treatment C) aligns with the work of Giatsis *et al*. (2015), who demonstrated that the rearing environment profoundly shapes gut microbial communities**.** They reported that stable aquatic microbiota can seed the

intestinal tract, influencing nutrient metabolism and immune homeostasis. By minimizing water replacement, Treatment C may have preserved environmental microbial stability**,** enabling synergistic microbial interactions between the water and the gut ecosystem.

Overall, these findings indicate that Effective Microorganisms, when combined with moderate water-change regimes, can optimize fish growth through microbial ecosystem management, reduce operational costs by lowering water use frequency, and support environmentally sustainable aquaculture production systems.

Conclusion

The study confirms that integrating Effective Microorganisms (EM) with a 10-day water-change cycle significantly enhances growth performance and gut microbial diversity in *Heteroclarias*. fingerlings. The dominance of beneficial bacteria particularly *Bacillus subtilis* and *Bacillus licheniformis* in the gut flora of EM-treated fish highlights the role in promoting digestive efficiency and immune competence. The improved FCR in EM-treated groups suggests a more balanced and productive microbial ecosystem. Overall, EM use offers a sustainable, low-cost, and eco-friendly approach to improving aquaculture productivity and microbial health while reducing water use and operational expenses.

Authors contributions

Conceptualization/investigation: OAG; Methodology: BRB; Formal analysis: EEP; Data curation: OKS; Visualization: ORC and C-OTJ

Conflict of interest

The authors declare they have no conflict of interest.

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