

## PHYSICOCHEMICAL CHARACTERISTICS OF EFFLUENT WATER FROM AFRICAN CATFISH (*Clarias Gariepinus*) TANKS FED FERMENTED PLANT-BASED DIETS

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**Received:** 05.01.2026; **Accepted:** 11.02.2026; **Available online:** 19.02.2026; **Published:** 30.03.2026

**Cite this article:** Nyamweha, B. R., Muhangi, C., Sunday, R., Muganyizi, D., Sserwadda, M., Tumwesigye, W., & Katuromunda, S. (2026). Physicochemical Characteristics of Effluent Water from African Catfish (*Clarias Gariepinus*) Tanks Fed Fermented Plant-Based Diets. *Trends in Ecological and Indoor Environmental Engineering*, 4(1), 60–69.

**Background:** Aquaculture intensification generates nitrogenous and phosphorus-rich effluents that threaten aquatic ecosystems. Fermented plant-based feeds are increasingly used to enhance nutrient digestibility and protein availability, yet their impact on effluent water quality remains poorly understood. Understanding how substrates such as banana, jackfruit seeds, and sweet potato influence ammonia, nitrite, BOD, and phosphates is critical for developing sustainable feeding strategies and minimizing environmental pollution in intensive African catfish (*Clarias gariepinus*) culture.

**Objectives:** This study aimed to evaluate the effects of solid-state fermented ripe banana, jackfruit seeds, and sweet potato tuber feeds on effluent water quality in African catfish (*Clarias gariepinus*), focusing on ammonia, nitrates, phosphates, BOD, copper, EC, and microbial composition to identify environmentally safer feed options. **Methods:** African catfish (*Clarias gariepinus*) fingerlings were stocked in 50 L glass aquaria and fed either fermented ripe banana, jackfruit seeds, sweet potato tubers, or commercial feed as control. Each treatment was triplicated in a completely randomized design. Effluent water was sampled weekly for four weeks to measure total ammonia nitrogen (TAN), nitrates, phosphates, biochemical oxygen demand (BODs), copper concentration, electrical conductivity (EC), pH, and microbial composition. TAN and nitrates were determined using colorimetric HS aqua test kits, phosphates and copper via Palin 7100 photometer, BODs with a magnetic stir BOD system, and microbial counts on nutrient agar. Statistical differences were assessed using Kruskal-Wallis and Dunn's post hoc tests ( $p < 0.05$ ). **Results:** Effluent water from tanks fed fermented banana and sweet potato exhibited lower total ammonia nitrogen ( $0.2\text{--}0.3 \text{ mg L}^{-1}$ ) and nitrates ( $0.01\text{--}0.12 \text{ mg L}^{-1}$ ) compared to jackfruit seeds (TAN  $1.3 \text{ p} < 0.05 \text{ mg L}^{-1}$ , nitrates  $0.75 \text{ p} < 0.05 \text{ mg L}^{-1}$ ) and commercial feed (TAN  $2.7 \text{ mg L}^{-1}$ , nitrates  $0 \text{ mg L}^{-1}$ ). Phosphate concentrations and biochemical oxygen demand (BODs) exceeded regulatory limits in all treatments except partial reduction in sweet potato tanks. Copper concentrations and electrical conductivity remained below permissible limits across all feeds. Microbial analysis revealed dominance of *Bacillus* and *Lactobacillus* species, with highest *Bacillus* counts in jackfruit seed tanks and *Lactobacillus* in banana tanks. Kruskal-Wallis tests confirmed significant differences ( $p < 0.05$ ) among treatments for TAN, nitrates, phosphates, BODs, copper, EC, and microbial counts. **Conclusion:** The study demonstrated the potential of specific fermented plant-based feed ingredients to mitigate nitrogen pollution in aquaculture systems. In particular, the inclusion of fermented ripe banana and sweet potato tubers in fish diets was shown to reduce ammonia and nitrite concentrations in culture water and effluent. However, high BOD and phosphate persisted, revealing a knowledge gap on nutrient release and effluent dynamics, guiding future sustainable feed research.

**Keywords:** sustainable aquaculture; African catfish (*Clarias gariepinus*); fermented plant-based feed; aquaculture effluent; total ammonia nitrogen (TAN); biochemical oxygen demand (BODs); nutrient loading; water quality management; solid-state fermentation.

### INTRODUCTION

Water quality remains a fundamental determinant of productivity, animal welfare, and sustainability in intensive aquaculture systems (Hambali et al., 2024; Symeonidou & Mente, 2024). In such systems, feed represents the primary external nutrient input, and its utilization efficiency directly influences both fish performance and the environmental footprint of production. A substantial proportion of dietary nutrients is not assimilated and is subsequently released into the culture water as dissolved and particulate wastes, primarily in the form of nitrogenous compounds and phosphorus (Kubiriza et al., 2024; White, 2013). These effluents may alter physicochemical conditions, stimulate eutrophication, and affect benthic community structure in receiving environments (Alvarado et al., 1997; Gonzalez-Gaya et al., 2022).

Intensive aquaculture operations, whether cage-based or land-based, generate organic solids from uneaten feed and faeces that accumulate in sediments and undergo microbial mineralization, resulting in elevated organic loads and nutrient release into surrounding waters (White, 2013; Kubiriza et al., 2024). In aquaculture effluent, nitrogen is released primarily as ammonia and its derivatives, along with phosphorus from feed residues and metabolic waste (Raza et al., 2025; Sun et al., 2024). Elevated concentrations of ammonia nitrogen and dissolved phosphorus are strongly linked to progressive eutrophication, depletion of dissolved oxygen, and alteration of physicochemical

conditions (Raza et al., 2025). These conditions can exacerbate hypoxia and shifts in aquatic ecosystem dynamics, affecting the survivorship, growth and physiological stress responses of cultured species. Unionized ammonia ( $\text{NH}_3$ ), which predominates at higher pH and temperature, is highly toxic to fish and has been associated with gill pathology, reduced growth, impaired immune function, and elevated mortality thresholds in various cultured species (Vera et al., 2023; Raza et al., 2025). Moreover, nutrient enrichment from aquaculture effluents can stimulate harmful algal blooms, which may further reduce oxygen availability and produce ichthyotoxic compounds, resulting in fish kills and long-term impacts on adjacent ecosystems (San Diego-McGlone et al., 2024; Zhang et al., 2025). The combined physicochemical pressures of organic loading, nitrogenous waste, and phosphorus input thus represent a critical challenge for sustainable aquaculture intensification.

The transition toward plant-based aquafeeds has intensified in response to sustainability concerns associated with the use and limited availability of fishmeal, which raises ecological, economic and resource-security issues in modern aquaculture. Recent advances indicate that plant-derived ingredients can significantly reduce reliance on marine proteins but often contain high levels of anti-nutritional factors (ANFs) and have lower nutrient digestibility compared to traditional fishmeal, which can compromise nutrient utilization and increase nutrient excretion into culture water (Ahmed et al., 2025). Microbial

fermentation technologies have been increasingly applied to plant ingredients to enhance their nutritional quality by reducing ANFs, increasing soluble protein content, and modifying biochemical composition, thereby improving protein availability and feed performance (Neves et al., 2024; Ahmed et al., 2025; Nandi et al., 2025). Fermentation can also increase the abundance of beneficial lactic acid bacteria and produce bioactive compounds that support gut health and may influence the interaction between dietary inputs and microbial communities in culture systems (Neves et al., 2024). However, while the nutritional performance and physiological benefits of fermented feeds have been studied in several fish species, their potential impact on effluent water quality, nutrient excretion patterns and subsequent environmental nutrient loading remains poorly understood, especially under intensive production regimes. Bridging this gap is essential for evaluating the broader sustainability and environmental footprint of incorporating fermented plant-based diets in aquaculture.

The solid-state fermented substrates evaluated in this study, banana wastes, jackfruit seeds, and sweet potato tubers, are nutrient-dense materials containing substantial crude protein, lipids, carbohydrates, and minerals (Intharathat et al., 2024; Ndyomugenyi & Ebong, 2016; Feng et al., 2024). Fermentation can increase nutrient availability and modify the biochemical composition of plant materials, potentially enhancing digestibility and altering nitrogen and phosphorus partitioning during metabolism. Consequently, the use of such substrates in aquafeeds may influence not only fish growth performance but also the quantity and form of nutrients released into culture water.

The fermented banana wastes contain approximately 32% crude protein, 6% crude fat, 6.13% crude fibre, 12.85% ash and 50–60% carbohydrates (Intharathat et al., 2024). Fermented jackfruit seeds contain 21.6% crude protein, 8.8% crude fat, 1.21% calcium and 0.84% phosphorus (Ndyomugenyi & Ebong, 2016), while fermented sweet potato tubers contain 10–20% crude protein, 15–30% crude fibre, 5–10% ash and 40–60% carbohydrates (Feng et al., 2024). Given their high nutrient density, these ingredients may contribute to altered nitrogenous and phosphorus loading in aquaculture systems depending on feed conversion efficiency, metabolic utilisation, and the balance between assimilated and excreted fractions.

Despite the growing adoption of fermented plant-based diets in the culture of African catfish (*Clarias gariepinus*), information remains limited regarding the physicochemical characteristics of effluents generated under such feeding regimes. Existing studies have largely focused on growth performance and feed efficiency, whereas the environmental implications of fermented ingredients, particularly their influence on biochemical oxygen demand (BOD), nutrient concentrations, trace elements, and overall water chemistry, remain insufficiently characterised. This represents a critical knowledge gap, as sustainable aquaculture intensification requires not only nutritionally efficient feeds but also a clear understanding of their environmental footprint.

Therefore, the present study aimed to determine how diets incorporating fermented ripe banana, jackfruit seeds, and sweet potato tubers affect the physicochemical quality of effluent water from African catfish tanks. Specifically, the study quantified biochemical oxygen demand (BOD), concentrations of phosphates, nitrates, and copper, and electrical conductivity as indicators of organic loading, nutrient enrichment, and potential pollution risk.

It was hypothesised that:

(i) the inclusion of fermented plant substrates would significantly

alter effluent nutrient profiles compared with conventional feeding regimes;

- (ii) differences in substrate composition would result in distinct patterns of nitrogen and phosphorus release;
- (iii) improved nutrient digestibility through fermentation could reduce organic loading and BOD, thereby mitigating potential environmental impacts.

By identifying relationships between fermented feed composition and effluent water characteristics, this study seeks to generate new insight into how alternative plant-based diets influence nutrient discharge dynamics in intensive aquaculture systems and to inform strategies for environmentally responsible feed formulation.

## MATERIALS AND METHODS

### Experimental site

The fish culture experiment was conducted at the Aquaculture Laboratory, and water analyses were performed at the Water Laboratory. Both laboratories are part of the Faculty of Agriculture and Environmental Sciences, Mountains of the Moon University, located in Fort Portal, Western Uganda. The university is situated at latitude 0° 39' 18.59" N and longitude 30° 17' 3.60" E, in a region characterized by proximity to Lake Saaka and surrounding volcanic hills.

### Setting up the experiment

African catfish (*Clarias gariepinus*) fingerlings were obtained from a local hatchery at Blue Valley Fish Farm, Kisinga, in the neighbouring district of Kasese, Western Uganda. The fingerlings were approximately 1 month old, with an initial mean body weight of 0.22 g and a mean body length of 2.74 cm. They were transported by road to the experimental site at Mountains of the Moon University and acclimated in glass aquaria prior to the start of the experiment.

The experimental tanks consisted of glass aquaria measuring 50 × 20 × 35 cm, each filled with 50 litres of Lake Saaka water and equipped with a single tube aerator. A total of 250 fingerlings were stocked in each tank. To mitigate ammonia accumulation, 100% of the water was exchanged weekly. The aquaria were partially shaded with newspaper to provide low-light conditions, as African catfish prefer reduced light for optimal feeding (Hildebrand et al., 2024).

Three experimental diets were tested: fermented ripe banana, fermented jackfruit seeds, and fermented sweet potato tubers, all provided in powdered form. These diets were compared with a commercially available fish feed used as the control. All fish were fed twice daily, with the control group receiving 5% of their fresh body weight per day. Each treatment was replicated three times in a completely randomized block design to ensure the reliability of the results.

### Preparation of treatment feeds

Fermented feed substrates of ripe banana, jackfruit seeds, and sweet potato tubers were prepared using the solid-state fermentation method as described by Nyamweha et al. (2025), with additional modifications to produce flour suitable for juvenile African catfish. The preparation involved several steps: collection and pre-treatment of raw materials, thermal processing, solid fermentation, and post-fermentation drying and milling.

### Collection and pre-treatment of raw materials

Mature banana fruits were harvested from the researchers' own plantation and placed in nylon bags to induce ripening. Jackfruit seeds and sweet potato tubers were obtained from the local

market in Kabundaire, Fort Portal, Uganda. All materials were thoroughly washed to remove soil and potential contaminants. The plant materials were then sliced into small pieces to increase the surface area for microbial colonization.

#### Thermal processing

Approximately 500 g of sliced plant material from each substrate was boiled in rainwater for 30 minutes to sterilize the material and facilitate nutrient availability for the target microbial inoculum (*Bacillus spp.*) (Nyamweha et al., 2025). After boiling, the substrates were drained and transferred to individual 10 L plastic buckets (Figure 1) for solid-state fermentation.



Figure 1. Solid fermentation of ripe banana, jackfruit seeds and sweet potato tubers in their respective plastic buckets for 10 days

#### Fermentation

The substrates were allowed to undergo solid-state fermentation for 10 days to encourage the growth of beneficial microbes, particularly *Bacillus spp.*, and the production of microbial protein suitable for fish nutrition.

#### Post-fermentation processing

Fermented substrates were sun-dried to approximately 15% moisture content and milled into fine flour using a clay mortar and pestle (Figure 2). The resulting flours were incorporated into the experimental diets as carbon sources. The carbon-to-nitrogen (C:N) ratio of the biofloc system was maintained at 10:1, following the protocols of Silva et al. (2017), Halim et al. (2025), and Sriyasak et al. (2022). The specific C:N ratios of fermented ripe banana, jackfruit seeds, and sweet potato were 3.12:1.71, 2.87:1.23, and 5.51:1.25, respectively, as determined by Nyamweha et al. (2025) using a combination of the loss-on-ignition and Kjeldahl methods. To achieve the target C:N ratio of 10:1, the carbon content was adjusted proportionally relative to nitrogen to calculate the amount of feed provided to the fish.



Figure 2. Flours of fermented plant-based feed ingredients: a – ripe banana, b – jackfruit seeds, c – sweet potato tubers

#### Data collection

Data on effluent water quality were collected from each experimental tank to evaluate the effects of the fermented plant-

based diets on key physicochemical parameters. All measurements were conducted according to manufacturer protocols using colorimetric kits and photometric methods.

#### Total ammonia nitrogen (TAN)

Total ammonia nitrogen ( $\text{NH}_3/\text{NH}_4^+$ ) concentrations were determined using the HS Aqua Test Kit (colorimetric method). Five millilitres of effluent water were transferred to a measuring cylinder, followed by the addition of 5 drops of reagent 1, and the cylinder was gently inverted several times to ensure thorough mixing. Subsequently, 5 drops of reagent 2 were added, and the sample was mixed again. Finally, 5 drops of reagent 3 were added, and the solution was allowed to develop colour for 15 minutes. The resulting colour was compared to the provided colour chart by placing the cylinder on the corresponding coloured circle until the closest match was observed.

#### Nitrites ( $\text{NO}_2^-$ )

Nitrite concentrations were measured using the HS Aqua Test Kit (colorimetric method). The measuring cylinder was rinsed prior to use, then filled with 5 mL of effluent water. Five drops of reagent 1 were added and the solution gently inverted several times. This was followed by the addition of 5 drops of reagent 2, and the cylinder was mixed again. The colour developed within approximately 3 minutes and was compared to the colour chart provided with the kit to determine the nitrite concentration.

#### Phosphate ( $\text{PO}_4^{3-}$ )

Phosphate concentrations in the effluent water were determined using a Palin Test Photometer 7100. Effluent water samples were first filtered through filter paper to remove suspended particles that could interfere with photometric readings. Ten millilitres of the filtered sample were transferred to a test tube, followed by the addition of one Phosphate No. 1 LLR tablet. The solution was gently shaken until complete dissolution. Next, one Phosphate No. 2 tablet was added and dissolved, after which the sample was allowed to stand for 10 minutes for full colour development. The test tube was then placed into the photometer's test cell holder, and the program for phosphate low range was selected. Readings were recorded from the photometer display. A blank sample consisting of deionized water was also analysed for comparison following the same procedure.

#### Biochemical oxygen demand (BOD)

Biochemical oxygen demand (BOD) of the effluent water was determined using standard procedures. A 250 mL sample of effluent water was manually collected from each tank and transferred into brown BOD bottles. Prior to sealing, the black absorption tubes on the bottles were filled with potassium hydroxide crystals to absorb any dissolved carbon dioxide that could interfere with measurements (Figure 3). The bottles were then sealed with specialized caps and placed on a magnetic stirrer connected to a power source for continuous agitation over a five-day incubation period. After incubation, BOD values were recorded directly from the display on top of the BOD bottle.

#### Electrical conductivity (EC) and pH

Electrical conductivity (EC) of the effluent water was measured using a Hach HQ40d multi-parameter meter. The probe was immersed in each tank, and readings were recorded directly from the device display.

pH measurements were conducted using a UWR International pH meter (Belgium). The probe was immersed in each tank, and the pH value was recorded twice per week.



Figure 3. Determination of BOD in effluent water from tanks fed fermented ripe banana, jackfruit seeds, and sweet potato tubers, compared with the control feed

#### Total copper

Total copper concentrations in the effluent water were determined using a Palin Test Photometer 7100. Test tubes were filled with 10 mL of sample or deionized water for the blank. One Coppercol No. 1 tablet was added to each tube and dissolved by gentle inversion. To remove air bubbles adhering to the tube walls, the test tubes were carefully inverted. One Coppercol No. 2 tablet was then added, crushed, and dissolved by mixing, with inversion repeated to eliminate bubbles. Copper concentrations were recorded in  $\text{mg L}^{-1}$  Cu from the photometer display.

#### Microbial composition of effluent water

The presence of *Bacillus* species in the effluent water was assessed using the nutrient agar method. Seven grams of nutrient agar were dissolved in 250 mL of rainwater and sterilized in an autoclave at 121 °C and 15 kPa. The agar was cooled for one hour and poured into sterile Petri dishes. After solidification, each plate was inoculated with 1000  $\mu\text{L}$  of water from the respective tank. Inoculated plates were incubated at 37 °C for 24 hours. Colony morphology was examined, and colonies were counted to estimate microbial abundance. Confirmation of *Bacillus spp.* presence was performed by applying hydrogen peroxide to colonies fixed on microscope slides.

#### Data analysis

All statistical analyses were conducted using STATA version 12. The Kruskal–Wallis test was employed to evaluate significant differences among treatments for total copper, total ammonia nitrogen (TAN), nitrites, BOD, electrical conductivity, phosphate, and microbial composition of effluent water samples, at a significance level of  $\alpha = 0.05$ .

Where significant differences were detected, Dunn's post hoc test was performed to determine which treatment groups differed statistically. P-values less than 0.05 were considered indicative of significant differences, while p-values greater than 0.05 were interpreted as non-significant. All results are presented as mean  $\pm$  standard deviation unless otherwise specified.

## RESULTS

#### Total ammonia nitrogen (TAN)

The concentrations of total ammonia nitrogen (TAN) in effluent water varied among treatments over the four-week experimental period (Table 1).

In week 1, TAN concentrations were 0.45  $\text{mg L}^{-1}$  in tanks fed fermented jackfruit seeds, 0.50  $\text{mg L}^{-1}$  in tanks fed fermented ripe banana, and 0.20  $\text{mg L}^{-1}$  in tanks fed fermented sweet potato tubers. The control feed produced TAN concentrations

comparable to the fermented jackfruit seed treatment (0.45  $\text{mg L}^{-1}$ ). No significant differences were observed among treatments during this week (Kruskal–Wallis test,  $P > 0.05$ ).

In week 2, the highest TAN was recorded in the control feed tanks (5  $\text{mg L}^{-1}$ ), followed by fermented jackfruit seeds (2  $\text{mg L}^{-1}$ ). TAN concentrations in tanks fed fermented ripe banana and sweet potato tubers were 0  $\text{mg L}^{-1}$ . Significant differences were observed among treatments (Kruskal–Wallis test,  $p < 0.05$ ), as indicated by differing superscripts in Table 1.

In week 3, TAN concentrations remained highest in the control feed tanks (5  $\text{mg L}^{-1}$ ), followed by fermented jackfruit seeds (0.5  $\text{mg L}^{-1}$ ). Tanks fed fermented ripe banana and sweet potato tubers exhibited the lowest TAN concentrations (0.2  $\text{mg L}^{-1}$ ). Significant differences among treatments were detected (Kruskal–Wallis test,  $p < 0.05$ ; Table 1).

In week 4, the highest TAN was observed in the control feed tanks (3  $\text{mg L}^{-1}$ ), followed by fermented jackfruit seeds (2  $\text{mg L}^{-1}$ ). The lowest TAN concentration was recorded in tanks fed fermented ripe banana (0  $\text{mg L}^{-1}$ ). Significant differences among treatments were observed (Kruskal–Wallis test,  $p < 0.05$ ; Table 1).

#### Nitrite ( $\text{NO}_2^-$ ) concentrations

Nitrite concentrations in the effluent water varied among treatments over the four-week experimental period (Figure 4).

In week 1, the highest nitrite concentration was observed in tanks fed fermented jackfruit seeds (1  $\text{mg L}^{-1}$ ), followed by tanks fed fermented sweet potato tubers (0.03  $\text{mg L}^{-1}$ ). Tanks fed fermented ripe banana and the control feed exhibited no detectable nitrite (0  $\text{mg L}^{-1}$ ). Significant differences were observed among treatments (Kruskal–Wallis test,  $p < 0.05$ ), as indicated by differing letters in Figure 4.

In week 2, nitrite concentrations were 0.75  $\text{mg L}^{-1}$  in tanks fed fermented jackfruit seeds, whereas no nitrite was detected in other treatments. Significant differences among treatments were observed (Kruskal–Wallis test,  $p < 0.05$ ; Figure 4).

In week 3, nitrite concentrations were generally lower than in previous weeks. Tanks fed fermented jackfruit seeds exhibited the highest concentration (0.25  $\text{mg L}^{-1}$ ), while all other treatments had no detectable nitrite. Significant differences among treatments were observed (Kruskal–Wallis test,  $p < 0.05$ ; Figure 4).

In week 4, nitrite production was highest overall compared with other weeks. Tanks fed fermented jackfruit seeds recorded 1  $\text{mg L}^{-1}$ , followed by tanks fed fermented ripe banana (0.5  $\text{mg L}^{-1}$ ), while the control feed tanks exhibited the lowest concentration. Significant differences among treatments were observed (Kruskal–Wallis test,  $p < 0.05$ ; Figure 4).

Table 1. TAN production from catfish fish tanks fed with fermented ripe banana, jackfruit seeds, sweet potato tubers and the control diet (Kruskal–Wallis test)

| Sampling periods | Control                | Ripe banana            | Jackfruit seeds        | Sweet potato           |
|------------------|------------------------|------------------------|------------------------|------------------------|
| Week 1           | 0.45±0.05 <sup>a</sup> | 0.51±0.03 <sup>a</sup> | 0.45±0.01 <sup>a</sup> | 0.25±0.04 <sup>a</sup> |
| Week 2           | 5.00±2.00 <sup>a</sup> | 0.00±0.00 <sup>b</sup> | 2.00±0.10 <sup>a</sup> | 0.00±0.00 <sup>a</sup> |
| Week 3           | 5.00±1.65 <sup>a</sup> | 0.20±0.06 <sup>b</sup> | 0.5±0.045 <sup>b</sup> | 0.2±0.06 <sup>b</sup>  |
| Week 4           | 3.00±1.50 <sup>a</sup> | 0.00±0.00 <sup>b</sup> | 2.00±0.70 <sup>a</sup> | –                      |

Note: superscripts stand for significant differences

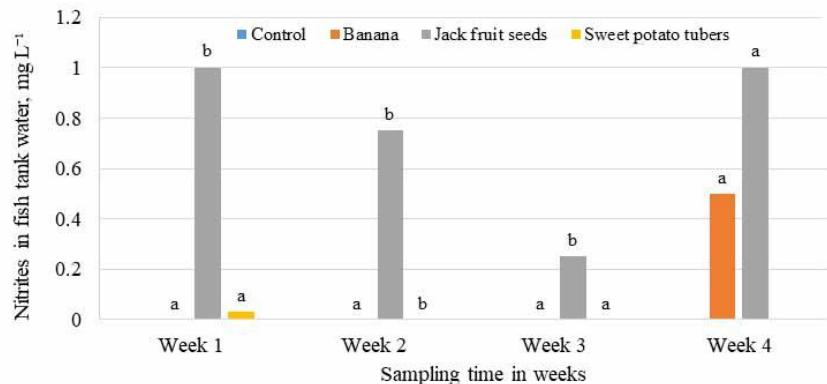


Figure 4. Nitrite production in fish tanks fed with fermented ripe banana, jackfruit seeds, sweet potato tubers and control feeds

#### Phosphate (PO<sub>4</sub><sup>3-</sup>) concentrations

Phosphate concentrations in effluent water differed among treatments (Figure 5). The highest concentration was observed in tanks fed the control feed (99.5 mg L<sup>-1</sup>), followed by tanks fed fermented sweet potato tubers (92.9 mg L<sup>-1</sup>), fermented jackfruit seeds (64.6 mg L<sup>-1</sup>), and the lowest concentration in tanks fed fermented ripe banana (61.8 mg L<sup>-1</sup>). Significant differences were detected among treatments (Kruskal–Wallis test,  $p < 0.05$ ), as indicated by differing letters in Figure 5.

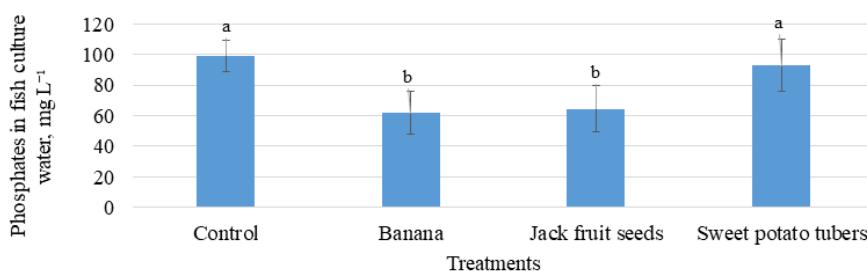


Figure 5. Phosphates produced from fish tanks fed tanks fed with fermented ripe banana, jackfruit seeds, sweet potato tubers and control feeds. Error bars representing standard deviation and different letters for significant differences

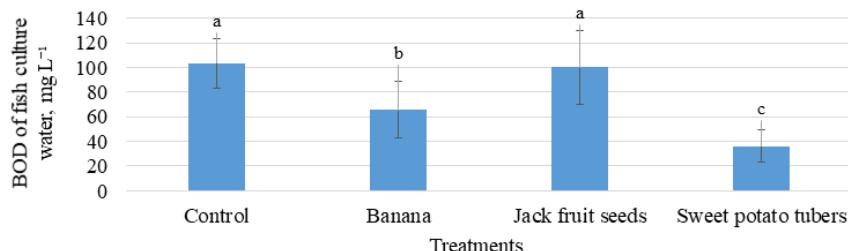


Figure 6. BOD in fish tanks fed with fermented ripe banana, jackfruit seeds, sweet potato tubers and control feeds

#### Electrical conductivity (EC)

Electrical conductivity (EC) of effluent water varied among treatments over the four-week experimental period (Figure 7).

In week 1, the highest EC was recorded in tanks fed fermented ripe banana (360  $\mu\text{S cm}^{-1}$ ), followed by control feed (306  $\mu\text{S cm}^{-1}$ ) and fermented jackfruit seeds (281  $\mu\text{S cm}^{-1}$ ). The lowest EC was observed in tanks fed fermented sweet potato

tubers ( $277 \mu\text{S cm}^{-1}$ ). Significant differences were detected among treatments (Kruskal–Wallis test,  $p < 0.05$ ), as indicated by differing letters in Figure 7.

In week 2, the highest EC was observed in control feed tanks ( $388 \mu\text{S cm}^{-1}$ ), while the lowest was in tanks fed fermented sweet potato tubers ( $223 \mu\text{S cm}^{-1}$ ). Significant differences among treatments were detected (Kruskal–Wallis test,  $p < 0.05$ ; Figure 7).

In week 3, EC was highest in control feed tanks ( $520 \mu\text{S cm}^{-1}$ ) and lowest in tanks fed fermented sweet potato tubers ( $225 \mu\text{S cm}^{-1}$ ). Significant differences among treatments were observed (Kruskal–Wallis test,  $p < 0.05$ ; Figure 7).

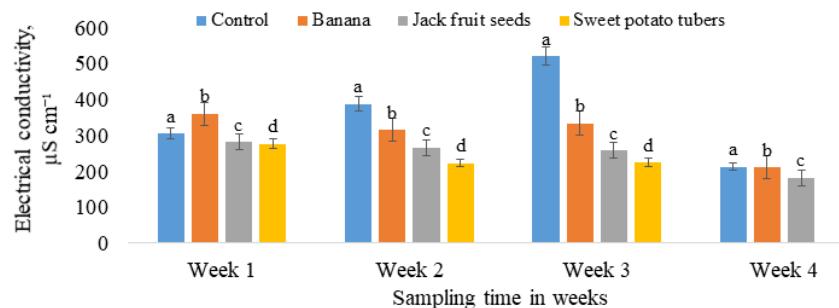


Figure 7. EC of water in fish tanks fed with fermented ripe banana, jackfruit seeds, sweet potato tubers and control feeds. Error bars representing standard deviation and different letters for significant differences

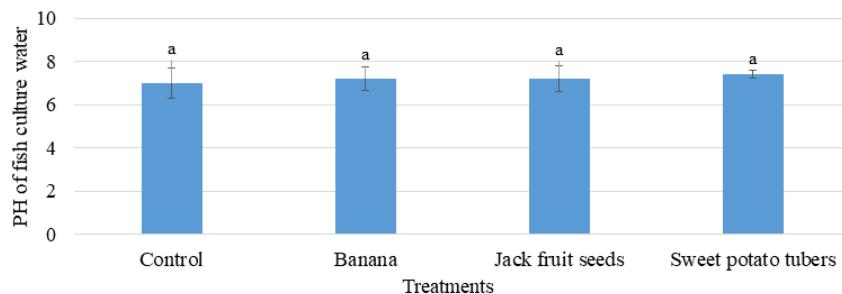


Figure 8. The pH of fish culture tanks fed with fermented ripe banana, jackfruit seeds, sweet potato tubers and control feeds. Error bars representing standard deviation and same letters indicating no significant differences

#### Total copper (Cu) concentrations

Total copper concentrations in effluent water differed among treatments (Figure 9). The highest concentration was recorded in tanks fed the control feed ( $0.92 \text{ mg L}^{-1}$  Cu), followed by tanks fed fermented ripe banana ( $0.32 \text{ mg L}^{-1}$  Cu), fermented jackfruit seeds ( $0.16 \text{ mg L}^{-1}$  Cu), and the lowest concentration in tanks fed fermented sweet potato tubers ( $0.02 \text{ mg L}^{-1}$  Cu). Significant differences were observed among treatments (Kruskal–Wallis test,  $p < 0.05$ ), as indicated by differing letters in Figure 9.

#### Microbial composition of effluent water

All water samples contained *Bacillus* and *Lactobacillus* species, with *Lactobacillus* dominating overall (Table 2).

The highest number of *Bacillus* colonies was observed in tanks fed fermented jackfruit seeds (23.5 colonies), whereas the lowest was recorded in tanks fed fermented ripe banana (3.5 colonies). Significant differences in *Bacillus* colony counts were detected among treatments (Kruskal–Wallis test,  $p < 0.05$ ), as indicated by differing superscripts in Table 2.

For *Lactobacillus*, the highest colony count was observed in tanks fed fermented ripe banana (201 colonies), while the lowest count was recorded in tanks fed fermented jackfruit seeds (83.5 colonies). Significant differences among treatments were observed (Kruskal–Wallis test,  $p < 0.05$ ; Table 2).

In week 4, the highest EC was recorded in control feed tanks ( $213 \mu\text{S cm}^{-1}$ ), whereas the lowest was observed in tanks fed fermented jackfruit seeds ( $183 \mu\text{S cm}^{-1}$ ). Significant differences among treatments were detected (Kruskal–Wallis test,  $p < 0.05$ ; Figure 7).

#### pH of effluent water

The pH of effluent water varied slightly among treatments (Figure 8). The highest pH was recorded in tanks fed fermented sweet potato tubers (7.41), while the lowest pH was observed in tanks fed the control feed (7.02). No significant differences were detected among treatments (Kruskal–Wallis test,  $p > 0.05$ ), as indicated by identical letters in Figure 8.

Additionally, *Staphylococcus* colonies were detected only in water samples from tanks fed fermented ripe banana, with 55.6 colonies exhibiting golden-yellow morphology. Significant differences in *Staphylococcus* colony counts among treatments were observed (Kruskal–Wallis test,  $p < 0.05$ ; Table 2).

#### DISCUSSION

##### Low TAN and nitrite concentrations in fish tanks fed with fermented substrates

The fermented substrates, ripe banana, jackfruit seeds, and sweet potato tubers, produced lower total ammonia nitrogen (TAN) compared to the control feed. On average, control feed generated  $2.7 \text{ mg L}^{-1}$  of TAN per week, whereas tanks fed fermented ripe banana, sweet potato tubers, and jackfruit seeds produced  $0.2$ ,  $0.1$ , and  $1.3 \text{ mg L}^{-1}$ , respectively. Consequently, TAN concentrations in the control feed and jackfruit seed treatments exceeded the recommended maximum limit of  $0.2 \text{ mg L}^{-1}$  for aquatic life (Ogbonna & Chinomso, 2010).

TAN excretion is influenced by feeding rate and stocking density (Mustapha & Akinshola, 2016; Fanani & Fata, 2022; Vajargah & Yalsuvi, 2022). In this study, each tank was stocked with 250 fingerlings and fed twice daily based on a C:N ratio of 10:1. While overfeeding cannot be ruled out, accumulation of organic matter likely contributed to elevated TAN in control and jackfruit seed treatments.

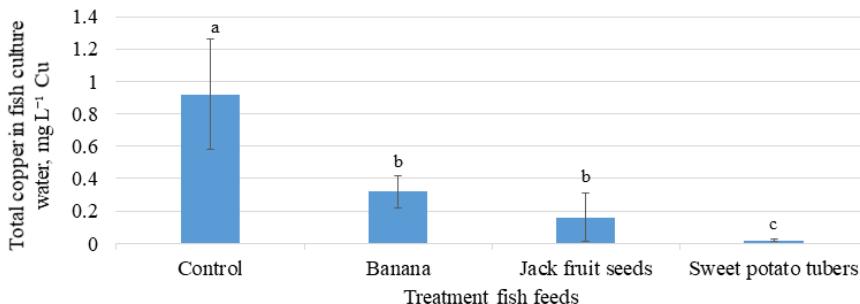


Figure 9. Total copper in water of fish tanks with fermented ripe banana, jackfruit seeds, sweet potato tubers and control feeds. Error bars representing standard deviation and different letters for significant differences

Table 2. Microbial composition of effluent water from the fish tanks fed with fermented ripe banana, jackfruit seeds, sweet potato tubers and control feeds

| Physical characteristics of microbial colonies on nutrient agar                       | Control feed                               | Ripe banana                                | Jackfruit seeds                              | Sweet potato tubers                           | Remarks  |
|---|--|--|--|---|--|
| Circular, flat and opaque white colonies, diameter 2–4 mm, irregular or ragged margin | 8.5±6.5 <sup>a</sup><br>microbial colonies | 3.5±2.1 <sup>a</sup><br>microbial colonies | 23.5±6.4 <sup>b</sup><br>microbial colonies  | 7.2±2.8 <sup>a</sup><br>microbial colonies    | These colonies are associated with <i>Bacillus subtilis</i> (Pandav et al., 2021)                                    |
| Small convex smooth and glistening colonies 2–5 mm                                    | 150±30 <sup>a</sup><br>microbial colonies  | 201±40 <sup>b</sup><br>microbial colonies  | 83.5±8.51 <sup>d</sup><br>microbial colonies | 121.5±8.51 <sup>c</sup><br>microbial colonies | These colonies are associated with <i>Lactobacillus</i> and <i>E. coli</i> (Meng et al., 2021; Hossain et al., 2021) |
| Golden yellow round smooth convex 1–3 mm  | 0±0.00<br>microbial colonies               | 55.6±13.44<br>microbial colonies           | 0.0±0.00<br>microbial colonies               | 0.0±0.00<br>microbial colonies                | Colonies associated with <i>Staphylococcus</i> (Afrin et al., 2019)  |

Note: different superscripts indicate significant differences

TAN represents both toxic ammonia and less toxic ammonium (Boyd, 2001), yet ammonia is particularly critical due to its toxicity, causing mortality in aquatic species at concentrations of 0.3–0.9 mg L⁻¹ in cold-water fish and 0.7–3.0 mg L⁻¹ in warm-water species (Boyd, 2018). Ammonia originates primarily from protein and amino acid catabolism, which explains the higher production in control feed and jackfruit seed treatments compared to fermented banana and sweet potato, which are lower in protein-rich nitrogen compounds (Davison, 2024).

Nitrite concentrations were generally low across all treatments, with fermented jackfruit seeds producing the highest (0.75 mg L⁻¹ per week), followed by fermented ripe banana (0.12 mg L⁻¹), sweet potato (0.01 mg L⁻¹), and control feed (0 mg L⁻¹). These differences may result from incomplete nitrogen cycling due to organic load (Zhang et al., 2025) and the activity of ammonia-oxidizing bacteria such as *Nitrosomonas* and *Nitrosococcus* in tanks with higher ammonia. Other nitrite-producing bacteria, including *E. coli*, *Klebsiella*, *Proteus*, and *Enterococcus*, could contribute to nitrite formation (Park & Rhee, 2024). Nitrite is a toxic nitrogen compound responsible for brown blood disease at high concentrations (Durborow et al., 1997; Kroupova et al., 2005). This underlines the importance of monitoring nitrite levels when using fermented plant-based feeds.

#### High phosphate concentrations and BOD in fish culture water

All treatments, including control feed, contained measurable phosphate concentrations, with the highest levels observed in control feed (99 mg L⁻¹) and fermented sweet potato (92 mg L⁻¹), followed by jackfruit seeds (64 mg L⁻¹) and ripe

banana (61 mg L⁻¹). These values exceed the Ugandan National Environment Standards (NEMA, 2020) maximum permissible limit of 5 mg L⁻¹.

High phosphorus levels in aquaculture effluent are linked to feed composition and overfeeding (Nathanailides et al., 2023; Sugiura, 2018). Solid-state fermentation may further enhance phosphorus and calcium bioavailability, as observed in other plant substrates (Duliński et al., 2017), likely contributing to elevated phosphate levels in the fermented feed treatments.

BOD values were also elevated, exceeding the maximum permissible limit of 50 mg L⁻¹ (NEMA, 2020), except in tanks fed fermented sweet potato (36 mg L⁻¹). The highest BOD occurred in control feed (103.3 mg L⁻¹), followed by fermented jackfruit seeds (100.3 mg L⁻¹) and fermented ripe banana (65.6 mg L⁻¹). Elevated BOD in control feed reflects high protein and fat content requiring extensive microbial decomposition (Boyd, 2008). In contrast, fermentation transforms plant substrates into simpler sugars, reducing oxygen demand during microbial breakdown (Rajczyk, 1993).

#### Copper concentration and electrical conductivity

Total copper was highest in control feed tanks (0.92 mg L⁻¹), followed by fermented ripe banana (0.32 mg L⁻¹), jackfruit seeds (0.16 mg L⁻¹), and lowest in sweet potato (0.02 mg L⁻¹). Copper is commonly present in fish feeds as a micronutrient for blood formation (El-Erian et al., 2023), and plants accumulate copper from soil, water, and air (Simončič et al., 2017). Only control feed tanks exceeded the Ugandan regulatory limit of 0.5 mg L⁻¹ (NEMA, 2020).

EC was highest in control feed tanks (356.7 µS cm⁻¹), followed by fermented ripe banana (305 µS cm⁻¹), jackfruit

seeds ( $247 \mu\text{S cm}^{-1}$ ), and sweet potato ( $241.7 \mu\text{S cm}^{-1}$ ). Elevated EC is associated with dissolved ions from feed decomposition and waste accumulation (United States Environmental Protection Agency [EPA], 2025). Potassium-rich substrates like banana may also contribute to EC (Hoy et al., 2022), and feed additives can enhance conductivity in commercial feeds (Wang et al., 2025). All measured EC values remained below the regulatory limit of  $1000 \mu\text{S cm}^{-1}$  (NEMA, 2020).

### Compliance with regulatory standards

Based on BOD, only effluent water from fermented sweet potato could be considered for discharge, yet its phosphate concentration ( $92 \text{ mg L}^{-1}$ ) exceeded the maximum limit by 18 times. Consequently, none of the effluents met Ugandan regulatory standards for discharge (NEMA, 2020), highlighting the need for treatment of effluent from fish tanks fed fermented plant-based diets before environmental release.

### Mitigation strategies for high BOD, phosphates, and EC

High phosphorus and BOD levels in aquaculture effluents are primarily due to overfeeding and organic matter accumulation (Nathanailides et al., 2023). Optimal feeding regimes based on body weight and stocking density, regular removal of uneaten feed, and routine water quality monitoring are recommended to mitigate environmental impacts.

### CONCLUSION

The results showed that none of the tested feeds produced effluent fully compliant with Ugandan wastewater discharge regulations, indicating that the development of a completely environmentally friendly feed remains a challenge. Nevertheless, significant differences were observed in key water quality parameters among the treatments. Effluents from tanks fed fermented ripe banana and sweet potato tubers exhibited substantially lower total ammonia nitrogen (TAN) and nitrite concentrations compared to commercial control feeds, remaining within ranges considered safer for African catfish. In contrast, effluents from jackfruit seed-fed tanks contained unsafe ammonia and nitrite levels, similar to or exceeding those produced by commercial feeds.

The study also revealed that despite the reduced nitrogenous waste, biochemical oxygen demand (BODs) and phosphate concentrations in all fermented plant feed treatments exceeded permissible limits, highlighting the need for further optimization of feeding regimes or post-treatment strategies prior to effluent discharge. Copper concentration and EC, however, remained below regulatory thresholds across all treatments.

These findings provide new insights into the environmental impact of fermented plant-based feeds in intensive aquaculture systems. Unlike previous studies that primarily focused on nutritional performance and growth of fish on fermented diets, this research fills a knowledge gap by demonstrating how specific plant substrates influence nitrogenous waste, BOD, phosphates, and overall water quality in African catfish culture. The results confirm the hypothesis that fermentation of low-protein plant substrates, such as banana and sweet potato, can reduce ammonia and nitrite excretion, offering a partial solution toward more sustainable aquaculture practices.

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Overall, the study demonstrated the potential of specific fermented plant-based feed ingredients to mitigate nitrogen pollution in aquaculture systems. In particular, the inclusion of fermented ripe banana and sweet potato tubers in fish diets was shown to reduce ammonia and nitrite concentrations in culture water and effluent. This underscoring that further refinement is required to fully comply with environmental standards.

Despite the partial mitigation of nitrogenous waste by fermented banana and sweet potato feeds, the persistence of high BOD and phosphate concentrations in the effluent highlights an important knowledge gap. The mechanisms by which different fermented plant substrates influence phosphorus release, organic load decomposition, and overall water quality in aquaculture systems remain insufficiently understood. Addressing this gap in the future is critical for developing truly environmentally safe feeds and for understanding the interactions between feed composition, microbial activity, and effluent characteristics in intensive fish culture.

### Author's statements

#### Contributions

Conceptualization: B.R.N., W.T.; Data curation: B.R.N.; Formal Analysis: B.R.N., S.K., M.S.; Investigation: B.R.N., R.S., C.M., D.M., S.K., M.S.; Methodology: B.R.N., S.K., W.T., M.S.; Project administration: B.R.N., S.K., M.S.; Supervision: B.R.N., M.S.; Validation: B.R.N., S.K., M.S.; Visualization: B.R.N.; Writing – original draft: B.R.N.; Writing – review & editing: B.R.N., S.K., M.S.

#### Declaration of conflicting interest

The authors declare no competing interests.

#### Financial interests

The authors declare they have no financial interests.

#### Funding

Not applicable.

#### Data availability statement

Data used for the study would be made available on request.

#### AI Disclosure

The authors declare that generative AI was not used to assist in writing this manuscript.

#### Ethical approval declarations

This study adhered to ethical guidelines for research involving human participants, as approved by the Institutional Research Committee. Informed consent was obtained from all individuals who participated in the study.

#### Additional information

#### Publisher's note

Publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

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