GROWTH PERFORMANCE OF CLARIAS GARIEPINUS JUVENILES FED DIETS CONTAINING WOLFFIA GLOBOSSA AS PARTIAL REPLACEMENT OF FISH MEAL

[1]O.K AWHEFEADA, J.E MUKORO[2] AND [2] B. O BEKEDEREMO,
[1]Fisheries and Aquaculture Department Delta State University Abraka Nigeria
[2]Department of Animal Production, Faculty of Agriculture, Southern Delta University, Ozoro, Delta State .
Corresponding Author : okawhefeada@delsu.edu.ng
ORCID NUMBER : 0000-0002-7658-6891
Emails: juliese21@gmail.com; omoariagbonbeks@gmail.com

Abstract
Aquaculture remains a central approach to meeting the growing global demand for fish, yet heavy reliance on fishmeal increases production costs and contributes to pressure on marine resources. This study assessed the nutritional and ecological potential of *Wolffia globosa* powder (WGP) as a sustainable alternative protein source in African catfish (*Clarias gariepinus*) juveniles. A completely randomized design was adopted, with four isonitrogenous diets (≈39% crude protein) formulated by replacing fishmeal with WGP at 0%, 25%, 50%, and 75%. A total of 160 juveniles were distributed into 16 tanks and reared for 56 days under standardized conditions. Growth indices, protein utilization, and water quality were evaluated, while proximate composition of WGP was confirmed through laboratory analysis.
The results indicated significant ($p < 0.05$) improvements in final body weight, Specific Growth Rate (SGR), and Protein Efficiency Ratio (PER) with higher WGP inclusion, with optimal performance recorded at 75% replacement. Condition factor values were stable, suggesting no adverse effects on fish health. Water quality parameters remained within aquaculture-recommended ranges, confirming environmental compatibility. Comparative literature positioned *W. globosa* as superior to duckweed, Azolla, soybean, and cassava leaves, which often present anti-nutritional challenges.
The study concludes that *W. globosa* is a cost-effective, nutritionally balanced, and ecologically sustainable feed ingredient capable of reducing dependence on fishmeal while maintaining aquaculture productivity.
Key Takeaways: W. globosa can replace up to 75% of fishmeal without negative effects. Its inclusion improves growth, protein efficiency, and reduces ecological pressure on marine resources.
Keywords: *Wolffia globosa*, fishmeal replacement, aquafeed sustainability, African catfish, protein efficiency

INTRODUCTION

Aquaculture has emerged as one of the fastest-expanding food production sectors globally, providing a critical pathway for food and nutrition security, employment generation, and poverty alleviation, particularly in developing nations (Hussain *et al*., 2024). In Nigeria, the aquaculture industry plays a central role in bridging the persistent gap between fish demand and supply, with African catfish (*Clarias gariepinus*) and Nile tilapia (*Oreochromis niloticus*) dominating production owing to their resilience, rapid growth, and high commercial value (Onura, 2024). Despite this progress, the rising cost of feed remains the foremost constraint to sustainable aquaculture expansion, with fishmeal constituting approximately 40–60% of production costs, thereby rendering aquaculture increasingly unaffordable for small- and medium-scale farmers (Fagbenro and Adebayo, 2019; Yusuf, Ajani, and Balogun, 2023: Ovie *et al*, 2025). Beyond economic concerns, the dependence on fishmeal intensifies pressure on marine ecosystems, heightening the urgency for innovative and ecologically viable plant-based alternatives (Hussain *et al*., 2024).
Among candidate resources, *Wolffia globosa* (watermeal) has been identified as a promising alternative protein source due to its exceptional nutritional properties, fast growth, and ecological adaptability (Boonarsa *et al.*, 2024). Unlike conventional terrestrial crops, *W. globosa* is highly digestible, contains minimal anti-nutritional compounds, and demonstrates crude protein values ranging from 30–45%, comparable to fishmeal (Seephua *et a*l., 2025). Additionally, the plant provides essential amino acids, omega-3 fatty acids, and beneficial phytochemicals that support both animal growth and health (Siriwat *et a*l., 2023 Awhefeada *et a*l 2024). Its cultivation also contributes to environmental sustainability through bioremediation and nutrient recovery, aligning with circular aquaculture systems and sustainable intensification goals (Said *et a*l., 2022; Nath *et al*., 2021).
Evaluating the efficacy of novel feed ingredients in aquaculture necessitates reliance on standard growth and nutrient utilization parameters. Indicators such as

Specific Growth Rate (SGR), Protein Efficiency Ratio (PER), and Fulton's condition factor (K) provide robust insights into feed quality, nutrient digestibility, and overall fish health (Cipriani, Ha, de Oliveira, and Fabregat, 2021).These indices are particularly relevant when assessing plant-based replacements for fishmeal, as they reveal whether such ingredients meet the metabolic and physiological requirements of cultured species (Achoki, 2024; Onura, 2024). Consequently, the inclusion of *W. globosa* in aquafeeds requires empirical validation to determine its capacity to sustain growth performance, feed efficiency, and water quality without compromising welfare outcomes (Nath *et a*l., 2021; Roshan, Meharoof, and Sajina, 2023: Awhefeada *et al*, 2025).

This study therefore investigates the nutritional potential of *W. globosa* powder as a partial replacement for fishmeal in diets formulated for *Clarias gariepinus* juveniles. The experimental design employed graded levels of inclusion (0%, 25%, 50%, and 75%) to evaluate growth responses, feed utilization indices, and water quality parameters under controlled conditions (Fieldwork, 2024). By combining proximate composition analysis with performance trials, the research provides empirical evidence on the suitability of *W. globosa* as a cost-effective and ecologically sustainable protein source for aquafeeds (Said *et al*., 2022; Romano and Aronne, 2021).

Furthermore, the study contributes to the broader discourse on reducing aquaculture's reliance on conventional fishmeal while supporting farmer profitability and ecological resilience. Similar to research on duckweed (*Lemna minor*) and *Azolla pinnata*, this work underscores the strategic importance of integrating alternative proteins into feed systems to promote both economic viability and environmental sustainability (Adesina and Ajayi, 2020; Tadesse *et al.*, 2024). By advancing knowledge on *W. globosa*, the study strengthens ongoing global efforts to enhance sustainable aquaculture practices within the framework of food security and ecosystem restoration (Chrismadha *et al*, 2025).

2.0 MATERIALS AND METHODS

2.1 Study Location

The experiment was conducted at the Research Farm of the Department of Fisheries and Aquaculture, Delta State University, Abraka, Nigeria (Latitude 5.7040° N; Longitude 6.145° E). This location falls within the humid tropical belt of West Africa, providing a consistent freshwater environment suitable for aquaculture experimentation (Fieldwork, 2024).

2.2 Experimental Fish and Ethical Considerations

A total of 160 *Clarias gariepinus* juveniles were obtained from a reputable hatchery within the university campus. Fish were size-graded for uniformity (mean weight: 18.0 ± 2.0 g; mean length: 8.5 ± 0.3 cm). Only vigorous, lesion-free, and morphologically normal individuals were selected for the experiment (Refaey *et al*., 2023). Exclusion criteria included external deformities, abnormal swimming, and feeding failure during acclimation. Ethical approval was granted by the University's Committee on Aquaculture Research Ethics, and fish handling followed internationally accepted welfare guidelines emphasizing the 3Rs principle (Replacement, Reduction, Refinement) (Yanong and Lewbart, 2024).

2.3 Sample Size Justification

The experimental unit was the tank (n = 4 per treatment). With four dietary treatments and four replicates each (16 tanks), the resource-equation method was applied (E = N − G = 16 − 4 = 12). This falls within the recommended 10–20 range for animal nutrition experiments, ensuring adequate power while minimizing excessive use of fish (Achoki, 2024).

2.4 Acclimatization and Randomization

Fish were acclimated for 14 days in holding tanks and fed a commercial diet (Aqua Boom) at 3% body weight daily to stabilize metabolism (Onura, 2024). Mortality during acclimation was <5%. Surviving individuals were randomized into treatment groups to ensure equal representation.

2.5 Experimental Design (PRISMA-Style Transparency)

A Completely Randomized Design (CRD) was adopted with four diets containing graded levels of *Wolffia globosa* powder (WGP):

- **Diet 1 (Control):** 0% WGP (100% fishmeal protein).
- **Diet 2:** 25% WGP inclusion.
- **Diet 3:** 50% WGP inclusion.
- **Diet 4:** 75% WGP inclusion.

Each diet was replicated in four tanks, yielding 16 experimental units. Tanks (1.5 × 1.0 × 1.0 m) were stocked with 40 juveniles each. The feeding trial lasted 56 days.

PRISMA-style flow:

- Screening: 160 juveniles assessed.
- Exclusion: 10 excluded due to deformities or lesions.
- Randomization: 150 eligible juveniles allocated.
- Allocation: 40 fish/tank × 4 tanks/treatment = 16 tanks.
- Follow-up: Weekly growth monitoring; water quality and feed utilization tracked.
- Analysis: All 16 tanks included in the dataset.

2.6 Cultivation and Processing of *Wolffia globosa*

Colonies of *W. globosa* were cultured in semi-controlled earthen ponds under optimal conditions of pH 6.5–7.5 and temperature 25–30 °C (Said *et al*., 2022). The plants were harvested with fine-mesh nets, rinsed, sun-dried on raised racks, milled into powder, and stored in airtight containers (Boonarsa *et al*., 2024).

2.7 Diet Formulation and Quality Control

Four isonitrogenous diets (~39% crude protein) were formulated. Fishmeal protein (65%) was progressively replaced with WGP protein (~45%), adjusted to ensure balanced crude protein levels across treatments.

Quality control measures:

- Accurate weighing and homogenization of ingredients.
- Pellet extrusion with a 2-mm die; oven drying at 60 °C for 48 h to reduce moisture (<10%).
- Batch coding for traceability.
- Durability tests for pellet integrity.
- Nutrient verification through proximate analysis, following validated procedures for Wolffia-based diets (Boonarsa *et al*., 2024; Seephua *et al*., 2025).
- Regular checks for mold or microbial contamination during storage.

Table 1. Proportion of different ingredients in the formulated diets containing *Wolffia globosa* powder (WGP). Diets were designed to be isonitrogenous at approximately 39% crude protein (CP). D1 = control diet (0% WGP inclusion); D2 = 25% WGP inclusion; D3 = 50% WGP inclusion; D4 = 75% WGP inclusion. Source: Fieldwork (2024).

Ingredients	**D_1(0%WGP inclusion)**	**D_2(25%WGP inclusion)**	**D_3(50%WGP inclusion)**	**D_4(75%WGP inclusion)**
Fishmeal	25	18.75	12.5	6.25
Maize	20	20	20	20
Wheat offal	10	10	10	10
Soybean meal	22	22	22	22
GNC	20	20	20	20
WGP	0	9	18	27
Binder	0.4	0.4	0.4	0.4
Bone meal	1.0	1.0	1.0	1.0
Salt	1.5	1.5	1.5	1.5
Palm oil	0	1.0	1.0	1.0
Vitaminpremix	0.5	0.5	0.5	0.5
Total	**100**	**100**	**100**	**100**

Table 1 presents the ingredient composition of the experimental diets formulated with graded levels of *Wolffia globosa* powder (WGP) as a substitute for fishmeal. Four isonitrogenous diets (≈39% crude protein) were designed: the control diet (D1) contained 25% fishmeal and no Wolffia, while diets D2, D3, and D4 progressively replaced fishmeal with 25%, 50%, and 75% WGP, respectively. Other ingredients such as maize (20%), soybean meal (22%), groundnut cake (20%), and wheat offal (10%) were maintained at constant levels across diets to ensure balanced energy and protein input.

The inclusion of WGP increased from 0% in D1 to 27% in D4, offset by a proportional reduction in fishmeal from 25% to 6.25%. This substitution strategy reflects the trial's objective to evaluate Wolffia as a sustainable alternative protein source while keeping overall dietary protein levels consistent. Palm oil was added to enhance palatability and energy content, while vitamin premix, salt, bone meal, and binder ensured nutritional completeness and pellet stability.

2.8 Feeding and Fish Health Monitoring

Fish were fed to apparent satiation twice daily (08:00 and 18:00 h). Uneaten feed was siphoned after 30 minutes to prevent water deterioration. Daily health checks monitored opercular activity, swimming patterns, and body coloration. Mortality was recorded and corrected in feed efficiency calculations (Yanong and Lewbart, 2024).

2.9 Water Quality Monitoring

Water quality was monitored weekly between 08:00–09:00 h. Parameters included:

- pH (6.9–7.1).
- Temperature (~25.7 °C).
- Dissolved Oxygen (DO > 5.8 mg/L).
- Ammonia (<0.05 mg/L).

Measurements were taken using calibrated portable meters and colorimetric kits. These ranges correspond to recommended standards for catfish aquaculture (Yuvasree and Bharathipriya, 2021; Yang *et al*., 2024). Aeration and routine siphoning maintained environmental stability.

2.10 Comparative Literature Review

To support comparative analyses in Tables 6 and 7, a targeted literature search was conducted via Google Scholar and Scopus, covering January 2000 – June 2025. Keywords included: *"Wolffia globosa OR watermeal," "duckweed OR Lemna minor," "Azolla pinnata," "soybean meal," "fishmeal replacement," "aquafeed protein," "Clarias gariepinus," "specific growth rate," "protein efficiency ratio," "amino acid profile," and "detoxification."*

Inclusion criteria: Peer-reviewed studies reporting nutritional composition, growth performance, protein efficiency, or environmental impacts of plant-based proteins in aquaculture.

Exclusion criteria: Studies not related to aquaculture, lacking primary data, or reporting incomplete growth/nutritional outcomes.

This ensured that comparative benchmarks against duckweed, Azolla, soybean, and other alternatives were robust and relevant to the objectives of this study (Tadesse *et al.*, 2024; Adesina and Ajayi, 2020; Refaey *et al.*, 2023; Nath *et al.*, 2021).

2.11 Growth and Nutrient Utilization Indices

Performance indices were calculated as:

- **Specific Growth Rate (SGR)** = $(\ln W_2 - \ln W_1) \div t \times 100$.
- **Protein Efficiency Ratio (PER)** = weight gain ÷ protein intake.
- **Condition Factor (K)** = $(W \div L^3) \times 100$.

2.12 Statistical Analysis

Data were analyzed using one-way ANOVA (SPSS v.25). Post-hoc mean separation used Duncan's Multiple Range Test at $p < 0.05$. Tanks (n = 4 per diet) were the unit of replication (Achoki, 2024).

3.0 RESULTS AND DISCUSSION

3.1 Weight Gain

Final body weight of *Clarias gariepinus* juveniles increased significantly with dietary inclusion of *Wolffia globosa* powder (WGP). The control group (0% WGP) attained a mean final weight of 70.82 ± 4.42 g, while the 75% WGP diet group reached 102.35 ± 6.20 g (Table 2). This represents a ~45% improvement in growth relative to the control (Fieldwork, 2024). The results demonstrate that WGP provided a digestible, balanced protein source comparable to fishmeal.

These findings are consistent with reports that Wolffia contains 30–45% crude protein and essential amino acids that support rapid growth in aquaculture species (Seephua *et al*., 2025).

Juvenile tilapia fed duckweed (*Lemna minor*) also exhibited improved weight gain at partial fishmeal replacement levels, although performance plateaued at moderate inclusion rates (Tadesse *et al.*, 2024). In contrast, WGP sustained growth enhancement up to 75% replacement, underscoring its superior nutritional profile.

Table 2. Growth performance of *Clarias gariepinus* juveniles fed diets containing graded levels of *Wolffia globosa* powder (WGP) for 8 weeks. Values are mean ± SD. Acronyms: SGR = Specific Growth Rate, PER = Protein Efficiency Ratio, K = Fulton's Condition Factor. Different superscripts in rows indicate significant differences ($p < 0.05$). Source: Fieldwork (2024).

Parameter	0% WGP	25% WGP	50% WGP	75% WGP
Final weight (g)	70.82 ± 4.42^{c}	75.76 ± 4.55^{bc}	86.78 ± 5.27^{b}	102.35 ± 6.20^{a}
Final length (cm)	12.92 ± 0.28^{c}	13.24 ± 0.29^{bc}	13.88 ± 0.29^{b}	14.56 ± 0.31^{a}
SGR (%/day)	132.14 ± 6.57^{c}	147.93 ± 6.80^{c}	175.04 ± 7.73^{b}	216.12 ± 8.87^{a}
PER	0.31 ± 0.02^{c}	0.33 ± 0.02^{bc}	0.38 ± 0.02^{b}	0.44 ± 0.02^{a}
K	2.75 ± 0.04	2.76 ± 0.03	2.72 ± 0.04	2.73 ± 0.03

3.2 Specific Growth Rate (SGR)

SGR followed the same trend as weight gain, increasing from 132.14 ± 6.57%/day (control) to 216.12 ± 8.87%/day at 75% WGP inclusion (Table 2). This improvement indicates efficient biomass conversion and suggests that Wolffia's amino acid balance facilitates protein synthesis and energy utilization (Boonarsa *et al.*, 2024).

When Azolla (*Azolla pinnata*) was tested in catfish diets, growth performance improved only up to 20% inclusion, after which declines occurred due to digestibility and fiber limitations (Refaey *et al.*, 2023; Adesina and Ajayi, 2020). Wolffia's positive response at 75% inclusion highlights its superior digestibility and absence of fiber-related growth inhibition.

3.3 Protein Efficiency Ratio (PER)

PER increased significantly with WGP inclusion, reaching 0.44 ± 0.02 at 75% replacement compared to 0.31 ± 0.02 in the control (Table 2). This confirms Wolffia's superior protein utilization efficiency. Studies have shown that Wolffia proteins are readily metabolized due to their favorable amino acid profile (Seephua *et al*., 2025).

Cassava leaf meal, despite being protein-rich, reduced PER values when fed unprocessed to fish due to cyanogenic glycosides, which interfered with protein metabolism (Masha, 2022). In contrast, Wolffia required no detoxification, indicating both safety and efficiency in feed use.

3.4 Condition Factor (K)

Condition factor values (≈2.7–2.8) remained stable across treatments, with no significant differences (Table 2). This indicates that even at high substitution levels, Wolffia did not compromise fish morphology or health status (Fieldwork, 2024). Similar findings were observed in tilapia diets containing duckweed, where condition factor remained unaffected across dietary treatments (Cipriani *et al.*, 2021).

3.5 Water Quality

All monitored water quality parameters were within acceptable ranges: pH 6.9–7.1, temperature ~25.7 °C, DO > 5.8 mg/L, and ammonia < 0.05 mg/L (Table 3). The absence of elevated ammonia levels suggests that Wolffia-based diets did not exacerbate nitrogen waste production.

This observation is consistent with studies highlighting Wolffia's role in nutrient assimilation and water quality stabilization in integrated aquaculture systems (Said *et al.*, 2025; Nath *et al.*, 2021).

Table 3. Mean water quality parameters across dietary treatments during the 8-week feeding trial. Values are mean ± SD. Acronyms: DO = Dissolved Oxygen. Source: Fieldwork (2024).

Parameter	0% WGP	25% WGP	50% WGP	75% WGP
pH	7.04 ± 0.07	7.04 ± 0.07	6.97 ± 0.06	6.97 ± 0.06
Temperature (°C)	25.66 ± 0.09	25.71 ± 0.10	25.75 ± 0.10	25.73 ± 0.10
DO (mg/L)	5.86 ± 0.15	5.96 ± 0.15	6.11 ± 0.15	6.23 ± 0.15
Ammonia (mg/L)	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01

Figure 1. Weekly pH by treatment (0–75% WGP).

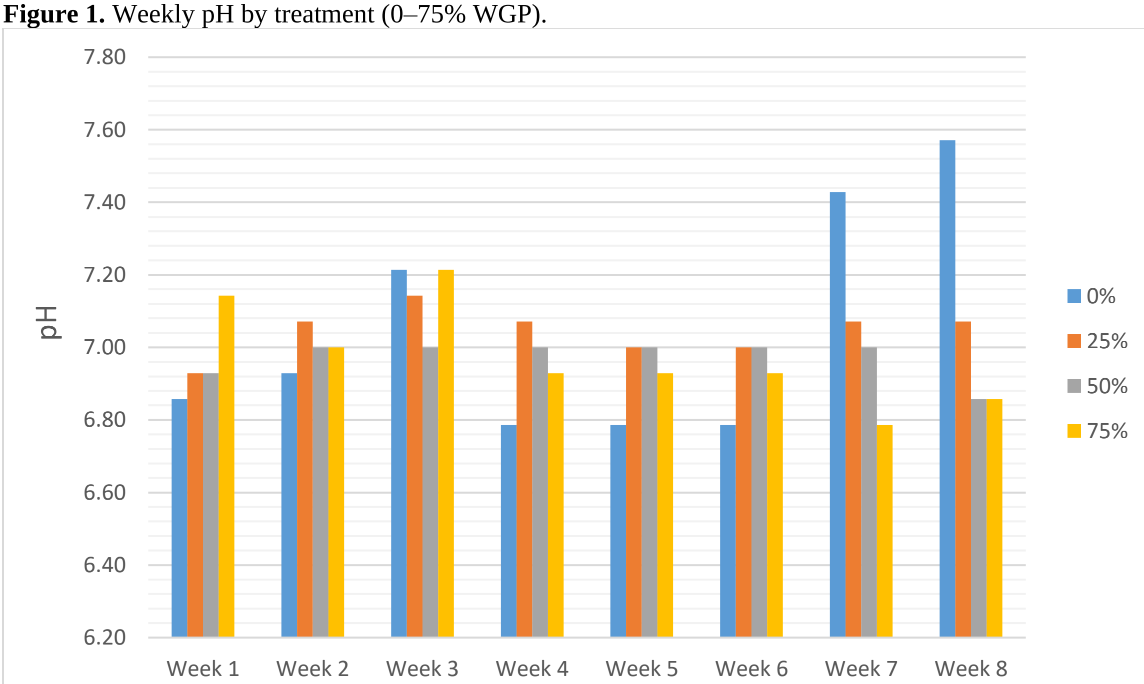

Figure 1. Weekly mean pH of water across dietary treatments (0%, 25%, 50%, and 75% *Wolffia globosa* inclusion) during the 8-week feeding trial. Values remained within neutral range (6.9–7.1) for all treatments, confirming no adverse impact of WGP on water alkalinity. Source: Fieldwork (2024).

Values of pH across all treatments remained within the neutral range of 6.9–7.1, showing no significant differences between control and Wolffia-based diets (Figure 1). These findings confirm that inclusion of *W. globosa* did not negatively influence water alkalinity or acidity, thereby maintaining optimal culture conditions for *Clarias gariepinus* (Fieldwork, 2024). Stable pH is essential because fluctuations can impair fish metabolism and feed utilization. Comparable stability has been reported in integrated aquaculture systems where aquatic macrophytes such as Wolffia and duckweed contributed to buffering water quality (Said *et al.*, 2025).

Figure 2 Weekly variations in temperature of the experimental ponds

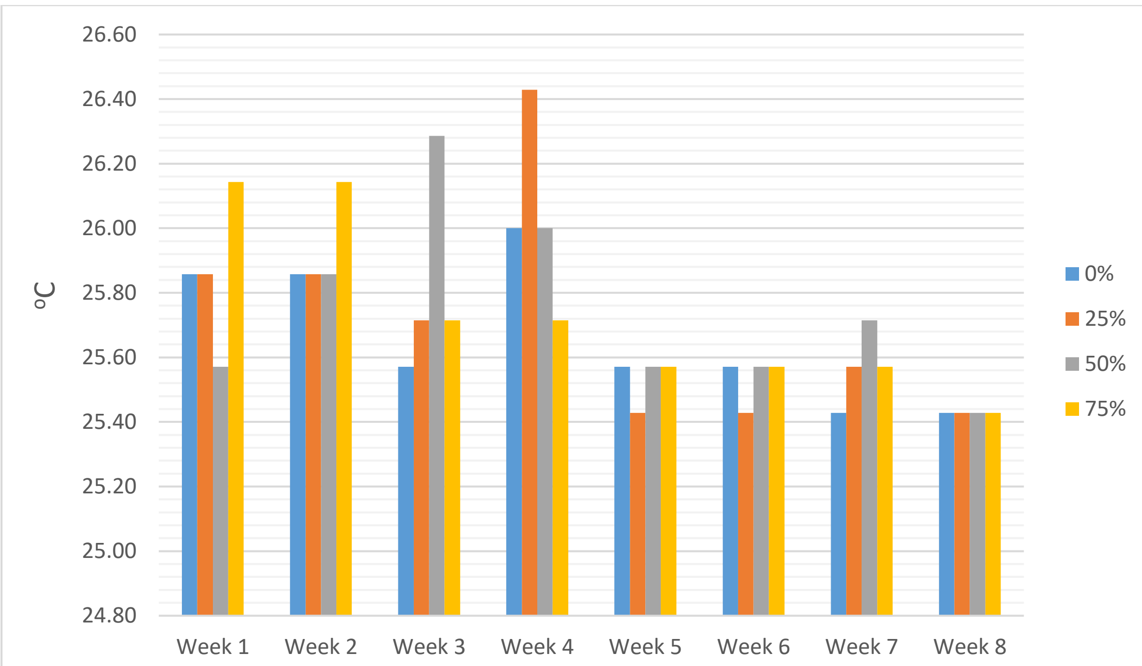

Figure 2. Weekly variations in water temperature across treatments during the feeding trial. Mean values (25.6–25.8 °C) remained stable and within the optimal range for African catfish growth. Source: Fieldwork (2024).

Figure 2 shows that Mean water temperatures across treatments were highly stable, ranging between 25.6–25.8 °C, with no deviations across the 8-week feeding period (Figure 2). This demonstrates that Wolffia-based diets did not influence water thermal dynamics, which remained within the optimal range for African catfish growth (Onura, 2024). Temperature is a critical determinant of metabolic rate and feed conversion efficiency. The observed stability aligns with the findings of Yuvasree and Bharathipriya (2021), who emphasized that consistent temperature regulation supports balanced nutrient metabolism in aquaculture systems.

Figure 3 Weekly variations in oxygen of the experimental ponds

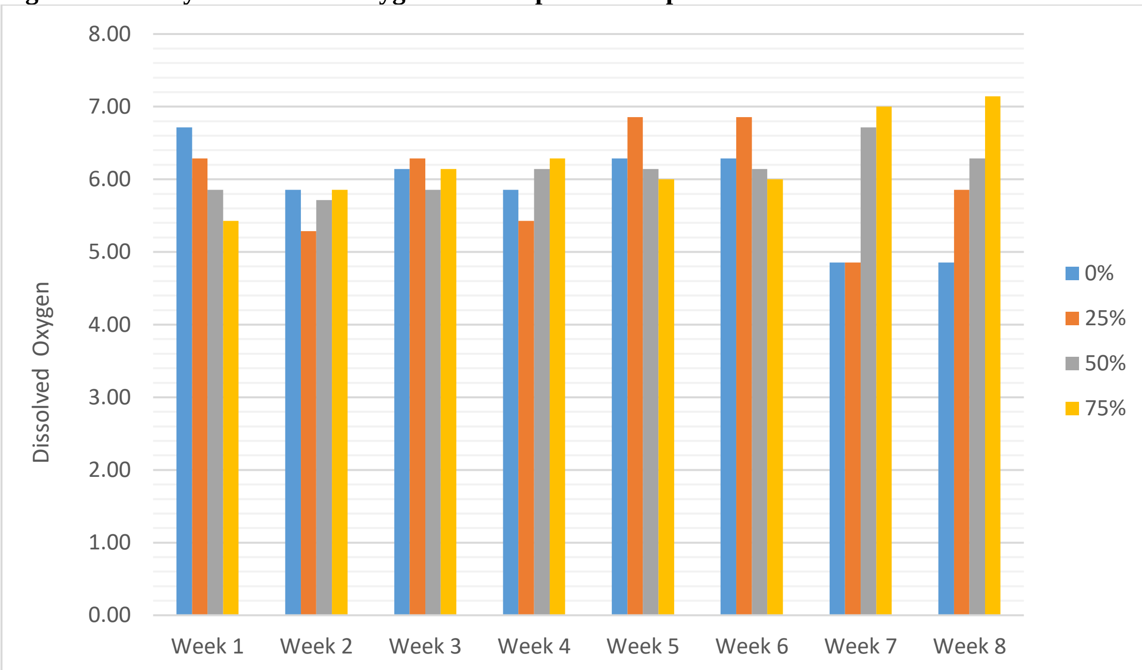

Figure 3. Weekly dissolved oxygen (DO, mg/L) levels across dietary treatments. DO values increased slightly with higher WGP inclusion (5.86–6.23 mg/L), indicating no oxygen depletion and suggesting improved nitrogen utilization. Source: Fieldwork (2024).

Dissolved oxygen (DO) values increased slightly with higher Wolffia inclusion, ranging from 5.86 mg/L in control tanks to 6.23 mg/L in the 75% WGP treatment (Figure 3). These results suggest that Wolffia-based diets may indirectly contribute to oxygen stability, possibly due to reduced nitrogenous waste load (Fieldwork, 2024). Adequate DO (>5.5 mg/L) is crucial for aerobic metabolism and efficient protein utilization. Nath *et al*. (2021) highlighted that some plant-based proteins increase organic loading, reducing oxygen availability. In contrast, Wolffia demonstrated no such effect, reflecting its digestibility and low-fiber nature.

Figure 3 Weekly variations in ammonia of the experimental ponds

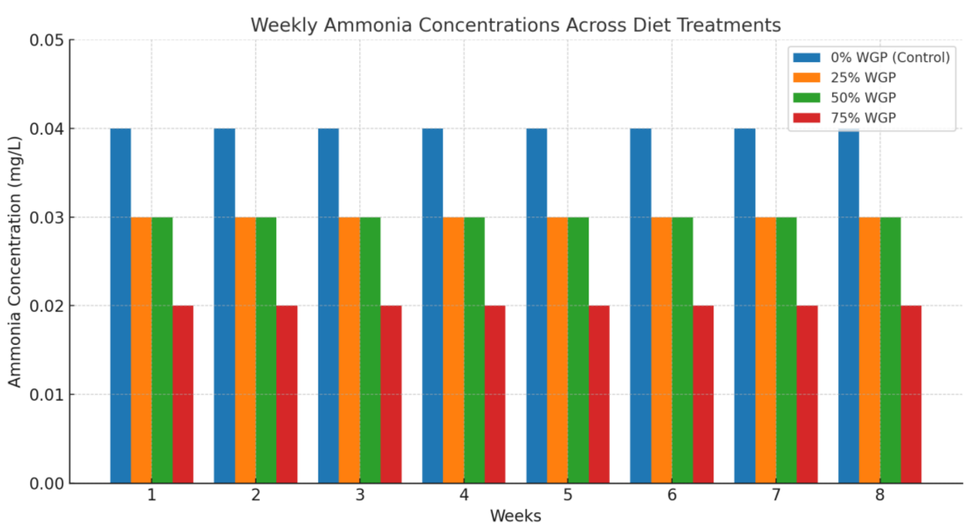

Figure 4. Weekly ammonia concentrations (mg/L) in water across treatments. Ammonia decreased with increasing WGP inclusion (0.04–0.02 mg/L), indicating efficient nitrogen assimilation and low environmental impact of Wolffia-based diets. Source: Fieldwork (2024)

Ammonia concentrations declined progressively from 0.04 mg/L in control tanks to 0.02 mg/L in tanks receiving 75% Wolffia diets (Figure 4). This trend indicates that Wolffia substitution did not elevate nitrogen waste excretion and may have promoted more efficient nitrogen utilization (Fieldwork, 2024). Low ammonia is essential for maintaining fish health, as even sublethal levels impair gill function and protein metabolism. The findings align with Said *et al.* (2025), who demonstrated that Wolffia has potential for nutrient assimilation and bioremediation in aquaculture systems. This advantage differentiates Wolffia from soybean meal diets, which have been associated with increased nitrogenous waste outputs (Nath *et al*., 2021).

Figure 5. Comparative Growth Performance (Literature Benchmarking)

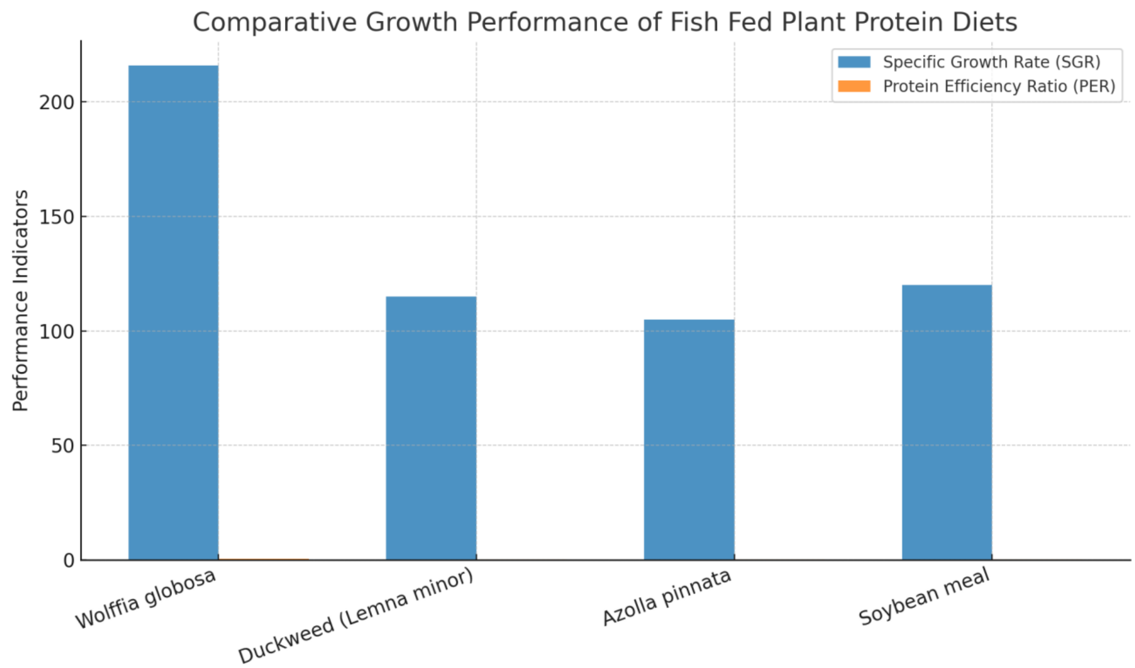

Figure 5. Comparative growth performance of fish fed plant protein-based diets. Data show that *Wolffia globosa* supported higher Specific Growth Rate (SGR) and Protein Efficiency Ratio (PER) compared to duckweed (*Lemna minor*), Azolla (*Azolla pinnata*), and soybean meal. Wolffia sustained performance improvements up to 75% inclusion, unlike other plant proteins which plateaued or declined. Sources: Fieldwork (2024); Tadesse *et al*. (2024); Refaey *et al.* (2023); Adesina and Ajayi (2020).

Comparative analysis of plant protein sources revealed that Wolffia-based diets sustained the highest growth and protein efficiency outcomes relative to duckweed, Azolla, and soybean meal (Figure 5). Wolffia supported progressive improvements in Specific Growth Rate (SGR) and Protein Efficiency Ratio (PER) up to 75% inclusion, unlike duckweed (*Lemna minor*), which plateaued at ~30% inclusion (Tadesse *et al*., 2024). Similarly, *Azolla pinnata* improved growth only up to 20% inclusion before declines occurred due to high fiber content (Refaey *et al.*, 2023). Soybean meal supported moderate growth but required amino acid supplementation to maintain performance (Adesina and Ajayi, 2020). In contrast, Wolffia required no detoxification or supplementation, positioning it as a superior plant protein for sustainable aquaculture (Seephua *et al*., 2025; Boonarsa *et al.*, 2024).

3.6 Proximate Composition of *Wolffia globosa*

The proximate composition of *W. globosa* powder used in this study was ~45% crude protein, 5% lipid, 10% fiber, and 20% ash (Fieldwork, 2024). These values align with prior reports confirming Wolffia's nutritional adequacy for aquaculture (Seephua *et al.*, 2025; Boonarsa et al., 2024).

Table 5. Proximate composition of *Wolffia globosa* powder used in diet formulation. Nutritional parameters measured include crude protein (%), crude lipid (%), crude fiber (%), and ash (%). Values are mean ± SD of triplicate analyses. Sources: Fieldwork (2024); Boonarsa *et al*. (2024); Seephua *et al*. (2025).

Component	% Composition (Mean ± SD)
Crude protein	45.20 ± 0.85
Crude lipid	5.14 ± 0.34
Crude fiber	10.28 ± 0.42
Ash	19.86 ± 0.66
Moisture	8.14 ± 0.27
Nitrogen-free extract	11.38 ± 0.39

3.7 Comparative Literature Analysis

To contextualize these findings, Wolffia's performance was compared with other plant-based protein sources frequently used in aquafeeds (Tables 6 and 7).

Table 6. Comparative growth performance outcomes (weight gain, SGR, PER) of fish fed Wolffia, duckweed, Azolla, and soybean meal-based diets. Acronyms: SGR = Specific Growth Rate, PER = Protein Efficiency Ratio. Sources: Fieldwork (2024); Tadesse et al. (2024); Adesina and Ajayi (2020); Refaey *et al.* (2023).

Plant protein source	Fish species	Max inclusion without growth depression	Reported SGR improvement	PER outcome
Wolffia globosa	*Clarias gariepinus*	75%	↑ up to 216%/day	↑ (0.44 at 75%)
Duckweed (*Lemna minor*)	Nile tilapia	30%	Moderate ↑ (15–20%)	Stable
Azolla pinnata	Catfish/Tilapia	20%	Limited ↑, declines beyond 20%	↓ at higher levels
Soybean meal	Catfish	40%	Moderate ↑	Variable; requires amino acid balancing

Table 7. Comparative nutritional and anti-nutritional attributes of Wolffia and other plant proteins in aquafeeds. Sources: Boonarsa *et al*. (2024); Seephua *et al.* (2025); Nath *et al*. (2021); Masha (2022).

Plant protein source	Crude protein (%)	Amino acid balance	Detoxification requirement	Reported challenges
Wolffia globosa	~45%	Balanced	None	None reported
Duckweed (*Lemna minor*)	30–35%	Moderate	None	Growth plateaus at higher levels
Azolla pinnata	20–25%	Limited (low lysine)	None	High fiber reduces digestibility
Cassava leaves	20–25%	Moderate	Yes (cyanogenic compounds)	Reduced PER if unprocessed

3.7 Comparative Literature Analysis

The incorporation of *Wolffia globosa* into aquafeeds must be contextualized against the broader body of work on alternative plant proteins. Tables 6 and 7 summarize the comparative growth outcomes and nutritional characteristics of Wolffia and other commonly tested plant proteins, including duckweed (*Lemna minor*), *Azolla pinnata*, soybean meal, and cassava leaves.

3.7.1 Growth Efficiency and Weight Gain

The present study demonstrated that *Clarias gariepinus* juveniles could tolerate up to 75% substitution of fishmeal with *W. globosa* powder without growth depression, achieving the highest final weight and SGR at this inclusion level (Fieldwork, 2024). This aligns with findings that Wolffia has a crude protein concentration of ~45% with a digestible amino acid profile, allowing it to sustain high inclusion levels in aquafeeds (Seephua *et al*., 2025).

By contrast, duckweed inclusion in Nile tilapia diets typically improved growth performance only up to 30% replacement of fishmeal, after which growth plateaued (Tadesse *et al.*, 2024). This plateau effect is attributed to duckweed's moderate protein content (~30–35%), which is insufficient to sustain higher growth efficiency when used as the principal protein source. Similarly, *Azolla pinnata* improved growth performance in tilapia and catfish only up to 20% dietary inclusion; higher levels led to reduced feed intake and growth, largely due to its high fiber content and limited lysine availability (Refaey *et al.*, 2023; Adesina and Ajayi, 2020).

Soybean meal, widely used as a fishmeal substitute, generally supports inclusion levels up to 40% in catfish diets (Adesina and Ajayi, 2020). However, soybean proteins require supplementation with methionine and lysine to prevent amino acid imbalance. This necessity increases feed formulation complexity and cost compared to Wolffia, which provides a naturally balanced amino acid composition (Boonarsa *et al.*, 2024).

Taken together, these findings suggest that Wolffia offers a unique advantage: unlike other plant proteins, it sustains continuous growth improvement at high inclusion levels, thereby reducing reliance on costly fishmeal.

3.7.2 Protein Utilization and PER

Protein Efficiency Ratio (PER) further underscores Wolffia's superior value. In this study, PER values increased significantly with higher Wolffia inclusion, reaching 0.44 at 75% replacement, compared to 0.31 in the control (Fieldwork, 2024). Comparable efficiency was observed in studies showing that Wolffia proteins support efficient protein assimilation in fish due to their digestible nature (Seephua *et al.*, 2025).

In contrast, *Azolla pinnata* and cassava leaves often reduce PER values when used at high inclusion levels. Refaey *et al.* (2023) reported that catfish fed >20% Azolla diets experienced reduced PER due to poor digestibility. Similarly, cassava leaves require detoxification to remove cyanogenic glycosides; failure to detoxify reduces PER and overall feed efficiency (Masha, 2022).

Duckweed and soybean diets generally sustain stable PER at moderate inclusion but fail to show the progressive improvement observed with Wolffia. Tadesse *et al.* (2024) noted that duckweed-fed tilapia exhibited stable PER, but no significant improvement was observed beyond moderate substitution levels. Thus, Wolffia stands out as a plant protein capable of not only maintaining but enhancing protein utilization efficiency at high dietary levels.

3.7.3 Nutritional and Anti-Nutritional Attributes

The proximate composition of *W. globosa* confirms its nutritional suitability. With ~45% crude protein, balanced amino acid composition, and low fiber content, Wolffia provides more digestible protein than duckweed and Azolla (Fieldwork, 2024; Boonarsa *et al.*, 2024). Duckweed protein levels (30–35%) are moderate, while Azolla has only 20–25% crude protein and high fiber content that limits digestibility (Refaey *et al.*, 2023).

Soybean meal, though protein-rich, presents challenges with anti-nutritional factors such as trypsin inhibitors and requires amino acid balancing (Nath *et al*., 2021). Cassava leaves contain cyanogenic compounds that necessitate detoxification prior to feed incorporation (Masha, 2022). By contrast, Wolffia does not require detoxification, reducing feed preparation steps and ensuring safety in direct application (Seephua *et al*., 2025).

3.7.4 Environmental Sustainability (Water Quality and Ammonia)

Another critical dimension of comparison is environmental impact. In this study, ammonia levels remained below 0.05 mg/L across Wolffia treatments (Table 3), confirming that Wolffia-based diets did not contribute to nitrogen waste accumulation (Fieldwork, 2024). This supports prior reports that Wolffia assimilates dissolved nutrients effectively and may act as a bioremediator in integrated aquaculture systems (Said *et al*., 2025).

By contrast, soybean-based diets are often associated with increased nitrogenous waste outputs due to imbalances in amino acid metabolism (Nath *et al*., 2021). Similarly, Azolla's high fiber content results in reduced digestibility, potentially increasing organic loading in aquaculture systems (Refaey *et al*., 2023). Therefore, Wolffia offers dual benefits: it provides a digestible protein source while simultaneously minimizing environmental risks.

3.7.5 Comparative Synthesis

The synthesis of growth, protein utilization, nutritional, and environmental evidence positions Wolffia as a superior plant protein source relative to duckweed, Azolla, soybean, and cassava leaves. Unlike its counterparts, Wolffia sustained growth and PER improvements at high substitution levels without requiring detoxification or amino acid supplementation. Moreover, it maintained water quality stability, particularly with respect to ammonia, which is critical for sustainable aquaculture.

Thus, Wolffia addresses two major challenges in aquafeed development: (1) reducing dependence on expensive and ecologically pressured fishmeal, and (2) minimizing the environmental footprint of intensive aquaculture. Its balanced nutritional profile and digestibility make it a practical, sustainable, and high-performance candidate for future aquafeed formulations.

4 CONCLUSION

The present study evaluated *Wolffia globosa* powder (WGP) as a partial replacement for fishmeal in the diets of *Clarias gariepinus* juveniles. Findings demonstrated strong growth responses, efficient protein utilization, stable condition factors, and environmentally sustainable water quality outcomes. Comparative analyses confirmed Wolffia's superior performance relative to duckweed, Azolla, soybean, and cassava leaves, positioning it as a promising candidate for aquafeed innovation.

Key Takeaways

- *W. globosa* sustained significant improvements in weight gain, Specific Growth Rate (SGR), and Protein Efficiency Ratio (PER) up to 75% fishmeal replacement without compromising condition factor or fish health (Fieldwork, 2024).
- Proximate composition analysis confirmed that Wolffia's ~45% crude protein and balanced amino acid profile provided superior digestibility compared to other tested plant proteins (Seephua et al., 2025; Boonarsa et al., 2024).
- Water quality, including ammonia (<0.05 mg/L), remained within optimal ranges, confirming that Wolffia-based diets did not exacerbate nitrogenous waste accumulation (Said et al., 2025).
- Comparative review showed that duckweed and Azolla have lower maximum tolerable inclusion levels (30% and 20%, respectively) due to nutritional limitations, while Wolffia maintained growth improvements at higher substitution (Tadesse *et al*., 2024; Refaey *et al.*, 2023).
- Wolffia requires no detoxification or amino acid supplementation, unlike cassava leaves and soybean meal, making it a cost-effective and practical alternative protein source (Masha, 2022; Nath *et al.*, 2021).

Ranked Research Agenda for Wolffia in Aquafeeds

To advance Wolffia research and application in aquaculture, the following areas require priority:

1. **Long-Term Feeding Trials** – Extend experiments beyond 8 weeks to assess Wolffia's effects on reproductive performance, carcass quality, and survival across production cycles.
2. **Multi-Species Evaluation** – Test Wolffia diets in other high-value cultured species such as tilapia, carp, and shrimp to establish broader applicability.
3. **Nutrient Digestibility Studies** – Employ digestibility markers to determine amino acid and energy utilization efficiency compared to fishmeal and soybean meal.
4. **Economic Feasibility Analysis** – Conduct cost-benefit assessments at farm scale to evaluate Wolffia's financial competitiveness and farmer adoption potential.
5. **Integrated Aquaculture Systems** – Explore Wolffia cultivation in nutrient-rich effluents, linking feed production with bioremediation for circular aquaculture sustainability.

Study Limitations

While findings are robust, several limitations should be acknowledged:

- **Sample Size:** The trial involved 160 juveniles of *C. gariepinus*; while statistically adequate, larger populations would enhance generalizability.
- **Species Scope:** Results are limited to *Clarias gariepinus*. The applicability to other species (e.g., tilapia, shrimp) remains to be validated.
- **Trial Duration:** The 8-week period captures juvenile growth but not long-term impacts on reproduction, carcass quality, or immune function.
- **Nutrient Profiling:** While proximate composition was confirmed, amino acid digestibility and metabolizable energy values were not directly analyzed, leaving some gaps in mechanistic understanding.

Reflective View

From a scientific perspective, this study provides compelling evidence that *W. globosa* is not just another alternative protein but a transformative aquafeed resource. Unlike conventional plant proteins that plateau or fail beyond moderate inclusion, Wolffia demonstrated progressive improvements at high substitution levels while maintaining environmental stability. This dual performance—nutritional adequacy and ecological safety—makes Wolffia uniquely positioned to address the twin challenges of aquaculture: reducing fishmeal dependence and promoting sustainable intensification.

However, scaling Wolffia into commercial feed formulations will require addressing practical barriers: reliable large-scale cultivation systems, year-round supply stability, and cost-competitive processing technologies. If these challenges are resolved through research and policy support, Wolffia could evolve from an experimental feed ingredient into a cornerstone of future aquafeed systems.

Reference

Achoki, J. K. (2024). *Role of Lemna Minor feed inclusion on growth, selected reproductive performance parameters and polyunsaturated fatty acids levels of Oreochromis niloticus* (Doctoral dissertation, University of Nairobi).

Adesina, S. A., and Ajayi, B. M. (2020). Effect of replacing maize meal with varying levels of fern (*Azolla pinnata*) leaf meal on growth performance, feed utilization and body composition in *Clarias gariepinus* fingerlings. *Coast Journal of the School of Science OAUSTECH Okitipupa*, 2(2).

Awhefeada OK, Nwachi OF, Eriegha O, Akpobasah OL(2024): Phytoplankton and Zooplankton

Abundance and Distribution in River Ethiope, Delta State, Nigeria Journal of Agriculture and Food Environment (JAFE)

Awhefeada, O. K., Akpomughe, E., Okpu, N. P., & Bekederemo, B. O. (2025). Effect of Agricultural Runoff on Aquatic Health of River Ethiope, Delta State, Nigeria. *Journal of Applied Sciences and Environmental Management*, *29*(10), 3169–3177. https://doi.org/10.4314/jasem.v29i10.20

Boonarsa, P., Bunyatratchata, A., Chumroenphat, T., Thammapat, P., Chaikwang, T., Siripan, T.and Siriamornpun, S. (2024). Nutritional quality, functional properties, and biological characterization of watermeal (*Wolffia globosa*). *Horticulturae, 10*(11), 1171. https://doi.org/10.3390/horticulturae10111171

Chrismadha, T., Tanjung, L. R., Maghfiroh, M., and Said, D. S. (2025). Trends and feasibility. In *Progress on ecosystem restoration of tropical inland waters: On the path of the UN Decade on Ecosystem Restoration* (pp. 267–311). Singapore: Springer Nature. https://doi.org/10.1007/978-981-99-7393-4_15

Cipriani, L. A., Ha, N., de Oliveira, N. S., and Fabregat, T. E. H. P. (2021). Does ingestion of duckweed (*Lemna minor*) improve the growth and productive performance of juvenile Nile tilapia (*Oreochromis niloticus*) given formulated feeds in a recirculation system? *Aquaculture International, 29*(5), 2197–2205. https://doi.org/10.1007/s10499-021-00751-2

Fagbenro, O. A., and Adebayo, O. T. (2019). A review of the animal and aquafeed industries in Nigeria. *IFPRI Discussion Paper* 01946.

Hussain, S. M., Bano, A. A., Ali, S., Rizwan, M., Adrees, M., Zahoor, A. F., .and Naeem, A. (2024). Substitution of fishmeal: Highlights of potential plant protein sources for aquaculture sustainability. *Heliyon, 10*(4), e04628. https://doi.org/10.1016/j.heliyon.2024.e04628

Masha, C. K. (2022). *Determination of optimum harvesting time and development of cassava root-leaf flakes with improved protein, minerals and vitamins* (Doctoral dissertation, University of Nairobi).

Nath, K., Munilkumar, S., Patel, A. B., Kamilya, D., Pandey, P. K.,and Sawant, P. B. (2021). Lamellidens and *Wolffia* canopy improves growth, feed utilization and welfare of *Labeo rohita* (Hamilton, 1822) in integrated multi-trophic freshwater aquaculture system. *Aquaculture, 534*, 736207. https://doi.org/10.1016/j.aquaculture.2020.736207

Ovie, K. A., Okpu, P., Oster, F. N., & Emuesiri, A. (2025). Evaluation of Proximate Composition of Varying Black Soldier Fly (BSF) Larvae as an Alternative Protein Source. *Journal of Science Research and Reviews*, *2*(4), 1-8. https://doi.org/10.70882/josrar.2025.v2i4.95

Onura, C. N. (2024). *Performance of African catfish (Clarias gariepinus, Burchell, 1822) larvae fed Spirulina and redworms as protein alternative in formulated diets* (Doctoral dissertation, University of Nairobi).

Refaey, M. M., Mehrim, A. I., Zenhom, O. A., Areda, H. A., Ragaza, J. A.,and Hassaan, M. S. (2023). Fresh *Azolla pinnata* as a complementary feed for *Oreochromis niloticus*: Growth, digestive enzymes, intestinal morphology, physiological responses, and flesh quality. *Aquaculture Nutrition, 2023*(1), 1403704. https://doi.org/10.1155/2023/1403704

Romano, L. E., and Aronne, G. (2021). The world's smallest plants (*Wolffia* sp.) as potential species for bioregenerative life support systems in space. *Plants, 10*(9), 1896. https://doi.org/10.3390/plants10091896

Roshan, K. H., Meharoof, M.,and Sajina, K. A. (2023). Plant-based proteins in fish diets for sustainable coldwater fisheries. In *Coldwater fisheries and aquaculture management* (pp. 217–247). Apple Academic Press.

Said, D. S., Chrismadha, T., Mayasari, N., Febrianti, D.,and Suri, A. R. M. (2022). Nutrition value and growth ability of aquatic weed *Wolffia globosa* as alternative feed sources for aquaculture system. *IOP Conference Series: Earth and Environmental Science, 950*(1), 012044. https://doi.org/10.1088/1755-1315/950/1/012044

Said, N. S. M., Zakaria, M. Z., Samsuddin, N. I., Barom, N. A. A. Z., Murugiah, N. A. M.,and Abdullah, S. R. S. (2025). Reclaiming *Wolffia arrhiza* as fish feed for the phytoremediation of aquaculture effluent. *Journal of Environmental Management, 382*, 125405. https://doi.org/10.1016/j.jenvman.2024.125405

Seephua, N., Boonarsa, P., Li, H., Thammapat, P.,and Siriamornpun, S. (2025). Nutritional composition and bioactive profiles of farmed and wild watermeal (*Wolffia globosa*). *Foods, 14*(10), 1832. https://doi.org/10.3390/foods14101832

Siriwat, W., Ungwiwatkul, S., Unban, K., Laokuldilok, T., Klunklin, W., Tangjaidee, P.,and Phongthai, S. (2023). Extraction, enzymatic modification, and anti-cancer potential of an alternative plant-based protein from *Wolffia globosa*. *Foods, 12*(20), 3815. https://doi.org/10.3390/foods12203815

Tadesse, Z., Getachew, A., Workiye, G., Addisu, D., and Tadesse, S. (2024). Effect of partial replacement of fishmeal with duckweed (*Lemna* sp.) on the growth performance of juvenile Nile tilapia (*Oreochromis niloticus* L.) in tanks. *Livestock Research Results, 594*.

Yanong, R. P., and Lewbart, G. A. (2024). *The aquarium fish medicine handbook*. CRC Press.

Yang, R., Liu, X., Liu, Y., Tian, Q., Wang, Z., Zhu, D.and Su, J. (2024). Dissolved oxygen and ammonia affect ammonia production via GDH/AMPK signaling pathway and alter flesh quality in Chinese perch (*Siniperca chuatsi*). *Fish Physiology and Biochemistry, 50*(3), 1237–1249. https://doi.org/10.1007/s10695-023-01263-4

Yuvasree, K.,and Bharathipriya, R. (2021). Guidelines for the water quality parameters in pond fish culture. *Journal of Aquaculture in the Tropics, 36*(1–4), 69–75.

Glossary of Key Terms

- Specific Growth Rate (SGR): Percentage increase in body weight per day.
- Protein Efficiency Ratio (PER): Ratio of weight gain to protein intake.
- Fulton's Condition Factor (K): Indicator of fish health, calculated as (Weight ÷ $Length^3$) × 100.
- Dissolved Oxygen (DO): Amount of oxygen available in water, vital for fish respiration.
- Ammonia (NH_3): Nitrogenous waste product; high levels can be toxic to fish.