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Functional Feed Development from *Alternanthera philoxeroides* for Herbivorous Fish: Effects on Growth Performance, Feed Efficiency, and Hematological Response



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ABSTRACT

This study investigates the potential of Alternanthera philoxeroides as a functional feed ingredient in gourami (Osphronemus goramy) aquaculture. The objective was to evaluate its effects on growth performance, feed conversion ratio (FCR), and hematological responses. A 60-day feeding trial was conducted using four diets with varying inclusion levels of A. philoxeroides: 0% (P0), 10% (P1), 20% (P2), and 30% (P3). Specifically, the P2 group achieved an SGR of 1.64 \pm 0.06%/day compared to 1.47 \pm 0.05%/day in the control, and an FCR of 1.39 \pm 0.04 versus 1.62 \pm 0.03 in the control, representing a statistically significant improvement (p < 0.05). Hematological analysis revealed that P2 also resulted in the highest erythrocyte and leukocyte counts, indicating enhanced physiological and immune status. Statistical analysis confirmed that P2 was significantly different (p < 0.05) from the control. The findings suggest that A. philoxeroides at a 20% inclusion level can be used to improve fish performance and health, while also offering a sustainable alternative protein source. These improvements not only demonstrate nutritional benefits but also highlight the feasibility of reducing feed costs while enhancing fish health in herbivorous aquaculture. Moreover, utilizing this invasive aquatic plant aligns with ecological and economic goals by converting waste biomass into value-added feed products.

1. INTRODUCTION

The sustainable development of aquaculture is critically dependent on the availability of cost-effective, nutritionally balanced, and environmentally friendly feed sources. Traditionally, fishmeal has served as the primary protein component in aquaculture feeds, attributed to its high nutritional quality. Nonetheless, factors such as rising costs, limited availability, and environmental concerns linked to fishmeal production are prompting researchers to investigate alternative feed ingredients, particularly those derived from plant sources [1, 2]. In fact, the rising demand for fish has exacerbated the economic pressures on aquaculture farms as their feeding costs increase [3]. Thus, a transition towards sustainable feed sources appears inevitable due to the ecological constraints on traditional fishmeal sourcing [4, 5]. Among the potential alternatives, *A. philoxeroides* (alligator

weed) has emerged as a promising candidate due to its high protein content, bioactive compounds, and abundance as an invasive species in freshwater systems.

Herbivorous fish, including species like gourami (O. goramy), are particularly well-equipped to thrive on plant-based diets due to their digestive physiology, which enables the effective utilization of complex carbohydrates and fibrous materials [6]. However, the challenge remains in formulating these plant-based diets to not only promote optimal growth but also enhance health and immune resilience among fish [7]. Incorporating bioactive compounds found in many aquatic plants—such as flavonoids, phytosterols, and phenolics—may serve as natural immunostimulants and antioxidants, thus enhancing feed efficiency and overall fish health [8-11]. While research into these ingredients is promising, comprehensive studies are still needed to ascertain their concrete effects on growth performance, feed conversion efficiency, and

hematological parameters in various fish species [12-14].

One particular plant that has garnered attention is A. philoxeroides, commonly recognized as alligator weed. Despite being classified as an invasive species, its remarkable nutritional value and biofunctional properties—such as antimicrobial and antioxidant activity—underscore its potential as a feed ingredient for herbivorous fish [15, 16] Its high protein and fiber content, in conjunction with its abundance and low cost, position it as a viable alternative in aquaculture feed formulations [6, 17]. Its dual advantages lie not only in its nutritional and immunostimulatory properties but also in its potential role in ecological management by repurposing invasive biomass into productive aquaculture inputs. However, empirical research focusing on the practical applications of A. philoxeroides, particularly in terms of evaluating its impacts on fish growth metrics and health indicators, remains scarce [18]. Therefore, this study specifically aimed to determine the optimal dietary inclusion level of A. philoxeroides for gourami (O. goramy) by evaluating its effects on growth performance, Feed Conversion Ratio, and hematological responses.

2. MATERIAL AND METHODS

2.1 Experimental design and fish rearing

This study was conducted to evaluate the effects of dietary inclusion of A. philoxeroides on growth performance, feed efficiency, and hematological response of herbivorous fish (Gourami, O. goramy). A total of 240 fish (initial average weight: \pm 25 g) were randomly distributed into 12 experimental aquaria (60 L) in a completely randomized design with 4 dietary treatments and 3 replicates each:

P0: Control diet (0% A. philoxeroides)

P1: Diet with 10% A. philoxeroides substitution

P2: Diet with 20% A. philoxeroides substitution

P3: Diet with 30% A. philoxeroides substitution

The proximate composition and main raw ingredients of the control and experimental diets are presented in Table 1 to demonstrate that all diets were formulated to be isonitrogenous and isolipidic.

Fish were acclimatized for 7 days before the experiment and fed the respective diets for 60 days. Water quality was monitored daily and maintained within optimal ranges (temperature $27-29^{\circ}$ C, DO > 5 mg/L, pH 6.8-7.5).

Table 1. Proximate composition and main ingredients of experimental diets containing different inclusion levels of *A. philoxeroides* for gourami (*O. goramy*)

Treatment	Crude Protein (%)	Crude Lipid (%)	Ash (%)	Moisture (%)	Crude Fiber (%)	Main Ingredients (g/kg)
P0 (0%)	32.1	5.4	9.6	8.7	10.1	Soybean meal, rice bran, corn gluten
P1 (10%)	32.0	5.5	9.8	8.6	10.4	Soybean meal, rice bran, corn gluten, <i>A. philoxeroides</i> (10%)
P2 (20%)	32.2	5.6	9.7	8.5	10.5	Same as above with 20% inclusion
P3 (30%)	32.1	5.4	9.9	8.4	10.7	Same as above with 30% inclusion

2.2 Feed preparation

Fresh A. philoxeroides was harvested from local aquatic habitats, washed, sun-dried, and ground into fine powder. The powder was used to substitute plant-based ingredients in isonitrogenous and isolipidic diets formulated to meet the nutritional requirements of gourami. Feed was pelletized using a manual extruder and dried at 50°C for 24 hours before storage at 4°C.

2.3 Growth performance

Fish in each aquarium were batch-weighed at the beginning and end of the trial. The specific growth rate (SGR) was calculated using the following equation:

$$SGR = \left(\frac{\operatorname{Ln} W_t - \operatorname{Ln} W_0}{t}\right) \times 100 \tag{1}$$

where, W_t is the final mean weight of the fish (g), W_0 is the initial mean weight of the fish (g), and t is the duration of the feeding trial (days).

2.4 Feed conversion ratio (FCR)

Feed intake was recorded daily, and FCR was calculated at the end of the experiment using the following equation:

$$FCR = \frac{F}{W_t + D - W_0} \tag{2}$$

where, F is the total feed consumed by the fish (g), W_t is the final mean weight of the fish (g), W_0 is the initial mean weight of the fish (g), and D is the total weight of dead fish during the experimental period (g).

2.5 Hematological analysis

At the end of the feeding trial, blood samples were collected from the caudal vein of three randomly selected fish per replicate using 1 mL syringes pre-loaded with EDTA as an anticoagulant. The blood samples were then analyzed to determine hematological parameters. The total erythrocyte count was measured using a Neubauer hemocytometer following dilution with Hayem's solution, while the total leukocyte count was assessed using the same type of hemocytometer, with Turk's solution serving as the diluent. Both erythrocyte and leukocyte counts were expressed as the number of cells per cubic millimeter (mm³) of blood.

2.6 Statistical analysis

All data were expressed as mean \pm standard deviation (SD). One-way ANOVA was performed to detect significant differences among treatments, followed by Duncan's multiple range test at a significance level of p < 0.05 using SPSS v26.

3. RESULTS

The trends in growth performance and feed efficiency among treatments are illustrated in Figure 1, highlighting the

superior performance of the P2 group. Hematological responses, including erythrocyte and leukocyte counts, are shown in Figure 2, further confirming the enhanced physiological status of P2.

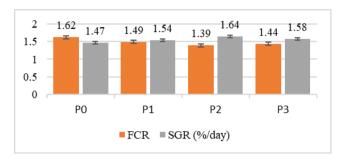


Figure 1. Specific growth rate (SGR) and feed conversion ratio (FCR) of Gourami (*O. goramy*) across the four dietary treatments (P0: 0% inclusion, P1: 10% inclusion, P2: 20% inclusion, P3: 30% inclusion of *Alternanthera philoxeroides*), with standard deviation (SD) represented as error bars

3.1 Growth performance

The specific growth rate (SGR) of gourami fed diets with different inclusion levels of A. philoxeroides is shown in Table 2 and Figure 1. Fish fed with the P2 diet (20% inclusion) exhibited the highest SGR value (1.64 \pm 0.06%/day), significantly (p < 0.05) higher than the control group (1.47 \pm 0.05%/day). The P1 (10%) and P3 (30%) groups also showed improved growth performance compared to the control, although not significantly different from P2.

3.2 Feed conversion ratio (FCR)

Table 3 and Figure 1 present the feed conversion ratio of

fish among treatments. The lowest FCR was recorded in the P2 group (1.39 \pm 0.04), indicating the highest feed efficiency, followed by P1 (1.49 \pm 0.05) and P3 (1.44 \pm 0.06). The control group (P0) showed the highest FCR (1.62 \pm 0.03), which was significantly different (p < 0.05) from P2.

3.3 Hematological response

Table 4 summarizes the total erythrocyte and leukocyte counts. Fish fed with *A. philoxeroides* diets (P1, P2, and P3) demonstrated a higher erythrocyte count compared to the control, with P2 yielding the highest value ($1.80 \pm 0.09 \times 10^6$ cells/mm³). Similarly, leukocyte counts increased in the treated groups, peaking in P2 ($12.7 \pm 0.5 \times 10^3$ cells/mm³), which was significantly different from P0 (p < 0.05). These results suggest a positive hematological response to the inclusion of *A. philoxeroides* in the diet.

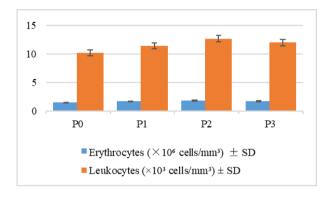


Figure 2. Total erythrocyte and leukocyte counts of gourami (*O. goramy*) after the 60-day feeding trial, comparing the four dietary treatments (P0-P3) containing varying levels of *A. philoxeroides*, with standard deviation (SD) shown as error bars

Table 2. Specific growth rate (SGR) of gourami over 60 days of dietary treatment

Treatment	Initial Weight (g)	Final Weight (g)	$SGR (\%/day) \pm SD$
P0	25.1 ± 0.3	60.8 ± 1.2	$1.47\pm0.05^{\rm a}$
P1	25.0 ± 0.4	63.2 ± 1.0	1.54 ± 0.04^{b}
P2	24.9 ± 0.2	66.5 ± 1.3	1.64 ± 0.06^{c}
P3	25.3 ± 0.3	64.0 ± 1.5	1.58 ± 0.07^{bc}

Note: Values with different superscript letters within the same column are significantly different (p < 0.05; Duncan's multiple range test).

Table 3. Feed conversion ratio (FCR) of gourami during the experimental period

Treatment	Feed Given (g)	Initial Biomass (g)	Final Biomass (g)	FCR ± SD
P0	500	1005	2432	$1.62\pm0.03^{\rm a}$
P1	500	1000	2528	$1.49\pm0.05^{\rm b}$
P2	500	996	2660	$1.39\pm0.04^{\rm c}$
P3	500	1012	2578	1.44 ± 0.06^{bc}

Note: Values with different superscript letters within the same column are significantly different (p < 0.05; Duncan's multiple range test).

Table 4. Total erythrocyte and leukocyte counts of gourami after 60 days of dietary treatment

Treatment	Erythrocytes (×10 ⁶ cells/mm ³) ± SD	Leukocytes ($\times 10^3$ cells/mm ³) \pm SD
P0	1.45 ± 0.08	10.2 ± 0.5
P1	1.65 ± 0.07	11.4 ± 0.6
P2	1.80 ± 0.09	12.7 ± 0.5
P3	1.70 ± 0.06	12.0 ± 0.4

4. DISCUSSIONS

4.1 Growth performance

The results of this study demonstrate that the inclusion of A.

philoxeroides in the diet significantly improved the growth performance of gourami, as reflected by the increased specific growth rate (SGR). The highest growth was observed in fish fed with the P2 diet (20% inclusion), indicating that this level

of substitution provides an optimal balance of nutritional benefit and palatability.

The enhancement in SGR can be attributed to the nutritional composition of *A. philoxeroides*, which is known to contain considerable amounts of crude protein, dietary fiber, and bioactive compounds such as flavonoids, saponins, and phytosterols [19, 20]. These compounds may contribute to improved feed utilization and digestive efficiency by enhancing enzymatic activity and gut morphology in herbivorous fish.

The findings align with previous studies on other plant-based feed additives. For instance, Armando et al. [21] reported that aquatic plants such as *Lemna sp.* and *Azolla* increased growth rates in herbivorous fish due to their nutrient density and fiber digestibility. Moreover, Islamy [10, 22] emphasized the potential of aquatic macrophytes as cost-effective protein sources for aquaculture feed, capable of reducing reliance on traditional plant or animal meals.

Interestingly, while all experimental groups (P1-P3) outperformed the control in terms of SGR, the increase plateaued beyond 20% inclusion. The slightly lower growth rate in P3 (30% inclusion) may reflect diminishing returns at higher substitution levels, possibly due to increased levels of antinutritional factors such as tannins, saponins, and oxalates. Tannins are known to form insoluble complexes with proteins, thereby reducing digestibility, while saponins can damage intestinal mucosa and impair nutrient absorption. Oxalates, on the other hand, may chelate essential minerals such as calcium and magnesium, limiting their bioavailability. The combined effects of these compounds likely contributed to the reduced growth performance observed in the P3 group, consistent with previous findings in other herbivorous fish fed high levels of unprocessed plant material [23]. This trend is consistent with prior findings indicating that excessive inclusion of unprocessed plant material in fish diets may lead to reduced feed intake and slower growth [24].

The observed optimal growth performance and feed utilization in the P2 group support the assertion that a 20% inclusion level of can constitute the most effective diet formulation among those evaluated in this study. This interpretation aligns with existing literature indicating that plant-based inclusions can yield diminishing returns or even detrimental effects at higher levels due to anti-nutritional factors (ANFs) that can increase with dosage [25, 26]. In particular, the 20% inclusion appears to provide a substantial supply of crude protein, dietary fiber, and bioactive compounds such as flavonoids and saponins, which may collectively enhance digestive enzyme activity and gut morphology, thereby improving nutrient absorption and growth performance [27]. Similar patterns have been reported in other plant-based substitution studies where moderate replacement levels supported favorable feed efficiency and growth relative to higher substitution levels that led to reduced intake and impaired feed conversion efficiency, nutrient digestibility, and overall growth performance [25, 26, 28].

At the 30% inclusion level (P3), the decline in growth is consistent with a dose-dependent increase in ANFs that can compromise nutrient utilization. Tannins and saponins, present in many plant tissues, are known to bind dietary proteins and disrupt intestinal integrity, respectively, which can reduce protein digestibility and impair nutrient absorption when concentrations are elevated [26, 29]. Moreover, oxalates present in certain plants can chelate minerals, reducing mineral bioavailability and further restraining growth under high

inclusion conditions [30]. This pattern aligns with evidence that excessive unprocessed plant material in aquafeeds can suppress feed intake and growth, likely due to reduced palatability and impaired nutrient bioavailability [31, 32]. Collectively, the P2 level appears to offer an optimal balance by capitalizing on the nutritional benefits of *A. philoxeroides* while mitigating the adverse effects of ANFs that intensify with greater dietary inclusion.

These findings are consistent with prior research across various fish species showing that plant-based diet components can support growth and feed utilization up to an optimal threshold, beyond which performance declines due to antinutritional and mineral-chelating effects, as well as potential impacts on gut morphology and enzyme activity. For instance, studies in tilapia and other teleosts have noted reduced growth and feed efficiency when high levels of plant-based ingredients substitute major portions of fish meal, especially when ANFs are not sufficiently mitigated (e.g., through processing or supplementation) [32-34]. Moreover, moderate plant inclusion can maintain or even enhance digestive capacity and growth when bioactive compounds positively influence gut physiology [27]. The current results, therefore, fit within this broader framework, supporting the 20% inclusion as the optimal threshold for balancing nutritional benefits against anti-nutritional drawbacks in diets based on A. philoxeroides.

Furthermore, the consistent alignment of our interpretation with analogous findings from plant-based replacement studies in diverse aquaculture systems highlights the generalizability of the principle that there exists an optimum inclusion level maximizing performance while mitigating ANF-related risks. This principle is reflected in meta-analytic syntheses of fishmeal replacement by plant proteins, emphasizing that performance outcomes depend critically on the degree of replacement, plant material quality, processing methods, and the inclusion of anti-nutritional mitigants or supportive minerals and amino acids. Thus, the present analysis indicating P2 as the optimal inclusion level is well-supported by cross-study evidence demonstrating that moderate plant inclusion can sustain superior growth and feed efficiency, while higher inclusions are associated with performance penalties driven by ANFs and limitations in mineral bioavailability. Overall, the growth response suggests that 20% inclusion of A. philoxeroides is a promising level for functional feed formulation in gourami culture, offering a balance between nutrient supply and fish acceptance. Beyond that threshold, the benefits may be offset by potential digestive challenges or reduced feed efficiency.

4.2 Feed conversion ratio (FCR)

The feed conversion ratio (FCR) is an essential parameter in aquaculture that quantifies feed efficiency by indicating the feed required to achieve a unit of biomass gain in fish. Research highlights that the incorporation of specific feed materials can significantly alter FCR outcomes. For instance, a study demonstrating improved FCR in Nile tilapia with marine microalgae inclusion noted that diets supplemented with microalgae resulted in higher growth rates and better nutritional profiles due to enhanced nutrient digestibility [7]. This aligns with findings related to *A. philoxeroides*, where a substantial inclusion in the diet can yield notable improvements in growth performance and FCR.

The beneficial effects on FCR attributed to A. philoxeroides

can be partially explained by its nutrient profile, particularly its phytosterols and flavonoids, which are acknowledged for their roles in enhancing digestive enzyme activity and promoting better nutrient absorption. These compounds may support gut health, which is vital for optimizing feed utilization, especially within herbivorous species [35]. Similar mechanisms have been documented where other plants, such as *Ocimum sanctum*, resulted in improved FCR due to better nutrient uptake and palatability, albeit their specific contributions are less conclusive for tilapia [36].

While the inclusion of *A. philoxeroides* at a certain level may enhance FCR, it is important to note that higher inclusion levels (such as 30%) could lead to diminished digestibility or the introduction of antinutritional factors [37]. This observation has been supported by studies showing that excessive plant material can negatively impact growth performance due to compounds such as tannins and saponins, which can impair protein assimilation [38]. This reinforces that a balance in dietary composition is crucial to maximizing feed efficiency without compromising fish health or growth performance.

Overall, the insights gained from various studies underscore the potential of *A. philoxeroides* and similar plant materials as functional feed ingredients to enhance FCR in herbivorous fish. Identifying optimal inclusion levels not only improves the economic viability of aquaculture practices but also supports sustainable feed strategies that leverage plants rich in beneficial phytochemical constituents [39, 40].

4.3 Hematological response

Hematological parameters such as erythrocyte and leukocyte count serve as key biomarkers in evaluating fish health and immune function. Recent studies indicate that dietary supplementation with various herbal materials and functional feeds can enhance these parameters, suggesting improved health and immunity in fish. Enhanced metabolic activity is crucial as it supports growth and maintains efficient oxygen delivery to tissues, especially in fish subjected to active feeding regimes or stressors [41].

The elevated leukocyte count, particularly in the 20% inclusion group, suggests immune-modulating properties of *A. philoxeroides*. Bioactive components such as flavonoids, phenolics, and saponins found in this plant may relate to improved immune responses. Compounds in these plants can stimulate the production and activation of various white blood cells, thereby bolstering nonspecific immunity [42]. Previous studies have indicated that dietary supplements, including herbal mixes, can induce increases in leukocyte counts and overall immune function across different fish species. For example, increases have been observed in Helostoma temminckii when administered herbal-based diets [43].

Interestingly, the P3 group did exhibit slight reductions in hematological values compared to the P2 group, suggesting a threshold beyond which the addition of plant material might interfere with digestibility or nutrient absorption, potentially due to the presence of antinutritional factors. Increased inclusion of certain herbs may lead to adverse effects, such as lower nutrient bioavailability or immune response modulation, underscoring the importance of optimal inclusion levels in aquaculture feeds [44].

Overall, these findings support the potential of *A. philoxeroides* as a functional feed ingredient, indicating it not only enhances growth but also contributes to better blood

health in fish, thereby promoting overall resilience and wellbeing within aquaculture systems. Such insights advocate for the incorporation of this plant in sustainable aquaculture, as its immunomodulatory effects could lead to healthier fish populations.

4.4 Environmental implications

Utilizing A. philoxeroides, commonly known as alligator weed, in aquaculture represents a strategic approach that can benefit fish growth and health while addressing the ecological impacts of this invasive species. The integration of A. philoxeroides into fish feed may enhance aquatic ecosystems while simultaneously managing the overgrowth issues associated with this plant in freshwater environments. By converting a problematic species into a resource, aquaculture can contribute to ecological sustainability goals.

Studies indicate that *A. philoxeroides* exhibits significant biomass accumulation in nutrient-rich environments, particularly when nitrogen and phosphorus are available [45]. This growth potential allows for large-scale harvesting and incorporation into fish feed, which can mitigate its ecological burden. Moreover, previous findings suggest that leveraging invasive species like *A. philoxeroides* in aquaculture could offset their negative ecological impacts while redistributing their nutritional value into fish diets [46].

Research by Harms et al. [47] highlights that *A. philoxeroides* demonstrates plasticity in response to nutrient availability, offering a competitive advantage in various environments. This adaptability implies that management strategies using *A. philoxeroides* can be refined to optimize fish growth while controlling its invasiveness in freshwater ecosystems. The nutrients provided by incorporating *A. philoxeroides* into fish feeds not only support fish health but also contribute to sustainable ecosystem management by controlling the growth of this invasive plant through strategic harvesting.

The large-scale adoption of *A. philoxeroides* in aquaculture shows promise, as it facilitates dual benefits—enhancing aquaculture productivity while contributing to ecological management. This approach aligns with broader sustainability goals, transforming invasive plant species from ecological threats into valuable resources [48]. By repurposing *A. philoxeroides*, it is plausible to alleviate its negative impacts on native flora and fauna, thus benefiting biodiversity conservation efforts in freshwater ecosystems.

Overall, the potential for *A. philoxeroides* as a sustainable ingredient in aquaculture addresses both growth and health parameters in edible fish and responds to the urgent need for effective environmental management of invasive species in aquatic systems. With widespread adoption, such strategies could significantly reduce the ecological burden associated with *A. philoxeroides* overgrowth, fostering its integration into sustainable aquaculture practices.

4.5 Functional feed perspective in sustainable aquaculture

The integration of *A. philoxeroides* into fish feed presents significant benefits that extend beyond basic nutrition. This invasive aquatic plant demonstrates immunostimulatory effects that underscore its potential as a biofunctional feed ingredient. As consumers and the aquaculture industry increasingly seek alternatives to antibiotics and synthetic growth promoters, the incorporation of *A. philoxeroides* aligns

with sustainable aquaculture principles. Recent research indicates that plant-based functional feeds can enhance fish welfare while promoting consumer safety and health [49, 50].

The immunostimulatory properties of *A. philoxeroides* stem from its rich bioactive compound profile, which includes flavonoids, phenolics, and saponins. These compounds can enhance immune responses in fish, making functional feeds derived from such plants advantageous for both growth and overall fish health in aquaculture systems that prioritize the reduction of chemical additives [51, 52]. For instance, studies have shown how dietary strategies incorporating natural ingredients can bolster innate immune responses in fish, providing an environmentally friendly approach to disease management.

The movement toward plant-based functional feeds resonates strongly with the One Health approach, which promotes the interconnection between human, animal, and environmental health [49]. By improving fish welfare and reducing reliance on antibiotics, *A. philoxeroides* showcases its dual role as both a functional feed ingredient and a management strategy for invasive species. This approach can help mitigate environmental impacts associated with *A. philoxeroides* overgrowth, subsequently enhancing ecosystem management [49].

Moreover, continual improvement in aquaculture welfare practices through the use of biofunctional ingredients can foster consumer confidence and safety in farmed fish products. As aquaculture faces increasing demand from a growing population, alternative feeding strategies that prioritize sustainability will be crucial [53, 54]. Thus, the development and adoption of functional feeds with *A. philoxeroides* not only meet the nutritional needs of farmed fish but also align with broader ecological sustainability goals, offering a holistic pathway for responsible aquaculture [54, 55].

Overall, A. philoxeroides stands out as a promising candidate for functional feed applications in aquaculture, representing a practical solution that encapsulates health, sustainability, and ecological management.

4.6 Practical recommendations for application

To effectively utilize *A. philoxeroides* as a functional feed ingredient in aquaculture, it is recommended that incorporation rates not exceed 20%. Such limitations are proposed based on research findings, indicating potential antinutritional factors that could arise from excessive inclusion rates. Current studies emphasize various pre-treatment methods—including drying, fermentation, and enzymatic hydrolysis—that may help mitigate these antinutritional effects and enhance the bioavailability of nutrients present in *A. philoxeroides* [56].

Research demonstrates that *A. philoxeroides* has a highwater content (over 90%), and understanding its biochemical composition is crucial for its optimal incorporation into fish diets. The main constituents of its dry matter, which include cellulose, hemicellulose, and lignin, suggest potential barriers to nutrient absorption that pre-treatment methods could alleviate, ultimately improving efficacy in feeding applications [56]. Implementing such methods can encourage aquaculture farmers to safely and efficiently utilize this underutilized biomass while avoiding the pitfalls related to antinutritional factors.

Additionally, studies focusing on submergence and nutrient availability have revealed that *A. philoxeroides* can thrive

under submerged conditions and responds positively to nutrient-rich environments. This adaptability highlights its potential for sustainable application within aquaculture systems [57]. Beyond addressing the nutrient needs of fish, adopting sustainable practices involving *A. philoxeroides* could foster environmental management strategies targeting invasive species, further supporting ecological balance in aquatic systems [58].

Overall, continued exploration of treatment methods for *A. philoxeroides* will reinforce its viability as a functional feed ingredient. By enhancing nutrient bioavailability and ensuring safe feeding practices, aquaculture systems can benefit from this approach while contributing to broader sustainability and ecological goals.

5. CONCLUSIONS

This study demonstrates that A. philoxeroides can be effectively utilized as a functional feed ingredient in the diet of herbivorous fish, particularly gourami (O. goramy). Dietary inclusion up to 20% significantly enhanced growth performance, improved feed conversion efficiency, and stimulated hematological responses—evidenced by increased erythrocyte and leukocyte counts. These results highlight the potential of A. philoxeroides to contribute to more sustainable and cost-effective aquaculture feed formulations. Beyond nutritional benefits, the use of this underutilized aquatic plant may also provide ecological and socioeconomic value by converting invasive biomass into a productive resource. Such an approach supports both environmental conservation and the economic empowerment of small-scale aquaculture producers. However, this study was limited to three performance parameters over a 60-day period. The long-term effects, potential antinutritional impacts at higher inclusion levels, and influences on fish reproduction and gut health remain unexplored. Future research should focus on processing techniques to enhance nutrient bioavailability and minimize potential drawbacks, as well as expanding trials to other herbivorous species and commercial farming conditions. The incorporation of A. philoxeroides at optimal levels offers a promising avenue for the development of sustainable, healthpromoting aquafeeds and provides a model for integrating ecological management with aquaculture innovation.

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NOMENCLATURE

Wo	Initial weight of individual fish, grams	
VV 0	(g)	
W_t	Final weight of individual fish, grams	
vv t	(g)	
t	Duration of the experiment, days	
F	Total feed consumed, grams (g)	
Во	Initial biomass (total fish weight),	
D0	grams (g)	
\mathbf{B}_{t}	Final biomass (total fish weight),	
Dţ	grams (g)	
D	Biomass of dead fish during	
D	experiment, grams (g)	
SGR	Specific Growth Rate, %/day	
FCR	Feed Conversion Ratio	
RBC	Total erythrocyte (red blood cell)	
RDC	count, ×10 ⁶ cells/mm ³	
WBC	Total leukocyte (white blood cell)	
WBC	count, ×10 ³ cells/mm ³	
SD	Standard Deviation	
P0-P3	Dietary treatments (0%–30% inclusion	
10-13	of 1 philoropoides)	

of A. philoxeroides)