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Dried Protein Curd from Invasive *Alternanthera philoxeroides* as a Sustainable Feed Ingredient for Nile Tilapia

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ABSTRACT

Alternanthera philoxeroides (alligator weed) is a widely distributed invasive aquatic plant with relatively high protein content, yet it remains largely underutilized for value-added applications. This study evaluated the feasibility of converting *A. philoxeroides* biomass into dried protein curd and its potential use as a sustainable aquaculture feed ingredient. Fresh plant material was processed through grinding, filtration, starch sedimentation, thermal treatment, acid coagulation, and spray drying to obtain protein curd. The resulting product was analyzed for proximate composition, essential amino acid (EAA) profile, and antioxidant activity. Four isonitrogenous diets containing 0%, 10%, 20%, and 30% dried protein curd were formulated and fed to juvenile Nile tilapia (*Oreochromis niloticus*) under controlled conditions. Protein curd production significantly increased crude protein content from 22.6% in raw leaves to 34.8% in the dried protein curd, while also improving the EAA profile, particularly lysine, leucine, and valine. The dried protein curd exhibited moderate antioxidant activity, with DPPH radical scavenging activity of 41.6% and an IC_{50} value of 2.18 mg mL^{-1}. Fish fed diets containing 20% protein curd showed the best growth performance, achieving the highest final weight (21.2 g), specific growth rate (2.66% day^{-1}), and the lowest feed conversion ratio (1.41). Overall, these findings demonstrate a viable strategy for converting invasive plant biomass into high-value aquafeed ingredients while supporting the development of more sustainable and diversified protein sources for aquaculture.

1. INTRODUCTION

Aquatic invasive plants are a serious environmental problem in freshwater ecosystems, especially in tropical and subtropical regions. These plants grow fast, spread aggressively, and disrupt hydrology, native biodiversity, fisheries, and agricultural systems. One of the most problematic aquatic invasive species is *A. philoxeroides* (alligator weed), which invades rivers, irrigation canals, wetlands, and agricultural lands in many countries, including Indonesia. Its strong phenotypic plasticity, clonal growth, and tolerance to environmental stress allow this plant to colonize a wide range of habitats and rapidly dominate invaded areas [1, 2]. Once established, alligator weed is difficult to control and often causes long-term ecological and economic impacts [3, 4].

Current management of *A. philoxeroides* mainly relies on mechanical removal, chemical herbicides, or biological control approaches. Mechanical and chemical controls are costly, labor-intensive, and usually provide only short-term solutions, while repeated application may increase environmental risks [5, 6]. Biological control and microbial agents show potential, but their effectiveness is often site-specific and slow to achieve large-scale suppression. Therefore, alternative strategies that combine invasive plant management with resource recovery are increasingly needed.

At the same time, global aquaculture production continues to expand and faces increasing challenges related to feed cost

and ingredient availability. Protein sources such as fish meal and soybean meal are the most expensive components in aquafeeds, and their use is limited by sustainability, competition with human food, and environmental concerns [7, 8]. This has encouraged intensive research on alternative and locally available protein sources, including agricultural by-products, aquatic weeds, and other unconventional feed ingredients [9, 10].

Previous studies have shown that several aquatic plants contain moderate levels of crude protein and bioactive compounds, suggesting potential use as aquafeed ingredients [10, 11]. *A. philoxeroides* biomass has also been reported to contain appreciable protein content and essential amino acids (EAA). However, direct use of raw or dried plant material in fish diets is limited by high fiber content, low digestibility, and the presence of antinutritional or allelopathic compounds, which may reduce feed utilization and growth performance [3, 12]. Processing methods such as fermentation or drying have been tested for aquatic weeds, but fish performance results remain inconsistent and strongly dependent on inclusion level and processing efficiency.

Multiple processing strategies have been explored to improve the nutritional quality of plant biomass used in aquafeeds. Fermentation is widely applied to reduce antinutritional factors and enhance digestibility through microbial metabolism, but it often requires controlled microbial cultures, longer processing times, and may produce variable results depending on fermentation conditions. Enzymatic hydrolysis can also improve protein availability and peptide bioactivity; however, the process typically involves higher operational costs due to the use of purified enzymes and controlled reaction conditions. In comparison, protein coagulation through protein curd production represents a relatively simple and scalable method that concentrates plant proteins while removing soluble carbohydrates, pigments, and a portion of fiber. This approach requires fewer specialized inputs and can be implemented using relatively straightforward processing steps, making it potentially more suitable for large-scale biomass valorization of invasive aquatic plants.

Protein curd production is a processing method commonly applied to legumes and oilseed crops to concentrate protein while reducing fiber and soluble carbohydrates. This method involves aqueous extraction, heat treatment, and acid coagulation to separate protein-rich fractions from undesirable components. Protein curd products generally show higher protein density and improved amino acid availability compared to unprocessed plant materials, making them more suitable for use in animal and aquaculture feeds. However, the application of protein curd technology to invasive aquatic plants, particularly *A. philoxeroides*, has received very limited attention, and information on its nutritional quality and biological effectiveness in fish diets is still scarce.

Therefore, this study aimed to valorize *A. philoxeroides* biomass by converting it into dried protein curd and evaluating its potential as a plant-based protein source for aquaculture feeds. The specific objectives were to assess the nutritional composition, EAA profile, antioxidant activity, and growth performance of Nile tilapia (*O. niloticus*) fed diets containing graded levels of *A. philoxeroides* protein curd. It was hypothesized that protein curd processing would improve protein quality and allow partial replacement of conventional plant protein sources without negatively affecting fish growth performance, while also providing an alternative and sustainable use for invasive aquatic plant biomass. To date, the application of protein curd technology to invasive aquatic plants such as *A. philoxeroides* has not been reported, and its potential as a nutritionally improved protein source for aquaculture feeds remains unexplored. This study addresses this gap by integrating invasive weed valorization with aquaculture feed development, offering a novel and sustainable approach for both invasive plant management and fish nutrition.

2. MATERIALS AND METHODS

2.1 Raw material collection

Wild *A. philoxeroides* was harvested from an agricultural field in Mojoroto District, Kediri, East Java, Indonesia. Only healthy plants at the vegetative growth stage were collected. The leaves were separated from coarse stems, transported to the laboratory in insulated containers, and processed immediately to minimize biochemical degradation.

2.2 Preparation of dried protein curd

The collected leaves were thoroughly washed under running tap water to remove soil and foreign materials. Cleaned leaves were blanched at 90 °C for 2 min to inactivate endogenous enzymes and reduce antinutritional compounds, followed by rapid cooling in an ice bath. Blanched leaves were homogenized using a laboratory blender with distilled water at a ratio of 1:5 (w/v). The resulting slurry was pressed and filtered through muslin cloth to obtain a green leaf extract. The filtrate was heated at 100 °C for 10 min to induce protein denaturation and then cooled to 75 °C. Protein coagulation was achieved by adjusting the pH of the extract to 4.5–4.6 using glacial acetic acid, followed by the addition of calcium sulfate ($CaSO_4$) at a concentration of 0.5–1.0 g L^{-1} to promote curd formation. This concentration range was selected based on preliminary coagulation trials and previous studies on leaf protein extraction, which reported that moderate Ca^{2+} concentrations enhance protein aggregation and curd firmness without excessive mineral deposition in the final product. The mixture was gently stirred once and allowed to stand for 15 min until protein curd formation was observed. The formed protein curd was separated by pressing to remove excess whey. The wet curd was subsequently dried using a laboratory-scale spray dryer operated at an inlet temperature of 160 ± 5 °C and an outlet temperature of 80 ± 3 °C. The feed suspension was supplied at a rate of approximately 5 mL min^{-1} using a peristaltic pump and atomized through a standard two-fluid nozzle system under compressed air. Drying was continued until a stable powder with low residual moisture content was obtained. The dried protein curd was milled into a fine powder and stored in airtight containers at room temperature prior to analysis and feed formulation. Protein curd yield was not quantified in terms of biomass recovery during the present laboratory-scale processing. The primary focus of this study was to evaluate the nutritional composition, amino acid profile, antioxidant properties, and biological performance of the resulting protein curd in Nile tilapia diets. Therefore, detailed mass balance analysis and recovery yield calculations were not conducted during this preliminary investigation. Future research should include a comprehensive evaluation of protein recovery efficiency and process yield to

better assess the techno-economic feasibility of large-scale production from invasive plant biomass.

The processing steps involved in converting *A. philoxeroides* leaves into dried protein curd, and its subsequent use in experimental diet formulation are illustrated in Figure 1.

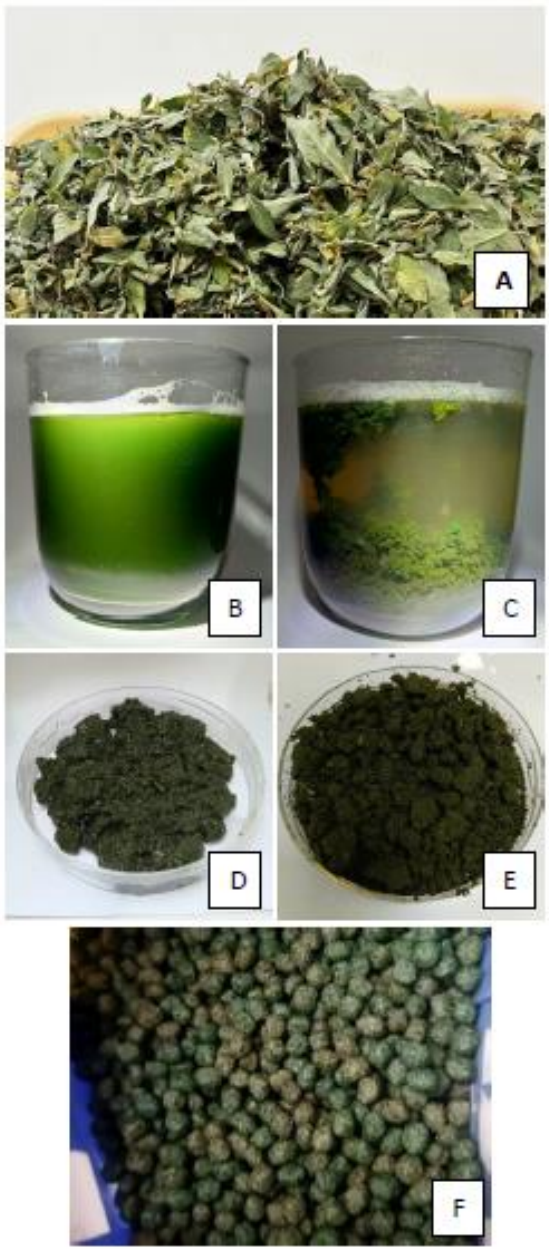

Figure 1. Processing stages for producing dried protein curd from *A. philoxeroides* and its application in experimental feed formulation: (A) dried leaves of *A. philoxeroides*; (B) leaf juice obtained after grinding and filtration; (C) coagulated leaf juice forming protein curd; (D) filtered wet curd; (E) dried protein curd powder; (F) pellets produced from the formulated experimental diets

2.3 Proximate composition analysis

Proximate composition of raw leaves, dried protein curd, and experimental diets was determined following standard AOAC procedures. Crude protein was analyzed using the Kjeldahl method (N × 6.25). Crude lipid was determined by Soxhlet extraction, moisture content by oven drying at 105 °C, ash content by muffle furnace combustion at 550 °C, and crude fiber by acid–alkali digestion. All analyses were conducted in triplicate.

2.4 Amino acid profile analysis

The amino acid profiles of dried protein curd of *A. philoxeroides* and the experimental diets were determined following acid hydrolysis and chromatographic analysis. Samples were first defatted using petroleum ether and then hydrolyzed with 6 N hydrochloric acid (HCl) at 110 °C for 24 h in sealed glass tubes under a nitrogen atmosphere to prevent oxidative degradation.

After hydrolysis, the samples were cooled to room temperature, filtered, and evaporated to dryness under reduced pressure. The residues were reconstituted in a suitable buffer solution and filtered through a 0.45 µm membrane filter prior to analysis. Amino acids were quantified using high-performance liquid chromatography (HPLC) equipped with a reversed-phase column after pre-column derivatization with o-phthalaldehyde (OPA). EAA and non-EAA were identified and quantified by comparison with external amino acid standards. Amino acid concentrations were expressed as a percentage of total protein. All analyses were performed in triplicate.

2.5 Antioxidant activity analysis

The antioxidant activity of dried protein curd of *A. philoxeroides* was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Dried protein curd powder was extracted with 80% methanol at a ratio of 1:10 (w/v) and shaken at room temperature for 24 h. The extract was filtered and used for antioxidant analysis. A volume of 1 mL of sample extract was mixed with 1 mL of 0.1 mM DPPH solution in methanol and incubated in the dark at room temperature for 30 min. The absorbance was measured at 517 nm using a UV–Vis spectrophotometer. Radical scavenging activity was calculated as a percentage of DPPH inhibition relative to a blank control. Results were expressed as percentage inhibition and IC_{50} values.

The DPPH radical scavenging assay was selected as a widely used and reliable method for evaluating antioxidant capacity in plant-derived extracts, particularly for preliminary screening of phenolic-rich matrices. This assay has been extensively applied in studies assessing antioxidant properties of plant-based feed ingredients and provides a rapid estimation of radical scavenging potential.

2.6 Total phenolic content

Total phenolic content (TPC) was determined using the Folin–Ciocalteu method. Briefly, 0.5 mL of sample extract was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent and 2.0 mL of 7.5% sodium carbonate solution. The mixture was incubated in the dark at room temperature for 30 min, and absorbance was measured at 765 nm. Gallic acid was used as the standard, and results were expressed as mg gallic acid equivalents (GAE) per g dry sample.

2.7 Experimental diet formulation

Four isonitrogenous experimental diets were formulated by incorporating dried protein curd of *A. philoxeroides* at 0%

(control), 10%, 20%, and 30% inclusion levels as partial replacements for soybean meal (Table 1 and Figure 2). Fish meal, corn meal, rice bran, fish oil, and a commercial vitamin–mineral premix were included to meet the nutritional requirements of the experimental fish species. All dry ingredients were thoroughly mixed and pelleted using a laboratory pelletizer through a cold-pelleting process without steam conditioning. The feed mash was moistened with a small amount of water to improve binding before extrusion through a 2-mm die. The produced pellets were dried at room temperature for 24 h to reduce moisture content and improve physical stability prior to storage.

Table 1. Formulation of experimental diets containing dried protein curd of *A. philoxeroides* (% dry matter basis)

Ingredient (%)	Control (0%)	AP-DPC 10%	AP-DPC 20%	AP-DPC 30%
Fish meal	25.0	23.0	21.0	19.0
Soybean meal	30.0	25.0	20.0	15.0
Dried protein curd of *A. philoxeroides*	0.0	10.0	20.0	30.0
Corn meal	15.0	15.0	15.0	15.0
Rice bran	20.0	17.0	14.0	11.0
Fish oil	5.0	5.0	5.0	5.0
Vitamin–mineral premix	5.0	5.0	5.0	5.0
Total	**100.0**	**100.0**	**100.0**	**100.0**

Note: AP-DPC = dried protein curd of *A. philoxeroides*. All diets were formulated to be isonitrogenous and prepared on a dry matter basis.

2.8 Feeding trial

The feeding trial was conducted for 8 weeks using a completely randomized design. Fish with similar initial body weights were randomly distributed into experimental tanks. Each dietary treatment was tested in triplicate. Fish were fed the experimental diets twice daily to apparent satiation. Water quality parameters, including temperature, dissolved oxygen, and pH, were monitored regularly and maintained within suitable ranges throughout the experimental period.

2.9 Experimental fish and acclimatization

Juvenile Nile tilapia (*O. niloticus*) were obtained from a local hatchery in East Java, Indonesia. Prior to the feeding trial, fish were acclimatized to laboratory conditions for 14 days and fed a commercial diet during the acclimation period. Only healthy and active fish with no visible signs of disease were selected for the experiment.

At the start of the trial, fish with similar initial body weights were randomly distributed into experimental tanks at an appropriate stocking density. During acclimatization and throughout the feeding experiment, fish were maintained under controlled conditions with continuous aeration. Water quality parameters, including temperature, dissolved oxygen, and pH, were monitored regularly and maintained within optimal ranges for tilapia culture.

All experimental procedures involving fish were conducted in accordance with institutional guidelines for the care and use of experimental animals and complied with standard ethical principles for aquaculture research.

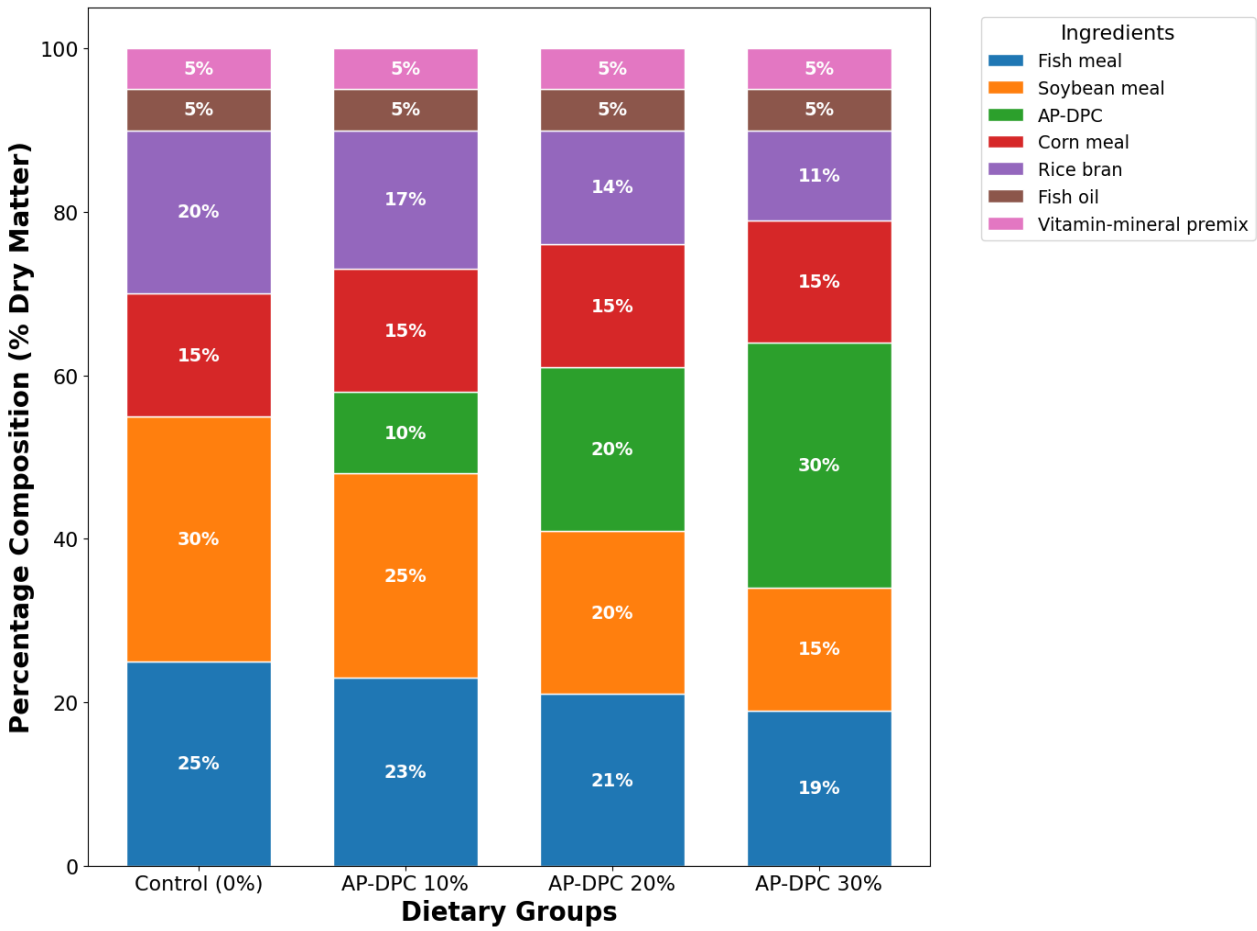

Figure 2. Experimental diet formulation comparison

2.10 Growth performance and feed utilization

At the beginning and end of the feeding trial, fish were weighed to determine growth performance. Parameters evaluated included weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and survival rate. Protein retention was calculated as dietary protein intake minus body protein gain.

2.11 Statistical analysis

All data were expressed as mean ± standard deviation. Statistical analysis was performed using one-way analysis of variance (ANOVA). When significant differences were detected, Tukey's multiple range test was applied to compare means at a significance level of $p < 0.05$.

3. RESULTS

3.1 Proximate composition of dried protein curd

The conversion of wild *A. philoxeroides* leaves into dried protein curd resulted in a marked improvement in nutritional quality, particularly crude protein content. As shown in Table 2 and Figure 3, crude protein increased significantly compared with raw leaves ($p < 0.05$), while crude fiber content decreased substantially after protein curd formation and drying.

Table 2. Proximate composition of raw leaves and dried protein curd of *A. philoxeroides* (% dry matter)

Parameter	Raw Leaves	Dried Protein Curd
Moisture	8.7 ± 0.4[a]	6.2 ± 0.3[b]
Crude protein	22.6 ± 0.9[a]	34.8 ± 1.2[b]
Crude lipid	4.3 ± 0.4[a]	6.1 ± 0.5[b]
Crude fiber	18.9 ± 1.1[b]	10.4 ± 0.7[a]
Ash	10.7 ± 0.6[a]	12.3 ± 0.8[b]

Note: Values are mean ± SD (n = 3). Different superscript letters within a row indicate significant differences ($p < 0.05$).

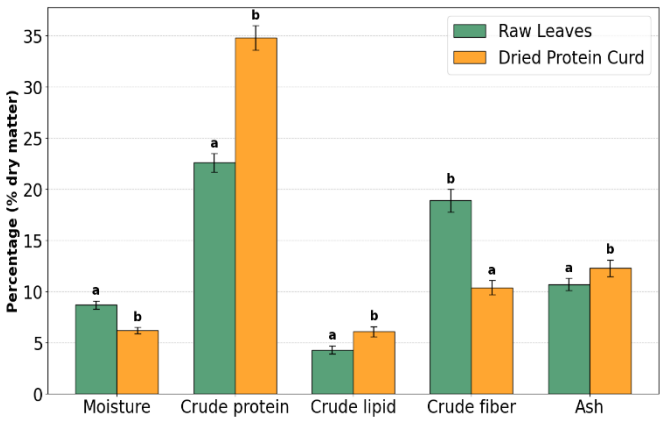

Figure 3. Proximate composition of raw leaves and dried protein curd of *A. philoxeroides*

3.2 Essential amino acid composition

The EAA composition of fresh leaves and dried protein curd of *A. philoxeroides* is presented in Table 3 and Figure 4. Overall, the protein curd exhibited a more concentrated and balanced EAA profile compared to the fresh leaves. Processing into protein curd resulted in noticeable increases in several nutritionally important amino acids, particularly lysine, leucine, valine, and threonine, which are critical for fish growth and protein synthesis.

Lysine content increased from 5.21% to 6.34% of total protein, reflecting the selective concentration of protein fractions during curd formation. Similarly, branched-chain amino acids (leucine, isoleucine, and valine) were moderately enriched in the dried protein curd. In contrast, methionine remained the most limiting amino acid in both fresh leaves and protein curd, although a slight improvement was observed after processing. The overall enhancement of EAA density suggests that protein curd production effectively improves the nutritional quality and applicability of *A. philoxeroides* protein for aquaculture feed formulations, particularly when used as a partial replacement for conventional plant protein sources.

Table 3. Essential amino acid (EAA) composition of fresh leaves and dried protein curd of *A. philoxeroides* (% of total protein)

Essential Amino Acid	Fresh Leaves (%)	Dried Protein Curd (%)
Arginine	6.12 ± 0.18	6.85 ± 0.22
Histidine	2.41 ± 0.09	2.78 ± 0.11
Isoleucine	3.84 ± 0.14	4.36 ± 0.16
Leucine	6.92 ± 0.21	7.88 ± 0.24
Lysine	5.21 ± 0.17	6.34 ± 0.20
Methionine	1.42 ± 0.06	1.58 ± 0.07
Phenylalanine	4.03 ± 0.13	4.52 ± 0.15
Threonine	3.67 ± 0.12	4.29 ± 0.14
Valine	4.18 ± 0.15	4.81 ± 0.17
Total EAA	37.80	43.41

Note: Values are mean ± standard deviation (n = 3).

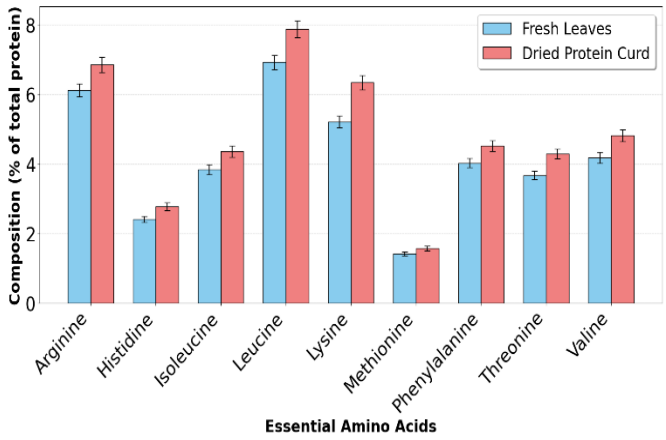

Figure 4. Comparison of essential amino acid (EAA) composition between fresh leaves and dried protein curd of *A. philoxeroides*

3.3 Proximate composition of experimental diets

All experimental diets were successfully formulated to be isonitrogenous. Crude protein content ranged from 32.1% to 34.2% across dietary treatments (Table 4). Increasing inclusion levels of dried protein curd resulted in a gradual increase in crude fiber and ash content, while lipid levels remained relatively stable.

Table 4. Proximate composition of experimental diets (% dry matter)

Parameter	Control	10%	20%	30%
Crude protein	32.1 ± 0.6[a]	32.8 ± 0.7[ab]	33.6 ± 0.8[b]	34.2 ± 0.9[b]
Crude lipid	7.9 ± 0.4	7.6 ± 0.3	7.4 ± 0.3	7.2 ± 0.4
Crude fiber	5.2 ± 0.3[a]	5.8 ± 0.4[b]	6.4 ± 0.4[c]	7.1 ± 0.5[d]
Ash	9.1 ± 0.4	9.4 ± 0.5	9.8 ± 0.5	10.2 ± 0.6

3.4 Growth performance and feed utilization

Growth performance of fish fed diets containing dried protein curd is presented in Table 5 and Figure 5.

Fish fed a diet containing 20% dried protein curd exhibited significantly higher final body weight, weight gain, and specific growth rate than the control group ($p < 0.05$). Feed conversion ratio was significantly improved at the 20% inclusion level, whereas performance declined slightly at 30% inclusion, although survival rates remained high across all treatments.

3.5 Protein utilization efficiency

Dietary inclusion of dried protein curd significantly affected protein utilization efficiency (Table 6). Protein retention and PER were highest in fish fed the 20% inclusion diet ($p < 0.05$). At 30% inclusion, both parameters showed a declining trend, suggesting reduced efficiency at higher substitution levels.

Table 5. Growth performance and feed utilization of fish fed experimental diets

Parameter	Control	10%	20%	30%
Initial weight (g)	5.02 ± 0.10	5.01 ± 0.12	5.03 ± 0.11	5.00 ± 0.09
Final weight (g)	18.4 ± 0.9[a]	19.7 ± 1.1[ab]	21.2 ± 1.3[b]	19.1 ± 1.2[a]
Weight gain (g)	13.4 ± 0.9	14.7 ± 1.1	16.2 ± 1.3	14.1 ± 1.2
SGR (% day⁻¹)	2.41 ± 0.08[a]	2.52 ± 0.09[ab]	2.66 ± 0.10[b]	2.48 ± 0.09[a]
FCR	1.58 ± 0.07[b]	1.49 ± 0.06[ab]	1.41 ± 0.05[a]	1.55 ± 0.07[b]
Survival (%)	96.7 ± 2.1	97.3 ± 1.5	96.0 ± 2.3	95.3 ± 2.7

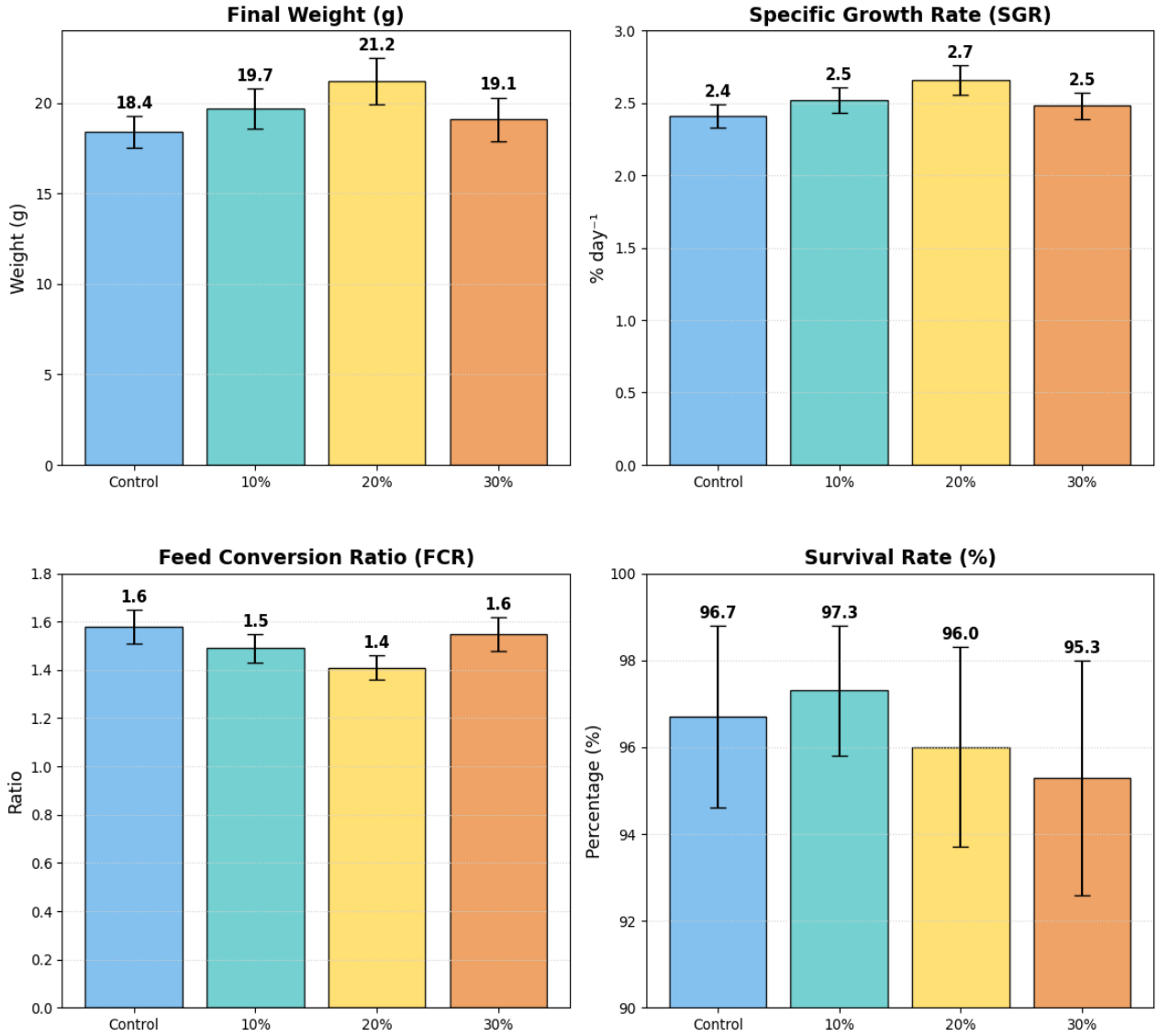

Figure 5. Growth performance and feed utilization across experimental diets

Table 6. Protein utilization parameters of fish fed experimental diets

Parameter	Control	10%	20%	30%
Protein retention (%)	28.6 ± 1.4[a]	30.2 ± 1.6[ab]	33.5 ± 1.8[b]	29.1 ± 1.5[a]
Protein Efficiency Ratio (PER)	1.72 ± 0.08[b]	1.84 ± 0.09[ab]	1.96 ± 0.10[a]	1.75 ± 0.09[b]

3.6 Antioxidant activity

Dried protein curd of *A. philoxeroides* exhibited measurable antioxidant activity (Table 7). The DPPH radical scavenging activity indicated moderate antioxidant capacity, which was retained after drying. TPC confirmed the presence of phenolic compounds associated with the leaf-derived protein matrix.

Table 7. Antioxidant properties of dried protein curd of *A. philoxeroides*

Parameter	Value
DPPH inhibition (%)	41.6 ± 2.3
IC_{50} (mg mL^{-1})	2.18 ± 0.12
Total phenolic content (mg GAE g^{-1})	18.4 ± 1.1

The antioxidant activity observed in the dried protein curd indicates a moderate radical scavenging capacity. The DPPH inhibition value of 41.6% and IC_{50} of 2.18 mg mL^{-1} fall within the range reported for several plant-derived feed ingredients, including leaf protein concentrates and aquatic plant extracts. Previous studies have reported DPPH inhibition values ranging from approximately 30–60% for plant-based protein materials used in aquafeeds, depending on the concentration of phenolic compounds and processing conditions. These results suggest that the protein curd derived from *A. philoxeroides* retains a measurable level of antioxidant compounds that may contribute to functional benefits in fish diets.

4. DISCUSSIONS

The results of this study demonstrate that processing *A. philoxeroides* biomass into dried protein curd can significantly improve its suitability as a feed ingredient for aquaculture. Compared to fresh plant material, the protein curd showed higher crude protein content and a more concentrated EAA profile, indicating that the aqueous extraction and coagulation process effectively separated protein fractions from fiber and other non-protein components. Similar improvements in protein concentration after processing have been reported for other plant-based feed ingredients used in aquaculture diets [7, 13].

The EAA composition of the *A. philoxeroides* protein curd showed relatively higher levels of lysine, leucine, and valine. Lysine is commonly identified as a limiting amino acid in plant-derived aquafeeds, and its improvement through protein curd processing represents an important nutritional advantage over raw plant biomass [14, 15]. Branched-chain amino acids such as leucine and valine are also known to play important roles in protein synthesis and energy metabolism, which may contribute to improved feed utilization in fish [16, 17].

However, methionine content remained relatively low even after protein curd processing. This limitation is consistent with previous studies on plant protein sources, where sulfur-containing amino acids are often deficient compared to fish meal-based diets [18]. This finding indicates that *A. philoxeroides* protein curd cannot fully meet the amino acid requirements of Nile tilapia when used as a sole protein source and should be combined with other protein ingredients or supplemented with crystalline amino acids, as commonly practiced in plant-based aquafeed formulations [19, 20].

In previous studies, biological processing methods such as microbial fermentation have frequently been applied to improve the nutritional value of plant-derived feed ingredients. Fermentation can enhance digestibility and reduce certain antinutritional factors through microbial enzymatic activity. However, fermentation typically requires longer processing times, controlled microbial cultures, and careful environmental regulation to maintain product consistency. In contrast, protein coagulation used in the present study represents a relatively rapid and controllable process that concentrates plant proteins through thermal denaturation and acid-induced precipitation. This approach allows separation of protein fractions from soluble carbohydrates and part of the fibrous matrix, thereby improving protein density and handling properties of the resulting ingredient. From a practical perspective, coagulation-based protein extraction may also offer advantages for scalability and process stability compared with biological fermentation systems, particularly when processing large volumes of invasive plant biomass.

The feeding trial results showed that dietary inclusion of *A. philoxeroides* protein curd up to moderate levels did not negatively affect growth performance, feed conversion ratio, or survival of Nile tilapia. This suggests that the protein curd was palatable and nutritionally acceptable for fish. Similar growth responses have been observed when processed plant proteins or alternative protein sources partially replaced soybean meal or fish meal in tilapia diets [19, 21, 22].

The absence of growth depression at moderate inclusion levels also suggests that protein curd processing may have reduced antinutritional or allelopathic effects commonly associated with raw aquatic plant biomass. The processing steps applied in this study may also contribute to the reduction of antinutritional compounds commonly present in aquatic plants. Blanching at high temperature can inactivate endogenous enzymes and facilitate the leaching of heat-labile compounds such as certain phenolics, tannins, and saponins. In addition, the subsequent extraction and acid-induced coagulation process separates soluble fractions from the protein-rich curd, potentially removing part of the antinutritional components that remain dissolved in the liquid phase. Similar reductions in plant secondary metabolites have been reported in studies involving leaf protein extraction and coagulation-based protein isolation. The partial removal of these compounds may improve palatability, nutrient availability, and overall feed utilization in fish. Although the present study did not directly quantify antinutritional factors, the processing sequence employed likely contributed to the acceptable growth performance observed in Nile tilapia fed diets containing the protein curd.

At higher inclusion levels, growth performance tended to plateau, which may be attributed to amino acid imbalance, residual fiber content, or reduced digestibility of plant-derived proteins. Previous studies have reported similar growth plateaus when plant protein inclusion exceeds optimal levels, even after processing, due to limitations in amino acid availability and digestive efficiency [23, 24]. This further supports the concept that *A. philoxeroides* protein curd is best

applied as a partial protein replacement rather than a complete substitute for conventional protein sources.

In addition to its nutritional value, the dried protein curd exhibited measurable antioxidant activity. Although antioxidant capacity was not the primary focus of this study, the presence of antioxidant compounds may provide additional functional benefits to fish, such as protection against oxidative stress. Plant-derived feed ingredients with antioxidant properties have been reported to improve immune response and stress resistance in cultured fish species [25, 26]. Further studies are needed to determine whether the observed antioxidant activity of *A. philoxeroides* protein curd can translate into measurable health benefits under intensive aquaculture conditions.

From an environmental and sustainability perspective, the valorization of *A. philoxeroides* biomass into a functional feed ingredient offers a dual advantage. It provides an alternative use for an invasive aquatic weed that is typically considered waste or a management burden, while simultaneously contributing to the diversification of sustainable protein sources for aquaculture. This approach aligns with circular economy principles and may help reduce the ecological and economic costs associated with invasive plant control. Transforming invasive biomass into value-added aquafeed ingredients represents a promising strategy to link invasive species management with sustainable aquaculture development. The findings of this study indicate that dried protein curd derived from *A. philoxeroides* has potential as a supplementary plant protein ingredient in Nile tilapia diets. However, its application should be carefully optimized in terms of inclusion level and amino acid balance. Further research on nutrient digestibility, long-term feeding effects, health responses, and economic feasibility is recommended before large-scale application in commercial aquaculture systems.

5. CONCLUSIONS

This study demonstrated the feasibility of utilizing the invasive aquatic plant *A. philoxeroides* as a sustainable protein source for aquaculture through a protein coagulation approach. The processing method successfully converted plant biomass into a concentrated dried protein curd with improved protein content and measurable antioxidant activity. The feeding trial results indicated that dietary inclusion of the protein curd supported normal growth performance and feed utilization in Nile tilapia (*O. niloticus*), with the 20% inclusion level producing the most favorable growth response. The processing steps, including blanching, extraction, and acid-induced coagulation, may also contribute to the reduction of anti-nutritional compounds, thereby improving the nutritional quality and safety of the resulting feed ingredient. Overall, the findings demonstrate a viable strategy for converting invasive aquatic plant biomass into a value-added aquafeed ingredient, supporting sustainable feed development and circular resource utilization in aquaculture systems.

Future research also should conduct extended feeding trials to assess growth performance, physiological health, and nutrient retention over prolonged cultivation periods. Research is needed on the best amounts of fermented *A. sessilis* to add to commercial diets, as well as how it affects the shape of the gut, the activity of digestive enzymes, and the immune response. Furthermore, economic analysis and pilot-scale fermentation studies are necessary to evaluate production viability and cost-effectiveness in agricultural settings. Research on the application of different microbial cultures or varying fermentation periods may further improve nutrient accessibility and functional characteristics. Solid-state fermentation of *A. philoxeroides* offers a potential and scalable way to make plant-based aquafeed ingredients that will last. This method has a lot of promise to help make aquaculture production systems that are more resilient, cost-effective, and ecologically friendly if it is improved and tested more.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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NOMENCLATURE

Symbol / Abbreviation	Description	Unit
A. philoxeroides	*Alternanthera philoxeroides* (alligator weed)	–
AP-DPC	Dried protein curd of *A. philoxeroides*	–
AOAC	Association of Official Analytical Chemists	–
ANOVA	Analysis of variance	–
$CaSO_4$	Calcium sulfate	g L^{-1}
CP	Crude protein	%
CF	Crude fiber	%
DM	Dry matter	%
DPPH	2,2-Diphenyl-1-picrylhydrazyl	–
EAA	Essential amino acids	% of total protein
FCR	Feed conversion ratio	–
GAE	Gallic acid equivalent	mg g^{-1}
HCl	Hydrochloric acid	N
HPLC	High-performance liquid chromatography	–
IC_{50}	Concentration required to inhibit 50% of DPPH radicals	mg mL^{-1}
PER	Protein efficiency ratio	–
pH	Potential of hydrogen	–
SD	Standard deviation	–
SGR	Specific growth rate	% day^{-1}
TPC	Total phenolic content	mg GAE g^{-1}
WG	Weight gain	g
w/v	Weight per volume ratio	–
°C	Degree Celsius	–