Biochemical Transformation, Functional Properties, and Digestibility of Alligator Weed Fermented with *Rhizopus Oligosporus* for Nile Tilapia

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ABSTRACT

This study examines the impact of solid-state fermentation of alligator weed using Rhizopus oligosporus on the chemical composition, antioxidant activity, antibacterial properties, and apparent digestibility in Nile Tilapia. Fermentation was conducted for 3 and 5 days, with unfermented material used as a control specimen. Fermentation significantly increased crude protein content and reduced crude fiber and antinutritional factors, including phytate, tannin, and oxalate. The fermented samples exhibited enhanced antioxidant capacity, as evidenced by increased phenolic content and radical-scavenging activity. Antibacterial activity against major fish pathogenic bacteria was also improved following fermentation. Digestibility trials showed significantly higher Apparent Digestibility Coefficients (ADCs) of dry matter, protein, and energy in diets containing fermented alligator weed, with the highest values observed after 5 days of fermentation. These results demonstrate that Rhizopus oligosporus fermentation effectively improves the nutritional quality, functional properties, and digestibility of alligator weed, indicating its potential application as a sustainable functional feed ingredient for Nile Tilapia aquaculture.

Keywords-alligator weed; antioxidant activity; digestibility; fermentation; life below water; Nile Tilapia

I. INTRODUCTION

The expansion of global aquaculture has increased the demand for sustainable and efficient feed ingredients, particularly for widely farmed species such as Nile Tilapia (*Oreochromis niloticus*). Conventional protein sources, including fish meal, face challenges related to high cost, limited availability, and environmental sustainability [1], prompting extensive research into alternative plant-based feed resources [2]. Although plant ingredients are generally more sustainable, their utilization in aquafeeds is often constrained by high fiber content and the presence of antinutritional factors [3], which reduce nutrient digestibility and growth performance in fish [4].

Aquatic macrophytes have received great attention as alternative feed ingredients due to their high biomass productivity and ability to utilize excess nutrients from aquatic environments [5]. However, their direct inclusion in fish diets remains limited due to the presence of structural carbohydrates and antinutritional compounds, such as phytate, tannins, and oxalates, which negatively affect mineral availability, protein utilization, and intestinal function [6]. These compounds are known to interfere with digestive enzymes and nutrient absorption, thereby reducing feed efficiency.

Fermentation is a widely used bioprocessing technique for enhancing the nutritional value of plant materials [7]. Solid-state fermentation using filamentous fungi has been shown to reduce antinutritional factors and enhance nutrient availability through enzymatic degradation of complex carbohydrates and phytate complexes [8]. *Rhizopus oligosporus*, commonly used in traditional fermented food products, such as tempeh, produces extracellular enzymes, including proteases, cellulases, and phytases, which can enhance protein content and digestibility while reducing fiber and antinutrient levels [9].

In addition to nutritional improvement, fermentation may improve the functional properties of plant-based feed ingredients. Fermented substrates can exhibit increased antioxidant activity due to the release of bound phenolic compounds and the formation of bioactive metabolites [10]. Furthermore, microbial activity associated with fermentation may contribute to antibacterial effects, which are beneficial in aquaculture systems where disease prevention and reduction of antibiotic use are major concerns.

Digestibility is an important factor determining the suitability of alternative feed ingredients for fish. Ingredient processing and enzymatic activity can significantly influence nutrient digestibility and intestinal function in Nile Tilapia and other cultured species [11]. Improving digestibility through fermentation is therefore essential to ensure efficient nutrient utilization and to minimize negative effects on gut health.

Alligator weed (*Alternanthera philoxeroides*) is an invasive aquatic plant with high biomass productivity and potential nutritional value. However, its application in aquafeeds has received limited attention, particularly following the use of fungal fermentation. Information regarding the effects of Rhizopus oligosporus fermentation on the chemical composition, antioxidant and antibacterial properties, and digestibility of alligator weed for Nile Tilapia is scarce.

In the context of this study, the term "functional properties" refers specifically to biological functional properties, including antioxidant and antibacterial activities that may contribute to fish health and disease resistance, rather than techno-functional feed properties such as water-holding capacity or oil-holding capacity. Therefore, the present study aims to evaluate the effects of solid-state fermentation of alligator weed using *Rhizopus oligosporus* on its chemical composition, antinutritional factors, biological functional properties (antioxidant and antibacterial activities), and apparent digestibility in Nile Tilapia.

II. MATERIALS AND METHODS

A. Raw Material Preparation

Fresh alligator weed (*Alternanthera philoxeroides*) was collected from freshwater wetlands in Kediri, East Java, Indonesia. The plant material was thoroughly washed with tap water to remove adhering soil and debris and then rinsed with distilled water. The cleaned biomass was chopped into small pieces (approximately 1–2 cm) and oven-dried at 60 °C for 48 h. It was subsequently ground into a fine powder using a laboratory grinder. The powdered material was stored in airtight containers at room temperature until further use.

B. Microorganism and Inoculum Preparation

Rhizopus oligosporus was obtained from a certified culture collection and maintained on Potato Dextrose Agar (PDA) at 30 °C. A spore suspension was prepared by flooding the agar surface with sterile distilled water and gently scraping the spores with a sterile loop. The spore concentration was determined with a hemocytometer and adjusted to approximately 1×10^6 spores mL^{-1}.

C. Fermentation Process

Solid-state fermentation was conducted by mixing alligator weed powder with sterile distilled water at a 1:1 (w/v) ratio to achieve a suitable moisture content. The substrate was autoclaved at 121 °C for 15 min and then cooled to room temperature before inoculation. The substrate was inoculated with *Rhizopus oligosporus* at a 10% (v/v) concentration and incubated at 30 °C for 3 and 5 days. The selection of 3 and 5 days of fermentation was justified based [12] reporting effective enzymatic activity and antinutrient reduction by *Rhizopus oligosporus* within this time frame, and to represent an early and extended fermentation phase without over-fermentation [12]. Unfermented alligator weed served as the control sample. After fermentation, samples were oven-dried at 50 °C, ground, and stored at −20 °C for subsequent analyses. The final moisture content of the substrate during solid-state fermentation was approximately 55–60%, which is within the optimal range for fungal growth under solid-state conditions. Each fermentation treatment (unfermented control, 3-day fermentation, and 5-day fermentation) was conducted in three independent fermentation batches, which were considered biological replicates (n = 3). Samples obtained from each independent batch were subsequently analyzed in triplicate for chemical composition, antinutritional factors, antioxidant activity, and antibacterial activity. The mean value of each batch was used for statistical analysis.

D. Proximate Composition Analysis

Proximate composition, including moisture, crude protein, crude lipid, crude fiber, and ash content, was analyzed following standard AOAC procedures [13]. Crude protein was determined using the Kjeldahl method (N × 6.25), crude lipid by Soxhlet extraction, crude fiber by acid–alkali digestion, and ash content by combustion at 550 °C. Nitrogen-free extract was calculated by difference.

E. Determination of Antinutritional Factors

Phytate content was determined using a colorimetric method based on ferric–phytate complex formation. Tannin content was measured using the Folin–Denis method, while oxalate content was analyzed by titration following acid digestion. All measurements were performed in triplicate.

F. Antioxidant Activity Analysis

Total phenolic content was determined employing the Folin–Ciocalteu method, and was expressed as mg Gallic Acid Equivalents (GAE) per gram of dry sample. Antioxidant activity was evaluated using the DPPH radical scavenging assay [14], and the percentage of inhibition was calculated. IC_{50} values were determined from the linear regression of inhibition percentage against extract concentration [15].

G. Antibacterial Activity Assay

Antibacterial activity was evaluated using the agar well diffusion method. Fermented and unfermented alligator weed samples were extracted with methanol at a 1:10 (w/v) ratio for 24 h by maceration at room temperature. The extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure to obtain crude extracts. Bacterial cultures of Aeromonas hydrophila, Streptococcus agalactiae, and Vibrio parahaemolyticus were adjusted to 10^8 CFU mL^{-1}, and were evenly spread on Mueller–Hinton agar plates. Wells (6 mm diameter) were filled with 100 µL of extract (100 mg mL^{-1}). Methanol was used as a negative control, while a standard antibiotic (oxytetracycline, 30 µg) served as a positive control. Plates were incubated at 37 °C for 24 h, and inhibition zones were measured in mm, excluding the well diameter.

H. Experimental Fish and Digestibility Trial

Juvenile Nile Tilapia (*Oreochromis niloticus*), with an average initial body weight of 12.5 ± 0.6 g, were used for the digestibility trial. Prior to the experiment, the fish were acclimated to laboratory conditions for two weeks and fed a reference diet. Experimental diets were formulated by replacing 30% of the reference diet with either fermented or unfermented alligator weed meal and by adding chromium oxide (Cr_2O_3) to each diet at a concentration of 0.5% as an inert internal marker.

Fish were randomly stocked in 200 L fiberglass tanks at a density of 15 fish per tank. The digestibility trial lasted 21 days, consisting of a 14-day dietary adaptation period, followed by a 7-day fecal collection period. Each dietary treatment was conducted in triplicate tanks (n = 3), with each tank considered as an independent experimental unit. The fish were fed to apparent satiation twice daily throughout the experimental period. Fecal samples were collected by gentle siphoning approximately 6 h after feeding to minimize nutrient leaching. Collected feces were immediately dried and stored at −20 °C until further chemical analysis.

I. Apparent Digestibility Coefficient Calculation

ADCs for dry matter, crude protein, and gross energy were calculated using the indicator method based on the relative concentrations of chromium oxide in the diets and feces. Chromium oxide (Cr_2O_3) concentration in experimental diets and fecal samples was determined deploying the acid digestion and spectrophotometric method described in [16], modified for fish digestibility studies. Briefly, dried diet and fecal samples were finely ground, digested with a mixture of nitric and perchloric acids until complete oxidation, and diluted with distilled water. Chromium concentration was quantified using a UV–visible spectrophotometer at 350 nm, and Cr_2O_3 content was calculated from a standard calibration curve prepared with analytical-grade chromium oxide. The ADC (%) was calculated using:

$$\mathrm{ADC}(\%) = 100 - \left[100 \times \left(\frac{\mathrm{Cr_2O_3}_{\mathrm{diet}}}{\mathrm{Cr_2O_3}_{\mathrm{feces}}}\right) \times \left(\frac{\mathrm{Nutrient_{feces}}}{\mathrm{Nutrient_{diet}}}\right)\right] \quad (1)$$

where Cr_2O_{3diet} and Cr_2O_{3feces} represent the chromium oxide concentration in the diet and feces, respectively, and $Nutrient_{diet}$ and $Nutrient_{feces}$ represent the concentration of the corresponding nutrient in the diet and feces.

J. Statistical Analysis

For all parameters, independent fermentation batches (n = 3) were considered biological replicates. Analytical measurements were performed in triplicate for each biological replicate, and the averaged values were used in the statistical analysis. All data were expressed as mean ± standard deviation. Statistical analysis was performed using one-way Analysis of Variance (ANOVA) to evaluate the effect of fermentation duration. Differences among treatment means were analyzed using Tukey's post-hoc test at a significance level of $p < 0.05$.

III. RESULTS

A. Proximate Composition

Fermentation with *Rhizopus oligosporus* significantly altered the chemical composition of alligator weed (Table I).

TABLE I. PROXIMATE COMPOSITION OF FERMENTED ALLIGATOR WEED (% DRY MATTER)

Parameter	Unfermented	3 days of fermentation	5 days of fermentation
Crude protein (%)	18.42 ± 0.31^c	21.76 ± 0.45^b	24.89 ± 0.52^a
Crude lipid (%)	2.11 ± 0.08^c	2.47 ± 0.09^b	2.93 ± 0.11^a
Crude fiber (%)	24.85 ± 0.62^a	20.14 ± 0.55^b	16.72 ± 0.48^c
Ash (%)	15.33 ± 0.29^a	14.87 ± 0.33^{ab}	14.25 ± 0.27^b
Nitrogen-free extract (%)	39.29 ± 0.74^b	40.76 ± 0.68^a	41.21 ± 0.71^a

The values in Tables I-V are expressed as mean ± SD of three independent fermentation batches (biological replicates, n = 3). Different superscript letters within the same row indicate significant differences (one-way ANOVA, Tukey's test, $p < 0.05$).

B. Antinutritional Factors

The concentrations of antinutritional compounds were significantly reduced following fermentation (Table II).

TABLE II. REDUCTION OF ANTINUTRITIONAL FACTORS

Parameter	Unfermented	3 days of fermentation	5 days of fermentation
Phytate (mg g^{-1})	14.26 ± 0.48^a	9.84 ± 0.36^b	6.31 ± 0.29^c
Tannin (mg g^{-1})	6.78 ± 0.27^a	4.39 ± 0.21^b	2.86 ± 0.18^c
Oxalate (mg g^{-1})	5.92 ± 0.24^a	3.87 ± 0.19^b	2.14 ± 0.13^c

The contents of phytate, tannin, and oxalate decreased markedly with increasing fermentation time, with the lowest values observed in the 5-day fermented treatment.

C. Antioxidant Activity

Fermented alligator weed exhibited significantly enhanced antioxidant properties compared to the unfermented control sample (Table III).

TABLE III. ANTIOXIDANT ACTIVITY OF FERMENTED ALLIGATOR WEED

Parameter	Unfermented	3 days of fermentation	5 days of fermentation
Total phenolic content (mg GAE g^{-1})	8.72 ± 0.34^c	12.85 ± 0.41^b	16.94 ± 0.53^a
DPPH scavenging activity (%)	32.18 ± 1.12^c	48.77 ± 1.45^b	61.39 ± 1.68^a
IC_{50} (mg mL^{-1})	4.87 ± 0.21^a	3.21 ± 0.18^b	2.14 ± 0.15^c

D. Antibacterial Activity

Fermentation significantly enhanced the antibacterial activity of alligator weed extracts against all tested fish pathogenic bacteria (Table IV).

TABLE IV. ANTIBACTERIAL ACTIVITY (INHIBITION ZONE DIAMETER, mm)

Test bacteria	Unfermented	3 days of fermentation	5 days of fermentation
Aeromonas hydrophila	6.12 ± 0.38^c	9.84 ± 0.42^b	13.27 ± 0.51^a
Streptococcus agalactiae	5.48 ± 0.29^c	8.76 ± 0.36^b	12.11 ± 0.47^a
Vibrio parahaemolyticus	6.34 ± 0.33^c	10.21 ± 0.39^b	14.06 ± 0.58^a

E. Apparent Digestibility in Nile Tilapia

The ADCs of dry matter, crude protein, and energy were significantly improved in fish fed diets containing fermented alligator weed (Table V).

TABLE V. ADCS (ADC, %) IN NILE TILAPIA

Parameter	Unfermented	3 days of fermentation	5 days of fermentation
ADC dry matter (%)	58.47 ± 1.42^c	68.92 ± 1.36^b	75.88 ± 1.51^a
ADC crude protein (%)	64.73 ± 1.28^c	76.45 ± 1.34^b	83.67 ± 1.22^a
ADC energy (%)	60.82 ± 1.37^c	71.16 ± 1.44^b	78.94 ± 1.49^a

The highest digestibility values were recorded in the 5-day fermented treatment, indicating improved nutrient availability following fermentation.

IV. DISCUSSION

The functional properties evaluated in this study were limited to biological activities, specifically antioxidant and antibacterial effects, which are relevant to fish health and disease prevention in aquaculture systems. The present study demonstrates that solid-state fermentation of alligator weed with *Rhizopus oligosporus* significantly improves its nutritional quality, functional properties, and digestibility for Nile Tilapia. These improvements underscore the effectiveness of fungal fermentation as a processing strategy for enhancing the nutritional value of plant-based feed ingredients that have inherent limitations.

The increase in crude protein content observed after fermentation can be attributed to the accumulation of microbial biomass and the synthesis of fungal proteins during substrate colonization. Similar protein-enhancing effects have been reported in fermented plant meals and legumes used in aquafeeds, where fermentation increases nitrogen availability and protein digestibility [17]. Additionally, the extracellular proteolytic enzymes produced by *Rhizopus oligosporus* may have contributed to partial protein hydrolysis, thereby improving nutrient accessibility. The significant reduction in crude fiber content following fermentation indicates effective degradation of structural carbohydrates by fungal cellulases and hemicellulases. High dietary fiber levels negatively affect feed intake and nutrient utilization in fish [18]. Therefore, fiber reduction is an important factor contributing to the improved digestibility observed in fish fed fermented alligator weed. Similar findings have been reported in [19], which evaluated fermented chickpea meals and other plant-based ingredients for Nile Tilapia diets [19]. Fermentation has also significantly reduced antinutritional factors such as phytate, tannins, and oxalates. The degradation of phytate is commonly associated with phytase activity during fermentation, which enhances mineral bioavailability and reduces the chelating effects of phytic acid [20]. The reduction of tannins and oxalates is particularly important, as these compounds can inhibit digestive enzymes and impair nutrient absorption [21].

The enhancement of antioxidant activity observed in fermented alligator weed is likely related to the release of bound phenolic compounds and the formation of bioactive metabolites during fermentation. Similar increases in antioxidant capacity have been reported for plant extracts and fermented feed additives used in aquaculture, where improved antioxidant status has been associated with enhanced physiological performance and stress resistance [22]. Increased antioxidant potential in feed ingredients may help mitigate oxidative stress in cultured fish, thereby supporting overall health.

The observed antibacterial activity against fish pathogenic bacteria further supports the functional value of fermented alligator weed. Fermentation-associated microbial metabolites, including organic acids and phenolic derivatives, have been shown to inhibit pathogenic microorganisms. The incorporation of feed ingredients with natural antibacterial properties aligns with current strategies aimed at reducing antibiotic usage and enhancing disease resistance in aquaculture systems [23].

Improvements in the ADCs of dry matter, crude protein, and energy confirm the positive impact of fermentation on nutrient utilization by Nile Tilapia. Enhanced digestibility can be attributed to the combined effects of fiber degradation, reduction of antinutrients, and enzymatic pre-digestion of macromolecules during fermentation. Processing methods and enzymatic activity significantly influence digestibility and intestinal function in Nile Tilapia [24]. Overall, the findings of the current study suggest that fermentation duration plays a significant role in determining the nutritional and functional quality of alligator weed, with longer fermentation periods yielding more pronounced improvements. The results support the feasibility of converting invasive aquatic plants into value-added feed ingredients through fungal fermentation, contributing to sustainable feed development and environmentally responsible aquaculture practices.

V. CONCLUSIONS

The present study demonstrates that solid-state fermentation of alligator weed using *Rhizopus oligosporus* effectively enhances its nutritional quality, functional properties, and digestibility for Nile Tilapia. Fermentation increased crude protein content while reducing crude fiber and key antinutritional factors, thereby improving nutrient availability. In addition, fermented alligator weed exhibited enhanced antioxidant and antibacterial activities, indicating its potential role as a functional feed ingredient. The Apparent Digestibility Coefficients (ADCs) of dry matter, protein, and energy were significantly improved in diets containing fermented material, with the most pronounced effects observed after extended fermentation. These findings suggest that fungal fermentation is a viable strategy for converting invasive aquatic plants into value-added feed resources, thereby supporting the development of sustainable and efficient aquaculture feed systems. The need for future studies on scale-up feasibility, long-term feeding trials, and economic evaluation of fermented alligator weed is evident.

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REFERENCES

[1] M. Gil *et al.*, "Sustainability of Alternatives to Animal Protein Sources, a Comprehensive Review," *Sustainability*, vol. 16, no. 17, 2024, Art. no. 7701, https://doi.org/10.3390/su16177701.

[2] N. Nirmal, C. F. Anyimadu, A. C. Khanashyam, A. E. A. Bekhit, and B. K. Dhar, "Alternative Protein Sources: Addressing Global Food Security and Environmental Sustainability," *Sustainable Development*, vol. 33, no. 3, pp. 3958–3969, 2025, https://doi.org/10.1002/sd.3338.

[3] G. Francis, H. P. S. Makkar, and K. Becker, "Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish," *Aquaculture*, vol. 199, no. 3–4, pp. 197–227, Aug. 2001, https://doi.org/10.1016/S0044-8486(01)00526-9.

[4] F. P. Martínez, L. Bermúdez, M. J. Aznar, and F. J. Moyano, "Evaluation of Enzyme Additives on the Nutritional Use of Feeds with a High Content of Plant Ingredients for Mugil cephalus," *Fishes*, vol. 4, no. 4, Dec. 2019, Art. no. 56, https://doi.org/10.3390/fishes4040056.

[5] R. N. Mandal and P. Bera, "Macrophytes Used as Multifaceted Benefits Including Feeding, Bioremediation, and Symbiosis in Freshwater Aquaculture—A Review," *Reviews in Aquaculture*, vol. 17, no. 1, Jan. 2025, Art. no. e12983, https://doi.org/10.1111/raq.12983.

[6] M. Samtiya, R. E. Aluko, and T. Dhewa, "Plant food anti-nutritional factors and their reduction strategies: an overview," *Food Production, Processing and Nutrition*, vol. 2, no. 1, Mar. 2020, Art. no. 6, https://doi.org/10.1186/s43014-020-0020-5.

[7] S. Dhiman, S. Kaur, B. Thakur, P. Singh, and M. Tripathi, "Nutritional Enhancement of Plant-Based Fermented Foods: Microbial Innovations for a Sustainable Future," *Fermentation*, vol. 11, no. 6, 2025, Art. no. 346, https://doi.org/10.3390/fermentation11060346.

[8] A. Álvarez *et al.*, "Solid-State Fermentation as a Biotechnological Tool to Reduce Antinutrients and Increase Nutritional Content in Legumes and Cereals for Animal Feed," *Fermentation*, vol. 11, no. 7, 2025, Art. no. 359, https://doi.org/10.3390/fermentation11070359.

[9] S. Kamalasundari, "Production, Quality and Acceptance of Tempeh prepared using Dolichos lablab (Indian bean) Legume," *Journal of Current Crop Science & Technology*, vol. 110, no. 1–3, 2023, Art. no. 134, https://doi.org/10.29321/MAJ.10.000718.

[10] Y.-S. Zhao *et al*., "Fermentation Affects the Antioxidant Activity of Plant-Based Food Material through the Release and Production of Bioactive Components," *Antioxidants*, vol. 10, no. 12, Dec. 2021, Art. no. 2004, https://doi.org/10.3390/antiox10122004.

[11] K. M. Eggink, P. B. Pedersen, I. Lund, and J. Dalsgaard, "Chitin digestibility and intestinal exochitinase activity in Nile tilapia and rainbow trout fed different black soldier fly larvae meal size fractions," *Aquaculture Research*, vol. 53, no. 16, pp. 5536–5546, 2022, https://doi.org/10.1111/are.16035.

[12] L. Mejias, D. Ruiz, E. Molina-Peñate, R. Barrena, and T. Gea, "Bacillus thuringiensis derived biopesticides from biowaste digestate at 290-L demonstration scale through solid-state fermentation," *Environmental Technology & Innovation*, vol. 37, Feb. 2025, Art. no. 103974, https://doi.org/10.1016/j.eti.2024.103974.

[13] R. A. Islamy, P. Senas, W. Isroni, N. B. Mamat, and Y. Kilawati, "Sea Moss Flour (E. cottonii) an an Ingredients of Pasta: the Aanalysis of Organoleptic, Proximate and Antioxidant," *Iraqi Journal of Agricultural Sciences*, vol. 55, no. 4, pp. 1521–1533, Aug. 2024, https://doi.org/10.36103/kzmmxc09.

[14] A. H. Al-Bagawi *et al*., "Phytochemical Analysis and Antioxidant Activity Assessment of Methanolic Extract from Jasmine Flowers," *Engineering, Technology & Applied Science Research*, vol. 15, no. 1, pp. 20258–20263, Feb. 2025, https://doi.org/10.48084/etasr.9493.

[15] A. Guetat, A. K. Alzahrani, M. H. Oueslati, A. E. M. Elhaj, J. Bouajila, and I. M. A. Shahhat, "Chemical Composition, Fatty Acids, Total Phenolics and Antioxidant Activity of the Desert Truffle Terfezia Boudieri Chatin in the Northern Region of Saudi Arabia," *Engineering, Technology & Applied Science Research*, vol. 14, no. 4, pp. 15016–15021, Aug. 2024, https://doi.org/10.48084/etasr.7470.

[16] A. Furukawa and H. Tsukahara, "On the Acid Digestion Method for the Determination of Chromic Oxide as an Index Substance in the Study of Digestibility of Fish Feed," *Nippon Suisan Gakkaishi*, vol. 32, no. 6, pp. 502–506, 1966.

[17] B. Çabuk *et al*., "Effect of Fermentation on the Protein Digestibility and Levels of Non-Nutritive Compounds of Pea Protein Concentrate," *Food Technology and Biotechnology*, vol. 56, no. 2, pp. 257–264, June 2018, https://doi.org/10.17113/ftb.56.02.18.5450.

[18] M. S. M. Nafees, M. S. Kamarudin, M. Karim, M. Z. Hassan, and C. R. de Cruz, "Effects of dietary fiber on growth, feed efficiency and nutrient utilization of tinfoil barb (Barbonymus schwanenfeldii, Bleeker 1853) fry," *Aquaculture Reports*, vol. 32, Oct. 2023, Art. no. 101743, https://doi.org/10.1016/j.aqrep.2023.101743.

[19] F. J. Valdez-González, R. Gutiérrez-Dorado, M. García-Ulloa, B. L. Cuevas-Rodríguez, and H. Rodríguez-González, "Effect of fermented, hardened, and dehulled of chickpea (Cicer arietinum) meals in digestibility and antinutrients in diets for tilapia (Oreochromis niloticus)," *Spanish Journal of Agricultural Research*, vol. 16, no. 1, Apr. 2018, Art. no. e0605, https://doi.org/10.5424/sjar/2018161-11830.

[20] M. Prajapati and H. Shah, "Impacts and Industrial Applications of Phytic Acid and Phytase," *Journal of Pure & Applied Microbiology*, vol. 16, no. 4, pp. 2292–2302, 2022, https://doi.org/10.22207/JPAM.16.4.16.

[21] S. A. Okaiyeto *et al*., "Anti-nutrients of plant-based food: physicochemical properties, effects on health and degradation techniques- a comprehensive review," *Journal of Future Foods*, Oct. 2025, https://doi.org/10.1016/j.jfutfo.2025.06.022.

[22] D. Ko, N. Medagoda, K.-S. Yun, and K.-J. Lee, "Effects of dietary supplementation of Achyranthes aspera extract on growth performance, digestibility, innate immunity, antioxidant capacity, and disease resistance of juvenile Pacific white shrimp, Penaeus vannamei," *Journal of the World Aquaculture Society*, vol. 55, no. 2, Apr. 2024, Art. no. e13021, https://doi.org/10.1111/jwas.13021.

[23] E. Ringø, X. Li, H. van Doan, and K. Ghosh, "Interesting Probiotic Bacteria Other Than the More Widely Used Lactic Acid Bacteria and Bacilli in Finfish," *Frontiers in Marine Science*, vol. 9, June 2022, Art. no. 848037, https://doi.org/10.3389/fmars.2022.848037.

[24] W. M. Santos *et al*., "Dietary protein modulates digestive enzyme activities and gene expression in red tilapia juveniles," *animal*, vol. 14, no. 9, pp. 1802–1810, Sept. 2020, https://doi.org/10.1017/S1751731120000543.