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Fermented *Alternanthera philoxeroides* in Tilapia Diets: Enhanced Nutrition, Antioxidant Properties, and Growth Performance

R Adharyan Islamy[1], Diana Aisyah[1], Ayu Winna Ramadhani[1], Naufal Fadhilah[1], Sulung Ilham Maulana Abduh[1], Avifah Trialvina Nur Azizah[1], Nurul Mutmainnah[2], Fitri Sil Valen[3], Ahmad Syazni Kamarudin[4], Veryl Hasan[4,5,6]*

[1] Department of Fisheries and Marine Resources Management, Faculty of Fisheries and Marine Sciences, Aquaculture (Kediri City Kampus), Brawijaya University, Kediri City 64111, Indonesia
[2] Doctoral Program of Agricultural Sciences, Faculty of Agriculture, Brawijaya University, Malang City 65145, Indonesia
[3] Faculty of Agriculture, Fisheries and Biology, UBB Integrated Campus, Bangka Belitung University, Bangka Regency 33172, Indonesia
[4] School of Animal Science, Aquatic Science and Environment, Besut Campus, Universiti Sultan Zainal Abidin, Besut 22200, Malaysia
[5] Department of Aquaculture, Faculty of Fisheries and Marine Science, Airlangga University, Surabaya 60113, Indonesia
[6] Research Group of Environmental and Fisheries Resources Management, Faculty of Fisheries and Marine Science, Airlangga University, Surabaya 60113, Indonesia

Corresponding Author Email: veryl.hasan@fpk.unair.ac.id

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ABSTRACT

This study evaluated the nutritional enhancement of *Alternanthera philoxeroides* meal (AFM) through *Saccharomyces cerevisiae* fermentation and its effects on growth performance, nutrient digestibility, antioxidant capacity, and immune responses of Nile tilapia (*Oreochromis niloticus*). Four iso-nitrogenous and iso-energetic diets containing 0%, 10%, 20%, and 30% fermented AFM were fed to tilapia juveniles for 56 days. Fermentation significantly improved the nutritional quality of AFM by increasing total phenolics and flavonoids, reducing tannins and structural fiber, and enhancing enzymatic activities associated with nutrient breakdown. These changes contributed to stronger antioxidant properties, as indicated by lower DPPH IC_{50} values and higher ABTS radical scavenging activity. Fish fed the 20% AFM diet exhibited the best performance, achieving the highest weight gain (WG, 2.68 ± 0.12 g), specific growth rate (1.81 ± 0.06% day^{-1}), and protein efficiency ratio (PER, 1.72 ± 0.05), along with the lowest feed conversion ratio (FCR, 1.08 ± 0.04). Immune indicators, including lysozyme activity and total immunoglobulin levels, were also significantly enhanced at this inclusion level. However, growth performance and immune responses declined at 30% AFM, likely due to excessive fiber content and reduced digestibility. Overall, *S. cerevisiae* fermentation effectively improved the nutritional and functional properties of *A. philoxeroides*, and a dietary inclusion level of 20% provided the optimal balance between growth performance and physiological health in tilapia. These findings highlight fermented *A. philoxeroides* as a promising functional ingredient, demonstrating its potential as a sustainable alternative to conventional protein sources in aquafeeds.

1. INTRODUCTION

The push for sustainable protein in aquaculture has never been more urgent. As traditional staples like fishmeal and soybean meal face mounting pressure from price volatility and environmental constraints, the industry is forced to look beyond conventional boundaries [1]. Nile tilapia (*Oreochromis niloticus*) is widely farmed in freshwater systems because of its high adaptability, making it suitable for evaluating alternative feed ingredients. Therefore, attention has shifted toward converting underutilized biomass, particularly invasive species, into functional feed sources without reducing fish performance.

A. philoxeroides stands out as a formidable invasive macrophyte, now firmly entrenched across tropical and subtropical freshwater systems. Its success is no accident; aggressive growth and a plastic ecological nature allow it to outcompete native species and complicate water management on a global scale. Recent molecular and transcriptomic insights reveal that this persistence is fueled by sophisticated defense mechanisms and high stress tolerance [2, 3]. However, from a circular economy perspective, these same survival traits suggest a hidden upside: the plant is a natural reservoir of diverse bioactive compounds and structural elements that, if properly harnessed, could be repurposed for higher-value applications rather than simply being managed as a nuisance.

Converting this raw biomass into viable feed is not without its hurdles. The primary bottlenecks remain its high fiber content, complex secondary metabolites, and inherently low digestibility—a common frustration when dealing with aquatic macrophytes [4]. These anti-nutritional factors, often reinforced by rigid cell wall structures, effectively "lock away" nutrients from monogastric animals, much like the constraints seen in various terrestrial plant sources [5, 6]. To unlock this potential, a targeted bioprocessing intervention is essential. By employing microbial fermentation, we can begin to dismantle these complex carbohydrates and neutralize anti-nutritional compounds, ultimately paving the way for a safer, more bioavailable ingredient in animal diets.

Microbial fermentation has recently re-emerged as a powerful toolkit for upgrading the functional profile of plant-based feeds. By leveraging microbial action, we can effectively dismantle complex carbohydrates and neutralize stubborn anti-nutritional factors that otherwise limit protein bioavailability. Beyond simple digestion, this process—as seen in cassava-based substrates—triggers a metabolic shift that enhances the overall safety and efficiency of the feed [7]. This isn't just about nutrient density; it's about biotransforming agricultural by-products into bioactive-rich ingredients with documented antioxidant benefits [8].

Within this fermentative landscape, *Saccharomyces cerevisiae* remains the gold standard. Far from being just a leavening agent, this yeast acts as a biological factory, secreting enzymes and organic acids that stabilize gut health and improve nutrient uptake. Its unique ability to bind phenolic compounds and boost their bioaccessibility during digestion is particularly relevant here [9]. When applied to plant or fruit-derived substrates, *S. cerevisiae* doesn't just process the material—it enriches it with probiotic-like properties [8]. In an aquaculture context, these yeast-driven benefits translate directly to better growth and heightened stress tolerance in fish, making it a logical choice for fortifying *A. philoxeroides* [10].

The use of unconventional biomass sources, including invasive plants, aligns with broader sustainability and circular bioeconomy concepts. Biomass sources have been explored not only for biofuel production but also as a nutrient-rich feedstock due to their high protein content and functional properties [11]. Similarly, freshwater plant biomasses have demonstrated beneficial effects when incorporated into fish diets, improving growth and nutrient utilization [12]. These examples highlight the feasibility of transforming low-value or waste biomass into high-value feed ingredients through appropriate processing technologies.

Incorporating fermented plant-based ingredients into aquafeeds also supports the development of functional diets aimed at improving fish health and resilience. Feed additives derived from fermentation can interact with the gut microbiota and immune system, contributing to improved physiological stability and disease resistance [10]. The increasing use of probiotics and fermented feed components in aquaculture reflects a shift toward preventive health management rather than reliance on chemotherapeutic interventions [13].

Despite the ecological abundance of *A. philoxeroides* and the well-documented benefits of yeast fermentation, we still lack a clear blueprint for its inclusion in tilapia diets. This study fills that gap. By evaluating how *S. cerevisiae* fermentation alters the nutritional profile of this invasive macrophyte, we aim to establish not just its feasibility, but its optimal inclusion level. Our focus goes beyond simple growth metrics; we delve into antioxidant capacity and health-related responses to ensure that *A. philoxeroides* serves as a truly functional and sustainable ingredient for modern aquaculture.

2. MATERIALS AND METHODS

2.1 Experimental design

We conducted the feeding trial over a 56-day period to ensure sufficient time for the nutritional effects of AFM to manifest in the growth and physiological responses of *O. niloticus*. This duration was selected based on standard protocols for tilapia nutritional studies. This study investigated the effects of dietary inclusion of *A. philoxeroides* meal (AFM) fermented with *S. cerevisiae* on growth performance, nutrient utilization, and antioxidant response in Nile tilapia (*O. niloticus*). Four experimental diets were formulated to contain 0%, 10%, 20%, and 30% fermented AFM, designated as Control, AFM-10, AFM-20, and AFM-30, respectively. All diets were formulated to be iso-nitrogenous (30% crude protein) and iso-energetic (3.7 kcal g^{-1}).

2.2 Preparation of fermented *A. philoxeroides* meal

Fresh alligator weed (*A. philoxeroides*) biomass was collected from aquatic habitats around Malang, Indonesia. The plant material was washed, chopped into 2–3 cm fragments, and oven-dried at 60 °C until constant weight. The dried material was ground (< 1 mm) and subjected to fermentation using *S. cerevisiae* (commercial baker's yeast). For the microbial transformation, we used high-density polyethylene (HDPE) containers with a 20-liter capacity (dimensions: 30 cm diameter, 45 cm height). Before starting the process, each container underwent a strict disinfection protocol using a 10% sodium hypochlorite solution, followed by multiple rinses with sterile distilled water to prevent any unwanted microbial interference during the *S. cerevisiae* fermentation.

Fermentation was conducted by mixing 1 kg of plant meal with 60% distilled water (w/w) relative to the dry substrate weight and inoculating with *S. cerevisiae* at a concentration of 10^7 CFU g^{-1}. The mixture was thoroughly homogenized and placed in sterile polypropylene fermentation containers that were tightly sealed to create a microanaerobic environment resulting from static culture conditions rather than strict anaerobic gas purging. Fermentation was carried out at 30 °C for 72 h, a duration selected based on preliminary trials and previous fermentation studies indicating that this period allows sufficient microbial growth and enzymatic degradation of structural carbohydrates while preventing nutrient losses from prolonged fermentation. No mechanical stirring was applied during incubation. The fermented product was subsequently oven-dried at 50 °C, pulverized, and stored at 4 °C until use.

2.3 Experimental diets

The ingredient composition and proximate analysis of the diets are presented in Table 1. Formulation included fishmeal, soybean meal, maize, and wheat middlings as major protein and energy sources. The fermented AFM replaced part of the soybean and maize fractions at inclusion levels of 10%, 20%, and 30%. Vitamin–mineral premix, fish and soy oils, and dicalcium phosphate were added to meet the nutritional requirements of herbivorous fish. All dry ingredients were homogenized, mixed with oil and water to form a consistent dough, and pelleted using a 2 mm die. Pellets were air-dried at room temperature and stored at 4 °C.

Table 1. Composition of experimental diets (g·kg^{-1}, as-fed basis) containing graded levels of fermented *Alternanthera philoxeroides* meal (AFM)

Ingredient (g·kg^{-1})	Control	AFM-10	AFM-20	AFM-30
Fishmeal	250	250	250	250
Soybean meal	200	190	180	170
Maize/energy	200	190	180	170
A. philoxeroides meal (AFM) — fermented	0	100	200	300
Wheat middling/binder	80	80	80	80
Fish oil	30	30	30	30
Soy oil	20	20	20	20
Vitamin-mineral premix	20	20	20	20
Dicalcium phosphate	10	10	10	10
Limestone	5	5	5	5
Total (g·kg^{-1})	1000	1000	1000	1000

Note: AFM = *Alternanthera philoxeroides* meal fermented with *Saccharomyces cerevisiae.* Ingredient proportions were adjusted to achieve comparable dietary protein and energy levels among treatments.

2.4 Fish and rearing conditions

Juvenile *O. niloticus* was obtained from a local hatchery in Kediri, East Java. Prior to the feeding trial, fish were acclimated to laboratory conditions for 14 days and fed a commercial diet. Fish were stocked in 80 L fiberglass tanks at a density of 15 fish per tank (three replicates per treatment) under a continuous aeration system. Each species was reared in separate systems but received identical dietary treatments. Water temperature (27–29 °C), pH (7.0–7.5), and dissolved oxygen (> 5 mg L^{-1}) were maintained throughout the 56-day feeding period. Approximately one-third of the water was renewed daily to maintain water quality.

2.5 Feeding regime

Fish were fed twice daily (08:00 and 16:00 h) to apparent satiation. Feed intake was recorded, and uneaten feed was siphoned, dried, and weighed to calculate the actual feed consumption. Growth and feed utilization were evaluated at the end of the 56-day trial.

2.6 Chemical analyses

Proximate composition of diets and ingredients was analyzed according to published research by Pratama et al. [14] and Islamy et al. [15]. Moisture was determined by oven drying (105 °C), crude protein by the Kjeldahl method (N × 6.25), lipid by Soxhlet extraction, ash by incineration (550 °C), and crude fiber by acid–alkali digestion. Nitrogen-free extract (NFE) and gross energy were calculated by difference. Phytochemical contents were assessed for total phenolics (Folin–Ciocalteu method, expressed as mg gallic acid equivalents g^{-1} DM), flavonoids (aluminum chloride colorimetric method, expressed as mg quercetin equivalents g^{-1} DM), and tannins (vanillin–HCl method). Antioxidant activity was evaluated by DPPH and ABTS radical scavenging assays [16], with results expressed as IC$_{50}$ (µg mL^{-1}) and µmol trolox equivalents g^{-1} DM, respectively.

2.7 Fermentation enzyme activity

Protease and cellulase activities were quantified following standard spectrophotometric protocols using casein and CMC substrates, respectively. Results were expressed in units per gram (U g^{-1}) of sample. Viable yeast counts were determined using yeast extract–peptone–dextrose agar (YPD) and expressed as log$_{10}$ CFU g^{-1}.

2.8 Amino acid and fatty acid profiles

Amino acids were analyzed using an automatic amino acid analyzer (HPLC, post-column derivatization with ninhydrin), while fatty acid methyl esters (FAMEs) were quantified using gas chromatography (GC-FID). Results were expressed as g·100 g^{-1} DM protein (amino acids) and g·100 g^{-1} DM total fatty acids (FAs).

2.9 Apparent digestibility and nutrient availability

Apparent digestibility coefficients (ADC) of protein, lipid, and energy were determined using 0.5% chromic oxide as an inert marker. Prior to fecal collection, fish were fed the experimental diets containing the marker for seven days to allow acclimation and stabilization of marker excretion. To assess digestibility, we collected fecal samples three times daily (at 08:00, 13:00, and 17:00 h), starting from the second week of the trial. For each replicate, we pooled approximately 2–3 grams (dry weight) of feces to ensure a representative sample for analysis. The chromium content in both diets and feces was determined using Acid Digestion followed by Flame Atomic Absorption Spectrometry (AAS), following the Integrated Research Laboratory Universitas Brawijaya standardized protocol.

Feces were collected 6 h post-feeding using a modified stripping method. Fish were first gently anesthetized with tricaine methanesulfonate (MS-222) to minimize handling stress. The abdominal region was then carefully massaged from the anterior to posterior direction to expel fecal material from the intestine. Care was taken to avoid contamination with urine or intestinal mucus. Collected feces from each replicate were pooled, freeze-dried, and stored at −20 °C until analysis of nutrient composition and chromium content. ADCs were calculated based on nutrient-to-marker ratios between feed and feces.

2.10 Growth performance and feed utilization

Growth performance and feed utilization were evaluated using standard indices to assess the response of fish to the experimental diets. Weight gain (WG) was calculated as the difference between the final and initial body weight. The specific growth rate (SGR, % day^{-1}) was determined based on the natural logarithmic change in body weight over the experimental period, expressed as a percentage per day. Feed utilization efficiency was assessed through the feed conversion ratio (FCR), calculated as the ratio of total feed intake to WG, and the protein efficiency ratio (PER), defined as the ratio of WG to protein intake. Survival rate (%) was calculated as the proportion of fish remaining at the end of the experiment relative to the initial stocking number. These indices collectively provide a comprehensive evaluation of growth performance, feed efficiency, and overall fish viability during the feeding trial.

2.11 Immunological and biochemical assays

The non-specific immune response was evaluated through respiratory burst activity and acid phosphatase (ACP) levels. We determined the respiratory burst activity of leucocytes using the Nitroblue Tetrazolium (NBT) reduction assay, following the spectrophotometric method. Briefly, the optical density of the formazan produced by stimulated cells was measured at 540 nm. For the humoral immune response, ACP activity in the serum was quantified using a commercial diagnostic kit (Sigma-Aldrich) based on the enzymatic hydrolysis of p-nitrophenyl phosphate. All physiological measurements were performed in triplicate to ensure analytical precision, with absorbance values recorded using a microplate reader (Thermo Scientific, USA).

2.12 Statistical analysis

All data were expressed as mean ± standard deviation (SD). Prior to statistical analysis, data normality and homogeneity of variance were verified using the Shapiro–Wilk test and Levene's test, respectively. When these assumptions were satisfied, differences among dietary treatments were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test. Statistical significance was accepted at $p < 0.05$. All statistical analyses were performed using SPSS v.26 (IBM, USA).

3. RESULTS

3.1 Diet composition and nutritional characteristics

The four experimental diets were formulated with graded inclusion levels of fermented *A. philoxeroides* meal (AFM) at 0%, 10%, 20%, and 30% (Table 1 and Figure 1), designated as Control, AFM-10, AFM-20, and AFM-30, respectively. The formulation strategy involved the progressive replacement of soybean meal and maize with AFM, while maintaining similar proportions of fishmeal and lipid sources (Table 1). As a result, all diets were designed to provide comparable protein (approximately 30% crude protein) and energy levels (approximately 3.7 kcal·g^{-1} DM).

Crude protein and lipid showed a slight decreasing trend with increasing AFM inclusion, while ash and crude fiber increased progressively (Table 2 and Figure 2). Specifically, crude protein decreased from 30.5 to 29.0 g·100 g^{-1} DM, and crude lipid from 8.0 to 7.2 g·100 g^{-1} DM between the control and AFM-30 diets. In contrast, ash content increased from 7.0 to 8.2 g·100 g^{-1} DM, and crude fiber from 3.5 to 5.6 g·100 g^{-1} DM. Nitrogen-free extract (NFE) and gross energy showed minor reductions across treatments (Table 2).

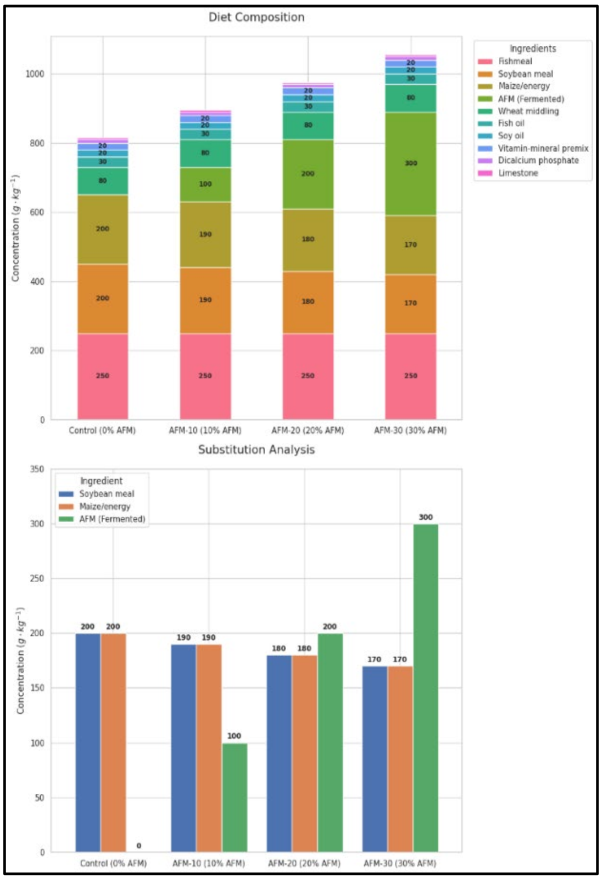

Figure 1. Experimental diet formulations showing the graded replacement of soybean meal and maize with fermented *A. philoxeroides* meal (AFM)

Table 2. Analyzed proximate composition of experimental diets expressed on a dry matter (DM) basis, with dry matter reported on an as-fed basis

Parameter	Control	AFM-10	AFM-20	AFM-30
Dry matter (g·100 g^{-1})	90.0	90.2	90.1	90.3
Crude protein (g·100 g^{-1})	30.5	30.2	29.8	29.0
Crude lipid (g·100 g^{-1})	8.0	7.8	7.5	7.2
Ash (g·100 g^{-1})	7.0	7.5	7.8	8.2
Crude fiber (g·100 g^{-1})	3.5	4.0	4.8	5.6
NFE (g·100 g^{-1})	51.0	50.5	50.1	49.0
Gross energy (kcal·100 g^{-1} DM)	380	375	370	365

Note: NFE (nitrogen-free extract) was calculated by difference [100 − (crude protein + crude lipid + ash + crude fiber)]. Gross energy was estimated based on standard conversion factors (or specify method if measured).

The magnitude of these compositional changes was relatively small, indicating that the diets remained broadly comparable despite the increasing inclusion of AFM. However, the progressive increase in ash and fiber fractions with higher AFM levels is evident from both Table 2 and Figure 2.

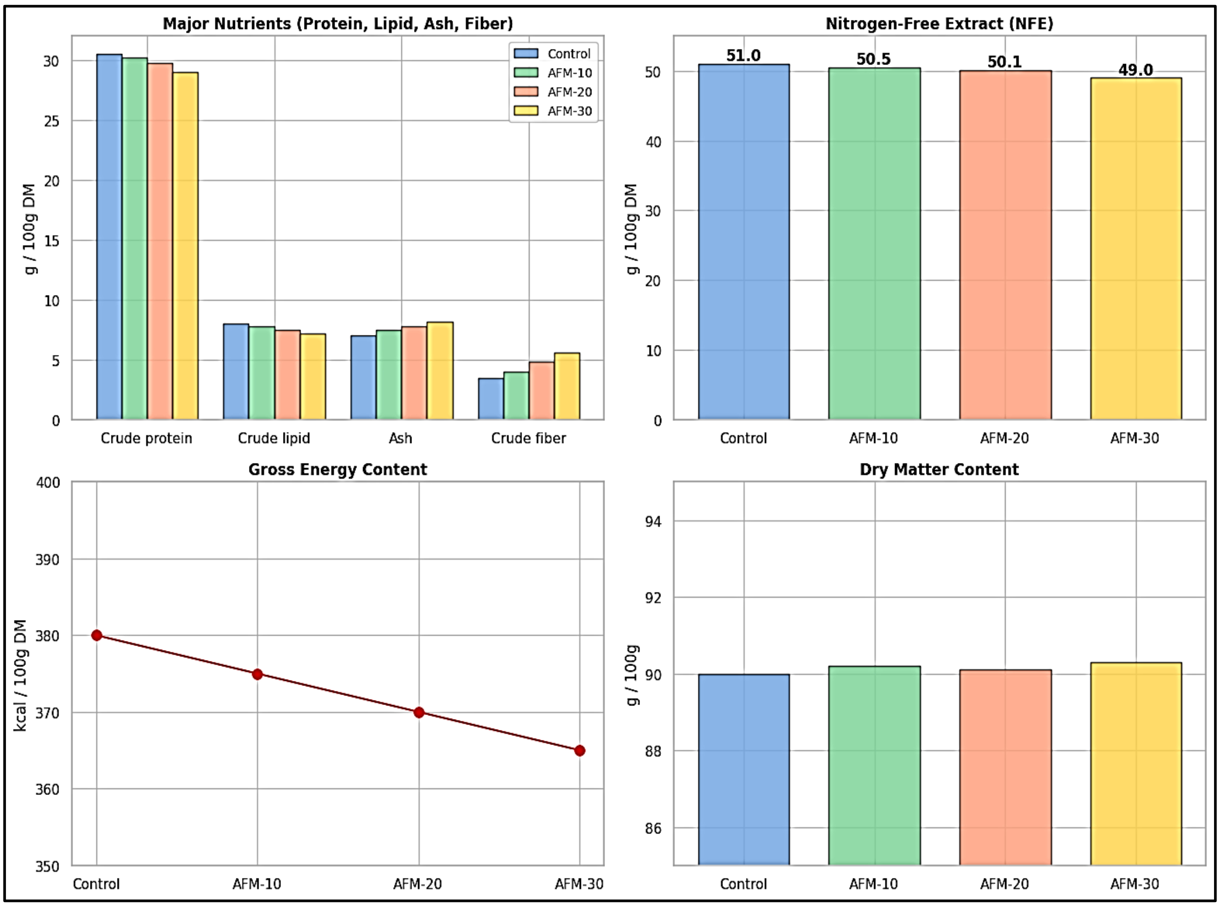

Figure 2. Nutritional profile of experimental diets (AFM inclusion)
Note: AFM = *Alternanthera philoxeroides* meal fermented with *Saccharomyces cerevisiae.*

3.2 Phytochemical composition and antioxidant activity

Fermentation markedly modified the phytochemical composition and antioxidant capacity of *A. philoxeroides* (Table 3 and Figure 3). The total phenolic content increased from 18.5 mg GAE·g^{-1} DM in the raw material to 22.0 mg GAE·g^{-1} DM after fermentation, representing an approximate 19% increase. A similar trend was observed for total flavonoids, which rose from 3.2 to 4.0 mg QE·g^{-1} DM. In contrast, tannin content declined from 2.1 to 1.4 mg·g^{-1} DM, indicating a reduction of polyphenolic compounds commonly associated with anti-nutritional effects. Changes in antioxidant activity were consistent with these compositional shifts. The DPPH IC$_{50}$ value decreased from 150 to 120 µg·mL^{-1}, reflecting stronger radical scavenging activity, while ABTS values increased from 85 to 110 µmol TE·g^{-1} DM.

The incorporation of fermented *A. philoxeroides* meal (AFM) into experimental diets resulted in measurable transfer of phytochemical constituents to the feed matrix. The control diet contained relatively low levels of total phenolics (2.0 mg GAE·g^{-1} DM) and flavonoids (0.4 mg QE·g^{-1} DM), whereas the AFM-20 diet showed higher concentrations (5.5 and 0.9 mg GAE·g^{-1} DM, respectively). A similar pattern was observed for tannins, which increased from 0.1 mg·g^{-1} DM in the control to 0.3 mg·g^{-1} DM in AFM-20, although absolute levels remained low compared to the raw plant material. Antioxidant activity of the diets followed the same direction. The DPPH IC$_{50}$ value decreased substantially from 900 µg·mL^{-1} in the control diet to 420 µg·mL^{-1} in AFM-20, indicating improved radical scavenging capacity, while ABTS activity increased from 8 to 22 µmol TE·g^{-1} DM.

Table 3. Phytochemical content & antioxidant activity (raw *A. philoxeroides*, fermented AFM, and diets)

Sample	Total Phenolics (mg GAE·g^{-1} DM)	Total Flavonoids (mg QE·g^{-1} DM)	Tannins (mg·g^{-1} DM)	DPPH IC$_{50}$ (µg·mL^{-1})	ABTS (µmol TE·g^{-1} DM)
Raw *A. philoxeroides*	18.5	3.2	2.1	150	85
AFM (fermented)	22.0	4.0	1.4	120	110
Control diet	2.0	0.4	0.1	900	8
AFM-20 diet	5.5	0.9	0.3	420	22

Notes: AFM = *Alternanthera philoxeroides* meal fermented with *Saccharomyces cerevisiae*. GAE = gallic acid equivalents; QE = quercetin equivalents; TE = trolox equivalents. Fermentation commonly increases extractable phenolics and antioxidant activity.

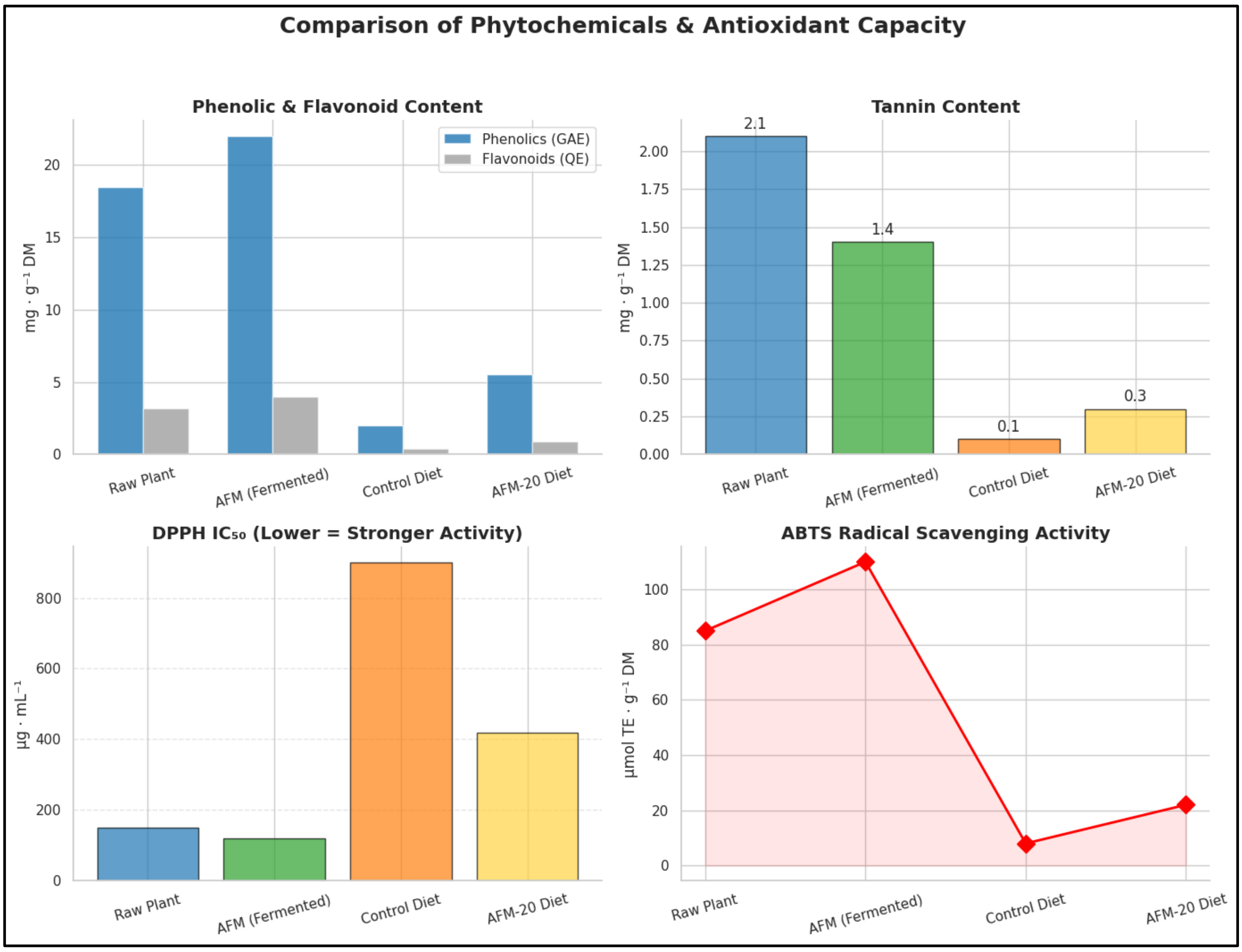

Figure 3. Transformation of phytochemicals and antioxidants via fermentation

3.3 Fermentation Indicators and Enzyme Activity

Fermentation was accompanied by a reduction in pH (6.8 → 5.2) and a modest decline in reducing sugars (12 → 8 mg g^{-1}), reflecting active microbial metabolism. Yeast viability reached 7.8 $\log_{10}$ CFU g^{-1}, while protease and cellulase activities increased more than sevenfold (0.2 → 1.5 U g^{-1} and 0.5 → 2.0 U g^{-1}, respectively), confirming enzymatic enhancement that likely improved digestibility and nutrient availability (Table 4).

Table 4. Fermentation characteristics and enzymatic activity of *Alternanthera philoxeroides* meal before and after fermentation

Parameter	Raw AF	AFM
pH	6.8	5.2
Moisture (%)	10	9
Viable yeast ($CFU\cdot g^{-1}$)	—	7.8
Protease activity ($U\cdot g^{-1}$)	0.2	1.5
Cellulase activity ($U\cdot g^{-1}$)	0.5	2.0
Reducing sugar ($mg\cdot g^{-1}$)	12	8

Note: AF = *Alternanthera philoxeroides* meal (unfermented); AFM = meal fermented with *Saccharomyces cerevisiae*. Viable yeast counts are expressed as $\log_{10}$ $CFU\cdot g^{-1}$. "—" indicates not detected.

3.4 Amino and fatty acid profiles

Fermented AFM contained slightly higher levels of essential amino acids such as lysine (3.8 $g\cdot 100\ g^{-1}$ DM protein), methionine (1.1 $g\cdot 100\ g^{-1}$ DM protein), and leucine (5.9 $g\cdot 100\ g^{-1}$ DM protein) compared with the raw meal (Table 5). The amino acid profiles of the Control and AFM-20 diets were highly comparable, indicating that the inclusion of fermented AFM at 20% did not substantially alter the overall amino acid balance of the formulated diets, despite the slight improvement observed in the amino acid composition of the fermented ingredient itself. The fatty acid profile showed modest improvements in unsaturated fatty acids, particularly α-linolenic acid (C18:3 n-3 = 10.5 $g\cdot 100\ g^{-1}$ DM FA), EPA (0.6 $g\cdot 100\ g^{-1}$ DM FA), and DHA (0.4 $g\cdot 100\ g^{-1}$ DM FA) (Table 6), supporting better nutritional quality for aquafeeds.

Table 5. Amino acid composition of raw and fermented *Alternanthera philoxeroides* meal and experimental diets ($g\cdot 100\ g^{-1}$ protein)

Amino Acid	Raw AF	AFM	Control Diet	AFM-20 Diet
Aspartic acid	6.0	6.4	5.8	6.0
Glutamic acid	9.5	10.2	8.8	9.2
Serine	3.0	3.2	2.9	3.0
Glycine	3.2	3.4	2.8	3.0
Threonine	2.8	2.9	2.6	2.7
Arginine	4.0	4.2	3.8	3.9
Alanine	4.2	4.5	4.0	4.1
Valine	3.4	3.6	3.2	3.3
Isoleucine	3.0	3.2	2.8	2.9
Leucine	5.6	5.9	5.4	5.5
Phenylalanine	3.0	3.1	2.8	2.9
Lysine	3.6	3.8	3.4	3.5
Histidine	1.6	1.7	1.5	1.6
Methionine	1.0	1.1	0.9	0.95

Note: AF = *Alternanthera philoxeroides* meal (unfermented); AFM = meal fermented with *Saccharomyces cerevisiae*. Values are expressed as g amino acid per 100 g crude protein.

Table 6. Fatty acid composition of raw and fermented *Alternanthera philoxeroides* meal and experimental diets ($g\cdot 100\ g^{-1}$ total fatty acids)

Fatty Acid	Raw AF	AFM	Control Diet	AFM-20 Diet
C16:0 (palmitic)	20.0	19.0	18.5	18.8
C18:0 (stearic)	3.5	3.2	3.0	3.1
C18:1 n-9 (oleic)	18.0	17.5	18.2	18.0
C18:2 n-6 (linoleic)	22.0	21.0	20.5	20.8
C18:3 n-3 (α-linolenic)	10.0	10.5	9.2	9.8
EPA (20:5 n-3)	0.5	0.6	1.5	1.4
DHA (22:6 n-3)	0.3	0.4	1.8	1.6
ΣSFA	25.0	24.0	24.0	24.1
ΣMUFA	20.5	20.0	20.5	20.4
ΣPUFA	34.5	34.0	31.0	31.8

Note: AF = *Alternanthera philoxeroides* meal (unfermented); AFM = meal fermented with *Saccharomyces cerevisiae*. Values are expressed as a percentage of total identified fatty acids. ΣSFA, ΣMUFA, and ΣPUFA denote the sum of saturated, monounsaturated, and polyunsaturated fatty acids, respectively. Totals may not equal 100% due to the presence of minor fatty acids not reported.

3.5 Apparent digestibility

ADC for protein, lipid, and energy declined slightly at higher AFM inclusion levels (Table 7). The control diet yielded ADC Protein = 85.0%, ADC Lipid = 92.0%, and ADC Energy = 88.5%, whereas AFM-30 recorded 75.0%, 85.5%, and 80.0%, respectively. Nevertheless, digestibility values in AFM-20 remained within acceptable limits for herbivorous fish species.

Table 7. Apparent digestibility coefficients (ADC, %) of protein, lipid, and energy in experimental diets

Diet	ADC Protein (%)	ADC Lipid (%)	ADC Energy (%)
Control	85.0	92.0	88.5
AFM-10	83.5	90.5	87.0
AFM-20	80.0	88.0	84.0
AFM-30	75.0	85.5	80.0

Note: ADC = apparent digestibility coefficient, calculated using an inert marker based on the ratio of marker and nutrient concentrations in diet and feces. Values are expressed as percentages.

A gradual decline in nutrient digestibility was observed with increasing AFM inclusion (Table 7). The control diet exhibited the highest ADC values for protein (85.0%), lipid (92.0%), and energy (88.5%). In contrast, the AFM-30 diet showed the lowest digestibility, with protein, lipid, and energy ADC values of 75.0%, 85.5%, and 80.0%, respectively. Intermediate values were recorded for AFM-10 and AFM-20, indicating a consistent downward trend across all measured parameters. The reduction in digestibility was more pronounced for protein and energy than for lipid. This pattern suggests that increasing levels of AFM may influence nutrient availability in the diets, although digestibility remained within a generally acceptable range across treatments.

3.6 Growth performance and feed utilization

The inclusion of fermented *A. philoxeroides* meal (AFM) influenced growth performance and feed utilization of *O. niloticus* over the 56-day feeding period (Table 8 and Figure 4). Final weight, weight gain (WG), and specific growth rate (SGR) increased from the control to AFM-20, followed by a decline at AFM-30. The highest WG (2.68 ± 0.12 g) and SGR (1.81 ± 0.06%$\cdot$day^{-1}) were observed in the AFM-20 group, which were significantly higher than those of the control and AFM-30 treatments ($p < 0.05$). Feed utilization parameters showed a similar pattern, with the lowest FCR (1.08 ± 0.04) and highest PER (1.72 ± 0.05) recorded in AFM-20. In contrast, AFM-30 resulted in reduced growth performance, with lower WG (1.99 ± 0.10 g) and higher FCR (1.45 ± 0.07). Feed intake remained relatively similar across treatments. Survival rates were high in all groups (95.0–98.0%) and were not significantly affected by dietary treatment ($p > 0.05$).

Table 8. Growth performance of *O. niloticus* fed diets containing fermented *A. philoxeroides* meal (AFM) for 56 days

Treatment	Control (0% AFM)	AFM-10	AFM-20	AFM-30
Initial weight (g)	1.20 ± 0.03	1.18 ± 0.02	1.22 ± 0.03	1.21 ± 0.04
Final weight (g)	3.45 ± 0.10	3.60 ± 0.12	3.90 ± 0.14	3.20 ± 0.15
Weight gain (g)	2.25 ± 0.09	2.42 ± 0.11	2.68 ± 0.12	1.99 ± 0.10
% Weight gain	187.5 ± 6.8	205.1 ± 7.2	219.7 ± 8.5	164.5 ± 6.3
SGR (%$\cdot$day^{-1})	1.67 ± 0.04	1.75 ± 0.05	1.81 ± 0.06	1.52 ± 0.05
Feed given (g$\cdot$fish^{-1})	10.0 ± 0.4	9.8 ± 0.3	9.6 ± 0.3	9.7 ± 0.4
Feed intake (g$\cdot$fish^{-1})	0.50 ± 0.02	0.49 ± 0.01	0.48 ± 0.02	0.49 ± 0.02
FCR	1.23 ± 0.06	1.16 ± 0.05	1.08 ± 0.04	1.45 ± 0.07
PER	1.58 ± 0.07	1.65 ± 0.06	1.72 ± 0.05	1.38 ± 0.06
Survival (%)	96.0 ± 1.0	97.0 ± 1.5	98.0 ± 1.0	95.0 ± 1.8

Notes: Values are means ± standard deviation (n = 3). SGR = Specific Growth Rate; FCR = Feed Conversion Ratio; PER = Protein Efficiency Ratio. Diets: Control (no AFM), AFM-10 (10% inclusion), AFM-20 (20% inclusion), AFM-30 (30% inclusion). Fish were fed twice daily to apparent satiation for 56 days.

3.7 Immunological indices

Feeding *O. niloticus* with diets containing fermented *A. philoxeroides* meal (AFM) was associated with changes in hematological and immune-related parameters (Table 9). Fish fed the AFM-20 diet showed higher lysozyme activity (16.2 ± 1.3 U$\cdot$mL^{-1}) compared with the control (12.5 ± 1.2 U$\cdot$mL^{-1}). Total immunoglobulin and serum total protein followed a similar pattern, increasing from 1.50 ± 0.12 to 1.92 ± 0.17 mg$\cdot$mL^{-1} and from 5.20 ± 0.30 to 5.80 ± 0.28 g$\cdot$dL^{-1}, respectively. Respiratory burst activity (NBT reduction) was also higher in AFM-20 (1.12 ± 0.08 OD_{660}) than in the control (0.85 ± 0.07 OD_{660}). In addition, ACP activity increased from 18.5 ± 1.6 to 23.0 ± 2.0 U$\cdot$mg protein^{-1}.

At the highest inclusion level (AFM-30), several parameters showed lower values compared with AFM-20, while ALT and AST were higher (40 ± 6 and 55 ± 7 U$\cdot$L^{-1}, respectively) than in the control and intermediate treatments. Survival was not affected (if applicable, add). Overall, the data indicate that responses peaked at the AFM-20 level, with reduced values observed at 30% inclusion.

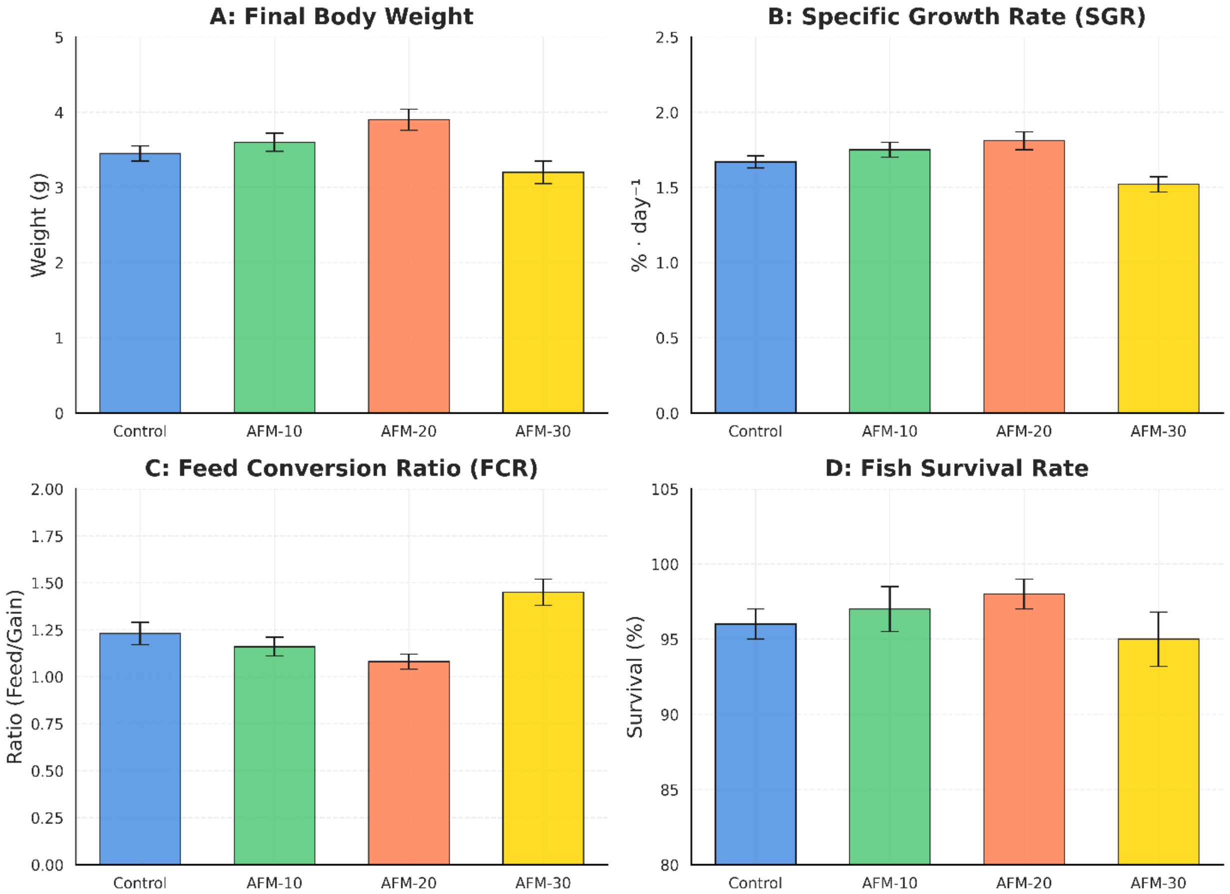

Figure 4. Growth performance of *O. niloticus* fermented AFM (56 days)
AFM = *Alternanthera philoxeroides* meal fermented with *Saccharomyces cerevisiae*.

Table 9. Hematological and immune-related biomarkers of *O. niloticus* after 56 days of feeding diets containing fermented *A. philoxeroides* meal (AFM)

Biomarker	Unit	Control (AFM-0)	AFM-10	AFM-20	AFM-30
Lysozyme activity	$U \cdot mL^{-1}$	12.5 ± 1.2	13.8 ± 1.0	16.2 ± 1.3	13.0 ± 1.1
Total immunoglobulin (Total Ig)	$mg \cdot mL^{-1}$	1.50 ± 0.12	1.65 ± 0.15	1.92 ± 0.17	1.60 ± 0.14
Serum total protein	$g \cdot dL^{-1}$	5.20 ± 0.30	5.45 ± 0.25	5.80 ± 0.28	5.10 ± 0.32
Respiratory burst (NBT reduction)	OD_{660} ($\times 10^{-2}$)	0.85 ± 0.07	0.95 ± 0.06	1.12 ± 0.08	0.88 ± 0.07
ACP (acid phosphatase)	$U \cdot mg\ protein^{-1}$	18.5 ± 1.6	20.2 ± 1.8	23.0 ± 2.0	19.0 ± 1.7
ALT (alanine aminotransferase)	$U \cdot L^{-1}$	35 ± 5	33 ± 4	30 ± 4	40 ± 6
AST (aspartate aminotransferase)	$U \cdot L^{-1}$	48 ± 6	46 ± 5	44 ± 5	55 ± 7

Note: Values are presented as mean ± standard deviation (n = 6). AFM = *Alternanthera philoxeroides* meal fermented with *Saccharomyces cerevisiae*. NBT = nitroblue tetrazolium assay; ACP = acid phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase.

4. DISCUSSION

Our results confirm that *Saccharomyces cerevisiae* fermentation does more than just 'clean up' the nutritional profile of *A. philoxeroides*—it fundamentally re-engineers its functional properties. We found that *O. niloticus* responded most favorably to a 20% inclusion level (AFM-20), where growth performance and immune markers reached their peak. This suggests a delicate balance: while fermentation unlocks the plant's potential, there is a clear physiological 'sweet spot' at 20% before the benefits begin to plateau or reverse.

This nutritional upgrade is largely driven by a significant shift in phytochemicals. By reducing tannin levels and boosting both phenolic and flavonoid concentrations (Table 3), the fermentation process likely dismantled the rigid plant cell walls that typically trap these compounds. This mirrors previous findings where yeast-mediated breakdown of biomass released bound phenolics, effectively turning 'waste' into an antioxidant powerhouse [17, 18]. The enhanced antioxidant activity observed in fermented AFM, reflected by lower DPPH IC_{50} values and higher ABTS activity, supports the role of fermentation in improving the functional value of plant-based feed ingredients. Antioxidant-rich diets are known to protect fish tissues from oxidative stress and contribute to improved health and performance [19, 20].

The increase in protease and cellulase activities after fermentation (Table 4) suggests that yeast metabolism played a key role in improving nutrient accessibility. Enzymatic degradation of complex carbohydrates and fiber is particularly important when using aquatic macrophytes, which are typically rich in structural polysaccharides that limit digestibility. Comparable increases in digestive enzyme activity and nutrient availability have been observed in fish fed diets supplemented with yeast or fungal-fermented ingredients

[21, 22]. Although crude protein and lipid levels in the diets slightly declined with increasing AFM inclusion (Table 2), the amino acid profile of fermented AFM showed modest improvements in essential amino acids such as lysine and methionine (Table 5), which are critical for protein synthesis and growth in tilapia.

ADC of protein, lipid, and energy decreased gradually as AFM inclusion increased (Table 7), particularly at the 30% level. This reduction is likely associated with increased dietary fiber and residual plant structural components that limit nutrient absorption. However, digestibility values at the 20% inclusion level remained within acceptable ranges for herbivorous fish species, indicating that fermentation partially alleviated the negative effects of fiber. Similar trends have been reported in tilapia and other freshwater fish fed raw or processed plant-based diets, where moderate inclusion improved utilization, while excessive levels reduced digestibility and growth [18, 23].

Growth performance data further confirmed the suitability of fermented AFM at moderate inclusion levels. Fish fed the AFM-20 diet achieved the highest WG, specific growth rate, and PER, along with the lowest FCR (Table 8). These improvements suggest enhanced nutrient assimilation and metabolic efficiency, likely driven by improved digestibility and the presence of yeast-derived functional compounds. Previous studies have shown that dietary *S. cerevisiae* and its fermented extracts can enhance growth performance, stress tolerance, and survival in tilapia under both laboratory and field conditions [9, 20]. In contrast, the decline in growth performance at the 30% inclusion level may reflect excessive fiber intake and reduced nutrient availability, which can impair feed efficiency.

Dietary inclusion of fermented AFM also significantly influenced immune responses in *O. niloticus*, with the strongest effects observed at the 20% inclusion level. Increased lysozyme activity, total immunoglobulin levels, serum protein concentration, respiratory burst activity, and acid phosphatase activity (Table 9) indicate stimulation of both humoral and cellular immune mechanisms. These responses are consistent with previous findings showing that yeast-based additives and β-glucan-rich ingredients enhance innate immunity and disease resistance in fish [24, 25]. Yeast-derived components are known to activate macrophages and neutrophils, resulting in increased phagocytic activity and oxidative burst responses [21, 26].

In addition to the improved antioxidant profile of fermented AFM, the immunostimulatory effects observed in this study may also be associated with bioactive components derived from *S. cerevisiae* during fermentation. Yeast cell wall components such as β-glucans, mannan-oligosaccharides (MOS), and chitin-derived polysaccharides are well known for their immunomodulatory properties in aquatic animals. These compounds can interact with pattern recognition receptors on immune cells, stimulating macrophage activation, phagocytosis, and respiratory burst activity. Furthermore, metabolites produced during yeast fermentation, including peptides, organic acids, and vitamins of the B-complex group, may enhance gut health and indirectly improve immune competence. The combined effects of these yeast-derived bioactive compounds and the antioxidant-rich phytochemicals of *A. philoxeroides* likely contributed to the enhanced humoral and cellular immune responses observed in fish fed the AFM-20 diet.

The improved immune status may also be linked to the enhanced antioxidant properties of fermented AFM. Phenolic compounds can modulate immune responses by reducing oxidative damage and maintaining immune cell integrity. Similar synergistic effects between antioxidants and immune stimulation have been reported in tilapia fed fermented herbal extracts and yeast-supplemented diets [17, 20]. The increase in serum total protein and immunoglobulin levels further suggests improved protein metabolism and antibody production, which are important indicators of overall health status.

At the highest inclusion level (30%), a slight reduction in immune parameters and elevated hepatic enzymes (ALT and AST) were observed, indicating possible mild hepatic stress. Excessive inclusion of fermented plant materials may impose metabolic burdens due to higher fiber content or residual secondary metabolites. Similar hepatic responses have been reported in tilapia exposed to dietary stressors or detoxification challenges, where liver enzyme activity increased as a physiological response [27]. This highlights the importance of optimizing inclusion levels even when using fermented and biologically improved ingredients.

Overall, the present findings indicate that fermentation of *A. philoxeroides* with *S. cerevisiae* effectively transforms an invasive aquatic plant into a nutritionally valuable and biologically active feed ingredient. The optimal inclusion level of 20% AFM supports improved growth, feed efficiency, antioxidant capacity, and immune responses in tilapia without inducing adverse physiological effects. This strategy aligns with sustainable aquaculture practices by reducing dependence on conventional feed ingredients and promoting the valorization of low-value or problematic biomass through fermentation-based bioprocessing [28].

From a practical standpoint, tapping into fermented *A. philoxeroides* offers a dual-purpose strategy for sustainable aquafeed. While often dismissed as a problematic invasive weed in freshwater ecosystems, this biomass is actually a low-cost, readily available resource that gains significant value through microbial fermentation. By converting this invasive species into a functional feed ingredient, production costs may be reduced while simultaneously supporting ecosystem management. However, scaling up the approach is not without challenges. Success will depend on the ability to standardize fermentation performance and maintain strict quality control. Therefore, the next logical step must involve pilot-scale systems and rigorous cost–benefit analyses to ensure that fermented *A. philoxeroides* is truly a viable alternative in commercial tilapia farming.

5. CONCLUSION

Overall, fermentation with *S. cerevisiae* successfully reclaims the nutritional value of *A. philoxeroides*, turning an invasive weed into a potent functional feed for *O. niloticus*. Our data shows that an inclusion level of 20% fermented meal (AFM) hits the "sweet spot"—maximizing growth and immunity without any trade-offs in digestibility or health. Beyond just feed efficiency, these results provide a concrete pathway for a circular bioeconomy in aquaculture by repurposing invasive biomass. Based on these outcomes, we recommend incorporating up to 20% AFM in practical tilapia diets as a reliable, eco-efficient alternative to conventional protein sources.

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NOMENCLATURE

AFM	Fermented *Alternanthera philoxeroides* meal	—
ADC	Apparent digestibility coefficient	%
ABTS	2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay	μmol TE·g^{-1} DM
ACP	Acid phosphatase activity	U·mg protein^{-1}
ALT	Alanine aminotransferase	U·L^{-1}
AST	Aspartate aminotransferase	U·L^{-1}
CFU	Colony-forming units	CFU·g^{-1}
CP	Crude protein	% DM
DPPH	2,2-Diphenyl-1-picrylhydrazyl assay	μg·mL^{-1} (IC_{50})
DM	Dry matter	%
FAME	Fatty acid methyl esters	—
FCR	Feed conversion ratio	—
GE	Gross energy	kcal·100 g^{-1} DM
Ig	Immunoglobulin	mg·mL^{-1}
NFE	Nitrogen-free extract	% DM
NBT	Nitroblue tetrazolium reduction assay	OD_{660}
PER	Protein efficiency ratio	—
SGR	Specific growth rate	%·day^{-1}
TE	Trolox equivalents	—
WG	Weight gain	g