



Fermented aquatic weed meal (FAWM) as a protein source in Asian Catfish *Clarias batrachus* diets: Impacts on growth, blood chemistry profile, liver and gut morphology and economic efficiency

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ABSTRACT

The global aquaculture industry is increasingly seeking sustainable alternatives to fishmeal (FM) due to its high price and shortfall in supply. In this context, fermented aquatic weed meal (FAWM) could emerge as a viable plant protein source for aquafeed. Four isoproteic diets [30 % crude protein (CP)] were formulated, incorporating 50 % total protein from FAWM comprising fermented *Azolla* diet (D₁), *Pistia* diet (D₂), and *Eichhornia* diet (D₃). The control diet (D₀) did not contain FAWM. At the end of the 90 days feeding trial, their growth performance, whole-body proximate composition, gut microbial load, haemato-biochemical indices, liver and gut health, and economic efficiency were determined. Fish fed with D₀ had significantly ($p < 0.05$) improved growth performance and feed utilization compared to other treatment groups. Meanwhile, the fish supplemented with D₁ diet exhibited significantly ($p < 0.05$) higher final weight (g), specific growth rate (%/day), weight gain (%), total biomass (g), and protein efficiency ratio among the FAWM dietary groups. The D₁ group also demonstrated the significantly ($p < 0.05$) highest whole-body CP (64.27 ± 0.40 %) and lower crude lipid (8.24 ± 0.28 %) compared to other test diets. The total bacteria (TB) and lactic acid bacteria (LAB) in the fish gut were found to be significantly ($p < 0.05$) higher in D₁ group. Furthermore, most of the hemato-biochemical indices of fish were significantly ($p < 0.05$) affected by FAWM inclusions, with few exceptions. The histological findings indicated that amongst the FAWM groups, D₁ fish exhibited improved intestinal health. Nonetheless, the gut of the control fish demonstrated substantially ($p < 0.05$) lower villi width and crypt depth than other treatments. The D₁ and D₂ diet groups had significantly improved liver health. Moreover, FAWM inclusion enhanced economic efficiency by considerably reducing farm feed cost (US\$/kg) and increasing return on investment (%). In summary, dietary inclusion of fermented *Azolla pinnata* (D₁) protein at 50 % in aquafeed promoted feed utilization, growth, health, and farm economics of Asian catfish fingerlings compared to other FAWM diets.

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1. Introduction

Aquaculture is a rapidly expanding industry in global food production, pivotal in fulfilling the rising demand for aquatic protein sources and mitigating the pressure on dwindling wild fish stocks (FAO, 2020). Asian catfish is a prominent candidate in the aquaculture sector due to its robust growth, wide adaptability, and high market acceptance. Moreover, this species is commercially valuable because of its high-profile nutrients (Thorat, 2017). Nevertheless, achieving sustainable growth and profitability in Asian catfish farming requires careful consideration in dietary formulation, particularly in selecting protein sources (Chandra Segaran et al., 2023). Protein is an essential macro-nutrient in the fish diet, accounting for a significant portion of the farm production costs (Kabir et al., 2019). As the aquaculture industry strives towards environmental and economic sustainability, the search for alternative, cost-effective, and eco-friendly protein sources from plant-based ingredients, such as aquatic weeds, assumes critical significance (Dawood and Kari, 2024; Kari et al., 2023).

Aquatic weeds are often considered a nuisance in aquatic ecosystems, but have recently gained interest from aquaculturists as promising and sustainable fish feed ingredients due to their nutritional profile, availability (Muhammad Anamul et al., 2023), and digestibility (Akan-kali and Elenwo, 2019). Dorothy et al. (2018) reported that aquatic weed meal (AWM) offers significant nutritional value [Crude protein (CP): 26.5–32.5 %] and is rich in amino acids, minerals, and vitamins making it a viable protein source for aquafeed formulation (Dorothy et al., 2018; Muhammad Anamul et al., 2023). Moreover, incorporating aquatic weeds into fish feed provides a multifaceted approach to environmental waste management, sustainability, and cost reduction in aquaculture (Tewari and Kaur, 2022). Nonetheless, the presence of antinutritional factors (ANFs), high crude fiber, and other harmful compounds in aquatic weeds adversely impacts the growth and health of aquatic organisms and limits their application in aquaculture feed development (Council, 2011; Elesho et al., 2021; Francis et al., 2001; Sadia et al., 2023; Vo et al., 2020). Therefore, researchers have proposed the semi-solidify fermentation (SSF) technology to reduce the ANFs and crude fiber levels in AWM to be used as an aquafeed ingredient (De Oliveira et al., 2022; Hossain et al., 2023; Jiang et al., 2023; Zakaria et al., 2022; Zulhisyam et al., 2020). SSF is a microbial process wherein microorganisms grow on a semi-solid substrate like molasses under controlled conditions in order to ferment organic materials. The process involves maintaining a semi-solid consistency of the fermented products, typically achieved by controlling moisture, pH, temperature (Srivastava et al., 2019) and the addition of specific microbial inoculant (Dawood and Koshio, 2020). The mechanism of SSF involves the action of microorganisms, such as bacteria, yeast, and fungi, which generate enzymes capable of breaking down complex polysaccharides and protein into simpler and more digestible forms (Colla et al., 2023). Furthermore, this approach is pivotal in bolstering the bioavailability of nutrients and increasing the palatability and digestibility of feedstuff ingredients (Dawood and Koshio, 2020).

Recent reports have demonstrated the successful incorporation of FAWM in the diets of various commercial important fish species. For instance, Common carp *Cyprinus carpio* treated with *Chaetomium globosum* fermented *Eichhornia crassipes* leaf meal as high as 15 g/kg exhibited beneficial effects on growth and health status (Malik et al., 2024). Baruah et al. (2018) reported that *Ipomoea aquatica* leaf meal fermented with *Aspergillus oryzae* at approximately 30–40 % in diets significantly bolstered the growth performance and feed utilization of Indian major carp *Labeo rohita*. Furthermore, different levels of fermented aquatic macrophytes had notable impacts on growth, survival, nutrient digestibility, whole-body biochemical composition, and immunity in Nile tilapia *Oreochromis niloticus* (Velásquez et al., 2015), Rohu *Labeo rohita* (Mandal and Ghosh, 2019), and African catfish *Clarias gariepinus* (Irabor et al., 2022). Nonetheless, there are limited studies on the relationship between dietary FAWM and the growth performance and health status of

C. batrachus in the aquaculture industry. Therefore, this study aimed to evaluate the effects of different dietary FAWM at 50 % total protein inclusion levels on the growth and health status of Asian catfish production in captivity.

2. Materials and methods

2.1. Preparation of fermented aquatic weeds meal (FAWM)

Raw mosquito fern (*Azolla pinnata*), water lettuce (*Pistia stratiotes*), and water hyacinth (*Eichhornia crassipes*) were collected from the haor basin of Habiganj, Sylhet, Bangladesh. The samples were sorted and exposed to sun-drying to reduce their moisture level to <10 %. Subsequently, they were processed into a fine powder and subjected to semi-solidify fermentation following the protocol established by Zulhisyam et al. (2020) and Nandi et al. (2023), with slight modifications. First, the aquatic weed meal fine powders were placed into separate airtight plastic containers and mixed with 0.001 % *Lactobacillus acidophilus* (Sigma, St. Louis, MO, USA) and 10 % molasses separately before adding other ingredients to the mixture and stored in an airtight plastic container for 21 days to facilitate the fermentation process.

2.2. Experimental diet formulation

The experimental diet ingredients and chemical composition are detailed in Table 1. Four experimental diets (30 % CP) were formulated to include 50 % total protein from FAWM: fermented *A. pinnata* (D₁), *Pistia stratiotes* (D₂), and *Eichhornia crassipes* (D₃), and control diet with 0 % FAWM (D₀). The proximate composition of each FAWM was estimated prior to diet preparation (Table 2). All the ingredients were finely ground, homogenized, and then formed into pellets using an extruder (Model: LM40-floating feed machine, Henan Lima Machinery Manufacture Co. Limited, China), with the feed measuring 2 mm in size. The formulated pellets were oven-dried overnight at 50 °C and stored at –20

Table 1

Feed formulation and proximate composition (dry matter basis) of the experimental diets.

Ingredient (%)	Diet (% FAWM)			
	D ₀	D ₁	D ₂	D ₃
Danish fish meal ^a	38	15	16	17
Fermented <i>Azolla pinnata</i>	0	43	0	0
Fermented <i>Pistia stratiotes</i>	0	0	45	0
Fermented <i>Eichhornia crassipes</i>	0	0	0	47
Soybean meal	12	10	10	10
Flour	20	16	13	10
Corn starch	19	5	5	5
Soybean oil	3	3	3	3
Palm oil	2	2	2	2
Vitamin and mineral premix ^b	3	3	3	3
CMC ^c	3	3	3	3
Total	100	100	100	100
Feed raw materials cost (US\$/MT)	1068.20	708.59	716.70	724.80
Proximate composition (%)				
Moisture	9.45	9.60	9.41	9.91
Crude protein	30.55	30.49	30.28	30.01
Crude lipid	7.45	7.29	7.30	7.12
Crude fibre	8.01	8.12	8.31	8.95
Crude ash	9.33	9.34	9.24	9.30
NFE ^d	35.21	35.16	35.46	34.71

Notes:

^a Danish fish meal: Crude protein, 62 %; crude lipid, 8.61 %; crude ash, 17.95 %; moisture, 11.08 %.

^b Vitamin and mineral premix (g/kg premix): Vitamins A, D, E, K, C, B₁, B₂, B₆, B₁₂, KCl, 90; KI, 0.04; CaHPO₄·2H₂O, 500; NaCl, 40; CuSO₄·5H₂O, 3; ZnSO₄·7H₂O, 4; CoO₄, 0.02; FeSO₄·7H₂O, 20; MnSO₄·H₂O, 3; CaCO₃, 215; MgOH, 124; Na₂SeO₃, 0.03; NaFl.

^c CMC: Carboxymethyl cellulose as binder.

^d NFE: Nitrogen free extract.

Table 2
Proximate composition (% dry matter) of different FAWM diets.

Proximate composition	Ingredients		
	Fermented <i>Azolla</i>	Fermented <i>Pistia</i>	Fermented <i>Eicchornia</i>
Crude protein	34.78	32.95	30.12
Crude lipid	9.20	8.30	5.30
Crude ash	13.00	11.00	16.30
Crude fibre	19.60	21.30	26.10
Moisture	13.60	14.40	13.92

°C for later use. In addition, the proximate composition analysis of test diets and FAWM ingredients was performed in triplicate according to AOAC (1990). Shortly, the examined samples were used for the following analyses: protein content using the Kjeldahl method (Model: BKD-8B, BIOBASE, China), lipid content by using the Soxhlet apparatus (Model: BFA-2, BIOBASE, China), moisture content by heating at 105 °C in an oven (Model: BOV-V30F, BIOBASE, China) until a constant weight was achieved, and ash content using a Muffle furnace (Model: MC5-12, BIOBASE, China) at 550 °C for 6 h.

2.3. Fish collection and rearing

A total of 1000 Asian catfish fingerlings (average weight: 3.50 ± 0.08 g) were purchased from BRAC fish hatchery, Sreemongal, Sylhet. The fingerlings were acclimatized in five glass aquariums (60 × 30 × 20 cm) for two weeks and fed twice daily with a basal diet (ACI Catfish Feed Ltd., Bangladesh; 35 % CP and 8 % crude lipid). Subsequently, 360 fish were randomly selected and distributed equally into 12 tanks, 90 L each (n = 30/tank). The formulated diets were provided to the fish at satiation twice daily (9.00 am and 5.00 pm) during a 90-day feeding trial. Water quality variables such as temperature, pH, and dissolved oxygen were measured using multiparameter probe (Model: HI 9828, Hanna Instrument, USA), while ammonia and nitrite levels were monitored by HACH kit (Model: HI 28049, HACH, USA). Siphoning was performed every three days interval to maintain water quality. Throughout the experiment: temperature = 29.86–30 °C, DO = 5.02–5.40 mg/L, pH = 7.06–7.49, ammonia = 0.12–0.15 mg/L, and nitrite: 0.09–0.12 mg/L were found.

2.4. Growth performance of Asian catfish

At the end of the feeding trial, ten fish from each tank were randomly harvested and anesthetized using MS₂₂₂ (0.1 g/L water). The growth parameters (total length, body weight, and total biomass) were recorded. Moreover, the fish mortality and total feed consumption were recorded daily. The growth performance of the fish was analyzed using the following formulae (Kabir et al., 2024; Sezu et al., 2024):

- Weight gain (%) = 100 X (Final weight-Initial weight)/Initial weight
- Specific growth rate (%/day) = 100 X ln (Final weight)- ln (Initial weight)/Days
- Feed conversion ratio (FCR) = Total feed consumed/Live weight gain
- Protein efficiency ratio (PER) = Live weight gain/Crude protein fed
- Survival rate (%) = 100 X Number of survive fish/Total number of fish rearing
- Condition factor (gcm⁻³) = 100 X Weight of body fish/(Total length of fish)³

2.5. Whole-body proximate composition analysis

Dried whole fish from each treatment group in triplicates were used for biochemical composition analysis according to the AOAC (1990) guidelines. Crude protein content was assessed using the Kjeldahl method (Model: BKD-8B, BIOBASE, China). In brief, approximately 0.5 g

of the sample was placed in test tubes. Each sample in the Kjeldahl tube was treated with a catalyst mixture (K₂SO₄:CuSO₄ = 9:1) and 25 ml of H₂SO₄. The Kjeldahl tubes were then placed in a Kjeldahl digestion unit, where the organic nitrogen was converted into ammonium sulfate through digestion at 350–380 °C with concentrated H₂SO₄ in the Kjeldahl flask. After that, about 300 ml of distilled water was added to each Kjeldahl tube. For the distillation process, 100 ml of 35 % NaOH solution was added to each tube. In the meantime, a 25 ml solution of 4 % boric acid, with 2–3 drops of an indicator (a mixture of bromocresol green and methyl red), was prepared in a conical flask. The distillation was carried out until 75 ml of the boric acid solution was collected in the conical flask. Following distillation, the solution in the conical flask was titrated with 0.1 N HCl until the solution turned pink. The initial and final readings of the HCl were recorded, and the crude protein content (% N × 6.25) of each sample was estimated.

Fat content was determined using a Soxhlet apparatus (Model: BFA-2, BIOBASE, China). First, about 3 g of each sample were weighed and placed in a thimble paper, which was then set into the Soxhlet apparatus. Acetone (150–180 ml) was added to the round-bottom flask of the apparatus. The system was maintained at around 70 °C for 3 h. During this process, acetone (Merck, KGaA, Germany) was evaporated and condensed, with its concentration gradually decreasing. The solvent containing lipids was siphoned into the flask and all lipid-extracted samples were collected. The solvent was transferred to a pre-weighed beaker and placed in an oven at 105 °C for 30 min to evaporate the acetone. After cooling in a desiccator for 15 min, the beaker was weighed again. The crude lipid (%) in the sample was then calculated based on the weight difference.

Ash content was performed employing a Muffle furnace (Model: MC5-12, BIOBASE, China). Briefly, each empty crucible was individually marked and weighed. About 2 g of each sample were placed into the crucible, which was then weighed again. The crucible with the sample was then placed in a muffle furnace at 550 °C for 6 h, followed by cooling overnight. After that, the crucible was transferred to a desiccator to further cool. Finally, the crucible with the sample was weighed again, and the percentage of ash content was calculated.

Moisture content of samples was determined by using a hot air oven (Model: BJPX-HGZ30L, BIOBASE, China). For this purpose, the weight of each marked empty crucible was recorded. About 2 g of each sample were added to the crucibles, which were then placed in a hot air oven at 105 °C for 24 hrs. Afterward, the crucibles were transferred to a desiccator for 15 min to cool. The final weight of each sample was measured, and the moisture percentage was calculated.

2.6. Gut microbial analysis of experimental fish

The intestinal lactic acid bacteria (LAB) and total bacterial (TB) loads were determined as per Nandi et al. (2023) with minor modifications. In short, the fish were ventrally dissected using sharp scissors and the body cavity of each fish was opened to extract the gut. After that, fish intestine (1 g) was taken and cut into smaller pieces before being vortexed (Vortex Mixer, Model: BJPX-VW, BIOBASE, China) with a sterile solution (1:9). Subsequently, the samples were diluted (10⁻¹ to 10⁻¹⁰) and plated on de Man, Rogosa and Sharpe (MRS) agar (HiMedia, India) and nutrient agar (HiMedia, India) for LAB and TB enumeration, respectively. The culture plates were incubated at 37 °C for 36 hours.

2.7. Blood hematology and serum biochemical assays

Fish from each replication group were randomly selected (n = 3) and anesthetized using MS₂₂₂ (0.1 g/L water). Following that, about 150 µl of blood was collected from the dorsal vein after being fasted for 12 h using a 1 cc syringe and preserved in heparinized tubes. Hematological profiles were analyzed using an automatic hematology analyzer (Mythic Vet 18, USA). Meanwhile, an additional 300 µl blood was collected from each tank containing fish and kept into cellular serum tubes. The blood

samples were then centrifuged at 3000 rpm for 10 min to isolate serum. Subsequently, around 150 μ l of serum was drawn in an automatic blood analyzer (Beckman Coulter AU680, USA) to evaluate the biochemical indices of fish such as albumin, glucose, total bilirubin, creatinine, serum glutamic pyruvic transaminase, alkaline phosphatase, serum glutamic oxaloacetic transaminase, cholesterol, alanine aminotransferase, total protein, aspartate aminotransferase, and globulin.

2.8. Histopathology of mid intestine and liver

Fish from each tank were randomly selected ($n = 3$), placed in separate tanks, and fasted for 72 h to empty their guts. After being anesthetized with MS₂₂₂ (0.1 g/L water, Syndel, Canada), the fish were dissected with sharp scissors from anus to head to collect their liver and mid gut tissues. A fresh and unused surgical blade was used for dissecting tissue samples to avoid any rough edges, squeezing, or abrasion and then preserved in 10 % neutral buffered formalin solution (Merck KGaA, Germany) for 72 h. The prepared tissue segments (2 mm thickness) were dehydrated using Carnoy's solution (a mixture of 60 % 2-propanol, 30 % chloroform and glacial acetic acid 10 %) and 2-propanol (Merck, Germany), cleaned with xylene (Merck KGaA, Germany), embedded in pure paraffin (Merck, Germany), and sectioned (6 μ m) by a microtome machine (Leitz, 1512, USA) for hematoxylin and eosin (H&E) staining (Sigma-Aldrich, Merck, Germany). The slides were observed under a light microscope (Olympus BX43, Tokyo, Japan) and visualized using Image J software (Wayne Rasband) to determine the crypt depth, width, and villi length.

2.9. Farm economic analysis

The cost of raw materials used in the feed formulation was determined by summing up the cost of each ingredient. Subsequently, the farm feed cost, total yield, farm revenue, farm raw margin and return on investment were analyzed to evaluate farm economics using the following formulae (Suma et al., 2023):

- i. Farm feed cost, FFC (US\$/kg) = FCR x raw materials cost
- ii. Total yield, TY (kg/m²) = Total biomass gain/ tank area
- iii. Farm revenue was determined on an estimated farm gate price of US\$ 7.38/ kg for Asian catfish, FR (US\$/m²) = TY \times 7.38
- iv. Farm raw margin, FRM (US\$/m²) = FR - (TY \times FFC)
- v. Return on investment, ROI (%) = 100 x FRM/ (TY \times FFC)

2.10. Statistical analysis

All the data were assessed for normality before conducting the statistical analysis. One-way analysis of variance (ANOVA) was performed using the Statistical Package for Social Sciences (SPSS) version 20.1 (IBM, USA) to ascertain the significant differences between the treatment groups, followed by Duncan's multiple range test. The significance level was set at $p < 0.05$, and the results were presented as mean \pm standard deviation (SD).

3. Results

3.1. Growth performance of Asian catfish

The growth performance of fish fed the control and FAWM diets is illustrated in Table 3. There were highly significant ($p < 0.05$) differences in the growth performance of fish among the various treatments. The control fish exhibited significantly ($p < 0.05$) superior growth and feed utilization than the other treatment groups. Nonetheless, among the FAWM dietary groups, the D₁ diet consumed fish recorded the significant ($p < 0.05$) highest FW (g), WG (%), TB (g), SGR (%/ day), and PER and the lowest FCR. Meanwhile, the D₃-treated fish demonstrated the significant ($p < 0.05$) lowest growth performance and the highest

Table 3

Growth and feed utilization parameters of *C. batrachus* fed with control and experimental diets for 90 days.

Parameters	Diets (% FAWM)			
	D ₀	D ₁	D ₂	D ₃
IW (g)	3.50 \pm 0.01	3.50 \pm 0.01	3.50 \pm 0.00	3.50 \pm 0.00
FW (g)	28.32 \pm 0.43 ^a	25.51 \pm 0.14 ^b	23.52 \pm 0.09 ^c	20.90 \pm 0.15 ^d
WG (%)	708.11 \pm 12.48 ^a	627.75 \pm 4.04 ^b	571.88 \pm 2.49 ^c	496.96 \pm 4.11 ^d
TB (g)	744.42 \pm 12.80 ^a	660.10 \pm 4.29 ^b	600.69 \pm 2.72 ^c	521.85 \pm 4.40 ^d
SGR (%/day)	1.88 \pm 0.02 ^a	1.79 \pm 0.01 ^b	1.72 \pm 0.01 ^c	1.61 \pm 0.01 ^d
FCR	1.30 \pm 0.02 ^d	1.51 \pm 0.01 ^c	1.72 \pm 0.01 ^b	1.81 \pm 0.01 ^a
PER	2.58 \pm 0.03 ^a	2.21 \pm 0.02 ^b	1.94 \pm 0.01 ^c	1.84 \pm 0.01 ^d
SR (%)	95.00 \pm 5.00	98.33 \pm 2.89	96.67 \pm 2.89	96.67 \pm 2.89
CF (g cm ⁻³)	0.58 \pm 0.01 ^c	0.75 \pm 0.00 ^b	0.85 \pm 0.00 ^a	0.84 \pm 0.01 ^a

Note: IW: Initial weight, FW: Final weight, WG: Weight gain, TB: Total biomass, SGR: Specific growth rate, FCR: Feed conversion ratio, PER: Protein efficiency ratio, SR: Survival rate, CF: Condition factor. Different superscript alphabets in each row denote statistical differences ($p < 0.05$). The data are presented as mean \pm SD.

FCR. The survival rate did not significantly ($p > 0.05$) differ between the treatment groups. Furthermore, D₀-fed fish had a remarkably ($p < 0.05$) lower CF (g cm⁻³) than the other experimental fish.

3.2. Gut microbial analysis of experimental fish

The bacterial quantification in the guts of experimental fish is presented in Table 4. The TB and LAB counts in the fish intestine fluctuated significantly ($p < 0.05$) among the treatment groups. In D₁ fish, both TB and LAB counts were significantly ($p < 0.05$) higher compared to other catfish groups, although TB counts did not significantly vary in the D₂ fish gut. On the other hand, fish in the control group had markedly ($p < 0.05$) lower TB and LAB in their intestine.

3.3. Whole-body proximate composition analysis

Table 5 displays the whole-body biochemical composition of Asian catfish. There were significant ($p < 0.05$) differences in the proximate composition, including crude protein, crude lipid, and moisture content, among the fish fed with different experimental diets. Interestingly, D₁ fish recorded significantly ($p < 0.05$) higher crude protein and lower crude lipid and moisture content compared to other treatment groups, although no significant variations were noted in the crude lipid values between the D₀ and D₃ treatments. However, the ash content of fish was not significantly affected ($p > 0.05$) by the FAWM diets.

3.4. Blood hematological assays

The hematological indices of Asian catfish treated with experimental diets are detailed in Table 6. The statistical analysis showed that fish fed

Table 4

The TB and LAB counts in the gut of experimental catfish ($n=3$).

Parameters	Diets (% FAWM)			
	D ₀	D ₁	D ₂	D ₃
TB (CFU/g intestine) X 10 ⁷	4.98 \pm 0.19 ^c	14.55 \pm 2.08 ^a	12.55 \pm 0.68 ^{ab}	11.43 \pm 0.48 ^b
LAB (CFU/g intestine) X 10 ⁵	1.03 \pm 0.17 ^c	7.66 \pm 0.61 ^a	6.19 \pm 0.06 ^b	5.99 \pm 0.11 ^b

Notes: TB = Total bacteria, LAB = Lactic acid bacteria. Different superscript alphabet in each row denote statistical differences ($p < 0.05$). The data are presented as mean \pm SD.

Table 5

Whole body proximate composition (% dry matter) of fish fed with different experimental diets (n=3).

Parameters	Diets (% FAWM)			
	D ₀	D ₁	D ₂	D ₃
Crude protein	62.65 ± 0.85 ^b	64.27 ± 0.40 ^a	62.36 ± 0.52 ^b	62.34 ± 0.58 ^b
Crude lipid	8.65 ± 0.66 ^{ab}	8.24 ± 0.28 ^b	9.01 ± 0.08 ^a	8.45 ± 0.23 ^{ab}
Crude ash	16.05 ± 0.14	16.21 ± 0.86	15.76 ± 0.41	16.39 ± 0.56
Moisture	10.68 ± 0.51 ^a	9.35 ± 0.27 ^b	10.50 ± 0.43 ^a	10.68 ± 0.67 ^a

Different superscript alphabets in each row denote statistical differences ($p < 0.05$). The data are presented as mean ± SD.

Table 6

Hematological indices of Asian catfish fed with different FAWM and control diets over 90 days of the experiment (n=3).

Parameter	Diets (% FAWM)			
	D ₀	D ₁	D ₂	D ₃
WBC (10 ⁹ /L)	541.60 ± 0.60	541.82 ± 0.43	542.21 ± 0.10	541.55 ± 0.55
NEU (%)	0.56 ± 0.02	0.60 ± 0.08	0.57 ± 0.10	0.54 ± 0.01
LYM (%)	0.32 ± 0.01 ^b	0.74 ± 0.11 ^a	0.64 ± 0.01 ^a	0.66 ± 0.05 ^a
MON (%)	0.04 ± 0.01	0.06 ± 0.04	0.04 ± 0.01	0.04 ± 0.03
EOS (%)	0.02 ± 0.01	0.02 ± 0.02	0.03 ± 0.02	0.04 ± 0.04
BAS (%)	0.06 ± 0.02 ^a	0.07 ± 0.02 ^a	0.03 ± 0.02 ^b	0.02 ± 0.01 ^b
RBC (10 ¹² /L)	2.54 ± 0.50 ^c	5.93 ± 0.16 ^a	6.03 ± 0.09 ^a	3.95 ± 0.04 ^b
HGB (g/L)	84.33 ± 3.06 ^b	91.00 ± 6.00 ^a	64.67 ± 1.53 ^c	47.99 ± 1.01 ^d
HCT (%)	0.20 ± 0.02 ^c	0.54 ± 0.10 ^a	0.37 ± 0.07 ^b	0.33 ± 0.05 ^{bc}
MCV (fL)	86.07 ± 1.1 ^a	80.43 ± 0.74 ^b	80.37 ± 0.65 ^b	84.23 ± 3.39 ^a
MCH (pg)	32.72 ± 0.49	31.35 ± 2.07	30.93 ± 4.68	33.95 ± 2.59
MCHC (g/L)	408.00 ± 15.13 ^{bc}	524.00 ± 4.36 ^a	394.00 ± 24.06 ^c	434.67 ± 12.70 ^b
RDW-CV (fL)	0.17 ± 0.05	0.18 ± 0.02	0.15 ± 0.02	0.16 ± 0.01
RDW-SD (fL)	60.37 ± 5.01	57.34 ± 1.16	53.07 ± 6.93	53.04 ± 6.96
PLT (10 ⁹ /L)	290.03 ± 8.26 ^b	290.37 ± 2.01 ^b	256.07 ± 12.94 ^c	313.47 ± 5.13 ^a
MPV (fL)	7.41 ± 0.62	7.81 ± 0.75	7.23 ± 0.93	6.70 ± 0.50
PDW (%)	16.83 ± 0.57 ^b	18.08 ± 0.85 ^a	17.69 ± 0.42 ^{ab}	18.23 ± 0.12 ^a
PCT (ml/L)	2.73 ± 0.45 ^a	2.26 ± 0.57 ^{ab}	1.93 ± 0.02 ^b	2.28 ± 0.31 ^{ab}

Note: WBC: White blood cell, NEU: Neutrophil, LYM: Lymphocytosis, MON: Monocytes, EOS: Eosinophil, BAS: Basophil, RBC: Red blood cell, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RDW-CV: Red cell distribution width-coefficient of variation, RDW-SD: Red cell distribution width-standard deviation, PLT: Platelet, MPV: Mean platelet volume, PDW: Platelet distribution width, PCT: Procalcitonin. Different superscript alphabets in each row denote statistical differences ($p < 0.05$). The data are presented as mean ± SD.

with the D₂ diet had notably ($p < 0.05$) elevated LYM, BAS, RBC, HGB, HCT, MCHC, and PDW levels. Conversely, the control group exhibited significantly ($p < 0.05$) lower levels of LYM, RBC, HCT, MCHC, and PDW. The HGB content was dramatically reduced in the D₃ group. However, other hematological traits, including WBC, NEU, MON, EOS, MCH, RDW-CV, RDW-SD, and MPV, were not significantly different ($p > 0.05$) between the treatments.

3.5. Serum biochemical assays

Table 7 demonstrates the serum biochemical profile of fish fed the FAWM and control diets. The results revealed that various dietary

Table 7

Blood biochemical parameters of fish when treated with different test diets for 90 days of the experiment (n = 3).

Parameters	Diets (% FAWM)			
	D ₀	D ₁	D ₂	D ₃
GLU (mmol/L)	3.67 ± 0.58 ^b	4.33 ± 0.58 ^b	9.00 ± 1.00 ^a	4.67 ± 0.58 ^b
CREA (mg/dL)	0.47 ± 0.06 ^b	0.20 ± 0.01 ^d	0.35 ± 0.01 ^c	0.83 ± 0.02 ^a
TBIL (mg/dL)	55.83 ± 0.76 ^a	35.70 ± 0.20 ^b	18.10 ± 0.10 ^c	12.03 ± 0.15 ^d
ALT (μ/L)	9.67 ± 0.58 ^{ab}	6.67 ± 0.58 ^c	10.67 ± 0.58 ^a	8.67 ± 0.58 ^b
BUN (mg/dL)	57.50 ± 0.50	48.83 ± 0.29	74.50 ± 0.50	86.40 ± 0.36
AST (μ/L)	7.50 ± 1.89 ^b	4.67 ± 0.58 ^d	20.67 ± 4.01 ^a	6.40 ± 1.85 ^c
ALB (g/dL)	4.67 ± 0.58 ^d	7.50 ± 0.50 ^b	8.67 ± 0.58 ^a	6.40 ± 0.36 ^c
ALKP (μ/L)	8.40 ± 0.36 ^a	8.10 ± 0.10 ^a	6.50 ± 0.20 ^b	5.73 ± 0.15 ^c
CHOL (g/dL)	9.03 ± 0.06 ^c	11.97 ± 0.06 ^b	5.60 ± 0.53 ^d	14.27 ± 0.25 ^a
TP (g/dL)	9.10 ± 0.10 ^d	25.50 ± 1.32 ^a	19.60 ± 0.53 ^b	14.50 ± 0.50 ^c
GLOB (g/dL)	6.50 ± 0.30 ^c	10.90 ± 0.10 ^a	5.67 ± 0.15 ^d	7.00 ± 0.10 ^b

Note: GLU: Glucose, CREA: Creatinine, TBIL: Total bilirubin, BUN: Blood urea nitrogen, ALB: Albumin, ALKP: Alkaline phosphatase, CHOL: Cholesterol, TP: Total protein, GLOB: Globulin, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase. Different superscript alphabets in each row denote statistical differences ($p < 0.05$). The data are presented as mean ± SD.

groups significantly ($p < 0.05$) impacted the serum biochemical composition of Asian catfish. Fish group that were consumed D₁ diet displayed substantial ($p < 0.05$) lower GLU, CREA, and AST contents and higher TP and GLOB levels compared to other dietary treatments, although the GLU level was statistically similar in the D₀ and D₃ groups. Meanwhile, the control group exhibited significantly ($p < 0.05$) higher TBIL, ALKP, and ALT, and lower ALB and TP than other test groups. The CHOL level differed significantly ($p < 0.05$) between the treatments, with the lowest value detected in the D₂ fish. Despite that, none of the dietary treatments influenced the fish BUN level.

3.6. Histopathology of mid-intestine and liver

Fig. 1 depicts the histological changes in the fish mid-intestine after being treated with FAWM and control diets. Morphological alterations were observed in the lamina propria, lamina epithelial mucosae, stratum compactum, goblet cells, and tunica muscularis. The control group exhibited improved intestinal health, characterized by more villi, increased goblet cells and a larger lamina propria. In contrast, 50 % FAWM protein supplementation was detrimental to the fish gut, except for the D₁ diet. The D₁ diet group displayed an effective epithelial barrier with increased goblet cells, expanded villi, and a well-developed stratum compactum in their gut. Conversely, the D₂ and D₃ diet groups exhibited irregular villi structure and a wider tunica muscularis rather than other treatments fish.

Fig. 2 illustrates the Asian catfish liver histology upon supplementation with various experimental diets, showcasing alterations in nuclei, vacuoles, sinusoids, erythrocytes, and other signs of hemorrhagic or degenerative activities. These findings indicated that *C. batrachus* fed the D₁ and D₂ diets had improved liver health, characterized by more nuclei and sinusoids and reduced vacuolar cytoplasm. On the contrary, hepatic disorders such as more vacuoles, degenerative nuclei, and erythrocyte infiltration were observed in the liver of fish fed the D₀ and D₃ diets.

3.7. Estimation of villi height, width, and crypt depth

Table 8 shows the morphological analysis of the midgut of fish that received various experimental diets. The inclusion of FAWM protein

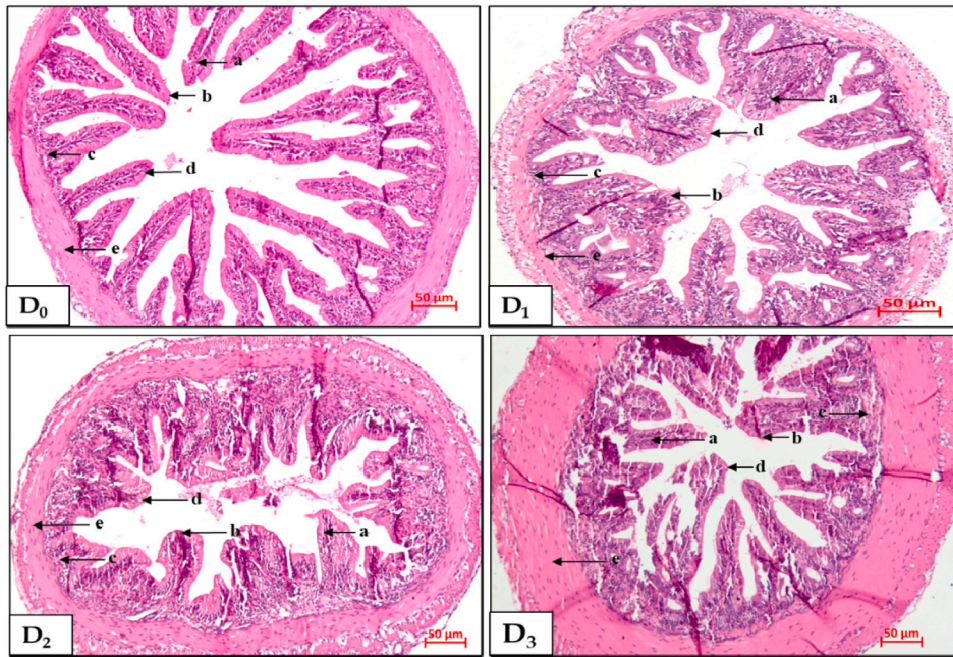


Fig. 1. Histopathological images of the mid intestine of Asian catfish fed with experimental diets. All micrographs were captured at 10X magnification and 50 μm scale bar. The histological variations were observed in (a) lamina propria, (b) lamina epithelial mucosae, (c) stratum compactum, (d) goblet cell, and (e) tunica muscularis.

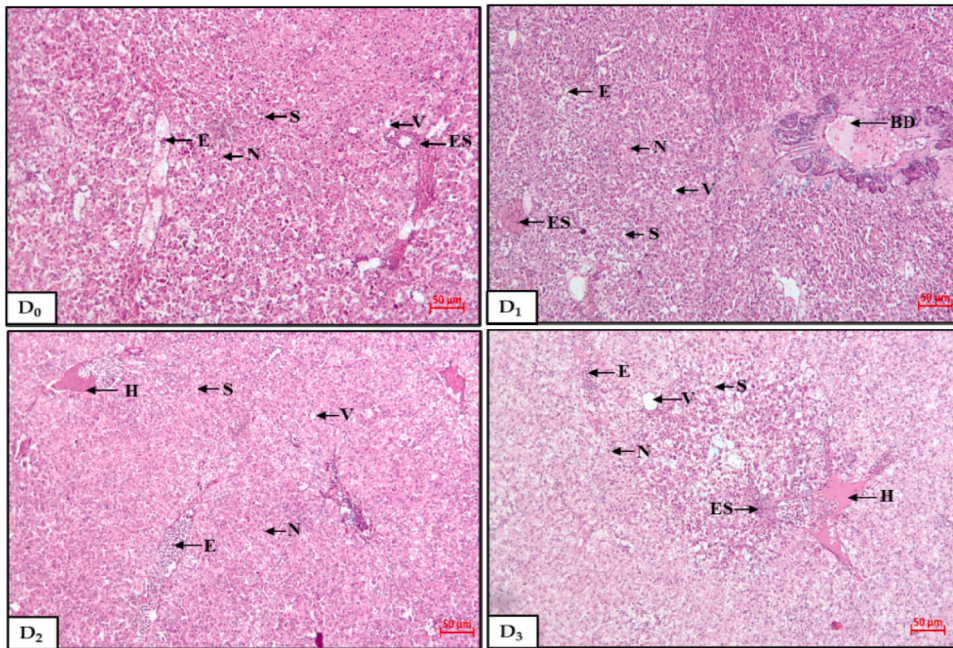


Fig. 2. Light microscopic investigation of Asian catfish liver fed various experimental diets for 90 days. The images were obtained at 10X magnifications and 50 μm scale bar. The morphological changes were observed in the nucleus (N), sinusoid (S), vacuole (V), erythrocyte (E), erythrocyte infiltration into blood sinusoid (ES), hemorrhage (H), and bile duct (BD).

significantly ($p < 0.05$) affected both villi width and crypt depth, with notably lower values observed in the control fish. However, there was no statistical variation in villi width found in the D₃ diet group. Nevertheless, villi length did not substantially ($p > 0.05$) differ between the treatment groups.

3.8. Farm economic analysis

The inclusion of 50 % FAWM protein boosted farm economic efficiency by extensively reducing the FFC (Table 9). Interestingly, the significantly ($p < 0.05$) lowest FFC was observed in the D₁ diet group, besides boasting significantly higher ROI. Moreover, TY, FR, and FRM were considerably ($p < 0.05$) higher in control group. However, amongst the FAWM dietary groups, D₁ diet fed fish showed noteworthy

Table 8

Mid-intestinal morphology of *C. batrachus* fed with different FAWM proteins (n = 3).

Parameters	Diets (% FAWM)				
	Intestinal region	D ₀	D ₁	D ₂	D ₃
Villi length	Mid gut	134.68 ± 49.24	137.78 ± 25.86	123.23 ± 22.68	113.69 ± 22.29
		4.36 ^b	10.58 ^a	5.35 ^a	7.66 ^b
Villi width	Mid gut	31.20 ± 4.36 ^b	42.93 ± 10.58 ^a	41.35 ± 5.35 ^a	29.72 ± 7.66 ^b
		14.59 ± 4.03 ^b	19.83 ± 2.38 ^{ab}	25.50 ± 7.58 ^a	23.20 ± 8.60 ^a
Crypt depth	Mid gut	14.59 ± 4.03 ^b	19.83 ± 2.38 ^{ab}	25.50 ± 7.58 ^a	23.20 ± 8.60 ^a

Different superscript alphabets in each row denote statistical differences (p < 0.05). The data are presented as mean ± SD.

Table 9

Farm economic efficiency resulting from Asian catfish feeding trial after 90 days (n=3).

Parameters	Diets (% FAWM)			
	D ₀	D ₁	D ₂	D ₃
TY (kg/m ²)	7.44 ± 0.13 ^a	6.60 ± 0.14 ^b	6.01 ± 0.03 ^c	5.22 ± 0.04 ^d
FFC (US\$/kg)	1.38 ± 0.02 ^a	1.07 ± 0.01 ^d	1.23 ± 0.00 ^c	1.31 ± 0.01 ^b
FR (US\$/m ²)	54.94 ± 0.95 ^a	48.72 ± 0.31 ^b	44.33 ± 0.20 ^c	38.51 ± 0.33 ^d
FRM (US \$/m ²)	44.66 ± 0.90 ^a	41.65 ± 0.33 ^b	36.94 ± 0.17 ^c	31.66 ± 0.24 ^d
ROI (%)	434.34 ± 6.66 ^d	589.08 ± 5.99 ^a	499.68 ± 1.96 ^b	461.75 ± 2.32 ^c

Note: TY, Total yield; FFC, Farm feed cost; FR, Farm revenue; FRM, Farm raw margin; ROI, Return on investment. Different superscript alphabets in each row denote statistical differences (p < 0.05). The data are presented as mean ± SD.

(p < 0.05) higher levels of these parameters.

4. Discussion

The FAWM is a promising and sustainable protein source for aqua-feed development due to its superior nutritional profile, abundance, and low production cost. In this study, different FAWM were incorporated in diets (50 % of the total protein) to determine the impacts on growth metrics, nutrient utilization, gut microbiota, biochemical composition, blood metrics, liver & intestinal health of Asian catfish, and assess the economic efficiency of the experimental feed. Fermenting aquatic weeds with 0.001 % *L. acidophilus* enhances their nutritional profile by converting polysaccharides into protein, reducing ANFs, and improving digestibility and absorption in fish (Hayati et al., 2023; Li et al., 2022). Numerous studies have demonstrated that using 50 % fermented plant ingredients or protein enhances fish productivity and overall health condition (Aragão et al., 2022; Kari et al., 2022; Mugwanya et al., 2023; Nandi et al., 2023; Rajaram et al., 2022; Seong et al., 2018; Zulhisyam et al., 2020). Thus, this study included 50 % protein from FAWM as the total protein in experimental diets.

This study finding's indicated that the fish supplemented with the D₀ diet exhibited the significantly highest growth performance and feed utilization. The results observed in control fish supplemented with the FM-based diet might be credited to its high protein content, balanced nutrient profile, optimal amino acid composition, and digestibility. Despite that, the D₁ diet group exhibited significantly (p < 0.05) higher FW, WG, TB, SGR, FCR, and PER than other treatments. These favorable outcomes are possibly due to the more digestible protein in fermented *Azolla*, enabling the fish to efficiently digest and absorb nutrients, thereby supporting their somatic growth. Liu et al. (2023) stated that fermentation of plant ingredients with *Lactobacillus* spp. improves fish growth and feed utilization by enhancing digestion, synthesizing nutrients, producing growth-promoting metabolites, modulating gut

microbiota, and reducing competition from pathogens. Likewise, fermented plant protein supplemented in other fish species have yielded promising outcomes, including *Barbonymus gonionotus* (Das et al., 2018), *O. niloticus* (Yousif et al., 2019), *Clarias gariepinus* (Irbor et al., 2022; Kari et al., 2022), *Oncorhynchus mykiss* (Davies et al., 2021), and *Labeo rohita* (Ferdous et al., 2023). Moreover, Nandi et al. (2023) reported that the SSF of plant-derived ingredients using 0.001 % *Lactobacillus* reduced ANFs, improved nutritional value, and boosted fish production when included optimally in their diet. Supplementation with beneficial microbes in fish diets can also improve the FCR of aquatic animals (Flefil et al., 2022; Kapka-Skrzypczak et al., 2012; Sadia et al., 2023; Veisi et al., 2023). In contrast, the D₃ diet group demonstrated lower growth performance compared to other treatment groups. The higher inclusion of dietary *Eicchornia* meal increased ANFs and fiber content, potentially reducing fish growth. Multiple reports (Gatlin III et al., 2007; Torstensen et al., 2008; Welker et al., 2016; Yu et al., 2015) have documented that high dietary plant protein lowered feed digestibility and palatability. In this study, the survival rate for all groups exceeded 95 %, with no significant differences, indicating that FAWM did not influence fish mortality. Abdul Kari et al. (2021) found similar results with African catfish fed various proportions of fermented soy pulp. Additionally, the CF was substantially higher in FAWM diet groups compared to the control, consistent with earlier findings (Lee et al., 2016; Zhou et al., 2011). A higher CF suggests that the fish are in good condition.

The fish gut microbial quantification results indicated that *Lactobacillus* fermentation significantly impacted the intestinal TB and LAB counts, particularly in the *Azolla* (D₁) diet group, which recorded the highest bacterial counts. *Azolla* is known to act as a prebiotic, containing substances that stimulate the growth and activity of beneficial microbes in the intestine (de la Cruz et al., 2023). The combination of *Lactobacillus* fermentation and *Azolla* treatment might have resulted in a synergistic effect, further enhancing both probiotic and prebiotic activities. This interaction probably led to the highest counts of beneficial bacteria, demonstrating improved intestinal health, defense mechanisms, and disease resistance in fish fed with a D₁ diet compared to other treatments. Chai et al. (2019) noted that during fermentation, microorganisms generate antimicrobial peptides that might have an inhibitory and killing effect on pathogenic microbes. In addition, delivering lactic acid bacteria into fish intestine through their feed has been proven effective in enhancing fish growth performance (Aliyu-A et al., 2019; de Oliveira et al., 2015; Ringø et al., 2018; Seal et al., 2018; Zulhisyam et al., 2020), health status (Abdul Kari et al., 2021; Kari et al., 2022), and disease resistance (Verma et al., 2018). Filannino et al. (2018) also reported that a higher LAB count in fish intestines increases lactic acid secretion, thus increasing digestive enzyme activities. Therefore, the current study's findings suggest that the dietary inclusion of FAWM protein (50 %) promotes beneficial microbiota growth in the distal intestine of fish.

The fish's whole-body biochemical profile is a critical indicator of food quality. The present study showed that FAWM significantly influenced the fish's whole-body composition, with the significantly highest CP detected in fish fed with fermented *Azolla* (D₁). Fermentation possibly improves protein bioavailability and digestibility, facilitating absorption and protein synthesis in the body. Similarly, Refaey et al. (2023) reported increased CP content in the whole-body of *O. niloticus* when supplemented with 20 % *Azolla* meal. Nonetheless, Ahmed et al. (2019) did not observe significant changes in fish CP levels when *Megalobrama amblycephala* were fed a diet formulated with plant protein. Furthermore, the considerably highest lipid content was recorded in fish provided with fermented *Pistia* (D₂). Reduced feed intake potentially compromises protein utilization, resulting in ineffective protein synthesis in the fish's body. This condition promotes protein conversion into lipids for storage. Different levels of *P. stratiotes* incorporated into fish diets also led to varying lipid content in *L. rohita* (Nisha and Geetha, 2017), while *Oncorhynchus mykiss* fed with 40–60 % *Camelina sativa* meal yielded similar outcomes. Suma et al. (2023) documented that fish with high protein and low-fat content present an outstanding dietary

option for human consumption. Meanwhile, fish ash content was not affected by FAWM diets, which is in line with earlier findings (Chen et al., 2020; Huang et al., 2023; Zhao et al., 2021). This stability is likely due to the similar mineral composition of FAWM compared to the control feed, coupled with the fish's inherent ability to regulate mineral levels despite dietary differences. Overall, these findings contribute to our understanding of optimal dietary strategies for enhancing fish quality and nutritional value.

Blood hematological assays are common tools for evaluating fish health and physiology (Fazio, 2019; Witeska et al., 2022). The hematological data indicated that the experimental diets did not significantly affect the WBC level, suggesting that FAWM protein had no adverse effects on fish immunity. Refaey et al. (2023) obtained corresponding result when Nile tilapia consumed different dietary inclusions of *Azolla* meal. The *Azolla* (D₁) diet fed fish group had the highest mean values for LYM and BAS. Both *Lactobacillus* and *Azolla* are known to have immunomodulatory effects (Bhattacharyya et al., 2016; Taverniti and Guglielmetti, 2011). These effects could bolster the fish's immune system, leading to increased LYM and BAS counts. Lymphocytes are key cells in the adaptive immune response, while basophils play roles in allergic and parasitic responses (Nandi et al., 2024). Fish fed a diet incorporating 50 % fermented soy pulp showed elevated lymphocyte counts in African catfish (Abdul Kari et al., 2021). Nevertheless, the control fish had lower RBC and HCT counts than the FAWM diet groups, indicating the possibility of FAWM-stimulated erythropoiesis. Abdul Kari et al. (2021) stated that WBC, RBC, and HCT are significant indicators of fish health and antinutritional toxicity of feed. The blood HGB count was significant and highest in fish fed with the D₁ diet. This result might be due to the combined effects of *Azolla*'s nutritional content, particularly its protein and iron bioavailability. The increased RBC and HGB could promote tissue oxygenation and fish growth, as supported by earlier studies (Esmaeili, 2021; Hossain et al., 2022). Numerous researches have highlighted variations in RBC, HGB, HCT, MCHC, PDW, and PCT levels in several fish species when fed various plant-protein diets (Dienye and Olumuji, 2014; Farhad et al., 2023; Zakaria et al., 2022), which aligns with the current findings. Meanwhile, the experimental diets did not affect other parameters such as NEU, MON, EOS, MCH, RDW-CV, RDW-SD, and MPV. Overall, these results reveal disparities in fish health and physiological states attributable to variations in hematological indices.

The experimental diets had notable impacts on fish's blood biochemical indices in the present study. For instance, the D₁ group recorded lower blood GLU than other treatments, but GLU levels in the D₀ and D₃ groups were statistically similar. *Azolla* contains various phytochemicals such as polyphenols, flavonoids, and alkaloids, which have been documented to regulate glucose metabolism (Kösesakal and Yıldız, 2019), potentially enhance insulin sensitivity, promote cellular glucose uptake, and inhibit liver glucose production, leading to reduced GLU. Nandi et al. (2023) reported similar observations in *H. fossilis* fed with graded levels of FWM. In this study, the fish supplemented with a D₁ diet exhibited significantly higher TP and GLOB contents. These outcomes were consistent with previous studies involving *O. niloticus* (Magouz et al., 2020), *C. gariepinus* (Abdul Kari et al., 2021), and *H. fossilis* (Nandi et al., 2023). Fermentation is likely to improve the digestibility of *Azolla* meal, thereby increasing the nutrient availability and absorption. As a result, protein utilization is enhanced, contributing to higher TP and GLOB. Elevated TP represents superior fish immunity (Rebl and Goldammer, 2018), while GLOB is a vital indicator of fish lipid catabolism and immune status (Khalil et al., 2018, 2022). These changes also indicate enhanced energy production and liver function in the D₁ diet group, which may boost the fish's immune system. However, the significantly elevated TBIL count in D₀ fish contrasts with earlier findings (Chaklader et al., 2020, 2021), implying a potential decline in liver function in the control group. In contrast, certain phytochemicals or microbial metabolites in the FAWM diets may support the liver's detoxification processes, resulting in better liver health.

The CHOL and ALB levels were highly significant across the various treatment groups, aligning with findings from previous studies (Abdul Kari et al., 2021; Rahman et al., 2023). CHOL is essential for the body's physiological functions, but elevated levels can lead to health problems. In contrast, ALB contents are an indicative of fish's health, as deviations from normal values can signal issues such as malnutrition and liver dysfunction. Additionally, the experimental diets had a substantial impact on other critical parameters, including CREA, AST, ALT, and ALKP, all of which serve as indicators of the fish's general health.

The gut morphological investigation conducted in this study provides insights into the fish's nutrient absorption and immune status (Abdel-Wareth et al., 2023; Dawood et al., 2020; Haygood and Jha, 2018). According to Wang et al. (2018), dietary nutrition is a primary factor influencing structural alterations in the fish's intestines. In the present study, the experimental diets substantially affected the fish's distal intestinal health at varying levels. For instance, the control group exhibited normal intestinal morphology, particularly in terms of villi structure, goblet cell arrangements, and tunica muscularis. This normal intestinal morphology in the control fish is likely due to the good quality protein, peptide, and EAAs, as well as the absence of ANFs in FM. Meanwhile, the D₁ diet group had an extended lamina propria with significantly increased villi width and crypt depth and more goblet cells than other FAWM treatment groups. These findings indicate that dietary *A. pinnata* improved the fish's gut health by potentially enhancing digestion and nutrient absorption. Goblet cells in the villi of the small intestine are crucial for mucus production, maintaining gut barrier function, supporting immune defense, facilitating nutrient absorption, and preserving gut homeostasis, thereby contributing to overall gut health and function. The supplementation of dietary plant protein has yielded promising outcomes in *Lateolabrax japonicus* (Zhang et al., 2018), *Micropterus salmoides* (Li et al., 2020), *Ameiurus nebulosus* (Matulić et al., 2020), *O. niloticus* (Ismail et al., 2022), *Scophthalmus maximus* L. (Li et al., 2022), and *Labeo rohita* (Palaniyappan et al., 2023). Earlier studies discovered that goblet cells are critical indicators of nutrient digestion and absorption (Pirarat et al., 2011). Elevated goblet cells are crucial for mucus secretion and combating ANFs (Mzengereza et al., 2022). The fish from the D₂ and D₃ groups exhibited abnormal gut morphology, characterized by microvilli disintegration, vacuole enlargement, increased connective tissue, and wider tunica muscularis. High crude fiber, ANFs, and other toxic elements in these dietary groups might cause these adverse impacts. Furthermore, the substantial thickening of the tunica muscularis in the D₃ diet group suggests higher muscle contraction due to the migration of unabsorbed food particles into the rectum (Nasruddin et al., 2014). In summary, incorporating 50 % protein from fermented *Azolla* meal in the *C. batrachus* diet promoted their nutrient absorption and health status compared to the other FAWM diets.

According to Lozano et al. (2017), the liver is a crucial indicator of the fish's overall health. Morphological evaluation of the liver tissues revealed that fish supplemented with D₁ and D₂ diets had increased nuclei, reduced vacuolation, and no apparent hemorrhage or hepatocyte dissociation. This improvement is likely attributed to the *Lactobacillus* fermentation of the feed ingredients, which modulates gut microbiota, reinforces the gut barrier, supports detoxification, reduces oxidative stress, and exerts anti-inflammatory effects. These findings are supported by Vo et al. (2020), Liu et al. (2021), and Han et al. (2022). In contrast, the D₀ and D₃ groups displayed poor liver health, characterized by increased vacuolation, extended hemorrhage, hepatocyte dissociation, and degenerative nuclei. These adverse outcomes may be attributed to the presence of indigestible nutrients in the fish diet. Abdul Kari et al. (2021) found analogous effects in African catfish liver when supplemented with 75 % –100 % fermented soy pulp meal. Additionally, substituting fish meal with 100 % plant protein led to more vacuole and fat accumulation in the liver of Gilthead Sea bream (Sitjà-Bobadilla et al., 2005).

The economic analysis revealed that FAWM protein significantly

reduced the FFC and increased ROI. FAWM offers economic benefits due to its locally available, low-cost production, contributing to reduced feed expenses and increased profitability in aquaculture operations. Precisely, the diet containing fermented *A. pinnata* had the lowest FFC among all dietary groups, highlighting its cost-effectiveness. Furthermore, the D₁ diet group recorded the highest ROI, suggesting that fermented *Azolla pinnata* is a highly economical protein source for aquaculture diets. These findings underscore the potential economic benefits of utilizing FAWM in aquaculture feeds to save cost and improve ROI. This information is valuable for aquaculturists seeking sustainable, cost-effective solutions to boost their farm productivity.

5. Conclusion

The study findings suggest that including 50 % FAWM protein in fish feed, particularly from fermented *A. pinnata*, significantly improved the growth performance, feed utilization, and health status of Asian catfish, while also boosting the farm's economic efficiency. Additionally, this study offers new opportunities for fish feed manufacturers to utilize FAWM-based protein to develop cost-effective diets for sustainable aquaculture, benefiting both the industry and end users.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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