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Partial replacement of fish meal with biologically fermented fish silage meal enhances nutrient utilisation in striped catfish (Pangasianodon hypophthalmus)

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Abstract

Fish-processing residues represent a promising resource for circular aquafeed production, with the potential to reduce dependence on conventional fish meal while improving nutrient-use efficiency. This study evaluated the effects of replacing the fish-meal fraction of diets for juvenile striped catfish (*Pangasianodon hypophthalmus*) with biologically fermented fish silage meal (FSM). Five diets were formulated to provide 0, 25, 50, 75, or 100% replacement of the fish-meal fraction and were tested in a completely randomised design. Fish were fed to apparent satiation three times daily. Apparent digestibility, nutrient retention, growth performance, digestive-enzyme activity, haematological and serum biochemical indices, water quality, ammonia excretion, and nitrogen- and phosphorus-related waste indicators were assessed. The FSM50 diet produced the highest apparent protein digestibility (97.57%), energy digestibility (81.65%), and protein retention (34.09%). It also yielded the lowest numerical feed-conversion ratio (1.13), while survival remained 100% in all treatments. Ammonia excretion was numerically lowest in FSM50 (0.012 mg TAN g-1 h-1). By contrast, complete replacement was associated with lower digestive-enzyme activities and higher total ammonia nitrogen, nitrite, and total suspended solids than the intermediate replacement treatments. Overall, replacing approximately half of the fish-meal fraction with biologically fermented FSM maintained growth and survival while improving selected indicators of nutrient utilisation. Further work using strictly isoenergetic and isonitrogenous diets, independent water circuits, and complete nutrient mass balances is required to confirm the environmental and production-scale benefits.

1 Introduction

Aquaculture is an increasingly important component of global food production, yet its continued expansion depends on feeds that are nutritionally adequate, economically viable, and environmentally responsible (Tacon and Metian, 2008; Amer et al., 2019). Feed quality directly affects growth, nutrient retention, feed-conversion efficiency, and the release of nitrogen and phosphorus into culture systems. Feed also represents one of the largest variable costs in intensive aquaculture, making the choice of dietary protein source a major determinant of production efficiency (Ismail et al., 2015). Fish meal remains a valuable ingredient because of its high protein content, balanced amino-acid profile, digestibility, and palatability; however, its supply and price are constrained by competing demand from aquaculture, livestock, pet-food, and other sectors. Reducing reliance on conventional fish meal is therefore a major priority in sustainable aquaculture nutrition (Sarker, 2023; Maksimenko et al., 2024).

The value of alternative aquafeed ingredients cannot be judged solely by their ability to sustain growth. Their nutrient composition, digestibility, amino-acid availability, effects on feed efficiency, physiological responses, and contribution to nutrient discharge must also be considered. Protein that is poorly digested or inefficiently retained is lost in faeces or catabolised and excreted as dissolved nitrogenous compounds. Consequently, improving protein digestibility and feed-conversion efficiency may enhance production while reducing the environmental burden associated with uneaten feed, faecal matter, ammonia, and phosphorus. Studies of alternative formulations for gilthead seabream have shown that

well-balanced diets containing non-conventional ingredients can maintain or improve nutrient utilisation, although the outcome depends strongly on ingredient quality, amino-acid balance, and processing method (Aragao et al., 2020).

The conversion of fish-processing by-products and low-value fish into feed ingredients is a practical circular-bioeconomy strategy. Heads, viscera, frames, skin, trimmings, and discarded fish often remain underutilised despite containing proteins, lipids, minerals, essential amino acids, and other potentially valuable compounds. Recovering these materials for aquafeed can reduce organic-waste disposal, improve resource-use efficiency, and lessen pressure on conventional feed resources. Accordingly, circular seafood production increasingly emphasises the valorisation of processing by-products rather than their treatment as waste (Cooney et al., 2023).

Fish silage is produced by liquefying and preserving fish or fish-processing residues through acidification, endogenous enzymatic hydrolysis, microbial fermentation, or combinations of these processes. In biologically fermented silage, lactic acid bacteria metabolise an added carbohydrate source and produce organic acids that lower pH, inhibit spoilage microorganisms, and stabilise the substrate. During ensiling, endogenous and microbial proteases partially hydrolyse proteins into smaller peptides and free amino acids, potentially increasing nutrient accessibility. However, excessive hydrolysis, prolonged storage, lipid oxidation, and the accumulation of free amino acids can reduce product quality. Temperature, fermentation duration, oxygen exposure, substrate composition, and starter culture are therefore critical determinants of silage stability and nutritional value (Sajib et al., 2020a, 2020b; Raeesi et al., 2021).

Recent evidence further shows that fermentation conditions and microbial selection strongly influence fish-silage quality. Microbial ensiling can stabilise fish waste and generate a protein-rich feed ingredient, whereas differences in raw material and carbohydrate source affect acidification, protein recovery, and storage stability (Ghosh et al., 2023). Native lactic acid bacteria may also perform differently from commercial starter cultures, indicating that strain selection should be matched to the substrate and the desired degree of hydrolysis (Ghosh et al., 2024). These findings support biological ensiling as a comparatively low-energy valorisation approach, particularly where conventional fish-meal processing is limited by capital or scale.

The nutritional value of fish silage nevertheless varies substantially with the raw material and processing method. Silages produced from fish viscera can provide digestible energy, essential amino acids, long-chain fatty acids, and minerals suitable for aquafeed. In juvenile tambaqui, acid and fermented viscera silages showed high apparent energy digestibility, while protein digestibility approached 92% in one fermented formulation (Santana et al., 2023). Feeding trials with fermented animal-derived silage have likewise shown that substantial fish-meal replacement can be achieved without necessarily impairing growth, digestive-enzyme activity, or tissue integrity when diets remain nutritionally balanced (Mbokane et al., 2022).

High digestibility, however, does not necessarily translate into efficient nutrient retention or optimal growth. Excessive replacement may alter indispensable amino-acid balance, dietary lipid and mineral concentrations, palatability, or the availability of nutrients required for tissue synthesis. Protein hydrolysis during silage production may also modify endogenous digestive-enzyme secretion because part of the dietary protein is already present as peptides and free amino acids. Graded inclusion studies are therefore needed to identify the level at which the nutritional advantages of silage are retained without compromising physiological condition, feed efficiency, or environmental performance. Current reviews consequently emphasise that fish silage should be treated as a biologically variable ingredient whose quality depends on raw material, fermentation control, preservation, drying, and final diet formulation (Maksimenko et al., 2024; Santana et al., 2023).

Striped catfish (*Pangasianodon hypophthalmus*) is an economically important freshwater species that is widely farmed in Southeast Asia. Its omnivorous feeding habit provides opportunities for the use of alternative protein ingredients, but formulated diets must still provide sufficient digestible protein, energy, indispensable amino acids, lipids, vitamins, and minerals to support rapid growth. Information remains limited on the combined effects of biologically fermented fish silage meal on digestibility, nutrient retention, digestive-enzyme activity, haematological and biochemical responses, growth, and nutrient-related waste indicators in this species. The present study addresses this gap through a graded fish-meal replacement trial that integrates nutritional, physiological, production, and environmental endpoints.

Accordingly, this study aimed to determine an appropriate level of fish-meal replacement with biologically fermented fish silage meal in diets for juvenile striped catfish. The response was evaluated using apparent nutrient digestibility, digestive-enzyme activity, nutrient retention, feed utilisation, growth performance, survival, haematological and serum biochemical indicators, ammonia excretion, and nitrogen- and phosphorus-related waste variables. We hypothesised that partial replacement would maintain or improve nutrient utilisation and growth, whereas excessive substitution would reduce nutrient retention and feed-use efficiency because of changes in ingredient composition and dietary nutrient balance.

2 Materials and methods

2.1 Experimental location and study design

The feeding trial and laboratory analyses were conducted at university fisheries facilities in Malang, East Java, Indonesia. Proximate composition and digestibility were analysed in a fish and animal nutrition laboratory, water-quality variables were analysed in a dedicated water-quality laboratory, and haematological measurements were performed in the fisheries laboratory. The experiment followed a completely randomised design with five dietary treatments and three replicate experimental units per treatment (15 aquaria in total). The feeding phase lasted 60 days, while water-quality monitoring continued until day 84.

2.2 Production of biologically fermented fish silage meal

Indian scad (*Decapterus spp.*) was cleaned and ground. For each 5-kg batch of ground fish, 150 mL of a lactic acid bacterial inoculum (approximately 10^9 CFU mL-1) and 20% molasses were added, and the mixture was fermented for 5 days. The inoculum was isolated from the digestive tract of Indian scad and cultured on de Man, Rogosa and Sharpe (MRS) agar under anaerobic conditions for 48–72 h. Colonies with characteristics consistent with lactic acid bacteria were purified and evaluated for cell morphology, Gram reaction, catalase activity, gas production from glucose, amylolytic activity, and carbohydrate-fermentation profiles using the API 50 CHL system. The fermented material was dried at 50°C for 48 h and milled to produce fish silage meal. Moisture, crude protein, crude lipid, and ash were determined according to standard AOAC procedures (Latimer, 2023). These measurements were selected as core indicators of the proximate nutritional composition of the silage, consistent with previous evaluations of fish silage and fish-derived feed ingredients (Shao et al., 2020). All chemical analyses were performed in triplicate, and results were expressed as percentages of the fish silage meal.

2.3 Experimental diets

Five experimental diets were formulated to replace 0, 25, 50, 75, or 100% of the fish-meal fraction with fish silage meal (FSM). The diets were designed to contain approximately 32% crude protein and a gross-energy-to-protein ratio of approximately 14 kcal g-1 protein. Chromium(III) oxide was included as an inert marker for digestibility determination. Ingredient composition and analysed nutrient content are presented in Table 1.

Table 1
Ingredient composition and analysed nutrient content of the experimental diets (g/kg)

Ingredient or variable	FSM0	FSM25	FSM50	FSM75	FSM100
Fish meal	44.0	33.5	22.0	12.0	0.0
Fish silage meal	0.0	12.5	22.0	33.0	44.0
Soybean meal	23.0	21.0	24.0	23.0	26.0
Wheat flour	21.0	21.0	20.0	20.0	18.0
Maize oil	3.0	3.0	3.0	3.0	3.0
Fish oil	3.0	3.0	3.0	3.0	3.0
Vitamin-mineral premix	3.0	3.0	3.0	3.0	3.0
Carboxymethyl cellulose	3.0	3.0	3.0	3.0	3.0
Chromium (III) oxide	0.6	0.6	0.6	0.6	0.6
Reported total	100.6	100.6	100.6	100.6	100.6
Crude protein (% dry matter)	31.69	32.16	32.47	32.81	33.55
Crude lipid (% dry matter)	11.04	11.65	11.7	11.69	12.1
Crude fibre (% dry matter)	1.49	1.43	0.17	1.31	1.23
Nitrogen-free extract (% dry matter)	38.69	39.12	41.6	42.98	44.8
Reported gross energy (kcal 100 g − 1)	439.3	452.16	464.53	472.03	487.57
Energy-to-protein ratio (kcal g − 1)	13.86	14.06	14.31	14.36	14.53

Values are percentages unless otherwise indicated. FSM, fish silage meal replacement level.

2.4 Fish rearing and feeding

Fish were acclimated to laboratory conditions for 2 weeks and were fed a commercial or reference diet containing approximately 30–32% crude protein. Before the experimental period, fish were fasted for 24 h, weighed, and randomly assigned to aquaria (60 x 40 x 40 cm; working volume 48 L). Experimental diets were offered to apparent satiation three times daily at 08:00, 13:00, and 18:00. All aquaria were aerated. Uneaten feed and settled waste were removed by siphoning twice daily, and approximately 20% of the water volume was replaced each day. Each aquarium had an independent recirculation loop.

2.5 Digestibility assessment and faecal collection

Fish were acclimated to the digestibility aquaria for 7 days before faecal collection. Following dietary adaptation, faeces were collected for approximately 20 days by siphoning at 2-h intervals to minimise nutrient leaching. Samples were dried at 60°C for 48 h and stored frozen until analysis. Moisture, ash,

crude protein, crude lipid, and crude fibre were determined using standard AOAC procedures. Chromium(III) oxide concentrations in diets and faeces were measured after wet digestion by spectrophotometry at 350 nm. Apparent digestibility coefficients were calculated from the ratios of marker and nutrient concentrations in the diets and faeces.

2.6 Growth, nutrient retention, and physiological measurements

Body mass was recorded at the beginning of the experiment and at 30-day intervals. Feed intake, specific growth rate, feed-conversion ratio, feed efficiency, survival, and nutrient-retention efficiencies were calculated using standard equations (Takeuchi, 1988; Watanabe, 1988). At the end of the trial, three fish from each experimental unit were sampled. Intestinal tissue (1 g) was homogenised in 10 mL of 0.05 M phosphate buffer (pH 7.0) and centrifuged at 12,000 x g for 20 min at 4°C using a refrigerated centrifuge (Model 3740, KUBOTA Corporation, Japan). The supernatant was used to determine protease, amylase, and lipase activities with commercial reagents or assay kits from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan), Kikkoman Biochemifa Company (Tokyo, Japan), and Sumitomo Bakelite Co., Ltd. (Tokyo, Japan), respectively. Muscle and hepatic glycogen concentrations, viscerosomatic index, and hepatosomatic index were also determined. Fish were anaesthetised with MS-222 (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) before blood was collected from the caudal vein. Haematocrit, erythrocyte count, leukocyte count, and haemoglobin concentration were measured using a Sysmex haematology analyser (Sysmex Corporation, Kobe, Japan). Serum glucose, cholesterol, and total protein were determined using commercial kits from FUJIFILM Wako Pure Chemical Corporation and a Hitachi automated chemistry analyser (Hitachi High-Tech Corporation, Tokyo, Japan).

2.7 Water quality and waste indicators

Water temperature, pH, and dissolved oxygen were monitored three times daily. Alkalinity, total ammonia nitrogen (TAN), unionised ammonia, nitrite, nitrate, phosphate, and total suspended solids (TSS) were measured at 2-week intervals using standard spectrophotometric methods. Absorbance was measured with a UV-Vis spectrophotometer (UV-1900i, Shimadzu Corporation, Kyoto, Japan). Nitrogen and phosphorus concentrations in uneaten feed and faeces were also determined as indicators of nutrient discharge during the experimental period.

2.8 Statistical analysis

The experimental design comprised five dietary treatments with three replicate aquaria per treatment. One-way analysis of variance (ANOVA) was performed in EZR version 3.5.1 (R Core Team, Vienna, Austria). Differences among treatment means were evaluated at $p < 0.05$ using the post hoc grouping reported in the corresponding tables. The aquarium was treated as the experimental unit.

2.9 Animal ethics

The study was conducted in accordance with applicable international, national, and institutional guidelines for the care and use of animals. The authors report that no formal ethical approval number was required for this study.

3 Results

3.1 Apparent digestibility and digestive-enzyme activity

Dietary replacement level influenced apparent nutrient digestibility and digestive-enzyme activity. Apparent protein digestibility was highest in FSM50 (97.57 ± 0.32%), and apparent energy digestibility also reached its maximum in this treatment (81.65 ± 0.97%). Apparent phosphorus digestibility was lowest in FSM100 (49.21 ± 0.57%). Protease activity was highest in FSM0 and decreased at all levels of fish-silage inclusion. By contrast, amylase activity was highest in FSM50, while lipase activity remained comparatively high in FSM0 and FSM50 but declined markedly in FSM75 and FSM100 (Table 2).

Table 2
Apparent nutrient digestibility and digestive-enzyme activities in striped catfish fed graded levels of fish silage meal

Variable	FSM0	FSM25	FSM50	FSM75	FSM100
Protein digestibility (%)	94.93 ± 0.11^{c}	96.08 ± 0.47^{b}	97.57 ± 0.32^{a}	96.45 ± 0.98^{b}	95.39 ± 0.29^{bc}
Energy digestibility (%)	76.22 ± 0.54^{b}	78.29 ± 0.91^{b}	81.65 ± 0.97^{a}	76.49 ± 1.28^{b}	75.92 ± 0.83^{b}
Phosphorus digestibility (%)	61.86 ± 1.68 ^{a}	62.95 ± 3.11 ^{a}	61.08 ± 0.83 ^{a}	64.17 ± 2.79 ^{a}	49.21 ± 0.57 ^{b}
Protease (U mg − 1 protein)	0.25 ± 0.01 ^{a}	0.09 ± 0.00 ^{d}	0.14 ± 0.01 ^{c}	0.08 ± 0.01 ^{d}	0.06 ± 0.00 ^{e}
Amylase (U mg − 1 protein)	8.51 ± 0.36 ^{b}	4.85 ± 0.07 ^{d}	11.76 ± 0.55 ^{a}	3.54 ± 0.11 ^{e}	1.87 ± 0.05 ^{f}
Lipase (U mg − 1 protein)	10.85 ± 0.29 ^{a}	6.70 ± 0.09 ^{c}	9.99 ± 0.37 ^{b}	3.23 ± 0.06^{d}	2.52 ± 0.08 ^{e}

Means ± SE (n = 3) followed by different superscripts in the same row are significantly different at p < 0.05 (DMRT).

3.2 Growth performance, nutrient retention, and ammonia excretion

Initial body weight was similar among treatments, ranging from 2.73 ± 0.13 to 2.84 ± 0.17 g. At the end of the feeding trial, final body weight ranged from 24.75 ± 0.24 to 25.93 ± 0.56 g. Fish fed FSM50 had the

highest numerical final body weight (25.93 ± 0.56 g) and weight gain (23.14 ± 0.41 g), whereas FSM100 produced the lowest numerical values (24.75 ± 0.24 and 21.93 ± 0.33 g, respectively). Specific growth rate varied only slightly among treatments (2.42–2.49% day-1), and survival was 100% in all groups. Feed intake also varied within a narrow range. Protein retention was significantly highest in FSM50 (34.09 ± 0.04%). This treatment also produced the lowest numerical feed-conversion ratio (1.13 ± 0.02), whereas FSM100 produced the highest numerical value (1.36 ± 0.04); however, the reported post hoc grouping did not indicate a significant treatment effect on feed-conversion ratio. Ammonia excretion was lowest in FSM50 (0.012 ± 0.003 mg TAN g-1 h-1) and increased significantly at the two highest replacement levels, reaching 0.020 ± 0.004 mg TAN g-1 h-1 in FSM100 (Table 3).

Table 3
Growth performance, nutrient retention, survival, and ammonia excretion of striped catfish

Variable	FSM0	FSM25	FSM50	FSM75	FSM100
Feed intake (g)	262.50 ± 15.80 [a]	253.50 ± 11.32 [c]	259.50 ± 13.75 [b]	261.00 ± 4.50 [a]	261.00 ± 11.91 [a]
Protein retention (%)	27.38 ± 1.47 [b]	28.48 ± 2.38 [b]	34.09 ± 0.04 [a]	26.91 ± 1.57 [b]	27.16 ± 1.50 [b]
Lipid retention (%)	9.15 ± 0.71 [b]	11.97 ± 0.60 [b]	18.23 ± 0.74 [a]	18.61 ± 1.45 [a]	10.92 ± 1.73 [b]
Energy retention (%)	5.34 ± 1.16 [a]	5.86 ± 0.08 [a]	5.63 ± 0.85 [a]	5.99 ± 1.91[a]	5.70 ± 0.60 [a]
Specific growth rate (% day − 1)	2.45 ± 0.05 [a]	2.49 ± 0.05 [a]	2.48 ± 0.04 [a]	2.43 ± 0.02 [a]	2.42 ± 0.60 [a]
Feed-conversion ratio	1.21 ± 0.00 [a]	1.22 ± 0.02 [a]	1.13 ± 0.02 [a]	1.24 ± 0.01 [a]	1.36 ± 0.04 [a]
Survival (%)	100.00 ± 0.00 [a]	100.00 ± 0.00 [a]	100.00 ± 0.00 [a]	100.00 ± 0.00 [a]	100.00 ± 0.00 [a]
Ammonia excretion (mg TAN g − 1 h − 1)	0.014 ± 0.002 [a]	0.013 ± 0.004 [a]	0.012 ± 0.003 [a]	0.016 ± 0.002 [b]	0.020 ± 0.004 [c]
Initial body weight (g)	2.84 ± 0.17	2.73 ± 0.13	2.79 ± 0.15	2.83 ± 0.06	2.81 ± 0.14
Final body weight (g)	25.85 ± 0.57	25.68 ± 0.22	25.93 ± 0.56	25.10 ± 0.18	24.75 ± 0.24
Weight gain (g)	23.01 ± 0.41	22.94 ± 0.26	23.14 ± 0.41	22.27 ± 0.17	21.93 ± 0.33

Means ± SE (n = 3) followed by different superscripts in the same row are significantly different at p < 0.05 (DMRT).

3.3 Somatic indices and blood variables

Viscerosomatic and hepatosomatic indices were highest in FSM50 and lowest in FSM100. Several haematological and serum biochemical variables differed among diets; however, their biological interpretation requires reference intervals that are appropriate for fish size, husbandry conditions, and analytical method. FSM50 showed the highest serum total protein, while serum glucose was lowest in FSM50 and highest in FSM100 (Table 4).

Table 4
Somatic indices, haematological variables, and serum biochemical variables

Variable	FSM0	FSM25	FSM50	FSM75	FSM100
Viscerosomatic index (%)	3.70 ± 0.14 ^{bc}	3.94 ± 0.07 ^{b}	4.30 ± 0.03 ^{a}	3.63 ± 0.30 ^{c}	2.80 ± 0.05 ^{d}
Hepatosomatic index (%)	2.83 ± 0.10 ^{b}	2.39 ± 0.09 ^{c}	3.17 ± 0.03 ^{a}	2.12 ± 0.13 ^{d}	1.33 ± 0.03 ^{e}
Haematocrit (%)	24.64 ± 0.45 ^{b}	25.46 ± 0.20 ^{a}	23.85 ± 0.37 ^{c}	25.94 ± 0.60 ^{a}	22.43 ± 0.50 ^{d}
Erythrocytes (10^5 cells mL − 1)	73.74 ± 0.48 ^{b}	73.10 ± 0.15 ^{b}	75.12 ± 0.40 ^{a}	75.41 ± 0.35 ^{a}	75.34 ± 0.39 ^{a}
Leukocytes (10^3 cells mL − 1)	68.64 ± 0.38 ^{b}	75.05 ± 0.73 ^{a}	63.32 ± 0.68 ^{d}	65.06 ± 0.07 ^{c}	69.20 ± 0.31 ^{b}
Haemoglobin (g dL − 1)	7.10 ± 0.20 ^{a}	6.73 ± 0.21 ^{a}	6.97 ± 0.21 ^{a}	6.50 ± 0.20 ^{a}	6.63 ± 0.15 ^{a}
Glucose (mg dL − 1)	36.13 ± 0.21 ^{abc}	33.30 ± 0.20 ^{abc}	26.33 ± 0.15 ^{ab}	41.37 ± 0.15 ^{bc}	44.33 ± 0.15 ^{c}
Cholesterol (mg dL − 1)	62.53 ± 0.21 ^{ab}	66.43 ± 0.15 ^{abc}	79.20 ± 0.10 ^{c}	78.40 ± 0.10 ^{bc}	65.47 ± 0.21 ^{abc}
Serum total protein (reported mg dL − 1)	23.40 ± 0.20 ^{ab}	31.33 ± 0.15 ^{ab}	36.30 ± 0.10 ^{b}	31.30 ± 0.10 ^{ab}	22.40 ± 0.10 ^{a}

Means ± SE (n = 3) followed by different superscripts in the same row are significantly different at $p < 0.05$ (DMRT).

3.4 Water quality and nitrogen- and phosphorus-related waste indicators

Temperature, pH, dissolved oxygen, and alkalinity remained within relatively narrow ranges across dietary treatments (Table 5). Nitrogen concentration in uneaten feed varied only modestly, whereas phosphorus concentration declined progressively as fish-silage replacement increased. Faecal nitrogen was lowest in FSM50 and FSM75, and faecal phosphorus was lowest in FSM100 (Table 6). Across repeated observations, FSM100 tended to show higher TAN, nitrite, and TSS, while FSM50 generally

showed comparatively low nitrite, nitrate, and phosphate concentrations (Fig. 1). Because these water-quality variables were measured repeatedly over time, a repeated-measures or mixed-effects analysis and expression of nutrient outputs as loads would provide a stronger basis for claims about waste reduction.

Table 5
Ranges of water-quality variables during the feeding trial

Variable	FSM0	FSM25	FSM50	FSM75	FSM100
Temperature (°C)	28.1–29.2	28.3–29.6	28.5–29.4	28.4–29.5	28.5–30.0
pH	7.2–7.3	7.1–7.2	7.3–7.6	7.2–7.4	7.4–7.6
Dissolved oxygen (mg L − 1)	4.7–5.2	4.8–5.2	4.7–5.4	4.8–5.3	4.7–5.5
Alkalinity (mg L − 1)	120–182	128–198	120–182	116–190	124–180
Water quality was monitored three times daily					

Table 6
Nitrogen and phosphorus concentrations in uneaten feed and faeces

Variable	FSM0	FSM25	FSM50	FSM75	FSM100
Nitrogen in uneaten feed (%)	3.17 ± 0.17 a	3.21 ± 0.16 a	3.12 ± 0.12 b	3.12 ± 0.11 b	3.12 ± 0.15 b
Phosphorus in uneaten feed (%)	0.59 ± 0.01 a	0.54 ± 0.04 b	0.46 ± 0.02 c	0.40 ± 0.03 d	0.37 ± 0.05 e
Faecal nitrogen (%)	1.47 ± 0.11 a	1.24 ± 0.23 b	0.95 ± 0.05 d	0.98 ± 0.24 d	1.13 ± 0.24 c
Faecal phosphorus (%)	1.35 ± 0.05 a	1.27 ± 0.02 b	1.34 ± 0.07 a	0.86 ± 0.02 c	0.63 ± 0.06 d

Means ± SE (n = 3) followed by different superscripts in the same row are significantly different at p < 0.05 (DMRT).

4 Discussion

4.1 Overall response to graded fish-meal replacement

The response to graded replacement of fish meal with biologically fermented fish silage meal was distinctly non-linear. Among the diets tested, FSM50 produced the strongest overall nutritional response, with significantly higher apparent protein and energy digestibility and significantly greater protein retention. Specific growth rate and survival, however, did not differ significantly among treatments. FSM50 also produced the lowest numerical feed-conversion ratio, but the post hoc grouping reported in

Table 3 indicates that this difference was not statistically significant. The evidence therefore supports FSM50 primarily as the most nutritionally efficient treatment rather than as a diet that significantly accelerated growth.

An intermediate optimum is consistent with the wider aquafeed literature, in which partial replacement often performs more favourably than complete substitution. Fermentation can improve nutrient accessibility, palatability, and functional characteristics of alternative ingredients, but outcomes depend on replacement level, species, ingredient composition, and diet formulation (Kari et al., 2022; Mbokane et al., 2022; Mugwanya et al., 2023). In gilthead seabream, appropriately balanced alternative formulations maintained or improved nutrient utilisation, whereas less suitable combinations reduced nutrient retention and increased losses (Aragao et al., 2020). The present response in striped catfish therefore suggests that fish silage meal complemented the nutritional contribution of fish meal at an intermediate replacement level but did not fully reproduce it when fish meal was completely removed.

The less favourable response to FSM100 probably reflects several interacting factors rather than a single nutritional limitation. Progressive replacement changed not only the protein source but also the analysed crude-protein, lipid, carbohydrate, and gross-energy contents of the diets. The treatments were therefore not strictly isoenergetic and isonitrogenous, and the observed effects cannot be attributed solely to fish silage meal. Complete replacement may also have altered indispensable amino-acid balance, peptide-size distribution, mineral load, lipid quality, pellet characteristics, or feeding attractiveness. Each of these factors can influence the performance of fermented and hydrolysed feed ingredients (Maksimenko et al., 2024; Sarker, 2023).

4.2 Apparent digestibility and digestive-enzyme responses

The high apparent protein digestibility observed across all diets, and particularly in FSM50, is biologically plausible because ensiling promotes endogenous and microbial proteolysis. During fermentation, intact proteins are progressively converted into soluble proteins, peptides, and free amino acids that may require less gastrointestinal hydrolysis before absorption. Studies of herring co-products have shown that both temperature and fermentation duration influence the degree of protein hydrolysis while also affecting lipid oxidation and non-enzymatic browning (Sajib et al., 2020a; Sajib and Undeland, 2020b). Fish-viscera silages have likewise shown high apparent digestibility of energy and protein in juvenile fish, confirming that properly prepared silage can provide readily accessible nutrients (Santana et al., 2023).

Greater hydrolysis is not invariably beneficial. Excessive proteolysis can increase the proportion of free amino acids and very small peptides, which may be absorbed rapidly but not necessarily in proportions that match the requirements for tissue-protein synthesis. When amino-acid supply is asynchronous or imbalanced, a greater fraction may be deaminated and used as an energy source rather than retained for growth. Fermentation conditions, carbohydrate source, starter culture, storage duration, oxygen exposure, and raw-material composition all influence this balance (Ghosh et al., 2023, 2024; Raeesi et al., 2021). The combination of lower protein retention and higher ammonia excretion at the highest

replacement level is therefore compatible with efficient digestion but less efficient post-absorptive nitrogen utilisation.

Digestive-enzyme activities also showed a non-linear response. Protease activity decreased after fish silage meal was introduced, whereas amylase activity peaked in FSM50 and lipase activity remained comparatively high in FSM0 and FSM50. Lower endogenous protease activity does not necessarily imply impaired digestion because partially hydrolysed dietary protein may reduce the requirement for luminal proteolysis. Previous studies have shown that fermented ingredients can alter digestive-enzyme secretion, with the direction and magnitude of the response depending on substrate, inclusion level, and fish species (Kari et al., 2022; Mugwanya et al., 2023). Nevertheless, the simultaneous reduction in protease, amylase, and lipase activities in FSM100, together with its poorer numerical feed conversion, suggests a broader change in digestive function or diet physical quality. Recent work with fermented fish silage similarly indicates that positive enzyme responses are most evident when fermentation is well controlled and the final diet remains nutritionally balanced (Zaza et al., 2026).

Interpretation of the enzyme data would be strengthened by additional characterisation of the ingredient and final pellets, including degree of protein hydrolysis, peptide-molecular-weight distribution, lipid-oxidation indices, amino-acid composition, pellet-water stability, and intestinal histomorphology. These measurements would help distinguish adaptive downregulation in response to pre-hydrolysed nutrients from a genuine reduction in digestive capacity.

4.3 Growth performance, feed utilisation, and nutrient retention

The lack of significant differences in specific growth rate, together with 100% survival across treatments, indicates that juvenile striped catfish tolerated a wide range of fish silage meal inclusion during the experimental period. Nutrient-retention data nevertheless revealed differences that were not apparent from growth alone. Protein retention was significantly highest in FSM50, while lipid retention was highest in FSM50 and FSM75. Similar growth can therefore arise from different patterns of nutrient deposition and catabolism. For this reason, alternative feed ingredients should be evaluated using digestibility, retention, and feed-efficiency metrics in addition to growth.

The numerical feed-conversion ratio of 1.13 in FSM50 was lower than those of the other treatments, but Table 3 does not indicate a significant treatment effect on this variable. Numerical rankings should therefore not be interpreted as statistically demonstrated improvements. Even so, the concurrent increases in protein digestibility and protein retention provide a plausible physiological basis for the favourable numerical feed-conversion response. Comparable studies have reported improved feed utilisation at intermediate levels of fermented ingredient replacement, whereas excessive substitution can impair performance because of amino-acid imbalance and other compositional constraints (Kari et al., 2022; Mbokane et al., 2022). Meta-analytical evidence likewise identifies replacement level and species as major moderators of responses to fermented protein sources (Mugwanya et al., 2023).

The balance between dietary protein and available energy is particularly relevant to these findings. Amino acids are retained efficiently only when adequate non-protein energy is available and indispensable amino acids are supplied in appropriate proportions. Otherwise, amino acids are oxidised, increasing ammonia production and reducing protein-use efficiency. In European grayling, dietary protein and lipid levels jointly affected feed efficiency, serum biochemistry, and ammonia excretion (Rahimnejad et al., 2021). Nitrogen-balance studies in common carp have similarly shown that feeding strategy and protein supply alter retained, excreted, and unaccounted nitrogen fractions (Mes et al., 2023). The higher ammonia excretion observed in FSM75 and FSM100 is therefore consistent with declining efficiency of absorbed nitrogen retention at high replacement levels.

The relatively narrow range of feed intake and the 100% survival observed across treatments indicate that all diets were broadly accepted and did not cause acute mortality. Feed intake should nevertheless be standardised per fish or unit biomass when treatment efficiency is compared, and the aquarium, rather than the individual fish, should remain the experimental unit for growth and feed-utilisation analyses.

4.4 Somatic indices and blood variables

The somatic-index and blood data indicate that diet composition influenced nutrient partitioning and physiological status, but these responses require cautious interpretation. Hepatosomatic and viscerosomatic indices were highest in FSM50 and lowest in FSM100. Higher values may reflect greater hepatic glycogen or lipid storage, digestive-organ mass, or overall nutrient deposition; without histology and tissue-composition data, they cannot be classified as beneficial or pathological. Conversely, the lower indices in FSM100 may indicate reduced energy reserves, altered organ development, or lower visceral tissue deposition rather than improved carcass quality.

The higher serum total protein and lower glucose in FSM50 may be consistent with favourable protein status and lower mobilisation of circulating glucose. Previous feeding studies have shown that alternative and fermented protein sources can influence serum proteins, glucose, haematological indices, and intestinal structure without necessarily reducing survival (Kari et al., 2022; Mbokane et al., 2022). Dietary supplementation with compounds such as L-arginine has also been reported to influence haematobiochemical variables and hepatic antioxidant activity in Nile tilapia (Kamal et al., 2024). Protein and lipid levels can similarly alter cholesterol, glucose, and enzyme activities in otherwise clinically normal fish (Rahimnejad et al., 2021). Accordingly, statistically significant differences in blood variables should not automatically be interpreted as evidence of either improved health or metabolic stress.

Haemoglobin did not differ significantly among treatments, and the absolute differences in haematocrit, erythrocyte count, and leukocyte count were relatively modest. This pattern suggests that oxygen-carrying capacity was broadly maintained. Stronger physiological interpretation would require baseline measurements, species- and size-specific reference intervals, validation of analytical units, and complementary indicators such as albumin, globulin, alanine aminotransferase, aspartate aminotransferase, antioxidant enzymes, and tissue histology. Recent evidence that controlled fermented-

silage diets can support immune and intestinal responses further underscores the value of combining blood chemistry with histomorphological assessment (Zaza et al., 2026).

4.5 Ammonia excretion, water quality, and nutrient-waste indicators

Ammonia-excretion and faecal-nitrogen patterns were broadly consistent with the nutrient-retention results. FSM50 produced the lowest numerical ammonia-excretion rate and one of the lowest faecal-nitrogen concentrations, while also producing the highest protein retention. However, ammonia excretion in FSM50 was not significantly different from FSM0 or FSM25 according to the reported post hoc grouping. The appropriate interpretation is therefore that moderate replacement maintained low ammonia excretion, whereas the significant increases at FSM75 and FSM100 indicate less efficient nitrogen utilisation at high replacement levels.

This response is consistent with the principle that nitrogen discharge is governed by feed intake, protein digestibility, amino-acid balance, energy availability, and the proportion of absorbed nitrogen retained in tissue. Diets that improve feed conversion and nutrient retention generally decrease nitrogen output per unit biomass produced, whereas poorly balanced or inefficiently utilised protein increases branchial ammonia excretion and faecal losses (Aragao et al., 2020; Mes et al., 2023; Rahimnejad et al., 2021). The lower faecal nitrogen concentration in FSM50 and FSM75 may therefore indicate improved apparent utilisation, but concentration data alone cannot establish total nitrogen load unless faecal dry-matter production is also quantified.

The water-quality results require particular caution. Temperature, pH, dissolved oxygen, and alkalinity remained within relatively narrow ranges, suggesting that major differences in fish performance were unlikely to be driven by gross deterioration of basic culture conditions. In contrast, TAN, nitrite, nitrate, phosphate, and TSS changed over time, with FSM100 tending to show higher TAN, nitrite, and TSS. These concentrations were influenced not only by fish metabolism but also by daily water replacement, siphoning, nitrification, solids removal, feed disintegration, and temporal accumulation. Environmental performance should therefore be expressed as a nutrient load, for example grams of nitrogen or phosphorus released per kilogram of feed consumed or biomass produced, rather than solely as concentration in aquarium water.

The higher TSS observed in FSM100 may have originated from faecal production, uneaten feed, or reduced pellet-water stability. Fish silage contains soluble and hydrolysed fractions that can increase nutrient leaching when pellet binding and drying are inadequate. Measurements of pellet durability, water stability, dry-matter loss, and nutrient leaching would help determine whether the observed solids were generated metabolically or released physically from the feed. More broadly, environmental assessments of sustainable feed ingredients require nutrient mass balances and system-level indicators because ingredient origin alone does not establish environmental superiority (Owsianiak et al., 2022).

4.6 Circular-economy relevance, study limitations, and future research

Converting low-value fish biomass and processing residues into fermented silage is consistent with circular-bioeconomy principles because it recovers protein, lipid, minerals, and bioactive compounds that might otherwise be discarded. Seafood by-product valorisation can reduce waste-management pressure and generate useful feed ingredients, provided that safety, nutritional quality, process stability, and economic feasibility are demonstrated (Cooney et al., 2023). Fish silage may be particularly relevant where conventional fish-meal production is constrained by scale, capital, or energy demand. Recent work continues to show that controlled fermentation can stabilise fish residues and improve their suitability for aquafeed production (Palekar et al., 2026).

Circularity, however, does not remove the need for rigorous ingredient characterisation. Variation in raw fish material, endogenous enzyme activity, microbial inoculum, carbohydrate addition, fermentation temperature, storage duration, and drying conditions can substantially alter amino-acid availability, lipid oxidation, microbial safety, and final feed quality (Ghosh et al., 2023, 2024; Maksimenko et al., 2024; Raeesi et al., 2021). Standardisation should therefore include fermentation pH kinetics, microbial counts, proximate and amino-acid composition, biogenic amines, peroxide and anisidine values, mineral and salt content, pathogen screening, and shelf-life assessment.

Several methodological limitations should be considered when interpreting the present findings. First, analysed nutrient and energy contents changed progressively among diets, confounding the effect of ingredient replacement. Second, only three replicate aquaria were used per treatment, limiting statistical power and reinforcing the aquarium as the valid experimental unit. Third, endpoints measured once are appropriately examined with one-way ANOVA or regression approaches for graded inclusion, whereas repeatedly measured water-quality variables require repeated-measures or mixed-effects models that include time, diet, and their interaction. Fourth, environmental claims should be supported by complete nitrogen and phosphorus mass balances rather than concentration data alone.

Taken together, the data provide the strongest support for replacing approximately 50% of the fish-meal fraction with biologically fermented fish silage meal. At this level, striped catfish showed the highest apparent protein and energy digestibility and the greatest protein retention while maintaining growth and survival. Complete replacement was less favourable because it was associated with lower digestive-enzyme activities, less efficient protein utilisation, and higher nitrogenous-waste indicators. Future studies should use strictly isoenergetic and isonitrogenous diets, balanced amino-acid profiles, independent water circuits, greater replication, pellet-quality testing, intestinal and hepatic histology, and complete nutrient mass balances. Such work is needed to determine whether the apparent intermediate optimum is reproducible and commercially relevant.

5 Conclusions

Biologically fermented fish silage meal can replace approximately 50% of the fish-meal fraction in diets for juvenile striped catfish without compromising growth or survival. Among the evaluated treatments, FSM50 produced the highest apparent protein and energy digestibility and significantly greater protein retention. It also produced the lowest numerical feed-conversion ratio while maintaining comparatively low ammonia excretion and faecal nitrogen concentrations. Complete replacement, in contrast, was associated with lower digestive-enzyme activities, lower phosphorus digestibility, less efficient protein utilisation, and higher nitrogenous-waste indicators. These findings support the use of biologically fermented fish silage meal as a partial rather than complete substitute for conventional fish meal. At an appropriate inclusion level, this strategy may support nutrient recovery from low-value fish biomass while reducing dependence on conventional marine-derived feed ingredients. Confirmation of environmental and commercial benefits will require trials with strictly isoenergetic and isonitrogenous diets, balanced amino-acid profiles, greater replication, and complete nitrogen and phosphorus mass balances.

Declarations

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Ethics Statement

The study was conducted in accordance with applicable institutional, national, and international standards governing research and animal welfare. An ethics self-assessment was completed through the Secretariat of the Research Ethics Committee, National Research and Innovation Agency of the Republic of Indonesia (BRIN). According to the official Letter of Notification issued, the research was determined not to require formal ethical clearance; therefore, no ethics approval number was assigned.

Clinical trial number

Clinical trial number: not applicable.

Competing interests

The authors declare that they have no relevant financial or non-financial interests to disclose.

Data availability

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Generative AI and AI-assisted technologies in the writing process

During preparation of this manuscript, the authors used ChatGPT (OpenAI) to improve language, organisation, readability, and consistency with journal formatting. The authors subsequently reviewed and edited the manuscript and take full responsibility for its content.

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Figures

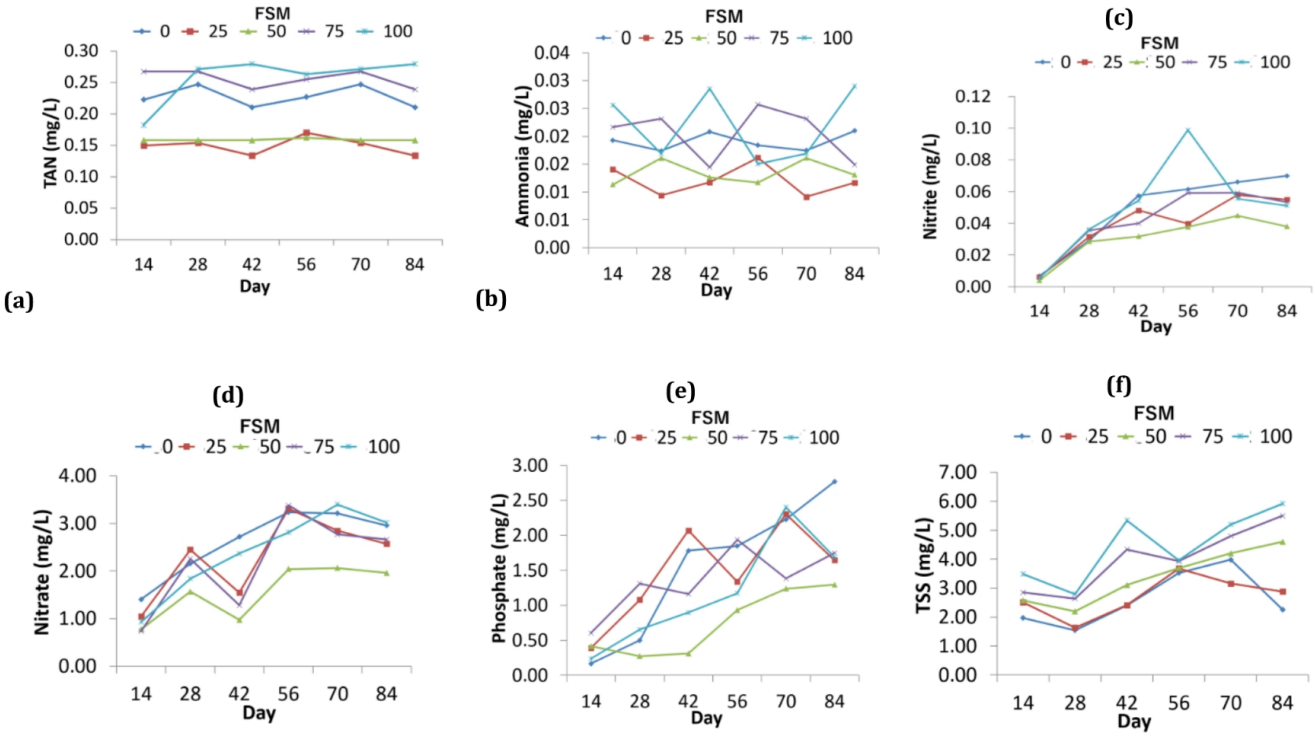


Figure 1

Changes in (a) total ammonia nitrogen, (b) unionised ammonia, (c) nitrite, (d) nitrate, (e) phosphate, and (f) total suspended solids during the striped catfish feeding trial