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Proximate Evaluation of Fermented Tuna Viscera Substituted with Fish Meal on Growth, FCR, Protein Retention, and Enzyme Activity of Striped Catfish Fry

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ABSTRACT

This study aimed to evaluate the effects of substituting fish meal with fermented tuna viscera meal on the growth performance, feed conversion ratio (FCR), protein retention, and protease enzyme activity in the striped catfish fry. The experimental diets incorporated different substitution levels (0, 10, 20, and 30%) of fermented tuna viscera meal. Parameters evaluated included survival rate, specific growth rate (SGR), feed conversion ratio (FCR), protein retention, and protease enzyme activity. Fry fed diets with 20% FTV substitution demonstrated the highest growth performance, reflected by the superior SGR (3.44%/day), lowest FCR (1.56 g/g), highest protein retention (26.04%), and protease activity (6.10 U/mg). Survival rates were high (>98%) and statistically similar across all treatments, indicating no detrimental effect of FTV inclusion. Results indicated that a 20% substitution of FM with FTV significantly enhanced feed efficiency, growth, and protein utilization in *Pangasius* fry. Thus, FTV represents a viable, cost-effective, and environmentally sustainable alternative protein source for Pangasius aquaculture.

INTRODUCTION

High-protein diets are essential in aquaculture to ensure optimal growth rates in farmed fish **(Hua *et al.*, 2019; Ndebele-Murisa *et al.*, 2024; Islamy *et al.*, 2025)**. Fish meal (FM), traditionally the predominant protein source in aquafeeds, is valued for its digestibility, palatability, and balanced amino acid composition **(Pratama *et al.*, 2020; Macusi *et al.*, 2023; Hussain *et al.*, 2024)**. Nevertheless, the rapid expansion of aquaculture has intensified demand for FM, leading to supply shortages and rising costs, which underscores the need for viable alternative protein sources **(Serdiati *et al.*, 2024)**. Given that feed costs represent up to 60–70% of total production expenditures in intensive systems **(Kaleem & Sabi, 2021)**, identifying affordable and sustainable FM substitutes is crucial for the long-term viability of the sector **(Shahin & Abdullah, 2023)**.

For striped catfish, a widely farmed species in Southeast Asia, fish meal is a key ingredient in commercial diets **(Arriaga-Hernández *et al.*, 2021; Samara *et al.*, 2024;**

Tran *et al.*, 2024) but also one of the main cost drivers, prompting interest in cheaper protein sources such as plant and animal by-products. While these alternatives can partially replace fish meal, most have nutritional or palatability limitations, and fish growth still tends to be at its best when a significant portion of the diet is fish meal **(Lee *et al.*, 2023)**.

One promising approach to reduce reliance on conventional fish meal is to utilize nutrient-rich waste from fish processing. Fish processing by-products (heads, frames, skin, viscera) can account for 50–80% of a fish's weight, and if recycled, can serve as high-quality feed ingredients. Tuna viscera is one such underutilized resource from tuna processing **(Hernández *et al.*, 2004)**. For instance, Indonesia produces around 613,000 metric tons of tuna per year, and viscera constitute roughly 5–7% of the fish's weight. These viscera are rich in protein but ~75–80% water, so they spoil rapidly if not stabilized.

Fermentation is an effective method to convert fishery wastes like tuna viscera into stable, digestible feed ingredients. Fermentation can be induced by adding acids or lactic acid bacteria to the ground viscera. The microbes hydrolyze complex proteins and lipids into simpler, more digestible molecules **(Sharma *et al.*, 2020)**. Fermentation preserves the nutritional value of the viscera and can even enhance it by generating additional nutrients such as B-vitamins. The resulting fermented tuna viscera meal is shelf-stable and palatable, making it a promising candidate to replace a portion of fish meal in aquafeeds.

Previous studies have reported encouraging results using fermented tuna by-products in aquafeeds. For example, a 10% dietary inclusion of fermented tuna viscera in olive flounder improved feed utilization with no loss of growth **(Lee *et al.*, 2009)**. Similarly, in *Pangasius* fry, replacing 20% of fish meal protein with fermented tuna innards led to equal or better growth and 100% survival compared to a fish meal-only diet. These findings underscore the potential of fermented tuna viscera to reduce reliance on wild-caught fish meal in aquafeeds.

Therefore, the present study evaluated the use of fermented tuna viscera as an alternative to fish meal in the diet of striped catfish fry. Diets were formulated with increasing levels of fermented tuna viscera replacing fish meal, and their impacts on fry growth, feed efficiency, and survival were assessed. The findings of this research will help determine the viability of fish waste-derived proteins as sustainable feed ingredients for striped catfish aquaculture.

MATERIALS AND METHODS

1. Raw materials

The raw material used in this study was tuna offal (*Pangasius* sp.), which had a protein content of 91.99% (dry basis), moisture content of 77.66%, fat content of 1.25%,

ash content of 2.04%, and crude fiber content of 0.94%. The tuna offal was sourced from the Malang central market.

The fermentation process utilized EM4, which contained 2.0×10^6 cells/mL of *Lactobacillus casei* and 3.5×10^6 cells/mL of *Saccharomyces cerevisiae*. Additionally, molasses was obtained from the Kebon Agung sugar factory in Malang. Other ingredients used in the feed formulation included fish meal, soybean meal, rice bran meal, tapioca flour, carboxymethyl cellulose (CMC), mineral mix, and vitamin mix.

The biological evaluation of the formulated feed was conducted using the striped catfish fry as the test species. The experimental fish were juveniles measuring 7–9cm in length, with an average weight of 3–4 grams. These fish were sourced from Cilangkap, Jakarta.

2. Preparation of fermented tuna viscera

The main components of tuna fish offal waste—specifically the intestines, liver, and gonads—were thoroughly washed and then drained for 30 minutes to reduce moisture content. After draining, the cleaned offal was finely chopped, weighed, and oven-dried at a temperature of 40°C. Once fully dried, the material was ground into a fine powder and was subjected to fermentation.

Fermentation was carried out using a mixture of molasses and EM4, which contains *Lactobacillus casei* at a density of 2.0×10^6 cells/ml and *Saccharomyces cerevisiae* at a density of 3.5×10^5 cells/ml. The ingredients were combined in a ratio of 1:1:3 (EM4:molasses:fish offal). The mixture was stirred thoroughly to ensure even distribution, transferred into a fermentation jar, and sealed tightly to prevent air entry. The fermentation process was allowed to proceed for two days.

3. Proximate analysis

The nutritional evaluation of the fermented tuna offal meal (Stage II) was conducted using proximate analysis, which included the determination of moisture content, crude protein, crude fat, ash content, and crude fiber following the **AOAC (1995)** guidelines. The following analyses were performed:

a. Moisture content analysis

The equipment used for this analysis included a porcelain crucible, an oven set at 105°C, a desiccator (containing silica gel), forceps, and an analytical balance. The moisture content was calculated using the following formula:

$$Moisture\ Content\ (\%) = \frac{Weight\ of\ fresh\ sample - Weight\ of\ dried\ sample}{Weight\ of\ fresh\ sample} \times 100$$

b. Crude protein analysis

The Kjeldahl method was used to determine crude protein content, consisting of three main stages: digestion, distillation, and titration.

1. **Digestion**
   - 1g of the sample was mixed with 0.25g selenium and 25mL of concentrated H_2SO_4 in a Kjeldahl flask.
   - The mixture was heated until a clear solution was obtained.

2. **Distillation**
   - The digested sample was transferred to a distillation flask, and 50mL of distilled water along with 20mL of 40% NaOH was added.
   - The resulting vapor was collected in a 10mL Erlenmeyer flask containing boric acid (H_3BO_3) and two drops of indicator solution (methyl red and bromocresol green).
   - The distillation was continued until 10mL of distillate was obtained, which turned blue-green in color.
3. **Titration**
   - The distillate was titrated using 0.1 N HCl until a pink endpoint was reached.
   - The volume of titrant used was recorded to determine nitrogen content (N), and the crude protein content was calculated using the formula:

$$Crude\ Protein\% = Nitrogen\ Content\ (\%) \times 6.25$$

c. Crude fat analysis

The Soxhlet extraction method was used to determine crude fat content.

1. A fat extraction flask was dried in an oven at 110°C, cooled in a desiccator, and weighed.
2. A 5g sample was wrapped in filter paper and extracted using hexane as a solvent in a Soxhlet apparatus.
3. Refluxing was carried out until the extract became clear, and the solvent was evaporated.
4. The extraction flask containing the extracted fat was dried in an oven at 105°C, cooled in a desiccator, and weighed.

The crude fat content was calculated using the following formula:

$$Crude\ Fat\ (\%) = \frac{Weight\ of\ extracted\ fat}{Weight\ of\ sample} \times 100$$

d. Ash content analysis

1. A porcelain crucible was dried in an oven at 110°C, cooled in a desiccator, and weighed.

2. A 5g sample was placed in the crucible and heated over a Bunsen burner until no more smoke was observed.
3. The sample was then incinerated in a muffle furnace at 400–600°C for 4–6 hours, or until white ash was obtained.
4. The ash was cooled in a desiccator and weighed.

The ash content was calculated using the following formula:

$$Ash\ Content\ (\%) = \frac{Weight\ of\ ash}{Weight\ of\ sample} \times 100$$

e. Crude fiber analysis

1. The fat extraction flask was dried in an oven, cooled in a desiccator, and weighed.
2. A 2.5–5g sample was dried and wrapped in filter paper, then extracted with diethyl ether for 6 hours using a Soxhlet apparatus.
3. The extracted sample was transferred to a 600mL Erlenmeyer flask and mixed with 200mL of boiling H_2SO_4 solution. The mixture was boiled for 30 minutes and then filtered through filter paper.
4. The residue was washed with boiling water and treated with 200 mL of NaOH solution, following the same procedure as with H_2SO_4.
5. The residue was filtered, washed with 10% K_2SO_4 solution, boiling water, and 95% ethanol, and dried in an oven at 110°C.
6. The dried residue was cooled in a desiccator and weighed.

The crude fiber content was calculated using the following formula:

$$Crude\ fiber\ (\%) = \frac{Weight\ of\ residue}{Weight\ of\ sample} \times 100$$

4. Experimental design

A feeding trial was conducted using *Pangasius* fry with an initial weight of approximately 5g. Fish were randomly distributed into tanks and were fed with four experimental diets containing different levels of FTVM substitution:

- A (0%): Control diet with 100% fish meal
- B (10%): 10% substitution of fish meal with FTVM
- C (20%): 20% substitution of fish meal with FTVM
- D (30%): 30% substitution of fish meal with FTVM

Each treatment was conducted in triplicate.

5. Growth performance and feed utilization

The following parameters were measured:

a. Survival rate (SR, %):

$$SR\ (\%) = \frac{Final\ nubmer\ of\ fish}{Initial\ number\ of\ fish} \times 100$$

b. Specific growth rate (SGR, %/day):

$$SGR = \left(\frac{\ln W_f - \ln W_i}{T} \right) \times 100$$

Where, W_f and Wi are the final and initial body weights, and T is the duration in days.

c. Feed conversion ratio (FCR, g/g):

$$FCR\ (\%) = \frac{Total\ feed\ intake\ (g)}{Total\ weight\ gain\ (g)}$$

d. Protein retention (%):

$$PR\ (\%) = \frac{Protein\ gain\ (g)}{Protein\ intake\ (g)} \times 100$$

6. Data analysis

All collected data were subjected to statistical analysis to determine significant differences among treatments. Growth performance parameters, including specific growth rate (SGR), feed conversion ratio (FCR), and protein retention, were analyzed using one-way analysis of variance (ANOVA). Tukey's post-hoc test was applied to identify differences between treatment means at a significance level of $P<0.05$. Additionally, proximate composition data of the experimental diets and fish body composition were analyzed to evaluate the nutritional impact of fermented tuna viscera inclusion. The statistical software SPSS was used for all computations. The results were expressed as mean ± standard deviation (SD). Correlation analysis was performed to examine the relationship between enzyme activity and protein retention, providing insights into the digestibility and bioavailability of the alternative protein source.

RESULTS

1. Proximate composition of experimental feeds

Proximate compositions of feed ingredients (fermented tuna viscera meal, fish meal, soybean meal, rice bran, and tapioca flour) were analyzed for dry matter, protein, lipid, ash, fiber, nitrogen-free extract (NFE), and energy content (Table 1).

Table 1. Proximate composition of feed ingredients (% dry matter basis)

Ingredient	Dry Matter (%)	Protein (%)	Lipid (%)	Ash (%)	Fiber (%)	NFE** (%)	Energy (Kcal/100g)
Fermented Tuna Viscera Meal	92.29	66.53	7.43	6.69	0.69	11.66	408.03
Fish Meal	89.15	58.22	5.73	22.75	3.50	9.80	324.56

Ingredient	Dry Matter (%)	Protein (%)	Lipid (%)	Ash (%)	Fiber (%)	NFE** (%)	Energy (Kcal/100g)
Soybean Meal	92.83	33.63	22.41	5.30	8.41	30.25	457.07
Rice Bran	89.72	12.02	8.69	13.44	29.79	36.06	271.96
Tapioca Flour	91.59	0.14	0.04	0.05	0.57	99.19	398.76

(**NFE: Nitrogen-free extract)

2. Experimental feed formulation

Four experimental diets were prepared with different substitution levels of FTV for FM: 0% (A), 10% (B), 20% (C), and 30% (D). The formulations are detailed in Table (2).

Table 2. Feed formulation for different substitution levels (%)

Ingredient	A (0%)	B (10%)	C (20%)	D (30%)
Fermented Tuna Viscera Meal	0.00	6.12	12.24	18.36
Fish Meal	30.92	23.19	15.46	7.73
Soybean Meal	32.11	32.11	32.11	32.11
Rice Bran	9.98	9.98	9.98	9.98
Tapioca Flour	11.59	11.61	11.63	11.65
Vitamin & Mineral	3.00	3.00	3.00	3.00
Fish Oil	0.50	0.50	0.50	0.50
CMC	11.89	13.48	15.08	16.67
Total	**100**	**100**	**100**	**100**

3. Proximate composition of tuna viscera before and after fermentation

Fermentation of tuna viscera led to slight enhancements in nutritional composition, notably in protein and lipid content, while reducing fiber. These changes indicate an improvement in digestibility and nutrient availability.

Table 3. Proximate composition of tuna offal and fermented tuna offal

Component	Tuna offal (%)	Fermented tuna offal (%)
Dry Matter	91.99	92.29
Crude Protein	66.29	66.53
Crude Fat	7.25	7.43
Crude Fiber	0.94	0.69
Ash	6.04	6.69
Nitrogen-Free Extract (NFE)	19.48	18.66

These improvements suggest that fermentation not only stabilizes the material but also enhances its nutritive value—particularly for aquafeed applications.

4. Growth performance and enzyme activity data

The inclusion of FTV had significant effects on the specific growth rate (SGR), feed conversion ratio (FCR), protein retention, and protease activity, with the best performance at 20% FTV substitution.

Table 4. Growth performance and feed utilization of *Pangasius* fry

Parameter	A (0%)	B (10%)	C (20%)	D (30%)
Survival Rate (%)	98.33 ± 2.89a	98.33 ± 2.89a	100 ± 0.00a	98.33 ± 2.89a
SGR (%/day)	2.96 ± 0.05a	3.14 ± 0.01b	3.44 ± 0.02d	3.21 ± 0.02c
FCR (g/g)	1.87 ± 0.04d	1.75 ± 0.01b	1.56 ± 0.02a	1.72 ± 0.01b
Protein Retention (%)	19.81 ± 2.63a	23.03 ± 1.41b	26.04 ± 0.58d	23.89 ± 1.28c
Protease Activity (U/mg)	3.21 ± 1.71a	4.38 ± 0.11b	6.10 ± 0.01d	4.65 ± 0.13c

The results confirm that moderate substitution of fish meal with fermented tuna viscera significantly enhances growth parameters, suggesting improved digestibility and utilization efficiency attributed to the fermentation process.

DISCUSSION

The fermentation of tuna viscera resulted in modest yet meaningful alterations in its proximate composition (Table 3), with important implications for its digestibility, palatability, and overall functional value in aquafeeds **(Jesebel & Erlinda, 2012)**.

A slight increase in crude protein content—from 66.29 to 66.53%—was observed following fermentation. Although numerically small, this increase suggests improved nitrogen retention and partial breakdown of non-protein nitrogen into more bioavailable peptides and free amino acids. This enhancement is likely due to microbial enzymatic activity during fermentation **(Wang *et al.*, 2024)**, which can: (1) produce bioactive peptides that may support gut health and immune function **(Fabbri *et al.*, 2024)** (2) reduce antinutritional factors that hinder protein utilization, and (3) hydrolyze complex proteins into simpler, digestible forms **(Wu *et al.*, 2025)**. These findings align with previous research showing that fermentation enhances the digestible protein fraction of fish waste, making it more suitable for carnivorous species such as the striped catfish **(Prakash *et al.*, 2023; Serra *et al.*, 2024)**.

A similar pattern was observed with crude fat, which slightly increased from 7.25 to 7.43%. This indicates effective lipid retention during fermentation, a critical factor for maintaining energy density in aquafeeds. Fermentation may also help stabilize lipids by reducing oxidation under anaerobic conditions and increasing the proportion of free fatty acids, which are more readily absorbed by fish **(Maksimenko *et al.*, 2024)**. Since dietary lipids serve as a major energy source and are essential for growth and physiological function **(Zhu *et al.*, 2025)**, improved lipid utilization may contribute to the enhanced feed conversion ratio (FCR) observed in this study.

The reduction in crude fiber—from 0.94 to 0.69%—is particularly beneficial, given fish have limited capacity to digest fibrous materials. High fiber content can hinder nutrient absorption and reduce digestive efficiency **(Nafees *et al.*, 2023)**. This reduction likely results from the partial hydrolysis of fibrous components during fermentation, which improves nutrient assimilation, reduces gut fill from indigestible material, and mitigates digestive irritation in fry **(Feng *et al.*, 2025)**.

An increase in ash content—from 6.04 to 6.69%—was also noted. This may reflect either the concentration of residual minerals or enhanced mineral preservation during fermentation. While excessive ash can dilute dietary energy, a moderate increase may improve the availability of essential minerals like calcium and phosphorus, both of which are vital for skeletal development. Microbial enzymatic activity under mildly acidic fermentation conditions may also enhance mineral solubility and bioavailability **(Sawant *et al.*, 2025)**.

The slight decrease in nitrogen-free extract (NFE), from 19.48 to 18.66%, suggests microbial consumption of soluble carbohydrates during fermentation. This reduction is beneficial, as carnivorous fish species like striped catfish fry have limited ability to metabolize carbohydrates efficiently **(Phan *et al.*, 2021)**. Lower NFE levels may reduce the risk of excessive fat deposition and promote more efficient energy use from protein and lipids, aligning with the species' natural metabolic profile.

Performance outcomes

The inclusion of fermented tuna viscera (FTV) in the diets of the striped catfish fry significantly improved growth performance and feed utilization parameters **(Lee *et al.*, 2003)**, particularly specific growth rate (SGR), feed conversion ratio (FCR), protein retention, and protease activity (Table 4). Among all treatment groups, fish fed a diet with 20% FTV substitution (Group C) achieved the most favorable outcomes across all indicators, suggesting that 20% is the optimal inclusion level for balancing nutrient enhancement with physiological tolerance.

Specific growth rate (SGR) increased in correlation with higher FTV inclusion, peaking in Group C at 3.44 ± 0.02%/day—a statistically significant improvement over the control group at 2.96 ± 0.05%/day ($P < 0.05$). This improvement likely results from enhanced digestibility and protein quality in FTV, as microbial activity during fermentation breaks down complex proteins into peptides and free amino acids **(Cruz-Casas *et al.*, 2021; Sawant *et al.*, 2025)**.

Feed conversion ratio (FCR) also improved, with the lowest FCR (1.56 ± 0.02) recorded in the 20% FTV group. This indicates more efficient feed-to-biomass conversion and is likely due to enhanced nutrient profiles, reduced fiber, and stimulated digestive enzyme activity. However, FCR slightly increased at the 30% inclusion level (1.72 ± 0.01), suggesting a potential upper limit beyond which palatability or nutrient balance may be compromised **(Fry *et al.*, 2018)**.

Protein retention mirrored SGR and FCR trends, peaking at 26.04 ± 0.58% in the 20% FTV group. This reflects efficient assimilation of dietary protein into body tissues, due to both high protein content and improved digestibility of FTV **(Nugroho *et al.*, 2020)**. These results are consistent with previous findings that support fermented animal byproducts as viable partial fishmeal replacements **(Samaddar *et al.*, 2015; El-Demerdash *et al.*, 2020; Luthada-Raswiswi *et al.*, 2021; Roslan *et al.*, 2024)**.

Protease activity was also at its highest in the 20% FTV group (6.10 ± 0.01 U/mg), indicating enhanced digestive enzyme production. This may be due to fermentation-derived peptides or bioactive compounds that stimulate digestive function **(Pham *et al.*, 2021)**. Potential mechanisms include: (1) peptide-driven stimulation of digestion, (2) shifts in gut microbiota favoring proteolytic bacteria **(Fathima *et al.*, 2022)**, and (3) increased activity of intestinal enzyme-secreting cells **(Fabbri *et al.*, 2024)**.

Importantly, survival rates exceeded 98% across all treatments, with no statistically significant differences. This confirms that FTV, even at inclusion levels up to 30%, does not negatively impact fish health or viability—an encouraging outcome for its practical use in commercial aquafeeds.

CONCLUSION

This study identified fermented tuna viscera (FTV) as a viable partial replacement for fish meal (FM) in diets formulated for *Pangasius hypophthalmus* fry. Among the tested substitution levels, 20% FTV inclusion yielded the most favorable outcomes in terms of growth performance and feed utilization. Fish fed this diet exhibited a significantly enhanced specific growth rate (SGR) and a reduced feed conversion ratio (FCR), indicating superior growth efficiency. Protein retention was the highest at this level, reflecting improved nutrient assimilation. The observed increase in protease activity further suggests enhanced digestive capacity, likely due to the presence of fermentation-derived bioactive compounds. Nutritional analysis demonstrated that FTV is rich in protein and lipids, has reduced fiber content, and possesses superior digestibility, underscoring its value as a functional feed ingredient. Importantly, all treatment groups maintained high survival rates (>98%), even at inclusion levels up to 30%, confirming the safety of FTV in aquafeed formulations. Overall, the results support the inclusion of FTV at 20% as the optimal substitution level, offering both nutritional and environmental benefits that align with the goals of sustainable aquaculture.

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