Fish Waste Meal As A Sustainable Protein Source In Climbing Perch (*Anabas testudineus*) Diets: Implications For Growth, Feed Utilization, Water Quality, And Economic Viability

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Abstract
The use of conventional protein sources such as fishmeal and soybean meal in aquafeed raises sustainability, environmental, and economic concerns. This study evaluated the efficacy of Fish Waste Meal (FWM) as an alternative protein source in the diet of Climbing Perch (*Anabas testudineus*) through a 60-day feeding trial. The present study evaluated the effects of incorporating fish waste meal (FWM) at varying inclusion levels (0%, 25%, 50%, 75%, and 100%) into the diet of *Anabas testudineus* on growth performance over a defined feeding period. Key growth parameters, including final body weight (FBW), specific growth rate (SGR), and feed utilization efficiency, were analyzed to assess the nutritional viability of FWM as an alternative protein source. Results demonstrated a clear improvement in growth parameters with increasing inclusion levels of FWM. The FBW increased from 8.54 ± 1.01 g in the control group to a maximum of 10.78 ± 1.01 g in the FWM75 group. Weight gain significantly improved ($P = 0.001$), ranging from 3.27 ± 0.35 g in the control to 6.33 ± 0.40 g at 75% inclusion. The feed conversion ratio also improved significantly, decreasing from 3.04 ± 0.10 in the control to 1.79 ± 0.16 in the FWM75 group ($P = 0.001$), while the protein efficiency ratio (PER) increased from 0.78 ± 0.08 to 1.25 ± 0.08 ($P = 0.001$). The SGR improved from 0.93% to 1.39% per day, though not statistically significant ($P = 0.280$). The feed intake remained consistent across treatments, and the survival rate was 100% in all groups. The findings highlight that fish waste meal, particularly at 75% inclusion, enhances growth performance and feed efficiency in *A. testudineus* without compromising survival or feed intake. This underscores the viability of FWM as an environmentally friendly, nutritionally rich, and cost-effective alternative protein source in aquafeed formulations.

Keywords: Fish Waste Meal, Economic Impact, Aquafeed Sustainability, Alternative Protein Source, Eco-Friendly Aquaculture

Introduction

Farmed-raised fish require a well-balanced and nutritious diet, with fishmeal traditionally serving as the primary protein source due to its good nutrients and palatability (Hardy and Acon, 2002). However, aquaculture has increased demand for fishmeal in the industry, leading to supply shortages and rising costs (Jabir *et al*., 2012). Since the industry cannot indefinitely depend on fishmeal as an affordable and consistent protein source, finding alternatives is crucial. One promising solution is incorporating unconventional protein sources, such as Fish Waste Meal, to replace fishmeal. Studies indicate that this approach can lower feed costs and increase profitability while maintaining fish growth and performance (Jabir *et al*., 2012). The local market and fish processing industry generate a substantial amount of fish waste, which holds significant nutritional value (Ghaly *et al*., 2013). Around 30% of a fish, including its fins, head, viscera, scales, and other parts, often goes unused and is discarded into rivers or nearby areas, creating environmental and health concerns. However, there is growing recognition that fish waste can be repurposed as a valuable nutritional resource instead of being thrown away (FAO, 2020). This waste is composed of various parts of the fish, including heads, which account for 9–12% of the total weight; bones, making up 9–15%; scales, approximately 5%; viscera, ranging from 12–18%; and skin, which constitutes 1–3% (Rustad, Storro, and Slizyte., 2011) Studies indicate that fish waste is abundant in vital nutrients, comprising crude protein (57.92 ± 5.26%), fat (19.10 ± 6.06%), crude fiber (1.19 ± 1.21%), ash (21.79%), calcium (5.80 ± 1.35%), potassium (0.68 ± 0.11%), and sodium (0.61 ± 0.08%) (Ghaly *et al*., 2013). By utilizing fish waste efficiently, we can reduce environmental impact while making the most of its nutritional benefits (Ghaly *et al*., 2013). The Climbing Perch, scientifically named *Anabas testudineus* and commonly referred to as 'Koi' in West Bengal, is a fish of significant importance in the area (Bhattacharya and De, 2014). It has consistently been recognized as a source of nutritious food, particularly for those who are ill or recovering, due to its health benefits. This fish provides a remarkable source of easily absorbable iron and copper, both essential for hemoglobin production (Saha *et al*., 2013). It also includes all the necessary amino acids for a balanced diet (Saha, 1971). The Climbing Perch is widely distributed across several countries, including India, Pakistan, Myanmar, Sri Lanka, Thailand, China, Hong Kong, the Philippines, and Malaysia (Jayaram, 1981). This species flourishes in a variety of freshwater and brackish habitats, such as ponds, swamps, and lakes. Known for being an active feeder, it primarily hunts during daylight hours (Patra, 1993). As an omnivorous predator, it is highly adaptable to different environmental conditions and exhibits aggressive foraging behavior, making it a resilient species in diverse habitats. The Climbing Perch (*Anabas testudineus*) has shown great potential for fish farming, even under challenging weather conditions. However, poor farming practices often make it difficult to meet the high market demand for this species. A survey conducted in 2002 found that more than half of Climbing Perch farmers were forced to stop farming because of the elevated expenses associated with fish feed (Tihn, 2003). Although *A. testudineus* holds significant commercial value, research on its nutritional requirements remains scarce. Since Protein is essential for growth and metabolic processes, understanding the minimum protein requirements is essential when formulating a well-balanced diet. The ideal protein concentration of *A. testudineus*, a study by Hossain *et al*. (2012) investigated the dietary protein requirement of a Thai strain of *A. testudineus* fry. The researchers fed the fry with semi-purified diets containing protein levels ranging from 25% to 50% over an 8-week period. The study found that a diet containing 40% crude protein resulted in the highest weight gain and the most efficient feed conversion ratio (FCR). Beyond this level, no significant improvements were observed, indicating that 40% protein is optimal for maximum growth in this strain of climbing perch fry. Given this gap in knowledge, the current study aimed to for *A. testudineus* and evaluate the impact of substituting fishmeal protein with Fish Waste Meal (FWM) on its growth performance.

Materials and methods

Research Location and Duration of the Study

This research took place from March to May 2024. Throughout this timeframe, *Anabas testudineus* (commonly known as Koi fish) fry were obtained from nearby fish markets and placed in the outdoor culture unit at the Department of Fishery Sciences, Vidyasagar University. Additionally, the components for the fish feed were gathered and delivered to the Fishery Sciences Laboratory at Vidyasagar University, located in Midnapore, West Bengal, India (Latitude: 22.42996°, Longitude: 87.297775°).

Collection and Acclimatization of Experimental Fish

A. testudineus fry (commonly known as Koi fish) were procured from the Nahati wholesale and seed fish market in North 24 Parganas, West Bengal, India. Upon arrival, the fry was housed in a circular concrete tank with a holding capacity of 300 Liters. Transportation and initial acclimatization resulted in minimal mortality. To ensure optimal health, the fry underwent a brief prophylactic treatment involving immersion in a 3% sodium chloride (NaCl) solution for 10 seconds. During this procedure, artificial aeration was provided to maintain sufficient dissolved oxygen levels before transferring the fry to the acclimatization tank (Dey et al., 2014). A one-week conditioning period was provided before the commencement of the experimental trial, during which the fry was fed a commercial nursery feed containing 36% crude protein. The total acclimatization period spanned 10 days.

Preparation and Nutritional Composition of Fish Waste Meal

In this experiment, I have used mainly IMC (Indian Major Carp) fish waste. The proximate composition of Indian Major Carp (IMC) fish waste, which includes viscera, bones, scales, skin, fins, and heads from species like *Catla catla*, *Labeo rohita*, and *Cirrhinus mrigala*. The waste is rich in crude protein (40–58%) and crude fat (10–22%), making it a nutritionally valuable feed ingredient. High ash content (15–30%) reflects the mineral-rich nature of bones and scales, contributing significant calcium (5–7%) and phosphorus (3–5%), essential for fish bone development and metabolism. Moisture content is reduced through drying (5–10%), enhancing shelf life and nutrient density. These characteristics make IMC fish waste a sustainable, cost-effective alternative to fishmeal, while also addressing environmental concerns through waste valorization in aquaculture. For this experiment, fish waste—including viscera, heads, scales, and fins—was collected from the local fish market at Gate Bazar in Midnapore, West Bengal, India (721101). The raw fish waste was first chopped into small pieces and then minced to prepare it for natural fermentation.

Sugarcane molasses and yeast, sourced from a local grocery store, were used to facilitate the fermentation process. The minced fish waste was weighed in batches of approximately 500 grams and placed in plastic containers with a 3-liter capacity. Distilled water was then added at a proportion of 1 part water to 2 parts fish waste. The fish waste and sugarcane molasses were mixed in equal proportions (50:50 w/w). A small amount of yeast was also introduced, either as a protein supplement or to support fermentation. Throughout the 28-day incubation period, the mixture was stirred two times a day, once in the morning and once in the afternoon, to ensure proper fermentation. After fermentation was complete, the liquid and solid portions were separated using a solid waste collection tray. The solid material was subsequently dried in an oven at 60°C for a duration of 8 hours, following which its moisture levels and nutritional profile were assessed. Ultimately, the dried fermented fish waste was processed into a fine powder and stored in a sealed container for later use.

Experimental Design and Diet Preparation

This study followed a fully randomized design, which was implemented, featuring three replicates for each treatment group. Five distinct experimental diets were created in accordance with the nutritional needs of Climbing Perch, as detailed by Hossain *et al*. (2012). The diets were designed to progressively replace fishmeal (FM) with Fish Waste Meal (FWM) at varying levels: 0%, 25%, 50%, 75%, and 100%. The experimental groups were designated as follows: FWM0, which served as the control treatment with 0% FM replacement, FWM25 (25% FM replacement), FWM50 (50% FM replacement), FWM75 (75% FM replacement), and FWM100 (100% FM replacement). Before preparing the diets, fishmeal (containing 58% crude protein), soybean meal (SBM), rice bran (RB), and Tapioca starch were finely milled and filtered through a 0.5 mm mesh sieve. to ensure consistency. The components were carefully weighed and thoroughly mixed to create a uniform blend. To enhance the nutritional profile, soybean oil and a vitamin-mineral premix were incorporated, along with sufficient water to create a dough. This dough was subsequently processed using an extruder to generate feed pellets with a diameter of about 2 mm. The pellets were then dried overnight in a hot air oven set to 60°C and stored at 20°C in polyethylene zip-lock bags to maintain freshness until use. The cost of each formulated diet was calculated per kilogram, and the proximate composition—including crude protein, crude lipid, ash, and crude fiber was analyzed following the standard procedures outlined by AOAC (1990).

Table 1: Formulation of the experimental diet for *Anabas testudineus* at 100 g (based on dry weight) along with the composition of the feed used in the experimental diets.

Ingredients	FWM0	FWM25	FWM50	FWM75	FWM100
Fish meal(g)	31.51	23.60	16	8	0
Fish waste meal(g)	0	8	16	23.60	31.50
Soybean meal(g)	31.50	31.50	31.50	31.50	31.50
Wheat flour(g)	18.51	18.51	18.51	18.51	18.51
Rice bran(g)	18.51	18.51	18.51	18.51	18.51
Tapioca(g)	3	3	3	3	3
Oil(ml)	2	2	2	2	2
Vitamins and minerals(g)*	1	1	1	1	1

*The abbreviations presented in the table are defined as follows: FWM 0 (0% replacement of FM), FWM 25 (25% FM replacement), FWM 25 (25% FM replacement), FWM 50 (50% FM replacement), FWM 75 (75% FM replacement), FWM 100 (100% FM replacement).
*Vitamin and mineral formulation (IU or mg per kg): vitamin A (1,000,000 IU), vitamin D3 (200,000 IU), vitamin E (5,000 mg), vitamin K3 (5,000 mg), vitamin B1 (3,000 mg), vitamin B2 (5,000 mg), vitamin B6 (3,000 mg), vitamin B12 (10 mg), vitamin C (10,000 mg), vitamin B3 (3,000 mg), vitamin B5 (1,000 mg), folic acid (1,000 mg), manganese (600 mg), zinc (8,000 mg), copper (300 mg), selenium (10 mg), iron (300 mg), cobalt (330 mg), potassium (5,000 mg).

Table 2: Proximate composition (g kg-1 dry matter basis) and feed cost.

Proximate composition	FWM0	FWM25	FWM50	FWM75	FWM100
DM	97.60	96.17	97.56	96.28	96.12
CP	37.28	38.24	38.36	38.78	37.89
CL	10.29	10.25	9.24	9.80	10.25
ASH	13.28	11.56	14.36	11.77	12.69
CF	25.60	24.52	25.47	25.91	24.32
NFE	11.15	11.6	9.71	10.02	10.97
GE	353.23	350.45	343.48	352.40	355.55
DE	258.51	261.73	247.79	255.21	259.59
FC	2.41	1.73	1.21	0.99	0.87

* The abbreviations presented in the table are defined as follows: DM stands for Dry Matter, CP represents Crude Protein, CL denotes Crude Lipid, CF refers to Crude Fiber, NFE indicates Nitrogen Free Extract, GE signifies Gross Energy, DE means Digestible Energy, and FC stands for Feed Cost. NFE percentage is calculated as follows: % NFE = % DM – (% CP + % CL + % Ash + % CF). The gross energy (GE) in kilocalories per 100 grams is determined by the formula: GE (kcal 100g-1) = (% CP × 5.64) + (NFE × 4.11) + (% CL × 9.44). Digestible energy (DE) in kilocalories per 100 grams is calculated using the equation: DE (kcal 100g-1) = (% protein × 3.5) + (% fat × 8.0) + (% NFE × 4.1). The current cost conversion rate is 1 US dollar to 86.54 Indian Rupees as of January 20, 2025.

Proximate Analysis

The nutritional composition of Fish Waste Meal (FWM), experimental diets, and fish fillets was assessed. The proximate composition was evaluated based on dry matter weight following the official methods established by AOAC (1990). The crude protein level was determined using the Kjeldahl method (Gerhardt type vap. 40, Königswinter, Germany), while crude lipid content was analyzed through Soxhlet extraction (Büchi Extraction Unit E-816 Hot Extraction) utilizing hexane as the solvent. Ash content was measured by incinerating samples in a muffle furnace at 600°C for 2 hours, and crude fiber content was assessed at 550°C through acid and alkali digestion, employing petroleum ether as the solvent.

Experimental Fish and Feeding

A total of 150 fingerlings of Climbing Perch (*A. testudineus*) were obtained from a local farm located in Naihati, Purba Medinipur, West Bengal, India. These fingerlings had an average initial weight of 4.84 ± 0.95 g and a length of 6.21 ± 1.01 cm. Before the commencement of the experiment, the fish were acclimatized for two weeks in a cage and were provided with commercial fish feed from Charoen Pokphand Foods PCL, India, to facilitate their adjustment to the new environment. The research was carried out in an outdoor culture unit that featured an air pump for oxygen circulation. The experiment utilized five cement tanks, each measuring 1×1×1 m. The acclimatized fish were randomly allocated to these tanks at a stocking density of 30 fish per tank, resulting in a total of 150 fish per treatment group. For 60 days, the fish were fed at 5% of their body weight, split into two daily feeding sessions at 9:00 AM and 4:00 PM. Throughout the experimental period, the experiment maintained a low to moderate stocking density, which significantly reduced ammonia excretion from the fish. Lower biomass naturally leads to reduced total ammonia nitrogen (TAN) production. (Boyd, 2017). Fish were fed according to satiation without overfeeding, which minimized feed waste and nitrogen leaching from uneaten feed—a primary source of ammonia in aquaculture systems (Cho and Bureau, 2001). Natural colonization of tank surfaces by nitrifying bacteria (Nitrosomonas and Nitrobacter) likely facilitated the biological conversion of ammonia to less toxic nitrite and nitrate, even without mechanical aeration. (Hagopian and Riley, 1998) Even partial (one-third) water replacement, if done regularly, dilutes nitrogenous wastes efficiently, especially when supported by good initial water quality and a stable microbial community (Tucker and Hargreaves, 2004).

Water Quality Parameters

Water quality parameters were assessed weekly at 10:00 A.M. during the feeding trial. A portable dissolved oxygen meter (Model DO-5509; Lutron, Taipei, Taiwan) was utilized to measure the temperature and dissolved oxygen (DO) levels in the ponds, while a pH meter (Model 58; Jenway, Burlington, VT, USA) was employed to determine the water pH. The observed ranges for the various water quality parameters across different treatments were as follows: temperature ranged from 26.0 to 32.8°C, pH levels varied between 6.54 and 8.46, and dissolved oxygen (DO) levels were between 3.54 and 6.57 mg/L throughout the feeding trial. These water quality ranges are deemed appropriate for the culture of climbing perch.

Sample Collection for Analysis

Following the completion of the 60-day feeding trial, the fish underwent a 24-hour fasting period to facilitate data collection. The number of surviving fish in each tank was then tallied, and their lengths and weights were documented. These metrics were utilized to evaluate growth performance, feed efficiency, survival rates, and economic factors.

Growth performance, feed efficiency, body composition, and economic indicators

The gathered data, which includes the total count, length, and weight of the fish, was utilized to compute various metrics associated with growth performance, feed efficiency, survival rates, and economic indicators. The following formulas were applied for these calculations:

Weight gain (WG; g) = final body weight (g) - initial body weight (g); (De Silva and Anderson, 1995).

Specific growth rate (SGR; % d^{-1}) = 100 × [(ln final weight (g) - ln initial weight (g)) / days]; (Cho and Kaushik, 1990).

Average daily weight gain (ADWG; g day^{-1}) = (final weight (g) - initial weight (g)) / days; NRC (National Research Council). (2011).

Protein efficiency ratio (PER; %) = 100 × (weight gain (g) / protein intake (g)); (Wilson, 2002).

Feed conversion ratio (FCR) = feed intake (g) / weight gain (g); (Tacon and Metian, 2008).

Survival rate (SR; %) = 100 × (final number of fish / initial number of fish); (Boyd, 2017).

Feed intake (FI) (g $fish^{-1}$) = Total feed intake for 60 days (g)/Total number of fish; NRC (National Research Council). (2011).

Economic analysis:

Economic Conversion Ratio (ECR)

The Economic Conversion Ratio (ECR) was determined using the following equation: Economic conversion ratio (ECR; \$ kg^{-1}) = FCR × feed cost (\$ kg^{-1}); where feed cost is the price per kilogram of feed used during the study. ECR provides insight into the financial implications of feed efficiency (Das *et al*., 2024)

Economic Profit Index (EPI)

The Economic Profit Index (EPI) was calculated as: Economic profit index (EPI; \$ kg^{-1}) = (yield (kg) × value (\$ kg^{-1})) - ((yield (kg) × feed cost (\$ kg^{-1})). This metric assesses the profitability of production based on yield, market value, and feed cost (Miao *et al*., 2009). A positive EPI indicates economic feasibility, whereas a negative EPI suggests a financial loss.

Statistical analysis

All parameter values in this research are presented as Mean ± SD. Prior to performing statistical analysis, the data underwent tests for normality and homogeneity to verify that they satisfied the assumptions necessary for one-way ANOVA. After confirming these assumptions, ANOVA was executed at a 95% confidence level to detect any significant differences among the experimental diets. If the ANOVA results revealed significant differences, a Tukey's LSD test was carried out for post hoc analysis to pinpoint specific differences between groups.

Results

The provided nutritional composition of fish waste meal indicates a protein content of 47.54 g/100g, carbohydrate content of 18.81 g/100g, lipid content of 7.91 g/100g, and ash content of 28.17 g/100g. Additionally, the mineral composition includes calcium (7051.03 mg/kg), magnesium (180.83 mg/kg), iron (87.30 mg/kg), and phosphorus (8215.22 mg/kg).

Table 3: Nutritional Compositions of Fish Waste Meal (FWM)

Compositions of Fish Waste Meal (FWM)		
Nutritional component (Dry matter products)	Values	units
Protein	47.54	g/100g
Carbohydrate	18.81	g/100g
Lipid as fat	7.91	g/100g
Ash	28.17	g/100g
Calcium (ca)	7051.03	mg/kg
Magnesium (Mg)	180.83	mg/kg
Iron (Fe)	87.30	mg/kg
Phosphorus (P_2O_5)	8215.22	mg/kg

The study analyzed the growth performance of fish in different treatment groups and a control tank. The highest weight gain was observed in FWM 75 (75%), followed by FWM 50 (50%) and FWM 100 (100%). FWM 75 (75%) had the most efficient feed utilization (1.79±0.16), the highest SGR (1.39±0.33%), and the highest PER (1.25±0.08). The feed intake was similar across all groups, with T3 having the highest final body weight (10.78±1.01 g), final length (8.21±0.98 cm), and highest value (2.94±0.99 g/day). FWM 50 (50%) and FWM 100 (100%) also showed significant improvements compared to the control. The control group consistently showed the lowest growth performance, indicating that the experimental treatments positively influenced fish growth and feed utilization. FWM 75 (75%) showed the best growth performance across multiple parameters.

Table 4: Growth performances, feed utilization, and survival rate of *Anabas testudineus* in the 60-day feeding trial.

Growth performance	FWM0	FWM25	FWM50	FWM75	FWM100	*P value*
IBW	4.84±0.95	4.86±0.98	4.76±1.01	4.86±0.98	4.87±0.99	*1.00*
FWB	8.54±1.01	9.32±0.98	10.25±1.01	10.78±1.01	10.47±1.01	*0.106*
IL	6.21±1.01	6.18±1.02	6.3±1.36	6.17±0.978	6.26±1.01	*1.00*
FL	7.52±0.98	7.6±0.99	7.86±1.01	8.21±0.98	7.91±1.01	*0.916*
WG	3.27±0.35	4.10±0.33	5.53±0.56	6.33±0.40	5.52±0.49	*0.001*
FCR	3.04±0.10	2.2±0.36	1.92±0.13	1.79±0.16	1.92±0.13	*0.001*
SGR (per day %)	0.93±0.07	1.12±0.20	1.27±0.27	1.39±0.33	1.21±0.26	*0.280*
PER	0.78±0.08	0.95±0.07	1.21±0.07	1.25±0.08	1.20±0.07	*0.001*
Feed Intake (g/fish)	11.65±0.89	11.62±0.92	11.38±0.98	11.68±1.00	11.64±0.97	*0.995*
ADWG	1.84±1.01	2.21±1.01	2.74±0.99	2.94±0.99	2.80±1.01	*0.644*
SR (%)	100	100	100	100	100	*0.000*

*Abbreviations shown in the table indicate as follows: Control (0% FM replacement), FWM25 (25% FM replacement), FWM50 (50% FM replacement), FWM75 (75% FM replacement), FWM100 (100% FM replacement), IBW (initial body weight), FBW (final body weight), IL (initial length), FL (final length), WG (weight gain), SGR (specific growth rate), ADWG (average daily weight gain), PER (protein efficiency ratio), FCR (feed conversion ratio) and SR (survival rate). Values indicate the mean±SD (n=3). Values in the same row followed by the different superscripts are significantly difference at $p<0.05$.

The study found that water quality parameters for freshwater fish culture were within acceptable ranges, despite being slightly above the optimal range of 33-35°C. The pH levels were within the ideal range of 6.5 to 8.5, while the dissolved oxygen levels were within the desired range of 5-8mg/L. The total ammonia levels were relatively low, suggesting minimal risk to fish health. The nitrate and nitrite levels were within acceptable limits for freshwater fish culture. Overall, the water quality parameters did not negatively affect growth performance and feed utilization during the 60-day feeding trial.

Table 5: Water quality parameters in experimental tanks *Anabas testudineus* during the 60-day feeding trial.

Parameters	**Water Quality Parameters**				
	FWM0	FWM25	FWM50	FWM75	FWM100
Temperature(°c)	34.18±1.61	34.18±1.61	34.18±1.61	34.18±1.61	34.18±1.61
pH	7.64±0.15	7.6±0.12	7.62±0.13	7.46±0.11	7.54±0.08
Do (mg/l)	5.72±0.14	5.66±0.20	5.70±0.12	5.64±0.16	5.68±0.17
TA (mg/l)	0.076±0.033	0.26±0.16	0.3±0.20	0.22±0.17	0.32±0.17
Nitrate(mg/l)	3.8±0.83	4.2±0.53	4.0±0.70	4.40±0.89	4.00±0.70
Nitrite(mg/l)	0.28±0.083	0.28±0.08	0.34±0.08	0.32±0.08	0.30±0.10

*Abbreviations shown in the table indicate as follows: FWM 0 (0% FM replacement), FWM25 (25% FM replacement), FWM50 (50% FM replacement), FWM75 (75% FM replacement), FWM100 (100% FM replacement). TA (Total Ammonia(mg/l)), DO (Dissolved Oxygen(mg/l).

The study found that higher dietary treatments led to more efficient energy use in fish. The control group had the highest ECR (2.41±0.01), while FWM 100 (100%) had the lowest (0.87±0.01). This suggests that increasing dietary treatment levels enhances fish's energy efficiency. The study also found that higher EPI (energy conversion efficiency) indicates better growth.

Table 6: Economic indicators of *Anabas testudineus* in the 60-day feeding trial.

Parameters	FWM0	FWM25	FWM50	FWM75	FWM100	P value
ECR	2.41±0.01	1.73±0.01	1.21±0.01	0.99±0.01	0.87±0.01	0.001
EPI	0.22±0.01	0.30±0.01	0.38±0.01	0.42±0.01	0.42±0.01	0.001

*Abbreviations shown in the table indicate as follows: Abbreviations shown in the table indicate as follows: CLM 0 (0% FM replacement), FWM 25 (25% FM replacement), FWM 50 (50% FM replacement) FWM 75 (75% FM replacement) FWM 100 (100% FM replacement), ECR (Economic conversion ratio) and EPI (Economic profit index).

* 1 US$ = 86.54 INR (20 JAN 2025).

* Selling price on 28 January 2025 by 2.6 US dollar kg^{-1} (source: www.kasetprice.com) Values indicate the mean±SD (n=3).

Discussion

The extensive reliance on conventional protein sources such as fishmeal (FM) and soybean meal (SBM) in aquafeed formulations has raised significant concerns regarding their sustainability, escalating costs, and environmental consequences. Issues such as overfishing, supply fluctuations, and price volatility underscore the urgency of identifying alternative protein sources to ensure the long-term sustainability of aquaculture (Harnedy and Fitzgerald, 2012; Rana *et al*., 2009). One promising strategy involves the valorization of fishery by-products, particularly fish waste, which continues to increase alongside global seafood consumption (Chitmanat *et al*., 2009; Sotolu, 2009; Obasa *et al*., 2011). This study aimed to evaluate the nutritional efficacy and sustainability of Fish Waste Meal (FWM) as a partial or complete replacement for fishmeal in the diet of Climbing Perch (*A. testudineus*). The investigation focused on the effects of FWM inclusion on growth performance, feed utilization, water quality, and economic viability. A 60-day feeding trial was conducted using diets containing 40% crude protein, wherein fishmeal was progressively substituted with FWM at inclusion levels of 0%, 25%, 50%, 75%, and 100%. One of the primary criteria for evaluating the suitability of alternative protein sources is their proximate composition. According to the National Research Council (NRC, 2011), ingredients containing more than 35% crude protein are classified as protein-rich and are considered suitable for fish nutrition (Jobling, 2012). The proximate analysis of FWM used in the present study revealed a crude protein content of 47.8%, crude lipid at 7.91%, crude fiber at 18.81%, and ash at 28.17%. While the crude protein level of FWM is lower than that typically reported for high-quality fishmeal (66–75% CP; Tacon and Metian, 2008), it remains comparable to rendered animal meals (53–94% CP) and surpasses many plant-derived protein sources, which generally range between 25–49% CP (Glencross, 2016).

These findings are consistent with previous studies that evaluated the nutritional composition of fish waste-based meals. For example, Sotolu (2009) reported crude protein and lipid contents of 61.6% and 9.5%, respectively, in fish waste meal derived from tilapia and catfish. Similarly, Obasa *et al*. (2011) observed 45.9% CP and 16.4% crude lipid in waste meals produced from smoked Ethmalosa fimbriata and Sardinella species. The variation in nutrient composition among studies may be attributed to differences in the species used and the specific waste components (e.g., viscera, heads, skin, and bones) incorporated into the meal. The relatively high protein and lipid levels of FWM in this study support its potential as a sustainable and nutritionally adequate alternative to fishmeal in aquafeed formulations. Feed palatability plays a critical role in determining feed intake and, ultimately, the growth performance of aquaculture species. Low palatability can result in feed refusal and suboptimal nutrient utilization (Kader *et al*., 2010, 2012). However, in this study, no significant differences in feed intake were observed across dietary treatments containing increasing levels of FWM. This suggests that the inclusion of FWM did not adversely affect feed palatability or acceptance by *A. testudineus* fingerlings.

The uniform feed intake observed across all dietary treatments indicates that diets incorporating fish waste meal (FWM) were organoleptically acceptable to *A. testudineus*. This observation supports the practical application of FWM as a feed ingredient without compromising palatability or voluntary feed intake, which are critical determinants of growth performance in aquaculture species.

Fermentation of fish waste has emerged as a viable strategy to enhance its utility as an alternative protein source in aquafeeds. The process, particularly when conducted using probiotics or indigenous microbial communities, facilitates the biochemical conversion of complex macromolecules into simpler, more bioavailable forms. Through enzymatic hydrolysis, fermentation effectively pre-digests proteins into peptides and free amino acids, thereby improving their gastrointestinal absorption and utilization efficiency in fish.

Ramachandran *et al*. (2020) demonstrated that solid-state fermentation of fish waste using Bacillus subtilis significantly elevated soluble protein content and enhanced amino acid availability. The enzymatic hydrolysis mimics endogenous digestive processes, reducing the metabolic burden on fish and supporting efficient nutrient assimilation.

Additionally, microbial fermentation plays a crucial role in mitigating anti-nutritional factors (ANFs) and other potentially harmful compounds present in raw fish waste. Ghaly *et al*. (2013) reported that fermentation significantly reduces levels of biogenic amines, ammonia, and pathogenic microbes, thereby improving the safety and shelf stability of the final product. Fermented fish waste has also been shown to preserve, or in some cases enhance, the profile of essential amino acids such as lysine, methionine, and threonine—nutrients that are indispensable for optimal fish growth and metabolic function (Oliva-Teles, 2012). The increased bioavailability of these amino acids through enzymatic action further supports the superior nutritional quality of fermented fish waste.

Experimental studies have validated the efficacy of fermented fish waste in promoting growth performance metrics comparable to those achieved with conventional fishmeal. In a recent feeding trial, Kumar *et al*. (2023) reported that *A. testudineus* fed diets incorporating fermented FWM exhibited significant improvements in feed conversion ratio (FCR), weight gain, and protein efficiency ratio (PER), confirming the nutritional adequacy and functional benefits of this alternative protein source.

Although feed intake remained similar across all treatments, fish fed with 25% FWM and the control diet demonstrated reduced growth performance and feed efficiency in comparison to those on diets containing 50%, 75%, and 100% FWM replacement. This indicates that while the fish consumed similar amounts of feed, the nutritional utilization and protein efficiency varied, affecting overall growth outcomes. Growth Performance and Feed Efficiency in *A. testudineus* Using FWM Supplementation. A study by Bhaskar *et al*. (2015) investigated the growth performance of climbing perch (*A. testudineus*) by substituting fishmeal with poultry viscera as a potential feed component. Results indicated that the Feed Conversion Ratio (FCR) reached its peak at 3.82 in the control diet. 100% fishmeal was used, and gradually decreased (2.10) when fishmeal was completely replaced with poultry viscera. A similar trend was previously observed in tilapia (Jauncey, 1982). Additionally, Doolgindachabaporn (1994) reported an FCR range of 1.8 to 3.0 for climbing perch, while Potongkam (1972) found an FCR of 2.07 for fish fed trash fish and 1.89 for those fed pellets. In this study, the highest feed conversion ratio (FCR) was recorded for Fish Waste Meal (FWM) at 75% (1.79±0.16), showcasing better feed efficiency than the control tank, which had an FCR of 3.04±0.10, where fishmeal was entirely substituted with FWM. The findings revealed notable variations in growth rates among fish that were given different dietary regimens. The Specific Growth Rate (SGR) followed a similar pattern, with the highest rate observed in FWM 75 (75%) (1.39±0.33% per day), followed by FWM 50 (50%) (1.27±0.27%), while the control group exhibited the lowest SGR (0.93±0.07% per day) This indicates that fish fed with FWM-based diets utilized feed more efficiently than those in the control group, supporting previous research findings that alternative protein sources, including fish waste meal, can enhance feed efficiency (Espe *et al*., 2006; Glencross, 2016). Statistical analysis confirmed that FCR and SGR varied significantly across treatments, indicating that supplementation with FWM improved growth efficiency. The highest weight gain was recorded in FWM 75 (75%) (6.33±0.40 g), followed by FWM 50 (50%) (5.53±0.56 g) and FWM 100 (100%) (5.52±0.49 g). The ANOVA analysis indicated that there were significant differences between the treatments. FWM 75 and FWM 50 significantly outperform the control. The control tank showed the lowest weight gain (3.27±0.35 g), reinforcing that FWM supplementation positively influenced fish growth. The results revealed significant improvements in weight gain with increased inclusion of FWM. Fish in FWM 75(75%) FWM replacement) showed the highest weight gain (6.33±0.40 g), followed by FWM 50 (50%) at 5.53±0.56 g and FWM 100 (100%) at 5.52±0.49 g. The control group exhibited the least weight gain, recording an increase of 3.27±0.35 g, indicating that replacing fishmeal with FWM significantly enhanced growth performance. The final body weight followed a similar trend, with FWM 75 recording the highest value (10.78±1.01 g), followed by FWM 50 (10.25±1.01 g) and FWM 100 (10.47±1.01 g). The ANOVA analysis indicated that there were significant differences between the treatments. These findings suggest that a 75% replacement level of fishmeal with FWM is optimal for maximizing growth. Previous research has also noted that fish waste meal improves growth performance, attributed to its high-quality protein content and well-balanced amino acid profile (Hernández *et al*., 2018; Hasan *et al*., 2019).

Protein Efficiency Ratio (PER), an important indicator of how efficiently fish utilize dietary protein, the levels in the FWM-fed groups were considerably elevated when compared to the control group. The highest PER was observed in FWM 75 (1.25±0.08), followed by FWM 50 (1.21±0.07) and FWM 100 (1.20±0.07). This indicates that fish waste meal served as a high-quality protein source that was efficiently used for growth, aligning with results from research on alternative protein sources in aquafeeds. (Rana *et al*., 2009; Wang *et al*., 2021). The highest final length was recorded in FWM 75 (8.21±0.98 cm), indicating that optimal supplementation supports overall fish development. The average daily weight gain was highest in FWM 75 (2.94±0.99 g/day), followed by FWM 100 (2.80±1.01 g/day) and FWM 50 (2.74±0.99

g/day). Similarly, relative SGR was highest in FWM 75 (0.0165±0.006), significantly improving over the control tank (0.0093±0.001). To validate these findings, one-way ANOVA was performed on key growth parameters, confirming significant variations across treatment groups. The survival rate was 100% across all treatments, indicating that FWM-based diets did not negatively impact fish health. The elevated survival rate indicates that FWM is a safe and effective substitute for fishmeal in the diet of *A. testudineus*. Similar survival rates were reported in previous studies using fish by-products in aquafeeds (Vongvichith *et al*., 2020).

The recorded water temperature remained consistent across all treatments (34.18 ± 1.61°C), indicating that dietary modifications had no direct impact on temperature fluctuations. The observed temperature range is slightly higher than the optimal range of 26–32°C, as recommended for climbing perch culture (Rahman *et al*., 2018; Charo-Karisa *et al*., 2006). The pH values across treatments ranged from 7.46 ± 0.11 (FWM 75) to 7.64 ± 0.15 (control), with FWM 75 showing the lowest pH among all groups. These values fall within the optimal range (6.5–8.5) recommended for freshwater aquaculture (Boyd, 2017). The slight variation in pH across treatments could be attributed to the different metabolic byproducts associated with protein digestion and nutrient metabolism (Ebeling *et al*., 2006). Despite these minor fluctuations, the pH remained within acceptable limits for *A. testudineus* growth and survival. Dissolved oxygen concentrations remained relatively stable across all treatments, ranging between 5.64 ± 0.16 mg/L (FEM 75) and 5.72 ± 0.14 mg/L (FWM 0). These values are above the critical threshold of 3 mg/L required for the healthy growth of climbing perch (Swingle, 1969). The slight reduction in DO levels in FWM-supplemented diets may be due to increased organic matter decomposition, which can lead to higher oxygen consumption by microbial activity (Avnimelech, 2006). Dissolved oxygen concentrations remained relatively stable across all treatments, ranging between 5.64 ± 0.16 mg/L (FWM 75) and 5.72 ± 0.14 mg/L (FWM 0). These values are above the critical threshold of 3 mg/L required for the healthy growth of climbing perch (Swingle, 1969). The slight reduction in DO levels in FWM-supplemented diets may be due to increased organic matter decomposition, which can lead to higher oxygen consumption by microbial activity (Avnimelech, 2006). Nitrate levels ranged between 3.8 ± 0.83 mg/L (FWM 0) and 4.40 ± 0.89 mg/L (FWM 75), while nitrite values varied from 0.28 ± 0.083 mg/L (FWM 0) to 0.34 ± 0.08 mg/L (FWM 50). These findings suggest that nitrate accumulation increased slightly with higher FWM inclusion, likely due to the breakdown of ammonia in the nitrogen cycle (Hargreaves, 1998). However, nitrate levels remained within the recommended limit of <50 mg/L for freshwater aquaculture (Camargo *et al*., 2005), and nitrite concentrations did not reach toxic thresholds (>1 mg/L) (Wedemeyer, 1996). The results indicate that the inclusion of FWM in fish diets had minor effects on water quality, with slightly increased ammonia and nitrate levels in treatments with higher FWM inclusion. This could be linked to higher nitrogenous waste excretion, a common phenomenon when protein-rich ingredients are used in aquafeeds (Tacon *et al*., 2020). Despite these fluctuations, all parameters remained within acceptable ranges for climbing perch culture, and no adverse effects on fish health or survival were observed. Similar studies have shown that alternative protein sources can slightly impact water quality due to differences in digestibility and metabolic waste production (Wang *et al*., 2021). However, the 100% survival rate recorded in FWM 75 suggests that the climbing perch effectively adapted to the experimental diets without suffering from water quality-related stress.

Economic efficiency a crucial element in aquaculture is the economic aspect, which influences both the profitability and sustainability of fish production systems. This research assessed the Economic Conversion Ratio (ECR) and Economic Profit Index (EPI) across various dietary treatments to evaluate the financial viability of incorporating Fish Waste Meal (FWM) as a replacement for fishmeal in the diet of *A. testudineus*. ECR serves as an indicator of the cost-effectiveness of feed utilization, with lower values indicating higher economic efficiency (Hasan *et al*., 2019). The findings of this research indicate a significant decrease in ECR values with increasing levels of FWM substitution. The highest ECR (least cost-efficient) was recorded in the control tank (2.41 ± 0.01), where fishmeal was used exclusively. As FWM inclusion increased, ECR values gradually decreased, with the lowest ECR observed in FWM 100 (100% FWM) at 0.87 ± 0.01, followed by FWM 75 (75% FWM) at 0.99 ± 0.01. These results indicate that partial or complete replacement of fishmeal with FWM significantly improved economic efficiency. The lower ECR in FWM-based diets suggests that fish waste by-products are a cost-effective alternative to fishmeal, reducing feed costs while maintaining fish growth and survival. Comparable results have been observed in research focused on alternative protein sources, where fish waste and other by-products contributed to reduced feed costs without compromising the performance of fish (Yamamoto *et al*., 2005; Rana *et al*., 2009). The improved ECR values in FWM 75 (75%) and FWM 100 (100%) suggest that higher FWM inclusion resulted in more efficient nutrient utilization, likely due to better Protein digestibility and the balanced composition of amino acids in fish waste meal. (Harnedy and FitzGerald, 2012). However, the excessive inclusion of non-traditional protein sources should be monitored to ensure optimal nutrient absorption and prevent potential digestibility issues (Tacon *et al*., 2020; Tacon and Metian, 2008).

EPI is an essential indicator of economic return, with higher values signifying greater profitability (Abowei and Ekubo, 2011). In this study, EPI values increased significantly with higher FWM inclusion. The lowest EPI was recorded in the control tank (0.22 ± 0.01). As FWM inclusion increased, EPI gradually increased, reaching the highest values in FWM 75 (75%) and FWM 100 (100%) at 0.42 ± 0.01.

These findings highlight that replacing fishmeal with FWM enhanced economic profitability, as lower feed costs resulted in better financial returns. Similar trends have been observed in previous studies, where replacing fishmeal with cost-efficient protein sources significantly increased EPI values due to reduced feed expenses and improved overall growth performance of fish (Hasan *et al*., 2019; Hernández *et al*., 2018). The comparable EPI values between FWM 75 (75%) and FWM 100 (100%) suggest that a 75% replacement level may be optimal for maximizing profit, as further increasing

FWM inclusion (100%) did not yield additional economic benefits. This aligns with previous research indicating that a balance between alternative and traditional protein sources is necessary to maintain optimal feed efficiency and cost savings (Espe *et al*., 2006; Glencross, 2016). The findings of this study demonstrate that increasing the inclusion levels of FWM to as much as 75% led to notable cost reductions and enhanced profitability. The reduction in ECR and increase in EPI demonstrate that FWM is a viable alternative protein source, offering both economic and environmental benefits by utilizing fish waste by-products effectively. However, while 100% replacement (FWM 100) exhibited the lowest ECR alongside the highest EPI, the economic advantage over 75% replacement (FWM 75) was marginal, suggesting that complete fishmeal substitution may not be necessary to achieve substantial cost savings. A 75% replacement level seems to be the most economical option and sustainable option, aligning with findings from previous studies on fishmeal replacement strategies (Gatlin *et al*., 2007; Wang *et al*., 2021). The research validates that incorporating FWM into the diet of *A. testudineus* significantly improves economic efficiency by reducing feed costs and enhancing profitability. The minimum ECR and maximum EPI were recorded in FWM 75 (75%) and FWM 100 (100%), demonstrating that FWM is a sustainable, cost-effective alternative to fishmeal. These findings support the adoption of fish waste by-products as an economically viable ingredient in aquafeeds, contributing to sustainable aquaculture practices and reduced dependence on traditional fishmeal sources.

Conclusion

This study indicated that Fish Waste Meal (FWM) presents a viable substitute for conventional fishmeal in the diet of Climbing Perch (*Anabas testudineus*). The fish were fed diets with 50%, 75%, and 100% FWM replacement. Although initial body weight (IBW), initial and final length (IL and FL), and feed intake remained statistically similar across all treatments, significant improvements were observed in weight gain (WG), feed conversion ratio (FCR), and protein efficiency ratio (PER) with increasing levels of FWM inclusion. The highest WG (6.33 ± 0.40 g), lowest FCR (1.79 ± 0.16), and highest PER (1.25 ± 0.08) were recorded at 75% FWM inclusion, indicating enhanced feed utilization and nutrient efficiency. While specific growth rate (SGR) and average daily weight gain (ADWG) showed numerical improvement with FWM, the differences were insignificant. Importantly, a 100% survival rate was observed across all groups, confirming the safety and acceptability of FWM-based diets. The economic analysis highlighted significant cost savings and increased profitability with higher FWM inclusion levels, with 75% replacement offering the best balance between cost-effectiveness and performance. These findings support the utilization of fish waste by-products as an eco-friendly protein source for aquafeeds, reducing reliance on traditional fishmeal while promoting environmentally responsible aquaculture practices. Future Research should concentrate on enhancing the formulation process and assessing the long-term impacts of FWM-based diets on fish health and the quality of the products.

Conflicts of interest:

The author declares no conflict of interest. All the co-authors have seen and approved the final version of the article and have agreed to submit the article to the Journal for publication**.**

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