



International Journal of Fauna and Biological Studies

Available online at www.faunajournal.com

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International
Journal of
Fauna and
Biological
Studies

E-ISSN 2347-2677

P-ISSN 2394-0522

Impact Factor (RJIF): 5.69

<https://www.faunajournal.com>

IJFBS 2025; 12(4): 109-115

Received: 16-06-2025

Accepted: 18-07-2025

Md. Tafiqul Islam

Faculty of Fisheries, Department
of Fisheries, University of
Rajshahi, Rajshahi-6205,
Bangladesh

Md. Shadin Ahmed Sojib

Faculty of Fisheries, Department
of Fisheries, University of
Rajshahi, Rajshahi-6205,
Bangladesh

Md. Al-Amin Sarker

Faculty of Fisheries, Department
of Fisheries, University of
Rajshahi, Rajshahi-6205,
Bangladesh

Incorporation of fermented mustard oilcake in fish feed and its effect on growth performance and economics of *Rui Labeo rohita* (Ham. 1822)

Md. Tafiqul Islam, Md. Shadin Ahmed Sojib and Md. Al-Amin Sarker

DOI: <https://www.doi.org/10.22271/23940522.2025.v12.i4b.1117>

Abstract

This study assessed the impacts of fermented mustard oilcake (MOC) on the diet of *Labeo rohita* on growth performance and economic returns. The three-month experiment was carried out in nine cages with three dietary treatments: T₁ (NFD), T₂ (50% FD), and T₃ (100% FD), each replicated three times. Solid-state fermentation of MOC was prepared using *Saccharomyces cerevisiae* to improve its nutritional quality, which decreases anti-nutritional factors. Water quality parameters remain within acceptable limits and were unaffected by the dietary treatments. Results indicated that fish in the T₂ group had significantly higher weight gain, specific growth rate (SGR), and average daily gain (ADG). Economically, T₂ achieved the highest cost-benefit ratio (0.53) and net profit, demonstrating better feed efficiency. Although T₃ showed moderate growth, the increased feed costs lessened profitability. Overall, a 50% inclusion of fermented MOC could improve growth and be economically feasible for *L. rohita*, promoting sustainable and cost-effective aquaculture practices.

Keywords: Feed cost, feed efficiency, sustainability, solid-state fermentation, profitability

1. Introduction

Aquaculture is the world's fastest-growing food production sector, increasing at a rate of 5.3% per year from 2001 to 2018 (FAO, 2020) ^[13]. This sector plays a significant role in providing income, employment, nutrition, animal protein supply, and earning foreign currency, contributing approximately 3.52% of the gross domestic product (GDP), 26.37% of agricultural resources, and 1.39% foreign currency of Bangladesh (DoF, 2020) ^[10]. One of the factors limiting the development of the aquaculture industry is the high price of aqua feed, which accounts for the largest percentage of production costs (Li *et al.*, 2015) ^[19].

Fish meal is widely employed as a protein source in the majority of formulated fish diets, and plays a crucial role in providing essential amino acid that promote fish growth and ensure their long-term well-being; however, it is considered a costly component (Haider *et al.*, 2018; Bjorndal *et al.*, 2024) ^[16, 7]. Therefore, owing to the limited supply of fish meal, it is necessary to replace it with cheaper alternative protein sources (Al-Thobaitia *et al.*, 2017) ^[3]. To reduce feed costs in commercial fish farming and ensure the sustainability of this industry, the level of fish meal in aquafeed manufacturing needs to be decreased. Considering alternative plant and animal protein sources for freshwater fish species is crucial. The most promising alternatives to fishmeal in carp diets are oilseed meals, such as mustard oil cake, linseed, and sesame oil cake (Hossain and Jauncey, 1989) ^[17]. Mustard oil cake is a promising alternative to animal protein as it contains 28-37% protein and is locally available, economically viable, and affordable to farmers in developing countries such as Bangladesh, for improving aquaculture productivity (Sarker *et al.*, 2015) ^[29]. However, certain compounds found in mustard oil cake contain anti-nutritional factors (ANFs), such as saponin (8-9%) and tannin (6-7%), which limit their use as fish feed components (Das *et al.*, 2022) ^[8]. These substances impair the digestibility of oilseeds and have negative effects when ingested (Jithender *et al.*, 2019) ^[18]. Therefore, optimizing processing methods to completely or partially remove ANFs from mustard oil would make it a viable component of fish diets. Fermentation is a cost-effective technique that breaks down complex compounds into simpler forms, making the fermented product more easily digestible than its original form (Setiarto, 2020) ^[30], and there is a notable reduction in fiber, lipids, ash, and phytic acid content (Flores-Miranda *et al.*, 2014) ^[14].

Corresponding Author:

Md. Al-Amin Sarker

Faculty of Fisheries, Department
of Fisheries, University of
Rajshahi, Rajshahi-6205,
Bangladesh

This could be the result of microorganisms breaking down toxins or anti-nutrients into less harmful substances (Shamna *et al.*, 2015) ^[31].

Rohu is a key aquaculture species in India, Bangladesh, Pakistan, and Myanmar (Burma). The excellent fecundity (2 lakh eggs/kg), external fertilization, and domestication of this species have made intensive culture feasible (Rasal and Sundaray 2020) ^[27]. It contains polyunsaturated fatty acids that are essential for human growth and development (Memon *et al.*, 2011) ^[21]. Carp production accounts for more than 72% of global freshwater aquaculture production, with *L. rohita* contributing approximately 15% (FAO, 2009) ^[12]. As a result, it has emerged as an important aquaculture species, contributing 3.7% of the global aquaculture production in 2018 (FAO, 2020) ^[13]. The use of fermented feed ingredients as partial replacements for fishmeal or soybean meal in aquaculture diets has emerged as a growing research area. Previous studies have reported that the incorporation of fermented linseed oil cake as a feed ingredient in the diets of the Indian major carp, *L. rohita*, achieved experimental success (Banerjee *et al.*, 2023) ^[5]; 40% fermented mohua oil cake was found to enhance growth and well-being during the pond culture of *L. rohita* (Das *et al.*, 2024) ^[9]. Few studies have explored the impact of fermented mustard oil cake (MOC) on rohu carp under controlled conditions in Bangladesh. Therefore, the present study aimed to evaluate the effects of dietary fermented mustard oil cake on the production performance, feed utilization, and economic analysis of *L. rohita*.

2. Materials and Methods

2.1 Study area and duration

The experiment was conducted in an experimental pond behind the hatchery building at the Department of Fisheries, University of Rajshahi, Bangladesh (latitude 22° 39.6' N and longitude 88° 38' 07.8" E). This experiment was conducted

for three months from August to October 2023.

2.2 Construction of cages

Nine experimental cages (each measuring 2×1×1 m³) were constructed for this experiment. The cage frames were made of iron rods and covered with a special knotless synthetic nylon net with a mesh size of 2 mm. The mesh size was suitable for the passage of water through the cage. The net was tied to the cage frames using nylon twine.

2.3 Solid-state fermentation of mustard oil cake

In the present study, we used baker's yeast containing *Saccharomyces cerevisiae* as the inoculum for the solid-state fermentation of mustard oil cake. Dried and finely powdered mustard oil-cake was subjected to SSF in a circular drum. Sterile water was added to obtain a final moisture level of 25% in the fermentation mix. Then, *S. cerevisiae* was added to the wet fermentation mix at a rate of 3 % and incubated under anaerobic conditions at 35 °C for 48 h. Wet samples were then collected and autoclaved at 105 °C for 30 min to end the fermentation process. Following a 24-hour drying process at 60 °C in a hot air oven, the fermented autoclaved samples were cooled, ground, packed, and stored at -18 °C until use.

2.4 Experimental diet preparation

The feed ingredients and their percent compositions in the experimental diets are shown in Table 1. The ingredients selected for the experimental diet were collected from a local market. First, all feed ingredients were finely ground using a grinder machine and sieved to less than 0.5 mm particle sizes. Then, an adequate amount of water was added to make a dough and pelletized (approximately 2 mm) using a hand pelletizer. The pelletized feed was then sun-dried for three days. Finally, the well-dried pellet feeds were stored in an airtight plastic container at 4°C until use.

Table 1: Percentage composition of different feed ingredients in the formulation of the experimental diet

Ingredients	Percentage (%)		
	T ₁ (NFD)	T ₂ (50% FD)	T ₃ (100% FD)
Fish meal	15	15	15
Mustered oilcake (fermented) ^a	0	18	36
Mustard oil cake	36	18	0
Wheat flour	5	5	5
Maize meal	10	10	10
Rice Bran	15	15	15
Soya bean meal	10	10	10
Soybean oil	7	7	7
Vitamin pre-mixture ^b	1	1	1
Choline chloride ^c	0.5	0.5	0.5
Vitamin E (50%)	0.5	0.5	0.5
Total	100	100	100

NFD=Non-fermented diet, FD=Fermented diet

^aFermented mustard oil cake by using baker's yeast (*Saccharomyces cerevisiae*) at a rate of 3% for 48 hrs under anaerobic conditions,

^bVitamin premix (mg/kg of premix): Vitamin A-156000 IU, vitamin D3-31200 IU, vitamin E-299, vitamin K3-26, vitamin B1-32.5, vitamin B2-65, vitamin B6-520, vitamin B12-0.16, nicotinic acid-520, folic acid-10.4, copper-130, iodine-5.2, manganese-780, and selenium-1.95. Renata Animal Health Pharma Co., Ltd., Bangladesh, supplied premix.

^cCholine chloride (Composition): Choline Chloride=60.96%,

Amine/Ammonia as (CH₃)₃N=0.0249%, Heavy Metal (As Pb) = ≤ 0.002%, Loss on Drying=0.73%, Fineness=90%, Choline Content (C₅H₁₅NO₂)=52.10%. Tangshan Finely Animal Care, China, supplied choline chloride].

2.5 Collection of fish, stocking and sampling

Rui (*L. rohita*) was selected for the study. This fish species was collected from Parila, Paba, Rajshahi, Bangladesh. The collected fish were transported to the experimental site in an oxygenated polythene bag. Before stocking, collected fish

were acclimatized in a hapa for 21 days. During this period, the fish was fed a commercial feed (Quality fish feed). Initial sampling was done before stocking the fish, and it was continued fortnightly. Final sampling was done after 3 months of rearing by a scoop net. Then, the fish were weighed using a digital balance.

2.6 Experimental design and feeding trials

The experiment consisted of three treatment groups: T₁ (control, NFD), T₂ (50% FD), and T₃ (100% FD). Each treatment was replicated thrice to minimize experimental error and enhance the reliability of the results. After acclimatization, a total of 180 fish were evenly distributed into nine experimental cages within a pond, assigned to three treatment groups (T₁, T₂, and T₃). Each group was fed twice daily (at 9:00 a.m. and 5:00 p.m.) at 5% of their body weight.

2.7 Water quality parameter analysis

To maintain optimum living conditions of the experimental fish, key physico-chemical parameters of the water were routinely monitored. Water samples were collected each morning (between 8:00 and 9:00 am) to assess some important water quality parameters like water temperature, DO, pH, CO₂, and alkalinity. Water temperature was measured using a Celsius thermometer and expressed as °C. Dissolved oxygen (DO) was determined by using a HACH kit; results were expressed in mg/L. Free carbon dioxide (CO₂) was measured via digital titration using phenolphthalein indicator powder pillows and 0.363 N sodium hydroxide, with concentrations also expressed in mg/L. pH was assessed using a pH indicator. Total alkalinity was determined by titration using N/50 sulfuric acid, methyl orange, and phenolphthalein indicators.

2.8 Growth performance and feed utilization parameters

The following formula was used for evaluating the growth performance and feed utilization of the experimental fish; Mean weight gain (g)=Mean final weight (g)-Mean initial weight (g)

$$\% \text{ Weight gain} = (\text{Mean final weight} - \text{Mean initial weight}) / (\text{Mean initial weight}) \times 100$$

$$\text{FCR} = \text{Food fed (dry weight)} / \text{Live weight gain}$$

$$\text{SGR} (\%, \text{ bwd}^{-1}) = \frac{[L_n (\text{final weight}) - L_n (\text{initial weight})]}{\text{Culture period (day)}} \times 100$$

$$\text{ADG (g)} = (\text{Mean final weight} - \text{Mean initial weight}) / \text{Culture period}$$

$$\text{Survival rate (\%)} = \times 100$$

$$\text{Yield (kg/ha)} = \text{fish biomass at harvest} - \text{fish biomass at stock}$$

2.9 Economic analysis

To calculate the economic return of the treatments, a basic economic analysis was conducted by the following formula:

$$\text{Benefit-Cost Ratio} = \sum \text{PV of all the Expected Benefits} / \sum \text{PV of all the Associated Costs.}$$

$$\text{Net Present Value} = \sum \text{PV of all the Expected Benefits} - \sum \text{PV of all the Associated Costs.}$$

2.10 Statistical analysis

Statistical analysis of collected data, one-way analysis of variance (ANOVA) was performed using the computer software SPSS (Statistical Package for Social Science, version 20.0). Significance was assigned at the 0.05 level.

3. Results

3.1 Water quality parameter

The water quality parameters observed in the study were temperature, DO, pH, CO₂, and Total alkalinity. Mean values for each parameter are shown in Table 2 (Figure 1). There were no significant ($p > 0.05$) differences in mean water quality parameters among the treatments. It was observed that the temperature, DO, pH, alkalinity and CO₂ ranged from 28.20 to 28.34 °C, 4.63 to 4.69 mg/l, 7.0 to 7.17, 143.33 to 147.67 mg/l, and 3.15 to 3.18 mg/l, respectively.

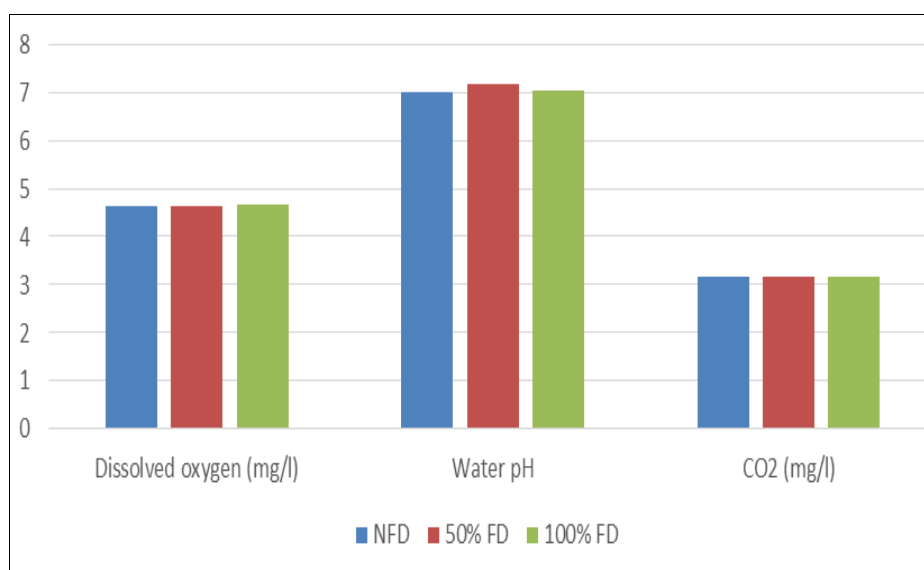


Fig 1: Mean water quality parameter under different treatment during the study

Table 2: Variation in the mean values of water quality parameters in different treatments during the study (Mean \pm SD), (N=3)

Parameters	Treatments		
	T ₁ (NFD)	T ₂ (50% FD)	T ₃ (100% FD)
Temperature (°C)	28.20 \pm 0.12 ^a	28.34 \pm 0.21 ^a	28.34 \pm 0.22 ^a
Dissolved oxygen (mg/l)	4.63 \pm 0.04 ^a	4.69 \pm 0.12 ^a	4.66 \pm 0.11 ^a
Water pH	7.00 \pm 0.11 ^a	7.17 \pm 0.06 ^a	7.03 \pm 0.08 ^a
Alkalinity(mg/L)	145.67 \pm 2.08 ^a	143.33 \pm 1.53 ^a	147.67 \pm 6.43 ^a
CO ₂ (mg/l)	3.18 \pm 0.06 ^a	3.15 \pm 0.06 ^a	3.15 \pm 0.05 ^a

Mean values in each row with the same superscripts have no significant differences ($p>0.05$)

3.2 Growth performance

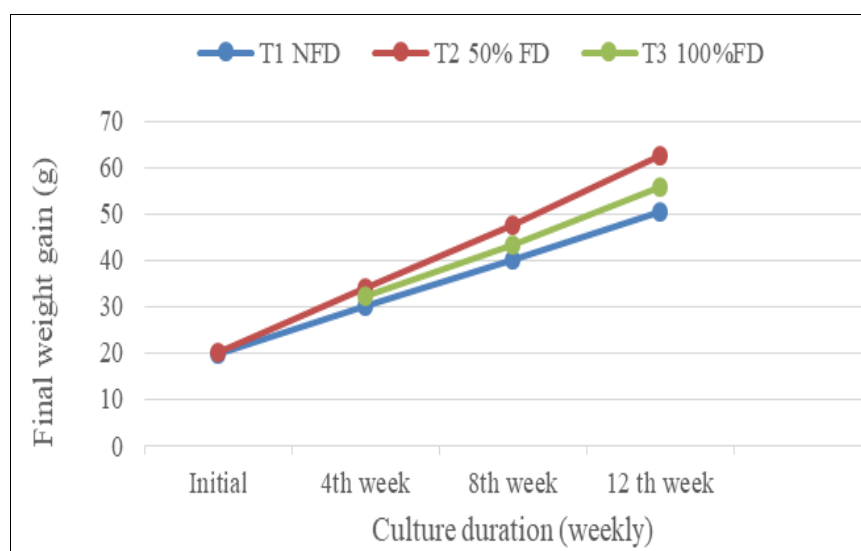
The mean growth performance and feed utilization of the experimental fish were determined in terms of initial weight, final weight, weight gain, food conversion ratio (FCR), survivability, specific growth rate (SGR) and average daily gain, are shown in Table 3. It was found that there was no significant ($p>0.05$) difference in mean initial weight among

the treatments. Significantly higher ($p<0.05$) mean weight gain, SGR, and ADG were in T₂ (42.44 \pm 0.7 g), (1.32 \pm 0.0), and (0.47 \pm 0.08), respectively. The recent study also revealed that there was a significant difference in FCR among the treatments, and the best FCR was observed in T₂ (1.90). Differences in mean final weight over time are shown in Figure 2.

Table 3: Mean growth performance of experimental fish after 90 days of rearing (N=3)

Parameters	Treatments		
	T ₁ (NFD)	T ₂ (50% FD)	T ₃ (100% FD)
Mean Initial Weight (g)	19.67 \pm 2.8 ^a	19.96 \pm 3.33 ^a	19.72 \pm 3.5 ^a
Mean final Weight (g)	50.30 \pm 8.5 ^c	62.40 \pm 9.3 ^a	55.90 \pm 7.3 ^b
Mean Weight Gain(g)	30.34 \pm 0.7 ^c	42.44 \pm 0.7 ^a	36.18 \pm 1.0 ^b
FCR	2.30 \pm 0.1 ^a	1.90 \pm 0.0 ^c	2.10 \pm 0.2 ^b
Survivability (%)	84.86 \pm 1.73 ^a	85.00 \pm 2.00 ^a	85.27 \pm 1.58 ^a
SGR (%/day)	1.06 \pm 0.0 ^c	1.32 \pm 0.0 ^a	1.20 \pm 0.0 ^b
ADG (g)	0.34 \pm 0.05 ^c	0.47 \pm 0.08 ^a	0.40 \pm 0.06 ^b

Mean values in each row with the different superscripts have significant differences ($p<0.05$)

**Fig 2:** Monthly weight gain trends in different treatments during the study

3.3 Economic analysis

A summary of the economic analysis of the experimental fish is shown in Table 4. The recent study found that significantly ($p<0.05$) higher production (3.12 kg/cage/cycle) and CBR

(0.53) were found in T₂ than in other treatments. It also revealed that significantly ($p<0.05$) higher production cost was in T₃ (579 tk/cage/cycle) and lower cost was in T₁ (491 tk/cage/cycle).

Table 4: Mean economic analysis of experimental fish at the end of three months (N=3)

Parameters	Treatments		
	T ₁ (NFD)	T ₂ (50% FD)	T ₃ (100% FD)
Total production kg/cage/cycle	2.52 \pm 0.0 ^c	3.12 \pm 1.1 ^a	2.79 \pm 1.1 ^b
Total cost tk/cage/cycle	491.66 \pm 1.5 ^c	561.14 \pm 1.6 ^b	579.60 \pm 6.1 ^a
Total income tk/cage/cycle	691.67 \pm 13.2 ^c	857.63 \pm 20.7 ^a	767.62 \pm 35.8 ^b
Profit	200.01 \pm 12.3 ^c	296.49 \pm 20.5 ^a	188.01 \pm 40.7 ^b
CBR	0.41 \pm 0.0 ^c	0.53 \pm 0.0 ^a	0.32 \pm 0.1 ^b

Mean values in each row with the different superscripts have significant differences ($p<0.05$)

4. Discussion

4.1 Water quality parameter

Understanding water quality variables, such as temperature, dissolved oxygen, pH, alkalinity, and carbon dioxide, is essential for effective aquaculture management. According to the results of the current study, water temperature was almost constant. Since temperature is a vital factor influencing biological functions, including digestion, metabolism, growth and development, reproduction, and overall physiological health, this small fluctuation had no adverse impacts on *L. rohita*. According to Abou *et al.* (2016) ^[1] and Samad *et al.* (2017) ^[28], who observed this steady, favorable temperature, suggest that nutritional interventions did not alter the water temperature in fish culture. Dissolved oxygen levels varied slightly but favorably among the dietary treatments, highlighting that a fermented diet has no potential negative effects on water DO. These findings support the compatibility of such diets with optimal aquaculture conditions and align with studies by Rahman *et al.* (2011) ^[26] and Wahab *et al.* (1995) ^[34]. Maintaining an ideal pH is crucial for fish survival and metabolic functions. The measured water pH across all treatments remained consistently near neutral. This pH stability matches established recommendations for freshwater fish culture and is supported by findings from Samad *et al.* (2017) ^[28] and Nwipie *et al.* (2015) ^[24]. The main sources of free carbon dioxide in water are air diffusion and respiration by aquatic organisms. Ekubo and Abowei (2011) ^[11] advise that for successful fish culture, CO₂ concentrations should be below 10 mg/l. According to Bhatnagar & Singh (2010) ^[6], the suitable range of free CO₂ for photosynthesis is 5-8 ppm; 12-15 ppm is sub-lethal to fish, and 50-60 ppm is lethal. Based on the above findings, it can be concluded that the free CO₂ levels in the experimental pond water were within a productive range of fish culture.

4.2 Growth performance

The possibility of using plant-derived proteins in conjunction with fish meal in aquafeeds has been studied by a range of researchers. Nevertheless, often containing high crude protein content, the use of these plant materials in aquaculture remains limited due to anti-nutritional effects that impair digestion and nutrient absorption (Mugwanya *et al.*, 2022; Siddaiah *et al.*, 2023) ^[22, 33]. A range of detoxification methods is being used to combat these issues by getting rid of these anti-nutrients. Among these, solid-state fermentation (SSF) has been recognized as an economically viable and ecologically sound biological detoxification technique that contributes to the biotransformation of plant waste, promoting sustainability and efficient nutrient recycling in aquaculture systems (Pandey *et al.*, 2001) ^[25]. Additionally, it has been anticipated that during fermentation, microbial production could boost the nutrient content (Ghosh *et al.*, 2015) ^[15].

The recent results show that the fermented mustard oil cake-based diet enhanced mean weight gain, SGR, and ADG in *L. rohita*, indicating an obvious advantage of fermented diets over the non-fermented diet (NFD). In addition to exhibiting better feed utilization in 50% FD diet groups, the lower feed conversion ratio also implies that fermentation improves metabolic and digestive utilizations of mustard oilcake. These findings are in line with previous research conducted on the same species by Mandal and Ghosh (2019) ^[20] found no negative effects when 20% bio-processed *Pistia* leaf in rui. Similarly, Bairagi *et al.* (2002) ^[4] showed that fermented duckweed (*Lemna polyrrhiza*) leaf meal was safe up to 30% in

L. rohita diets. Moreover, Banerjee *et al.* (2023) ^[5], who found no negative effects on growth, carcass composition, and feed utilization when 30% solid-state fermented linseed oil cake was included in *L. rohita* diets. Furthermore, Das *et al.* (2024) ^[9] discovered that in *L. rohita* fingerlings, up to 40% SSF mahua oil cake promoted the optimal growth, nutritional utilization, and immunological response. The findings are further in agreement with some previous research in different species, where Ngugi *et al.* (2016) ^[23] reported that using a fermented diet improves growth performance of tilapia without compromising economic benefit and Alom *et al.* (2025) ^[2] found that 50% fermented mustard oil cake has no negative effect on tilapia growth while increasing overall productivity and net profit. Conversely, the current study found that fish fed non-fermented diets performed poorly in terms of growth, most likely due to anti-nutritional factors and the high fibre content of raw mustard oil cake (Mandal *et al.*, 2019) ^[20].

4.3 Economic analysis

In aquaculture, fermented feed has emerged as a viable, affordable, and sustainable alternative to conventional fishmeal-based diets. The economic benefits of fermented diets were increased in the T₂ group, which showed an increase in total fish production and a favorable cost-benefit ratio (CBR), resulting in an enormous net profit. These findings emphasize that, despite the potentially higher initial cost of incorporating fermented ingredients compared to raw materials, improved growth performance and feed conversion efficiency significantly enhance overall profitability, resulting in a higher return on investment (Ngugi *et al.*, 2016; Shimul *et al.*, 2024) ^[24, 32]. On the other hand, the T₃ group, which involved a higher inclusion level of fermented mustard oilcake, experienced the highest total cost but did not achieve a proportionate increase in profit. However, this suggests that exceeding a 50% inclusion rate may not be economically advantageous for *L. rohita* under these experimental conditions.

5. Conclusion

In summary, this study demonstrates that the strategic incorporation of fermented mustard oilcake in the diets of *L. rohita* significantly enhances growth performance and improves economic returns. The 50% fermented mustard oil cake inclusion in T₂ emerged as the most advantageous, highlighting the potential of fermentation as a method to upgrade plant-based protein sources to high-quality aquaculture feed development. Future research should focus on optimizing fermentation conditions to improve the nutritional quality and digestibility of mustard oilcake.

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