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# Efficacy of using plant ingredients as partial substitute of fishmeal in formulated diet for a commercially cultured fish, *Labeo rohita*

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Reliance on fish meal can be reduced by incorporating plant-based ingredients, making aquaculture more economical, sustainable and environmental friendly. In this study, the efficacy of plant protein ingredients (PPI) such as mustard oil cake (MOC), soybean meal (SBM) and rice bran (RB) as Partial substitute of fishmeal (FM) was investigated for a commercially important fish, *Labeo rohita* in cages for 90 days. Three experimental diets, labeled as Diet 1, Diet 2, and Diet 3, were formulated to be isonitrogenous (with protein content ranging from 32.20 to 32.29%) and iso-caloric (with gross energy ranging from 4.12 to 4.17 kcal/g). These diets contained different proportions of PPI (45, 68, and 79%) and FM (46, 23, and 11%, respectively). Square-shaped cages with a volume of 1m<sup>3</sup> (1 m × 1 m × 1 m) were stocked with 40 fish/m<sup>3</sup> each with an average initial weight of 52.97 g in triplicates. Fish were hand-fed to apparent satiation twice daily for 7 days a week at a feeding rate of 5% in the initial month and 3% for the rest of the culture period. 50% of the caged fish was sampled monthly to monitor growth performance and at the termination of the experiment, all the fish was harvested to measure production economics performance. The results indicated improved growth performance and higher feed utilization at Diet 2, yielding significantly ( $p < 0.05$ ) higher fish production compared to Diet 3, while these parameters were insignificant with Diet 1. By replacing FM with PPI, the total feed cost compared to Diet 1 was reduced to 20.62 and 32.76% for Diet 2 and Diet 3, respectively. The replacement of 50% FM in Diet 2 also yielded a 15.61% higher total economic net return than the Diet 1 group. However, a higher inclusion rate of PPI in Diet 3 potentially reduced fish growth, with a consequent decline of 41.61% total economic net return compared to the Diet 1 group. In conclusion, the replacement of 50% FM in Diet 2 compared to Diet 1 returned a higher benefit–cost ratio (1.72) among the feeding groups. Therefore, this FM replacement experiment suggested a 50% FM replaced diet as an unconventional, cost-effective, and readily available novel protein source without compromising the inherent nutritional quality of fish and feed in the

cage culture of *L. rohita*. The results could be widely applicable to the fast-growing approach of cage culture technology across Asia and beyond.

#### KEYWORDS

feed formulation, growth metrics, proximate chemical composition, dietary protein sources, Indian major carps

## Introduction

The increasing trend of fish feed cost poses a challenge in intensive culture systems (Haider et al., 2016; Iqbal et al., 2020a; Bjørndal et al., 2024), with dietary protein being identified as the costliest component in the production of manufactured fish food. Generally, the cost of feed constitutes around 56.45–58.49% of the total production expenses in aquaculture industry (Hossain et al., 2022). In specific cases, it may even escalate to 60–70% (Khan M. A. et al., 2018; Khan N. et al., 2018; Prodhan and Khan, 2018; Hossain et al., 2020a,b). This is primarily attributed to the heightened requirements for protein, contributing significantly to the overall costs. Fishmeal (FM) is widely employed as a protein source in the majority of formulated diets and is considered as the costliest component in fish diets (Moniruzzaman and Fatema, 2022). Additionally, it plays a crucial role in providing essential nutrients that promote fish growth and ensure their long-term well-being (Batool et al., 2018; Haider et al., 2018; Bjørndal et al., 2024). FM is well balanced with respect to essential amino acids, fatty acids and minerals, has a low carbohydrate content, and is free of anti-nutritional factors with high palatability and digestibility (Gatlin et al., 2007; Bhuyain et al., 2019). As a result, the rapid growth of aquaculture demands for higher FM (NRC (National Research Council), 2011). Small pelagic fishes such as anchovies, sardines, mackerel, capelin, and menhaden are known to contribute about 90% of produced FM worldwide (Tacon and Metian, 2009). However, FM resources are finite, continuous pressure on these fish species' natural stock due to overfishing is likely to increase the scarcity and price of FM in the near future (Hardy, 2008; Savonitto et al., 2021). There is also acute scarcity in the supply of FM because of the equally high demand of this protein source from other animal husbandry practices and uncertainty in collecting wild trash fish, which is the primary source of FM (Naylor et al., 2000). Therefore, a higher cost and fluctuating FM supply necessitate replacing FM with cheaper, alternative protein sources with acceptable amino acid composition (Santigosa et al., 2011; Köprücü and Sertel, 2012; Al-Thobaitia et al., 2018). In this case, the more affordable and alternative FM replacement options could include plant protein ingredients (PPI), animal byproducts, and other novel protein feedstuffs (Kishawy et al., 2021).

The effectiveness of various PPI as a partial and complete replacement of FM in aquafeeds has been investigated by several researchers (Suprayudi et al., 2015; Aziza and El-Wahab, 2019) whereas, soybean, barley, corn, cottonseed, wheat, mustard oil cake, rice bran etc. can replace FM and are widely used in aquafeeds (El-Saidy and Gaber, 2002; Gatlin et al., 2007; Zamal et al., 2008; Koumi et al., 2009; Brinker and Friedrich, 2012; Khan et al., 2013; Ibrahim and Ibrahim, 2014). However, the inclusion of PPI (>50%) are sometimes reported to reduce the growth performance compared to that of fish fed FM-based diets (Collins et al., 2013; Yaghoubi et al.,

2016; Turchini et al., 2019). Because PPI are possessing anti-nutritional factors and indigestible carbohydrates, protein digestion and absorption of amino acids are less efficient in fish (Lall and Anderson, 2005). On the other hand, using only FM to the diets sometimes results in the waste of excessive protein which increases the load of nitrogen and phosphorus in the water and deteriorate the water quality in fish pond (Hardy, 2010). Study shows that the partial replacement of FM by PPI can reduce Phosphorous (Ketola and Harland, 1993) and Nitrogen excretion (as ammonia) by reducing protein levels (Cheng et al., 2003). Therefore, additional research is needed to adequately determine the inclusion rate of PPI in the partial replacement of FM in fish diet which could be readily available, cheap and environmentally friendly (Hernández et al., 2016; Hossain et al., 2021). Studies indicate that diets allowing for the partial or complete substitution of FM can be feasible through a meticulous formulation process (Espe et al., 2006; Kousoulaki et al., 2012).

*Labeo rohita*, locally known as rohu, is a culturally and economically significant fish species in Bangladesh, playing a vital role in aquaculture. Its vigorous biology and adaptability make it a preferred species for sustainable fish farming, contributing significantly to economic livelihoods, nutritional security, and the overall resilience of local communities (Jewel et al., 2020a; Pervin et al., 2020). The significance of developing aquaculture for *L. rohita* with low-cost feed in Bangladesh cannot be overstated as it is one of the sustainable practices. Because, by utilizing affordable feed options, aquaculture becomes more accessible to a broader spectrum of farmers, fostering widespread participation and contributing to poverty reduction. *L. rohita*, being a major cultivable species in the country, ensures that the production of this fish with low-cost feed provides an affordable protein source, positively impacting the nutritional well-being of the population. Additionally, the adoption of cost-effective feed formulations reduces the overall production costs, enhancing the competitiveness and profitability of aquaculture ventures in Bangladesh (Akter et al., 2018; Pervin et al., 2020; Jewel et al., 2023a).

In Bangladesh, high feed costs in aquaculture industry impose a significant economic burden on rural farmers, leading to reduced profitability and limiting the accessibility of aquaculture activities. Additionally, these elevated costs can contribute to food security concerns by potentially increasing fish prices and impacting the affordability of this essential protein source for local communities.

A primary factor contributing to this is the rising expense of fish feed ingredients, particularly the cost of FM in Bangladesh. Hence, replacing fish meal with plant-based protein sources has the potential to decrease costs. Considering the availability and crude protein content, a combination of Mustard oil cake (MOC), soybean meal (SBM) and rice-bran (RB) can be a suitable replacement option for FM. It is widely prevalent and extensively utilized as a component in

aquafeed across the country. Because, mustard is one of the major oilseed crops occupying 78% of the cultivated area and contributing nearly 62% of the cultivated area of the total oilseed production in Bangladesh (Bangladesh Bureau of Statistics (BBS), 2003). MOC serves as a relatively good source of crude protein (Bhuyain et al., 2019). Additionally, it is more cost-effective compared to other oil cakes in Bangladesh. Moreover, SBM stands out as the most frequently employed plant ingredient, boasting high protein content (approximately 48% crude protein) and a relatively stable amino acid profile (Ye et al., 2019; Meng et al., 2020; Pervin et al., 2020). RB or polish, a by-product of rice, is abundantly available throughout the year in Bangladesh. Several studies also demonstrated that RB contain 13–15% protein and 11–12% lipid (Saunders, 1990; Alencar and Alvarenger, 1991; Nyirenda et al., 2000) which signifies its role as a suitable ingredient to be used for FM replacer. However, research on low-cost feed development using locally available ingredients specially using PPI for *L. rohita* culture in Bangladesh is very limited. Hence, this study aims to (i) assess the nutritional effectiveness of MOC, SBM, and RB as a replacement for FM in fish diets, (ii) investigate the economic impact of different FM replacement levels in a cage culture system (CSS), (iii) analyze the proximate composition of harvested fish for nutritional quality assessment, and (iv) conduct an economic analysis to determine the profitability of using PPI as a FM substitute in *L. rohita* culture. The insights gained from the development of low-cost aquafeeds can be extended to advance sustainable aquaculture practices for various species. This not only contributes to economic empowerment and food security but also emphasizes a commitment to responsible and inclusive aquaculture practices in Bangladesh and other developing nations.

## Materials and methods

### Study area and installation of cages

The experiment was conducted for 90 days, from February to May 2017, in nine experimental cages at the Department of Fisheries, University of Rajshahi, Bangladesh. A total of three ponds were used for installing these nine cages, whereas each had three cages. The cages were square-shaped, with a volume of 1 m<sup>3</sup> (1 m × 1 m × 1 m) and built with metallic frames fully wrapped with a nylon net of 1 cm mesh size. Cages for each treatment were landed securely at a fixed place in a well-prepared fish pond with the help of bamboo poles. The cages were arranged in one column and firmly fixed by the bamboo poles set longitudinally and vertically. The cages were kept floating in pond water, keeping about 1 m distance from the pond bottom. Fish was hand-fed with a floating feeding tray attached inside each cage, facilitating the regular feeding of fish.

### Feed formulation

The feed ingredients with their percent compositions used in the experimental diet formulation and per kg feed production cost of the prepared diets are shown in Table 1. The selected ingredients for this experiment were collected from the local market. The feed ingredients (finely ground and sieved) were weighed accordingly, thoroughly mixed with a mixture, moistened with water to form the dough, and pelletized using a manual food grinder with a diameter of 2 mm. Three

TABLE 1 Formulation and proximate composition of experimental diets.

Ingredients (%)	Diet 1	Diet 2	Diet 3
Fishmeal	46	23	11
MOC (water treated)	0	23	35
Wheat flour	10	10	10
Rice bran	25	15	8
Soybean meal	10	20	26
Soybean oil	7	7	7
Vitamin premix <sup>a</sup>	1.5	1.5	1.5
Choline chloride	0.5	0.5	0.5
Vitamin E (50%)	0.1	0.1	0.1
Total	100.1	100.1	100.1
Feed formulation cost	44 BDT/kg	35 BDT/kg	31 BDT/kg
Proximate composition (Mean ± SEM)			
Crude Protein	32.29 ± 1.33	32.20 ± 1.50	32.25 ± 1.25
Lipid	9.80 ± 0.86	9.79 ± 1.01	9.73 ± 0.95
Moisture	6.33 ± 0.75	6.44 ± 0.66	6.62 ± 0.48
Ash	12.24 ± 1.03	12.21 ± 0.89	12.33 ± 1.12
Gross energy (kcal/g)	4.12 ± 0.52	4.15 ± 0.36	4.17 ± 0.46

<sup>a</sup>MOC, mustard oil cake, (Diet 1 (46% FM), Diet 2 (23% FM), and Diet 3 (11% FM). Crude protein (%) in FM = 57.26, MOC = 33.06, wheat flour = 11.80, rice bran = 14.50, soybean meal = 37.73, soybean oil = 46.30.

<sup>a</sup>Vitamin premix (mg/kg of premix): vitamin A-156000IU, vitamin D3-31200IU, 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. Premix was supplied by Renata Animal Health Pharma Co. Ltd. Bangladesh. Gross energy calculated according to NRC (1993).

diets were formulated with the selected ingredients. Three diets were prepared using the selected ingredients. Diet 1 contained 46% FM with a crude protein (CP) content of 32.29%. Additionally, the FM concentration in Diet 1 (46%) was decreased to 50 and 75% in Diet 2 (FM 23%) and Diet 3 (FM 11%), respectively. To compensate for the reduced CP in these two diets, various combinations of PPI were included. Diet 2 and 3 are containing 68 and 79% plant protein and are lower in animal-derived protein sources (FM 23 and 11% in diet 2 and 3, respectively). Finally, the prepared sinking pelleted feeds were sun-dried for 3 days and stored in airtight polythene bags at room temperature until feeding.

### Experimental setup and fish sampling

A total of three ponds were used for this experimental setup. Three cages were used for one specific experimental diet. Each cage was considered as replicate and therefore, each experimental diet of each pond was consisting of three replicated cages positioned as a row. Three treatments were assigned as Diet 1 (FM 46%), Diet 2 (FM 23%), and Diet 3 (FM 11%). The fingerlings with an initial average body weight of 52.97 g were collected from local vendors and released at a stocking density of 40 fish/m<sup>3</sup> in each cage. Fish were hand-fed to apparent satiation twice daily (9:00 am and 4:00 pm) for 7 days a week throughout the study period. The feeding rate was 5% in the initial month and 3% for the rest of the culture period. Every day, feed given to the fish was weighed, and the uneaten pellets were removed from the feeding tray at least 2 h after the feed was given. The uneaten feed

weight was estimated daily, and feed intake was calculated for each feeding group by subtraction between the weight of daily feed given and feed uneaten. Sampling (50% fish from each cage) was done monthly to monitor growth performance and to adjust the feeding ration accordingly. At the final harvest, all the fish in each cage were collected, their final growth and production were measured, and economics was calculated.

## Proximate composition of diets and fish

Diets and fish muscles were analyzed to measure crude protein, lipid, moisture, ash, and carbohydrate according to the steps followed by the Association of Official Analytical Chemists (AOAC (Association of Official Analytical Chemists), 2005). Ten fish were initially used for the analysis of proximate composition. Protein, lipid, moisture and ash content of the stocked fish were 11.07, 2.40, 84.60, and 1.07%, respectively. Furthermore, at final harvest, three fish were randomly selected from each feeding group, weighed, and sacrificed and the muscle tissue was collected. Crude protein was determined by the Kjeldahl method using the automatic Kjeldahl system; lipid by petroleum ether extraction using the Soxhlet method; ash by combustion at 550°C for 24h, moisture by oven drying at 105°C for 24h to a constant weight. A bomb calorimeter was used to determine the gross energy content of the diet. All the samples were analyzed in triplicates. The proximate composition of the formulated diets analyzed in the present study is presented in Table 1.

## Water quality monitoring

Water quality parameters viz. water temperature (WT), hydrogen ion concentration (pH), dissolved oxygen (DO), ammonia (NH<sub>3</sub>), and total alkalinity (TA) were studied fortnightly between 9:00 am to 10:00 am. WT was recorded with the help of a Celsius thermometer, while pH was measured using a pH indicator paper (Lojak). However, DO, TA and NH<sub>3</sub> concentrations were determined with the water quality testing kit (HACH kit FF-2, United States).

## Fish growth and production performances

After 90 days of the culture, all fish biomass was harvested from the cages. The following parameters were used to monitor fish growth and production performance during the sampling and after the harvesting.

$$\text{Weight gain (g)} = \text{Mean final weight (g)} - \text{Mean initial weight (g)}$$

$$\text{Specific growth rate (SGR\%, bw / d)} = \frac{\left[ \frac{\text{Ln}(\text{finalweight}) - \text{Ln}(\text{initialweight})}{\text{Cultureperiod (day)}} \right] \times 100}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed fed (dry weight)}}{\text{Live weight gain (g)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Live weight gain (g)}}{\text{Crude protein fed (g dry weight)}}$$

$$\text{Survival rate (\%)} = \frac{\text{No. of fish harvested}}{\text{No. of fish stocked}} \times 100$$

$$\text{Yield (kg / m}^3\text{)} = \text{Weight of fish harvested}$$

## Economic analysis

An economic analysis was performed to estimate the net economic return and benefit–cost ratio of the experimental diets used for the culture of *L. rohita* in CCS. The prices were expressed in Bangladesh Taka (BDT). The unit cost for cage preparation was BDT 150. Fingerlings were purchased as BDT 6/pieces while the selling price was BDT 200/kg in Diet 1, Diet 2, and BDT 180/kg in Diet 3. The following equation was used according to Asaduzzaman et al. (2010).

$$R = I - (FC + VC)$$

Where, R = net economic return, I = income from *L. rohita* sale, FC = fixed/common costs, VC = variable costs.

The benefit–cost ratio was determined as:

$$\text{Benefit - cost ratio (BCR)} = \text{Total economic return} / \text{Total cost}$$

## Statistical analysis

The data obtained were presented as means ± standard deviation (SD). One-way analysis of variance (ANOVA) was performed using SPSS (Statistical Package for Social Science, ver. 20.0) to determine the effect of diets in different treatments. Detected differences were compared by Duncan's multiple range test (DMRT), considering a significance level of  $p < 0.05$ . The percentages and ratios were analyzed using arcsine transformed data before conducting the one-way analysis of variance (ANOVA).

## Results

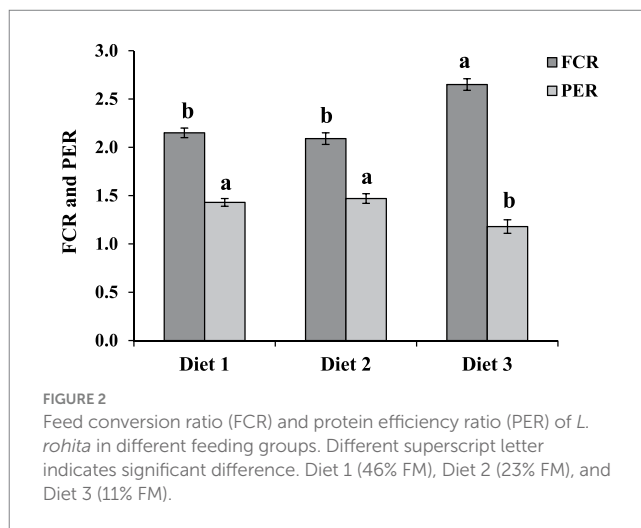
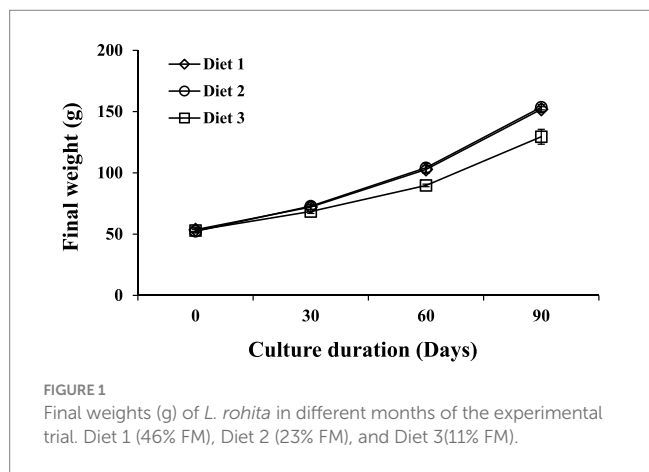
### Growth performance evaluation

The growth performance of *L. rohita* fed on varying fish feeds based on FM concentrations reared in triplicate treatments is shown in Table 2. The fish growth increment comparison based on monthly intervals is presented in Figure 1. The growth of *L. rohita* varied significantly among the Diets, with Diet 2 fed group showing a significantly higher final weight (153.62 ± 2.18g) gain in comparison with Diet 3 group (129.50 ± 6.09g). The maximum weight gain (101.30 ± 3.02g) in *L. rohita* was observed in Diet 2 group, nonetheless, showing significant difference from Diet 3 group. Similarly, a significantly ( $p < 0.05$ ) higher SGR was observed in Diet

TABLE 2 Growth performance of *L. rohita* in different feeding groups.

Parameters	Diet 1	Diet 2	Diet 3	P-value
Initial weight (g)	53.60 ± 1.59 <sup>a</sup>	52.32 ± 1.30 <sup>a</sup>	53.00 ± 1.91 <sup>a</sup>	0.648
Final weight (g)	151.91 ± 2.09 <sup>a</sup>	153.62 ± 2.18 <sup>a</sup>	129.50 ± 6.09 <sup>b</sup>	0.000
Weight gain (g)	98.31 ± 2.48 <sup>a</sup>	101.30 ± 3.02 <sup>a</sup>	76.50 ± 4.88 <sup>b</sup>	0.000
SGR (%/day)	1.16 ± 0.03 <sup>a</sup>	1.20 ± 0.04 <sup>a</sup>	0.99 ± 0.04 <sup>b</sup>	0.001
Survival rate (%)	95.83 ± 1.44 <sup>a</sup>	95.00 ± 2.50 <sup>a</sup>	94.17 ± 2.89 <sup>a</sup>	0.702

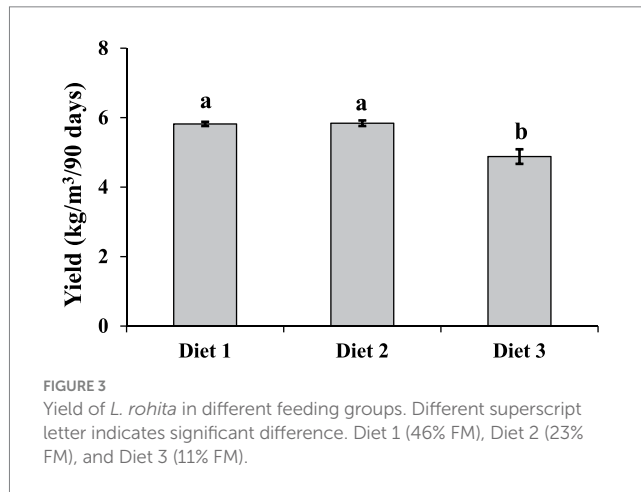
Mean values with the same superscript in the same row indicate non-significant differences ( $P < 0.05$ ).



2 ( $1.20 \pm 0.04\%$  bw/day) followed by Diet 1 ( $1.16 \pm 0.03\%$  BW/day) and Diet 3 ( $0.99 \pm 0.04\%$  BW/day). However, final weight, weight gain and SGR were showing insignificant differences among the Diet 1 and Diet 2 group. Furthermore, the survival of fish did not vary significantly among the treatments.

### Feed utilization

Feed utilization parameters examined for the experimental treatments are shown in Figure 2. During this study, FCR was significantly lower in Diet 2 group ( $2.09 \pm 0.06$ ) followed by Diet 1 ( $2.15 \pm 0.05$ ) and Diet 3 ( $2.65 \pm 0.06$ ). Furthermore, the FM replacement in the different treatments significantly affected PER of fish. A considerably higher PER was recorded in Diet 2 ( $1.47 \pm 0.05$ ) and lower in Diet 3 ( $1.18 \pm 0.07$ ).



### Fish yield

During the present study, the highest yield was obtained from Diet 2 group ( $5.84 \pm 0.08$  kg/m<sup>3</sup>/90 days), followed by the Diet 1 ( $5.82 \pm 0.06$  kg/m<sup>3</sup>/90 days) and Diet 3 group ( $4.88 \pm 0.21$  kg/m<sup>3</sup>/90 days). There was no significant difference ( $p < 0.05$ ) between the yields of the Diet 1 and Diet 2 group, while the yield of *L. rohita* in Diet 3 group was significantly different from both treatments (Figure 3).

### Proximate composition of fish

The final carcass composition assessment of fish fed on different feed types formulated with varying levels of FM replacement is

presented in Table 3. There were no significant differences ( $p < 0.05$ ) between Diet 1 and Diet 2 group, while Diet 3 group was significantly different ( $p < 0.05$ ) from Diet 1 and Diet 2 groups. The protein and lipid contents were higher in the Diet 1 group ( $14.45 \pm 0.27$  and  $4.85 \pm 0.04\%$ ) followed by Diet 2 ( $14.38 \pm 0.06$  and  $4.78 \pm 0.03\%$ ) and Diet 3 group ( $13.68 \pm 0.02$  and  $4.36 \pm 0.05\%$ ), respectively. However, the moisture content turned out to be significantly ( $p < 0.05$ ) higher in Diet 3 ( $80.14 \pm 0.25\%$ ) and lower in Diet 1 ( $79.13 \pm 0.36$ ). In the same pattern, the ash content was significantly higher in Diet 3 ( $1.48 \pm 0.02\%$ ) and lower in Diet 1 ( $1.24 \pm 0.02\%$ ). However, the carbohydrates did not vary considerably among the treatments.

TABLE 3 Proximate composition of *L. rohita* in different feeding groups.

Parameters	Diet 1	Diet 2	Diet 3	P-value
Protein (%)	14.45 ± 0.27 <sup>a</sup>	14.38 ± 0.06 <sup>a</sup>	13.68 ± 0.02 <sup>b</sup>	0.003
Lipid (%)	4.85 ± 0.04 <sup>a</sup>	4.78 ± 0.03 <sup>b</sup>	4.36 ± 0.05 <sup>c</sup>	0.006
Ash (%)	1.24 ± 0.02 <sup>c</sup>	1.38 ± 0.02 <sup>b</sup>	1.48 ± 0.02 <sup>a</sup>	0.000
Moisture (%)	79.13 ± 0.36 <sup>b</sup>	79.46 ± 0.18 <sup>b</sup>	80.14 ± 0.25 <sup>a</sup>	0.011

Mean values with the same superscript in the same row indicate insignificant differences ( $p > 0.05$ ).

TABLE 4 Benefit–cost analysis of *L. rohita* in different feeding groups after 90 days of culture period.

Variables	Price rate (BDT*)	Diet 1	Diet 2	Diet 3
Fixed cost				
Net cage (1 m <sup>3</sup> )		649.98	649.98	649.98
Other materials cost for cage supporting (bamboo, rope, anchors, bricks)	150 BDT/unit	150.00	150.00	150.00
Subtotal		799.98	799.98	799.98
Cost in one cycle**		133.33	133.33	133.33
Variable cost				
Fish fingerling	6 BDT/pieces	240.00	240.00	240.00
Feed cost	Diet 1 = 44 BDT/kg; Diet 2 = 35BDT/kg; Diet 3 = 31 BDT/kg	354.20	281.75	238.39
Labor and fish harvesting		25.00	25.00	25.00
Total cost		752.53	680.08	636.72
Financial return				
Fish sale as the total return		1164.52 <sup>b</sup>	1167.23 <sup>a</sup>	877.61 <sup>b</sup>
Total net return		411.99 <sup>b</sup>	487.15 <sup>a</sup>	240.89 <sup>c</sup>
BCR		1.55 <sup>b</sup>	1.72 <sup>a</sup>	1.38 <sup>c</sup>

Mean values with the same superscript in the same row indicate non-significant differences ( $P > 0.05$ ).

\*BDT, Bangladeshi Taka, 1 USD = BDT 84.72 (2017). The market price of *L. rohita* was BDT 200/kg in Diet 1, Diet 2 and BDT 180/kg in Diet 3.

\*\*Assuming durability of each cage for six culture cycles.

## Economic performance evaluation

A comparison of economic returns from the fish groups treated with varying degrees of FM replacement is shown in Table 4. The highest cost was estimated from the Diet 1 group (BDT 752.53), followed by Diet 2 (BDT 680.08) and Diet 3 group (BDT 636.72). However, significantly higher total economic return as the fish sale of Diet 2 (BDT 1167.23) followed by Diet 1 (BDT 1164.52) and Diet 3 (BDT 877.61). The total net economic return was significantly higher in Diet 2 (BDT 487.15), while the lowest was Diet 3 (BDT 240.89). Furthermore, the Diet 2 group provided a considerably higher BCR (1.72) than the other two treatments.

## Water quality assessment

The mean values of water quality parameters recorded from the three experimental treatments during the study period are displayed in Table 5. Formulation of feed based on varying replacement levels of FM did not affect the water quality of CCS, indicating no significant impact of increased plant protein on the suitability of water quality.

## Discussion

This study attempted to investigate replacing FM with PPI for *L. rohita* reared in CCS. Significantly higher finishing weight of fish was achieved in Diet 2 group and lower in Diet 3. Weight gain and SGR alluded to substantially higher performance in Diet 2 that declined in, Diet 3. The replacement of animal protein sources (FM) up to a certain level with plant protein sources was not detrimental, as was evident in the findings of Furuya et al. (2004) and Lin and Luo (2011). Even a 50% replacement of FM in diets has been reported to be favorable for the overall fish growth performance (Viola et al., 1982; Jahan et al., 2012). However, replacing 75% animal-source protein reduced fish growth in the present experiment in Diet 3. Incorporating PPI higher than the sub-optimal level might negatively affect fish growth. Although, we have not measured the level of anti-nutritional factors (ANFs) present in PPI in our study. However, we presume that the greater PPI might increase toxic components (ANFs) and imbalance the amino acid profile responsible for intestinal irritation and reduced growth (Olvera-Novoa et al., 2002). This understanding corroborates the earlier outcomes by Hua and Bureau (2012), who opposed the total replacement of FM protein with plant protein as it could be detrimental to the cultured organisms. Complete FM

TABLE 5 Water quality parameters in different feeding groups (Mean  $\pm$  SD).

Parameters	Diet 1	Diet 2	Diet 3	p-value
Temperature ( $^{\circ}$ C)	25.78 $\pm$ 0.09 <sup>a</sup>	25.86 $\pm$ 0.15 <sup>a</sup>	25.96 $\pm$ 0.10 <sup>a</sup>	0.236
pH	7.04 $\pm$ 0.02 <sup>a</sup>	7.04 $\pm$ 0.05 <sup>a</sup>	7.05 $\pm$ 0.03 <sup>a</sup>	0.990
DO (mg/l)	5.07 $\pm$ 0.05 <sup>a</sup>	5.02 $\pm$ 0.05 <sup>a</sup>	5.08 $\pm$ 0.04 <sup>a</sup>	0.344
NH <sub>3</sub> (mg/l)	0.13 $\pm$ 0.01 <sup>a</sup>	0.13 $\pm$ 0.00 <sup>a</sup>	0.12 $\pm$ 0.01 <sup>a</sup>	0.252
Total alkalinity (mg/l)	79.99 $\pm$ 0.70 <sup>a</sup>	80.50 $\pm$ 1.28 <sup>a</sup>	80.61 $\pm$ 0.65 <sup>a</sup>	0.695

Mean values with the same superscript in the same row indicate insignificant differences ( $P > 0.05$ ).

replacement is also reported to decrease protease activities in the intestine and hepatopancreas in Juvenile Tilapia (Lin et al., 2010). Jalili et al. (2013) also found reduced digestive enzyme activities and subsequent lower growth in rainbow trout fed the diet with 75 and 100% FM replacement. In the present study, 75% replacement of FM by PPI in Diet 3 may be the reason for the reduced growth performance of *L. rohita* to the other treatments.

The proportion of protein and non-protein energy sources is necessary while preparing a balanced diet. An excess protein in the diet causes higher ammonia, affecting fish growth performance (Kaushik and Medale, 1994). When adequate non-protein energy sources are available in the diet, it could minimize the use of protein as an energy source and enhance fish's growth performance (Iqbal et al., 2020b). Carps are the most efficient exploiters of carbohydrates (Kumar et al., 2005). The intrusion of carbohydrates in the form of PPI in Diet 2 could impart a protein-sparing effect that may enhance the feed utilization by fish. The protein-sparing effect of suboptimal levels of carbohydrates was also reported in silver barb by Mohanta et al. (2007). An appropriate level of carbohydrate in the diet can reduce protein degradation and amino acid oxidation which results in improved growth (Frick et al., 2008). However, several studies also reported that dietary carbohydrate beyond the optimal level can cause lower growth and feed utilization in fish (Tan et al., 2009; Gao et al., 2010; Yu et al., 2022). Therefore, lower growth and feed utilization in Diet 3 group was lower despite of the higher carbohydrate level. Therefore, replacing FM in the diet did not affect fish survival. Jahan et al. (2007) found no significant difference between the treatments regarding survival rate in a partial replacement experiment of FM with SBM for the fry of *Cirrhinus cirrhosus*. Replacement of FM in fish diets can significantly affect the total fish yield, whereas the higher production was recorded in Diet 2 group with a 50% replacement of FM. However, the higher inclusion rate of PPI in Diet 3 caused a significant reduction in the total yield.

The FCR decreased in the fish of Diet 2 compared to the Diet 1 fish and increased significantly in Diet 3. The earlier investigations have argued that low FCR indicates higher feed utilization efficiency, balancing bioavailability and partitioning dietary nutrients toward growth (Angelidis et al., 2005). Zamal et al. (2009) reported a high-energy diet produced a lower FCR and higher nutrient retention in the fish body. Therefore, 50% FM replacement in feed increased the feed efficiency, but it decreased in growing proportion for PPI in the feed (Diet 3). The present findings are supported by the conclusions of Devi et al. (1999), who reported relatively better (lower) FCR in *L. rohita* fingerlings fed on a diet including 20% SBM.

Similarly, they found a higher FCR while increasing the proportion of SBM up to 40 and 60%. PER is related to the dietary

protein intake and its conversion into fish weight gain (Koumi et al., 2009). The fish fed on varying levels of a FM replacement diet showed a significantly higher PER in Diet 2 and lower in Diet 3. The lower PER obtained in Diet 3 might be due to the imbalance of amino acid profile as affected by the higher inclusion rate of PPI, which was also supported by Espe et al. (2008). Plant protein sources generally have lower biological value and palatability properties (Estevez et al., 2011), which may be responsible for lower feed utilization in Diet 3 group. However, a balanced proportion of FM and PPI in Diet 2 might provide essential amino acids and increase fish feed utilization. The study by Espe et al. (2006, 2010) and Estevez et al. (2011) also showed that the palatability of a plant protein-based diet could be enhanced by adding essential amino acids and other animal proteins protein sources.

Replacement of 50 and 75% FM in Diet 2 and Diet 3 resulted in a 20.62 and 32.76% reduction in total feed cost, while 15.61% higher net economic return was obtained from Diet 2 compared to the Diet 1 group. A study conducted by Khan et al. (2013) reported that 24% of feed formulation costs could be reduced by the replacement of FM with rice polish (up to 20%) and MOC (up to 22%) without changing the nutritional quality in an experiment on *Oreochromis niloticus*. However, a higher inclusion rate of PPI in Diet 3 reduced the fish growth and subsequent reduction of 41.61% net economic return compared to Diet 1 in the present study. Finally, the replacement of 50% FM provided a higher BCR (1.72) compared to the other treatments. Apart from fish feed types, various environmental factors such as water temperature, turbidity, pH and ammonia play a crucial role in the CSS that must be considered (Ara et al., 2020, 2023; Jewel et al., 2020b, 2023b; Bashak et al., 2021).

Replacement of fish meal with FM at different levels significantly impacted the muscle protein and lipid content of *L. rohita*. Fish in Diet 2 retained a higher protein level compared to other treatments. However, lipid content was reduced considerably in Diet 3 compared to the Diet 1 group, potentially due to a higher inclusion rate of PPI in the diet. A similar observation by Devi et al. (1999) has reported higher protein and lower lipid levels in the muscle tissues of *L. rohita* fingerlings fed with a plant protein-based diet (SBM-based rations) compared to the Diet 1 group (0% SBM). Contradictory results were observed by Khan M. A. et al. (2018) and Khan N. et al. (2018). They reported that incorporating plant protein by replacing FM did not significantly affect the whole-body composition of Indian major carps, *Catla catla*, *L. rohita* and *C. cirrhosus*. A significant effect of FM replacement on the proximate composition of fish during the present study may be linked to anti-nutritional factors in PPI, although several studies reported the influence of FM replacement with plant protein sources on the proximate composition of fish (Olli and Krogdahl, 1995; Elangovan and Shim, 2000).

## Conclusion

The present study affirmed that 50% replacement of FM by PPI could be economically efficient in reducing the feed formulation cost by approximately 20.45% without changing the proximate composition (nutritional quality) of *L. rohita* reared in the CSS. A replacement of 50% FM with PPI also increased the total net return by 15.61%, which was the most economical among all the experimental treatments for the cage culture of *L. rohita* in ponds. This study can also be extended to other leading aquaculture fish species to test the potential of using PPI as an alternative protein source of FM to reduce the increasing pressure on FM demand. This approach aligns with sustainable practices by promoting environmentally friendly alternatives and encouraging knowledge-sharing among practitioners. Therefore, the development of aquaculture for *L. rohita* using low-cost feed will not only supports economic empowerment and food security but also embodies a commitment to responsible and inclusive aquaculture practices in Bangladesh or other developing nations. However, in the current investigation, ANFs of PPI were not examined, which may restrict the acceptability of the designed diet. As a result, the current study recommends that the PPI's ANFs be examined before to their usage in feed composition.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

## Ethics statement

The animal study was approved by University of Rajshahi, Bangladesh. The study was conducted in accordance with the local legislation and institutional requirements.

## Author contributions

SA: Data curation, Writing – original draft. AH: Data curation, Writing – original draft. A-AS: Supervision, Writing – review & editing. UA: Software, Writing – review & editing. SI: Software,

Writing – review & editing. PS: Data curation, Funding acquisition, Writing – review & editing. BP: Writing – review & editing. TA: Writing – review & editing. MH: Resources, Software, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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