Assessment of Growth Responses, Nutrient Utilization, and Carcass Composition in *Labeo rohita* (Hamilton, 1822) Fed Diets Enriched with Varying Dietary Lipid Sources

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Abstract
This study evaluated the effects of varying dietary lipid sources on growth performance, feed utilization, and carcass composition of *Labeo rohita*. Three experimental diets, including a control diet (CD), a low omega fat diet (LOFD), and a high omega fat diet (HOFD), were prepared and fed to fish for three months. Water quality parameters remained within the optimal range for fish culture throughout the experiment, with no significant differences among treatments. Growth performance indices, including final weight, weight gain, average daily gain (ADG), specific growth rate (SGR), and feed conversion ratio (FCR), were significantly influenced by dietary lipid source. The LOFD group achieved the highest growth performance and feed utilization efficiency. Proximate composition analysis revealed significantly higher crude protein and lipid content in the LOFD group, and no notable changes in ash content among the dietary groups. These findings indicate that moderate lipid in combination with vegetable oil and omega fat optimizes growth and carcass composition in *L. rohita*, providing valuable insights for formulating efficient and sustainable aquafeeds.

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Keywords: Growth Performance, Omega Fat, Soybean Oil, Rui Fish.

Introduction

Bangladesh is blessed with abundant water resources, offering excellent potential for fish culture. Bangladesh's aquaculture industry accounts for more than 57% of total fish production, reaching 4.621 million tons from both freshwater and saltwater sources [1]. Fish is a highly nutritious food that supplies the energy required for humans [2] and offers its consumers an optimal combination of essential fatty acids and all the essential amino acids [3]. Wild fisheries are intrinsically prone to overexploitation, and although they possess a natural capacity for regeneration, the magnitude of these resources is inherently finite, and their availability is increasingly constrained [4]. Therefore, intensive fish farming is an effective and sustainable technique of increasing food production and meeting the increasing demands of a growing population [5].

A healthy diet is related to food and is crucial for all organisms to obtain energy. Fish require additional nutrients in their diet since they are cold-blooded animals, and artificial diets can provide them with these nutrients [6]. Dietary lipid plays a vital role as the energy source for the fish. Lipids are constituents of the bio membrane structure, supplying fat-soluble vitamins (precursors for eicosanoids, hormones, and vitamin E) as well as enzyme cofactors [7]. Lipid can be added to formulated feed to benefit from the protein-sparing effect and reduce feed costs [8]. Fish oil (FO) contains an excellent amount of n-3 LC-PUFA, particularly EPA (eicosapentaenoic acid; 20:5n-3) and DHA (docosahexaenoic acid; 22:6n-3), which makes it an indispensable lipid source in aquaculture diets [9]. Fish also require EPA and DHA through diet [10]. Fish diets ought to include an appropriate proportion of dietary lipids since excessive lipid inclusion might harm mitochondrial structure and stunt growth [11], impair the quality of the carcass composition of fish [12].

Rohu (*Labeo rohita*) is a key aquaculture species in India, Bangladesh, Pakistan, and Myanmar (Burma). This species' excellent fecundity (2 lakh eggs/kg), external fertilization, and domestication made intensive culture feasible [13]. It carries polyunsaturated fatty acids that are essential for human growth and development [14]. Carp production accounts for more than 72% of global freshwater aquaculture production, with *L. rohita* contributing around 15% [15]. As a result, it has emerged as an important aquaculture species, which made up around 3.7% of global aquaculture production in 2018 [16]. Some previous studies were performed based on dietary protein and lipid optimization of rui by using de-oiled rice bran [17]; dietary lipid level of rui [18]; plant-origin meal [19]. Still, there is a lack of comprehensive studies focusing on the specific effects of various dietary lipid sources in the diets of *L. rohita*. Thus, this study aims to fill these gaps by investigating the effects of varying fish oil concentrations along with vegetable oil in a practical diet on growth performance, feed utilization, and proximate composition of *L. rohita.*

Materials and Methods

Study Area and Duration

The experiment was conducted in an experimental pond, behind the hatchery building, Department of Fisheries, University of Rajshahi (latitude $22^{0}22\ ^{0}39.6$" N and longitude $88\ ^{0}38^{0}\ 07.8$" E). This experiment was conducted for three months (90 days) from July to October 2023.

Construction of Cages

A total of nine experimental cages (Each volume was 2×1×1 m^3) were constructed for this experiment. The cages' frames were made of iron rods and covered with a special knotless synthetic nylon net with a mesh size of 5 mm. The mesh size was suitable for water passing through the cage. The net was tied in cage frames with nylon twines.

Experimental Diet Preparation

The feed ingredients with their percent composition of the experimental diets are shown in Table 1. The selected ingredients for this experimental diet were collected from the local market. A fish oil commercial named Omega-Fat (marketed by Bonafide Agrovet Limited, Bangladesh), and soyabean oil (marketed by Sonargaon Seeds Crushing Mills Ltd, Bangladesh) were selected as a dietary lipid source. It was collected from a local store, Salim Enterprise, Greater Road, Rajshahi City Corporation, Rajshahi. Firstly, all the feed ingredients were finely ground by using a grinder machine and sieved to less than 4 mm particle sizes. Then add an adequate amount of water to make a dough and pelletize (approximately 2 mm) by using a hand pelletizer. The pelletized feed was then sun-dried for 3 days. Finally, the well-dried pellet feeds were stored in an airtight plastic container at 4 °C until use. Analyses for the proximate composition of feed were determined before setting up the experiment. The proximate composition of experimental diets is shown in Table 2.

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

Ingredients	Percentage (%)		
	CD	LOFD	HOFD
Fish meal	20	20	20
Mustered oilcake	36	36	36
Wheat flour	5	5	5
Maize meal	5	5	5
Rice Bran	15	15	15
Soya bean meal	10	10	10
Soybean oil[a]	7	3.5	0
Omega fat[b]	0	3.5	7
Vitamin pre-mixture[c]	1	1	1
Choline chloride	0.5	0.5	0.5
Vitamin E (50%)	0.5	0.5	0.5
Total	100	100	100

[CD = Control Diet, LOFD= Low omega fat diet, HOFD= High omega fat diet;

[a]Soyabean oil=SFA-15.32g, MUFA-21.25g, PUFA-63.43g, Vitamin A-1.5-3.0mg according to manufacturer Sonargaon Seeds Crushing Mills Ltd, Bangladesh;

[b]Omega fat= Crude fat-99.4%, EPA-16%, DHA-11%, Vitamin H Biotin-12%, Total omega fatty acid-33% according to manufacturer ASIFAC Vietnam provided by Bonafide Agrovet Limited, Bangladesh;

[c]Vitamin 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 the premix.]

Table 2: Proximate composition of the experimental diet

Experimental diet	Moisture (%)	Ash (%)	Crude fibre (%)	Crude Fatt (%)	Crude Protein (%)
CD	10.86	6.56	6.12	13.86	28.14
LOFD	10.36	5.86	6.27	14.56	29.66
HOFD	9.96	6.2	5.44	15.12	28.24

Collection of Fish, Stocking, and Sampling

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 of fish and continued fortnightly. Final sampling was done

after 3 months of rearing by a scoop net. Then, the weighing of fish by a digital balance. For proximate composition analysis, fish from each of the three experimental groups were fasted for 24 hours before sampling. From each replicate, three fish were randomly selected, filleted, and oven-dried at 60 °C until properly dehydrated. The dried samples were then ground into a fine powder, sealed in plastic zipper bags, and stored at –20 °C until analysis.

Experimental Conditions and Feeding Trials

After acclimatization, a total of 180 fish with an average body weight of 98.61 ± 0.46 g were evenly distributed into nine experimental cages, assigned to three treatment groups: T_1 (control diet, CD), T_2 (low omega fat diet, LOFD), and T_3 (high omega fat diet, HOFD). Each treatment was replicated thrice to minimize experimental error and enhance the reliability of the results. Each group was fed twice daily (at 9:00 a.m. and 5:00 p.m.) at 5% of their body weight using the three experimental diets.

Evaluation of growth performance parameter

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)

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

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

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

Proximate composition analysis

The proximate composition of fish and experimental diets was analyzed following the standard procedures outlined by the Association of Official Analytical Chemists International, AOAC [20]. All analyses were performed in triplicate. Moisture content was determined by oven-drying the samples at 105 °C for 24 hours. Crude protein was measured using the Kjeldahl method, with protein content calculated as Nitrogen × 6.25. The crude lipid content was assessed using the Soxhlet method. Crude ash was determined by incinerating the samples in a muffle furnace at 550°C for 24 hours.

Monitoring of water quality parameters

To maintain optimal living conditions of the experimental fish, key physico-chemical parameters of the water were routinely monitored. Water samples were collected weekly in the morning (between 8:00 and 9:00 am) to assess some important water quality parameters like water temperature, DO, pH, CO_2, and alkalinity. Water temperature was measured using a Celsius thermometer and expressed in degrees Celsius (°C). Dissolved oxygen (DO) was determined by using a HACH kit; results were expressed in mg/L. Free carbon dioxide (CO_2) 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 paper. Total alkalinity was determined by titration using N/50 sulfuric acid, methyl orange, and phenolphthalein indicators.

Statistical analysis

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

Result

Water quality parameter

The water quality parameters observed in the study were temperature, DO, pH, CO_2, and Total alkalinity. Mean values for each parameter are shown in Table 3. There were no significant (P>0.05) differences in mean water quality parameters assessed among the feeding groups. It was observed that the temperature, DO, pH, alkalinity and CO_2 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 respectively.

Table 3: Variation in the mean values of water quality parameters in different treatments during the study (Mean ± SD)

Parameters	CD	LFOD	HFOD
Temperature (°C)	28.20±0.12[a]	28.34±0.21[a]	28.34±0.22[a]
Dissolved oxygen (mg/l)	4.63±0.04[a]	4.69±0.12[a]	4.66±0.11[a]
Water pH	7.00±0.11[a]	7.17±0.06[a]	7.03±0.08[a]
Alkalinity(mg/L)	145.67±2.08[a]	143.33±1.53[a]	147.67±6.43[a]
CO2 (mg/l)	3.18±0.06[a]	3.15±0.06[a]	3.15±0.05[a]

[Mean values in each row with the same superscripts have no significant differences (P>0.05)]

Growth performance

The mean growth performance of the experimental fish was determined in terms of initial weight, final weight, weight gain, food conversion ratio (FCR), specific growth rate (SGR) and average daily gain (ADG) under different treatments, as shown in Table 4. 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 the LOFD group (156.150 g), (1.13) and (1.74) respectively. The recent study also revealed that there was a significant difference in FCR among the treatments and the best FCR was found in the LOFD group (2.83). The variation in growth over time in different treatment groups is shown in Figure 1.

Table 4: Mean growth performance of experimental fish after 90 days of rearing fed experimental diets (Mean ± SD) (n=3)

Parameters	CD	LFOD	HFOD
Mean Initial Weight (g)	98.61±0.65^{a}	98.15±0.52^{a}	99.1±0.61^{a}
Mean final Weight (g)	211.50±10^{c}	254.30±18^{a}	237.85±14^{b}
Mean Weight Gain(g)	112.88±3.11^{c}	156.15±2.76^{a}	138.75±0.44^{b}
FCR	3.57±0.08^{a}	2.83±0.04^{c}	3.15±0.01^{b}
SGR (% bwd-1)	0.91±0.02^{c}	1.13±0.01^{a}	1.04±0.01^{b}
ADG (g)	1.25±0.03^{c}	1.74±0.03^{a}	1.54±0.00^{b}

[Mean values in each row with the different superscripts have significant differences (P<0.05)]

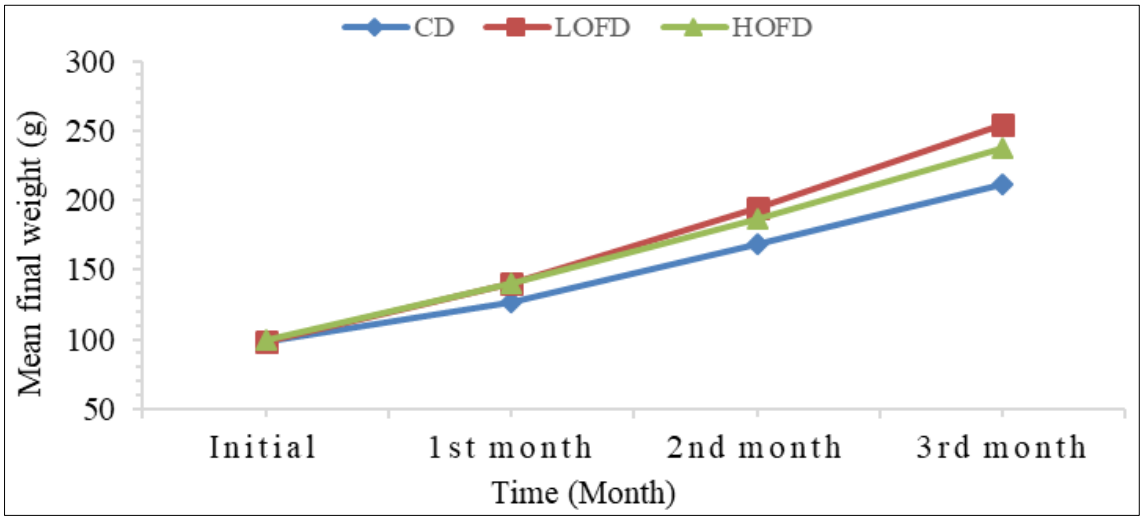

Fig 1: Mean monthly final weight of *L. rohita* fed different diets over time

Proximate composition analysis

The proximate composition of the experimental fish was evaluated to identify the quality and changes in fish body composition as a result of various experimental diets. The overall composition of the experimental fish is summarized in Table 5. The present finding demonstrates that *L. rohita* had the maximum crude protein content (59.04±0.13) when fed LOFD, followed by HOFD and CD diets. On the other hand, fish lipid contents were generally higher in fish fed HOFD and LOFD than in fish groups fed CD. The ash amounts differed modestly among the dietary groups.

Table 5: Proximate composition of *L. rohita* fed different experimental diets after 3 months of rearing (n=3)

Components	CD	LOFD	HOMD
Crude protein	56.21±0.12^{c}	59.04±0.13^{b}	57.65±0.11^{c}
Crude lipid	9.14±0.37^{c}	12.05±0.24^{a}	13.56±0.18^{b}
Ash	12.32±0.02^{b}	11.56±0.15^{c}	12.86±0.35^{a}
Crude fibre	0.17±0.05^{a}	0.19±0.28^{a}	0.15±0.12^{a}
Moisture	4.86±0.23^{a}	4.56±0.18^{a}	3.88±0.21^{b}

[Mean values in each row with the different superscripts have significant differences (P<0.05)]

Discussion

Water quality management

The formulation of feeds with varying dietary lipid sources had no significant effect on water quality parameters across the treatments, suggesting that different dietary lipid sources did not compromise water quality suitability. Throughout the experimental period, all measured water quality parameters remained within the optimal range recommended for fish culture [21].

Growth performance

This study evaluated the influence of dietary lipid sources on the growth performance and carcass composition of *L. rohita*. The LFOD group attained a significantly higher final weight, whereas the CD group recorded the lowest. The recent finding aligns with Gandotra *et al.* [18], who reported that *L. rohita* fed diets with moderate lipid content achieved greater final weight gain. This suggests that moderate lipid levels optimize growth, likely due to the protein-sparing effect, wherein lipids reduce the demand for protein as an energy source, allowing more protein to be allocated for growth [22, 23]. Weight gain and ADG indicated markedly superior performance in the LOFD group, while the CD group showed a decline. Comparable findings in the same species were reported [18]. Similar trends have been documented in other species [24, 25]. The specific growth rate (SGR) measures fish growth over a given period, while the feed conversion ratio (FCR) means the ratio of the total dry weight of feed consumed to the total wet weight gain of the animal over a given period. It indicates how efficiently the feed is converted into body mass. In *L. rohita*, both SGR and FCR were significantly influenced by varying dietary lipid sources. SGR and FCR were significantly improved in the LOFD group, indicating enhanced feed utilization efficiency. Similar trends were reported in *L. rohita* [26] and in *Cyprinus carpio* [27].

Proximate composition

The proximate composition analysis of *L. rohita* indicated significant variations in crude protein, lipid, and moisture contents among fish fed different experimental diets, reflecting the influence of dietary lipid sources on carcass nutritional quality. The crude protein and crude lipid are critical parameters for assessing fish flesh quality, as they directly affect consumer value and overall fish health. In the recent study, the highest crude protein content was recorded in fish fed the LOFD diet, followed by HOFD and CD. This suggests that the lipid source in the LOFD diet promoted better protein retention in *L. rohita*. Similar results were reported by Zeb *et al.* [26], who observed higher carcass protein levels in *L. rohita* when fed diets rich in both lipid and protein. In contrast, Kim *et al.* [28] found trends in juvenile parrotfish (*Oplegnathus fasciatus*), where protein-rich diets enhanced carcass protein deposition. Moisture content also differed significantly among treatments, exhibiting an inverse relationship with body fat. Higher moisture levels corresponded with lower lipid content and vice versa. This inverse relationship between moisture and fat has been well-documented in *L. rohita* [26] and other carp species [29]. This is possibly due to the increase in dietary lipid levels, which leads to higher lipid deposition in the fish muscle [18, 30]. Body ash content of *L. rohita* varied slightly among the treatments, showing no obvious influence of varying dietary lipid sources. In consistent ash content varies only slightly among fish fed different lipid sources, suggesting that dietary lipid type has a limited direct effect on mineral retention or absorption [31, 32].

Conclusion

The present study demonstrated how different dietary lipid sources affected the growth performance, feed utilization, and carcass composition of *L. rohita*. Recent findings showed that moderate lipid levels, as in the LOFD diet, resulted in better growth performance and enhanced feed conversion efficiency. Proximate composition analysis indicated higher protein and lipid retention in the LOFD groups, while ash content was unaffected by the lipid source. These results suggest that combining vegetable oil with omega fats at moderate lipid levels in diets can improve growth and flesh quality in *L. rohita* culture, providing practical benefits for aquaculture production.

Ethical statement

This animal study received ethical approval from the University of Rajshahi, Bangladesh, and was carried out in compliance with institutional guidelines and local regulations.

Conflict of interest

The authors confirm that there are no financial, non-financial, professional, or personal conflicts of interest that could have affected the outcomes of this study.

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