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COMPARATIVE ANALYSIS OF GROWTH PERFORMANCE AND PROXIMATE BODY COMPOSITION IN *Hypophthalmichthys molitrix* AND *Labeo rohita* FED DIET WITH RICE PROTEIN MEAL

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ABSTRACT:

The present study evaluated the role of rice protein meal (RPM) as a cost-effective and widely available plant-based protein source in the diets of *H. molitrix* (Silver Carp) and *L. rohita* (Rohu). 360 fingerlings were randomly assigned to 18 aquaria (20 fingerlings/aquaria) after a 1-week of acclimation period. There were three-dietary groups, T0: control, T1:RPM5%, and T2:RPM10%, each with triplicates. The trial lasted for 60 days. Results showed that treatment T2 exhibited significantly higher (weight, Protein efficiency ratio, Specific growth rate) performance. T2 showed higher weight gain (3.53 ± 0.05^a) compared to T0 and T1 in Rohu ($P < 0.0001$), and silver carp had significantly higher weight gain in T1 (2.42 ± 0.08^b) and T2 (2.61 ± 0.09^a) treatments compared to the T0 group (2.31 ± 0.06^c). In Rohu, PER and SGR were also observed to be higher ($P < 0.001$) in treatment groups, particularly in T2, than control group, and also observed comparable outcomes in silver carp. Proximate body composition analysis revealed that T2 had significantly ($P < 0.05$) higher moisture, crude protein, and ash content. Notably, Rohu in the T0 group exhibited higher crude fat levels; similarly, silver carp in T2 showed similar results. T2 was most effective in promoting sustainable growth and improving nutrient utilization in both species.

KEYWORDS: Silver Carp, Rohu, Rice Protein Meal, Growth Parameter, Body Composition.

1. INTRODUCTION

During the past few decades, the aquaculture industry has grown substantially to become an essential component of worldwide nutrition security and economic growth. The aquaculture industry stands as a critical branch of food manufacturing because it delivers inexpensive yet effective protein sources, thus preserving the fish populations in their natural ecosystem (Jolly *et al*., 2023). The aquaculture farming sector produces Silver carp and Rohu as primary species because these fish grow quickly while thriving in different environments with excellent nutritional benefits. The continuous rise in fishmeal-based diet expenses along with environmental issues has created a need for finding sustainable alternatives in aquafeed production (Noor & Harun, 2022).

Studies show that rice protein meal (RPM) stands as a suitable plant-based protein source because it contains high protein levels (40-70%) and effective digestibility, combined with affordable costs. RPM functions as an alternative protein source for fishmeal through substantial interest in aquaculture because it supports sustainability and fish species growth as well as physiological well-being (Haider *et al*., 2024). Few studies have examined plant-based protein diets for fish feeding, although researchers have evaluated their effects on various cultivated species, especially for silver carp and Rohu. Various aquatic species benefit from the research findings about RPM integration in aquafeeds. Studies conducted on tilapia and catfish consumption demonstrate that RPM serves as a suitable fishmeal substitute capable of achieving 50% removal without causing growth parameter issues (Lin *et al*., 2022). Studies exploring the reaction of carp species to diets incorporated RPM remain scarce, especially when measuring their growth rate alongside nutrient absorption effectiveness. The effective use of RPM as a protein source by Silver carp and Rohu species holds great potential to boost the economic profitability of aquaculture operations because these are fundamental commercial fish species (Napier *et al*., 2020).

Silver carp and Rohu serve major functions in freshwater aquaculture because each species produces economic value and provides high-protein content while using plant-based food sources (Yang *et al*., 2024). Silver carp maintains importance as an aquarium plankton controller while Rohu stands out as the most market-desirable fish for commercial farming. The research requires clarification about how RPM supplementation in diets affects growth metrics and nutritional aspects because animals display distinct eating patterns and metabolic patterns (Mitra *et al*., 2023).

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Scientific studies conducted in the past showed that fishmeal replacement through soybean meal and cottonseed meal maintains growth performance at equivalent levels (Diao *et al*., 2024). The proteins found in plants introduce anti-nutritional factors (ANFs) that negatively affect fish digestive capacity, alongside nutrient uptake and general metabolic functions. RPM serves as a promising fish feed ingredient because it derives from rice starch processing and provides essential amino acids together with high-quality proteins (Rohani *et al*., 2023). Several research projects investigate how RPM promotes developmental growth alongside body composition in various fish species. The research on fish nutrition within aquaculture has continually stressed that protein sources determine how fish grow, together with their metabolic health. The most essential macronutrient in a fish diet is protein because it determines tissue development while impacting total physiological performance (Senarathna *et al.,* 2024). Previous usage of fishmeal stood as the central protein feed for aquaculture operations until market expenses rose while environmental worries emerged, thus creating an urgent demand for sustainable substitutes. RPM demonstrates several benefits as a plant-based alternative because its high protein absorbability combines with its suitable amino acid distribution to support fish metabolic needs (Zheng *et al*., 2024). Fish diet efficiency evaluations rely on body weight gain (BWG) measurements with specific growth rate (SGR) measurements as the primary indicators for protein source assessment. Information regarding nutrient use and metabolic performance is obtained through body composition measurements involving crude protein and lipid content and ash and moisture analysis. Knowledge from a direct comparison of silver carp and Rohu metabolism of dietary proteins will lead to better feed formulations, along with enhanced freshwater aquaculture efficiency.

The study investigates how the dietary combination of RPM at two inclusion rates (5% and 10%) affects growth performance and body composition in silver carp and Rohu. The research examines the dietary treatments' impact on body weight and specific growth rate (SGR) and protein efficiency ratio (PER), and the proximate body composition elements of protein content, lipid percentage, and moisture and ash quantities.

2. MATERIALS AND METHODS

Experimental Fish and Rearing Conditions:

Three hundred fingerlings consisting of 180 *H. molitrix* (Silver Carp) and 180 *L. rohita* (Rohu) were obtained from Punjab Fish Seed Hatchery in Pakistan. The study started with silver carp fish having an average weight of 2.6 ± 0.00 g and Rohu fish measuring 3.0 ± 0.00g.

Sodium chloride (NaCl) was applied (obtained from Jinshen Pakistan) to create a bath that acted as a protective measure against fungal infections and ectoparasitic infestations for fingerling health management (Asad *et al*., 2025a). Acclimatization of fingerlings took place under laboratory conditions at the Fish Nutrition Laboratory of the Department of Zoology within Government College University, Faisalabad, for one week before experimentation. Fingerlings spent their acclimatization period in aquaria measuring (30 × 10 × 45cm) that received tap water while receiving non-stop aerated water from the capillary system of aerators. The fingerlings received a control diet made with 32% crude protein while consuming 4% of their body weight daily (Asad *et al*.,2025a).

The experimental aquaria were separated into three dietary areas, including a control group and two treatment groups (T1 RPM5% and T2 RPM10%), which consisted of triplicate. Water volumes in the rearing aquaria were kept optimal through daily replacement of 30% water and regular siphoning out of waste material.

Experimental Condition:

The measured water quality parameters included temperature at 26.91 ± 0.37°C and dissolved oxygen at 6.50 ± 0.36 mg/L, together with pH at 7.58 ± 0.20 and total alkalinity at 402.10 ± 10.57 ppm. A Hanna HI98194 multiparameter meter tracked this water quality data during the feeding period.

Experimental Diets:

For this experiment, researchers designed control diet together with two separate test diets. All required ingredients used in diet development are organized in Table 1. Habib Rice Products Ltd., Karachi, Pakistan, distributed (STD 50MF-2000) to the market as RPM. Experimental feed material composition was examined rigorously according to methods present in the Association of Official Agricultural Chemists guidelines (Alemu & Wudu, 2024). The researcher stored manufactured diets inside sealed plastic buckets in a cool room before they reached their usage date.

Table 1: Ingredients and proximate composition of the basic diet.

Ingredients (g)	Control	RPM 1 (5%)	RPM2 (10%)
Soybean meal	32	29	24
Wheat bran	26	24	24
Yellow corn	17	17	17
Rapeseed meal	12	12	12
Cellulose	7	7	7
Sunflower oil	5	5	5
Vitamin premix	1	1	1
Rice protein powder	-	5	10
Total	100g	100g	100g

Experimental Design and Ethical Considerations:

The research procedures followed all ethical standards established for animal care in investigative processes. The experimental method relied on a completely randomized design (CRD) to maintain research objectivity. The researchers executed handling procedures with minimal stress on fish while performing ethical, humane euthanasia at the end of the study.

Growth and Feed Efficiency Study:

At every fortnight, total body length and weight were measured to monitor the growth performance of fish throughout 60 days using the established method (Al Sulivany *et al*., 2024). The observation of fish growth occurred every two weeks for tracking growth patterns to improve feeding plans. The ethics committee of the Government College University, Faisalabad, Pakistan, approved the animal study protocol for scientific purposes. Approval number is GCUF/ERC/504. Researchers used a ruler to determine fish length by aligning the snout with the first mark of the measuring scale, and they measured final weight individually through an Ohaus Electronic Balance (88–1220/02A, United States).
The following parameters were calculated:

Weight Gain (g/day): WG = (FW- IW)

Specific Growth Rate (%) = [(ln (FW) − ln (IW))/ t] × 100

FW= Final Weight, IW = Initial Weight

Protein Efficiency Ratio (%) = WG/Dry weight of protein (Wiszniewski *et al*., 2022)

Condition Factor (K) = (100 × Weight) / Length³(cm3) (Hvas *et al*., 2022)

Analysis of Proximate Nutritional Composition:

The proximate nutritional analysis of the flesh began with the removal of the sample, followed by washing with distilled water. Analytical procedures for test diets and fish body proximate composition followed methods described by the Association of Official Analytical Chemists (AOAC, 2016). The electric oven (Thermostat Oven DHG-9202) dried 5g of fish flesh at 105°C to estimate the moisture content (Ran *et al*., 2015; Abass *et al*., 2018). A mortar and pestle were used to break the dried samples, and then lipid content was determined through a chloroform-methanol extraction procedure on the Soxhlet HTz 10,454 apparatus (Hassaan *et al*., 2018). The micro-Kjeldahl analysis determined crude protein content as a method (Abarike *et al*., 2018). A method for ash measurement requires drying two grams of sample and incinerating it at 550°C inside a (Nabertherm) muffle furnace for complete combustion to assess the ash weight remaining (Elumalai *et al*., 2020).

A set of formulas calculated the nutritional composition of the samples:

Dry matter =100-moisture (%)

Moisture = $\frac{W1-W2}{Wt.of sample} \times 100$

W1=Weight before sampling, W2=Weight after Sampling

Ash = $\frac{W1-W2}{Wt.of sample} \times 100$

Crude Fat(%) = $\frac{W1-W2}{Wt.of sample} \times 100$ (Biancacci *et al*., 2022)

N2(%) = $\frac{Volume of 0.1 NH2SO4 used \times 0.001 \times 250}{weight of sample \times 10}$ ×100 (Sarkar *et al*., 2021).

Crude protein(%) = %N2×6.25 (Muqier *et al*., 2023).

NEF%(Nitrogen Free Extract) = 100-Protein%+Fat%+Moisture%) (Reda *et al*., 2022).

GE (Gross Energy)= (5.64×Protein%) + (9.44×lipids%) +4.11×NEF% (Adineh *et al*., 2021).

Statistical Analysis

The statistical analysis included ANOVA using SPSS (version 20) to compute both mean ± SE values, while Tukey's HSD test determined ($p<0.05$) differences between means (Steel &Torrie, 1996).

3. RESULTS AND DISCUSSION

Growth Performance:

The study looked into how different RPM concentrations affected the growth performance of two fish species: *L. rohita* and *H. molitrix*. In the experimental diet, one control and two treatment groups (5% & 10% RPM) were added. When RPM was included in the diets, both species' growth parameters were substantially enhanced. They showed a significant ($P< 0.05$) improvement in body length and body weight in comparison to the control diets. When both species were compared, *L. rohita* showed better results in weight gain (3.53±0.05[a]) as compared to *H. molitrix* (2.61±0.09[a]) when fed at 10% RPM.

Furthermore, RPM supplementation increased Rohu's specific growth rate (SGR) and protein efficiency ratio (PER), indicating improved feed consumption and general health (Table 2). The degree of growth response in silver carp was different from that of Rohu, despite the fact that they also showed improvements in SGR and PER.

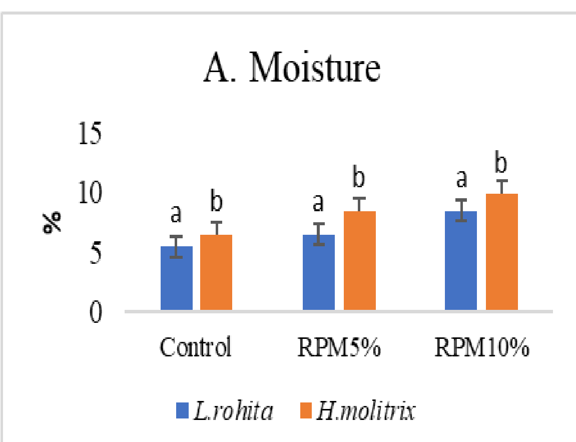
Table 2: Effect of control and RPM-treated diets on growth performances (Mean ± SE) of *L. rohita* and *H. molitrix.*

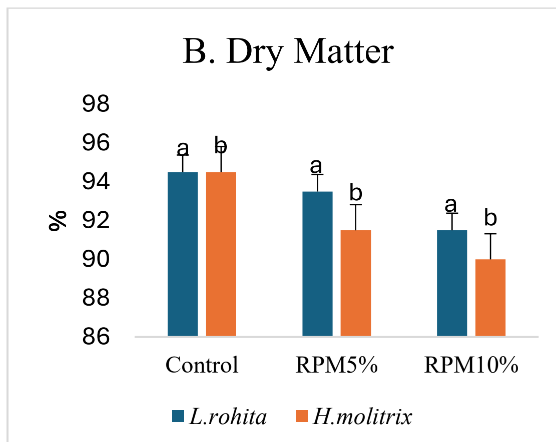
Growth Parameter	***L. rohita***				***H. molitrix***			
	Control	T1	T2	P value	Control	T1	T2	P value
IW (g)	3.00±0.02^{a}	3.01±0.04^{a}	3.01±0.06^{a}	NS	2.68±0.00^{a}	2.68±0.02^{a}	2.67±0.01^{a}	NS
FW (g)	5.1±0.01^{c}	5.8±0.05^{b}	6.5±0.002^{a}	P < 0.0001	4.9±0.03^{c}	5.1±0.05^{b}	5.3±0.07^{a}	P < 0.0001
WG (g)	2.12±0.03^{c}	2.79±0.01^{b}	3.53±0.05^{a}	P < 0.0001	2.31±0.06^{c}	2.42±0.08^{b}	2.61±0.09^{a}	P < 0.001
FBL (cm)	7.0±0.06^{c}	7.4±0.08^{b}	7.9±0.090^{a}	P < 0.0001	7.0±0.002^{c}	7.2±0.01^{b}	7.4±0.06^{a}	P < 0.010
CF (K)	1.8±0.00^{c}	1.7±0.01^{b}	1.6±0.03^{a}	P < 0.001	1.7±0.09^{c}	1.6±0.07^{b}	1.5±0.6^{a}	P < 0.02
PER	0.06±0.05^{c}	0.08±0.06^{b}	0.11±0.04^{a}	P < 0.0001	0.06±0.04^{c}	0.07±0.01^{b}	0.08±0.00^{a}	P < 0.03
SGR	0.8±0.02^{c}	1.1±0.03^{b}	1.2±0.08^{a}	P < 0.001	3.8±0.02^{b}	4.0±0.05^{b}	4.3±0.04^{a}	P < 0.001

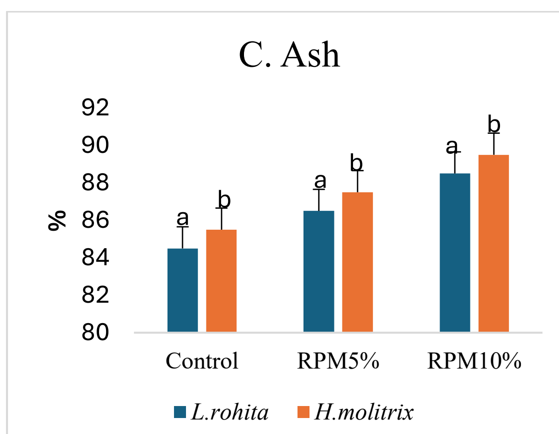
Different superscripts indicate significant variations between dietary treatments. N.S. indicates non-significant (P > 0.05), significant (P < 0.05), very significant (P < 0.01), and very highly significant (P < 0.0001). Initial weight is denoted by IW, final weight by FW, weight gain by WG, final body length by FBL, condition factor by CF (K), specific growth rate by SGR, and protein efficiency ratio by PER.

Body Composition:

The findings in Figure 1 provide information on the proximate chemical composition of both species fish body meat. The control group showed the lowest moisture and ash level, while the 10% RPM showed the highest value of moisture and ash (P<0.05) in both fish species. On the other hand, the control group showed the highest dry matter retention (P<0.05). Meanwhile, treatments T1 and T2 displayed the lowest dry matter retention. The highest crude protein level (30.75 and 34.61) in fish flesh was observed in T2, which was fed with 10% RPM in both *L. rohita* and *H. molirix*, respectively. The highest crude fat level was observed in the control group, while the lowest level was in T2 in both fish species. The NFE (nitrogen free extract) and GE (gross energy) showed the highest value in the control group and the lowest in T2 in both fish species.







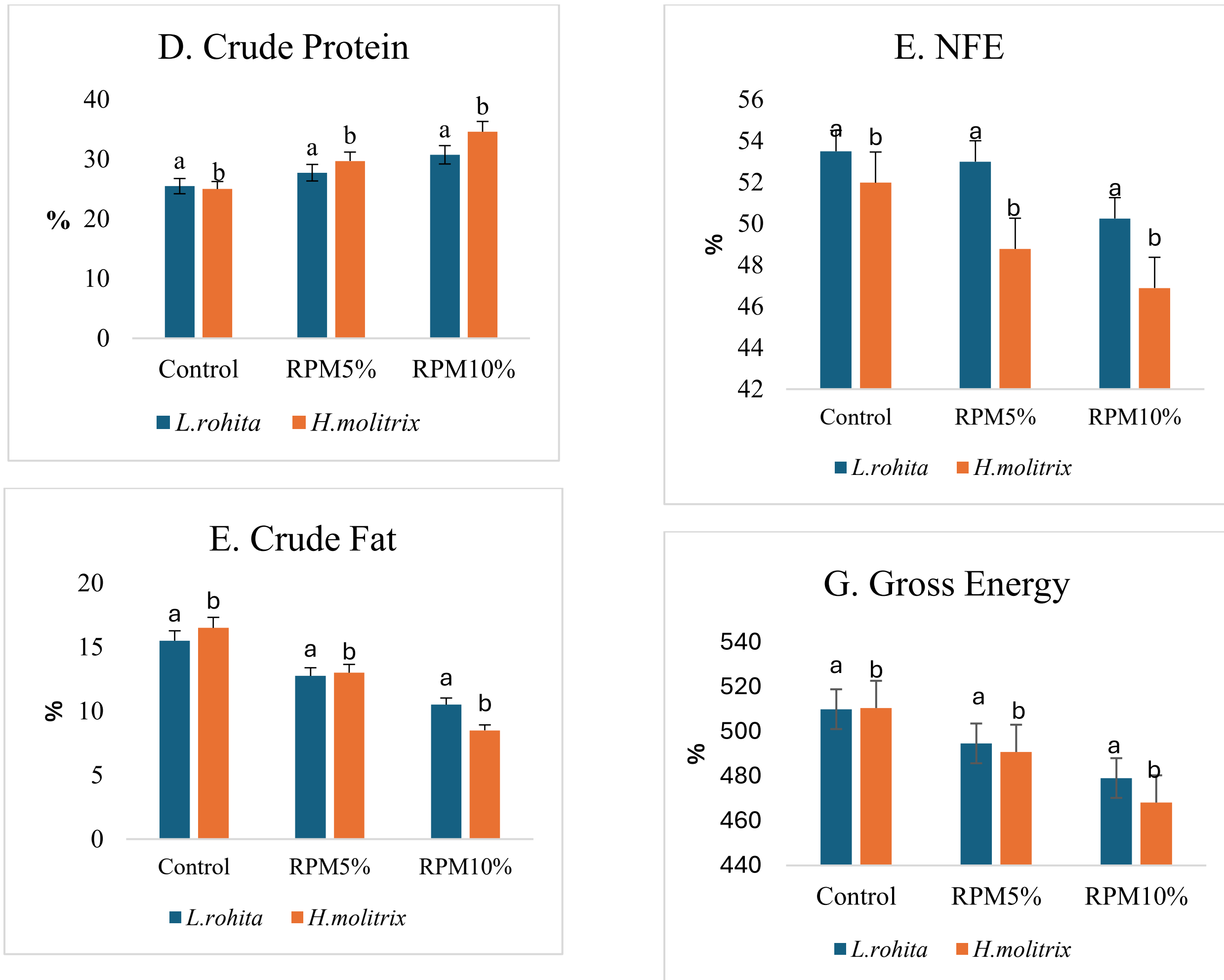


Figure 1: Proximate body composition of *L. rohita* and *H. molitrix* fed on control and RPM-treated diets. The symbols' a and 'b' denote statistically significant differences (P<0.05). A: Moisture (%), B: Dry matter (%), C: Ash (%), D: Crude protein (%), E: Crude fat (%), F: NFE?? (%) and G; Cross energy.

4. DISCUSSION

Aquaculture is a growing industry facing several challenges, including disease control, water quality management, and sustainable feed production (Asad *et al*., 2024; Asad *et al*., 2025b). For sustainable feed production, rice protein meal was incorporated in the fish feed that is cost-effective, environment friendly and showed good results on fish health. Throughout feeding trials that explore alternate diets, evaluating the health and well-being of fish becomes especially crucial. Positive results were shown when RPM was incorporated into the feed. The current research examines the potential impacts on fish by adding varying amounts of RPM into their feed. RPM exhibits a higher rate of growth compared to the control group over the current growth research. Irrespective of all the groups, the RPM2 group gained the largest amount of weight. This increase in weight gain is the root cause of the high amount of crude protein in the diet of (RPM2) RPM2-treated groups. Our findings are in accordance with those of Abdel Rahman *et al*. (2021) in tilapia and Abbas *et al*. (2021) in *Labeo rohita*, showing that a higher nutritional protein content is advantageous for proper fish growth. Similar conclusions were drawn by Zhang *et al*. (2023) and Muqier *et al*. (2023), who found that while providing tilapia diets comprising a substantial quantity of plant proteins improved their growth, plant protein had no negative influence on fish growth.

Fish growth is considered in terms of an increase in volume, and the volume is represented by weight (Asad *et al*., 2023). The potential advantages of RPM as an economically practicable food supplement in aquaculture are illustrated by the considerably higher specific growth rate (SGR) and protein efficiency ratio (PER) observed in the treatment groups of (T2) compared to the control groups. Similar results were reported by Hassan *et al*. (2021) and Abidin *et al*. (2022), adjusting the protein content of *(Chanos chanos)* diets significantly affected a number of growth parameters, feed utilization, along specific growth rates. In contrast, Mohammadi *et al*. (2020) noticed a considerable decrease in growth parameters that were the result of rising plant protein levels. This was justified by the argument because there were numerous anti-nutritional factors (ANFs), and a number of key amino acid deficiencies would restrict the utilization of diverse sources of plant protein in feed used in aquaculture. Wis *et al*. (2022) presented some results in rainbow trout and concluded that growth was unaffected by dietary protein levels.

Fluctuations in demand, food and oxygen availability, stocking density, age, and environmental factors like temperature could have been the cause. Singha *et al*. (2021) obtained similar findings and ultimately concluded that fish and other animals' growth is a phenotypic indication of muscular hyperplasia that is influenced by atmospheric and nutritional variables. Each of the scientists' research yields a different outcome. Several variables are considered, including the methodology of this research, the use of different fish species, and, at the conclusion, varying amounts of plant protein.

In both species, it was shown that in the treated groups' body composition levels were significantly higher than those of the control groups. (T2) was shown to have the most important value of moisture and ash compared to the control group in both species. According to Asad *et al*. (2025a) and Dawson *et al*. (2018), black sea bass had noticeably greater whole-body moisture and ash concentrations. The treatment group for body composition showed the highest value for crude protein, as that of the control group in both fish (Silver carp and Rohu) species. Similar results for crude protein, ash, and moisture were found by Abdel Rahman *et al*. (2021) in tilapia. Also, Nasr *et al*. (2021) observed identical findings for crude protein, concluding that plant protein in fish feed was beneficial for body composition. Han *et al*. (2022) observed similar findings in yellow catfish, showing that plant-based protein diets had the greatest crude protein concentration, subsequent to the control. The findings showed that fat levels drop as protein levels rise. The results obtained by Abasubong *et al*. (2019) in the blunt-snout bream agreed with it. However, contrasting findings have been reported. Hassan *et al*. (2021) in Asian sea bass and Assi *et al*. (2020) in tilapia found that all-treatment groups that fed RPM-based diet revealed a much greater fat content, a significantly reduced protein content, and a significantly higher level of ash in the control group. The main factor influencing fish body composition is the meal's content, and fish body composition differs greatly between species and within an individual fish. The findings showed that the treatment groups' dry matter, crude lipid, NFE, and gross energy levels turns decreased in comparison to the control group. Similar outcomes were noted by Yuanfa *et al.* (2023); there were no appreciable variations in body composition across treatments in terms of moisture, crude protein, or crude ash. The findings of Muniasamy *et al*. (2019) on the impact of meals on crude protein absorption were incongruous. These results indicated that the proximate composition of body meat in *H. molitrix* and *L. rohita* is greatly influenced by the diet's composition, particularly the amount of RPM consumed. It concluded that RPM had a pronounced effect on the growth performance and body composition of *H. molitrix* and *L. rohita*. For both species (T2)10% was more effective on the overall health condition.

Conclusion and Recommendation:

The findings show that RPM has significantly improved effects on fish growth and body composition of both species, silver carp and Rohu, particularly the best results revealed in treatment with 10% (T2) level, followed by 5% (T1) least but better than the control groups. RPM is a prospective component for the production of both species due to its cost-effective nature and environmental favorable. The experimental findings showed that the inclusion of RPM in the diet had accelerated their rate of growth. RPM is easily accessible and reasonably priced in its locality, and is recommended for use by farmers. Maintain a high muscle protein level for a healthy body composition. To avoid deficiencies or growth retardation, keep an eye on the health and behavior of the fish. If purchased from reputable vendors, it can be an affordable source of protein. To save money on transportation and promote regional farming, consider local availability. To optimize fish growth, quality, quantity, and health, they must be aware of the optimal practices for feeding RPM through a dedicated awareness platform. An awareness platform is required to teach local fish farmers about the optimal feeding methods, and farmers should take part in seminars and awareness campaigns that emphasize the advantages of RPM. This is required to achieve the maximum possible benefits of these activities. This allows fish to reach their maximum potential. Revealing how to use it best can help farmers increase feed efficiency, attain long-term sustainability in their farming methods, and optimize growth rates, quality, quantity, and health levels.

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Author Contribution

A.M., was responsible for writing the original draft preparation and formal analysis; A.L., resources and data curation; R.F., data curation and formal analysis; F.A., handled conceptualization and visualization; S.S., provided supervision; S.N., managed statistics and validation; A.N., writing review and editing; and R.J., was responsible for the software.

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