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Substitution of fishmeal with groundnut meal and its effect on growth, feed conversion efficiency, carcass composition and digestive enzyme activity of fingerling *Labeo rohita*

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Abstract
The effects of replacing fishmeal (FM) with groundnut meal (GNM) in the diets of *Labeo rohita* fingerling (6.32 ± 0.38 cm; 2.36 ± 0.02 g) were evaluated by conducting an 8-week feeding trial. The diets with 18 kJ g^{-1} gross energy and 35% crude protein were prepared by gradually replacing FM with GNM on a protein-to-protein basis at levels of 0%, 20%, 40%, 60%, 80% and 100%. Diets were fed to different groups near apparent satiation. No evident effect was noted by the replacement of FM with GNM up to 40% on live weight gain, specific growth rate, feed conversion ratio, protein retention efficiency and whole-body carcass composition. Digestive enzyme activities were not affected up to 40% FM replacement ($P > 0.05$) but declined significantly at 60%, 80% and 100% replacement ($P < 0.05$). The findings suggest that FM can be substituted by GNM up to 40% in formulating nutritionally balanced, cost-effective commercial feeds for *Labeo rohita* fingerling without hampering growth performance, feed utilization and carcass quality.

Keywords: Groundnut meal, fishmeal, *Labeo rohita*, cost-effective.

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Introduction
Aquaculture has emerged as one of the fastest-growing food production sectors worldwide, achieving the highest growth rate among global animal protein industries over the past decade (FAO, 2024). This rapid expansion has been driven by global population growth, increasing demand for high-quality animal protein, and stagnation in capture fisheries production (Yadav et al., 2025). As aquaculture production intensifies to meet rising protein demands, the need for nutritionally balanced and cost-effective feeds has increased substantially (Hodar et al., 2020; Davoudi-Sefidkohi et al., 2025). Feed alone accounts for more than 60% of total operational costs in aquaculture, making feed formulation a critical determinant of economic viability and sustainability (Mugwanya et al., 2022; Cui et al., 2025).

Fishmeal (FM) has traditionally been the primary protein source in aquafeeds, due to its superior nutritional profile, high palatability, balanced amino acid composition, rich omega-3 fatty acids, taurine content, and the presence of unidentified growth-promoting factors (Gatlin et al., 2007; NRC, 2011; Hardy and Kaushik, 2021; Song et al., 2026). Despite these advantages, the sustainability of fishmeal is increasingly questioned. Approximately 71% of global fishmeal production is derived from wild-caught small pelagic fishes such as sardines, anchovies, and herrings, while the remainder originates from processing by-products (Boyd et al., 2022). Projections indicate that by 2033, nearly 83% of total fishmeal production will be utilized by the aquaculture sector (FAO, 2024). This escalating demand exerts significant ecological pressure on marine ecosystems, particularly forage fish populations that play essential roles in aquatic food webs (Naylor et al., 2021; Monteiro et al., 2025). Furthermore, fluctuating supply and rising costs of fishmeal pose economic challenges for aquaculture producers (Hodar et al., 2020; Wang et al., 2021; Liu et al., 2025).

In response, considerable research efforts have focused on identifying sustainable and economically viable alternatives to fishmeal. Plant-based protein sources have gained prominence due to their wide availability, lower cost, and relatively stable supply chains (Tacon and Metian, 2008; Hardy, 2010; Turchini et al., 2019; Qian et al., 2024). Among plant-derived ingredients, oilseed cakes and meals have shown substantial potential as alternative protein sources in aquafeeds. These by-products of the edible oil industry are abundantly available and cost-effective, enhancing their suitability for large-scale feed production (Ramachandran et al., 2007; Jannathulla et al., 2019; Kumar et al., 2024).

Groundnut meal (GNM) is a particularly promising feed ingredient. Groundnut meal is a by-product of groundnut oil extraction

after husk removal (NRC, 2011) and contains a high crude protein content ranging from 41–56%, depending on processing methods (Neto et al., 2015; Zhu et al., 2022). It is especially rich in arginine and exhibits good palatability, making it a widely utilized plant protein source in aquatic animal diets (Variath and Janila, 2017; Farooq et al., 2025). Global groundnut production averaged 54 million metric tons between 2016 and 2023, indicating substantial availability and economic feasibility (Gelaye and Luo, 2024; FAO, 2024). However, groundnut meal contains certain anti-nutritional factors, such as phytate, trypsin inhibitors, and tannins. It may exhibit amino acid imbalances, particularly lower lysine levels compared to soybean meal (Liu et al., 2012; Zehra et al., 2020; Zhu et al., 2022). These limitations may affect nutrient digestibility, growth performance, and physiological responses when included at higher replacement levels.

Numerous studies have investigated the effect of fishmeal replacement with groundnut meal in feeds for various aquatic species such as *Heteropneustes fossilis* (Ansal et al., 2018), *Litopenaeus vannamei* (Yue et al., 2012; Liu et al., 2012; Jannathulla et al., 2018), *Oreochromis niloticus* (Agbo et al., 2011; Duodu et al., 2018; Apollo et al., 2022; Zehra et al., 2020), *Oreochromis mossambicus* (Yıldırım et al., 2014), Oncorhynchus mykiss (Acar and Türker, 2018; Farooq et al., 2025), *Labeo rohita* (Ghosh et al., 2015), *Portunus trituberculatus* (Cui et al., 2025) and *Huso huso* (Sefidkohi et al., 2025).

Carps dominate freshwater aquaculture production in Southeast Asian countries, particularly India. Among Indian major carps, rohu (*Labeo rohita*) is one of the most commercially important species due to its rapid growth rate, high consumer acceptance, and strong market demand. Rohu ranks among the top finfish species in inland aquaculture production, contributing significantly to national fish output (Mir et al., 2015; Siddiqua and Khan, 2022; Yadav et al., 2025). Given its economic importance, optimising feed formulation and production for fingerling rohu is essential to ensure sustainable and profitable aquaculture practices.

Therefore, the present study was undertaken to evaluate the effects of graded replacement of fishmeal with groundnut meal on growth performance, carcass composition, and digestive enzyme activities in *Labeo rohita* fingerlings. These findings are expected to contribute to the development of cost-effective and sustainable feed production for carp aquaculture.

Materials and Methods

2.1 Feed Preparation

Six different isoproteic (CP 350 g kg-1), isocaloric (18.0 kJ g-1) and isolipidic (70 g kg-1) diets were prepared. Dietary ingredients were finely ground and sieved to obtain a uniform particle size. Half of the crude protein (17.5%) was contributed by FM, and the remaining half (17.5%) was contributed by other ingredients in the diet. Fishmeal was replaced with groundnut meal at the replacement level of 0% (17.5% CP), 20% (14.0% CP), 40% (10% CP), 60% (7% CP), 80% (3.5% CP), and 100% (0% CP) and the diets were designated as GNM0, GNMM20, GNMM40, GNM60, GNM80 and GNM100, respectively. The required quantities of each ingredient were weighed using a balance (Precisa 120A, 0.1 mg sensitivity; Oerlikon AG, Zurich, Switzerland) and mixed until a homogenous mixture was obtained. After that, the required amount of distilled water was added, and the mixture was pressure-cooked for 20 minutes to form a dough. After the cooked mixture was cooled to room temperature, oil was added and mixed for 4 min in a blender (Hobart Corporation, Troy, OH, USA), followed by mixing the vitamin and mineral premixes at 40 °C. The resulting dough was forced through the extruder (2-mm die), the strands were dried at 40 °C to reduce the moisture content to 10%, crumbled, sieved (500 µm) and stored at −4 °C.

2.2 Fish acclimatization and experimental design

Induced-bred rohu fingerlings were sourced from KGF Aquaculture and Fish Hatchery, Aligarh. Prior to stocking, the fish received prophylactic treatment in a $KMnO_4$ solution (1:3000) and were then placed in circular tanks (600 L capacity; 0.91 m in height and 1.22 m in diameter). Before commencing the feeding trial, fish were acclimated for two weeks on the GNM0 feed.

2.3 Feeding trial

Fingerlings of rohu were randomly distributed into 18 circular polyvinyl tanks (55 L capacity) with three replicates per dietary treatment. Each tank was stocked with 30 fish and supplied with continuous water flow. The experiment was conducted under a 12 h light and 12 h dark cycle. Fish were fed two times daily (09:00 and 17:00 h) to apparent satiety, and unconsumed ration was collected immediately, dried, and weighed to determine feed utilization. Body weight was measured weekly after anaesthetizing fish with MS-222 (100 mg L^{-1}; Sigma, St. Louis, MO, USA). The feeding trial was conducted for eight weeks.

2.4 Sample collection and analysis

Nine fish from the replicates of each dietary group were sacrificed at the end of the feeding trial, pooled and six subsamples were taken for carcass composition analysis (n = 3 × 6). The viscera and liver were excised and weighed to evaluate viscerosomatic

index (VSI) and hepatosomatic index (HSI).

Table 1 Experimental diet formulation and proximate composition (% dry matter).

Ingredients (g kg^{-1})	GNM0	GNM20	GNM40	GNM60	GNM80	GNM100
Fishmeal	28.23	22.58	16.93	11.92	5.64	0
Groundnut Meal	0	7.77	15.55	23.33	31.11	38.88
Rice distiller dried grains	19.09	19.09	19.09	19.09	19.09	19.09
Mustard oil cake	13.33	13.33	13.33	13.33	13.33	13.33
Wheat middlings	20.71	20.71	20.71	20.71	20.71	20.71
Tapioca starch	13.93	11	9.0	6.2	4.1	1.5
Mineral mix[b]	1.5	1.5	1.5	1.5	1.5	1.5
Vitamin mix[c]	2	2	2	2	2	2
Oil premix[a]	1.0	1.3	1.7	2.1	2.5	2.9
Analysed crude protein	34.85	34.92	35.00	34.89	34.97	35.00
Analysed crude lipid	7.23	7.14	7.27	7.36	7.29	7.38
Gross Energy (kJ g^{-1})[d]	18.19	18.10	18.27	18.32	18.48	18.56

a Cod liver oil, Universal Medicare Private Limited, Mumbai, India and refined corn oil, Nashiel Chemical Private Limited, Ahmedabad, India. ₆Mineral mixture (g kg-1): calcium biphosphate 135.7; calcium lactate 326.9; ferric citrate 29.7; magnesium sulphate 132.0; potassium phosphate (dibasic) 239.8; sodium biphosphate 87.2; sodium chloride 43.5; aluminium chloride. 6H2O 0.154; potassium iodide 0.15; cuprous chloride 0.10; manganous sulphate. H2O 0.80; cobalt chloride. 6H2O 1.00; zinc sulphate. 7H2O 4.0; Loba Chemie, India, Halver (2002). c Vitamin mixture (30 g kg− 1 of diet; 10 g vitamin mix +20 g α-cellulose): choline chloride 5.00; inositol 2.00; ascorbic acid 1.00; niacin 0.75; calcium pantothenate 0.5; riboflavin 0.2; menadione 0.04; pyridoxine hydrochloride 0.05; thiamine hydrochloride 0.05; folic acid 0.015; biotin 0.005; alphatocopherol 0.4; vitamin B12 0.0001; Loba chemie, India. d Estimated value of the basal diet on Gallenkamp ballistic bomb calorimeter.

2.5 Proximate Composition Analysis

The whole-body proximate composition at the start and end of the trial was evaluated following the procedures described by AOAC (2015). Crude protein (N×6.25) was quantified using an automatic analyser (Kjeltec Tecator TM Technology 2300, Hoganas, Sweden). Moisture content was measured by drying the samples in a hot-air oven at 102 ± 1 °C (Yorko Instruments, New Delhi, India). Lipid content was estimated through the solvent-extraction method (Socs Plus SCS 4, Pelican Equipments, Chennai, India), while ash content was obtained by incinerating the samples in a muffle furnace at 650 °C for 6 hours (S.M. Scientific Instrument Pvt. Ltd., Jindal Company, New Delhi, India). Gross energy was analyzed by a ballistic bomb calorimeter (CBB 330 010L, Gallenkamp, Loughborough, UK).

2.6 Digestive enzyme assay

Protease, amylase, and lipase activity were determined as per Moore and Stein (1948), Bernfeld (1955) and Seligman and Nachlas (1963), respectively.

2.7 Growth performance assessment

The growth of the fish was measured using the indices:

Absolute weight gain (g $fish^{-1}$) = Final body weight (g $fish^{-1}$) - Initial body weight (g $fish^{-1}$).

Live Weight Gain (LWG %) = Final body weight (g $fish^{-1}$) - Initial body weight (g $fish^{-1}$) / Initial body weight (g $fish^{-1}$) x 100.

Specific growth rate (SGR% day^{-1}) = (ln final body weight (g) - ln initial body weight (g) / No. of days of the experiment × 100.

Feed conversion ratio (FCR) = Dry feed fed (g) / Wet weight gain (g).

Protein efficiency ratio (PER) = Weight gain (g) / Protein intake (g).

Protein retention efficiency (PRE%) = Protein gain (g) / Protein intake (g) x 100.

Hepatosomatic index (HSI%) = Liver weight (g) / Body weight (g) x 100.

Viscero Somatic Index ratio (VSI) = Viscera weight (g) / Body weight (g) x 100.

2.8 Statistical analyses

Data were analyzed using one-way ANOVA, with normality and variance homogeneity confirmed via Shapiro–Wilk and Levene's tests, respectively. Tukey's HSD test identified significant differences ($P < 0.05$). Broken-line regression was applied to model LWG, SGR, PRE and protease against FM-replaced GNM levels. Statistical analyses were performed using Origin Software

(v10.1.5.32).

Results

3.1 Growth Performance

Growth parameters of fingerling *L. rohita* fed diets with graded replacement of fishmeal (FM) by ground meal (GNM) are summarised in Table 2. Live weight gain (LWG%), specific growth rate (SGR), and protein retention efficiency (PRE) did not differ significantly ($P > 0.05$) up to 40% replacement, while further replacement at 60, 80 and 100% caused a significant reduction ($P < 0.05$). Feed conversion ratio (FCR) remained unaffected up to 40% FM replacement, beyond which it significantly increased ($P < 0.05$). Broken-line analysis of LWG, SGR and PRE against FM replaced levels indicated optimum substitution at 40%, 40% and 39.70%, respectively (Fig.1, Fig.2 and Fig.3).

Table 2 Growth performance of fingerling *Labeo rohita* fed diets containing varying replacement levels of fishmeal with groundnut meal.

Parameter	GM0	GM20	GM40	GM60	GM80	GM100
Initial body weight/fish (g)	2.38±0.01	2.34±0.05	2.28±0.02	2.34±0.03	2.39±0.02	2.4±0.03
Final body weight/fish (g)	15.58±0.32^{a}	15.00±0.43^{a}	14.79±0.29^{a}	13.25±0.38^{b}	10.53±0.41^{b}	10.21±0.32^{c}
Absolute weight gain (g fish^{-1})	13.20±0.21^{a}	12.66±0.32^{a}	12.51±0.46^{a}	10.91±0.36^{b}	8.14±0.29^{b}	7.81±0.28^{c}
Live weight gain (%)	554.62±14^{a}	541.02±12^{a}	548.68±18^{a}	466.23±20^{b}	340.58±16^{c}	325.41±19^{c}
SGR (% day^{1})	3.35±0.04^{a}	3.31±0.06^{a}	3.33±0.07^{a}	3.09±0.04^{b}	2.64±0.05^{bc}	2.58±0.03^{c}
FCR	1.58±0.11^{c}	1.60±0.15^{c}	1.63±0.18^{c}	1.74±0.14^{c}	1.87±0.09^{b}	1.98±0.13^{a}
PER	1.80±0.05^{a}	1.78±0.07^{a}	1.75±0.04^{a}	1.64±0.06^{b}	1.52±0.08^{c}	1.44±0.05^{d}
PRE (%)	30.68±0.68^{a}	29.91±0.45^{a}	29.86±0.53^{a}	26.18±0.68^{b}	23.02±0.61^{b}	22.47±0.80^{c}
HSI (%)	2.46±1.1^{a}	2.62±1.31^{a}	2.71±1.42^{a}	2.50±1.84^{a}	2.49±1.32^{a}	2.60±1.88^{a}
VSI (%)	4.44±0.53^{a}	4.36±0.24^{a}	4.47±0.63^{a}	4.56±0.41^{a}	4.38±0.53^{a}	4.45±0.66^{a}

*Mean values of 3 replicates ± SEM; **mean values sharing the different superscripts in the same column are significantly different ($P<0.05$)

3.2 Carcass Composition and Biometric Indices

Carcass composition of fish fed experimental diets is presented in Table 3. Whole-body protein and lipid content remained unchanged up to GNM40 but declined significantly ($P<0.05$) at GNM60, GNM80 and GNM100. Carcass ash contents showed no significant differences ($P > 0.05$) in fish fed with all diets. No significant difference ($P > 0.05$) was observed in moisture content till GNM40; however, a significant ($P < 0.05$) increase in moisture content was evident in fish fed with GNM60, GNM80 and GNM100 diets. Somatic indices such as hepatosomatic index (HSI) and viscerosomatic index (VSI) did not differ significantly ($P > 0.05$) among all dietary treatments (Table 2).

Table 3 Carcass compositions (wet basis) fingerling *Labeo rohita* fed diets containing varying replacement levels of fishmeal with groundnut meal.

Parameter (%)	GM0	GM20	GM40	GM60	GM80	GM100
Protein	16.15±0.12^{a}	15.95±0.27^{a}	16.2±0.16^{a}	15.18±0.19^{b}	14.28±0.25^{bc}	14.64±0.21^{c}
Ash	3.41±0.25^{a}	3.28±0.18^{a}	3.35±0.16^{a}	3.31±0.26^{a}	3.42±0.21^{a}	3.26±0.16^{a}
Moisture	73.68±0.09^{b}	73.35±0.05^{b}	73.72±0.04^{b}	74.86±0.08^{a}	75.53±0.03^{a}	75.72±0.08^{a}
Lipid	4.64±0.09^{a}	4.57±0.12^{a}	4.72±0.03^{a}	3.85±0.14^{b}	3.42±0.06^{b}	3.29±0.04^{b}

*Mean values of 3 replicates ± SEM; **mean values sharing the different superscripts in the same column are significantly different ($P<0.05$)

3.3 Digestive Enzyme Activities

Intestinal digestive enzyme activities of *L. rohita* fingerling are presented in Table 4. Protease, lipase, and amylase activities were not significantly affected ($P > 0.05$) up to 40% replacement of FM by GNM; thereafter, significant reductions ($P < 0.05$) were

observed at 60%, 80%, and 100% replacement levels. Broken-line analysis of intestinal protease activity against FM replacement showed maximum activity of these enzymes up to 40% replacement level, indicating an optimum replacement of 40% of the dietary protein contributed by FM (Fig.4).

Table 4. Digestive enzyme activities of fingerling *Labeo rohita* fed diets containing varying replacement levels of fishmeal with groundnut meal.

Enzyme	GNM0	GNM20	GNM40	GNM60	GNM80	GNM100
Amylase (U mg protein^{-1})	22.87±0.93^{a}	23.11±0.87^{a}	23.19±0.84^{a}	19.66±0.93^{b}	18.17±0.79^{b}	16.25±0.69^{c}
Lipase (U mg protein^{-1})	3.74±0.16^{a}	3.67±0.19 ^{a}	3.59±0.15^{a}	2.66±0.11^{b}	2.48±0.09^{b}	1.77±0.14^{c}
Protease (U mg protein^{-1})	32.70±1.20^{a}	31.85±0.95^{a}	32.25±1.22^{a}	27.01±1.30^{b}	24.52±0.99^{b}	22.51±1.00^{c}

*Mean values of 3 replicates ± SEM; **mean values sharing the different superscripts in the same column are significantly different (P<0.05)

Discussion

Growth Performance

The increasing scarcity and rising cost of FM, coupled with stagnation in global FM production, have intensified the search for sustainable, economically viable alternative protein sources for aquafeeds. Developing nutritionally balanced diets incorporating alternative ingredients is essential to sustain aquaculture growth while maintaining fish performance. Formulated feeds must provide adequate levels of essential nutrients to meet species-specific requirements, as proper nutrient balance plays a fundamental role in supporting growth, metabolism, and overall physiological development (Gamboa-Delgado and Márquez-Reyes, 2018; Howlader et al., 2023; Cai et al., 2024). Among plant-based protein sources, GNM has attracted attention due to its relatively high crude protein content and availability (Yuan et al., 2020; Cui et al., 2025).

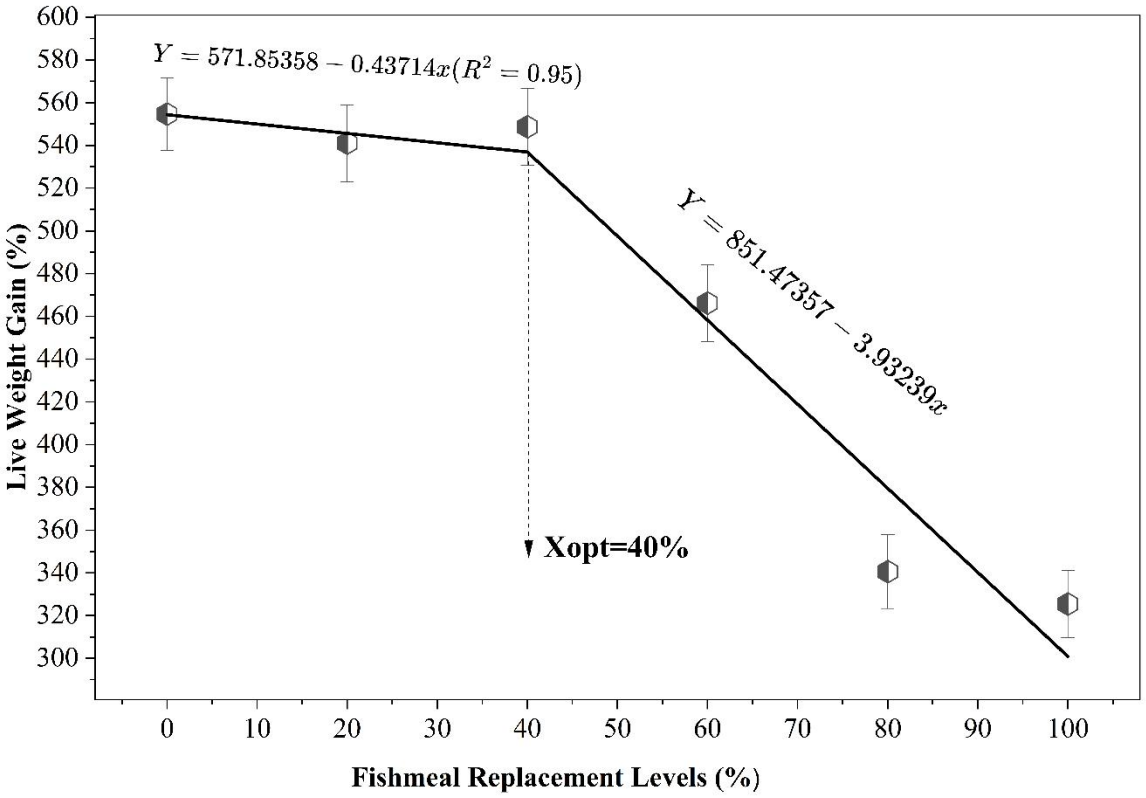

Fig.1 Broken-line relationship of live weight gain to dietary fishmeal replacement levels. Each point represents the mean of three replicates per treatment.

Growth performance is widely recognised as a primary indicator of dietary protein quality and nutritional adequacy in aquaculture species (Roques et al., 2020; Cui et al., 2025). Parameters such as weight gain rate (WGR), specific growth rate (SGR), protein efficiency ratio (PER), protein retention efficiency (PRE), and feed conversion ratio (FCR) are reliable indicators for evaluating dietary effectiveness (Cai et al., 2022; Cai et al., 2024). In the present study, absolute weight gain, SGR, PER and PRE remained stable up to 40% replacement of FM with GNM. However, beyond this replacement level, significant declines in these parameters were observed, accompanied by an increase in FCR.

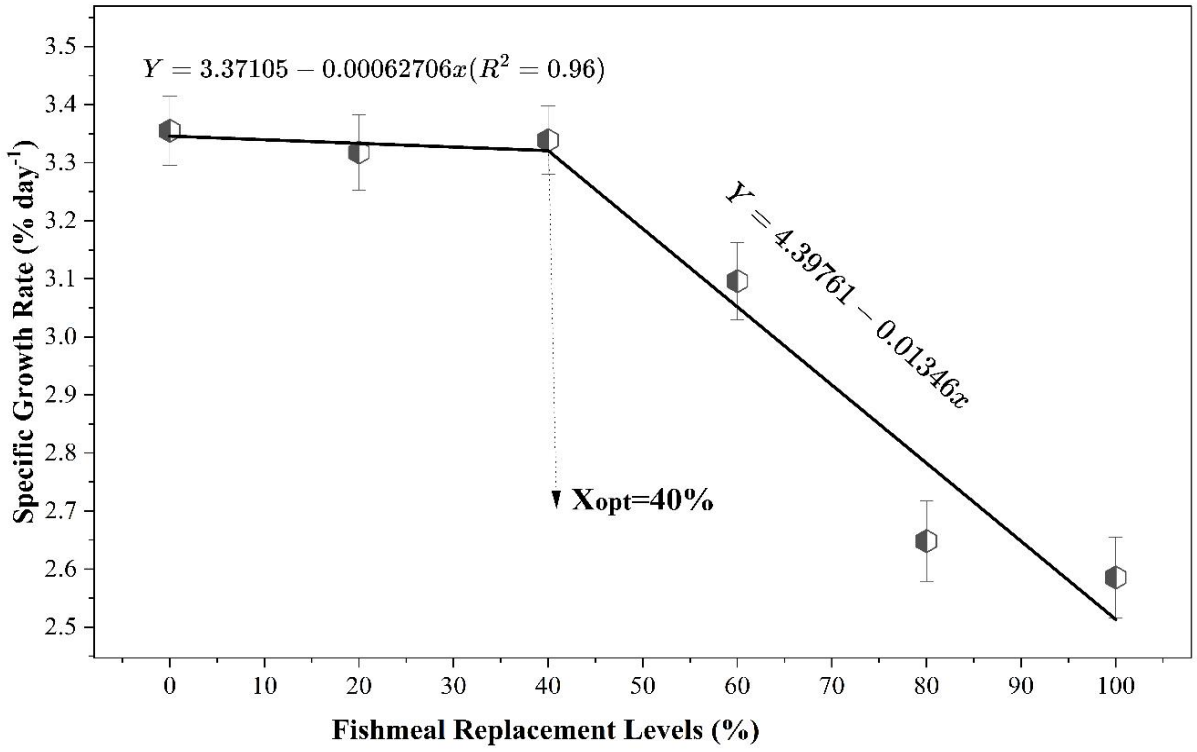

Fig.2 Broken-line relationship of specific growth rate to dietary fishmeal replacement levels. Each point represents the mean of three replicates per treatment.

These findings indicate that while moderate incorporation of GNM can sustain growth in *L. rohita*, higher substitution levels compromise feed efficiency and growth performance. Ghosh and Kaushik (2015) reported that up to 45% of FM can be replaced with fermented GNM in *L. rohita* without hampering growth performance, while Zhu et al. (2022) reported that up to 22% FM replacement with GNM did not impair growth in juvenile hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂). Similarly, rainbow trout (*Oncorhynchus mykiss*) maintained comparable weight gain at 10% GNM substitution (Acar and Turker, 2018), while Mozambique tilapia (*Oreochromis mossambicus*), Nile Tilapia (*Oreochromis niloticus*) and swimming crab *Portunus trituberculatus* tolerated replacement levels up to 20% without adverse growth effects (Yildirim et al., 2014; Zehra et al., 2020; Cui et al., 2025). Reduced growth performance at higher replacement levels has also been observed in red claw crayfish (*Cherax quadricarinatus*), suggesting limited tolerance in certain species (Qian et al., 2021). These variations indicate that optimal FM replacement levels with GNM are strongly influenced by species-specific digestive capacity and metabolic adaptability.

The decline in growth observed beyond 40% replacement in the present study may be attributed to several nutritional constraints associated with plant-derived proteins. GNM typically exhibits an imbalanced essential amino acid profile, particularly with respect to lysine and methionine, which are critical for protein synthesis and tissue accretion (Farooq et al., 2025). Additionally,

residual antinutritional factors and reduced palatability may negatively influence feed intake and nutrient utilization efficiency. Increased FCR at higher replacement levels further supports the hypothesis of diminished nutrient digestibility and metabolic utilization.

Overall, the findings suggest that GNM can effectively replace fishmeal up to 40% in diets for *L. rohita* without compromising growth performance. However, excessive substitution leads to reduced growth efficiency, emphasising the importance of optimising replacement levels and balancing essential amino acids when formulating plant-protein-based aquafeeds.

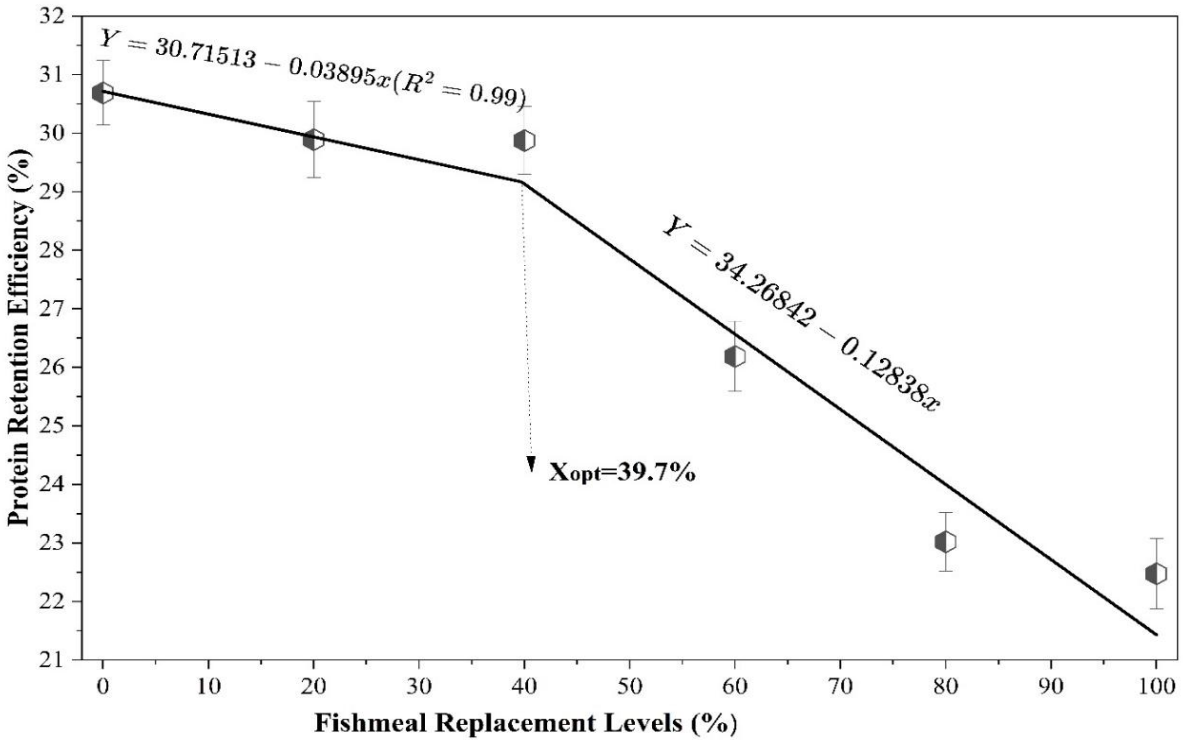

Fig.3 Broken-line relationship of protein retention efficiency to dietary fishmeal replacement levels. Each point represents the mean of three replicates per treatment.

Proximate Composition

The proximate composition of fish, comprising moisture, protein, fat, and ash, represents the fundamental nutritional makeup (96-98%) of fish tissue. It is a critical indicator of both nutritional quality for human consumption and physiological health for fish, with composition varying based on species, diet, season, and life stage. The proximate composition of a fish, such as the percentage of moisture (water), crude protein, crude fat (lipid), and ash (minerals) is the crucial indicator of its nutritional status, energy reserves, and metabolic efficiency in utilizing nutrients from its diet. It reveals how efficiently a fish has converted feed into tissue, reflecting its health, physiological state, and environmental conditions. Carcass composition is altered by numerous endogenous and exogenous factors that often indicate the quality of cultured fish species (Ahmed et al., 2022; Majeed et al., 2025). Protein and ash content are mainly regulated by internal factors, whereas lipid level is influenced by both internal and external factors. Carcass protein and lipid are considered key indicators of fish quality and are important for processing and storage (Ahmed et al., 2023). In this study we observed that after 40% replacement levels of FM with GNM the body protein and lipid content of fish decreased while its moisture content increased, this could be attributed to lower quality of plant protein as compared to high quality of FM (Ahmed et al 2023), similar effects have been reported in species *Scophthalmus maximus* (Wang et al., 2016), *Lateolabrax japonicus* (Liang et al., 2017), *Cyprinus carpio* (Imtiaz et al., 2023), *L. rohita* (Ghosh et al., 2015), *Litopenaeus vannamei* (Liu et al., 2012), *Oncorhynchus mykiss* (Farooq et al., 2025) and *Heteropneustes fossilis* (Hossain et al., 2023). However, there was no difference in the total ash content of the body, which is consistent with the study reported in *Oncorhynchus mykiss* (Farooq et al 2025).

Hepatosomatic index (HSI) and viscerosomatic index (VSI) did not differ significantly among treatments, even at 100% FM replacement. Similar findings were reported in *Huso huso* (Davoudi-Sefidkohi et al., 2025) and *Epinephelus fuscoguttatus* ♀ *Epinephelus lanceolatus*♂ (Zhu et al., 2022) where the replacement of FM with GNM meal did not affect somatic indices. Yadav et al. (2025) and Badran et al. (2024) also showed stable HSI and VSI in *L. rohita* and *Oreochromis niloticus* fed different plant protein meals.

Digestive Enzymes

Evaluation of digestive enzyme activity is a crucial indicator of growth performance in in vivo feeding trials. These enzymes catalyse the breakdown of complex dietary components into simpler, absorbable molecules, thereby enhancing their assimilation within the intestinal tract (Yadav et al., 2025). Insufficient digestive enzyme activity leads to incomplete degradation of feed components, which may result in poor digestion and nutrient deficiencies, ultimately compromising overall health (Ahmadifar et al., 2019; Zhang et al., 2025). This study assessed the activities of amylase, lipase, and protease to determine the effect of a gradual replacement of FM with GNM on digestive enzyme function in *L. rohita*. Amylase, lipase, and protease activity showed no significant changes up to GNM 40. However, a further increase in the replacement of FM at GNM 60, GNM80 and GNM100 significantly reduced the activity of these enzymes.

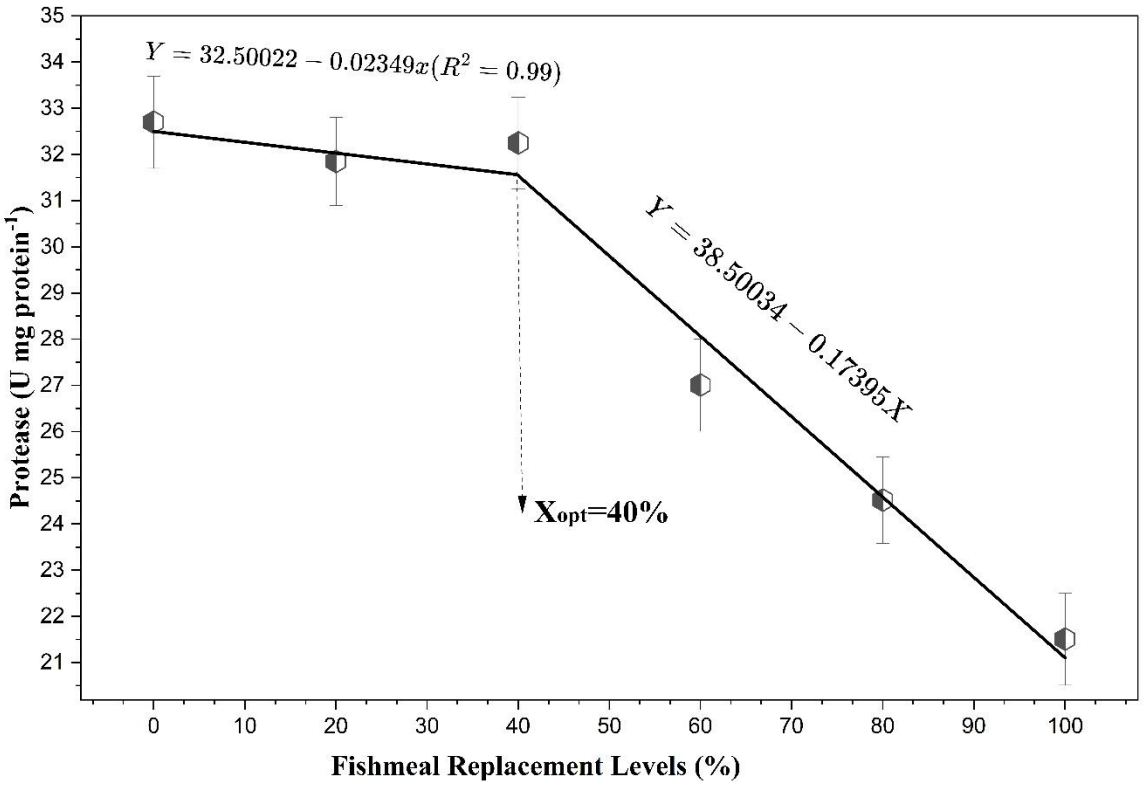

Fig.4 Broken-line relationship of intestinal protease activity to dietary fishmeal replacement levels. Each point represents the mean of three replicates per treatment.

Digestive enzyme activity influences growth performance parameters and feed utilization efficiency (Li et al., 2017; Huang et al.,2025), which in fish is influenced by the feed formulation and ingredients (Wang et al., 2022; Huang et al, 2025). Increased enzyme activity is associated with improved growth performance, better nutrient assimilation, and more efficient protein and lipid utilisation (Magouz et al., 2020), which in the present study was evident in fish fed up to 40% FM replaced by GNM and declined with further substitution at 60, 80 and 100%. The reduction in digestive enzyme activities beyond this threshold may primarily be attributed to the anti-nutritional factors (ANFs) present in GNM. GNM reported to contain major ANFs, including tannins, trypsin

inhibitors and phytic acid (Zhu et al., 2022). Dietary tannins are known to interfere with protein and dry matter digestibility by inhibiting proteolytic enzymes and forming indigestible complexes with dietary proteins (Krogdahl, 1989; Ghosh et al., 2015; Farooq et al., 2025). Such interactions can impair digestive efficiency and ultimately contribute to growth retardation. More specifically, tannins exert growth-inhibitory effects by suppressing digestive enzyme activities, particularly proteases. The inhibitory effects of plant tannins on digestive enzymes have been documented in carp and salmonids (Maitra and Ray, 2003; Mandal and Ghosh, 2010; Zhu et al., 2022; Wang et al., 2024), although the extent of inhibition varies depending on the fish species and the plant source used. In general, while plant-based protein sources are increasingly incorporated into aquafeeds, their inherent ANFs may adversely affect fish physiology (Chikwati et al., 2012; Jannathulla et al., 2019; Han et al., 2022; Feng et al., 2026). These compounds can hinder digestion by inhibiting digestive enzyme activities or by binding to nutrients, thereby reducing their bioavailability and overall feed utilization efficiency (Liu et al., 2025; Yadav et al., 2025). Therefore, the higher replacement levels of FM with GNM in the present study likely intensified the effects of these ANFs, resulting in reduced digestive enzyme activity and the subsequent decline in growth performance in fingerling *L. rohita*. which is consistent with the study reported on *L. rohita* (Gosh et al 2015), *Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂ (Zhu et al 2022) and *Portunus trituberculatus* (Cui et al., 2025).

Conclusion

The present study demonstrated that groundnut meal (GNM) can effectively replace fishmeal (FM) in the diets of *Labeo rohita* fingerlings up to 40% without adversely affecting growth performance, feed utilization, carcass composition and digestive enzyme activities. However, replacement levels beyond 40% resulted in reduced growth, poorer feed efficiency, and decreased digestive enzyme activities, likely due to amino acid imbalance and the presence of anti-nutritional factors in GNM. These limitations could potentially be overcome through nutritional strategies such as amino acid supplementation (e.g., lysine and methionine), phytase supplementation to improve phytate phosphorus availability, and processing techniques such as fermentation to reduce anti-nutritional factors. Therefore, with appropriate dietary modifications, groundnut meal holds a strong potential as a sustainable and economical alternative protein source to reduce reliance on fishmeal.

5. Statements and Declarations

Author Contributions: The first author, Shigufta Ali, conducted the feeding trial and substantially contributed to the writing of the manuscript, statistical analysis, and the interpretation of the data. The second author, Mukhtar Ahmad Khan, provided expert assistance and is a scientific advisor for designing this study. He also contributed to the drafting of the paper.

Conflict of Interest Declaration: The authors declare that they have no conflict of interest.

Ethical Approval: All the experimental procedures, including the animal experimentation, were approved by the institutional ethical committee of the Department of Biochemistry, Aligarh Muslim University, Aligarh, India (registration no: CCSEA/BC/P-11/2024-2025).

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