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Effects of substituting groundnut oil cake with *Prosopis cineraria* seed meal on growth, physio-metabolic, and haemato-biochemical responses of *Labeo rohita*

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Abstract

Background The search for alternative aqua feed ingredients is continuous and the costliest ingredients are replaced to cut down the feed cost and make the aquaculture venture a profitable one. A 60-day feeding trial was conducted to determine the effects of substituting groundnut oil cake (GNOC) with *Prosopis cineraria* seed meal (PSM) on the growth, physio-metabolic, and immunological responses of *Labeo rohita* fingerlings. Four iso-nitrogenous (30.36% crude protein) and iso-caloric (17.64 MJ GE kg-1) diets were formulated with varying inclusion levels: 0, 50, 75, and 100 g PSM kg^{-1}, denoted as Cs, PSM_5, $PSM_{7.5}$, and PSM_{10}, respectively. One hundred twenty (120) fingerlings (average weight 12.70 ± 0.02 g) were randomly distributed in four groups in triplicates using a completely randomized design.

Results Increasing dietary PSM levels enhanced growth and nutrient utilization indices, with the most significant improvement observed in the $PSM_{7.5}$ and PSM_{10} groups. Intestinal protease enzyme activities improved significantly ($P < 0.05$) with higher dietary PSM levels, while lipase and amylase activities remained unaffected. Similarly, the activity of the protein (aspartate aminotransferase and alanine aminotransferase) and carbohydrate metabolic enzymes (lactate dehydrogenase and malate dehydrogenase) significantly ($P < 0.05$) elevated in higher PSM-fed groups. However, carcass composition, oxidative stress enzymes (superoxide dismutase and catalase) activity, and haematological biochemical parameters remained unaffected ($P > 0.05$) due to feeding of varying levels of PSM.

Conclusion PSM can be incorporated up to 100 g kg^{-1} as a substitute for GNOC in *L. rohita* diet without compromising growth and immunity. The outcome of this study may reduce the cost of aquafeed for carp culture in the context of sustainable aquaculture practices.

Keywords *Prosopis cineraria*, Growth, Oxidative and haematological parameters, Groundnut oil cake, *Labeo rohita*

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Background

Aquaculture plays a vital role in ensuring global food security as aquaculture is one of the fastest-growing food-producing areas. The rapid growth can be credited to global population growth, increasing demand for animal protein, and a stable fisheries sector [28]. India is a significant hub for major carp production, contributing about 5% of total carp production over the past decade [28]. Carps are the predominant cultured fish species in Southeast Asian countries, particularly India [96]. Among carps, rohu (*Labeo rohita*) is an important candidate species for aquaculture due to its higher growth rate, consumer preference, and better market demand. Inland and aquaculture production of rohu was ranked at 9th position, accounting for 5.1% of the total finfish aquaculture production [25]. In aquaculture, feed represents the largest portion of expenses in commercial aquaculture operations, constituting approximately 60% of total operating costs [21, 42, 81].

In India, 80–85% of farmers use groundnut oilcake (GNOC) and de-oiled rice bran (DORB) based farm-made feed for aquaculture [89]. Groundnut (*Arachis hypogaea*) is a leguminous (Fabaceae family) crop renowned for its edible oilseeds. In India, groundnut production declined from 971 lakh tonnes in 2014–15 to 670 lakh tonnes in 2019–2020 [92], probably influenced by unpredictable rainfall patterns and subpar productivity. Groundnut seeds contain approximately 25–30% crude protein and 35–50% oil content [62]. After oil extraction, the residual material, known as groundnut oil cake (GNOC), contains 43.4–51.6% protein, 0.68–11.3% ether extract, 29.8–36.2% carbohydrate, 1.2–2.7% crude fibre, and 4.4–5.9% ash [70]. Notably, cysteine, methionine, and lysine are identified as the most limiting amino acids in GNOC [38]. GNOC is commonly used as feedstock for cattle, aquatic animals, and other faunas [18] and is also blended with varying proportions of DORB in farm-made fish feeds [72]. However, it is important to address the anti-nutritional factors (ANF) such as tannins, phytates, and trypsin inhibitors in groundnut, which necessitates adequate processing before incorporating it into fish feed [39, 56]. Nevertheless, recent price hikes and an inadequate supply of GNOC have led to its substitution with unconventional plant feedstuffs in aquafeeds.

Forty-four different Prosopis species are cultivated in arid and semi-arid regions across various countries in Asia, Africa, and the Americas [37, 102]. *Prosopis cineraria* (Khejri), a resilient member of the Leguminosae family, thrives in dry and semi-dry regions globally, including India [37]. This plant is cultivated in the northwest (Haryana, Rajasthan, Punjab, Delhi, Uttar Pradesh and Gujarat), central (Madhya Pradesh) and southern regions (Karnataka, Andhra Pradesh, Telangana) of India [102]. This plant is known for its nitrogen-fixing ability and thrive in dry conditions commonly found in arid and semi-arid regions [71]. The biomass production for the Khejri seeds can vary from 113.25 to 250.0 tonnes per hectare. [79]. The seeds of the khejri plant contain 31 to 37% crude protein, making them a nutrient-dense source of amino acids, fatty acids including linolenic acid, oleic acid, stearic acid, and palmitic acid, minerals, and vitamins [35, 49, 73]. Additionally, the seeds are embraced by phytochemical complexes like alkaloids, tannins, steroids, terpenes, and flavonoids [80, 87, 102]. *P. cineraria* has been studied as a dietary supplement for promoting the growth of animals like sheep, rabbits, poultry birds, and fish in Asia and Africa [53, 54, 102, 104]. The fresh and tender pods of *P. cineraria* are typically harvested from March to May and providing farmers an excellent chance to collect seeds from ripe pods from June to July [46]. By harvesting of the seeds during this optimal period, farmers can effectively utilize these seeds in fish feed throughout the entire culture phase. The natural availability of these seeds allows farmers to reduce feed costs. For those seeking commercial options, seeds can also be purchased year-round at prices ranging from 30 to 40 rupees per kg. Nevertheless, the availability of conventional fish feed ingredients is diminishing, accompanied by a steady increase in prices of conventional feed ingredients. From now on, there's a pressing necessity to explore affordable, alternative ingredients to mitigate feed expenses. In this context, *P. cineraria* seed meal (PSM) emerges as a promising alternative, which might serve as a non-conventional protein source for carp fish diets. Using PSM as a partial substitute for GNOC in carp diets is expected to reduce the overall cost of fish production. Consequently, this study aims to evaluate the impact of *P. cineraria* seed meal on the growth, physiological, metabolic, and haemato-biochemical responses of rohu fingerlings.

Materials and methods

Collection and preparation of seed meal

P. cineraria seeds were sourced from the local market in Jaipur, Rajasthan. Upon procurement, the seeds underwent a cleaning process and dried afterwards in an oven at 48 °C for 24 h at the Division of Aquaculture, ICAR-Central Institute of Fisheries Education (ICAR-CIFE), Mumbai. Following drying, the seeds were finely ground into meal, sifted through a 90 µ mesh sieve, and stored in an airtight polyethylene bag at room temperature.

Feed preparation

Four diets were formulated to ensure iso-nitrogenous levels and iso-caloric contents (300 g kg^{-1} CP and 17.64 MJ GE kg^{-1}) respectively, as outlined in Table 1.

Table 1 Proximate, anti-nutritional factors and amino acids composition of *Prosopis cineraria* seed meal (PSM)

Nutrients (g kg^{-1})	PSM	GNOC
Dry matter	911.40	904.70
Crude protein	301.20	444.30
Ether extract	42.00	59.90
Crude fibre	64.40	86.80
Nitrogen free extract	534.70	344.40
Total ash	57.60	64.60
Gross energy (MJ kg ^{1})	19.07	20.27
Anti-nutritional factors		
Tannin (g 100 g^{-1})	1.41 ± 0.04	0.37 ± 0.006
Phytate (g kg^{-1})	0.88 ± 0.06	6.71 ± 0.11
Saponin (g 100 g^{-1})	0.20 ± 0.03	0.57 ± 0.009
Alkaloid (g 100 g^{-1})	0.11 ± 0.01	0.18 ± 0.007
HCN (mg 100 g^{-1})	3.02 ± 0.39	4.43 ± 0.12
Trypsin Inhibitors (TIU mg^{-1} protein)	52.01 ± 1.18	69.76 ± 0.80

Amino acids (g kg^{-1} PSM)			
Essential amino acids (g kg^{-1})		**Non-essential amino acids (g kg^{-1})**	
Arginine	38.33	Alanine	16.31
Histidine	2.43	Glycine	19.61
Isoleucine	3.75	Hydroxyproline	42.21
Leucine	18.49	Serine	17.37
Lysine	11.80	Glutamic acid	66.93
Methionine	0.98	Aspartic Acid	38.40
Phenylalanine	8.36	Cysteine	0.04
Tryptophan	ND	Tyrosine	5.80
Threonine	4.19		
Valine	6.20		
Methionine + Cysteine	0.98		
Phenylalanine + Tyrosine	8.36		

Data expressed as Mean, mean ± SE ($n = 3$), *ND* Not detected

PSM Prosopis cineraria seed meal, *GNOC* Groundnut oilcake

The control diet utilized groundnut oil cake without PSM. Three experimental diets were prepared by gradually substituting groundnut oil cake with varying levels of PSM. Each feed ingredient was meticulously weighed according to the formulation, excluding additives and oil. The weighed ingredients were thoroughly mixed to ensure a homogeneous blend and then steam-cooked in a pressure cooker at 121 °C for 20 min to facilitate proper starch gelatinization before cooling to room temperature. Subsequently, oils and feed additives were weighed and evenly incorporated into the cooked mixture. The mixture was then passed through a pelletizer (Uniextrude: Single screw extruder) to produce pellets of uniform size (with a diameter of pellet die: 2 to 2.5 mm). The pellets were spread on trays, dried at room temperature and filled in polythene bags, labelled, and stored at room temperature.

Amino acid profiling PSM

A 50 mg sample of PSM and experimental diets were subjected to hydrolysis with 6 M HCl for 24 h under vacuum at 110 °C. Subsequently, pre-column derivatization was performed using phenyl isothiocyanate before quantification using High-performance liquid chromatography (HPLC). The analysis of both total and free amino acids was carried out using an HRL-CMS-114 instrument (Model:1290 Infinity UHPLC System,1260 infinity Nano HPLC with Chipcube, 6550 iFunnel Q-TOFs, Agilent Technologies, USA) equipped with a flame ionization detector (FID) and an automatic liquid sampler, following the method described by Devappa and Swamylingappa [25], at the Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology (IIT), Mumbai, Maharashtra.

Proximate and anti-nutritional factors compositions

Methods of AOAC [12] were followed to analyze the proximate composition of whole fish and experimental diets. Tannin, trypsin inhibitor, phytates, and saponin contents in PSM and GNOC were determined by spectrophotometric methods by Makkar et al. [55], Gao et al. [36] and Hiai et al. [44], respectively. Hydrogen cyanide and alkaloid contents of PSM and GNOC were quantified by alkaline titration and gravimetric method [13, 43], respectively.

Feeding trial

Rohu, *L. rohita* fingerlings were procured from a fish pond at the Aquaculture Research and Seed Unit in Udaipur. These fingerlings underwent a period of acclimatization to laboratory conditions in a 2000 L quarantine tank, maintained at 27 ± 1 °C, for 20 days while being fed a control diet. Following acclimatization, the fish were randomly distributed into twelve FRP rectangular tanks (capacity 490 L, volume 250 L water), following a completely randomized design. Ten fingerlings, with a mean body weight of 12 ± 0.50 g, were stocked in each tank. Fish were fed at satiation level twice daily. Approximately 10–15% of the water from all tanks was exchanged daily, along with the removal of fecal matter. The physicochemical parameters of the experimental water were monitored fortnightly over 60 days according to standard methods outlined by APHA [14]. The range of water quality parameters was as follows: temperature ranged from 26.10 to 26.98 °C, pH ranged from 7.87 to 8.25, dissolved oxygen ranged from 5.78 to 6.53 mg L^{-1}, alkalinity ranged from 92.05 to 101.50 mg L^{-1} as $CaCO_3$, ammonia ranged from 0.011 to 0.045 mg L^{-1} as NH_3-N, nitrite-N ranged from 0.01 to 0.06 mg L^{-1}, and nitrate–N ranged from 0.12 to 0.39 mg L^{-1} as NO_3-N. Notably, free carbon dioxide was not detected all over the research period.

Growth and feed utilization

Survival, growth, conversion, nutrient utilization, and body indices were calculated by using the following formula [47].

i. Weight gain (g) = FW—IW
ii. Weight gain (%) = [(FW-IW)/IW] *100
iii. Specific growth rate (SGR, %/day) = [{(Ln (FW)—Ln (IW)}/Experiment days] *100
iv. Feed conversion ratio (FCR) = FC (DW)/BWG (WW)
v. Feed efficiency ratio (FER) = BWG (WW)/ FC (DW)
vi. Protein efficiency ratio (PER) = NWG (WW)/Protein intake
vii. Hepatosomatic index (HSI, %) = [LW/WF] *100
viii. Viscero-somatic index (VSI, %) = [VW/WF] *100
ix. Survival (%) = Total no. of fishes catch*100/Total no. of fishes stocked

Where, FW: Final weight (g); IW: Initial weight (g); DW: Dry weight (g); WW: Wet weight (g); FC: Feed consumed (g); BWG: Body weight gain (g); NWG: Net weight gain (g): LW: Liver weight (g); VW: Viscera weight (g); WF: Weight of fish (g).

Apparent digestibility coefficients (ADC)

An inert marker, i.e. chromium oxide (Cr_2O_3), was used to assess the apparent digestibility coefficients for dry matter, protein, and lipid. For this purpose, initially, fish were acclimatized for 10 days. For digestibility trials, the feeding rate was 1.5% body weight, and the feeding frequency was only once a day (10:00 am) for 15 days. After 6 h, the fecal matter was siphoned, stored at −20 °C, and lyophilized for 24 h. Furukawa and Tsukahara [33]' spectrophotometric method was used to quantify the Cr_2O_3 content in feed and fecal matter and ADC for dry matter, and nutrients were calculated based on the following formulas-

ADC of dry matter of diet (%) = $100 \times \{1\text{-}(D_{Cr})/ (F_{Cr})\}$.

ADC of nutrients of the diets (%) = $100 \times [1 - \{(N_D/N_F) \times (F_{Cr}/D_{Cr})\}]$.

Where N_D is the percentage of nutrients in the diet, N_F is the percentage of nutrients in the feces, D_{Cr} is the percentage of chromic oxide in the diet, and F_{Cr} is the percentage of chromic oxide in feces.

Enzymes assay activities

Tissue collection

After completion of the feeding experiment, fish were starved for 24 h. Two fish from each replicate were randomly collected and anesthetized with clove oil at a concentration of 50 µL/L. Blood was then drawn from the caudal vein (Vena caudalis) of three sedated fish using a medical syringe (1.0 ml) that had been previously rinsed with 2.7% ethylene diamine tetra-acetic acid (EDTA) disodium salt, serving as an anticoagulant. The blood samples were promptly transferred to Eppendorf tubes containing a thin layer of EDTA and shaken thoroughly to prevent haemolysis. The collected blood was immediately used for respiratory burst activity and haematological study. Similarly, blood was then drawn from the caudal vein (Vena caudalis) of three sedated fish using a medical syringe and transferred to a 1 ml Eppendorf tube. The blood was allowed to clot for 1 h and centrifuged at 5000 rpm for 10 min (4 ^{0}C). Yellow-straw-colored serum was carefully collected, transferred to another Eppendorf tube, and kept at −20 ^{0}C for further biochemical analysis.

Meanwhile, the same four fish were dissected to obtain tissue samples from the liver, muscle, gill, and intestine. Each tissue sample was weighed and homogenized in chilled 0.25 M sucrose to create a 5% homogenate. The homogenate was then centrifuged at 8000 g (Thermo Fisher Lectron LED centrifuge, Langenselbold, Germany) for 10 min at 4 °C. The sample supernatant was collected and stored at −20 °C. The protein content of the intestinal, hepatic, branchial, and muscular tissues was determined using the Bradford method [17].

Digestive enzymes

Intestinal protease, lipase, and amylase activities were assayed as per Drapeau [26], Cherry and Crandall [19], and Rick and Stegbauer [78], respectively. Protease activity was determined by the casein digestion method. Amylase activity was quantified using the dinitrosalicylic acid (DNS) method, and activity was assayed with 2% (w/v) starch solution as the substrate.

Metabolic enzymes

Lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) activities in liver and muscle tissues were evaluated by following the protocol outlined by Wróblewski and Ladue [101] and Ochoa [64], respectively. Alanine aminotransferase (ALT) activity in the liver and muscle tissues of *L. rohita* was determined following the procedure specified by Wooton [100]. Similarly, aspartate aminotransferase (AST) activity in liver and muscle tissues was assessed using the same methodology as for ALT activity.

Oxidative enzymes

Catalase (CAT) and superoxide dismutase (SOD) activities in liver and gills tissues were measured following the protocol detailed by Takahara et al. [95] and Misra and Fridovich [58], respectively.

Haemato-biochemical study

After the 60 days of feeding experiment, six fish from one treatment (2 fish/replicate) were anesthetized (2-phenoxyethanol, 1 ml L^{-1}), immediately blood was taken after rinsing with the syringe with 2.7% EDTA and kept in tubes (thin EDTA layer) and mixed gently to avoid haemolysis. Serum biochemical indices were assayed by commercial kits from Erba Diagnostics, India, and respiratory burst activity as outlined by Stasiak and Baumann [94]. Blood samples collected in anticoagulant were analyzed for haematological parameters using an automatic blood analyzer (Biorad, California, USA).

Statistical analysis

Shapiro–Wilk and Levene's tests were applied to check the normality and variance homogeneity of data before the ANOVA test. The results (parametric data) were compared at a significance level of 5% using one-way analysis of variance (ANOVA), followed by post hoc comparisons conducted with DMRT using SPSS 22.0 for Windows. Additionally, orthogonal-polynomial contrasts were employed to examine overall, linear, and quadratic trends of parameters concerning PSM levels in the diets. Considerable variation among different dietary treatments was confirmed with DMRT (Duncan's multiple range test).

Results

Proximate, anti-nutritional factors and amino acid compositions

The proximate composition analysis of the *P. cineraria* seed meal (PSM) revealed the following composition: 301.20 crude protein, 42.00 ether extract, 64.40 crude fiber, 57.60 total ash, and 534.70 g kg^{-1} nitrogen-free extract (Table 2). PSM contains trypsin inhibitor, 52.01 TIU mg^{-1} protein, tannin 1.41 g 100 g^{-1}, phytates 0.88 g kg^{-1}, saponin 0.20 g 100 g^{-1}, alkaloids 0.11 g 100 g^{-1}, and hydrogen cyanide 3.02 mg 100 g^{-1} contents. Essential amino acids (EAA) such as arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine of PSM were 38.33, 2.43, 3.75, 18.49, 11.80, 0.98, 8.36, 4.19, and 6.20 g kg^{-1}, respectively. Whereas PSM contained nonessential amino acids (NEAA) such as alanine, glycine, hydroxyproline, serine, glutamic acid, aspartic acid, cysteine, and tyrosine. The concentrations of nonessential amino acids of PSM were found to be 16.31, 19.61, 42.21, 17.37, 66.93, 38.40, 0.04, and 5.80 g kg^{-1}, respectively (Table 2).

Growth performance

The inclusion of dietary PSM did not significantly ($P > 0.05$) affect the linear, overall, and quadratic trend of growth performance and nutrient utilization parameters in rohu fingerlings, as indicated by parameters such as WG, WG%, SGR, FCR, FER, PER, and ANPU (Table 3). Similarly, HSI and VSI values did not significantly ($P > 0.05$) differ among the experimental groups fed with PSM diets. Furthermore, the survival percentage did not vary ($P > 0.05$) between the PSM and control groups, indicating no toxicity of PSM in fish (Table 3).

Whole body composition

The analysis of whole-body composition indicated that the linear and quadratic effects of dietary PSM inclusion did not significantly influence ($P > 0.05$) the moisture,

Table 2 Feed, proximate and essential amino acids composition of experimental diets

Ingredients (g kg^{-1})	[1]Treatments			
	C_S	PSM_5	$PSM_{7.5}$	PSM_{10}
Fish meal[¥]	50.00	50.00	50.00	50.00
Soybean meal[¥]	300.00	300.00	300.00	300.00
Groundnut oil cake[¥]	165.00	127.00	114.00	92.00
PSM[£]	0.00	50.00	75.00	100.00
De-oiled rice bran[¥]	300.00	300.00	300.00	300.00
Wheat flour[¥]	80.00	55.00	50.00	50.00
Maize flour[¥]	43.68	56.68	49.68	46.68
Fish oil[¥]	15.00	15.00	15.00	15.00
Sunflower oil[¥]	15.00	15.00	15.00	15.00
Vitamin mineral premix[©]	20.00	20.00	20.00	20.00
Butylated hydroxytoluene[€]	0.20	0.20	0.20	0.20
Choline chloride[€]	1.00	1.00	1.00	1.00
Carboxy methyl cellulose[€]	10.00	10.00	10.00	10.00
Vitamin C[€]	0.12	0.12	0.12	0.12
Total	1000.00	1000.00	1000.00	1000.00
Proximate composition (g kg^{-1} dry matter basis)				
Dry matter	917.22	912.77	917.11	919.06
Crude protein	303.21	302.09	302.82	301.32
Crude fibre	82.77	84.53	85.41	86.87
Ether extract	57.72	55.56	56.30	58.29
[2]Nitrogen free extract	460.84	464.55	462.43	459.48
Total ash	95.46	93.28	93.04	94.04
[3]Gross energy (MJ kg^{-1})	17.67	17.64	17.63	17.62
Essential amino acids (g kg^{-1} diet)				
Arginine	29.97	29.05	26.43	22.40
Histidine	7.29	7.09	5.96	4.73
Isoleucine	13.38	12.89	10.67	8.28
Leucine	22.54	22.65	19.49	16.03
Lysine	17.94	17.35	14.23	10.93
Methionine	4.46	4.55	4.02	3.43
Phenylalanine	14.73	14.53	12.38	10.00
Tryptophan	ND	ND	ND	ND
Threonine	12.01	12.06	10.39	8.60
Valine	14.83	14.57	12.34	9.90

[1] C_S, 0 g kg^{-1} PSM; PSM_5, 50 g kg^{-1} PSM; $PSM_{7.5}$, 75 g kg^{-1} PSM; PSM_{10}, 100 g kg^{-1} PSM

[¥] Purchased from local animal feed ingredient dealer, Mumbai, India

[£] PSM- *Prosopis cineraria* seed meal Procured from Jaipur, Rajasthan, India

[€] Procured from Himedia Pvt. Ltd., Mumbai, India

[©] Composition of vitamin mineral mix (Agrimin Forte) (quantity kg^{-1}): Vitamin A- 55,00,000 IU; vitamin D3- 11,00,000 IU; vitamin B2- 2,000 mg; vitamin E- 750 mg; vitamin K- 1,000 mg; vitamin B6- 1,000 mg; vitamin B12- 6 mcg; calcium pantothenate- 2,500 mg; nicotinamide- 10 g; choline chloride- 150 g; Mn- 27,000 mg; I- 1,000 mg; Fe- 7,500 mg; Zn- 5,000 mg; Cu- 2,000 mg; Co- 450 mg; L-lysine- 10 g; DL- methionine- 10 g; selenium- 125 mg procured from Virbac, India

[2] Nitrogen free extract (g kg^{-1}) = {1000—[Crude protein (g kg^{-1}) + Ether extract (g kg^{-1}) + Total ash (g kg^{-1}) + Crude fibre (g kg^{-1})}

[3] Gross energy (GE) was calculated according to gross caloric values of using the values of 23.6, 39.5, and 17.2 kJ/g for crude protein, crude fat, and total carbohydrate, respectively (Brett, 1973)

ND, Not determined

crude protein, crude lipid, total carbohydrate, and total ash contents in *L. rohita* fingerlings (Table 4). Across experimental groups, the values ranged from 73.12% to 74.17% for moisture, 16.11% to 16.53% for crude protein, 4.25% to 4.47% for crude lipid, 1.26% to 2.04% for total carbohydrate, and 4.02% to 4.13% for total ash.

Digestive enzyme activities and apparent digestibility coefficients (dry matter and nutrients)

In the current study, fish fed with dietary PSM exhibited significantly higher ($P < 0.05$) protease activity compared to the control group, with linear and quadratic variations observed between the control and PSM-fed groups (Table 5). Conversely, amylase and lipase activities did not differ significantly ($P > 0.05$) between the control and PSM-fed groups. The coefficients of digestibility (ADC) for dry matter, protein, and lipid did not vary ($P > 0.05$) in *L. rohita* fingerlings when fed with dietary PSM (Table 5).

Metabolic enzyme activities

Aspartate aminotransferase activity in the liver and muscle, as well as Alanine aminotransferase activity in muscle, were significantly ($P < 0.05$) influenced by dietary PSM levels, while hepatic ALT activity did not ($P > 0.05$) exhibit significant variation among different dietary treatments (Table 6). Aminotransferase (AST and ALT) activities were notably higher at inclusion levels of 75 and 100 g kg^{-1} PSM compared to the control and 50 g kg^{-1} PSM inclusion levels. There was no significant ($P > 0.05$) difference in muscle LDH activity, while hepatic LDH significantly ($P < 0.05$) increased with increasing the levels of PSM (Table 6). Hepatic MDH activity did not vary significantly ($P > 0.05$) between control and PSM-fed groups, whereas MDH activity in muscle was increased with dietary PSM inclusion ($P < 0.05$) in diet.

Antioxidant enzyme activities

The activities of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) in the gill and liver did not exhibit significant differences ($P > 0.05$) in *L. rohita* fingerlings fed with dietary PSM (Table 7).

Haemato-biochemical haematological parameters

The linear, overall, and quadratic effect of dietary PSM inclusion did not significantly ($P > 0.05$) affect the serum protein profile (total protein, albumin, globulin, and A: G ratio), glucose content, and NBT activity of rohu fingerlings (Table 8). Similarly, contrast analysis revealed that the quadratic, linear, and overall trends of haematological parameters in *L. rohita* fingerlings were not significantly affected ($P > 0.05$) by feeding different experimental diets with PSM (Table 9).

Table 3 Growth performance and nutrient utilization parameters of *L. rohita* fingerlings fed with experiments diets with PSM for 60 days

[1]Treatments	[2]WG (g)	[3]WG%	[4]SGR (% day^{-1})	[5]FCR	[6]FER	[7]PER	[8]ANPU (%)	[9]HSI (%)	[10]VSI (%)	Survival (%)
Cs	13.42	105.32	1.20	2.41	0.42	1.37	26.03	1.45	3.25	100
PSM_5	12.64	99.47	1.15	2.55	0.39	1.30	23.44	1.38	3.55	100
$PSM_{7.5}$	13.33	105.06	1.20	2.49	0.40	1.33	23.70	1.35	3.82	100
PSM_{10}	13.04	102.45	1.18	2.45	0.41	1.36	25.37	1.33	3.25	100
Contrast analysis										
***P* values**										
Overall	0.211	0.255	0.256	0.304	0.298	0.319	0.282	0.565	0.107	-
Linear	0.709	0.758	0.772	0.78	0.725	0.872	0.718	0.752	0.725	-
Quadratic	0.373	0.468	0.46	0.103	0.099	0.094	0.071	0.124	0.201	-

Data expressed as Mean ($n = 3$); Mean values in the same column with different superscript differ significantly ($P < 0.05$)

[1] C_S, 0 g kg^{-1} PSM (*Prosopis cineraria* seed meal); PSM_5, 50 g kg^{-1} PSM; $PSM_{7.5}$, 75 g kg^{-1} PSM; PSM_{10}, 100 g kg^{-1} PSM

[2] WG, Weight gain

[3] WG%, Weight gain percentage

[4] SGR, Specific growth rate

[5] FCR, Feed conversion ratio

[6] FER, Feed efficiency ratio

[7] PER, Protein efficiency ratio

[8] ANPU, Apparent net protein utilization

[9] HSI, Hepato-somatic index

[10] VSI, Viscero-somatic index

Table 4 Whole body composition (% wet weight basis) of *L. rohita* fingerlings fed with different experiments diets with PSM for 60 days

[1]Treatments	Moisture	Crude protein	Ether extract	Total ash	Total carbohydrate
Cs	73.55	16.11	4.57	4.02	1.75
PSM_5	74.02	16.13	4.41	4.03	1.40
$PSM_{7.5}$	74.17	16.12	4.31	4.13	1.26
PSM_{10}	73.12	16.53	4.25	4.06	2.04
Contrast analysis					
***P* values**					
Overall	0.464	0.808	0.456	0.886	0.125
Linear	0.891	0.858	0.782	0.852	0.794
Quadratic	0.128	0.329	0.221	0.195	0.346

Data expressed as Mean ($n = 3$); Mean values in the same column with different superscript differ significantly ($P < 0.05$). [1]C_S, 0 g kg^{-1} PSM (*Prosopis cineraria* seed meal); PSM_5, 50 g kg^{-1} PSM; $PSM_{7.5}$, 75 g kg^{-1} PSM; PSM_{10}, 100 g kg^{-1} PSM

Discussion

The pressing need for cost-effective feed alternatives in intensive fish farming is underscored by the fact that feed costs represent 50 to 60% of production expenses [68]. This situation compels a thorough investigation into sustainable and locally available options to replace traditional feeds [88]. While plant proteins have been explored for animal feed, their high levels of indigestible fractions and anti-nutritional factors often limit their effectiveness [41]. Additionally, as urbanization reduces grazing land, conventional feed resources are increasingly hard to come by, posing a significant challenge to growing livestock and fish populations [83]. Among the potential plant proteins, legume seeds present an exciting opportunity due to their impressive nutrient profiles, including rich protein, lipid, and carbohydrate content [15]. Yet, legume seeds also contain anti-nutritional factors such as trypsin inhibitors, alkaloids, tannins, and

Table 5 Digestive enzymes activities of *L. rohita* fingerlings fed with different experiments diets with PSM for 60 days

[1]Treatments	[2]Amylase	[3]Protease	[4]Lipase	[5]ADC	[6]ADCP	[7]ADCL
Cs	11.11	0.31^{a}	0.68	69.06	83.20	87.47
PSM_5	9.61	0.35^{a}	0.73	69.15	82.28	86.95
$PSM_{7.5}$	11.32	0.48^{b}	0.64	68.36	81.01	86.57
PSM_{10}	11.81	0.37^{ab}	0.62	68.15	80.42	86.15
Contrast analysis						
***P* values**						
Overall	0.127	0.047	0.393	0.834	0.769	0.923
Linear	0.832	0.023	0.679	0.423	0.319	0.514
Quadratic	0.225	0.031	0.715	0.874	0.937	0.973

Data expressed as Mean ($n=3$), Mean values in the same column with different superscript differ significantly ($P<0.05$)

[1] C_S, 0 g kg^{-1} PSM (*Prosopis cineraria* seed meal); PSM_5, 50 g kg^{-1} PSM; $PSM_{7.5}$, 75 g kg^{-1} PSM; PSM_{10}, 100 g kg^{-1} PSM

[2] Amylase activity is expressed as micromole of maltose released/min/mg protein

[3] Protease activity is expressed as micromole of tyrosine released/min/ mg protein

[4] Lipase activity is expressed as unit/mg protein

[5] ADC, Apparent digestibility coefficient;

[6] ADCP, Apparent digestibility coefficient of crude protein; [6]ADCL, Apparent digestibility coefficient of crude lipid/ether extract

Table 6 Protein and carbohydrate metabolic enzyme activities of *L. rohita* fingerlings fed with different experimental diets with PSM for 60 days

[1]Treatments	[2]AST		[3]ALT		[4]LDH		[5]MDH	
	Liver	**Muscle**	**Liver**	**Muscle**	**Liver**	**Muscle**	**Liver**	**Muscle**
Cs	4.01^{a}	11.81^{a}	4.96	2.94^{b}	4.34^{a}	0.82	0.86	2.34^{a}
PSM_5	4.07^{a}	14.59^{b}	4.84	3.22^{b}	3.72^{a}	1.01	0.86	2.63^{a}
$PSM_{7.5}$	4.89^{b}	15.42^{b}	4.99	1.90^{a}	4.47^{a}	0.96	1.01	3.20^{b}
PSM_{10}	5.43^{b}	14.49^{b}	4.91	1.64^{a}	5.69^{b}	1.03	1.09	3.37^{b}
Contrast analysis								
***P* values**								
Overall	0.004	0.011	0.965	< 0.001	0.006	0.302	0.175	0.004
Linear	0.013	0.024	0.893	< 0.001	0.015	0.854	0.902	0.023
Quadratic	0.009	0.015	0.349	0.006	0.011	0.278	0.206	0.009

Data expressed as Mean ($n=3$); Mean values in the same column with different superscript differ significantly ($P<0.05$)

[1] C_S, 0 g kg^{-1} PSM (*Prosopis cineraria* seed meal); PSM_5, 50 g kg^{-1} PSM; $PSM_{7.5}$, 75 g kg^{-1} PSM; PSM_{10}, 100 g kg^{-1} PSM

[2] AST, Aspartate aminotransferase specific activity is expressed as nano moles of oxaloacetate released/min/mg protein at 37^0C

[3] ALT, Alanine aminotransferase specific activity is expressed as nano moles of sodium pyruvate formed/min/mg protein at 37^0C

[4] LDH, Lactate dehydrogenase specific activity is expressed as units/ min/mg protein at 37^0C

[5] MDH, Malate dehydrogenase specific activity is expressed as units/ min/ mg protein at 37^0C

hemagglutinins, which can negatively impact digestion, nutrient utilization, and growth performance in animals [32]. Notably, the pods and seeds of *P. cineraria* are recognized for their excellent nutritional profile and have been studied as beneficial dietary supplements and an ingredient for enhancing the growth of various animals, including sheep, rabbits, poultry birds, and fish, in both Asia and Africa [53, 54, 102, 104].

With the intensification of aquaculture practices focused on enhancing food security, there is mounting pressure to find unconventional feed ingredients, including agro-industrial waste [6, 45, 63, 76]. Plant-based meals from leaves, pods, and seeds are emerging as viable alternatives that do not compete with other livestock feed resources. Previous studies underscored the potential of plant-based meals as alternative ingredients for aquafeed, particularly for *L. rohita* [47, 59, 65, 74, 82]. In light of the challenges arising from the shortage and competition for conventional animal feeds, this study aims to explore the potential of partially replacing traditional feeds with

Table 7 Antioxidant enzyme activities of *L. rohita* fingerlings fed with different experimental diets with PSM for 60 days

[1]Treatments	[2]SOD		[3]CAT	
	Liver	Gill	Liver	Gill
Cs	10.22	7.87	0.57	1.99
PSM_5	10.05	7.48	0.63	2.31
$PSM_{7.5}$	9.16	8.37	0.70	1.87
PSM_{10}	8.82	8.40	0.59	1.78
Contrast analysis				
***P* values**				
Overall	0.054	0.343	0.183	0.096
Linear	0.879	0.902	0.287	0.822
Quadratic	0.347	0.454	0.782	0.348

Data expressed as Mean ($n=3$); Mean values in the same column with different superscript differ significantly ($P<0.05$)

[1] C_S, 0 g kg^{-1} PSM (*Prosopis cineraria* seed meal); PSM_5, 50 g kg^{-1} PSM; $PSM_{7.5}$, 75 g kg^{-1} PSM; PSM_{10}, 100 g kg^{-1} PSM

[2] SOD, Superoxide dismutase activity is expressed as 50% inhibition of epinephrine auto-oxidation/min/mg protein

[3] CAT, Catalase activity is expressed as nanomoles of H_2O_2 decomposed/min/mg protein

unconventional alternatives. Given the challenges posed by the scarcity and competition for conventional animal feeds, the present study was undertaken to investigate the partial replacement of traditional feeds with unconventional alternatives.

The proximate composition and chemical analysis of the PSM revealed that PSM contains ANF trypsin inhibitor, tannin, phytates, saponin, alkaloids, and hydrogen cyanide, which are in agreement with the findings reported by Yadav et al. [102]. The observed ANF values in this study were within the acceptable range for the fish, as described by Francis et al. [32]. ANF contents in PSM were significantly lowered compared to GNOC except for tannin content. Consequently, no additional treatments were implemented to reduce the ANF content in the seed meal. The amino acid profile, digestibility and low ANF are the favorable indicators for the plant protein. In this study, PSM comprised high arginine, leucine & lysine contents among EAA and low methionine levels. Meanwhile, glutamate, aspartate, and hydroxyproline contents were highest in PSM among NEAA. It has been observed that proximate, ANF and amino acid composition of PSM showed variations with previous studies [7, 73]. These differences in the chemical composition of PSM could be attributed to different factors such as variations in soil properties, environmental temperatures, seed ripening stages, and climatic conditions [73]. Furthermore, the amino acid composition of PSM closely resembled values reported by other researchers for seed meal of *P. cineraria* and other congeneric species like *P. juliflora and P. Africana* [2, 5, 11, 22, 98, 103, 104]. Similarly, discrepancies in proximate composition and amino acid profiles of plant proteins were dependent on plant species, agronomic practices, environmental conditions, and protein content [40, 88, 97].

The biological evaluation of plant proteins is assessed through the determination of growth indices under an in vivo feeding trial. In this experiment, dietary PSM inclusion did not significantly affect the growth performance and nutrient utilization parameters in rohu fingerlings. This suggests that the growth rates, feed conversion, and protein retention remained consistent regardless of PSM inclusion. It infers that *L. rohita* fingerlings well-utilized dietary PSM and can be included @ 10% in rohu diet. Rohu fingerlings accepted experimental

Table 8 Serum biochemical indices of *L. rohita* fingerlings fed with different experimental diets with PSM for 60 days

[1]Treatments	Glucose (mg/dL)	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	[2]A: G	[3]NBT
Cs	69.71	3.12	1.62	1.49	1.09	1.14
PSM_5	68.3	3.16	1.57	1.59	0.99	0.98
$PSM_{7.5}$	74.02	3.07	1.55	1.51	1.03	1.04
PSM_{10}	64.25	3.14	1.53	1.62	0.95	1.07
Contrast analysis						
***P* values**						
Overall	0.191	0.654	0.319	0.327	0.222	0.669
Linear	0.767	0.921	0.898	0.919	0.886	0.926
Quadratic	0.301	0.245	0.233	0.187	0.434	0.262

Data expressed as Mean ($n=3$); Mean values in the same column with different superscript differ significantly ($P<0.05$)

[1] C_S, 0 g kg^{-1} PSM (*Prosopis cineraria* seed meal); PSM_5, 50 g kg^{-1} PSM; $PSM_{7.5}$, 75 g kg^{-1} PSM; PSM_{10}, 100 g kg^{-1} PSM

[2] A: G, Albumin to globulin ratio

[3] *NBT*, Nitrobluetetrazolium activity (value) is expressed as OD_{620} nm

Table 9 Haematological indices of *L. rohita* fingerlings fed with different experimental diets with PSM for 60 days

[1]Treatments	[2]Hb (g/dL)	[3]RBC count ($\times 10^6$/mm^3)	[4]PCV (%)	[5]MCV (fl)	[6]MCH (pg)	[7]MCHC (g/L)	[8]TLC count ($\times 10^3$/mm^3)
Cs	10.05	2.24	30.3	119.7	46.8	30.95	31.51
PSM_5	9.8	2.22	28.8	121.5	44.2	36.5	27.15
$PSM_{7.5}$	8.95	2.08	28.25	112.1	40.65	36.25	26.9
PSM_{10}	9.35	2.02	26.9	113.45	46.6	41.05	30.71
Contrast analysis							
***P* values**							
Overall	0.061	0.149	0.099	0.098	0.073	0.091	0.174
Linear	0.789	0.898	0.854	0.776	0.566	0.672	0.692
Quadratic	0.231	0.255	0.196	0.187	0.095	0.878	0.341

Data expressed as Mean ($n=3$), Mean values in the same column with different superscript differ significantly ($P<0.05$)

[1] C_S, 0 g kg^{-1} PSM (*Prosopis cineraria* seed meal); PSM_5, 50 g kg^{-1} PSM; $PSM_{7.5}$, 75 g kg^{-1} PSM; PSM_{10}, 100 g kg^{-1} PSM

[2] *Hb* Hemoglobin

[3] *RBC* Red blood cell

[4] *PCV* Packed cell volume

[5] *MCV* Mean corpuscular volume

[6] *MCH* Mean corpuscular haemoglobin

[7] *MCHC* Mean corpuscular hemoglobin concentration

[8] *TLC* Total leucocyte cell

diets well, and no difference was observed in feed intake. It might be associated with lower tannin contents of experimental diets. No differences in growth and protein retention can be explained as the lower concentrations of ANF present in the experimental diets did not produce any negative effects on protein accretion and nutrient utilization of rohu. Similarly, Yadav et al. [102] confirmed that *P. cineraria* seed meal serves as a viable protein source for rohu fingerlings. However, research on the use of *P. cineraria* seed meal in fish and other animal's diets is limited. Therefore, insights from related congeneric species, such as *P. juliflora* and *P. africana,* are relevant for contextualizing the results of this study. Previous studies have shown that including 20% mesquite meal (*P. juliflora*) in the diet enhances weight gain and feed conversion in Nile tilapia [84]. Likewise, studies on poultry showed that dietary inclusion of 10–20% *P. juliflora* meal exhibited growth performance comparable to control groups [40]. Conversely, Bhatt et al. [15] reported a decline in growth rates and feed conversion in *L. rohita* fingerlings fed with *P. juliflora* seed meal. The author stated that higher ANF content and nutrient deficiency in *P. juliflora* seed meal were responsible for lower growth.

Moreover, unconventional plant proteins comprise several ANF and, when consumed by fish or other animals can lead to inflammation in digestive organs and glands (liver) and alter the weights of vital organs. However, in this experiment, HSI and VSI values remained consistent among all groups, suggesting that the digestive physiology of the intestine was unaffected by dietary PSM inclusion. These results align with previous studies that investigated fermented sweet potato leaf meal as a substitute for de-oiled rice bran and pigeon pea (*Cajanus cajan*) leaf meal in the diet of *L. rohita* fingerlings [45, 74]. Additionally, the survival percentage did not differ between the PSM and control groups, indicating no toxicity associated with PSM. Similarly, the pullet chicks that were fed a diet containing *Prosopis africana* seed meal did not exhibit any mortality [11]. Conversely, poultry fed with seeds of *Prosopis laevigata* demonstrated significantly lower growth performance compared to the control group [8]. Other non-conventional plant protein sources, such as sesame seed meal and defatted *Jatropha curcas* kernel meal, did not cause any toxicity to monosex Nile tilapia (*O. niloticus*) juvenile and rohu, (*L. rohita)* fingerlings, respectively [27, 69]. Collectively, these findings suggest that PSM can be safely incorporated into fish diets without adverse effects on growth, nutrient utilization, or survival rates.

Previous studies have underscored the relationship between whole-body composition, growth performance, and digestive enzyme activities in fish [60]. In the present study, the inclusion of dietary PSM did not exert a significant impact on the carcass composition of *L. rohita* fingerlings, aligning with findings reported by Sena et al. [84] in Nile tilapia. Similarly, Gaber et al. [34] found no distinct variation in carcass composition when substituting corn meal with date stone in Nile tilapia fingerlings. Moreover, the incorporation of *P. juliflora* pods did not alter the carcass characteristics of rabbits, as indicated

by Adamu et al. [1]. Bohnenberger et al. [16] also documented that varying levels of cassava leaves protein concentrate did not influence the biochemical composition variables of Nile tilapia. Furthermore, the incorporation of fermented sweet potato leaf meal and mixed leaf meal likewise failed to modify the carcass composition of *L. rohita*, as reported by Jayant et al. [45] and Mondal et al. [59].

Assessment of digestive enzyme activities becomes an essential parameter to indicate the growth performance of an animal under in vivo feeding trials. These enzymes hydrolyze the complex nutrients from feed into simpler, absorbable forms, facilitating their absorption by the intestine [91]. Nowadays, incorporating plant-based protein ingredients into fish feed is a common practice. However, the inherent ANF in these plant-based proteins can negatively affect the physiology of fish species [20, 103]. These ANF may hinder the digestion process by inhibiting the activities of digestive enzymes or interacting with nutrients, thereby reducing their bioavailability [66]. In this feeding trial, fish that were fed diets containing 75 and 100 g kg^{-1} of dietary PSM exhibited significantly higher protease activity compared to other groups. However, there was no significant difference in protease activity in the group fed GNOC and PSM_5. It suggests that an increase in protease activity is related to lower trypsin inhibitor content in PSM compared to GNOC. Similar to these findings, protease activities have been identified as indicators of growth in Atlantic cod [52].

In contrast, amylase and lipase activities did not differ significantly, indicating that the experimental diets likely contained ANF within tolerable limits and did not impede the digestion process. These findings are consistent with those of other studies [9, 57, 75]. However, it's noteworthy that Bhatt et al. [15] reported negative effects on digestive enzyme activities when feeding *P. juliflora* meal to *L. rohita* fingerlings. The authors indicated that reported decreases in digestive enzyme activities were associated with higher ANF levels in treatment groups compared to control. This discrepancy may be attributed to differences in experimental conditions, such as the composition and processing of the PSM used, as well as variations in the developmental stages of *P. cineraria* and different species of the genus *Prosopis*. Apparent digestibility coefficients (ADC) are the indicator of the digestive capacity of an animal. Interestingly, ADC of dry matter and nutrients were not affected by the inclusion of dietary PSM, which might correlate with the insignificant digestive enzyme activities and protein accretion (growth) observed in rohu fingerlings.

Aminotransferase activities, specifically AST and ALT, were notably higher at inclusion levels of 75 and 100 g kg^{-1} PSM compared to the control and 50 g kg^{-1} PSM inclusion levels. Higher AST and ALT activities in muscle in PSM-fed groups might be explained as PSM-based diets typically contain lower amino acid contents than groundnut-based diets. Therefore, the efficiency of AST and ALT enzyme activities was enhanced to synthesize new nonessential amino acids for protein synthesis. Several researchers reported no variations in aminotransferase activities in fish when fed with plant proteins [23, 30]. Jiang et al. [48] observed a positive correlation between AST activity in hepato-pancreas and growth rates in common carp (*Cyprinus carpio).* The author highlighted that absorbed amino acids undergo transamination to produce new amino acids, which are required for protein accretion, which supports our findings of elevated AST activity in muscle tissue. Additionally, Deng et al. [24] reported no significant differences in aminotransferase activities between control and dietary treatments in tilapia fed with rubber seed meal. These findings collectively suggest that higher AST and ALT activities in the muscle of PSM-fed groups may represent a compensatory mechanism to meet the amino acid requirements for protein synthesis. Despite the lower amino acid content in PSM-based diets, the fish seem to adapt enzymatically to utilize available amino acids for growth and muscle development efficiently.

It has been observed that toxins or ANF in plant-proteins-based ingredients negatively influenced the antioxidative system in fish [67]. Higher dietary ANF or imbalanced dietary nutrients can enhance the production of more oxygen free radicals as well as augment the oxidative enzyme system to capture the free radicals. In this study, SOD and CAT activities did not exhibit any significant differences between GNOC- and PSM-fed groups. It suggests that no oxidative-induced stress is induced even at higher inclusion levels. Similarly, insignificant SOD activity was observed in tilapia fed with rubber seed meal [23] and rohu fingerlings fed with rubber protein isolate [30] and jatropha protein isolate [31]. Conversely, SOD and CAT activities were enhanced in rohu fingerlings fed with fermented jatropha protein concentrate [86]. Phulia et al. [69] also found no considerable difference in SOD and CAT activities in rohu fingerlings fed with fermented Jatropha (*Jatropha curcas*) kernel meal. These findings collectively suggest that the inclusion of PSM in the diet did not elicit oxidative stress in *L. rohita* fingerlings, as evidenced by the consistent activities of SOD and CAT.

When sufficient oxygen is available, pyruvate enters the Krebs cycle. However, in cases of tissue oxygen debt, pyruvate is converted to LDH, accompanied by the conversion of NADH to NAD^+, to maintain the tissue's redox potential [61]. Thus, LDH plays a crucial role in sustaining the glycolysis cycle by providing NAD^+. The higher hepatic LDH activity observed at 10% inclusion of PSM

in fish may be associated with a higher concentration of anti-nutritional factors (ANFs) and/or nutrient imbalance. These results align with the findings of Shamna et al. [85] when *L. rohita* fingerlings were fed with jatropha (*Jatropha curcas*) protein isolate, and a similar trend was observed when fed with fermented jatropha protein isolate. Higher MDH activity in muscle may be correlated with higher AST activity in the liver (r = 0.96) and muscle (r = 0.77) for the production of oxaloacetate to synthesize new nonessential amino acids or for energy production through gluconeogenesis. However, growth and other stress enzymes did not vary with higher inclusion of dietary PSM, indicating no observed stress condition in rohu in response to PSM inclusion. Similarly, Anand et al. [9] noted elevated hepatic LDH and MDH activity in common carp, *Cyprinus carpio*, when fed with sesbania (*Sesbania aculeata*) leaf meal, while Phulia et al. [69] found no significant difference in LDH and MDH when fed with fermented Jatropha kernel meal.

No differences were seen in the serum protein, glucose, and NBT activity of rohu fingerlings fed with dietary PSM inclusion. It infers that fish are in good health condition and dietary PSM does not elicit any detrimental effect on fish health. Similarly, total protein and albumin content were in agreement with similar nutrient utilization in all fed groups. It suggests that there was no impaired protein metabolism in the liver. Nevertheless, the globulin concentration in rohu fingerlings did not vary with dietary PSM levels, which signifies that the dietary PSM does not suppress the immune system. Similarly, no differences were observed in haemato-biochemical parameters of rohu, *L. rohita* fingerlings fed with detoxified *Jatropha curcas* protein isolate and rubber (*Ficus elastica*) protein isolate [29, 51, 99].

The respiratory burst activity (NBT) forms the basis of a highly potent antibacterial system, which means an increased NBT can be correlated with increased activity of phagocytic cells and better immunity [10]. A significant reduction in NBT was recorded in mirror carp (*Cyprinus carpio*) fed earthworm meal-based diets compared to the control and SBM-fed groups [77]. In this study, NBT did not significantly differ among the dietary treatments and control groups. This suggests that the innate immune system of rohu fed with PSM-based diets was not altered or compromised, and the reason may be due to the low level of ANFs present in the diets. Furthermore, feeding dietary PSM to rohu fingerlings did not alter the serum glucose concentration across the various treatment groups, which is consistent with the findings of Slawski et al. [90] and Fawole et al. [31]. Authors reported that feeding plant proteins to fishes did not influence the haemato-biochemical parameters [90] and Fawole et al. [31].

Haematological indices serve as valuable indicators of animal health status [4, 50]. Contrast analysis revealed that haematological parameters were not significantly affected by dietary PSM inclusion. This suggests that the erythropoiesis processes were not affected after feeding PSM to rohu, which can be attributed to the low level of ANFs present in the diets. Also, Slawski et al. [90] recorded no variation in the blood haematocrit and haemoglobin concentrations in rainbow trout fed with rapeseed protein concentrate as a replacement for fishmeal. The authors stressed that the similarities observed in the blood Hb, Hct and plasma metabolites among the various groups indicate equal nutritional status. These results are consistent with the findings reported in rohu fingerlings fed with rubber protein isolate [29]. However, in contrast, Soltan et al. [93] found that replacing fishmeal protein with a mixture of plant proteins in tilapia diets resulted in a decrease in haematocrit value. Ahmad [3] reported that haematological parameters, including RBC count, Hb concentration, and WBC count, were not affected by dietary sweet potato leaf meal in rohu fingerlings.

Conclusion

The current study demonstrates that incorporating *P. cineraria* seed meal (PSM) at a level of 100 g kg^{-1} can effectively substitute groundnut oil cake (GNOC) in the diet of *L. rohita* fingerlings without adversely affecting growth performance, nutrient utilization, digestive enzyme activities, or physio-metabolic responses. These findings support the feasibility of utilizing PSM as a sustainable alternative protein source in aquafeed formulations for *L. rohita* fingerlings. By replacing GNOC with PSM, aquafeed producers can reduce reliance on traditional protein sources while maintaining or even improving the nutritional quality and health status of farmed fish. Overall, the results of this study contribute to the development of environmentally friendly and economically viable aquafeed formulations that promote sustainable aquaculture practices. The results of the feeding trial revealed that 100 g kg^{-1} dietary inclusion of *P. cineraria* seed meal (PSM) is possible without encumbering the growth rate, nutrient utilization, digestive enzyme activities, or physio-metabolic responses in rohu, *L. rohita* fingerlings. Further research is warranted to investigate the detoxification of PSM using different strategies, long-term effects and optimal inclusion levels of PSM in *L. rohita* diets, as well as its potential application in other fish species and aquaculture systems.

Abbreviations

ALT	Alanine aminotransferase
ANF	Anti-nutritional factors
ANOVA	One-way analysis of variance
AST	Aspartate aminotransferase
CAT	Catalase

CP	Crude protein
DNS	Dinitro- salicylic acid
DORB	Deoiled rice bran
EDTA	Ethylene diamine tetra-acetic acid
EAA	Essential amino acids
FID	Flame ionization detector
FRP	Fibreglass reinforced plastics
GE	Gross Energy
GNOC	Groundnut oil cake
HCl	Hydrochloric acid
HPLC	High-performance liquid chromatography
LDH	Lactate dehydrogenase
MDH	Malate dehydrogenase
MJ	Millijoule
NEEA	Nonessential amino acids
PSM	*Prosopis cineraria* Seed meal
SOD	Superoxide dismutase

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Authors' contributions
Rohitash Yadav; Methodology, Formal analysis, Writing – original draft. Manish Jayant; Statistical analysis, Writing-review and editing. Prasanta Jana; Statistical analysis, Writing-review and editing. Nitesh Kumar Yadav; Writing-review and editing. Narinder K. Chadha; Conceptualization, Supervision, Validation, Writing-review and editing. Ved P. Saini; Supervision, Formal analysis, writing–review and editing. Paramita B. Sawant; Writing-review and editing. Manohar L. Ojha; Writing-review and editing.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The ethical procedures for the Animal Care as guided by the Ethical Committee of ICAR-CIFE, Mumbai, India was strictly adhered to conduct the current study.

Competing interests
The authors declare no competing interests.

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