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Improvement in Performance of Rohu (*Labeo rohita*) Fingerlings Fed with Isocaloric and Isonitrogonus Pumpkin Seed Meal Based Diet

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MU conducted the feeding trial and prepare the manuscript. MMS planned and supervised all material for research. SMH helped in manuscript preparing. SD helped in writing the manuscript. DH and WA helped in review and editing the manuscript.

Key words
***Labeo rohita*, Pumpkin seed meal, Fish meal (FM), Growth rate, Hematological profile**

ABSTRACT

Pumpkin seeds (*Cucurbita pepo*) and their derivatives have certain beneficial effects for farmed fish. Because of its great adaptability in diet, it is being investigated as a valuable option for commercial aquaculture feed in Asia. A trial study was performed to check the effects of pumpkin seed meal on rohu (*Labeo rohita*) fingerlings. This study aimed to investigate the effects of replacing the diet of *L. rohita* with fish meal (FM) and pumpkin seed meal (PSM) on the growth rate, carcass composition, and hematological profile. Six isonitrogenous and isocaloric meals (PSM-0%, PSM-15%, PSM-30%, PSM-45%, PSM 60%, and PSM-75%) were used in the experiment. Fingerlings of *L. rohita* were divided into three groups and fed for 80 days with PSM-based diets at 4% of their body weight. The group fed with a 45% PSM-based diet shows the maximum growth performance showing 21.23g weight gain, 383% weight gain %, 1.75 SGR and 98% survival rate. The fish fingerlings fed with the 45% PSM showed 3.12×10^6mm^3 RBCs, 67.75 PLT, 8.92 g/100ml haemoglobin, 31.03% packed cell volume. This study shows that 45% PSM when replaced with FM had a beneficial impact on the growth, carcass composition and hematological parameters in *L. rohita* fingerlings.

INTRODUCTION

As a result of current trends, experts predict that the global population will reach between 7.3 and 11.2 billion by 2100 (Kasun, 2018). Countries in Asia and Africa are mainly home to expanding nations, with populations exceeding 75% (Singh *et al*., 2019). The expanding global population and variables such as evolving socioeconomic factors will exert additional pressure on the world's resources to meet the need for larger quantities and diverse varieties of food (Henchion *et al*., 2017). Aquatic foods exhibit significant diversity, with more than 3500 species and taxonomic groups. They are generated under numerous conditions, including wild catch fisheries and aquaculture, in marine and inland environments, and through small to industrial-scale production (Golden *et al*., 2021).

Aquaculture is the most rapidly expanding sector in the world for providing food. It accounts for approximately 50% of the worldwide consumption of edible fish (Bostock *et al*., 2010). Currently, around 50% of the demand for edible fish is fulfilled through aquaculture. By 2030, this percentage is anticipated to increase to 60-70% (Subasinghe *et al*., 2009). Presently, customer demand for safe and high-quality farmed fish is rising, prompting researchers to enhance efforts in developing safe fish feed additives and supplements to replace conventionally utilized synthetic hormones and chemotherapy treatments (Dawood *et al*., 2018).

Traditionally, fish meal and fish oil have been crucial components of the formulated diet for carnivorous and omnivorous species in aquaculture. Fish meal (FM) is valuable for two significant reasons. First, it has a favorable amino acid profile for most aquatic species. Second, it is highly palatable, making the final feed appealing and

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encouraging feed intake (Jackson, 2006). However, the cost of formulating fish feed is the aquaculture industry's biggest obstacle (Sarfaraz *et al*., 2020).

Plant sources of proteins have become highly valued by aquaculture producers and nutritionists due to their accessibility, environmentally benign nature, sustainable production, environmental safety, and comparatively reduced costs compared to FM protein (Abdel *et al*., 2022). Plant based diets fulfill the scarcity of FM (Sarfaraz *et al*., 2020). Pumpkin seed cake (PSC) is a residual product of the pumpkin seed oil extraction processing sector and is classified as an agro-industrial residue. Pumpkin seed meal (PSM) is obtained through the mechanical extraction of seeds using a continuous screw press. PSM is an additional byproduct that can be utilized as a feed additive (Rabrenović *et al*., 2014).

PSM has a high protein content, roughly 40% crude protein, and significant amounts of unsaturated fatty acids. The pumpkin, scientifically known as *Cucurbita*, is a member of the Cucurbitaceae family. In Pakistan, pumpkin seeds are discarded as garbage after being utilized for cultivation. The current experimental study aimed to investigate the impact of the locally accessible protein source, pumpkin seed meal, on the overall performance of *Labeo rohita* fingerlings. The recent study was conducted to determine the cost-effective feed and its optimum level as a protein substitute for *L. rohita*. It also determines the effect of PSM on growth performance, hematological indices, and carcass composition of *L. rohita* fingerlings.

MATERIALS AND METHODS

The experiment was conducted in the Animal Research Station, Zoology Department, Division of Science and Technology (DSNT), University of Education Township Campus, Lahore.

Fish and conditions for experiment

L. rohita fingerlings were obtained from Mananwan Fish Hatchery in Lahore, Punjab, Pakistan. Prior to the experiments, the fingerlings were acclimated to the experimental conditions for a duration of fifteen days (Allan *et al*., 2000). The fish were housed in uniquely designed ponds. The fingerlings were provided a baseline diet once daily throughout this adaptation phase. Same experimental design was followed as explained by Shahzad *et al*. (2025).

Formation of feed pellets

After the extraction of pumpkin seed oil from pumpkin seeds, a dried de-fatted cake was produced, which was procured from a local market in Lahore. Table I showed all the ingredients with specific ratio utilized in the formation of all test diets. It was pulverized using a pestle and mortar into a fine powder to facilitate passage through a 0.3mm mesh sieve. Feed preparation was done by following method as explained by Shahzad *et al*. (2025).

Experimental design

Six experimental diets were formulated using PSM as the test ingredient. A control diet (lacking PSM) and five PSM based diets (incorporating PSM levels of 15%, 30%, 45%, 60%, and 75%) were administered to 15 fingerlings in each of three replicate water tanks at 4% of their live wet body weight for 80 days. The uneaten diet was removed

Table I. Ingredients composition (%) of test diets (TD) for *L. rohita* fingerlings.

Ingredients	TD I (Control)	TD II (15%)	TD III (30%)	TD IV (45%)	TD V (60%)	TD VI (75%)
Pumpkin	0	5.7	11.4	17.1	22.8	28.5
Fish meal	38	32.3	26.6	20.9	15.2	9.5
Rice polish	14	13	12	11	10	9
Wheat bran	19	15.75	14.5	13.25	10	6.75
Corn gluten 30%	0	6	10	14	20	26
Maize flour	20	18	16	14	12	10
Fish Oil	5	5.25	5.5	5.75	6	6.25
Vitamin*/ mineral premix**	2	2	2	2	2	2
Ascorbic acid	1	1	1	1	1	1
Chromic acid	1	1	1	1	1	1

* Vit. D3: 3,000,000 IU, Vit. A: 15,000,000 IU Vit. C: 15,000 mg, Vit. B6: 4000 mg, Vit. E:30000 IU. Vit. B2: 7000 mg Vit. B12: 40 mg. Folic acid: 1500 mg, Vit. K3: 8000 mg Ca pantothenate: 12,000 mg, Nicotinic acid: 60,000 mg. ** Mg: 55 g , Ca: 155 g, Se: 3 mg, Na: 45 g P: 135 g Cu: 600 mg, Mn: 2000 mg, Co: 40 mg, Fe: 1000 mg Zn:3000 mg I: 40 mg. CP, crude protein; CF, crude fat; GE (kcal/g), Gross energy (kcal/g)

from each tank following a 40-min feeding session. The tanks were meticulously cleaned to eliminate any residual feed particles prior to being filled with water. Subsequently, feces were accurately collected by opening valves I and II on the fecal collection tube to prevent fecal disruption. Fecal samples were desiccated in an oven at 65°C for 3-4 h and subsequently stored at room temperature for chemical analysis. To assess growth efficiency parameters, hematology, and proximate body composition in *L. rohita* fingerlings, all test diets containing PSM were compared with one another and with a control group (without pumpkin seed meal) utilizing a complete randomized design (CRD). Three fingerlings from each tank were selected for blood sample collection after 80 days of the experiment and were sedated with a 150 mg/mL methane sulfonate solution. Blood samples were obtained using a heparinized syringe from the caudal vein of anesthetized fish and thereafter dispatched to the laboratory for the assessment of hematological indices.

Growth study

Fingerlings with an average weight of 7.97 g were introduced into each tank. To assess the growth performance of *L. rohita* fingerlings biweekly, the fish were weighed over the entire experimental period. The growth parameters, including feed conversion ratio (FCR), specific growth rate (SGR), and percentage weight increase of *L. rohita* fingerlings, were assessed. Standard formulas were used to evaluate the growth performance of fingerlings as mentioned in our previous paper (Shahzad *et al*., 2021).

Chemical analysis of body composition

The moisture content of the meals, feces, and corpse was determined by oven drying for 12 h at 105°C. The content of crude protein was assessed utilizing a micro Kjeldahl apparatus, whereas crude fat was quantified by the Soxhlet extraction method (Shahzad *et al*., 2025). Crude fiber was evaluated by measuring the loss on ignition of dried lipid-free residues following digestion with 1.25% H_2SO_4 and 1.25% NaOH (Sana *et al*., 2024). The ash was measured in the electric furnace at 650°C for 12 h with consistent heating (Eyela-TMF 3100). An oxygen bomb calorimeter was employed to assess the sample's gross energy (Shahzad *et al*., 2025). The following formula was utilized to ascertain total carbohydrates.

Total carbohydrate (%) = 100 - (protein% + moisture% + fat% + Ash % + crude fiber %)

Analysis of hematological parameters

The micro-hematocrit technique was employed for hematocrit analysis. A designated chamber for Neubauer hemocytometer counting was calibrated for red blood cells (RBCs) and white blood cells (Blaxhall and Daisley, 1973). The hemoglobin estimation was done as per method by Wedemeyer (1977). The formula employed to calculate mean cell volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) as explained by Sana *et al*. (2024).

Apparent digestibility constant (ADC)

A standard formula is used to test diets nutrient digestibility coefficients (%) (NRC, 1993).

$$\mathrm{ADC}(\%) = 100 - 100\,\frac{\%\text{ marker in diet } \times \%\text{ neutrients in feces}}{\%\text{ marker in feces } \times \%\text{ nutrient in diet}}$$

Statistical analysis

The test diets growth statistics, carcass composition, and hematological indices were analyzed using a one-way analysis of variance. Duncan test was employed to evaluate the differences among treatments at a significance threshold of $P<0.05$. The SPSS software was utilized for statistical analysis.

RESULTS

Effect on growth

Table II shows the overall growth parameters, i.e., weight gain %, FCR, SGR, feed intake, and weight gain (g) of the *L. rohita* fingerlings fed the PSM-based test diets. Results showed that fish fed with the PSM-based diet gained more weight than the control group. The initial weight of the fingerlings was statistically similar for all of the test diets in the experiment. All the growth parameters of the fingerlings started to increase when fed with the 0% PSM-based diet, and the growth parameters reached the maximum rate when fingerlings fed with the 45% PSM-based diet. Also, there were higher levels of PSM-based diets (60% and 75%), but there was no such significant increase in the growth parameters at these levels. It is clear that for the *L. rohita* fingerlings, 45% level of the PSM showed the best growth performance compared to the control group (having no replacement of the fish meal) and all of the test diets.

Carcass composition analysis

Carcass composition of fish fed with PSM is represented in the Table III. PSM based diets enhance the absorption of various nutrients in *L. rohita* fingerlings. Highest contents of the crude protein (19.12%) and gross energy (2.78 %) were found in the fingerlings of group 4 (test diet III) fed with the 45% replacement of fish meal with the PSM. These value found to be significantly different ($P < 0.05$) from the control group values (containing no PSM)

Table II. Effect of replacing the fish meal with the Pumpkin seed meal on growth parameters of the *L. rohita* fingerlings.

Levels	TD I (0%)	TD II (15%)	TD III (30%)	TD IV (45%)	TD V (60%)	TD VI (75%)	Std. error	Polynomial orthogonal contrast		
								Com-bined	Linear	Quad
Initial weight (g)	5.56±0.19	5.58±0.27	5.59±0.11	5.55±0.20	5.57±0.17	5.54±0.19	0.03805	1	0.843	0.872
Final weight (g)	19.51±0.97^{d}	21.67±0.94^{c}	24.48±0.96^{b}	26.78±1.00^{a}	24.33±0.80^{b}	22.32±0.74^{c}	0.59161	0.000***	0.000***	0.000***
Weight gain (g)	13.95±1.09^{d}	16.09±1.12^{c}	18.89±0.85^{b}	21.23±0.82^{a}	18.76±0.90^{b}	16.78±0.93^{c}	0.59543	0.000***	0.000***	0.000***
Initial wt*100	251.25±26.63^{d}	289.21±31.41^{bc}	338.08±8.92^{b}	382.77±7.71^{a}	337.17±23.89^{b}	303.43±26.75^{bc}	11.0871	0.000***	0.002**	0.001**
Wt gain/70	0.20±0.02^{d}	0.23±0.02^{c}	0.27±0.01^{b}	0.30±0.01^{a}	0.27±0.01^{b}	0.24±0.01^{c}	0.00827	0.000***	0.000***	0.000***
Feed intake (g)	0.31±0.01^{d}	0.34±0.02^{cd}	0.36±0.02^{abc}	0.39±0.00^{a}	0.37±0.01^{ab}	0.35±0.02^{bc}	0.00658	0.002**	0.001**	0.001**
FCR= Feed intake/ Wt gain	1.56±0.05^{d}	1.49±0.03^{d}	1.34±0.06^{ab}	1.28±0.06^{a}	1.39±0.02^{bc}	1.48±0.05^{cd}	0.02509	0.000***	0.006**	0.000***
SGR	1.39±0.08^{d}	1.51±0.09^{cd}	1.64±0.02^{ab}	1.75±0.02^{a}	1.64±0.06^{ab}	1.55±0.07^{bc}	0.03011	0.000***	0.002**	0.000***
Survival rate (%)	94.00±5.89	95.83±3.61	98.04±3.40	98.04±3.40	96.08±3.40	91.79±3.72	0.95645	0.411	0.604	0.045
Length (cm)	13.32±0.42^{ab}	13.64±0.47^{a}	13.24±0.25^{ab}	12.45±0.24^{c}	12.96±0.28^{bc}	13.87±0.28^{a}	0.12979	0.003**	0.952	0.003
Conditional factor	0.83±0.04^{c}	0.86±0.09^{c}	1.05±0.02^{b}	1.39±0.10^{a}	1.12±0.03^{b}	0.84±0.03^{c}	0.05053	0.000***	0.002***	0.000***

Means within columns with various superscripts (a-d) differ considerably at P<0.05Data are three replicates mean (± symbol shows standard deviations).

Table III. Effect of replacing the fish meal with the pumpkin seed meal on carcass composition of the *L. rohita* fingerlings.

	TD I	TD II	TD III	TD IV	TD V	TD VI	Std error	Polynomial orthogonal contrast		
								Com-bined	Linear	Quad
Protein (%)	13.96±0.36^{d}	14.91±0.26^{c}	17.24±0.33^{b}	19.12±0.47^{a}	16.86±0.39^{b}	14.60±0.72^{cd}	0.443	0***	0***	0***
Fat (%)	9.01±0.^{50a}	8.74±0.33^{ab}	8.12±0.56^{bc}	7.79±0.28^{c}	7.36±0.59^{c}	8.16±0.43^{abc}	0.16221	0.009**	0.002**	0.027*
Gross energy (Kcak/g)	1.15±0.12^{e}	1.38±0.12^{de}	2.09±0.16^{b}	2.78±0.18^{a}	1.89±0.18^{bc}	1.68±0.27^{cd}	0.13248	0***	0***	0***
Ash (%)	6.07±0.22^{a}	5.71±0.37^{ab}	5.39±0.41^{b}	5.15±0.26^{b}	5.68±0.28^{ab}	5.56±0.32^{ab}	0.09272	0.059*	0.082*	0.018*
Crude fiber (%)	1.23±0.07^{abc}	1.23±0.08^{ab}	1.06±0.12^{bc}	1.05±0.11^{c}	1.15±0.10^{abc}	1.25±0.07^{a}	0.02697	0.067*	0.793	0.008**
Carbohydrates (%)	2.78±0.25	2.72±0.36	2.53±0.20	2.37±0.19	2.69±0.32	2.83±0.29	0.0666	0.374	0.988	0.059*
Moisture (%)	65.80±0.54^{a}	65.31±0.62^{a}	63.56±1.12^{b}	61.74±0.51^{c}	64.37±0.93^{ab}	65.92±1.48^{a}	0.40029	0.001**	0.387	0***

a-d Means within columns with dissimilar superscripts differ quietly at P <0.05. The data are the means of three replicates of fifteen fingerlings each. (±shows standard deviations).

and also from the other groups fed with the different levels of PSM based test diets. The maximum value of fat (9.01%), ash (6.07%) and moisture (65.80%) were observed in the control group. The maximum value of crude fiber (1.23%) was observed in the group 2. This value found to be similar with group 1 and significantly different with group 4. The maximum value of carbohydrate (2.23%) found in the group 6.

Hematological indices

Table IV shows the hematological indices of *L. rohita* fingerlings fed with the PSM-based diet. It is clear from the recorded values of hematological indices that fish fingerlings fed with PSM based diet have significant difference from the control group. The results showed that RBCs (3.12×10^{6} mm^{-3}), PLT (67.75), Hb (8.92 g/100mL), PCV (31.03%) were observed maximum in group 4

Table IV. Effect of replacing the fish meal with the pumpkin seed meal on the hematological parameters of *L. rohita* fingerlings.

	TD I	TD II	TD III	TD IV	TD V	TD VI	Std. error	Polynomial orthogonal contrast		
								Com-bined	Linear	Quad
RBC ($10^6 mm^{-3}$)	1.79±0.20^c	1.99±0.13^{bc}	2.89±0.12^a	3.12±0.15^a	2.26±0.24^b	2.24±0.22^b	0.12056	0***	0.003**	0***
WBC ($10^3 mm^{-3}$)	7.26±0.37^a	6.76±0.27^b	6.37±0.15^{bc}	6.33±0.07^c	6.41±0.20^{bc}	6.71±0.15^{bc}	0.08993	0.002**	0.004**	0***
PLT	61.33±0.94^c	66.23±0.86^a	67.34±0.97^a	67.75±0.67^a	64.34±0.75^b	61.20±0.91^c	0.66626	0***	0.179	0***
Hb(g/100mL)	5.90±0.30^c	6.44±0.38^c	8.05±0.44^b	8.92±0.28^a	7.56±0.44^b	6.32±0.33^c	0.26908	0***	0.004**	0***
PCV (%)	21.32±0.87^d	26.37±0.79^c	28.54±0.86^b	31.03±0.75^a	26.48±0.86^c	20.38±0.92^d	0.92783	0***	0.655	0***
MCHC (%)	27.71±1.92^b	24.41±0.71^c	28.20±0.71^{ab}	28.74±0.25^{ab}	28.60±2.57^{ab}	31.00±1.44^a	0.55856	0.005**	0.002**	0.131
MCH (pg)	33.22±2.89	32.36±2.10	27.91±2.04	28.60±0.55	33.87±5.53	28.51±4.25	0.88607	0.155	0.275	0.367
MCV (fl)	120.13±11.10^a	132.57±7.38^a	98.91±5.84^b	99.52±2.42^b	118.10±10.99^a	92.06±13.81^b	3.94143	0.001**	0.002**	0.745
Ht (%)	24.41±0.91^d	27.98±0.67^c	30.46±0.60^{ab}	31.75±0.91^a	29.58±0.94^b	27.56±0.78^c	0.59423	0***	0***	0***
Lymphocyte (%)	26.71±0.65^a	24.56±0.60^{bc}	23.51±0.80^{cd}	22.52±0.59^d	23.57±0.99^{cd}	25.32±0.58^b	0.36114	0***	0.008**	0***
Eosinophile (%)	1.87±0.09^a	1.60±0.12^{bc}	1.45±0.10^{cd}	1.26±0.12^d	1.49±0.12^{bc}	1.70±0.13^{ab}	0.05189	0***	0.026*	0***
Monocyte (%)	2.85±0.15^a	2.37±0.10^c	2.27±0.09^{cd}	1.96±0.15^d	2.46±0.19^{bc}	2.73±0.31^{ab}	0.07981	0.001**	0.468	0***
Neutrophile (%)	68.56±0.64^d	71.47±0.45^{bc}	72.77±0.76^b	74.26±0.87^a	72.48±0.95^b	70.25±0.63^c	0.46932	0***	0.003**	0***

PLT, platelets; Hb, hemoglobin; PCV, packed cell volume; Ht, hematocrit (± shows standard deviations). $^{a-d}$ Means within columns with dissimilar superscripts differ quietly at $P < 0.05$. The data are the means of three replicates of fifteen fingerlings each.

when fish fingerlings were fed with the test diet having 45% replacement of fish meal with the PSM. The highest value of WBCs ($6.33 \times 10^3 mm^{-3}$) found maximum in group 1 and significantly different in group 4. The maximum value of MCHC (31%) found maximum in group 6 followed by group 4. This maximum value of MCHC% is highly dissimilar from group 2. The maximum value of MCV observed in group 1 followed by the group 5. The maximum value of Ht (31.75%) observed maximum in group 4 followed by group 3. This value is significantly different from the group 1.

DISCUSSION

Effect of PSM based diet on growth performance

The current study is consistent with the findings of Sezgin and Aydin (2021), who reported that 66% of PSM-based diets have the highest values of FW, WG, WG%, SGR while replacing the dietary soya bean meal with PSM. A 63-day feeding trial evaluated the effects of replacing SBM with PSM as an alternative feed ingredient on growth performance, feed utilization, parameters, and the fatty acid profile of carp fingerlings. Four experimental diets were designed in 0% (control), 33% (PSC33), 66% (PSC66) and 100% (PSC100). An equivalent percentage of PSC protein replaced SBM protein. That study indicated that a PSM-based diet could be replaced with the dietary soya bean meal without any negative impact on the growth performance of *Cyprinus carpio* fingerlings. A 10-week feeding experiment was performed to determine the impacts of partial substitution of SBM with PSM in *Oreochromis niloticus* diets on water quality and growth rate. One hundred and fifty tilapia fish (average weight, 11.93 ± 0.17 g) were randomly allocated to five diets. The first diet (the basal diet) contained 420 g of SB per kg of feed. The remaining four diets, namely, D1, D2, D3, and D4, had SB partially replaced by PSM at 10%, 20%, 30%, and 40%, respectively. Mounes *et al*. (2024) discovered that PSM-based diets at a 40% level have significantly improved the specific growth indicators and the feed conversion ratio in *O. niloticus*.

Current findings are pretty different from Lovatto *et al*. (2017). The study aimed to determine the growth and activity of proteolytic digestive enzymes of silver catfish (*Rhamdia quelen*) fed by replacing increased fish meal in the diet with either phosphorylated protein concentrate or PSM. Five experimental diets were formulated with levels: 0 (control), 25 (25% PSM and 25% PPCPS) and 50% (50% PSM and 50% PPCPS). Who explained that the nutritional value of PSM could be increased by the chemical process called phosphorylation of pumpkin seeds. According to their findings, the 50% phosphorylated PSM level has increased silver catfish's growth performance.

Effect of PSM-based diet on carcass composition of L. rohita *fingerlings*

The maximum protein content (19%) and gross energy (2.78 Kcal/Kg) were found in group 4, fed with a 45% replacement of fish meal with a PSM-based diet. The maximum values of fat (9%), ash (6%), and moisture (65.80%) were observed in the control group when fed with fish meal. The maximum value of crude fiber (2.83%) and carbohydrates (2.83%) was observed in group 6, fed with 75% replacement of fish meal with PSM-based diet. According to Khattab *et al*. (2023) the body moisture content was decreased significantly when feeding on the experimental diet. The fish fed the control meal achieved higher crude protein content than the rest of the meals. It is clear from the results that there is an inverse relationship between the fish body moisture content and the protein content. Pumpkin seed is highly recommended to decrease the level of CHO in the blood owing to its high sterol content (Rabrenović *et al*., 2014).

Sezgin and Aydin (2021) reported that *C. carpio* muscle was not significantly affected by the replacement of dietary SBM with PSC, which was in agreement with our studies of the replacement of fish meal with PSM in *L. rohita* fingerlings. Mounes *et al*. (2024) reported that when *O. niloticus* was fed with pumpkin (*Cucurbita maxima*) seed meal at a high level, there was an increase in the ash and crude lipid content in fishes. These findings are quite different from current studies.

Effect of PSM-based diets on hematological indices of L. rohita *fingerlings*

The hematology profile can estimate the quality of fish diet. Fish health is particularly determined in by studying their blood profile. According to Fazio (2019), hematological indices are a powerful diagnostic tool for estimating fish health, nutrition, and other quality parameters. The present study reveals that *L. rohita* fingerlings showed significantly improved levels of RBCs (3.13×106 mm^{-3}), PLT (67.75), and hemoglobin (8.92 g/100mL) when fed a 45% replacement of fish meal with a PSM-based diet. Vakili *et al*. (2025) evaluated the effects of a PSM-based extract combined with diet on hematologic factors in rainbow trout (*Oncorhynchus mykiss*). Blood factors were measured on days 0, 30, and 60. The results showed that treatment group 2 with a PSM-based extract (4mg/Kg) showed a significant increase in hematological parameters such as RBC and PLT.

A study was conducted by Sezgin and Aydın (2021) The hematological and serum biochemical parameters of *C. carpio* after 63 days of feeding trial RBC, Hct, MCV, MCH, MCHC, MPV and PDW were not affected by dietary treatments ($P > 0.05$). However, Hb concentration with the PSC33 and PSC100 diets was significantly higher than that in fish fed the control diet ($P < 0.05$). Similarly, a study by Musthafa *et al*. (2017) in Oreochromis mossambicus demonstrated that a diet containing Cucurbita mixta seed meal at 4–6 g/kg^{-1} enhanced innate immunity and increased resistance against Aeromonas hydrophila infection. In sustainable aquaculture, enhancement of the immune function of fish by using medicinal plants with various therapeutic potential active substances is valuable in terms of increasing fish resistance to pathogens. Hematological parameters can vary according to rearing conditions, immunological status, and the composition of the basic diet, and they are reliable markers of fish's general health condition (Eslamloo *et al*., 2012).

CONCLUSION

This research concluded that replacing PSM with fish meal improved the growth performance and hematological indices of *L. rohita* fingerlings. It was also clear that 45% inclusion levels of PSM resulted in the best performance of fingerlings. So, partially substituting a Pumpkin meal for a Fish meal as a traditional source of protein in the diet of *L. rohita* fish is certainly beneficial and cost-effective.

DECLARATIONS

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Statement of conflict of interest

We confirm that there is no conflict of interest related to this publication. We affirm that the original copy has been perused and endorsed by all the authors listed there and that there is no one else left behind who fulfilled the criteria of authorship however is not listed. We further proclaim that the order of authors mentioned has been supported by us all.

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