EFFICACY OF WATERMELON SEED MEAL-BASED DIET ON GROWTH, DIGESTIBILITY, AND HEMATOLOGICAL INDICES OF ROHU (*Labeo rohita*) FINGERLINGS

M. M. Shahzad[1*], Z. Hussain[1], S. M. Hussain[2], R. Gull[1], M. Y. Zahoor[3], S. Amin[1] and M. S. Khan[1]

[1] Department of Zoology, Division of Science and Technology, University of Education, Township, Lahore, Pakistan
[2] Department of Zoology, Government College University Faisalabad, Faisalabad, Pakistan
[3]Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore, Pakistan.
[*]Corresponding Author's Email: drmudassarshahzad@gmail.com

ABSTRACT

Rising demand for fish products and the high cost of fish meal (FM) as well as soybean meal (SBM) in aquafeeds highlight the need for alternative protein sources. In Pakistan, watermelon is widely cultivated, and its seeds are commonly discarded. In this study, watermelon seeds were used to prepare fish feed. This experiment comprised six treatments, each with three replicate tanks, containing fifteen fish per tank. Test diets (TDI-TDVI) included different levels of watermelon seed meal (WMSM) as 0%, 20%, 40%, 60%, 80%, and 100%. Fingerlings with an initial average weight of 7.21 g were transferred to tanks with a 70 L water-holding capacity and were fed twice a day for 70 days. The fecal samples were obtained daily for chemical analysis. Fish fed with a 60% WMSM-based diet (TD-IV) achieved the maximum growth indices: final weight (29.27 g), weight gain (21.72 g), weight gain percentage (289%; increase from initial weight), specific growth rate (1.51), and survival rate (98%). However, nutrient digestibility varied: GE peaked at 40% WMSM, while CP and EE digestibility were highest at 60% WMSM. The highest hematological indices (RBC: $3.12\times106\ mm^{-3}$, Hb: 8.27 g/dL, PLT: 69.38×10^3 /µL, Hct: 36.13%) were observed at 60% WMSM, whereas PCV peaked at 40%. From these findings, it was concluded that WMSM can replace soybean meal with an optimal inclusion level between 40% and 60%, depending on the performance parameter considered, without compromising the health of fish.

Keywords: Watermelon seed meal, Soybean meal replacement, Growth performance, Nutrient digestibility, Hematological indices, *Labeo rohita*

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INTRODUCTION

The rapidly expanding global population is expected to elevate overall food demand by 35–56% by 2050, highlighting aquaculture as an important source of animal protein. As aquaculture grows to fulfill this demand, reliance on nutrient-rich fish meal in feed formulations has correspondingly risen to facilitate efficient growth and production (Muringai *et al.*, 2021; van Dijk *et al*., 2021). Although soybean meal is widely used as a plant-based substitute, its rising cost and anti-nutritional factors limit its effectiveness in feed formulations. Therefore, there is a need to explore alternative plant protein sources, such as watermelon seed meal (WMSM), to ensure sustainable and cost-effective aquafeed production (Hussain *et al*., 2024; Macusi *et al*., 2023).

Watermelon (*Citrullus lanatus*) is a tropical and semi-tropical crop grown all over the world and belongs to the Cucurbitaceae family (Niu *et al*., 2025). Watermelon seeds are a substantial source of protein, comparable to other oilseed proteins such as those present in soybeans (Nissar *et al*., 2025). Due to its substantial protein content, WMSM represents a promising plant-based protein ingredient for use in aquafeed formulations (Jimoh *et al.*, 2020a). *C. lanatus* is an oilseed that comprises 30.6 grams of crude protein, some minerals, lipids, and vitamins in addition to being high in 10 essential amino acids (Ombugadu *et al*., 2021; Ghosh *et al.*, 2021). Due to its high protein content, balanced amino acids, and cost-effectiveness, WMSM has been evaluated as an alternative protein source in several fish species. However, there is currently inadequate data on the ideal inclusion level in *L. rohita* fingerlings, notably in terms of growth, nutrient digestibility, and hematological responses (Jimoh *et al.*, 2020a). *Labeo rohita*, commonly known as rohu, is an omnivorous fish species found in rivers, lakes, or other slow waters across Asia (Ranjan *et al*., 2018). Rohu stands out as a major carp species and is widely farmed in Pakistan, and is a favorite among consumers because of its good flavor and rapid growth; it also has high demand in most tropical fish markets (Memon *et al*., 2025; Gupta *et al*., 2021).

This study was designed to analyze the effect of replacing soybean meal with WMSM with varying levels of watermelon seed meal (WMSM; 0–100%) in the diets of Rohu (*L. rohita*) fingerlings with respect to growth performance, feed utilization, nutrient digestibility, and hematological indices, to determine the optimal inclusion level of WMSM as an alternative protein source under local aquaculture conditions.

MATERIALS AND METHODS

Experimental design: The feeding trial was conducted in the Fish Nutrition Laboratory, University of Education, Township Campus, Lahore, Pakistan. Fingerlings with an initial average weight of 7.21 g were purchased from a local hatchery. Before the experiment, fingerlings were acclimatized to the experimental conditions for fifteen days and fed with a basal diet twice daily. Water quality metrics such as temperature (25–27°C), dissolved oxygen (5–6 ppm), and pH (6–8) were regularly monitored during the acclimatization period. Throughout the experimental period, the feeding trial was conducted under a natural photoperiod of approximately 13 h light and 11 h dark. For the experimental work, specially designed V-shaped tanks with a capacity of 70 L were used to allow efficient collection of fecal samples and minimize nutrient loss into the water. During the trial period, the fish were fed experimental diets twice a day. During the entire experiment, O_2 was supplied via an air pump connected to air stones/diffusers. Before the feeding trial started, fish were bathed with 0.5% NaCl solution for 60-120 seconds as a safe prophylactic treatment to reduce external pathogens and minimize stress.

Formulation of diet: Watermelons (*Citrullus lanatus)* were purchased from local watermelon merchants. A stainless-steel knife was used to cut the watermelons into smaller pieces. After separation from the pulp, the seeds were thoroughly washed with distilled water to remove any adhering residues and impurities. The cleaned seeds were then uniformly spread out on aluminum foil and sun-dried for five days (average daytime temperature ≈30–35 °C). After being dried, the seeds were ground into fine powder using an electric grinder and kept in sealed containers at 4°C until further use. Despite the variation in soybean meal proportions among diets, all experimental diets were formulated with WMSM replacing soybean meal (SBM) protein at 0, 20, 40, 60, 80, and 100% of the SBM protein contribution to be isonitrogenous and isoenergetic. With the increasing inclusion of WMSM, the proportions of soybean meal, corn gluten, maize flour, and fish meal were adjusted correspondingly to maintain consistent crude protein and gross energy levels across all diets. Minor increases in fish meal and corn gluten were made to balance essential amino acids and compensate for differences in protein quality among ingredients, while maize flour was adjusted to stabilize dietary energy. The corresponding actual inclusion levels of WMSM in the test diets were 0, 8, 16, 24, 32, and 40 g per 100 g of diet, as shown in Table 2.

The diets containing WMSM were fed to 6 treatments of fingerlings. Fifteen fish were kept in each tank, and triplicate tanks were used for each group. The fingerlings were fed five percent of their live body weight, and the trial period was seventy days. A completely randomized design (CRD) was used to assess the overall performance of fish by using WMSM in all test diets.

Table 1. Analysis of the composition of WMSM added to the diets

Parameter	**% (Dry Matter Basis)**
Moisture	9.96±0.9
Crude Protein	29.71±0.72
Crude Lipid	9.84±0.15
Ash	5.18±0.29
Crude Fiber	4.51±0.09
Nitrogen-Free Extract (NFE)	40.80±0.98
Gross Energy (kcal/kg)	5020±24

* *Data are means of 03 replicates (± SD).*

Table 2. Ingredients (g/100 g diet) composition of WMSM -based test diets formulated at graded levels of SBM protein replacement for rohu fingerlings

Ingredients	Test Diet-1 (0% Replacement of SBM)	Test Diet -II (20% Replacement of SBM)	Test Diet -III (40% Replacement of SBM)	Test Diet -IV (60% Replacement of SBM)	Test Diet -V (80% Replacement of SBM)	Test Diet -VI (100% Replacement of SBM)
WMSM	0	8	16	24	32	40
Soybean Meal	40	32	24	16	8	0
Fish meal	12	12.5	13	13.5	14	14.5
Wheat Bran	17.5	17.5	17.5	17.5	17.5	17.5
Maize Flour	5	4	3	2	1	0
Corn Gluten	16	16.5	17	17.5	18	18.5
Fish Oil	5.5	5.5	5.5	5.5	5.5	5.5
Vit-Min premix	2	2	2	2	2	2
Ascorbic acid	1	1	1	1	1	1
Chromic oxide	1	1	1	1	1	1

[*] Vitamin A: 15,000,000 IU; Vitamin D3: 3,000,000 IU; Vitamin E: 30,000 IU; Vitamin B2: 7,000 mg; Vitamin B6: 4,000 mg; Vitamin B12: 40 mg; Vitamin C: 15,000 mg; Folic acid: 1,500 mg; Calcium panto-thenate: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:2,000 mg; Co:40 mg; Fe:1,000 mg; Zn:3,000 mg; I:40 mg.

All diets had identical levels of fish oil, vitamins, minerals, ascorbic acid, and chromic oxide with the adjusted proportions of WMSM, SBM, FM, Maize flour, and corn gluten.

Table 3: Analyzed Nutrient Composition (% DM) of WMSM -based test diets

	Test Diet -I	Test Diet-II	Test Diet-III	Test Diet-IV	Test Diet-V	Test Diet-VI
Dry Matter	90.1 ± 0.2	90.3 ± 0.3	90.5 ± 0.1	90.4 ± 0.2	90.2 ± 0.3	90.6 ± 0.2
Protein in diet	32.19 ± 0.44	32.06 ± 0.76	32.20 ± 0.37	32.18 ± 0.41	32.20 ± 0.35	32.16 ± 0.49
Fat in diet	6.00 ± 0.18	5.99 ± 0.25	6.02 ± 0.32	6.00 ± 0.21	5.98 ± 0.30	5.99 ± 0.17
GE in diet	3.96 ± 0.14	3.97 ± 0.11	3.96 ± 0.16	3.96 ± 0.13	3.97 ± 0.11	3.96 ± 0.11
Ash	8.5 ± 0.3	8.7 ± 0.2	8.6 ± 0.4	8.4 ± 0.3	8.8 ± 0.2	8.9 ± 0.3
Crude Fiber	5.2 ± 0.2	5.5 ± 0.3	5.8 ± 0.2	6.1 ± 0.3	6.4 ± 0.2	6.7 ± 0.3
Nitrogen-Free Extract (NFE)	38.11	37.75	37.38	37.32	36.62	36.25
Calculated GE	3961 ± 14	3961 ± 11	3960 ± 16	3962 ± 14	3970 ± 11	3963 ± 11
Analyzed GE	3915 ± 22	3984 ± 18	3941 ± 25	3955 ± 20	3962± 19	3952 ± 21

Data are means of 03 replicates (± SD).

Feed Pellets Formation: The feed ingredients listed in Table 2 were obtained from a wholesale market and processed finely enough to pass through a 0.3 mm filter. In accordance with the feed formula, the other required amounts of components, such as vitamins, mineral premix, and chromic oxide, were all weighed and thoroughly mixed manually. Chromic oxide was included in the diets at 1% as an inert marker for digestibility determination. Fish oil was also added gradually when other feed components were being mixed. For dough formulation, 15 mL of distilled water was added, and mixed for another 5 minutes until a uniform dough was obtained. After that, the resulting dough was passed through a feed pelleting machine to formulate the feed pellets of 2 millimeters in diameter. The formulated pellets were dried for approximately 72 hours. After formulation, the six test diet pellets were kept at 4°C. Each of the six experimental diets was prepared using the same protocol.

Sample Collection and Feeding Protocol: The test diets were fed to *L. rohita* fingerlings twice daily. After the feeding period (120 min), surplus feed was drained from the tanks by opening the tank valves, and the tanks were gently rinsed with tap water to remove remaining feed particles. By using specially designed V-shaped tanks for rapid settling and collection of feces, fecal leaching was minimized. To minimize contamination of feces with uneaten feed, a settling period of 30 min was allowed before fecal collection. Feces were collected by opening valve I and valve II subsequently from the fecal collecting tube of each replicated tank using a settling column collection method, ensuring that only settled fecal material was obtained. Fecal material was collected carefully to prevent fecal breakage and to reduce the leaching of nutrients into water. Feces were dehydrated in an oven at 70°C before being stored for further chemical analysis.

Analysis of Hematological Parameters: After completing a 70-day trial, three fingerlings were sampled from each tank for hematological analysis. Fish were fasted for 12 hours before sampling to avoid post-prandial interference and sedated using Tricaine Methanesulfonate (MS-222) at 150 mg L^{-1}. After sedation, blood samples were taken from their caudal vein using a heparinized syringe. After that, the blood samples were transferred to a laboratory to be analyzed using hematological parameters. Hemoglobin (Hb) concentration was measured in the laboratory using a spectrophotometer following the Wedemeyer & Yasutake (1977) method. The hematocrit was calculated by using the microhematocrit method following Witeska *et al.* (2022), which outlines current standard procedures in fish hematology. By means of a Neubauer counting hemocytometer in the appropriate chamber, the RBCs were measured (Fazio, 2019). Three RBC indices were calculated using the following formulas.

$$\text{MCV} = \text{PCV} / \text{RBC} \times 10$$

$$\text{MCHC} = \text{Hb} / \text{PCV} \times 10$$

$$\text{MCH} = \text{Hb} / \text{RBC} \times 10$$

Study of Growth Parameter: The average initial weight of fish in each tank was 7.21g. Throughout the experiment, the average mass of each tank's fish was noted after two weeks. Using the given formulas, growth parameters for rohu fingerlings were calculated (NRC, 1993).

$$\text{SGR\% (per day)} = \frac{[\text{ln. (Final Weight)} - \text{ln. (Initial Weight)}]}{\text{Trial duration}} \times 100$$

$$\text{Weight gain \%} = \frac{\text{(Final Weight} - \text{Initial Weight)}}{\text{Initial Weight}} \times 100$$

$$\text{FCR} = \frac{\text{Total dry Feed Intake (g)}}{\text{Wet weight gain (g)}}$$

$$\text{FI (dry basis)} = \text{Given feed weight - Collected feed after feeding session}$$

Nutrient Digestibility Coefficient %: The nutrient digestibility coefficient (%) was calculated using the given formula.

$$\text{ADC}(\%) = 100 - 100 \times \frac{\%Marker\ in\ Diet\ \times \%\ Nutrients\ in\ Feces}{\%Marker\ in\ Feces\ \times \%\ Nutrients\ in\ Diet}$$

Analysis of Gross Energy: The Gross Energy (GE) of the experimental diets and feces was determined by direct combustion using an oxygen bomb calorimeter, following standard procedures (Table 3). Additionally, the GE of the diets was estimated using the following equation to compare with the measured values, following the method of Bell (1963), as applied in recent aquaculture nutrition studies (Abozaid *et al*., 2025).

GE (%) = (Crude Protein% × 5.65) + (Ether extract (EE) % × 9.4) + (NFE% × 4.15)

Whereas, nitrogen-free extract (NFE) was calculated as:

NFE = 100 - (Moisture + CP + EE + Ash + Crude Fiber)

Statistical Analysis: Data concerning fish growth, hematological parameters, and nutrient digestibility were analyzed using one-way analysis of variance (ANOVA). The Duncan test was used to compare means, with $p < 0.05$ considered significant. In addition to one-way ANOVA, polynomial contrast analysis (linear and quadratic) was performed to assess trends across WMSM inclusion levels. The level of significance was $P < 0.05$.

RESULT

The growth performance of fish fed diets containing WMSM is shown (Table 4). The final weight of fish in all triplicates differed significantly from their starting weight after a 70-day trial. The highest values of final weight (29.27 g), weight gain (21.72 g), weight gain % (289%), specific growth rate (1.51), and survival rate (98%) were recorded at TD-IV. The lowest values of FW (22.42 g), WG (14.83 g), WG % (196%), SGR (1.21), and survival rate (93%) were recorded at TD-VI. WG and WG% were significantly affected by dietary treatments ($P < 0.05$). WG and WG% were highest in TD-IV (superscript "a") and were significantly higher than TD-I and TD-VI, statistically comparable to TD-III and TD-V. Specific growth rate (SGR) followed a similar trend, with TD-IV showing the highest value and being significantly different from the control and 100% WMSM diets. Final weight (FW) also differed significantly among treatments ($P < 0.05$), with the highest FW recorded in TD-IV (60% WMSM), which was significantly higher than TD-I, TD-II, and TD-VI but not significantly different from TD-III and TD-V. Polynomial contrast analysis revealed that a highly significant quadratic (Q) effect was observed for FW, WG, WG%, FCR, and SGR ($P < 0.01$), demonstrating a non-linear response with optimal growth performance occurring at intermediate WMSM inclusion levels, particularly at 40–60% replacement. Additionally, a significant linear (L) effect of increasing WMSM inclusion on feed intake (FI), feed conversion ratio (FCR), and specific growth rate (SGR) ($P < 0.05$), indicating a general improvement in these parameters with increasing WMSM levels.

All values of the survival rate are significantly similar to each other. Among the diets, the highest (least efficient) value of feed conversion ratio (1.71) was recorded at TD-I; similarly, the lowest value of FCR (1.28) was found at TD-IV. The results showed that replacing 60% soybean meal with WMSM can effectively improve the growth performance of fish.

Table 4. Growth performance of rohu fingerlings fed WMSM diets for 70 days. Values are represented as mean ± SD (n = 3)

Levels (%)	Diets	IW (g)	FW (g)	WG (g)	WG (%)	FI (% body weight/day)	FCR	SGR (%/day)	Survival rate (%)
0%	**TD-I**	7.57 ± 0.58^{a}	23.98 ± 0.6^{c}	16.41 ± 1.03^{de}	218.24 ± 29.94^{bc}	0.40 ±0.02^{a}	1.71 ± 0.06^{a}	1.28 ± 0.10^{bc}	92.67 ± 7.1^{a}
20%	**TD-II**	7.57 ± 0.48^{a}	24.98 ± 0.7^{c}	17.42 ± 0.55^{cd}	230.81 ± 16.37^{bc}	0.39 ±0.02^{a}	1.56 ± 0.04^{b}	1.33 ± 0.06^{bc}	92.67 ± 0.3^{a}
40%	**TD-III**	7.54 ± 0.54^{a}	27.72 ± 0.9^{b}	20.18 ± 1.24^{ab}	269.03 ± 34.70^{ab}	0.39 ±0.02^{a}	1.35 ± 0.04^{cd}	1.45 ± 0.10^{ab}	95.05 ± 4.2^{a}
60%	**TD-IV**	7.55 ± 0.56^{a}	29.27 ± 0.7^{a}	21.72 ± 1.13^{a}	289.17 ± 33.80^{a}	0.40 ±0.02^{a}	1.28 ± 0.03^{d}	1.51 ± 0.10^{a}	97.62 ± 4.1^{a}
80%	**TD-V**	7.58 ± 0.55^{a}	26.48 ± 0.9^{b}	18.90 ± 0.36^{bc}	249.95 ± 13.57^{ab}	0.37 ±0.02^{a}	1.39 ± 0.06^{c}	1.39 ± 0.04^{ab}	95.05 ± 4.2^{a}
100%	**TD-VI**	7.59 ± 0.56^{a}	22.42 ± 0.8^{d}	14.83 ± 1.05^{e}	196.58 ± 26.81^{c}	0.33 ±0.01^{b}	1.57 ± 0.08^{b}	1.21 ± 0.10^{c}	92.86 ± 7.1^{a}
SEM		0.11	0.58	0.59	9.24	0.01	0.04	0.03	1.10
L		0.955	0.652	0.684	0.818	0.000^{***}	0.000^{***}	0.740	0.674
Q		0.928	0.000^{***}	0.000^{***}	0.001^{**}	0.013^{*}	0.000^{***}	0.001^{**}	0.276
P Value		1.000^{***}	0.000^{***}	0.000^{***}	0.013^{*}	0.002^{**}	0.000^{***}	0.011^{*}	0.805

^{abcd}Means within columns with different superscripts (a–e) differ significantly at $P < 0.05$.

Data are means of three replicates (± SD). SGR = specific growth rate; IW = initial weight; WG = weight gain; FW = final weight; FCR = feed conversion ratio; FI (% body weight/day) = feed intake; L = linear; Q = quadratic; SEM = standard error of the mean. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$**.** Survival rate did not differ significantly among treatments (NS, $P > 0.05$).

Table 5. Digestibility (%) of crude protein (CP), Ether extract (EE), and gross energy (GE) in rohu juveniles fed WMSM

Diets	Levels (%)	CP Digestibility (%)	Ether extract (EE) Digestibility (%)	GE Digestibility (%)
TD-I	0%	50.36 ± 0.68^{f}	52.53 ± 0.89^{f}	51.55 ± 0.90^{f}
TD-II	20%	57.62 ± 0.97^{d}	62.65 ± 0.73^{d}	60.71 ± 0.96^{c}
TD-III	40%	67.31 ± 0.92^{b}	69.49 ± 0.98^{b}	69.55 ± 0.64^{a}
TD-IV	60%	72.55 ± 0.70^{a}	73.62 ± 0.90^{a}	63.21 ± 0.73^{b}
TD-V	80%	62.72 ± 1.00^{c}	67.25 ± 0.91^{c}	56.53 ± 0.86^{d}

TD-VI	100%	53.74 ± 0.72^{e}	57.74 ± 0.92^{e}	54.18 ± 1.00^{e}
	SEM	1.8676195	1.7404959	1.4658512
	P value	0.000^{***}	0.000^{***}	0.000^{***}
	L	0.000^{***}	0.000^{***}	0.191
	Q	0.000^{***}	0.000^{***}	0.000^{***}

$^{a-e}$Means in each column with different superscripts (a–f) differ significantly at $P < 0.05$. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$**.** Data are means of 3 replicates (± SD), each based on 15 fingerlings. CP = crude protein; GE = gross energy; Q = quadratic; L = linear; SEM = standard error of the mean.

The values of the ADC of CP, EE, and GE are presented in Table 5. The highest value of CP digestibility, 72.55%, was obtained at test diet IV, and the lowest value of CP digestibility, 50.36%, was obtained at test diet I. The highest value of Ether extract (EE) digestibility (73.62%) and the lowest value of EE digestibility (52.53%) were found at TD-IV (60% replacement) and TD-I, respectively. According to GE digestibility, the highest value (69.55%) was obtained at diet-III, and the lowest value of GE digestibility (51.55%) at TD-I. Gross energy (GE) digestibility exhibited a significant quadratic effect ($P < 0.001$), with the highest value observed at 40% WMSM inclusion, suggesting an optimal inclusion level for energy utilization. CP and EE digestibility showed significant linear and quadratic responses to increasing dietary WMSM levels ($P < 0.05$), indicating improved nutrient utilization up to 60% inclusion followed by a decline at higher levels.

Blood indices of fish fed diets containing WMSM are presented (Table 6). The results showed that red corpuscles (3.12×10^{6} mm^{-3}), platelets (69.38), Hb (8.27 g/100 ml), MCHC (35.6%), and Hct (36.13%) were observed to be higher in rohu given experimental diet-IV, having 60% replacement of SBM with WMSM. PCV (28.13%) values were highest at TD-III; MCH (68.51 pg) and MCV (169.4 fL) were highest at TD-V. The minimum values of RBCs (1.41×106 mm^{-3}), PLT (59.57), Hb (5.02 g/100 ml), PCV (22.40%), MCHC (26.5%), MCH (41.34 pg), MCV (99.71 fL), and Hct (25.39%) were obtained at control diet-I. According to the above-mentioned values of RBCs, PLT, PCV, mean corpuscular hemoglobin concentration (MCHC), and MCH are significantly similar to each other. But the values of MCV and Hct are significantly different from each other.

Table 6. Hematological indices of rohu fingerlings fed WMSM-based diets

Level (Test Diets)	**WMSM%**								
	0(TD-I)	**20(TD-II)**	**40(TD-III)**	**60(TD-IV)**	**80(TD-V)**	**100(TD-VI)**	**SEM**	**L**	**Q**
RBCs (10^{6}mm^{-3})	1.41 ± 0.14^{c}	2.24 ± 0.24^{b}	2.56 ± 0.18^{b}	3.12 ± 0.49^{a}	2.13 ± 0.19^{b}	1.53 ± 0.19^{c}	0.1509	0.809	0.000^{***}
PLT (10^{3} /µL)	59.57 ± 0.73^{d}	61.48 ± 0.89^{c}	68.49 ± 0.81^{a}	69.38 ± 0.89^{a}	65.75 ± 0.89^{b}	68.60 ± 0.65^{a}	0.92661	0.000^{***}	0.000^{***}
Hb (g/dL)	5.02 ± 0.11^{d}	6.45 ± 0.30^{c}	7.80 ± 0.26^{a}	8.27 ± 0.37^{a}	7.07 ± 0.20^{b}	6.37 ± 0.43^{c}	0.26354	0.000^{***}	0.000^{***}
PCV (%)	22.40 ± 0.84^{d}	25.48 ± 0.91^{bc}	28.13 ± 0.89^{a}	26.87 ± 0.98^{ab}	24.41 ± 0.99^{c}	22.58 ± 0.95^{d}	0.54178	0.437	0.000^{***}
MCHC (%)	26.5 ± 0.78^{d}	29.4 ± 0.87^{c}	32.7 ± 0.76^{b}	35.6 ± 0.92^{a}	32.4 ± 0.61^{b}	30.4 ± 0.91^{c}	0.70811	0.000^{***}	0.000^{***}
MCH (pg)	41.34 ± 0.73^{d}	58.95 ± 0.94^{b}	55.46 ± 0.76^{c}	60.37 ± 0.97^{b}	68.51 ± 0.77^{a}	60.40 ± 1.07^{b}	2.00130	0.000^{***}	0.000^{***}
MCV (fl)	99.71 ± 1.00^{f}	105.4 ± 0.94^{d}	103.3 ± 0.92^{e}	151.5 ± 0.84^{b}	169.4 ± 0.87^{a}	131.4 ± 0.81^{c}	6.41255	0.000^{***}	0.000^{***}

$^{a-d}$Means within columns having different superscripts (a–f) are significantly different at $P < 0.05$. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$**.** Data are means of three replicates (± SD). RBCs = red blood cells; WMSM = watermelon seed meal; Hb = hemoglobin; PCV = packed cell volume; PLT = platelet count; MCV (fL) = mean corpuscular volume; MCHC = mean corpuscular hemoglobin concentration; Q = quadratic; L = linear; SEM = standard error of mean

DISCUSSION

This study evaluates the impact of substituting soybean meal with different proportions of WMSM on the growth performance, nutrient digestibility, and hematological responses of *L. rohita* fingerlings during a 70-day feeding trial. The results showed that fish fed 60% WMSM (TD-IV) had the highest values for final weight (FW), weight gain (WG), weight gain percentage (WG%), and specific growth rate (SGR). However, not all performance parameters peaked at the same inclusion level. For example, gross energy (GE) digestibility was highest at 40% WMSM (TD-III), rather than 60%, and other hematological parameters, such as hematocrit (PCV), were likewise highest in TD-III. These variations indicate that different physiological systems may react differently to WMSM inclusion, and that 60% is not always optimal for all metrics. The moderate level of WMSM inclusion probably enhanced growth due to the increased amino acids availability and nutrient utilization. However, the lower performance at higher inclusion levels can be explained by higher fiber content and anti-nutritional compounds like phytate, which decrease the level of digestion of the nutrient. Higher energy digestibility at 40% suggests higher efficiency of energy utilization at a moderate level of inclusion. This reflects a quadratic response, with optimal growth at moderate inclusion and reduced performance at higher levels.

Findings from earlier studies on WMSM substitution in aquafeeds have shown considerable variability, with some reporting reduced performance at high inclusion (Tiamiyu *et al.*, 2014; Hassan *et al.*, 2017) and others finding no negative impact (Jimoh *et al*., 2020b, 2022). However, significant inconsistencies in growth indices were noted. The disparities may be attributed to various factors, including species-specific nutritional needs and digestive capacities, as nutrient utilization efficiency significantly differs among fish species. Moreover, differences in the processing methods of WMSM, including drying techniques, particle size, and the extent of lipid oxidation, can affect protein availability and palatability. Variations in basal diet composition, specifically the energy-protein ratio and amino acid balance, may also influence growth responses and feed utilization efficiency. These factors likely explain the observed discrepancies among studies and highlight the necessity of improving ingredient processing and diet formulation when incorporating WMSM into aquafeeds.

The present results indicate that diets with 60% WMSM made the fish digest CP and EE significantly higher, while the highest GE digestibility occurred at 40% WMSM (TD-III). At 60% WMSM inclusion level, CP digestibility reached 72.55%, and EE digestibility was 73.62%, whereas the highest GE digestibility value, 69.55%, was found at 40% WMSM inclusion. The relatively low GE digestibility values (51–69%) may be partially due to nutrient leaching during fecal collection or the higher fiber content of WMSM, rather than indicating actual digestive inefficiency. The variation in peak digestibility responses indicates that distinct nutrient fractions react differently to escalating WMSM inclusion. Gross energy digestibility reached its highest level at 40% inclusion, possibly owing to an ideal equilibrium between digestible carbohydrates and lipids before an increase in dietary fiber. On the other hand, the digestibility of CP and EE peaked at 60%, which may indicate increased lipolytic and proteolytic activity at moderate WMSM levels. By reducing enzyme–substrate interaction and speeding up gastrointestinal transit time, the gradual increase in dietary crude fiber and residual anti-nutritional components may have decreased nutrient availability at higher inclusion levels (≥80%). Such variations may result from differences in fiber content, lipid composition, and anti-nutritional factors that affect energy availability. Moderate inclusion levels may improve digestive enzyme function, whereas excessive inclusion may dilute digestible nutrients (Bonvini *et al*., 2018). Tiamiyu *et al.* (2014) similarly reported that 10% inclusion of WMSM in *C. carpio* diets provided the best FCR and SGR, while increasing WMSM levels led to inverse growth trends.

Ombugadu *et al.* (2021) and Jimoh *et al.* (2015) conducted experiments to evaluate the effect of WMSM on the apparent digestibility coefficient (ADC) of nutrients in *C. gariepinus* and Nile tilapia fingerlings. Ombugadu *et al*. (2021) found that 36% WMSM inclusion resulted in the highest growth, while Jimoh *et al*. (2015) found no significant difference in protein ADC up to 30% WMSM. Fagbenro *et al.* (2013) also found similar results. But these results are not in line with the given results. The study by Babale (2016) found that using WMSM instead of soybean meal in five diets improved fish feeding results, with a 25% inclusion level recommended for optimal results. Current study and previous studies could be attributed to a diversity of causes, including diverse growth rates, the existence of changed levels of nutrients in nutritive treatments, species variants, fish size differences, and the physical ability of cultured fish to alter diets into absorbable nutrients (Naeem and Ishtiaq, 2011; Mugo-Bundi *et al*., 2015).

The results showed that feeding fish with 60% WMSM (TD-IV) significantly improved several blood parameters compared to other treatments. However, not all hematological parameters peaked at 60%; for instance, PCV was highest in TD-III (40%), indicating that different blood parameters respond differently to WMSM levels. The higher oxygen-carrying ability and increased aerobic metabolism may be the cause of the increasing RBC count and hemoglobin concentration shown up to 60% WMSM inclusion, which probably contributed to the better growth performance noted in TD-IV. Healthy *L. rohita* fingerlings typically show RBC counts of $2.5–4.5 \times 10^6$ mm^{-3} (Hemprabha & Arya, 2025), indicating that the extreme values at 80–100% WMSM may reflect nutritional stress rather than adaptive responses. However, a compensatory hematological response, defined by fewer but larger erythrocytes with a greater hemoglobin content, is probably responsible for the peak in MCH (68.51 pg) and MCV (169.4 fL) at 80%

inclusion. Such increases in erythrocyte volume are often associated with physiological adaptation to maintain efficient oxygen transport under varying nutritional conditions. Blood indices often reflect responses to nutritional stress rather than growth itself; hematological parameters may not directly indicate growth performance accurately. Therefore, rather than better nutritional efficiency, greater MCH and MCV at higher WMSM levels may suggest adaptive oxygen-transport pathways. Overall, the hematology results demonstrate that WMSM can replace fish meal in *L. rohita* diets without inducing physiological stress, particularly at moderate to high inclusion levels. This highlights WMSM's potential as a sustainable and economical protein source for fish farming. Although hematological values enhanced with WMSM inclusion, immune-specific measures (such as lysozyme, complement activity, and phagocytosis) were not examined in this study; therefore, no inferences about immunological improvement can be derived. Further research is needed on the long-term effects of WMSM on fish growth and immunity.

Similar studies have shown that WMSM has variable effects on fish hematology. Jimoh *et al*. (2015) investigated WMSM inclusion in Nile tilapia diets and found no significant differences in hematological parameters, which contrasts with the present findings. The differences between their findings and others could be due to factors such as fish species, sizes, and statistical techniques. The water quality, altered culture system (Tucker *et al*., 1989), size, sex, and age (Ranzani-Paiva *et al*., 1989) of the fish, as well as other aspects, could be the reasons for these differences from previous studies (Saleh, 2020). Collectively, these findings demonstrate how sensitive fish hematology is to experimental settings, highlighting the necessity of interpreting results within the particular context of each study.

Conclusion: This study illustrates that watermelon seed meal (WMSM) can effectively replace soybean meal in the diets of *L. rohita* fingerlings without adversely affecting growth, health, or nutritional absorption. Among all dietary treatments, the 60% WMSM inclusion level yielded the highest final weight, weight gain, specific growth rate, and survival, indicating greater growth-promoting efficacy. However, nutrient digestibility responses were not uniform; gross energy digestibility peaked at 40% WMSM, while crude protein and EE digestibility were highest at 60% WMSM. Hematological measures such as RBC, hemoglobin concentration, hematocrit, and platelet levels improved at higher inclusion levels, but PCV was highest at 40%, indicating that different physiological systems respond optimally to different WMSM levels and suggesting that WMSM did not cause stress or compromise fish health at moderate to high inclusion levels. Thus, these findings support the use of WMSM as a sustainable, cost-effective alternative protein source in feed formulations, with 40% for optimal energy utilization, 60% for optimal growth performance, and protein utilization. An inclusion level of 40–60% WMSM is recommended, depending on whether the focus is on energy utilization (40%) or growth and protein digestibility (60%).

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