EFFECTS OF LYCOPENE SUPPLEMENTATION ON GROWTH, BLOOD PROFILE, AND MINERAL DIGESTIBILITY IN *Cirrhinus mrigala* FED CANOLA MEAL-BASED DIETS

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ABSTRACT

Lycopene is a lipophilic natural antioxidant that effectively neutralizes oxygen and free radicals. The present study was conducted to investigate the effects of lycopene supplemented canola meal-based diets on growth, nutrient digestibility, hematology, carcass composition, and mineral digestibility of *Cirrhinus mrigala* fingerlings. Six concentrations of lycopene supplementation (0%, 1%, 2%, 3%, 4%, and 5%) were prepared. A feeding rate of 5% live body weight was given to the fish for three months, stocked in tanks (n=270; average weight= 5.31 ± 0.039 g). The results showed that weight gain % (288.49±9.82%), FCR (1.332±0.03), protein content (77.50±0.87%), and gross energy (75.42±0.94%) were significantly higher in the group fed 3% lycopene supplemented diets, while crude fat digestibility (77.94±1.43%) was maximum at 2% lycopene level. Furthermore, the hematological parameters were significantly improved at Test-diet IV (3% lycopene supplementation). Moreover, the body composition parameters and mineral digestibility (Ca, K, Fe, Cu, Zn, Na, and P) were substantially enhanced when *C. mrigala* were fed the Test-diet IV. In conclusion, the supplementation of 3% lycopene in canola meal-based diets is recommended for *C. mrigala* fingerlings, enhancing growth performance, nutritional digestibility, and blood parameters.

Keywords: Lycopene, aquaculture, fishmeal, canola meal, supplementation

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INTRODUCTION

Aquaculture plays an important role in the food production industry as it provides a major source of high-quality animal protein and seafood. This industry plays a significant role in improving nutrition, providing job opportunities, increasing income worldwide and contributing to food security both directly and indirectly (Anderson *et al*., 2017). The demand for aquaculture has increased threefold due to the growing human population. Fish is considered the best source of omega-3 and high-protein products, and constitutes a healthy part of the human diet. Fish is essential to meet the requirement for animal protein, and aquaculture provides almost half of the fish for human consumption (Khalili *et al*., 2021). Additionally, seafood contains high levels of vitamins and minerals, such as calcium, phosphorus, selenium, and vitamin D (Khalili and Sampels, 2018).

Fishmeal is an important component of aquaculture, leading substantially to global food security and livestock nutrition worldwide (Talas *et al*., 2014; Macusi *et al*., 2023). Fishmeal is a flour-like substance having high protein content, essential amino acids, high palatability, and no antinutritional factors (Zhang *et al*., 2018; Mugwanya *et al*., 2023). Due to increasing demand and inadequate supply of fishmeal, the fishmeal prices have become substantially high, almost two-fold over the past decade (Lin *et al*., 2022). Therefore, the supply of fish oil and fishmeal cannot be sustained due to the expansion of aquaculture production (Hodar *et al*., 2020).

Thus, plant-based diets are used as a viable and sustainable alternative for fishmeal in fish diets. These additives are highly nutritious, inexpensive, readily available, and improve fish production (FAO, 2022; Kuebutornye *et al*., 2024). It reduces the discharge of nutrients such as nitrogen and phosphate, enhancing the growth performance of fish and reducing the cost of feeds (Chakraborty *et al*., 2019). Canola meal contains 38%-46% crude protein, which is analogous to herring meal protein and soybean meal (Shafaeipour *et al*., 2008). It is a useful substitute for fishmeal and soybean due to its high protein content, cost-effectiveness, and digestibility (Yigit and Olmez, 2011). Furthermore, it contains adequate amount of amino acid content (methionine, lysine, and cysteine), minerals, and vitamins (choline, biotin, folic acid, niacin, riboflavin, thiamine) (Mohammadi *et al*., 2016). Several studies such as Iqbal *et al*. (2022), estimated that the growth, carcass, immune and serum biochemistry significantly improved in farmed tilapia fed with canola meal based diets.

Lycopene is a natural, eco-friendly pigment present in foods such as oranges, watermelons, and vegetables like *Punica granatum*, *Psidium guajava*, *Cucurbita papaya, Solanum lycopersicum, and Citrullus lanatus* (Ashraf *et al*., 2022). In addition, non-red foods like *Prussian asparagus, Petroselinum crispum*, and marine halophilic organisms also contain lycopene. Lycopene exhibits a strong antioxidant and anti-inflammatory effect, regulates body metabolism and immunity, and possesses anti-cancer properties (Dawood *et al*., 2020; Chen *et al*., 2023). It contains a singlet oxygen with a constant rate of 2–10 times more than α-tocopherol and β-carotene (Munde *et al*., 2017). It has the capacity to scavenge free radicals (Ali *et al*., 2021), influence fatty acid oxidation (Landrier *et al*., 2023), and regulate inflammatory factor expression (Ba *et al*., 2023). Due to its high-quality antioxidant potential and improved immunological responses, lycopene is extensively used in aquatic fish feeds (Tahir *et al*., 2024).

A high-quality balanced diet improves the production, welfare, and quality of farmed fish in aquaculture. The *Cirrhinus mrigala,* commonly known as Mrigal carp, is a bottom feeder and is considered a dominant species in freshwater aquaculture. *C. mrigala* has been extensively cultivated with *Catla catla* and *Labeo rohita* in a polyculture system (FAO, 2024; Hussain *et al*., 2011). Mrigal carp is a globally consumed species owing to its traditional medicinal uses, including weight reduction, enhanced sexual potency, improved cognitive function, and therapeutic oil against respiratory ailments (Muhammad *et al*., 2018). Its fast-growth, high nutritional value, and adaptability has increased the attention of aquaculturists around the world. The present research aimed to investigate the growth, nutrient digestibility, hematology, carcass composition, and mineral digestibility of *C. mrigala* fingerlings fed lycopene supplemented canola meal-based diets.

MATERIALS AND METHODS

In the current study, *C. mrigala* fingerlings fed different concentrations of canola meal-based diets supplemented with lycopene to evaluate the growth, nutrient digestibility, blood profile, carcass composition, and mineral digestibility.

Ethical Statement: The research design was approved by the Ethics Review Committee (ERC) of the Khwaja Fareed University of Engineering and Information Technology, Rahim Yar Khan, Pakistan and was conducted in adherence with the ARRIVE guidelines.

Fish and Experimental Conditions: The *C. mrigala* fingerlings (5.31 ± 0.039 g) were procured by Government Fish Seed Hatchery in Rahim Yar Khan, Pakistan. Before the onset of the current experiment, fish were acclimated in cemented tanks to accept the new environmental conditions for fourteen days. During this period, the water was changed regularly. Before the beginning of feeding trial, the fingerlings were given a sodium chloride (5 g per liter) bath solution to avoid external parasites and mycosis (Rowland, 1991). The fingerlings were fed with commercial feed until apparent satiety during this phase. The pH (7.5 ± 0.30), the temperature (26.45 ± 0.46 °C), and the oxygen content (6.56 ± 0.04 mg L^{-1}) of the water were checked regularly. Via a capillary system, aeration was supplied to each experimental tank for twenty-four hours. At seven in the morning, the fingerlings were hand-fed at five percent of their live body weight.

In order to perform a nutrition analysis, the residual feed was collected after feeding and dried in an oven at 60°C to calculate feed intake. The experimental tanks were refilled with tap water after being cleaned to get rid of any suspended particles. The waste was eliminated approximately two hours after feeding by opening the valves of the tanks. The feces were collected, processed into a fine powder, dried in the oven at 60°C, and kept at -4°C until analysis.

Experimental Design: Six dietary treatments were formulated in triplicate, with canola meal used as a basal diet. The treatments were supplemented with graded levels of lycopene (0, 1, 2, 3, 4, and 5%) referred to as T1, T2, T3, T4, T5, and T6, respectively. For each experimental diet, 15 fingerlings were stocked in each replicate (n=270 fingerlings). Using a completely randomized methodology, the experimental trial continued for 60 days.

Feed ingredients and Formulation of experimental diets: Feed ingredients were purchased from the local market, which were chemically analyzed using the procedures described by Arsalan *et al*. (2023). Each feed ingredient was finely ground and sieved through a 0.5 mm mesh screen. Table 1 shows the diet formulation and proximate composition of diet. Fish oil was included gradually as the feed ingredients were mixed for ten to twenty minutes using an electric mixer. 10–15% water was added to make the dough properly, and a pelleting machine was used to make feed pellets (Lovell *et al*., 1989). To measure digestibility, an inert marker, chromic oxide was added to the experimental diets at a rate of 1%.

Lycopene was incorporated into the feed pellets by adding lycopene (2 g) first into fish oil and then the distilled water (100 mL). Following this, lycopene solution was sprayed at required concentrations (0, 1, 2, 3, 4, and 5%) (Girao *et al*., 2012) and allowed these pellets to dry at room temperature. Pellets were broken into desired sizes, placed in airtight bags, and kept in the refrigerator at 4°C (Arsalan *et al*., 2023a).

Sample Collection: After feeding session, the valves were opened to clean the tanks thoroughly, and the uneaten diet was drained out from the tanks by manual siphoning, and filtered fresh water was supplied to each tank. For chemical analysis, the samples (feed, feces and body sample) were collected, crushed, dried in an oven, and stored.

Table 1. Formulation of experimental diets (%)

Ingredients	Test Diet-I (control)	Test Diet-II	Test Diet-III	Test Diet-IV	Test Diet-V	Test Diet-VI
Canola meal	50	50	50	50	50	50
Fish meal	15	15	15	15	15	15
Wheat flour*	17	16	15	14	13	12
Rice polish	8	8	8	8	8	8
Fish oil	6	6	6	6	6	6
Vitamin premix	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix	1.0	1.0	1.0	1.0	1.0	1.0
Ascorbic Acid	1.0	1.0	1.0	1.0	1.0	1.0
Chromic oxide	1.0	1.0	1.0	1.0	1.0	1.0
Lycopene	0	1	2	3	4	5
Proximate composition of experimental diets						
Crude Protein	30.63±0.02	30.55±0.04	30.67±0.03	30.53±0.03	30.53±0.02	30.66±0.02
Gross energy	3.53±0.02	3.53±0.01	3.54±0.02	3.52±0.01	3.52±0.02	3.53±0.02
Crude fat	8.22±0.01	8.23±0.02	8.24±0.01	8.24±0.02	8.25±0.02	8.24±0.03

* Lycopene was used at the expense of wheat flour

Chemical Analysis of Feed and Feces: The experimental diet and fecal samples were homogenized using a pestle and mortar, and standard procedures were applied to analyze these samples chemically (AOAC, 1995). The total protein content was calculated by using a micro Kjeldahl apparatus; the moisture content was estimated in a furnace through drying at 105°C for 12 hours; the total fat content was determined by the petroleum ether extraction method using a Soxtec HT2 1045 system. Ash was obtained by igniting the feed samples at 650°C for twelve hours in a muffle furnace (Eyela-TMF 3100), and the gross energy was calculated using an oxygen bomb calorimeter. 1.25% sulfuric acid and 1.25% sodium hydroxide were used to break down dried residues without lipids to produce crude fiber (CF).

Chromic Oxide Estimation: Chromic oxide was determined by the acidic digestion method using an ultraviolet-visible spectrophotometer (UV-VIS 2001), and absorbance was measured at 350 nm (Divakaran *et al*., 2002). After oxidation, a perchloric solution was used to determine the amount of Cr_2O_3 from the diet and fecal samples.

Growth Study: At the start and completion of the experimental trial, the fingerlings were collectively weighed from each tank to determine their growth rate. Using the established formulae by Arsalan *et al*. (2023b), the fingerling's growth performance was evaluated.

Calculation of Digestibility: Using the method outlined by NRC (1993), the amount of nutrients digested in experimental diets was calculated directly to determine how many nutrients were taken by the fish and how many are excreted through the feces in relation to intake.

The formula is as follows:

$$\text{ADC (\%)}=[100-(100\times\frac{\%\text{ marker in diet}\times\%\text{ nutrient in feces}}{\%\text{ marker in feces}\times\%\text{ nutrient in diet}})]$$

Blood Collection and Hematological Analysis: Tricaine methanesulfonate (MS-222) at a 150 mg L^{-1} solution was used to immobilize the fish to collect the blood samples after 60 days (Wagner *et al*., 1997). Blood samples were sent to the laboratory for hematological examination. Cyanmethemoglobin technique was used to measure the hemoglobin content according to Wedemeyer and Yasutake's (1977) method. Blood cells were calculated using the haemocytometer accompanied by the Neubauer counting chamber (Blaxhall and Daisley, 1973). Mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and PVC (packed cell volume) were estimated by using standard formulae by Khalid *et al*. (2022).

Carcass Analysis: After the completion of the experimental trial, three fish were chosen randomly from each tank, and standard protocols were followed to assess the proximate composition of experimental diets and feces (AOAC, 1995). Protein content was evaluated using a micro Kjeldahl instrument, and the moisture content was ascertained by dehydrating for 12 hours at 105°C in a furnace. Petroleum ether extraction was obtained using the Soxtec HT2 1045 apparatus. Ash was burned in a muffle furnace (Eyela-TMF 3100) at 650 for 12 hours in order to preserve mass.

Estimation of minerals: For this purpose, the wet digestion method was used, in which nitric acid and perchloric acid were boiled in a 2:1 ratio to digest the feeding and fecal samples. Upon boiling, 10 mL $HClO_4$ was poured into the flask and boiled again until the volume reduced to 1 mL. After the proper dilution, the mineral contents were calculated using an Atomic Absorption Spectrophotometer (AOAC, 1995). A calorimetric analysis of the phosphorus was performed at 350 nm using the UV/VIS spectrophotometer. However, a flame photometer (Jenway PFP-7, UK) was used to determine sodium and potassium digestibility.

Statistical Analysis: The growth performance, nutritional digestibility, carcass composition, blood profile, and mineral status of the test diets were examined using the One Way Analysis of Variance (Steel and Torrie, 1960). Tukey's Honest Significant Difference was used to assess the mean differences with a significance level of $p<0.05$ (Snedecor and Cochran, 1989). The statistical analysis was performed using version 6.303 of the Costat Computer Package (PMB 320, Monterey, CA, 93940 USA).

RESULTS

Growth Performance: Tables 2 and 3 show the growth results of *C. mrigala* fingerlings fed canola meal-based test diets with varying levels of lycopene supplementation (0%, 1%, 2%, 3%, 4%, and 5%). Lycopene supplementation significantly improved FCR and SGR (1.332±0.038 and 1.938±0.036) recorded at 3% level, respectively. The highest weight gain (15.36±0.366 g) in *C. mrigala* was also observed at 3% lycopene supplementation level. However, optimal growth parameters were observed at 3% lycopene level (Test Diet-IV).

Table 2. Growth indices (weight gain %, SGR) in *C. mrigala* fingerlings fed with varying lycopene concentrations.

Diet	Lycopene Levels	Initial Weight (g)	Final Weight (g)	Weight gain (%)	SGR
Test Diet – I (Control)	0%	5.24±0.03	12.16±0.12^{e}	131.91±0.75^{e}	1.401±0.005^{e}
Test Diet – II	1%	5.31±0.02	16.63±0.18^{c}	213.19±4.61^{c}	1.630±0.02^{c}
Test Diet – III	2%	5.34±0.04	18.65±0.23^{b}	248.94±3.74^{b}	1.785±0.01^{b}
Test Diet – IV	3%	5.32±0.05	20.69±0.31^{a}	288.49±9.82^{a}	1.938±0.03^{a}
Test Diet – V	4%	5.34±0.03	14.29±0.26^{d}	167.61±5.77^{d}	1.406±0.03^{d}
Test Diet – VI	5%	5.34±0.04	11.45±0.13^{f}	114.28±3.05^{f}	1.088±0.02^{f}

^{abcdef} Mean values in columns with distinct superscripts are statistically different ($P\leq 0.05$).

Table 3. Growth indices (weight gain, feed intake, FCR) in *C. mrigala* fingerlings fed with varying lycopene concentrations.

Experimental Diets	Lycopene Levels	Weight Gain	Feed Intake	FCR=Feed intake/Weight gain
Test Diet -I (Control Diet)	0%	6.92±0.08^{f}	0.310±0.006^{f}	2.698±0.06^{e}
Test Diet -II	1%	11.32±0.20^{c}	0.305±0.007^{c}	1.886±0.07^{c}
Test Diet -III	2%	13.31±0.21^{b}	0.345±0.005^{b}	1.818±0.03^{b}
Test Diet -IV	3%	15.36±0.36^{a}	0.292±0.006^{a}	1.332±0.03^{a}

Test Diet -V	4%	8.95±0.27[d]	0.293±0.005[d]	2.292±0.03[d]
Test Diet- VI	5%	6.11±0.14[e]	0.289±0.004[e]	3.312±0.09[f]

[abcdef] Mean values in columns with distinct superscripts are statistically different ($P \leq 0.05$).
Values that have the same superscript don't differ much.

Nutrient Digestibility: Table 4 illustrates the apparent digestibility coefficients of crude protein, gross energy, and crude fat for the diets supplemented with lycopene in *C. mrigala*. The highest crude protein digestibility (77.50±0.87%) was observed at 3% lycopene level, while crude fat digestibility (77.94±1.43%) was maximum at 2% lycopene level. The optimal level for maximal digestion of gross energy (75.42±0.94%) was 3% lycopene, with significantly different digestibility measurements compared to other levels ($P<0.05$).

Table 4. The apparent digestibility coefficients of crude protein, gross energy, and crude fat for various diets enriched with lycopene in *C. mrigala* fingerlings.

Experimental Diets	Lycopene Levels	Crude Protein Digestibility	Gross Energy Digestibility	Crude Fat Digestibility
Test Diet-I (Control Diet)	0%	47.79±1.90[e]	55.03 ± 1.92[e]	66.32±0.82[e]
Test Diet -II	1%	64.28±0.86[c]	72.69 ± 0.73[c]	69.05±1.47[d]
Test Diet -III	2%	73.21±0.95[b]	74.78 ± 0.35[b]	77.94±1.43[a]
Test Diet -IV	3%	77.50±0.87[a]	75.42 ± 0.94[a]	73.19±1.66[b]
Test Diet- V	4%	56.02±0.44[d]	60.20 ± 1.32[d]	70.11±1.27[c]
Test Diet- VI	5%	33.24±0.72[f]	40.16 ± 0.36[f]	58.72±4.02[f]

[abcdef] Mean values in columns with distinct superscripts are statistically different ($P \leq 0.05$).
Values that have the same superscript don't differ much.

Hematology: The highest values for RBCs (3.63±0.08 $\times 10^6$/mm³), WBCs (8.94±0.13 $\times 10^3$/mm³), Hb (8.86±0.11 g/100 mL), PCV (28.47±0.37%), MCHC (31.12±0.13%), MCH (24.41±0.24 pg), and MCV (78.45±0.81 fl) were observed in Test Diet-IV with 3% lycopene level (Table 5). The values decreased at higher lycopene levels (4% and 5%). The results indicated that 3% lycopene supplementation had a positive effect on hematological parameters. The 5% lycopene level showed the lowest values for all parameters.

Table 5. The blood profile of *C. mrigala* fingerlings fed various diets enriched with lycopene concentrations.

Experimental Diet	Lycopene Levels	RBC (10^6/mm³)	WBC (10^3/mm³)	Hb (g/100ml)	PVC (packed cell volume) (%)	MCHC (%)	MCH (pg)	MCV (fl)
Test Diet-I (Control Diet)	0%	2.44±0.11[e]	8.03±0.03[e]	6.56±0.05[e]	21.77±0.02[e]	30.13±0.20[f]	26.92±1.00[f]	89.33±3.94[f]
Test Diet-II	1%	3.11±0.07[c]	8.34±0.04[c]	7.74±0.04[c]	25.64±0.63[c]	30.19±0.62[c]	24.87±0.47[c]	82.36±0.16[c]
Test Diet-III	2%	3.24±0.04[b]	8.43±0.17[b]	8.03±0.23[b]	27.67±1.17[b]	29.02±0.40[b]	24.77±0.40[b]	85.38±2.56[b]
Test Diet-IV	3%	3.63±0.08[a]	8.94±0.13[a]	8.86±0.11[a]	28.47±0.37[a]	31.12±0.13[a]	24.41±0.24[a]	78.45±0.81[a]
Test Diet-V	4%	2.94±0.27[d]	8.20±0.12 [d]	7.21±0.07[d]	23.23±0.38[d]	31.04±0.21[d]	24.65±2.03[d]	79.38±6.02[d]

Test Diet-VI	5%	1.67±0.20^{f}	7.10±0.06^{f}	6.20±0.09^{f}	18.27±0.11^{f}	33.93±0.29^{e}	37.47±5.27^{e}	110.33±14.52^{e}

^{abcdef} Mean values in columns with distinct superscripts are statistically different (P≤ 0.05).

Carcass Composition: The highest protein content (15.16±0.06%) and ash content (2.19±0.05%) were observed in the diet having 3% lycopene level (Table 6). The lowest fat content (3.03±0.12%) was also observed in Test Diet-IV with 3% lycopene level. The control diet (0% lycopene) had a lowest protein content of 13.75±0.13%. Moisture content was almost non-significant among all test diets.

Table 6. Carcass Composition of *C. mrigala* fed with varying lycopene levels.

Test diets	**Lycopene levels**	**Protein**	**Fat**	**Ash**	**moisture**
Test Diet-I (Control)	0%	13.75±0.13^{f}	4.80±0.04^{a}	2.08±0.06^{d}	79.36±1.05^{b}
Test Diet-II	1%	14.27±0.21^{e}	4.18±0.13^{b}	2.20±0.05^{c}	79.33±1.26^{b}
Test Diet-III	2%	14.84±0.07^{b}	3.29±0.05^{e}	2.37±0.04^{b}	79.42±0.83^{b}
Test Diet-IV	3%	15.16±0.06^{a}	3.03±0.12^{f}	2.43±0.05^{a}	79.39±0.61^{b}
Test Diet-V	4%	14.63±0.08^{c}	3.40±0.08^{d}	2.19±0.02^{c}	79.77±2.14^{a}
Test Diet-VI	5%	14.35±0.11^{d}	3.79±0.15^{c}	2.10±0.02^{d}	79.28±1.17^{c}

^{abcdef} Mean values in columns with distinct superscripts are statistically different ($P \leq 0.05$).

Minerals Digestibility (%): The optimal mineral digestibility was observed at 3% lycopene level, with significant differences ($P<0.05$) compared to other levels (Table 7). The highest digestibility values for calcium, ferrous, potassium, copper, zinc, sodium, and phosphorus were 66.55±4.63%, 75.37±2.85%, 73.45±2.11%, 49.23±0.21%, 72.08±0.38%, 62.88±2.15%, and 77.41±2.53%, respectively. Test Diet –IV which contains 3% lycopene supplementation, showed the maximum mineral digestion as compared to other experimental diets.

Table 7. The mineral digestibility of *C. mrigala* fingerlings fed various diets enriched with lycopene concentrations.

Experimental Diets	**Lycopene Levels**	**Calcium (Ca) %**	**Ferrous (Fe) %**	**Potassium (K) %**	**Copper (Cu) %**	**Zinc (Zn) %**	**Sodium (Na) %**	**Phosphorus (P) %**
Test Diet-I (Control Diet)	0%	26.50±4.51^{e}	39.19±0.92^{e}	32.62±1.90^{e}	36.14±0.12^{e}	31.47±0.97^{e}	23.17±2.81^{e}	33.98±4.81^{e}
Test Diet -II	1%	45.46±4.12^{c}	52.14±0.93^{c}	56.09±3.48^{c}	45.52±0.38^{c}	53.63±4.71^{c}	40.56±0.44^{c}	53.08±2.43^{c}
Test Diet-III	2%	54.61±4.30^{b}	66.19±4.88^{b}	65.79±2.95^{b}	47.72±0.12^{b}	62.63±1.06^{b}	54.00±1.78^{b}	64.36±3.36^{b}
Test Diet -IV	3%	66.55±4.36^{a}	75.37±2.85^{a}	73.45±2.11^{a}	49.23±0.21^{a}	72.08±0.38^{a}	62.88±2.15^{a}	77.41±2.53^{a}
Test Diet- V	4%	30.56±0.49^{d}	45.75±4.78^{d}	43.13±3.76^{d}	41.46±0.07^{d}	44.34±4.48^{d}	32.69±2.30^{d}	43.79±3.48^{d}
Test Diet -VI	5%	21.17±1.07^{f}	23.86±4.87^{f}	22.95±1.63^{f}	33.15±0.92^{f}	21.47±1.19^{f}	19.51±0.30^{f}	24.65±0.81^{f}

^{abcdef} Mean values in columns with distinct superscripts are statistically different ($P \leq 0.05$).

DISCUSSION

The current study showed that the growth indices (weight gain, SGR, FCR) were maximum at 3% lycopene supplementation level as compared to other levels. While at higher lycopene levels (4% and 5%) detrimental effects on growth performance were observed. In line with our research, Khalid *et al*. (2022) reported that 40 mg/kg lycopene supplemented canola meal based diet improved the growth of *L. rohita* fingerlings. According to Zhou *et al*. (2024), dietary lycopene supplementation (200–300 mg/kg) enhanced the growth performance of hybrid grouper fed high lipid based diet. Furthermore, dietary lycopene effectively ameliorated the growth performance of common carp after chronic glyphosate exposure (Tahir *et al*., 2024). Moreover, Wu *et al*. (2024) investigated that inclusion of 20 mg/kg lycopene in the diets of broilers increases the growth performance. This may be due to lycopene's ability to neutralize singlet oxygen and scavenge the free radicals, thus improving growth and oxidative stress (Wu *et al*., 2024). In contrast to our findings, Girao *et al*. (2012) found that lycopene supplementation did not affect the growth performance (weight gain, intake and SGR) of Nile tilapia.

In the current study, the maximum nutrient digestibility was observed at 3% lycopene supplementation level; gross energy (75.42%) and crude protein (77.50%) content, while the highest crude fat (77.94%) content was observed at 2% dietary lycopene level. Similar to the current findings, Khalid *et al*. (2022) observed that the highest digestibility of the crude proteins (72%), gross energy (65%), and fat content (77%) were recorded at 40 mg/kg lycopene supplemented diets in *L. rohita*. Furthermore, Sweed *et al*. (2025) suggested that lycopene's antioxidant properties alleviate the oxidative stress thus improving the metabolic activities that are involved in the nutrient digestion and utilization. Additionally, another study indicated that lycopene improves the digestive enzyme activities which in turn enhances the feed efficiency and nutrient absorption (Sarker *et al*., 2021).

Hematological parameters are important indicators of fish health monitoring, reflecting pathological and physiological changes due to diet, disease, and environmental stressors (Shahzad *et al*., 2016; Fazio, 2018). In the present study, a significant improvement was observed in the hematological indices of fingerlings fed with lycopene at 3% compared with the control diet. Similar to our findings, Khalid *et al*. (2022) reported that supplementing 40 mg/kg lycopene in the diets of *L. rohita* showed maximum blood profile (RBCs, WBCs, Hb) while higher inclusion level had no effect on hematological indices. Furthermore, Tahir *et al*. (2024) suggested that lycopene supplementation effectively alleviated the toxicity in blood indices of common carp after chronic glyphosate exposure. Moreover, Yonar *et al*. (2020) suggested that lycopene ameliorate the hematological changes in common carp against trichlorfon effects. Lycopene boosts the antioxidant capacity due to scavenging the free radicals and activating the nuclear factor erythroid 2–related factor 2 (Nrf2) pathway thus improving the blood profile and balancing the oxidative stress (Long *et al*., 2024; Sweed *et al*., 2025).

Lymphocytes and white blood cells (WBCs) act as the primary body defense mechanism that fight against foreign invaders. WBCs play a significant role in immunological responses, the ability of fish to resist diseases, and to cope effectively with stress or toxins (Douglass and Janes, 2010). In the current study, WBC ($8.94 \pm 0.03 \times 10^3/mm^3$) was upregulated with 3 % lycopene supplementation, and then downregulated thereafter. A high WBC count is typically linked to a microbial infection, a foreign body, or an antigen in the bloodstream (Oyawoye and Ogunkunle, 1998). As the amount of lycopene in the diet increased, the hemoglobin levels decreased because higher levels of additional nutrients have negative impacts on blood parameters (Dienye and Olumuji, 2014). In the current study, fish fed a diet containing 3% lycopene-treated canola meal had the highest values of PCV, MCHC, MCH, and MCV. A decrease in PCV concentration indicates the presence of a toxic substance, such as haemagglutinin, which disrupts the normal blood production and function (Oyawoye and Ogunkunle, 1998).

In this research, the carcass composition of *C. mrigala* was improved when fed 3% lycopene supplemented canola meal-based diet. To our knowledge, limited studies have investigated the effects of dietary lycopene on carcass traits in aquatic species. However, Wu *et al*. (2024) investigated that dietary lycopene supplementation enhances the meat quality in broiler. Furthermore, Wen *et al*. (2022) reported that inclusion of lycopene in the diets improved the meat quality of finishing pigs.

Lycopene is added to fish diets to mitigate the negative impact of antinutritional factors such as phytate, glycoside, oxalate, and tannin, resulting in enhancing the bioavailability of vital minerals, including phosphorus, protein, calcium, and zinc in aquatic species (Jonathan, 2015). In the current study, the minerals digestibility was observed at 3% lycopene supplemented canola meal-based diets in *C. mrigala* fingerlings. The reason may be the lycopene likely improves mineral digestibility in fish by reducing oxidative stress in the gut, enhancing nutrient absorption, and protecting gut health, thus boosting mineral uptake. According to Wu *et al*. (2024), lycopene may improve intestinal morphology by boosting digestive enzymes and alkaline phosphatase secretion. Alkaline phosphatase, a marker of intestinal health, enhances nutrient absorption. Limited studies have investigated the effects of dietary lycopene on mineral digestibility in aquatic species.

Conclusion: This study demonstrates that canola meal-based diets supplemented with lycopene enhance growth, nutrient digestibility, carcass, hematological indices and mineral digestibility in *C. mrigala* fingerlings. Our findings indicated that 3% lycopene supplementation is a valuable additive in aquaculture feeds, which significantly enhances the production and health of *C. mrigala* fingerlings. Therefore, it focuses on providing a sustainable, economical, and cost-effective dietary method, which potentially reduces feed costs and leads to the expansion of alternative plant-based aquafeeds for various fish species.

Conflict of interest: The authors declare that there is no conflict of interest exists.

Animal welfare statement: The authors strictly adhered to the guidelines and regulations set forth by the committee, ensuring the highest standards of animal care and welfare.

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