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Original Article

Evaluation of silkworm pupa meal on growth, body indices, digestive enzymes and histopathology of *Cirrhinus mrigala* fingerlings

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Abstract
The world's growing population needs food, and aquaculture; a fast growing food producing sector, provides it. Fish meal (FM) is a major feed source in the aquaculture industry, but its low profitability and scarcity have prompted a quest for high-protein substitutes. Because silkworm pupa meal is inexpensive and has a high protein (50–60%) concentration, it was utilized as a substitute for fish feed. The goal of the current study was to determine the ideal proportion of silkworm pupa meal (SPM) to substitute for FM while creating an affordable feed for *Cirrhinus mrigala* fingerlings (average total length: 6 cm; average weight: 8 g). The fingerlings were fed six test diets (0, 20, 40, 60, 80, and 100%) at a rate of 4% of their live wet body weight twice a day. Diets contained chromium oxide as an inert marker. The feeding trial lasted for ninety days. The fingerlings' weight was measured every 14 days to calculate growth metrics (weight gain percentage, FCR, and SGR). Following the conclusion of the trial, intestinal histology, digestive enzyme function, and body indices were examined. Highest growth rate (WG%: 333%, SGR: 1.64, FCR: 1.26, CF: 1.50) and best body indices (hepatosomatic index: 1.95, viscerosomatic index: 6.43) values were observed at 40% replacement of FM. Intestinal histological analysis and digestive enzyme activity demonstrated that SPM can be readily substituted up to 40% without affecting the general performance of *C. mrigala* fingerlings and aid in creating an economical and environmentally friendly diet.

Keywords: body indices; *Cirrhinus mrigala*; digestive enzymes; fishmeal; histopathology; silkworm pupa; specific growth rate

1 | INTRODUCTION

With nine billion people on the Earth by 2050, there will be a greater need for food (Hashem 2022). Nutritionists are therefore looking for sustainable and natural dietary substitutes (Glausiusz 2022). As the world's population grows, requirement of food particularly animal proteins has also gone high. Aquaculture is a contemporary requirement since aquatic life provides humans with a practical and essential source of protein (Hussain *et al.* 2024). One important source of high-quality human proteins is fish aquaculture (Stankus 2021). Today, the primary issue aquaculture is facing is the steadily rising demand for fish for human consumption (Hussain *et al.* 2024). Since the 1980s, the size of wild fish capture fisheries has peaked (FAO 2022). Fishmeal (FM), a strong protein source, has been used in aquafeeds to promote the rapid growth and overall health of farmed fish (Hussain *et al.* 2024). Nowadays, aquaculture's potential to grow is limited by the high cost of aquafeeds including FM, which is seen as costly and unsustainable. However, since aquafeed production has increased, the price of raw materials like FM has also increased quickly (FAO 2018). Since FM is uncommon and expensive, research is being done on other sources with higher protein content and comparable nutritional advantages (Daniel 2018). Even though aquaculture uses plant-based sources, excessive usage of plant-based feeds is prohibited since they include certain anti-nutritional components (Hussain *et al.* 2024).

Because of their nutritional similarities to FM, many insect meals (IM) have recently become attractive protein substitutes in aquafeeds (Henry *et al.* 2015). The use of insects as aquafeed components has been extensively studied (Mastoraki *et al.* 2020a, 2020b; Wang *et al.* 2022). Insects have a far better growth ratio, use less room and energy, and are easier to develop and reproduce than other organisms (van Huis 2022). Sericulture is a long-standing industry with a unique characteristic that originated in Asia (Zhao *et al.* 2023) and it produces and sells four types of natural silk worldwide. The phrase "silk" is commonly used to refer to the silk created by mulberry silkworms because it is the most significant of them and makes up to 90% of the world's production (Sheikh *et al.* 2018). The primary product of the reeling business is silkworm pupae; 70% of the world's production, or over 400,000 tons, are produced in China per year (Zhao *et al.* 2023). Silkworm pupae are natural protein sources that are essential for development and use, even though they are abundant in protein and unsaturated fatty acids (Sadat *et al.* 2022). The protein content of silkworm pupae ranges from 48 to 94.98% DM, depending on their nutritional composition (Hăbeanu *et al.* 2023). Due to their high biodegradability, these young silkworm pupae damage the environment and emit an unpleasant odor in the surrounding area. The ecology in areas that produce silk may be negatively impacted when large amounts of pupae are discarded. Therefore, using these amazing resources to feed animals and poultry is a crucial strategy to reduce the environmental impact of the silk industry (Sheikh *et al.* 2018).

Cirrhinus mrigala is the third most major carp commonly known as mori in Pakistan, after Rohu (*Labeo rohita*) and thaila (*Catla catla*) (Mishra 2020). Its diet typically includes a variety of various feed items and protein sources. As a result, feed costs rose, nitrogen consumption became out of balance, and nitrogen excretion increased in the aquaculture system. Since the nutritional requirements of various developmental stages vary, *C. mrigala* has a basic protein requirement of 28 – 33% (Alam 2020). It is found in different freshwater bodies throughout Pakistan and its neighboring nations (Bais 2018). It is found in rivers, lakes, and growing regions throughout Indian subcontinent, and under the right circumstances, it can grow to a considerable size. The current study examined growth characteristics, body indices, digestive enzyme activity, and histological examination of *C. mrigala* fingerlings in order to determine the optimal doses of SPM to replace FM.

2 | METHODOLOGY

2.1 Experimentation site

Current study was done to evaluate the effect of silkworm pupa meal on performance of *C. mrigala* fingerlings. Research trial was done at Animal research station, Department of Zoology, University of Education, Lahore.

2.2 Fish and experimental conditions

Cirrhinus mrigala juveniles were brought from Manawa Fish Hatchery, Lahore, Punjab, Pakistan and acclimated to experimental set up for two weeks and were fed on basal diet during this period (Allan and Rowland 1992). The fingerlings were housed in specially designed aquarium tanks with a 100 L water storage capacity. Regular observations of water quality parameters such as temperature (25 – 27°C), dissolved oxygen (5 – 6 ppm), and PH (6 – 8) were made. Throughout the experiment, an air pump was utilized to deliver oxygen to the capillary system. The fingerlings were treated with a 0.5% saline solution for one to two minutes prior to the feeding trial in order to eradicate any pathogens (Rowland and Ingram 1991).

2.3 Experimental design

Silkworm pupa meal (SPM) was used as test diet for formulation of six experimental diets. One control diet and five SPM based diets were fed to 15 fingerlings in each triplicate water aquarium. Treatment span was about 90 days.

2.4 Silkworm pupa meal preparation

Dried silkworm pupae was purchased from sericulture department, Lahore. Firstly, top layer of silk thread (cocoon) was removed from pupa and cleaned pupa was then completely sun dried. Dried pupa was ground into powder and stored in air tight plastic container.

2.5 Formation of feed pellets

Feed ingredients as explained in Table 1, were procured from commercial market in Lahore and ground properly to pass through a sieve of 0.3mm. Prior to experiment, chemical composition of all ingredients was analyzed using standard methods as explained by AOAC (1995).

Chromium oxide was used at 1% as an inert marker. After adding fish oil, all feed components were properly combined for 5 – 10 minutes. During the process, 10 – 15% distilled water was added gradually to mix all of the feed components and create the appropriate dough, which was then shaped into pellets using a pelleting machine. All prepared diets were dried at 105°C and stored at 4°C.

TABLE 1 Composition of ingredients (%) in test diets.

Ingredients	Test Diet-I (0%)	Test Diet-II (20%)	Test Diet-III (40%)	Test Diet-IV (60%)	Test Diet-V (80%)	Test Diet-VI (100%)
Silkworm pupa meal	0	7.6	15.2	22.8	30.4	38
Fish meal	38	30.4	22.8	15.2	7.6	0
Wheat bran	22	21	20	19	18	17
Maize flour	23	21	19	17	15	13
Corn gluten	10	13	16	19	22	25
Fish oil	3	-	-	-	-	-
Vitamin Premix*	1	-	-	-	-	-
Min. Premix**	1	-	-	-	-	-
Ascorbic acid	1	-	-	-	-	-
Chromic oxide	1	-	-	-	-	-
Protein (%)	29.89	29.91	29.90	29.93	29.94	29.92
Lipid (%)	6.88	6.89	6.87	6.92	6.90	6.93
Gross Energy (Kcal g^{-1})	3.2	3.5	3.6	3.1	3.3	3.7

* Vitamin D3: 3,000,000 IU; Vitamin A: 15,000,000 IU; Vitamin C: 15,000 mg; Vitamin B6: 4000 mg; Vitamin E: 30000 IU; Vitamin B2: 7000 mg; Vitamin B12: 40 mg; Folic acid: 1500 mg; Vitamin 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.
Data are three replicates' means (± show standard deviation)
Mg = magnesium; P = phosphorus; Cu = Copper, Ca = Calcium; Fe = Iron; Na = Sodium; K = Potassium

2.6 Procedure for feeding and collecting samples

At 4% of live wet body weight *C. mrigala* juveniles were fed. After two hours of feeding the remaining diet in aquariums was removed from each aquarium. After thoroughly cleaning the aquariums to get rid of any feed particles, water was added again.

2.7 Study of growth parameters

In each aquarium with average weight (8 ± 0.5g) of 15 fingerlings were retained. During the trial period, after every fourteen days fingerlings were weighed to analyze growth parameters. As per formulas proposed by Shahzad *et al.* (2021) calculation of growth parameters was done.

2.8 Analysis of body indices

Indices of liver and intestine analysis of three juvenile from all group were dissected, measured and quantified using the standard procedures (Babalola *et al.* 2022).

Hepatosomatic index (HIS, %) = liver weight / final body weight × 100

Viscerosomatic index (VSI, %) = viscera weight / final body weight × 100

2.9 Digestive enzyme analysis

Fish from each replicate tank were sampled for intestine tissue. Immediately after collecting intestine, it was homogenized with 0.25 M sucrose buffer (5% w/v) and centrifuged at 10000 rpm for 30 minutes. The supernatant was collected in a 15 mL tube and kept at –20°C. The supernatant was utilized as an enzyme extract. Protease activity was evaluated using the casein digestion method (Drapeau 1976). The dinitro-salicylic acid approach (Rick and Stegbauer 1974) was used to assess α-amylase activity on carbohydrates, while Zamani *et al.* (2009) described the method for determining lipase activity.

2.10 Histological preparation

After completion of trial, one sedated fish from each aquarium (3 fish per group) was randomly selected for histopathological examination. Intestine of *C. mrigala* juvenile was dissected and placed in 10% formalin fixative solution. For analysis of histopathology of fish intestine a method described by Luna (1968) was followed. For determination of any abnormalities in intestine a light microscope was used.

2.11 Data analysis

One-way Analysis of Variance (ANOVA) will be used to compare the data of all parameters using SPSS software (Steel and Torrie 1996). Duncan's new multiple range test was performed to do various comparisons having significance level at $p < 0.05$.

3 | RESULTS

In the current study, growth parameters (FW, WG, WG%, FCR, SGR, and CF) were studied in *C. mrigala* fingerlings using SPM as FM replacement, as explained in Table 2. The results showed that the growth of *C. mrigala* juveniles increased when fed with SPM. The initial weight (8.46 g) of *C. mrigala* juveniles was significantly similar. It was clearly noticed that maximum growth in *C. mrigala* fingerlings was observed at 40% replacement of FM. Maximum values of FW (37.16 g), WG (28.66 g), WG% (333%) and weight gain fish^{-1}day^{-1} (0.41g) were seen at test diet III having 40% replacement of FM while minimum values of FW (26.38 g), WG (17.83 g), WG% (208%) and weight gain fish^{-1}day^{-1} (0.25 g) were observed at test diet VI having 100% replacement of FM. All *C. mrigala* juveniles survive at same rate throughout experimental trial. Highest values of SGR (1.64) and condition factor (1.50) were observed at test diet III while lowest values of SGR (1.01) and condition factor (0.97) were seen at 100% replacement of FM with SPM. *Cirrhinus mrigala* juveniles fed at 40% replacement of FM have best FCR value (1.26) while maximum FCR value (1.81) was noticed at test diet VI. All these values differ significantly from each other and from control diet as well. All these values differ significantly from each other and from control diet as well. Majority of growth indices (WG, WG%, SGR, CF, FW) were significantly different in combined and quadratic tests while slightly non-significant in linear tests.

Body indexes analysis of *C. mrigala* fingerlings fed SPM replacing FM was presented in Table 3. Both HSI and VSI values differ significantly in all groups ($p < 0.05$). Highest values of HSI (1.95) were observed at test diet III while lowest HSI values (1.03) were noticed at 100% replacement of FM. All values of HSI differ significantly from each other. Highest values (13.53) of VSI were depicted in test diet VI while lowest values (6.43) were noticed at 40% replacement of FM. Values of VSI at test diet VI and control diet were slightly non-significant to each other. Statistically, it was evident that values of HSI and VSI differ significantly in linear, quadratic and combined test.

Digestive enzymes activities in intestine of *C. mrigala* fingerlings changes significantly when fed with diet having inclusion of SPM as shown in Table 4. Protease (38.33) activity was maximum at 40% replacement of FM while lowest protease level (18.33) was observed at control diet having 0% SPM. Lipase (36) and Amylase (42.33) activity was maximum at test diet VI followed by 39.33 and 34.33 for amylase and lipase at control diet and test diet V respectively. Significant differences were observed in these values. It was observed after statistical analysis that values of protease, lipase and amylase differ significantly in all tests (linear, combined and quadratic).

TABLE 2 Growth parameters of *Cirrhinus mrigala* fingerlings fed silkworm pupa meal (SPM).

TD	SPM	IW	FW	WG	WG%	WG fish^{-1} day^{-1}	Feed Intake	FCR	Survival rate	SGR	Length	CF
1	0	8.43± 0.20	28.79± 1.53^{de}	20.36± 1.70^{de}	241.78± 25.21^{de}	0.29± 0.02^{de}	0.50± 0.02^{bc}	1.71± 0.21^{cd}	94.00± 5.89	1.24± 0.02^{d}	11.10± 0.08^{b}	1.06± 0.04^{de}
2	20	8.45± 0.08	33.01± 1.98^{bc}	24.56± 2.03^{bc}	290.76± 25.57^{bc}	0.35± 0.03^{bc}	0.57± 0.02^{a}	1.63± 0.18^{bcd}	95.83± 3.61	1.43± 0.02^{c}	11.08± 0.03^{b}	1.23± 0.05^{c}
3	40	8.51± 0.07	37.16± 1.06^{a}	28.66± 1.04^{a}	333.44± 6.34^{a}	0.41± 0.01^{a}	0.52± 0.00^{b}	1.26± 0.05^{a}	98.04± 3.40	1.64± 0.03^{a}	10.94± 0.04^{b}	1.50± 0.07^{a}
4	60	8.49± 0.10	34.57± 1.39^{ab}	26.08± 1.49^{ab}	307.35± 21.19^{ab}	0.37± 0.02^{ab}	0.50± 0.01^{bc}	1.34± 0.09^{ab}	98.04± 3.40	1.53± 0.04^{b}	11.16± 0.04^{c}	1.35± 0.04^{b}
5	80	8.53± 0.09	31.33± 1.66^{cd}	22.80± 1.75^{cd}	267.60± 23.48^{cd}	0.33± 0.03^{cd}	0.48± 0.05^{bc}	1.47± 0.27^{abc}	96.08± 3.40	1.31± 0.07^{d}	12.24± 0.02^{a}	1.12± 0.08^{d}
6	100	8.56± 0.05	26.38± 1.41^{e}	17.83± 1.46^{e}	208.41± 18.22^{e}	0.25± 0.02^{e}	0.46± 0.01^{c}	1.81± 0.11^{d}	91.79± 3.72	1.01± 0.07^{e}	12.19± 0.04^{a}	0.97± 0.04^{e}
	StE	.02359	.91737	.92429	10.88154	.01320	.00970	.05864	.95663	.05044	.04457	.13217
p-value	*L*	.135	.021	.021	.024	.021	.002	.918	.604	<0.001	.049	<0.001
	Q	.982	<0.001	<0.001	<0.001	<0.001	.034	.001	.045	<0.001	<0.001	<0.001
	C	.727	<0.001	<0.001	<0.001	<0.001	.004	.013	.411	<0.001	<0.001	<0.001

Mean values within columns with various superscripts (a – e) differ considerably at $p < 0.05$.
Data are three replicates' mean (± shows Standard Deviations). TD = Test diets; IW = Initial weight; WG = Weight gain; FW = Final weight; FCR = Feed Conversion Ratio; SGR = Specific Growth Rate; CF = condition factor
StE = Standard Error; L = Linear, Q = Quadratic, C = Combined

The histopathological observation in *C. mrigala* fingerlings fed with different levels of SPM showed slight variations as depicted in fig 1 (I, II, III, IV, V, VI). At 0% replacement of SPM section reveals normal villous structures showing overall no shortening and fusion of mucosal folds, there is a little amount of brown material deposition in lumen, there is moderate chronic inflammatory cell infiltrate which was insignificant with test diet II and test diet V. It was noticed at 40% replacement of FM that there was no deposition of any material in lumen while no shortening of mucosal folds and moderate inflammation in cells was noticed. Severe mucous production and goblet cell production was noticed in cells of fingerlings when fed with test diet IV, V and test diet VI. Certain variation in mucosal folds and muscle thickness were also observed at 100% replacement of FM.

TABLE 3 Body indices of *Cirrhinus mrigala* juveniles fed silkworm pupa meal (SPM).

TD	SPM	HSI	VSI
1	0	1.14±0.11[e]	12.69±0.33[de]
2	20	1.45±0.03[c]	11.03±1.47[cd]
3	40	1.95±0.03[a]	6.43±0.50[a]
4	60	1.72±0.02[b]	8.38±0.06[b]
5	80	1.34±0.07[d]	10.76±1.49[c]
6	100	1.03±0.03[f]	13.53±1.00[e]
	StE	.07810	.62004
p-value	*L*	<0.001	<0.001
	Q	<0.001	<0.001
	C	<0.001	<0.001

Mean values within columns with various superscripts (a – f) differ considerably at $p < 0.05$.
Data are three replicates' mean (± shows Standard Deviations). TD = Test diets; HSI = hepato-somatic index; VSI = viscero-somatic index. StE = Standard Error; L = Linear; Q = Quadratic; C = Combined.

TABLE 4 Digestive Enzymes activity of *Cirrhinus mrigala* juveniles fed silkworm pupa meal (SPM).

TD	SPM	Protease	Amylase	Lipase
1	0	18.33±0.58[e]	39.33±0.58[d]	12.67±1.53[f]
2	20	24.67±0.58[d]	36.67±0.58[c]	22.33±0.58[e]
3	40	38.33±0.58[a]	29.67±0.58[a]	28.33±0.58[d]
4	60	35.00±1.00[b]	31.33±0.58[b]	31.33±0.58[c]
5	80	30.67±1.15[c]	36.33±0.58[c]	34.33±0.58[b]
6	100	25.67±0.58[d]	42.33±0.58[e]	36.00±1.00[a]
	StE	1.63210	1.06173	1.94071
p-value	*L*	<0.001	<0.001	<0.001
	Q	<0.001	<0.001	<0.001
	C	<0.001	<0.001	<0.001

Mean values within columns with various superscripts (a – f) differ considerably at $p < 0.05$.
Data are three replicates' mean (± shows Standard Deviations). TD = Test diets; StE = Standard Error; L = Linear; Q = Quadratic; C = Combined.

4 | DISCUSSION

Numerous researchers have examined the effects of SPM as a substitute for FM in diets for various species, and it is thought to be a viable feed ingredient with high-quality protein (Shakoori *et al.* 2015). The purpose of the current study was to examine how SPM, as opposed to FM, affected the growth, feed utilization, and survival of *C. mrigala* fingerlings.

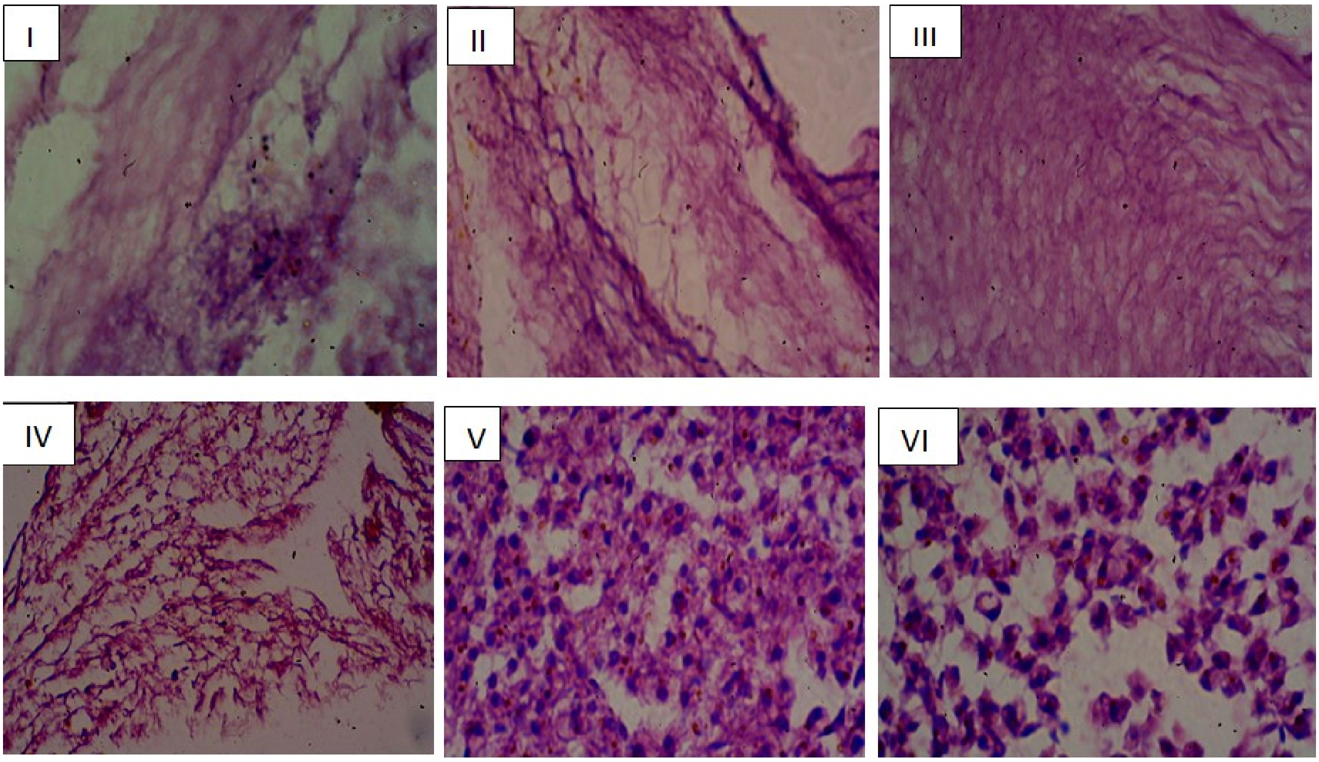

FIGURE 1 Histopathological examination of liver of *Cirrhinus mrigala* juveniles fed with different inclusion levels of silkworm pupa meal (SPM).

It was determined that SPM may be given to *C. mrigala* juveniles' diets up to 40% without affecting their growth, survival, or feed conversion ratios. Studies reveal that addition of SPM in fish diets result in better growth and ability of fishes to resist different infections increases (Shakoori *et al.* 2015). Similar findings were also reported by Mahato *et al.* (2023), who used silkworm pupa meal in diets of rainbow trout and discovered that SPM can be added to the diets of rainbow trout up to 35% without effecting growth of fingerlings. Findings of Ji *et al.* (2015) were also consistent with the current research; they showed that SPM can be added to Jian carp (*Cyprinus carpio* var. *jian*) diets up to 50%, resulting in the best FCR values and maximum growth rates. Higher levels of SPM in diets result in poor growth and maximum FCR. Similar to the current study, Karthick Raja (2019) found that SPM can be increased to major carps' diets up to 50%. In accordance with the survival findings of other researchers, including Shakoori *et al.* (2016) and Mahato *et al.* (2023), who investigated the impact of SPM on rainbow trout fingerling survival, the current study explains that SPM has no effect on fish survival and that all values were non-significant to one another in all groups. However, contrary, Salem *et al.* (2008) showed that when Nile tilapia were fed a diet containing 66% SPM, the fingerlings performed at their best, whereas higher protein levels caused stunted growth. Quite different from current study, Sathishkumar *et al.* (2021) explained the use of SPM in diets of GIFT tilapia and concluded that SPM can be incorporated into diets of tilapia up to 66% replacing FM. Quite different results were noticed by Shakoori *et al.* (2016) who explained that FM can be replaced with SPM at about 10% in rainbow trout. Completely different results from ours were observed by Olaniyi and Babasanmi (2013) who have study on African catfish, fed a diet having different levels of SPM. They discovered that SPM can be included up to 100% in cat fish diets without having any adverse effect on growth. In contrast to current study, Lee *et al.* (2012) depicted that SPM can be added into diets of juvenile olive flounder (*Paralichthys olivaceus*) at about 10% replacement of FM without having any bad impact on growth.

It was concluded from current experiment that hepato-somatic and viscero-somatic index of *C. mrigala* fingerlings fed SPM at varying levels were maximum at 40% replacement of FM. Analysis of body indices was primarily done to show the fish's metabolic activity, liver energy reserves, exposure to contaminants, feeding intensity, overall health, and fillet production capacity. SPM improves food absorption and decreases waste by increasing the activity of digestive enzymes including amylase and protease. Improved digestion promotes leaner body mass, increased muscle deposition, and optimal growth of internal organs (Xu *et al.* 2018). There is no research on the use of SPM in *C. mrigala* fingerling diets. The addition of insect meal (black army fly) to largemouth bass diets causes a fluctuation in HSI and VSI values, according to a similar discovery made by Peng *et al.* (2021). Wang ShuWen *et al.* (2015) found conflicting results, stating that the body indices of Nile tilapia were unaffected by the addition of SPM to their meals. Mastoraki *et al.* (2022), who investigated several insect meals and their incorporation in gilthead seabream (*Sparus aurata*) diets, observed different findings from the current study. HSI and VSI readings were found to be non-significant throughout all trial tests.

Current study was done using SPM at different inclusion levels replacing FM and its effect was studied on enzymes activity in intestine of *C. mrigala* fingerlings. In aquaculture diets, SPM has shown potential as a substitute for conventional fish meal, especially when it comes to increasing fish digestive enzyme activity (Vishnu *et al.* 2024). Methionine and lysine, two important amino acids that are frequently lacking in fish diets, are abundant in SPM. The synthesis and activity of digestive enzymes are indirectly influenced by this balanced amino acid profile, which promotes ideal development and metabolic processes (Hăbeanu *et al.* 2024). It was concluded that both protease and lipase activity was best at 40% replacement of FM. It was reported by Wang ShuWen *et al.* (2015) that 50% inclusion of SPM in diets of Nile tilapia results in increased activity of enzymes but higher levels stops their activity that come in line with the recent study. Nandeesha *et al.* (2000) reported that as the levels of SPM in diets of common carps increases the protease activity also increases but up to 50% replacement, quite similar to recent study. Protein deposition increases in the body of mirror carp as 50% SPM was added into its diet as FM replacement; nearly similar to current work (Ji Hong *et al.* 2012). Increased protease activity in cells of *C. mrigala* fingerlings was inversely related to lipase and amylase values. Ji *et al.* (2015) explained that when SPM was replaced with FM at different levels in diets of Jian carp, it was noticed that proteases work at their peak at 60% replacement of FM while as the level of SPM goes up there was a decline in protease activity.

In the current study, *C. mrigala* fingerlings fed SPM instead of FM underwent histopathological investigation. It was found that at high levels of SPM, there was chronic inflammation in the cells and increased production of mucous and goblet cells, while at lower levels, there were specific depositions in the lumen. There was no deposition and only a mild irritation in the fingerling cells after 40% FM replacement. Similar to the current work, Ji *et al.* (2015) found that extreme mucosal fold shortening, specific lymphoid aggregation, and severe chronic inflammation were all present in mirror carp at high levels of FM substitution with SPM, especially at 80% and high levels. Different substitution levels of proteins affect the formation of intestinal microvilli (e.g. rainbow trout, Caballero *et al.* 2002; sea bream, Caballero *et al.* 2003; *Cyprinus carpio*, Ostaszewska *et al.* 2010) that was quite similar to recent study. It was observed that several other insect meals including black soldier fly meal can improve intestinal histo-morphology in certain aquatic animals and became a promising feed source for aquaculture industry (Cummins *et al.* 2017). Ji *et al.* (2015) observed different results: mucosal folds were more shaped in mirror carp at control diet and 50% substitution of FM with SPM, whereas more debris was observed in fish cells at 60% replacement. Overall, it was determined that SPM can be introduced to *C. mrigala* fingerling meals at a rate of 40% without negatively affecting juvenile growth or performance. This makes the diet for fishes both economical and environmentally benign. Different fish species, different feed formulation techniques, and varied climatic circumstances could all be the cause of differences between the current study and earlier findings.

5 | CONCLUSIONS

From current experimental work, it was concluded that replacing FM with cost-effective and environment friendly insect meal based diet *i.e.* SPM at rate of 40% resulted in improved overall performance (growth, body indices, digestive enzymes activity and histopathology) in *C. mrigala* fingerlings. This research holds significant implications for multiple stakeholders in the aquaculture sector as it will reduce their reliance on FM, which helps address overfishing and aligns with global sustainability goals. It offers economic benefits by developing cost-effective SPM-based feed, making fish farming more profitable and accessible, especially for small-scale fish farmers in Pakistan. Additionally, the results can be directly applied by feed manufacturers and fish farmers, offering practical solutions for the aquaculture industry.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

AUTHORS' CONTRIBUTION

F Yasin conducted the feeding trial and prepared manuscript. MM Shahzad planned and supervised and provided all materials for research. Z Hussain and SM Hussain helped in manuscript preparing. SZ Hussain Shah and W Abbas helped in writing, review, and editing the manuscripts.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on a reasonable request from the corresponding author.

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