Pakistan J. Zool., pp 1-8, 2025. DOI: https://dx.doi.org/10.17582/journal.pjz/20241203040103

Effect of Silkworm Pupa Meal on Growth, Body Indices, Digestive Enzymes and Histopathology of Rohu Fingerlings

Fatima Yasin[1], Muhammad Mudassar Shahzad[1]*, Zawar Hussain[1], Syed Makhdoom Hussain[2], Rizwan Khurshid[3] and Asma Choudhry[1]

[1]*Department of Zoology, Division of Science and Technology, University of Education, Township, Lahore, Pakistan*
[2] *Department of Zoology, Government College University, Faisalabad, Pakistan*
[3]*Insect Breeder, Sericulture Officer, Forest Department, Lahore*

Article Information
Received 03 December 2024
Revised 05 April 2025
Accepted 18 April 2025
Available online 24 July 2025 (early access)

Authors' Contribution
FY conducted the feeding trial and prepared manuscript. MMS planned and supervised and provided all materials for research. ZH and SMH helped in manuscript preparing. RK provided all material for trial. AC helped in writing, review, and editing the manuscript.

Key words
Rohu, Digestive enzymes, Body indices, Histopathology, Silkworm Pupa

ABSTRACT

Aquaculture being the rapidly expanding food production sector meets the global need of food for rapidly expanding human population. Major feed source in aquaculture sector is fish meal (FM) but unprofitable cost and limited supply of FM lead to search for alternatives, high-protein feed sources. Silkworm pupa meal (SPM) being low in cost and high protein (50-60%) content was used as an alternative feed source for fishes. Current study was done to evaluate the best percentage of SPM to be used as replacement of FM while making a cost-effective feed for rohu fingerlings. Six test diets (0, 20, 40, 60, 80 and 100%) were prepared for fingerlings and fed to them twice a day at 4% of their live wet body weight for 90 days. Chromium oxide was used in diets as an inert marker. After every 14 days weight of the fingerlings were taken to determine growth parameters (weight gain %, FCR, SGR). At the end of experimental period, body indices, digestive enzyme activity and intestinal histopathology were analyzes. Highest growth rate (WG%: 388%, SGR: 1.76, FCR: 1.15, CF: 1.50) and body indices (HSI: 2.16, VSI: 8.38) values were observed at 60% replacement of FM. Digestive enzymes activity and intestinal histopathological examination explained that SPM can be easily replaced to an extent of 60% without effecting overall performance of rohu fingerlings and help in forming an environment friendly and cost-effective diet.

INTRODUCTION

By 2050, there will be nine billion people on the planet, increasing the demand for food (Hashem, 2022). As a result, nutritionists are searching for natural and sustainable dietary alternatives (Glausiusz, 2022). The need for food, especially animal proteins, is increasing along with the global population. Since aquatic life offers humans a necessary and practical supply of protein, aquaculture is a modern necessity (Hussain *et al*., 2024). Fish aquaculture is a major source of high-quality human proteins (Stankus, 2021). The constant increase in the demand for fish for human use is the main problem facing aquaculture today (Hussain *et al*., 2024). The extent of wild fish capture fisheries has reached its peak since the 1980s (FAO, 2022). Aquafeeds have relied on fishmeal (FM), a robust protein source, to support the fast growth and general well-being of farmed fish (Hussain *et al*., 2024). These days, FM is viewed as expensive and unsustainable, and the high cost of aquafeeds containing FM restricts aquaculture's ability to expand. However, the cost of raw materials like FM has rapidly climbed along with the growth in aquafeed production (FAO, 2018). Other sources with a higher protein content and similar nutritional benefits are being researched because FM is rare and costly (Daniel, 2018). Although plant-based sources are used in aquaculture, excessive use of plant-based feeds is discouraged due to the presence of some anti-nutritional components (Hussain *et al*., 2024).

Many insect meals (IM) have lately become appealing protein alternatives in aquafeeds because of their nutritional similarities to FM (Henry *et al*., 2015). Many studies have been conducted on the use of insects as components in aquafeed (Mastoraki *et al*., 2020a, b; Wang *et al*., 2022b). Insects are easier to develop and reproduce than other creatures, use less space and energy, and have

* Corresponding author: drmudassarshahzad@gmail.com
0030-9923/2025/0001-0001 $ 9.00/0

a significantly better growth ratio (Van Huis, 2022). An industry with a long history and a distinctive feature that started in Asia is sericulture (Zhao *et al*., 2023). Around the world, four types of natural silk are manufactured and sold. The word "silk" is frequently used to refer to the silk made 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). Silkworm pupae are the main output of the reeling industry; China produces over 400,000 tons of fresh silkworm pupae annually, accounting for 70% of global production (Zhao *et al*., 2023). Despite being high in protein and unsaturated fatty acids, silkworm pupae are natural protein supplies that are crucial for development and use (Sadat *et al*., 2022). The nutritional makeup of silkworm pupae reveals that their protein concentration varies between 48 and 94.98% DM (Hăbeanu *et al*., 2023). These new silkworm pupae are extremely biodegradable, which pollutes the environment and gives the nearby area a foul smell. When pupae are disposed of in vast quantities, the ecosystem in silk-producing regions may suffer. Thus, one key way to lessen the impact of the silk business on the environment is to use these wonderful resources to feed animals and poultry (Sheikh *et al*., 2018).

Labeo rohita is the most extensively farmed carp species in South Asia. Current study was done to evaluate the best levels of SPM replacing FM to study growth parameters, body indices, digestive enzymes activity and histopathological examination of rohu fingerlings. By increasing the effectiveness of nutrient digestion and absorption from their meal, digestive enzyme activity is essential for boosting fish growth. These enzymes help fish better absorb nutrients through their intestinal walls by dissolving food into smaller, more absorbable components.

MATERIALS AND METHODS

Experimentation site

Experiment was performed at Animal research station, Department of Zoology, University of Education, Lahore.

Fish and experimental conditions

Rohu juveniles were brought from Manawa Fish Hatchery, Lahore, Punjab, Pakistan and were adapted to the experimental conditions for fourteen days. Fingerlings were housed in specially designed aquarium tanks with a 100L water storage and were fed a basal diet during the acclimation period (Allan and Rowland, 1992). Regular observations of water quality parameters such as temperature, dissolved oxygen (Jenway 970), and pH (Jenway 3510) 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).

Silkworm pupa meal (SPM) preparation

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 (average weight: 8.0±0.5g) in each triplicate water aquaria for 90 days. 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.

For preparation of feed pellets, feed ingredients listed in Table I were analyzed using standard methods as explained by AOAC (1995). The ingredients were then ground and mixed with fish oil for 5-10 min adding 10-15% distilled water to prepare dough, which was then shaped into pellets using a pelleting machine. All prepared diets were dried and stored at 4°C. The rohu juveniles were fed at 4% of live wet body weight for two hours. The unused diet in the aquaria was removed after each feeding and the aquaria replenished with fresh water.

Study of growth parameters

Fingerlings were weighed after every fourteen days to analyze growth parameters such as weight gain, feed conversion ratio (FCR), specific growth rate (SGR), condition factor survival rate as per formulae explained by NRC (1993):

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

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

$$\text{SGR} = \frac{(\text{ln.final wt.of fish} - \text{ln.initial wt.of fish})}{\text{Trial day}} \times 100$$

$$\text{Condition factor (CF\%)} = \frac{\text{Final body weight (g)}}{\text{Length (cm)}^3} \times 100$$

$$\text{Feed intake fish}^{-1}\text{day}^{-1} = \frac{\text{dry feed consumed}}{[(\text{final BW} + \text{initial BW})/2]/\text{days}}$$

$$\text{Survival rate} = \frac{\text{final fish number}}{\text{initial fish number}} \times 100$$

For body indexes evaluation, the liver and intestine of three sedated fish from each group were dissected, weighed, and quantified using the usual procedure.

$$\text{Hepatosomatic index (HIS,\%)} = \text{liver weight}/\text{final body weight} \times 100$$

$$\text{Viscerosomatic index (VSI,\%)} = \text{viscera weight}/\text{final body weight} \times 100$$

Table I. 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	3.2	3.5	3.6	3.1	3.3	3.7

* 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. Data are three replicates' means (± show standard deviation). Mg, magnesium; P, phosphorus; Cu, Copper; Ca, Calcium; Fe, Iron; Na, Sodium; K, Potassium.

Digestive enzyme analysis

At the end of the experimental period, fish wee dissected and their intestines were taken out. The intestine was homogenized in 0.25 M sucrose buffer (5% w/v) and centrifuged at 10000 rpm for 30 min to get supernatant for estimation of protease according to Drapeau (1976), α-amylase activity according to Rick and Stegbauer (1974) and lipase activity according to Zamani *et al*. (2009).

Histopathological preparation

For histopathological analysis the intestine of rohu juvenile was taken out, and then fixed in 10% formalin and then processed for histological section cutting following the routine protocol. Histological section (6-8 µm thick) were stained in haematoxylin and eosine.

Statistical analysis

One-way analysis of variance (ANOVA) will be used to compare the data of all parameters using SPSS software (Steel *et al*., 1996). Duncan's new multiple range test will be used to do various comparisons having significance level at $P<0.05$.

RESULTS

Effect on fish growth

Table II shows effect of SPM as replacement of FM on growth parameters (FW, WG, WG%, FCR, SGR, CF) of rohu fingerlings. From results it was evident that growth of rohu juveniles increases when fed with SPM. Maximum growth of rohu fingerlings was observed at 60% replacement of FM with SPM and growth declines as SPM levels increase. Highest values of FW, WG, WG% and WG/ fish/ day were noticed at TD-IV while lowest values of FW, WG, WG% and WG/ fish/ day were observed at 100% replacement of FM. All rohu juveniles survived at same rate throughout the experimental trial. The maximum values of SGR and condition factor were noticed at TD-IV while minimum values of SGR and condition factor were observed at 100% replacement of FM. Fingerlings fed at TD-IV have best FCR values while maximum FCR values were noticed at 100% replacement of FM. All these values differ significantly from each other and from control diet as well. Majority of growth indices (WG, WG%, FCR, SGR, CF, FW) were significantly different in combined and quadratic tests while slightly non-significant in linear tests. Overall it was evident that 60% replacement of FM with SPM in diets of rohu juveniles resulted in best growth.

Effect on body indices

Table III shows the body indices of rohu fingerlings fed SPM replacing FM. Both HSI and VSI values differ significantly in all groups (P<0.05). Among all trial diets it was noticed that TD-IV had maximum values of HSI while lowest values of HSI were noticed at control diet. All values of hepato-somatic index differ significantly from

Table II. Growth parameters of rohu fingerlings fed SPM.

	TD-I	TD-II	TD-III	TD-IV	TD-V	TD-VI	St. E	p-value		
								L	Q	C
IW (g)	8.43±0.20	8.45±0.08	8.51±0.07	8.49±0.10	8.53±0.09	8.56±0.05	0.023	.135	.982	.727
FW (g)	29.53±1.42^{d}	32.32±1.19^{c}	37.16±1.06^{b}	41.47±0.89^{a}	35.55±1.30^{b}	25.28±0.93^{e}	1.295	.216	.000	.000
WG (g)	21.09±1.41^{d}	23.87±1.15^{c}	28.66±1.04^{b}	32.98±0.95^{a}	27.03±1.25^{b}	16.72±0.98^{e}	1.296	.169	.000	.000
WG%	250.21±17.49^{d}	282.47±12.82^{c}	336.87±12.06^{b}	388.59±14.69^{a}	316.93±12.88^{b}	195.49±12.58^{e}	15.284	.103	.000	.000
WG/fish/day	0.30±0.02^{d}	0.34±0.02^{c}	0.41±0.01^{b}	0.47±0.01^{a}	0.39±0.02^{b}	0.24±0.01^{e}	0.0185	.169	.000	.000
Feed intake	0.47±0.00^{d}	0.47±0.01^{d}	0.52±0.00^{c}	0.54±0.01^{b}	0.56±0.00^{a}	0.39±0.00^{e}	0.0136	.002	.000	.000
FCR	1.58±0.11^{ab}	1.37±0.05^{cd}	1.26±0.05^{de}	1.15±0.04^{e}	1.46±0.08^{bc}	1.65±0.09^{a}	0.0441	.170	.000	.000
SR (%)	94.00±5.89	95.83±3.61	98.04±3.40	98.04±3.40	96.08±3.40	91.79±3.72	0.956	.604	.045	.411
SGR	1.39±0.06^{d}	1.49±0.04^{c}	1.64±0.03^{b}	1.76±0.03^{a}	1.59±0.03^{b}	1.20±0.05^{e}	0.044	.020	.000	.000
Length	11.10±0.08^{d}	11.08±0.03^{d}	11.16±0.04^{d}	10.94±0.04^{e}	12.24±0.02^{c}	12.19±0.04^{c}	0.132	.000	.000	.000
CF	1.06±0.04^{de}	1.23±0.05^{c}	1.35±0.04^{b}	1.50±0.07^{a}	1.12±0.08^{d}	0.97±0.04^{e}	0.045	.049	.000	.000

Means within columns with various superscripts (*a-e*) differ considerably at p<0.05. Data are three replicates' mean (± shows Standard Deviations). SPM, silkworm pupa meal; IW, initial weight; WG, weight gain; FW, final weight; FCR, feed conversion ratio; SGR, specific growth rate; CF, condition factor. SR, servival rate; St. E, standard error; L, Linear, Q, Quadratic; C, Combined.

Table III. Body indices of rohu juveniles fed SPM.

T.D	SPM (%)	HSI	VSI
I	0	1.43±0.05^{e}	13.32±0.78^{a}
II	20	1.61±0.04^{d}	11.03±1.47^{b}
III	40	1.95±0.03^{b}	9.50±0.73^{bc}
IV	60	2.16±0.02^{a}	8.38±0.06^{c}
V	80	1.72±0.02^{c}	10.76±1.49^{b}
VI	100	1.67±0.07^{cd}	13.53±1.00^{a}
	St. E	.05808	.49786
p-value	L	.000	.000
	Q	.000	.000
	C	.000	.000

Means within columns with various superscripts (*a-e*) differ considerably at p<0.05. Data are three replicates' mean (± shows Standard Deviations). HIS, hepato-somatic index; VSI, viscero-somatic index. St. E, standard error; L, Linear; Q, Quadratic; C, Combined.

each other. Highest values of VSI were depicted in TD-VI while lowest values were noticed at 60% replacement of FM. Values of VSI at TD-VI and control diet were non-significant to each other. Statistically, it was evident that values of HSI and VSI differ significantly in linear, quadratic and combined test. It was obvious that body indices perform best at TD-IV having 60% replacement of FM.

Effect on enzyme levels

Digestive enzymes activities of rohu fingerlings were significantly effected when fed on SPM. Protease and lipase activity was maximum at TD-IV followed by TD-III (Table IV). Lowest values of protease and lipase were noticed at 0% replacement of FM. Maximum values of amylase were observed at 80% replacement of FM followed by TD-III, while the lowest values were observed at at 60% replacement of FM. It was observed after statistical analysis that values of protease, lipase and amylase differ significantly in all tests (linear, combined and quadratic). It was concluded that best digestive enzyme activities were noticed at 60% replacement of FM.

Table IV. Digestive enzymes activity in intestine of rohu fingerlings fed SPM.

T.D	SPM (%)	Protease (U/mL)	Amylase (U/mL)	Lipase (U/mL)
I	0	14.33±0.58^{e}	16.33±0.58^{e}	12.33±1.53^{e}
II	20	28.33±0.58^{d}	44.33±0.58^{c}	28.33±1.53^{d}
III	40	39.33±0.58^{b}	50.00±1.00^{ab}	37.67±0.58^{b}
IV	60	46.00±1.00^{a}	39.33±0.58^{d}	44.67±0.58^{a}
V	80	38.67±0.58^{b}	50.67±0.58^{a}	36.33±0.58^{b}
VI	100	34.67±1.53^{c}	48.67±1.15^{b}	34.33±0.58^{c}
	St. E	2.45645	2.89717	2.46652
p-value	L	.000	.000	.000
	Q	.000	.000	.000
	C	.000	.000	.000

Means within columns with various superscripts (*a-e*) differ considerably at p<0.05. Data are three replicates' mean (± shows Standard Deviations). St. E, standard error; L, Linear; Q, Quadratic; C, Combined.

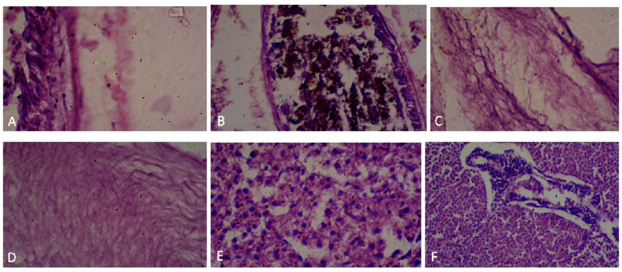

Fig. 1. Histopathological examination of intestine of rohu fingerlings fed with different inclusion levels of SPM. **A**) control diet explaining shortening of villous structure; **B, C**) 20% and 40% replacement of FM, respectively, mucosal folds seems fused and signs of material deposition in lumen. **D**) 60% FM replacement showed significantly different results. **E, F**) maximum replacement levels 80% and 100%, respectively showing highest deposition of material in lumen.

Effect on histological structure of intestine

Figure 1 shows histopathological changes in intestine of rohu fingerlings fed on SPM. At 0% replacement of SPM section compared with TD-I and II showed overall shortening of villous structure, fusion of mucosal folds, deposition of brown material in lumen and infiltration of moderate chronic inflammatory cell. It was noticed at 60% replacement of FM that there was no deposition of any material in lumen while shortening of mucosal folds and moderate inflammation in cells was noticed. Overall no major histopathological changes were noticed in fingerlings fed SPM.

DISCUSSION

Effect of SPM as FM replacement in diets have been studied by different researchers on different species and considered as promising feed ingredient having good quality protein (Shakoori *et al*., 2015). Current study was done to investigate the effect of SPM on growth, feed utilization and survival of rohu fingerlings replacing FM. It was concluded that SPM can added upto 60% in diets of rohu juveniles without effecting growth, survival and 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). Findings in line with the current research work, Ji *et al*. (2015) demonstrated that SPM can be added to the diets of Jian carp to an extent of 50% giving maximum growth rates and best FCR values. Addition of SPM above 60% results in reduced growth and higher FCR values. Similar to the recent study, Salem *et al*. (2008) depicted that adding 66% of SPM in diets of Nile Tilapia result in best performance of fingerlings while stunted growth observed at higher levels of protein. Karthick (2019) discovered that SPM can be added upto 50% in diets of major, quite similar to current study. Current study explain that SPM does not effect the survival of fish and all values were non-significant to each other in all groups which come in line with the survival results of different researchers; Shakoori *et al*. (2016) and Mahato *et al*. (2023) who studied effect of SPM on survival of rainbow trout fingerlings. 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 upto 66% replacing FM which is similar to current research work. Different from current study, a study done by Mahato *et al*. (2023) using silkworm pupa meal in rainbow trout concluded that SPM can be added to the diet of rainbow trout upto 35% without effecting the growth of fingerlings. 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 were observed by Olaniyi and Babasanmi (2013) who have study on African cat fish, fed a diet having different levels of SPM. They discovered that SPM can be included upto 100% in cat fish diets without having any adverse effect on growth. In contrast to current study, it was depicted by Lee *et al*. (2012) 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 ad viscero-somatic index of rohu fingerlings fed SPM at varying levels were maximum at 60% replacement of FM. Body indices analysis was basically done to indicate metabolic activity of fish, energy reserves in their liver, contaminant exposure, feeding intensity, health of fish and its ability to produce fillets. No work has been done using SPM in diets of rohu fingerlings. Quite similar observation was done by Peng *et al*. (2021) who explained that addition of insect meal (black soldier fly) in diets of largemouth bass results in variation of HSI and VSI values. Varying conclusion was observed by Wang *et al*. (2015) who said that inclusion of SPM in diets of Nile tilapia does not effect the body indices of fish. Different results from current study were noticed by Mastoraki *et al*. (2022) who worked on different insect meal and their inclusion in diets of gilthead seabream (*Sparus aurata*). They discovered that values of HSI and VSI were non-significant throughout all trial test. Similar to the recent reference it was explained by Ji-Hong *et al*. (2012) that inclusion of SPM in diets of common carp does not effect body indices of juveniles.

Current study was done using SPM at different inclusion levels replacing FM and its effect was studied on enzymes activity in intestine. It was concluded that both protease and lipase activity was best at 60% replacement of FM. Fish with higher levels of digestive enzyme activity grow more quickly because they are better able to break down, absorb, and convert nutrients into body mass and energy. Fish grow more effectively and are healthier as a result. Similar to current research it was explained by Ji *et al*. (2015) 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. It was reported by Wang *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 diet increases the protease activity also increases but upto 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).

Histopathological examination of rohu fingerlings fed SPM replacing FM was done in current study and it was observed that chronic inflammation in cells and certain lymphoid aggregates were present at high levels of SPM while at lower levels certain depositions were present. Because the type and quality of feed directly affect the health, growth, and general well-being of the fish, it is important to anticipate changes in the histopathology of fish after giving them specific feed. Histopathological analysis, which entails looking at tissue samples under a microscope, can show how feed affect the internal organs, tissues, and overall structure of fish.

At 60% replacement of FM slight inflammation and no deposition was noticed in cells of fingerlings. Likewise, to current study Ji *et al*. (2015) reported that at high levels of FM replacement with SPM in mirror carp particularly at 80% and high levels extreme shortening of mucosal folds were noticed and certain lymphoid aggregates along with severe chronic inflammation were also present. Different substitution levels of proteins as in rainbow trout (Caballero *et al*., 2002), sea bream (Caballero *et al*., 2003), *Cyprinus carpio* (Ostaszewska *et al*., 2010) effect the formation of intestinal microvilli that was quite similar to recent study. Different results were noticed by Ji *et al*. (2015) that at control diet and 50% replacement of FM with SPM in mirror carp the mucosal folds were more in shape and at 60% replacement more debris was noticed in the cells of fish. It was also reported that several other insect meals including black soldier fly meal can improve intestinal histo-morphology in certain aquatic animals upto 60% inclusion in diets becoming promising feed source for aquaculture sector (Cummins *et al*., 2017). Overall it was concluded that SPM can be added in to diets of rohu fingerlings at 60% without having any bad impact in growth or performance of juveniles and make a cost-effective and environment friendly diet for fishes. Variations of current study from previous data may be due to different fish species, different method of feed formulation, varying environmental conditions.

CONCLUSION

From current experimental work, it was concluded that replacing fish meal with cost-effective and environment friendly insect meal based diet i.e SPM at rate of 60% resulted in improved overall performance (growth, body indices, diegestive enzymes activity and histopathology) in rohu fingerlings.

DECLARATIONS

Acknowledgements

We are very grateful to University of Education management for providing us with a platform and guidance for the research purpose.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Allan, G.L. and Rowland, S.J., 1992. Development of an experimental diet for silver perch (*Bidyanus bidyanus*). *Austas. Aquacult*., **6**: 39-40.

AOAC (Association of Official Analytical Chemists), 1995. *Official methods of analysis, 15th ed.* Association of Official Analytical chemists, Washington, D.C. USA., pp. 1094.

Caballero, M.J., Izquierdo, M.S., Kjørsvik, E., Montero, D., Socorro, J., Fernandez, A.J. and Rosenlun, G., 2003. Morphological aspects of intestinal cells from gilthead seabream (*Sparus aurata*) fed diets containing different lipid sources. *Aquaculture*, **225**: 325–340. https://doi.org/10.1016/S0044-8486(03)00299-0

Caballero, M.J., Obach, A., Rosenlund, G., Montero, D., Gisvold, M. and Izquierdo, M.S., 2002. Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, **214**: 253–271. https://doi.org/10.1016/S0044-8486(01)00852-3

Cummins Jr, V.C., Rawles, S.D., Thompson, K.R., Velasquez, A., Kobayashi, Y., Hager, J. and Webster, C.D., 2017. Evaluation of black soldier fly (*Hermetia illucens*) larvae meal as partial or total replacement of marine fish meal in practical diets for Pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture*, **473**: 337-344. https://doi.org/10.1016/j.aquaculture.2017.02.022

Daniel, N., 2018. A review on replacing fish meal in aqua feeds using plant protein sources. *Int. J. Farm. Allied Sci*., **6**: 164-179.

Drapeau, G.R., 1976. Protease from *Staphyloccus aureus*. In: *Methods in enzymology* (ed. L. Lorand) (Vol 45), *Proteolytic enzymes* Part B, Academic Press, New York, pp. 469-475. https://doi.org/10.1016/S0076-6879(76)45041-3

FAO, 2018. *The state of world fisheries and aquaculture*. Meeting the sustainable development goals. Food and Agriculture Org.

FAO, 2022. *The world fisheries and aquaculture towards blue transformation.* FAO. Rome, Italy.

Glausiusz, J., 2022. Global population is crashing, soaring and moving. *Nature*, **604**: 33-34. https://doi.org/10.1038/d41586-022-00926-6

Hăbeanu, M., Gheorghe, A. and Mihalcea, T., 2023. Silkworms pupae as protein source for pigs. A review. *Sci. Pap. Ser. D. Anim. Sci*., **66**.

Hashem, S.M., 2022. Impact of mulberry silkworm (*Bombyx mori* L.) pupae powder on physicochemical properties and consumer preference of ready-to-eat smoked fish paste. *Ain Shams Eng. J*., **43**: 571-582. https://doi.org/10.21608/asejaiqjsae.2022.271008

Henry, M., Gasco, L., Piccolo, G. and Fountoulaki, E., 2015. Review on the use of insects in the diet of farmed fish: past and future. *Anim. Feed Sci. Technol*., **203**: 1–22. https://doi.org/10.1016/j.anifeedsci.2015.03.001

Hussain, S.M., Khurram, F., Naeem, A., Hussain Shah, S.Z., Sarker, P. K., Naeem, E., Arsalan, M.Z., Riaz, D., Yousaf, Z., Faisal, M. and Amjad, M., 2024. A review on the prospects and potentials of fishmeal replacement with different animal protein sources. *Int. Aquat. Res*., **16**.

Ji-Hong, J.H., Cheng, X.F.C.X., Li, J.L.J., Zhang, J.L.Z.J. and Liu, C.L.C., 2012. *Effect of dietary replacement of fish meal protein with silkworm pupae on the growth performance, body composition, and health status of* Cyprinus carpio *var. specularis fingerlings*. https://doi.org/10.3724/SP.J.1231.2012.27945

Ji, H., Zhang, J.L., Huang, J.Q., Cheng, X.F. and Liu, C., 2015. Effect of replacement of dietary fish meal with silkworm pupae meal on growth performance, body composition, intestinal protease activity and health status in juvenile Jian carp (*Cyprinus carpio* var. Jian). *Aquacult. Res*., **46**: 1209-1221. https://doi.org/10.1111/are.12276

Karthick Raja, P., Aanand, S., Stephen Sampathkumar, J., Padmavathy, P., 2019. Silkworm pupae meal as alternative source of protein in fish feed. *J. exp. Zool*., **7**: 78-85.

Lee, J., Choi, I.C., Kim, K.T., Cho, S.H. and Yoo, J.Y., 2012. Response of dietary substitution of fishmeal with various protein sources on growth, body composition and blood chemistry of olive flounder (*Paralichthys olivaceus*, Temminck & Schlegel, 1846). *Fish Physiol. Biochem*., **38**: 735-744. https://doi.org/10.1007/s10695-011-9555-3

Mahato, I.S., Timalsina, P., Paudel, K., Shrestha, A., Bhusal, C. and Kunwar, P., 2023. *Effects of replacing dietary shrimp meal and soybean meal with silkworm* (Bombyx mori) *pupae meal on growth performance of rainbow trout* Oncorhynchus mykiss. https://doi.org/10.22271/fish.2023.v11.i6a.2874

Mastoraki M., Mollá Ferrándiz P., Vardali S.C., Kontodimas D.C., Kotzamanis Y.P., Gasco L., Chatzifotis S. and Antonopoulou E., 2020a. A comparative study on the effect of fish meal substitution with three different insect meals on growth, body composition and metabolism of European sea bass (*Dicentrarchus labrax* L.). *Aquaculture*, **528**: 735511. https://doi.org/10.1016/j.aquaculture.2020.735511

Mastoraki M., Vlahos N., Patsea E., Chatzifotis S., Mente E. and Antonopoulou E., 2020b. The effect of insect meal as a feed ingredient on survival, growth, and metabolic and antioxidant response of juvenile prawn *Palaemon adspersus* (Rathke, 1837). *Aquacult. Res*., **51**: 3551–3562. https://doi.org/10.1111/are.14692

Mastoraki, M., Katsika, L., Enes, P., Guerreiro, I., Kotzamanis, Y.P., Gasco, L., Chatzofotis, S. and Antonopoulou, E., 2022. Insect meals in feeds for juvenile gilthead seabream (*Sparus aurata*): Effects on growth, blood chemistry, hepatic metabolic enzymes, body composition and nutrient utilization. *Aquaculture*, **561**: 738674. https://doi.org/10.1016/j.aquaculture.2022.738674

Nandeesha, M.C., Gangadhara, B., Varghese, T.J. and Keshavanath, P., 2000. Growth response and flesh quality of common carp, *Cyprinus carpio* fed with high levels of non-defatted silkworm pupae. *Asian Fish. Sci*., **13**: 235–242. https://doi.org/10.33997/j.

afs.2000.13.3.005

National Research Council, 1993. *Nutrient requirements of fish*. National Academies Press, Washington, DC, 114.

Olaniyi, C.O. and Babasanmi, G.O., 2013. Performance characteristics of African Cat fish (*Clarias gariepinus*) fed varying inclusion levels of silk worm pupae (*Anaphe infracta*). *Bhutan J. Anim. Sci.*, **42**: 76-80. https://doi.org/10.3329/bjas.v42i1.15787

Ostaszewska, T., Dabrowski, K., Kamaszewski, M., Grochowski, P., Verri, T., Rzepkowska, M. and Wolnicki J., 2010. The effect of plant protein-based diet supplemented with dipeptide or free amino acids on digestive tract morphology and PepT1 and PepT2 expressions in common carp (*Cyprinus carpio* L.). *Comp. Biochem. Physiol. A*, **157**: 158–169. https://doi.org/10.1016/j.cbpa.2010.06.162

Peng, K., Xiao, H.F., Mo, W.Y., Wang, G.X. and Huang, Y.H., 2021. *Effects of replacing fish meal with black soldier fly larvae meal on growth performance, physical indexes, body composition and nutrient retention rates of* Micropterus salmoides. *Chi. J. Anim. Nutr.,* **33**: 6340-6348. https://doi.org/10.3969/j.issn.1006267x.2021.11.034

Rick, W. and Stegbauer, H.P., 1974. Amylase measurement of reducing groups. In: *Methods of enzymatic analysis* (eds. H.V. Bergmeyer), Vol. 2, 2nd ed. Academic Press, New York, USA, pp. 885–889. https://doi.org/10.1016/B978-0-12-091302-2.50074-8

Rowland, S.J. and Ingram, B.A., 1991. Diseases of Australian native fishes. *Fish. Bull.*, **4**: 1.

Sadat, A., Biswas, T., Cardoso, M.H., Mondal, R., Ghosh, A., Dam, P., Nesa, J., Chakraborty, J., Bhattacharjya, D., Franco, O.L., Gangopadhyay, D. and Mandal, A.K., 2022. Silkworm pupae as a future food with nutritional and medicinal benefits. *Curr. Opin. Fd. Sci.*, **44**: 100818. https://doi.org/10.1016/j.cofs.2022.100818

Salem, M., Khalafalla, M.M.E., Saad, I.A.I. and El-Hais, A.M.A., 2008. Replacement of fish meal by silkworm *Bombyx mori* pupae meal in Nile tilapia, *Oreochromis niloticus* diets. *Egypt. J. Nutr. Feeds*, **11**: 611-624.

Sathishkumar, G., Felix, N. and Prabu, E., 2021. Growth performances and nutrient utilization efficiency of GIFT tilapia reared in floating net cages fed with bioprocessed silkworm pupae meal. *Aquacult. Nutr.*, **27**: 2786-2797. https://doi.org/10.1111/anu.13408

Shakoori, M., Gholipour, H. and Naseri, S., 2015. Effect of replacing dietary fish meal with silkworm (*Bombyx mori*) pupae on hematological parameters of rainbow trout *Oncorhynchus mykiss*. *Comp. Clin. Pathol.*, **24**: 139-143. https://doi.org/10.1007/s00580-013-1872-8

Shakoori, M., Gholipour, H., Naseri, S. and Khara, H., 2016. Growth, survival, and body composition of rainbow trout, when dietary fish meal is replaced with silkworm pupae. *Fish. Aquat. Life*, **24**: 53-57. https://doi.org/10.1515/aopf-2016-0006

Sheikh, I.U., Banday, M.T., Baba, I.A., Adil, S., Nissa, S.S., Zaffer, B. and Bulbul, K.H., 2018. Utilization of silkworm pupae meal as an alternative source of protein in the diet of livestock and poultry: A review. *J. Ent. Zool. Stud.*, **6**: 1010-1016.

Stankus, A., 2021. State of world aquaculture 2020 and regional reviews: FAO webinar series. *FAO Aquacult. Newsl.*, **63**: 17-18.

Steel, R.G. and Torrie, J.H., 1996. *Principles and procedures of statistics*. 3rd Ed. McGraw Hill International Book Company, Inc. New York. USA. pp. 336-352.

van Huis, A., 2022. Edible insects: Challenges and prospects. *Entomol. Res.*, **52**: 161-177. https://doi.org/10.1111/1748-5967.12582

Wang, W.S., Zhi, H.X., Li, L.L., Jun, C.Y., Jin, B.F. and Yun, L.Y., 2015. Effects of replacement of fish meal with silkworm pupae on growth performance, body composition and serum biochemical indices of genetically improved farmed tilapia (*Oreochromis niloticus*). *Chinese J. Anim. Nutr.*, **27**: 2774-2783.

Wang, T., Wang, X., Shehata, A.I., Wang, R., Yang, H., Wang, Y., Wang, J. and Zhang, Z., 2022b. Growth performance, physiological and antioxidant capacity responses to dietary fish meal replacement with insect meals for aquaculture: A case study in red claw crayfish (*Cherax quadricarinatus*). *Aquacult. Res.*, **53**: 3853–3864. https://doi.org/10.1111/are.15892

Zamani, A., Hajimoradloo, A., Madani, R. and Farhangi, M., 2009. Assessment of digestive enzymes activity during the fry development of the endangered Caspian brown trout Salmo caspius. *J. Fish Biol.*, **75**: 932–937. https://doi.org/10.1111/j.1095-8649.2009.02348.x

Zhao, X., Kuang, Z., Yang, J., Bilal, M. and Yang, R., 2023. Effect of fermented silkworm (*Bombyx mori*) pupae on growth performance, immune index and meat quality in Lingnan yellow broiler chickens. *Pakistan J. Zool.*, **55**: 1891-1898. https://doi.org/10.17582/journal.pjz/20220217030205