

Assessment of fermented silkworm pupae as fish meal for rohu (*Labeo rohita* H.)

Sumalini Bora^{1*}, K A Murugesh¹, P Priyadharshini¹,
S Aanand² & P Radha³

¹Department of Sericulture, FC&RI, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu 641301, India

²Erode Bhavanisagar Centre for Sustainable Aquaculture, Bhavanisagar, Erode, Tamil Nadu 638451, India

³Department of Forest Biology and Tree Improvement, FC&RI, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu 641301, India

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A sixty-day feeding trial was conducted to investigate whether solid-state fermentation (SSF) could enhance the nutritional profile of silkworm pupae (SWP) for use as a potential aqua feed ingredient of rohu fingerlings. Seven iso-nitrogenous experimental diets were formulated with 40% crude protein, where fishmeal (FM) was replaced with fermented SWP at levels of 15%, 20%, 25%, 30%, and 35%. Two additional diets were used as controls: one without SWP and another with 20% deoiled SWP (DSWP 20). Rohu fingerlings with an average initial weight of 5.16 ± 0.12 grams were stocked in tubs and fed twice daily at 5% of their body weight for 60 days. The results showed that fish fed a diet containing 30% fermented SWP meal exhibited the fastest growth, achieving a final weight of 11.22 ± 0.26 grams. This diet also demonstrated the highest specific growth rate (SGR) and protein efficiency ratio (PER) compared to the other groups. The improved growth performance may be attributed to the reduction of harmful substances in the SWP during the fermentation process, which enhanced the nutritional quality of the feed. Overall, the findings suggest that fermented SWP can be a valuable ingredient in aqua feed, particularly for rohu fingerlings. The successful replacement of FM with 30% fermented SWP meal in the diet resulted in better growth performance, indicating the potential of SWP as a sustainable and cost-effective feed ingredient. These results have implications for the development of more sustainable and environmentally friendly aquaculture practices.

Keywords: Fermentation, Fermented silkworm pupae, Fish feed, *Labeo rohita*, Silkworm pupae, Rohu

Carp is the primary aquaculture species, contributing over 71.9% to global freshwater production¹, with rohu being a particularly important cultivable fish. Successful aquaculture growth and intensification

heavily rely on aquafeed nutrition. The nutritional value of fish feed is mainly determined by the amount of protein in its ingredients²⁻⁴. Fishmeal (FM) has traditionally been the go-to protein source in fish feed^{5,6}. It is made by cooking, pressing, drying, and grinding fish or fish waste, widely used in aquaculture and livestock diets due to its excellent amino acid profile. FM serves as a rich source of essential nutrients like omega-3 fatty acids, vitamins, and minerals. The rising demand for animal and fish feed has increased the cost of fishmeal⁷. As of mid 2025, global 65% protein fishmeal costs around ₹1.44 lakh per metric tonne (approx. US \$1,688/t), and in India domestic prices have ranged between ₹1,35,000 and ₹1,50,000/t in recent years. As a result, there is an increasing demand for alternative protein sources to decrease the use of FM in fish feed. Although various animal and plant-based alternatives have been studied, none has been as effective as FM due to their inherent limitations⁸. However, terrestrial animal byproducts or insects are promising alternatives due to their high protein content, favourable amino acid profiles, low carbohydrate content, and absence of antinutritional factors^{4,8}.

While animal waste can be valuable source of protein in aquaculture feeds, their exclusive use can often lead to suboptimal animal productivity due to deficiencies or excesses in essential nutrients⁹. Combining animal waste with FM in practical diets has been proposed as a way to reduce costs without affecting fish growth or nutritional value^{4,10}.

Fermentation, a cost-effective and environmentally friendly biotechnological process, offers a solution to the problems in fish feed formulation¹¹⁻¹³. It improves both the nutritional value and digestibility of feed ingredients¹⁴. However, the greater digestibility and swift uptake of dietary proteins as free amino acids (FAA) can cause hyperaminoacidemia in various stomachless fish species, leading to protein loss and reduced growth rates¹⁵. Insects, such as silkworm pupae (SWP, *Bombyx mori*), represent a significant biomass and are valuable as a protein source¹⁶. Fermentation of these insects further enhances their protein content and minimises unpleasant odors¹⁷. In the sericulture industry, SWP are treated as waste and

*Correspondence:

E-mail: sumalinibora@gmail.com

are often discarded into the environment, leading to water and land pollution.

Taking these aspects into consideration, the present study is undertaken to evaluate the extent to which fermented SWP can replace fish meal (FM) in the diet of *Labeo rohita*.

Materials and Methods

Fermentation of silkworm pupae

SWP were obtained from a silk reeling center in Coimbatore District, where they were usually discarded after the silk reeling process. Collected pupae were thoroughly cleaned and sun-dried. The weight of the SWP was reduced by 80%. Subsequently, the dried pupae were ground into powder using a blender. Fermentation of deoiled silkworm pupae (DSWP) was performed following the protocol described by earlier researchers¹⁸ Briefly, 100 g/kg of baker's yeast (*Saccharomyces cerevisiae*, Instant Dried Yeast, Angel Yeast, China) and 50 g/kg of *Lactobacillus casei* (Yakult Danone India Pvt.; cell density: 0.1×10^9 cells/mL) were added to the DSWP. Subsequently, 700 mL/L of distilled water was incorporated into the mixture and thoroughly homogenized. The mixture was then sealed and incubated for fermentation under controlled conditions (29°C, pH 6.2) for 3 days. Upon completion, the fermented silkworm pupae (FSPW) were dried in a hot air oven. The dried fermented SWP were subsequently stored in an airtight container for further analysis. Biochemical analyses, including assessments of crude protein, moisture content, carbohydrate content and ash content were performed on the sample.

Preparation of experimental feeds

The five experimental feeds were prepared using appropriate quantities of finely ground rice bran,

groundnut oil cake, FM, fermented SWP and vitamin and mineral mix and water as a binding agent. Other two feeds which served as a control were formulated using the same ingredients except in one feed the FM was substituted with 20% deoiled SWP (DSPM 20) and in another there was no inclusion of SWP. In first feed the fish meal was replaced by 15% of fermented SWP (FSPM 15) and similarly in FSPM 20, FSPM 25, FSPM 30 and FSPM 35 the fermented SWP was included by 20, 25, 30 and 35% respectively. The feeds were pelleted using a grinder with a 1 mm mesh diameter and were then dried, labelled and used to feed the fish. The protein content of the formulated diets was kept at 40%. The ingredients used in the experimental diets are shown in Table 1.

Proximate analysis

Samples such as SWP, deoiled pupae, fermented SWP, and experimental feeds were analysed for crude protein¹⁹, ash²⁰, crude fiber²⁰, and lipids (Soxhlet with n-hexane). All analyses were conducted in six replicates (n= 6) and results were presented as mean \pm SD.

Collection, feeding and growth performance of the fingerlings

Healthy *Labeo rohita* fingerlings were obtained from Erode Centre for Sustainable Aquaculture located in Bhavanisagar, Erode District, Tamil Nadu, India in well-aerated plastic bags containing oxygen. The fish underwent a 20-day acclimatisation period within plastic tubs in order to adjust to their new environment and ensuring their health through a controlled, fish meal-based diet. Fingerlings measuring approximately 8.01 ± 0.01 cm in length and weighing around 5.16 ± 0.12 g were distributed into $60 \times 40 \times 40$ cm tubs, with ten fish per tub.

Each experimental group consisted of four replicates, and the fish in each replicate were fed

Table 1 — Ingredient composition and proximate content of experimental diets

Ingredients	FSPM 15	FSPM 20	FSPM 25	FSPM 30	FSPM 35	DSPM 20	FSPM 0
Fish meal	38.25	36	33.75	31.5	29.25	24	45
SWP	6.75	9	11.25	13.5	15.75	6	-
Ricebran	25	25	25	25	25	37.5	25
Groundnut oil cake	26	26	26	26	26	28.5	26
Vitamin premix	2	2	2	2	2	2	2
Mineral premix	2	2	2	2	2	2	2
Crude protein (%)	38.92 \pm 0.20	38.93 \pm 0.04	39.01 \pm 0.09	39.26 \pm 0.09	39.93 \pm 0.06	35.48 \pm 0.64	38.73 \pm 0.03
Crude fat (%)	9.17 \pm 0.03	9.26 \pm 0.08	9.28 \pm 0.07	9.29 \pm 0.02	9.35 \pm 0.01	7.97 \pm 0.28	9.12 \pm 0.01
Moisture (%)	9.50 \pm 0.05	9.49 \pm 0.06	9.44 \pm 0.05	9.43 \pm 0.19	9.42 \pm 0.07	8.57 \pm 0.09	9.53 \pm 0.03
Ash (%)	13.22 \pm 0.06	12.01 \pm 0.54	11.44 \pm 0.01	11.10 \pm 0.08 ^d	9.91 \pm 0.11	11.17 \pm 0.05	13.66 \pm 0.06
Crude fibre (%)	6.29 \pm 0.08	6.67 \pm 0.07	6.82 \pm 0.04	6.87 \pm 0.02	7.21 \pm 0.01	4.92 \pm 0.07	6.12 \pm 0.40

twice a day at 5% of their body weight, with feedings occurring at 8:00 AM and 4:00 PM for a period of 60 days. Waste materials, including uneaten feed, droppings, and other particles, were removed from the tubs daily using a siphon. Twenty five percent of the water was being replaced each day and complete water was changed once in five days. The fish were weighed after every 15 days and daily rations were adjusted accordingly. The observations were recorded to determine growth metrics and feed efficiency measures. The observations were

Weight gain (g): Determined by subtracting the initial average body weight (W1) from the final average body weight (W2) for each tank; Relative growth rate (g/day): Determined by dividing the weight gain by the experimental period in days (t); Specific growth rate (SGR) (% per day): Computed using the formula $[(\ln W2 - \ln W1) / t] * 100$, where \ln represents the natural logarithm; Protein efficiency ratio (PER): Determined by dividing the gain in wet body weight (g) by the amount of protein consumed (g); Feed conversion ratio (FCR): Determined by dividing the total feed intake (g) by the gain in wet body weight (g); Feeding rate (%): it is calculated as 5% of the body weight; Feed efficiency: the feed efficiency rate is calculated as the body weight gain divided by feed given; Survivability (%): the percentage is calculated by final fish obtained by initial fish stocked.

Statistical analysis

The data were examined using a one-way analysis of variance (ANOVA) with "SPSS" statistical software. Statistical significance was set at a 5% level ($P < 0.05$). Duncan's multiple range test ($P < 0.05$) was employed to identify significant differences between treatments.

Results and Discussion

Proximate composition

The chemical composition of mulberry SWP varied significantly by processing method (Table 2).

Fermented SWP had significantly higher crude protein (69.88 ± 0.42) and ash content (6.58 ± 0.35) and lower crude fat ($3.81\% \pm 0.72$) compared to non-deoiled and deoiled pupae. Non-deoiled pupae had significantly higher crude fat ($27.35\% \pm 0.07$) and moisture ($7.59\% \pm 0.06$) whereas deoiled pupae exhibited significantly higher ash content ($6.28\% \pm 0.56$) and crude fiber ($6.33\% \pm 0.11$).

Fermentation of SWP (SWP) with brewer's yeast (*Saccharomyces cerevisiae*) was taken up for nutrient enrichment and as a feed for rohu fry. In this study, the crude protein content of SWP improved from 46.51% to 69.88% under yeast fermentation with a change in other nutrient composition too. In the research done in 2015, it was seen that there was an increase in the protein content in sunflower meal from 30.70% to 38.40% when fermented with yeast for 48 hours²¹. In another research, it was observed that in the fermentation of poultry by products, protein and ash concentrations increased from 58.16% to 63.72% and 6.17% to 6.33% respectively whereas lipid (19.12 to 17.94 percent), moisture (8.61 to 7.36 percent) and fiber (3.56 to 3.42 percent) content degraded²². Researchers found that fermentation of soybean meal resulted in reduced crude fiber (8.84% to 6.13%) and ash content (8.62% to 7.45%) and increased crude protein (46.81% to 55.21%), crude lipid (1.84% to 1.93%) and moisture (9.35% to 9.64%) content. This shows a change in the nutrient profile after fermentation²³. The *L. casei* and *S. cerevisiae* have been shown to improve the nutritional value of feed ingredients²⁴. It was observed that the rise in the increase in protein content after fermentation is attributed to a decrease in the carbon ratio of the total mass. During the fermentation process, microorganisms utilise carbohydrates for energy, producing carbon dioxide as a by product. Consequently, the nitrogen in the fermented mixture becomes more concentrated, which results in a higher proportion of protein within the overall mass²⁵.

Table 2 — Proximate composition of silkworm pupae

Mulberry SWP	Crude protein (%)	Crude fat (%)	Moisture (%)	Ash (%)	Crude fiber (%)
Non deoiled	46.51± 0.63c	27.35± 0.07a	7.59± 0.06a	4.1 ± 0.18c	3.03± 0.02b
Deoiled	59.01± 1.39b	4.16± 0.082b	4.10± 0.11c	6.28 ± 0.56a	6.33± 0.11a
Fermented	69.88± 0.42a	3.81± 0.72c	5.16± 0.04b	6.58 ± 0.35a	3.02± 0.02b
SE(d)	0.74	0.51	0.063	0.323	0.054
CD(P=0.05)	1.85	1.28	0.158	0.805	0.134

[Values are expressed in mean ± SD with six replications (n=6). Means followed by different small superscript in a column are statistically different at $P \leq 0.05$]

Growth parameters

The study examined the effects of various diets on growth parameters, feed efficiency, and survivability of rohu fingerlings (Table 3). The results indicate significant differences across the various dietary treatments. The relative growth rate during the experiment is illustrated in Fig. 1. The graph shows the relative growth rates of rohu fingerlings over 60 days under various dietary treatments. All diets resulted in positive growth, with FSPM 30 showing the highest growth rate by day 60, followed closely by FSPM 25 and FSPM 35. FSPM 20 had moderate growth, while DSPM 20 and the control diet (FSPM 0) had the lowest growth rates throughout the study. The differences in growth rates between the higher-performing diets (FSPM 25, FSPM 30, FSPM 35) and the lower-performing ones (FSPM 15, DSPM 20, and the control) became more evident after 30 days, indicating that higher levels of FSPM are more effective for promoting growth in rohu fingerlings.

The initial weights of the fingerlings were fairly consistent across the different diet groups, ranging from 5.16 ± 0.12 g to 5.76 ± 0.04 g. However, by the end, the final weights varied significantly. The highest final weights were observed in the FSPM 30 (12.56 ± 0.26 g) and FSPM 25 (11.75 ± 0.14 g) groups, while the control group (FSPM 0) had the lowest final weight (7.59 ± 0.22 g).

Weight gain was highest in the FSPM 30 group (7.26 ± 0.27 g), followed closely by the FSPM 25 groups (6.59 ± 0.32 g). These values were significantly greater than the control group (2.34 ± 0.20 g) and the other treatment groups, indicating that diets with 25-30% fishmeal replacement yielded the best growth results.

The SGR was highest in the FSPM 30 group (7.40 ± 0.05 %/day) and FSPM 35 group (6.90 ± 0.40 %/day). The control group exhibited the lowest SGR (2.80 ± 0.07 %/day), emphasising the improved growth performance of fingerlings fed diets with higher of fishmeal replacement.

The FCR was lowest in the FSPM 30 group (0.014 ± 0.004), indicating the most efficient conversion of feed into body mass. The control group had a significantly higher FCR (0.17 ± 0.031), reflecting less efficient feed utilisation. The PER was also highest in the FSPM 30 group (0.69 ± 0.03), indicating better protein utilisation, while the control group had the lowest PER (0.37 ± 0.01).

The feeding rate was highest in the FSPM 30 group (0.494 ± 6.25 %) and lowest in the control group

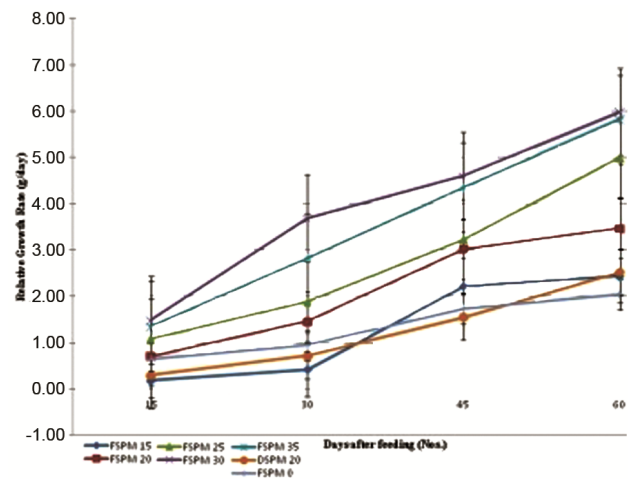


Fig. 1 — Growth performance of Rohu fingerlings under varying inclusion levels of fermented SWP.

Table 3 — Growth performance of rohu fingerlings under varying inclusion levels of fermented SWP

Parameter	Diet							CD	SE (d)
	FSPM 15	FSPM 20	FSPM 25	FSPM 30	FSPM 35	DSPM 20	FSPM 0		
Initial weight (g)	5.76±0.04	5.42±0.37	5.16±0.12	5.30±0.08	5.23±0.08	5.46±0.38	5.25±0.03	0.30	0.15
Final weight (g)	7.69±0.13	8.50±0.24	11.75±0.14	12.56±0.26	11.45±0.30	9.20±0.01	7.59±0.22	0.30	0.15
Weight gain (g)	1.93±0.15 ^d	3.08±0.60 ^c	6.59±0.32 ^a	7.26±0.27 ^a	6.22±0.18 ^b	3.74±0.36 ^d	2.34±0.20 ^d	0.49	0.23
Growth rate (%/ day)	4.43±0.04 ^d	5.85±0.08 ^c	6.20±0.14 ^b	7.40±0.05 ^a	6.90±0.40 ^a	5.20±0.11 ^d	2.80±0.07 ^d	0.10	0.06
Feed conversion ratio	0.147±0.019 ^b	0.118±0.009 ^c	0.043±0.005 ^f	0.014±0.004 ^g	0.066±0.002 ^e	0.097±0.025 ^d	0.17±0.031 ^a	0.026	0.012
Protein efficiency ratio	0.42±0.04 ^c	0.48±0.01 ^d	0.63±0.01 ^b	0.69±0.03 ^a	0.58±0.06 ^c	0.50±0.03 ^d	0.37±0.01 ^f	0.05	0.02
Feeding rate (%)	0.351±2.48 ^f	0.399±5.40 ^c	0.474±2.47 ^b	0.494±6.25 ^a	0.469±4.30 ^c	0.422±20.46 ^d	0.349±9.42 ^g	18.60	8.89
Feed efficiency (g)	6.05±1.11 ^f	7.32±0.41 ^c	11.10±0.66 ^b	12.40±0.76 ^a	9.89±0.24 ^c	8.59±1.52 ^d	4.78±0.62 ^g	1.25	0.61
Survivability (%)	80.8±0.33 ^d	83±1.63 ^c	83.9±1.25 ^c	87.8±0.58 ^a	85.5±0.38 ^b	82.9±0.80 ^c	79.6±0.49 ^d	1.3	0.64

[Values are expressed in mean ± SD. Means followed by different small superscripts in a column are statistically different at $P \leq 0.05$]

($0.349 \pm 9.42\%$). Feed efficiency was greatest in the FSPM 30 group (12.40 ± 0.76 g), followed by the FSPM 25 group (11.10 ± 0.66 g), with the control group showing the lowest feed efficiency (4.78 ± 0.62 g).

The survivability rate was highest in the FSPM 30 group ($87.8 \pm 0.58\%$) and lowest in the control group ($79.6 \pm 0.49\%$). This suggests that diets incorporating higher amounts of fishmeal alternatives not only boost growth and feed efficiency but also increase the overall survival rate of fingerlings. In conclusion, the FSPM 30 diets were the most effective in promoting growth, feed efficiency, and survivability, while the control group performed the poorest in all measured parameters.

This study demonstrated consistent weight gain across all treatments during the experiment, indicating that the rohu fingerlings responded well to all diets. The growth parameters of the fish in all groups with substituted fishmeal outperformed those in the control group. The growth rate was greater in the groups fed diets containing a mix of FM and fermented SWP, with the highest growth observed in the FSPM 30 diet. This suggests that a 30% replacement level achieved the optimal balance between nutritional enrichment and digestibility, providing the best synergy with fishmeal and maximising growth, feed efficiency, and survivability, without the negative effects observed at higher inclusion levels. In contrast, the growth rate was lower in the groups given diets consisting entirely of FM (FSPM 0) or defatted SWP (DSPM 20). These results demonstrate that partially substituting FM with fermented SWP led to better growth performance compared to feeding only FM or defatted SWP.

Little work has been done pertaining to the inclusion of SWP in fish feed for optimum growth of fishes. A study showed that the inclusion of sericulture waste resulted in better growth and conversion with the diet containing deoiled SWP, compared to the traditional feed blend of rice bran and mustard oil cake (1:3)²⁶. Earlier workers found that optimum inclusion of fermented SWP silage 30.2-30.9% protein levels gave better survival and growth parameters in carps fishes¹⁷. Inclusion of chicken viscera upto 35% gave good result in survival rate in rohu fingerlings²⁷. A similar study was done where fish meal was substituted by fermented fish offal in the diet of rohu. They reported that, incorporation of fermented fish offal in the diet of

rohu (*L. rohita*) by 30% to 35% protein gave the best growth with high feed intake, growth and body composition²⁸. Incorporating high levels of fermented cottonseed meal (over 30%) in the diets of rainbow trout (*Oncorhynchus mykiss*) and Nile tilapia (*Oreochromis niloticus*) has been shown to improve growth performance^{29,30}. A study demonstrated that replacing 20% of fishmeal protein with fermented soybean meal in the diet of juvenile rockfish, *Sebastes* sp., did not negatively impact growth performance. Conversely, replacing fishmeal with higher amounts of fermented soybean meal or pea protein meal (30-40%) in black sea bream diets resulted in decreased growth rates³¹. It was found that when fish meal is substituted with a fermented blend of SWP, rapeseed and wheat in minor carp (*Cyprnis carpio*), it shows better and fast growth³². Moreover in a research conducted on efficiency of fermented sunflower meal (FSFM) as a replacement for groundnut oil cake in *L. rohita* larvae, they found that 30% replacement of FSFM showed better growth with maximum SGR and PER³³. Due to the varying methodologies, including differences in experimental conditions and diet formulations, it is challenging to directly compare the results of these studies.

Conclusion

This study investigated the use of fermented silkworm pupae waste protein (SWP) as a substitute for fishmeal (FM) in the diet of *Labeo rohita* fingerlings. Results revealed that diets containing fermented SWP, particularly at a 30% inclusion level (FSPM 30), significantly enhanced growth performance, feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), and survival rates compared to conventional FM-based diets. The findings suggest that fermented SWP can effectively replace up to 30% of fishmeal without compromising fish health or performance. Given its abundance as a sericultural byproduct, fermented SWP offers a cost-effective and economically feasible alternative to fishmeal. Moreover, the simplicity of the fermentation process and its reliance on widely available microbial strains highlight its potential for scale-up and adoption in commercial aquaculture. This highlights its potential as a sustainable, eco-friendly protein alternative in aquaculture. Further studies should explore long-term impacts and broader application across fish species.

Conflict of interest

The authors have declared that no competing interests exist.

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