Effect of Silkworm pupae (*Bombyx mori*) as a fishmeal substitute on the growth parameters of Pangasius (*Pangasius pangasius*) fingerlings

**ABSTRACT**

This study aimed to determine the impact of silkworm pupae (*Bombyx mori*) as a fishmeal substitute on the growth parameters in Pangasius (*Pangasius pangasius*) fingerlings. Four treatments (T1, T2, T3 and T4) and one control (C) were set up with different percentages of silkworm pupae concentrations, which were 25, 50, 75, and 100 percent incorruption in basal diet. Each treatment has three replications and 27 percent crude protein diet was given to experimental fishes at the rate of 5 percent of body weight. The results showed significant (*p<0.005*) differences in different growth parameters like net weight gain (NWG), percentage weight gain (PWG), specific growth rate (SGR), net length gain (NLG), and feed conversion rate (FCR). The best result was found in treatment T3 and followed by T2, T1, C, and T4. This results in the viability of silkworm pupae as a replacement for fishmeal by T3, T2, and T1. This study will open the doors of new era in feeding management in fisheries sector.

**Keywords:** Digestibility, Pellets, Autoclave, Cocoon and Sericulture

**1.0 Introduction**

Fisheries are one of the fastest-growing fish food-producing sectors worldwide. In 2022, the global production of fisheries and aquaculture had reached 223.2 million tonnes, comprising 185.4 million tonnes of aquatic animals and 37.8 million tonnes of algae, representing a 4.4 percent increase from 2020. The majority (62%) of aquatic animals were caught and farmed in marine areas, with fisheries accounting for 69 percent and aquaculture 31 percent; the remaining 38 percent were sourced from inland waters, primarily through aquaculture (84%) and to a lesser extent, capture fisheries (16%). The majority (70%) of global aquatic animal production came from Asian countries, with China being the largest producer (36%), followed by India (8%), Indonesia (7%), Vietnam (5%), and Peru (3%) (**FAO, 2024**).

In India, the total production of aquatic animals from both marine and inland production is 175.45 for the year 2022 to 2023, where 44.32 lakh tonnes from marine and 131.13 lakh tonnes from inland. India contributed 8.92 percent in 2022 to 2023 the World's Fish Production. Andhra Pradesh has always been the topmost production state of fish in India, followed by West Bengal, the second. Andhra Pradesh has mostly been from inland fish production, whereas Karnataka has come first in marine fish production in 2022 to 2023. Fishmeal (FM) is a critical ingredient in the manufacture of commercial fish feed due to its balanced amino acid composition, high digestibility, and palatability, which enhances the uptake, digestion, and absorption of nutrients in fish (**Miles and Chapman, 2006**). The dwindling supply of wild-caught fish, coupled with the escalating demand for high-quality aquaculture feed, has rapid decline in availability, making it a less reliable and increasingly expensive ingredient for fish feed manufacturers. Shortage in fish meal results in the high price of fish feed (**Ayoola, 2010; Rana *et al*., 2009**), which demands low-cost alternative feed ingredients such as plant-based proteins, insect meal, and other low-cost options, to ensure the long-term viability and environmental sustainability of aquaculture practices. Silkworm pupae (SWP) have high-grade nutritional properties, including high protein content, low ash, and a balanced amino acid profile. Lysine and methionine are particularly high in silkworm pupae meal. Silkworms are typically raised for the production of silk yarn. The pupae remaining after reeling silk, pupae become a waste product that is often been discarded in an open environment or used as a fertilizer (**Wei *et al*., 2009**). The disposal of large quantities of pupae can create a serious environmental impact (**Wang *et al*., 2010**). Therefore, utilizing these pupae as a protein source in aquaculture feed formulation is a promising approach to reducing the environmental footprint of the sericulture industry, as it valorizes a previously underutilized by-product and decreases the industry’s reliance on resource-intensive fishmeal and fish oil. Therefore, the present study analysed the effect of fishmeal replacement with silkworm pupae meal (SPM) on growth performance, feed utilization for advanced fingerling in *Pangasius pangasius*.

**2.0 Materials and Methodology**

**2.1 Experimental site and location**

The research work was done in the laboratory department of Aquaculture, School of Agriculture, Sanjeev Agarwal Global Education (SAGE) University and Bhopal.

**2.2 Procurement of silkworm pupae meal**

Dried silkworm pupae were purchased from the district Sericulture Office, East Khasi Hills, Shillong, and Government of Meghalaya. The pupae taken out from the cocoon were sun-dried and ground into a powder form using a grinder. After grinding the silkworm pupae, the powdered form was stored in an air-tight container for future use.

**2.3 Experimental setup**

The experimental fishes (*P. pangasius*) were purchased from Prayash fish farm, Narmada Puram, Bhopal, Madhya Pradesh, and acclimatized for two weeks. During the acclimatization, the fish were fed a basal diet at 27 percent crude protein and 5 percent of their body weight. The water quality parameters (water temperature, pH, dissolved oxygen, and alkalinity) were checked every 3 days for 50% and 100% weekly. The experiment was conducted for 45 days with a setup of four treatments and one control with three replication, control diets (C) and the other four were treatment tanks (T1, T2, T3, and T4). The fingerlings of uniform size were distributed in five tanks with 05 nos. each treatment. The basal diet ingredients like fishmeal, rice bran, mustard oil cake, wheat flour, vitamin premix, and vegetable oil; (25:25:22:22:1:5) were prepared for the control diet, and the treatment tanks feed together with the basal diet, and silkworm pupae were prepared at 25%, 50%, 75%, and 100% to evaluate the effect of silkworm pupae in the growth of *P. pangasius* fingerlings, the combination of diet in different were mentioned in table 1.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **S.**  **No.** | **Diet Ingredients**  **(g/100g)** | **C**  **(Basal Diet)** | **T1**  **(25%)** | **T2**  **(50%)** | **T3**  **(75%)** | **T4**  **(100%)** |
| **1.** | **Fishmeal** | 37.5 | 28.12 | 18.75 | 9.37 | 0 |
| **2.** | **Rice bran** | 37.5 | 37.5 | 37.5 | 37.5 | 37.5 |
| **3.** | **Mustard oil cake** | 33 | 33 | 33 | 33 | 33 |
| **4.** | **Wheat flour** | 33 | 33 | 33 | 33 | 33 |
| **5.** | **Vitamin premix** | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| **6.** | **Vegetable oil** | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 |
| **7.** | **Silkworm pupae** | 0 | 9.37 | 18.75 | 28.12 | 37.5 |
|  | **Total** | 150gm | 150gm | 150gm | 150gm | 150gm |

**Table1. Experimental Fish Feed Composition**

**2.4 Water Quality Parameters:**

The selected water quality parameters were analysed at the interval of 7 days of different parameters like water temperature (0C), pH, dissolved oxygen (mg/l), alkalinity (mg/l) were analyzed according to **APHA. 2005**.

**2.5 Growth Parameter**

The growth parameter was examined at an interval of every 15-day gap. Throughout the experimentation, the growth parameters of experimental fish, *viz.* Body weight and total length of fish were measured. Based on the following formula, the weight of each fish, net weight gain (NWG), percentage weight gain (PER), specific growth rate (SGR), feed conversion ratio (FCR), and net length gain (NLG) were recorded.

1. Net Weight gain (g) = Final Weight Gain (g) – Initial Weight Gain (g)
2. Net Length gain (g) = Final Length Gain (g) – Initial Length t Gain (g)

1. Percentage weight gain (%) = Mean Final Weight- Mean Initial Weight

X 100

Mean Initial Weight

1. Specific growth rate (SGR):

SGR = (ln Wt- ln Wo) x 100

D

Where,

W = Initial weight of live fish (g)

W = Final weight of live fish (g)

D = Duration of feeding (days)

1. Feed conversion ratio (FCR) = Feed given (g) / Weight gain (g)

**Statistical Analysis**

The data were analysed using one-way analysis of variance (ANOVA) to assess significant differences among treatment group means. A *p-value* of less than 0.05 was considered statistically significant and all data were presented as mean ± standard error. All statistical analyses were performed using SPSS software 16.0.

**3.0 RESULT:**

At the end of the experiment, the relationships between NLG, NWG, PWG, SGR, and FCR were significantly (p<0.05) impacted by the diet contains with silkworm pupae as a substitute for fishmeal. NLG, NWG, PWG, SGR, and FCR showed the best result in treatment T3, followed by T2, T1, Control (C), whereas the lowest result was found T4 (Table 2, Fig. 1.0). The highest NLG value (*p < 0.05*) was observed in fish fed diet T3 (5.86±0.051), followed by T2, T1, and C, with the lowest (p < 0.05) in fish fed T4 (4.32±0.051) (Table 2). The highest NWG value (p < 0.05) was observed in fish fed diet T3 (34±0.346), followed by T2, T1, and C, with the lowest (p < 0.05) in fish fed T4 (24.9±0.491) (Table 2). The highest PWG value (p < 0.05) was observed in fish fed T3 (86.07±0.005), followed by T2, T1, and C, with the lowest (p < 0.05) in fish fed T4 (60.73±0.005) (Table 2). The highest SGR value (p < 0.05) was observed in fish fed T3 (1.37±0.010), followed by T2, T1, and C, with the lowest (*p < 0.05*) being observed in fish fed T4 (1.05±0.010) (Table 2). The better FCR significantly (p<0.05) was observed in fish fed T3 (2.61±0.005), followed by T2, T1, and C, with the lowest FCR observed in fish fed T4 (3.7±0.057) (Table 2).

The growth difference of each growth parameter from 0-45 days is shown below in each table. NLG length variation of Pangasius fed with SWP as a substitute for FM showed a non-significant (p > 0.05) at first, on 0-15 days and 16-30 days. However, showed significant (p < 0.05) differences on 31-45 days and 0-45 days (Table 1, Fig. 1.0). NWG of Pangasius body showed significant (p<0.05) differences during the entire observation of the experiment (Table 2, Fig. 1.0). PWG of Pangasius also showed significant (p < 0.05) differences during the entire observation of the experiment (Table 2, Fig. 1.0). SGR of Pangasius per day ranges within 0.94-1.6 during the entire experiment and showed significant (p<0.05) differences within 0-15 days,16-30 days, and 0-45 days; however, non-significant (p>0.05) in 31-45 days (Table 2, Fig. 1.0). FCR of the Pangasius showed significant (p<0.05) differences during the entire experiment (Table 2, Fig. 1.0).

**3.1 Water parameter**

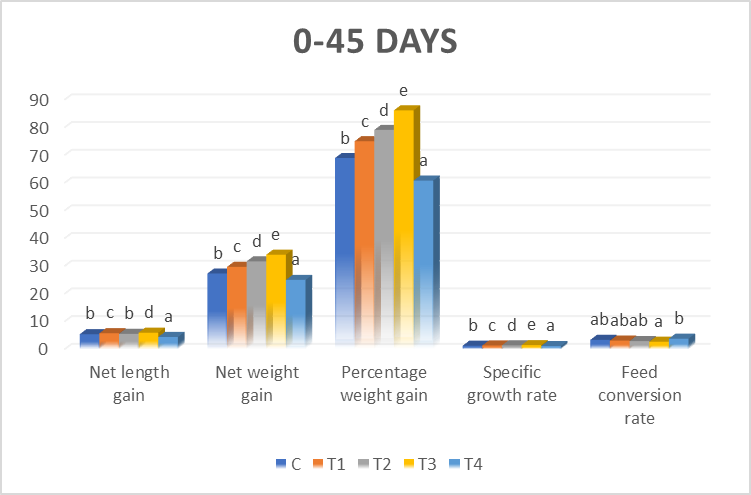
The water temperature was slightly different among the tanks (Table 3), and it ranged from 25 to 27 °C, which was optimal for fish production. Water pH (Table 3) ranged from 7.6 to 8.8. Dissolved oxygen (Table 3) concentrations were highest in T3 (8.7 mg/L), followed by T2 (8.5 mg/L), T1 (8.3 mg/L), T4 (8 mg/L), and C (8 mg/L). The total alkalinity was observed in the range of 373 to 384 (Table 3).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatment** | **Net length gain** | **Net weight gain** | **Percentage weight gain** | **Specific growth rate** | **Feed conversion rate** |
| C | 5.32b±0.051 | 27.2b±0.346 | 68.86b±0.005 | 1.16b±0.010 | 3.27ab±0.005 |
| T1 | 5.46b±0.051 | 29.6c±0.346 | 74.93c±0.005 | 1.24c±0.010 | 3ab±0.577 |
| T2 | 5.66c±0.051 | 31.6d±0.057 | 79d±0.577 | 1.29d±0.010 | 2.85ab±0.005 |
| T3 | 5.86d±0.051 | 34e±0.346 | 86.07e±0.005 | 1.37e±0.010 | 2.61a±0.005 |
| T4 | 4.32a±0.051 | 24.9a±0.491 | 60.73a±0.005 | 1.05a±0.010 | 3.7b±0.057 |

**Table 2.** Overall growth parameter of Pangasius fed with diets SWP as a replacement for FM

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatment** | **Water temperature (oC)** | **pH** | **Dissolved Oxygen (mg/lit.)** | **Alkalinity (mg/lit.)** |
| C | 25 | 8.1 | 8 | 380 |
| T1 | 26.3 | 7.9 | 8.3 | 376 |
| T2 | 26.5 | 8.5 | 8.5 | 381 |
| T3 | 27 | 8.8 | 8.7 | 384 |
| T4 | 26 | 7.6 | 8 | 373 |

**Table 3.** Water parameters were observed during the entire experiment.



**Fig. 1 Growth Parameters of Pangasius**

**4.0 Discussion**

After analyzing the replacement of FM with SWP, it was observed that NLG, NWG, PWG, SGR, and FCR growth progress occurred in Pangasius. However, in the experiment, the growth parameters of fish fed T3, T2, T1, and C showed a better weight gain than those of fish fed T4. This indicated that 100% replacement of FM with SWP or FM alone was not efficacious for digestion in Pangasius. This was in line with the growth rate observed by **Kurbanov *et al***. (**2015**), which was higher in group fed the diets with mixed fishmeal and silkworm pupae protein (SPP) (the highest was FM50) and lower was observed in the group fed 100 % of SPP or fishmeal (denoted as FM0 and FM100, respectively). The study showed that partial replacement of fishmeal with SPP can enhance higher growth performance than feeding on single fishmeal. **Hossain *et al*.**(**1992**) recommended that the feed conversion ratio, protein efficiency ratio, and apparent net protein utilization in the diet of catfish (*Heteropneustes fossils*) using SWP can be replaced up to 75% of fishmeal without affecting the growth rate. **Mahata *et al***. (**1994**), whose experiment involved silver barb (*Barbonymus gonionotus*) fingerlings, indicated that replacing FM with 38% SWP performed better than a fish meal-based diet.

The highest value of the SGR in the present results was obtained from the diet T3, having 75% of SWP, and the range of SGR in the present study was from 1.05 to 1.37% day-1. **Karthik *et al***. (**2020**) reported that the SGR of Rainbow shark (1.80 to 2.54% day-1) showed better results when fed with a diet containing 30% of SWP. **Begum *et al***. (**1994**) reported that rohu’s SGR (1.64% day-1) performed better when fed with a 50% SWP diet. **Habib *et al***. (**1994**) found that the SGR values (2.06 to 2.12% day-1) of *Clarius batrachus* were well-performed and higher when fed with SWP diet as compared to fish meal, bloodworm, and poultry byproduct meal supplemented diets (1.94 to 1.99% day-1). However, it was observed that SWP was a more affordable and effective protein source than poultry byproduct meal and blood meal in the diet of *C. batrachus*. Similarly, ***Salem et al***. (**2008**) in Nile tilapia (1.2 to 1.425 day-1) and **Ji *et al*.** (**2015**) in Jian carp (2.10 to 2.32% day-1) contained a close range of SGR values when fed diets containing different levels of FM substituted with SWP.

Feed conversion ratio (FCR) is a crucial growth determinant, as feed costs account for a substantial portion of the total operational expenses in aquaculture (**Amin *et al*., 2005**). The FCR value of the present results was found to range from 2.61 to 3.7. **Habib *et al*.** (**2001**) observed FCR values of 1.95 to 2.70 in *C. batrachus* when fed with the SWP diet. **Nandheesa *et al*.** (**1988**) observed that FCR (2.07 to 2.48) was better when fed the catla-rohu hybrid with the diet involving SWP. **Nandheesha *et al*.** (**1990**) reported that the FCR was better when fed common carp fry with SWP diet. Similarly, **Jayaram and Shetty** (**1980**) in *L. rohita* fingerlings and **Oso and Iwalaye** (**2014**) in *C. gariepinus* juveniles found a better FCR when fed with diets containing different levels of SWP.

In our present study, T2 and T3 (respectively SWP 50% and SWP75%) substitutes of FM with SWP showed significantly better growth performance than the control diet (C) and T4 (SWP 100%). **Begun *et al*.** (**1994**) in rohu and **Nandheesa *et al*.** (**2000**) in common carp studies showed that replacement of FM with SWP was best performed in 50% FM replacement. The present study found that fish fed diets with 50% and 75% substitution of FM with SWP had better weight gain compared to those fed a diet with C, T1, and T4 (respectively FM 100%, SWP 25%, and SWP 100%). **Karthick Raja *et al*.** (**2019**) found that fish fed diets with 40% or 50% fishmeal replacement with silkworm pupae had significantly lower weight gain compared to those fed diets with 30% replacement. Higher levels of SWP in the diet led to a notable decline in the fish growth rate. Similar results were observed in Jian carp (**Ji *et al*.,** **2015**) and Nile tilapia (**Salem et al., 2008**). The reduced growth rate may be attributed to the high chitin content (5-15%) in SWP, which fish struggle to digest effectively (**Cummins *et al*., 2017**). Additionally, studies have shown that replacing fishmeal with insect meal increases chitin levels, negatively impacting protein digestibility (**Longvah *et al*., 2011**) and lipid digestibility (**Kroeckel *et al*., 2012**). **Salem *et al*.** (**2008**) found that silkworm pupae meal can be effectively substituted for fishmeal up to 66.66% in Nile tilapia diets, yielding positive effects on growth performance, protein utilization, feed conversion, and economic returns. **Khatun et al.** (**2005**) claimed that improved fish growth was observed when silkworm pupae meal replaced fishmeal at a rate of 6-8%. Optimal growth was achieved in *C. gariepinus* when 25% of fishmeal protein was replaced with Bombyx mori. (**Kurbanov et al., 2015**). Pacific white prawns (*Litopenaeus vannamei*) thrived on a diet with 75% fishmeal replaced by silkworm pupae, showing good growth and immune response (**Rahimnejad *et al*., 2019**).

**Conclusion**

Silkworm pupae protein is rich in essential amino acids like methionine, phenylalanine, and valine, which aligns with the amino acid needs of fish. Based on the findings of this study, it can be inferred that silkworm pupae (SWP) are a viable substitute for fishmeal (FM) in the diet of Pangasius. Notably, replacing 75% of fishmeal with SWP yielded the most efficient results. However, as the proportion of SWP in the diet increased beyond this point, growth rates decreased, and feed conversion ratios (FCR) worsened. Despite this, SWP offers a significant advantage in terms of cost, being cheaper than FM. Therefore, incorporating SWP into the diet of rainbow sharks could provide farmers with a cost-effective alternative protein source, potentially enhancing their yields and improving the economic viability of their operations.

**Availability of data and Materials**

The data will be provided upon request to the journal.

**Ethical Statement:**

In the present study, rohu were collected from the School of School, Sanjeev Agrawal Global Educational (SAGE) University, and Bhopal India). Ethical approval, specimen collection, and maintenance were performed in strict agreement with all the recommendations India.

**Conflict of interest**

The authors state that there are no conflicts of interest regarding the publication of this research paper.

**Disclaimer (Artificial Intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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