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# Impact of dietary protein supplements on the biological performance of mulberry silkworm (Bombyx mori L.)

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#### Abstract

The mulberry silkworm (Bombyx mori L.) serves as an important lepidopteran model organism in nutritional and developmental biology. Its growth, development, and reproduction are strongly influenced by dietary composition, particularly protein availability. The present investigation was conducted to evaluate the effect of dietary protein-treated mulberry leaves on the biological traits of B. mori under controlled conditions at the Experiential Learning Programme Unit on Commercial Sericulture, College of Agriculture, Latur, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, during March-April 2025. The bivoltine double hybrid CSR2 strain was reared on V-1 mulberry variety in a Completely Randomized Design with three replications. Supplementation of mulberry leaves with various plant-derived protein sources significantly altered larval and pupal biology. Among the treatments, mulberry leaves fortified with soya flour at 0.5% concentration yielded the most favourable results. Larvae fed on this diet exhibited a significantly reduced larval duration of 28.63 days and pupal duration of 8.67 days, compared to 31.13 days and 11.33 days, respectively, in the control (distilled water treated leaves). These findings demonstrate that protein enrichment accelerates the developmental cycle of B. mori, likely by improving protein assimilation efficiency and energy metabolism during the critical larval and pupal stages. Reproductive performance was also markedly enhanced under protein supplementation. The highest moth emergence (97.07%), fecundity (515.33 eggs per female), and egg hatching percentage (97.30%) were obtained with soya flour supplementation. Conversely, the control treatment recorded significantly lower values for moth emergence (84.80%), fecundity (440.33 eggs), and egg hatching (89.30%). These improvements suggest that dietary proteins play a direct role in enhancing reproductive physiology, possibly by modulating vitellogenin synthesis and other egg-associated biochemical pathways. Overall, the study establishes that dietary protein fortification of mulberry leaves exerts a profound influence on the biological traits of B. mori. Soya flour at 0.5% proved to be the most effective supplement, reducing developmental duration and improving reproductive efficiency. The untreated control consistently produced inferior biological outcomes, underscoring the significance of protein nutrition in silkworm biology.

**Keywords:** *Bombyx mori*, dietary proteins, larval biology, pupal duration, reproductive efficiency, nutritional physiology

#### Introduction

Silkworm (*B. mori*) is a monophagous lepidopteron insect that is reared for silk production under a special controlled environment (Fambayun *et al.* 2022) <sup>[8]</sup>. Silkworm derives all essential nutrients from mulberry leaf for growth and development (Chanotra, and Bhat 2021) <sup>[5]</sup>. The growth and development of silkworm larvae is greatly influenced by the mulberry leaves' quality (Prasad and Madhavi 2020; Murugesh *et al.* 2022; Saha *et al.* 2022) <sup>[25, 23, 31]</sup>. The production of cocoons is also affected by different factors including environmental conditions, planting and pruning and soil characteristics that affect mulberry leaf quality (Samami *et al.* 2019) <sup>[32]</sup>. The dietary and nutritional requirements of the silkworm including water, vitamins, carbohydrates, proteins, fats and ascorbic acid are met by digestion and assimilation of mulberry leaves (He *et al.* 2021; Rajan *et al.* 2022) <sup>[12, 28]</sup>. Furthermore, proteins present in mulberry leaves contribute to more than 70% of silk synthesis in silkworms, thereby playing a crucial role in determining cocoon yield. (Alipanah *et al.* 2020) <sup>[2]</sup>.

Nutritional supplementation plays a crucial role in improving the growth and productivity of silkworms. Additives enriched with vitamins, amino acids, and proteins have been reported to significantly enhance the economic traits of Bombyx mori (Amalarani et al., 2011) [3]. Soy protein, being a rich dietary source, has been recognized for its role in enhancing larval growth and improving the economic traits of Bombyx mori. Daily supplementation of soy protein significantly contributes to increased quality and quantity of silk cocoon production (Kamaraj et al., 2017) [16]. Larvae of B. mori have been successfully reared on semi-artificial diets supplemented with various protein sources such as soybean, mushroom, corn flour, and their mixtures. Among these, corn flour-based diets during the fifth instar recorded the highest values for larval duration, larval weight, silk gland weight, pupal weight, cocoon and shell weight, as well as the number of eggs deposited, while also resulting in the lowest mortality percentage (Mona Mahmoud, 2013) [21].

The growth and development of silkworms are strongly influenced by the nutritional quality of mulberry leaves (Masthan et al., 2011) [22]. This nutritional value can be further enhanced through enrichment with supplementary nutrients. Studies have demonstrated that honey supplementation improves both biological performance and economic traits of Bombyx mori (Saad et al., 2014; Kamel et 2017: Thulasi and Siva Prasad, Alagumanikumaran and Prema, 2016; Gad, 2013) [30, 17, 33, 1, <sup>9]</sup>. Protein-rich food sources are recognized for enhancing the economic traits and supporting the growth of silkworms, with soy protein being a notable example (Rahman, 2018). Bombyx mori utilizes all amino acids in egg albumen, especially the essentials during growth and development, to synthesize silk fiber (Islam et al., 2020) [13]. To assess this, the effect of different dietary protein-treated mulberry leaves was investigated on the life cycle of the mulberry silkworm (Bombyx mori L.).

#### **Materials and Methods**

The study was conducted during March-April 2025 at the Experiential Learning Programme Unit, Department of Agricultural Entomology, College of Agriculture, Latur, Maharashtra, to evaluate the effect of protein-treated mulberry leaves on economic traits of *Bombyx mori*.

#### **Materials**

Disease-free layings (DFLs) of mulberry silkworm were fed on mulberry variety V-1 leaves sourced from the Instructional-cum-Research Farm, Department of Agricultural Entomology, College of Agriculture, Latur. Essential equipment included plastic rearing trays (36"  $\times$  24"  $\times$  3"), iron stands, chopping boards, knives, bamboo sticks, feathers, cleaning nets, collapsible plastic mountages (2  $\times$  0.95 m²), paraffin papers, foam pads, and a digital electronic balance for weighing larvae, pupae, cocoons, and shells.

#### **Experimental treatments**

Eight treatments involved mulberry leaves sprayed with 0.5% protein supplements derived from soya, groundnut, pigeon pea, pea, gram, black gram, green gram flours, and distilled water as a control. Leaves were sprayed, air-dried, and fed to larvae until pupation.

#### Rearing method

DFLs of CSR2 bivoltine double hybrid silkworms were obtained from SSPC, Latur. Eggs at the blue stage were incubated in black boxes to ensure uniform hatching. After hatching, 100 chawki larvae per treatment per replication were transferred to separate trays. Early instars were fed tender mulberry leaves, while later instars received mature leaves treated with dietary proteins. Bed size, spacing, and (Krishnaswami, 1978; Nalwandikar *et al.*, 2017) [19, 24]. Environmental conditions Environmental conditions were maintained at 22-28 °C temperature, 60-90% relative humidity, with 14-16 hours light and 8-10 hours darkness, monitored using thermohygrographs. Rearing trays were disinfected using 2% formalin and 0.3% lime powder before, during, and after rearing. Leaves were chopped according to larval preferences and fed at 6, 10, 16, and 21 hours daily. Larvae were not disturbed during moulting; beds were dusted with Vijetha (4 kg/100 DFLs) post-moulting and cleaned

Cocoons were harvested on the fifth day. Three random samples of 10 cocoons per treatment were selected to measure cocoon weight, filament length, and moth emergence.

regularly. Feed quantity was adjusted as larvae grew.

#### **Observations Recorded**

The biological parameters recorded in the study were: larval duration (hatching to onset of spinning), pupal period (spinning to moth emergence), moth emergence (%) calculated as (number of moths emerged  $\div$  total cocoons) × 100, fecundity expressed as the number of eggs laid per female moth, and egg hatching (%) determined as (number of hatched larvae  $\div$  total eggs) × 100.

#### Statistical analysis

Data on biological and economic traits were subjected to analysis of variance (ANOVA) using OPSTAT software. Differences among treatments were tested for significance at the 5% probability level by the F-test, following the procedures described by Gomez and Gomez (1984) [10].

#### **Experimental design**

The experiment was arranged in a Completely Randomized Design (CRD) with eight treatments and three replications. Each treatment involved 100 silkworms per replication.

#### **Result and Discussion**

### 1.1 Effect of different dietary protein treated mulberry leaves on larval duration of mulberry silkworm (*Bombyx mori* L.)

The results on effect of different dietary protein treated mulberry leaves on the larval duration of mulberry silkworm are presented in (Table 1 and depicted in Fig. 1). The data showed that duration of silkworm larvae varied in between 28.63 to 31.13 days. Larval duration ranged from 28.63 to 31.13 days. The shortest duration (28.63 days) was observed in  $T_1$  (0.5% soya protein), closely followed by  $T_2$  (0.5% groundnut flour) at 28.73 days. Other shorter durations were seen in  $T_7$  (green gram-29.20 days) and  $T_6$  (black gram-29.50 days). Moderate durations were recorded in  $T_3$  (pigeon pea-29.83 days),  $T_5$  (gram flour-30.30 days), and  $T_4$  (pea flour-30.90 days). The longest duration (31.13 days) occurred in the control ( $T_8$ ) with distilled water.

The results align with Kamaraj et al. (2017) [16], showing that Tasar silkworm larvae developed two days earlier with sova solution spray compared to sova powder and control treatments. Similarly, Hamzah and Megahed (2017) [11] found that larval duration significantly decreased with protein-supplemented diets. The shortest duration (25.16 days) was with 1.5g casein milk, while the longest (27.98 days) was with 0.5g curcuma, compared to 28.98 days in the untreated group. Mahmoud et al. (2013) [21] illustrated that the larvae of B. mori fed on semi-artificial diet containing soybean protein during 5th instar recorded highest larval duration. Krishanan et al. (1995) [18], they showed that the hydrolyzed soya protein (P-soyatase) supplementation decreased the larval duration. Roychoudhury et al. (1994) [29] exhibited that the larval duration of B. mori was found to be little reduced for larvae reared on artificial food as compared to larvae reared on leaf.

### 1.2 Effect of different dietary protein treated mulberry leaves on pupal duration of mulberry silkworm (*Bombyx mori* L.)

The results on effect of different dietary protein treated mulberry leaves on the pupal duration of mulberry silkworm are presented in (Table 2 and depicted in Fig. 2). The data showed that the duration of silkworm pupae was observed in range of 8.67 days to 11.33 days. Pupal duration ranged from 8.67 to 11.33 days. The shortest duration was observed in  $T_1$  (0.5% soya flour) at 8.67 days followed by  $T_2$  (groundnut flour-9.10 days),  $T_7$  (green gram-9.50 days),  $T_6$  (black gram-9.60 days), and  $T_3$  (pigeon pea-9.70 days). Longer durations were recorded in  $T_5$  (gram flour-10.43 days) and  $T_4$  (pea flour-10.57 days). The control ( $T_8$ ) showed the highest pupal duration of 11.33 days.

The results of present investigation are in harmony with findings of Borgohain (2015) [4] who concluded that fortification of mulberry leaves with royal jelly, dietary proteins, amino acids, vitamin B3 and vitamin B6 exhibited positive effects on the growth and development of *B. mori* silkworm. Mahmoud *et al.* (2013) [21] observed insignificant differences on the pupal stage duration when larvae of *B. mori* fed on semi-artificial diet containing soybean protein during 5<sup>th</sup> instar. Also, El-Hattab (2002) [7] who reported that the different source of protein affects the periods of pupal durations.

## 1.3 Effect of different dietary protein treated mulberry leaves on moth emergence of mulberry silkworm (Bombyx mori L.)

The results on effect of different dietary protein treated mulberry leaves on the percent moth emergence of mulberry silkworm are presented in (Table 4 and depicted in Fig. 3). The data indicated that the percent moth emergence of mulberry silkworm ranged from 84.80 to 97.07 percent. Moth emergence ranged from 84.80% to 97.07% (Table 4; Fig. 3). The highest emergence (97.07%) was observed in T<sub>1</sub> (0.5% soya flour), followed by T<sub>2</sub> (0.5% groundnut flour) with 94.57%, both significantly higher than other treatments. Among other treatments, moth emergence was 94.20% in T<sub>7</sub> (green gram), similar to T<sub>6</sub> (black gram-92.90%) and followed by T<sub>3</sub> (pigeon pea-91.77%). T<sub>5</sub> (gram flour-88.80%) was at par with T<sub>4</sub> (pea flour-86.60%), while the lowest emergence (84.80%) was observed in the control

 $(T_8)$ .

These results align with Kalokhe *et al.* (2024) <sup>[15]</sup>, who reported the highest moth emergence (94.66%) in larvae fed on leaves treated with 1.5% Baker's yeast. Similarly, Jaybhay (2018) <sup>[14]</sup> observed maximum moth emergence (96.55%) with mulberry leaves treated with 0.5% soya flour, compared to distilled water.

## 1.4 Effect of Dietary Supplement Treated Mulberry Leaves on Fecundity of Mulberry Silkworm (*Bombyx mori* L.)

The effect of different protein-supplemented mulberry leaves on silkworm fecundity is presented in Table 5 and Fig. 4. Fecundity ranged from 440.33 to 515.33 eggs per female moth. The highest fecundity (515.33 eggs) was recorded in T<sub>1</sub> (0.5% soya flour), followed by T<sub>2</sub> (groundnut flour-509.67 eggs), both significantly superior to other treatments. Moderate fecundity was observed in T<sub>6</sub> (black gram-497.33), T<sub>7</sub> (green gram-489.00), T<sub>3</sub> (pigeon pea-481.00), and T<sub>5</sub> (gram flour-475.33). T<sub>4</sub> (pea flour-467.00) and the control (T<sub>8</sub>-440.33 eggs) recorded the lowest values. These results align Jaybhay (2018) [14] reported the highest fecundity (484.33 eggs/female) with mulberry leaves treated with 0.5% soya flour. Kamel and Megahed (2017) [17] found maximum fecundity with casein milk (355 eggs/female) and soybean flour (350 eggs/female), while curcuma treatment recorded the lowest (260 eggs/female). Mahmoud et al. (2013) [21] observed that the larvae of B. mori fed on semiartificial diet containing soybean protein during 5th instar recorded highest number of eggs deposited per female. Krishanan et al. (1995) [18] who confirmed that hydrolysed protein (P-soyatose) at 2.00 percent concentration recorded maximum fecundity of B. mori.

## 1.5 Effect of Dietary Protein Treated Mulberry Leaves on Egg Hatching Percentage of Mulberry Silkworm (Bombyx mori L.)

The egg hatching percentage ranged from 89.27% to 97.30% (Table 6; Fig. 5). The highest hatching (97.30%) was observed in larvae fed mulberry leaves treated with 0.5% soya flour, comparable to treatments with 0.5% green gram (96.27%) and 0.5% groundnut flour (95.87%). Moderate hatching rates were recorded with 0.5% black gram (94.33%), pigeon pea (93.83%), and gram flour (92.80%). The lowest hatching occurred in 0.5% pea flour (90.83%) and the control (89.30%).

These findings align with Kumar *et al.* (2023) <sup>[20]</sup>, who reported increased hatchability from 70.6% in control to 83.6% with 5% Spirulina supplementation. Hossain *et al.* (2022) observed hatch rates above 93% with sericin-supplemented diets, while Hamzah and Megahed (2017) <sup>[11]</sup> reported 99.4% hatchability with 1.5g soybean protein, and the lowest with curcuma treatment. More or less similar results were found by Kamel *et al.* (2017) <sup>[17]</sup> who reported that in the mean hatchability were 98.14, 97.94 & 96.94% when larvae were fed on mulberry leaves mixed with casein milk, soybean flour and curcuma protein, respectively. These results agree with the findings of Radjabi (2010) <sup>[26]</sup> who showed that asparagine had no clear effect of on egg characteristics but alanine amino acid in 1 percent could significantly increase hatchability.

**Table 1:** Effect of different dietary protein treated mulberry leaves on larval duration, pupal duration, moth emergence, fecundity and egg hatching percentage of mulberry silkworm

Tr. No.	Treatment details	Mean larval duration (days)	Mean pupal duration (days)	Moth emergence (%)	Mean fecundity (eggs per female moth)	88
T <sub>1</sub>	Mulberry leaves treated with protein @ 0.5 percent of Soya Flour	28.63	8.67	97.07 (80.47) *	515.33	97.30 (80.53) *
$T_2$	Mulberry leaves treated with protein @ 0.5 percent of Ground Nut Flour	28.73	9.10	94.57 (76.70) *	509.67	95.87 (78.24) *
T <sub>3</sub>	Mulberry leaves treated with protein @ 0.5 percent of Pigeon Pea Flour	29.83	9.70	91.77 (73.51) *	481.00	93.83 (75.68) *
T <sub>4</sub>	Mulberry leaves treated with protein @ 0.5 percent of Pea Flour	30.90	10.57	86.60 (68.73) *	467.00	90.83 (72.37) *
T <sub>5</sub>	Mulberry leaves treated with protein @ 0.5 percent of Gram Flour	30.30	10.43	88.80 (70.43) *	475.33	92.80 (74.45) *
T <sub>6</sub>	Mulberry leaves treated with protein @ 0.5 percent of Black Gram Flour	29.50	9.60	92.90 (74.86) *	497.33	94.33 (76.43) *
<b>T</b> 7	Mulberry leaves treated with protein @ 0.5 percent of Green Gram Flour	29.20	9.50	94.20 (76.15) *	489.00	96.27 (78.88) *
T <sub>8</sub>	Mulberry leaves treated with distilled water	31.13	11.33	84.80 (67.07) *	440.33	89.30 (70.96) *
	C.D.	1.06	0.63	5.04	23.84	2.89
	S.E. (m)	0.35	0.21	1.67	7.88	0.96
	S.E. (d)	0.49	0.29	2.36	11.15	1.35
<b>Φ</b> Γ	C.V. (%)	2.04	3.64	3.16	2.82	1.766

<sup>\*</sup>Figures in parenthesis are mean arcsine transformed values

#### **Conclusions**

The overall results indicate that fortifying mulberry leaves with protein supplements specifically soya flour, groundnut flour, pigeon pea flour, green gram flour, gram flour, and black gram flour positively influences the growth and development of Bombyx mori larvae, as well as cocoon and silk production. This enhancement is likely attributable to increased protein availability during the larval stages, which significantly affects subsequent pupal and adult stages. Among the treatments, mulberry leaves enriched with 0.5% soya flour yielded the most favourable outcomes, including reduced larval and pupal duration, higher moth emergence, increased fecundity and hatching rates, improved effective rate of rearing, and superior cocoon and silk quality parameters such as single cocoon weight, cocoon yield, filament length, filament weight, and denier. These findings confirm soya flour as the optimal protein supplement for sericulture applications. Therefore, the application of mulberry leaves treated with 0.5% soya flour is strongly recommended as a dietary supplement to enhance the biological performance of B. mori.

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