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# Influence of dietary calcium chloride on development and commercial performance of *bombyx mori*.

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## ABSTRACT

The present study was conducted to evaluate the effect of calcium supplementation on the growth, survival, and commercial traits of the mulberry silkworm, *Bombyx mori* L. (FC1 × FC2 hybrid). The experiment was carried out under controlled laboratory conditions using calcium concentrations of 100, 150 and 200 ppm along with a control group. The treated group received the mulberry leaves dipped into different concentrations of calcium chloride 100 ppm, 150 ppm and 200 ppm in distilled water, once a day and the rest of the day larvae fed with normal leaves without any treatment, while the control group received the normal leaves without any treatment. The parameters recorded during the study were larval growth parameters including length and weight during the fourth and fifth instar and also recorded the disease incidence, larval survival percentage, cocoon weight, pupal weight, shell weight, and shell ratio. The results indicated that calcium supplementation significantly enhanced larval growth, survival and cocoon characteristics compared to the control. Among the tested concentrations, 100 ppm calcium consistently produced the highest larval length and weight, maximum cocoon and pupal weight, and improved shell ratio, while also reducing disease incidence. These findings demonstrate that calcium-enriched mulberry leaves can positively influence silkworm development and silk productivity, suggesting its potential application in improving sericulture output.

**Keywords:** silkworm, mulberry, calcium, enriched and parameters.

## Introduction

Sericulture is a key component of the economy in Jammu & Kashmir, where the fertile land and favourable climatic conditions support the cultivation of mulberry and rearing of bivoltine silkworms. In 2012–13, the state produced 115 MT of cocoons (Anonymous, 2012). Despite a gradual increase in cocoon production over the years, the industry has yet to regain its former prominence and realize its full economic potential. The nutritional quality of mulberry leaves plays a critical role in silkworm growth, silk yield and disease resistance (Ravi Kumar, 1988). Silkworm nutrition encompasses the essential substances required for growth and metabolic functions, obtained from ingested food and other nutrients synthesized through biochemical pathways (Takano and Arai, 1978; Hamano *et al.*, 1986; Zhang *et al.*, 2002).

Mulberry leaf quality is influenced by several factors, including variety, season, irrigation, fertilizer or manure application, temperature, photoperiod, soil characteristics, water table, pruning practices, leaf maturity and harvesting methods (Bongale and Chaluvachari, 1993; Purohit and Kumar, 1996; Rachotaiah *et al.*, 2002). Proper nutrition is essential for *B. mori* L. to achieve optimal growth and silk production. The nutritional value of mulberry leaves directly affects cocoon quality and silkworm yield.

Studies have highlighted the importance of leaf components and nutrient supplementation in improving larval growth and silk productivity (Hason *et al.*, 2004; Kanafi *et al.*, 2007; Brandão *et al.*, 1995).

Fortifying mulberry leaves with minerals and other nutrients enhances their nutritional content, as minerals serve as cofactors in enzymatic systems and help maintain osmotic balance in larval cells. Larval health and nutrition are therefore key determinants of silk production. Research has demonstrated that minerals, along with amino acids such as methionine and L-serine, and vitamins including C and B-complex, significantly enhance larval and pupal growth, ultimately improving silk quantity and quality (Kahn *et al.*, 2010; Banu *et al.*, 2004; Bhattachary, 2005). Enhancing mulberry leaves through such supplementation represents a promising approach to improving the overall performance of silkworms and the economic output of sericulture.

## 2. Materials and Methods

The study was carried out at the Sericulture Research Laboratory under the Division of Sericulture, Sher-e-Kashmir University of Agricultural Sciences and Technology (SKUAST), Jammu, during the spring seasons of 2023 and 2024. A bivoltine double hybrid strain of the silkworm, *B. mori* L., specifically the FC1 × FC2 hybrid, was used in the investigation. The hybrid eggs were procured from the Division of Sericulture, SKUAST-J Chatha. The eggs were incubated under controlled environmental conditions, maintaining a temperature of 25 ± 1°C and relative humidity of 80 ± 5% until hatching. After hatching, the larvae were transferred to rearing trays and managed following the standard procedures described by Krishnaswami (1978).

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DOI: <https://doi.org/10.21276/AATCCReview.2025.13.04.902>

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The experiment was designed as a completely randomized design (CRD) with four treatments, each replicated three times. The treatments were as follows: T1 (Calcium 100 ppm), T2 (Calcium 150 ppm), T3 (Calcium 200 ppm) and T4 (Control). Calcium was individually supplemented to the silkworms through mulberry leaves with the aim of improving silk production in *B. mori*. The materials and methods employed in the study are described in detail below.

#### Geographical features of the experimental site:

The experimental location is the Chatha campus of SKUAST-J, situated on the peripheries of Jammu city at approximately 32°39'10.5" N latitude and 74°48'25.6" E longitude. The area lies in the subtropical foothills of the Shivalik range, with an elevation ranging from 300 to 370 m above mean sea level, resulting in gently undulating terrain. The main Chatha campus covers about 578 acres, serving as the centre for academic, research and experimental agricultural activities, supplemented by additional lands across the university's multi-campus system.

#### Climate

The Chatha campus experiences a subtropical climate characterized by hot summers, a defined monsoon season and relatively mild winters. Summers are generally hot and dry, while the monsoon season brings moderate to heavy rainfall, supporting agricultural operations. Winters are cool, occasionally influenced by cold northern winds, but are less severe than in higher Himalayan regions. These climatic conditions provide a diverse range of growing environments, making the site suitable for research on various crops and production systems.

#### Experimental Details

Number of Treatments: 4

#### Experimental Design: Completely Randomized Design (CRD)

#### Treatment Details:

- T1: Calcium 100 ppm
- T2: Calcium 150 ppm
- T3: Calcium 200 ppm
- T4: Control

#### Number of Replications: 3

#### Number of Larvae per Replication: 100

After hatching, the larvae were removed from the stock culture and provided with an adequate supply of fresh mulberry leaves. The experiment was conducted using larvae from the fourth and fifth instar. Following the first instar, the larvae were acclimatized to laboratory conditions and divided into four experimental groups, including a control group. During the study period, larvae were fed four times daily, and appropriate disinfection practices were maintained. Each of the 12 trays contained 100 larvae, arranged in three replicates per treatment. The control group was fed mulberry leaves treated with distilled water only.

Calcium chloride, sourced from Shiv chemicals, Jammu, was used as the experimental material. It was dissolved in distilled water and prepared at concentrations of 100 ppm, 150 ppm, and 200 ppm. Fresh mulberry leaves were dipped in the respective calcium solutions and allowed to air-dry under laboratory conditions for 30 minutes before feeding. The treated leaves were provided once daily to the fourth and fifth instar larvae, while the control group received leaves dipped in distilled water. This feeding regimen continued until the larvae reached the seriposition.

For all treatments, the following parameters were recorded: larval weight, length, larval survival %, disease incidence, cocoon weight, shell weight, shell ratio and pupal weight. The collected data were statistically analysed using ANOVA.

#### Results and Discussion

#### Effect of Calcium on Larval Growth

**Table 01: Effect of mulberry leaves supplemented with different concentration of Calcium chloride on larval growth parameters of silkworm larvae of 4<sup>th</sup> instar**

Treatments	Concentration (ppm)	Larval length of 4 <sup>th</sup> instar (cm)		Larval weight of 4 <sup>th</sup> instar (g)	
		Initial	Final	Initial	Final
Calcium	100	2.74±0.01 <sup>a</sup>	4.47±0.009 <sup>d</sup>	2.37±0.01 <sup>a</sup>	11.17±0.02 <sup>a</sup>
	150	2.74±0.01 <sup>a</sup>	4.28±0.000 <sup>c</sup>	2.36±0.01 <sup>a</sup>	10.71±0.02 <sup>c</sup>
	200	2.74±0.00 <sup>a</sup>	4.24±0.007 <sup>b</sup>	2.36±0.01 <sup>a</sup>	10.54±0.01 <sup>b</sup>
Control	-	2.74±0.01 <sup>a</sup>	4.05±0.007 <sup>a</sup>	2.37±0.01 <sup>a</sup>	9.82±0.01 <sup>a</sup>

\*Each value is mean± S.E of the three replication

\*Value followed by different letters in column is significantly different by Tukey's HSD test.

The impact of calcium supplementation on the growth of fourth and fifth instar larvae of *B. mori* L. is presented in Tables 1 and 2. In the fourth instar, the initial larval length and weight were uniform across all treatments, ranging from 2.74 ± 0.00 to 2.74 ± 0.01 cm and 2.36 ± 0.01 to 2.37 ± 0.01 g, respectively. After treatment, the larvae fed with calcium-supplemented mulberry leaves showed a significant increase in both length and weight compared to the control. The highest growth was observed at 100 ppm calcium, with a final length of 4.47 ± 0.009 cm and weight of 11.17 ± 0.02 g, followed by 150 ppm and 200 ppm treatments. The control group recorded the lowest final larval length (4.05 ± 0.007 cm) and weight (9.82 ± 0.01 g).

**Table 02: Effect of mulberry leaves supplemented with different concentration of Calcium chloride on larval growth parameters of silkworm larvae of 5<sup>th</sup> instar**

Treatments	Concentration (ppm)	Larval length of 5 <sup>th</sup> instar (cm)		Larval weight of 5 <sup>th</sup> instar (g)	
		Initial	Final	Initial	Final
Calcium	100	4.90±0.007 <sup>d</sup>	7.50±0.007 <sup>d</sup>	11.68±0.02 <sup>d</sup>	41.14±0.17 <sup>d</sup>
	150	4.79±0.007 <sup>c</sup>	7.21±0.003 <sup>c</sup>	11.05±0.03 <sup>c</sup>	39.83±0.06 <sup>c</sup>
	200	4.76±0.009 <sup>b</sup>	7.16±0.008 <sup>b</sup>	10.86±0.02 <sup>b</sup>	38.87±0.15 <sup>b</sup>
Control	-	4.42±0.009 <sup>a</sup>	6.90±0.009 <sup>a</sup>	10.16±0.03 <sup>a</sup>	35.04±0.12 <sup>a</sup>

\*Each value is mean± S.E of the three replication

\*Value followed by different letters in column is significantly different by Tukey's HSD test.

Similarly, in the fifth instar, calcium supplementation significantly enhanced larval growth. Larvae fed 100 ppm calcium exhibited the highest final length ( $7.50 \pm 0.007$  cm) and weight ( $41.14 \pm 0.17$  g), whereas the control group showed the lowest final length ( $6.90 \pm 0.009$  cm) and weight ( $35.04 \pm 0.12$  g). The 150 ppm and 200 ppm treatments also resulted in improved growth, but their effects were slightly lower than the 100 ppm treatment, indicating that 100 ppm calcium is the most effective concentration for promoting larval development. The results demonstrate that calcium plays a vital role in larval metabolism, exoskeleton development, and enzymatic activities, which are crucial for growth.

Calcium supplementation positively influenced larval survival percentage and reduced disease incidence. The lowest disease incidence ( $6.60 \pm 0.05\%$ ) and highest survival rate ( $94.15 \pm 0.07\%$ ) were recorded in the 100 ppm treatment, while the control group showed the highest disease incidence ( $11.45 \pm 0.05\%$ ) and the lowest survival rate ( $89.30 \pm 0.08\%$ ). The results suggest that calcium enhances larval immunity and overall health, reducing vulnerability to infections and stress during rearing.

### Effect of calcium on Cocoon and Pupal Traits

Table 04: Effect of mulberry leaves supplemented with different concentration of Calcium chloride on the economic parameters

Treatments	Concentration (ppm)	Cocoon Weight (g)	Shell weight (g)	Pupal weight (g)	Shell ratio (%)
Calcium	100	$2.31 \pm 0.01^e$	$0.45 \pm 0.00^d$	$1.79 \pm 0.00^d$	$20.69 \pm 0.05^e$
	150	$2.21 \pm 0.01^d$	$0.42 \pm 0.00^c$	$1.74 \pm 0.00^c$	$18.56 \pm 0.05^b$
	200	$2.16 \pm 0.01^c$	$0.40 \pm 0.00^{ab}$	$1.70 \pm 0.01^b$	$18.70 \pm 0.04^b$
	-	$1.99 \pm 0.01^a$	$0.39 \pm 0.00^a$	$1.54 \pm 0.00^a$	$17.71 \pm 0.06^a$

\*Each value is mean  $\pm$  S.E of the three replication

\*Value followed by different letters in column is significantly different by Tukey's HSD test.

Calcium supplementation significantly improved cocoon and pupal parameters. The highest cocoon weight ( $2.31 \pm 0.01$  g), shell weight ( $0.45 \pm 0.00$  g), pupal weight ( $1.79 \pm 0.00$  g), and shell ratio ( $20.69 \pm 0.05\%$ ) were observed at 100 ppm calcium, while the control group recorded the lowest values (cocoon weight  $1.99 \pm 0.01$  g, shell weight  $0.39 \pm 0.00$  g, pupal weight  $1.54 \pm 0.00$  g, shell ratio  $17.71 \pm 0.06\%$ ). The increase in these parameters can be attributed to enhanced larval nutrition and metabolic activity due to calcium supplementation, which promotes silk gland development and better cocoon formation.

### Discussion

Overall, the results indicate that calcium fortification of mulberry leaves positively affects the growth, health, and commercial traits of silkworm larvae. The 100 ppm calcium treatment consistently produced the best outcomes across all parameters, suggesting it as the optimal concentration for improving larval growth, survival, cocoon weight, pupal weight, and shell ratio. Higher concentrations (150–200 ppm) also improved performance compared to the control but were less effective than 100 ppm, indicating a threshold beyond which additional calcium does not provide further benefits.

The enhanced larval growth, survival and cocoon quality observed with calcium supplementation may be due to its crucial role in physiological and biochemical processes, including enzyme activation, cuticle formation and osmotic regulation. These findings are consistent with previous studies highlighting the importance of mineral supplementation in improving silkworm productivity.

### Conclusion

Calcium supplementation through mulberry leaves has a significant positive effect on the growth, survival and commercial traits of *B. mori* L. larvae. The study revealed that the 100 ppm calcium concentration was the most effective, resulting in the highest larval length and weight, improved

### Effect of calcium on Larval Survival and Disease Incidence

Table 03: Effect of mulberry leaves supplemented with different concentration of Calcium chloride on larval Survival and disease incidence &survival % of silkworm larvae

Treatments	Concentration (ppm)	Larval survival (%)	Disease incidence (%)
Calcium	100	$94.15 \pm 0.07^d$	$6.60 \pm 0.05^e$
	150	$90.89 \pm 0.13^b$	$9.86 \pm 0.10^b$
	200	$92.36 \pm 0.18^c$	$8.39 \pm 0.08^e$
	-	$89.30 \pm 0.08^a$	$11.45 \pm 0.05^a$

\*Each value is mean  $\pm$  S.E of the three replication

\*Value followed by different letters in column is significantly different by Tukey's HSD test.

survival rate, reduced disease incidence and better cocoon and pupal characteristics, including cocoon weight, shell weight, pupal weight and shell ratio. Higher concentrations (150–200 ppm) also improved performance but were less effective than 100 ppm. These results indicate that calcium plays a vital role in silkworm metabolism, development and silk production. Incorporating optimal calcium levels into mulberry leaf feeding strategies can enhance sericulture productivity and may provide economic benefits for silk growers. In conclusion, calcium-enriched mulberry leaves, particularly at 100 ppm, significantly improve the growth, health and commercial traits of *B. mori* larvae, demonstrating its potential for enhancing silk production in sericulture practices.

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