



Evaluation of honey induced nutritional modulation on larval energetics and economic performance in *Bombyx mori* L. (FC1 × FC2)

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Abstract

Silkworm *Bombyx mori* L. the backbone of global silk production, shows significant improvements in growth and silk yield when its diet is nutritionally enriched. Honey contains rapidly assimilable carbohydrates, amino acids, vitamins, minerals and bioactive compounds that enhance digestive metabolism and silk gland activity in the silkworm. The present study evaluated the effects of 1%, 2% and 3% honey-enriched mulberry leaves on larval energetics and economic traits of the bivoltine hybrid FC1 × FC2. Significantly improvements were observed in all treated groups, with 2% honey producing the highest cocoon weight (2.30 g), shell percentage (22.20 %), filament length (1400 m), renditta (5.05 kg) and fecundity (865 eggs). While 3% honey yielded the greater larval (4.78 g) and pupal weights (1.78 g), it did not translate into proportional gains in silk traits. The results indicate a physiological threshold wherein moderate honey supplementation (2%) achieves optimal nutrient assimilation and silk gland efficiency. These findings support honey as an accessible, natural nutritional enhancer to improve biological and commercial performance of bivoltine hybrids in sericulture.

Keywords: *Bombyx mori*, honey enrichment, larval energetics, cocoon traits, silk yield

Introduction

The mulberry silkworm *Bombyx mori* L. is a domesticated lepidopteran of major economic importance, forming the biological foundation of sericulture across Asia. Its exceptional ability to convert mulberry foliage into commercially valuable silk protein makes larval nutrition a critical determinant of cocoon yield, fibre quality and overall productive efficiency. Although mulberry leaves constitute the primary diet, their nutrient composition fluctuates with season, cultivation practices and leaf maturity, potentially limiting the metabolic efficiency and silk productivity of the larvae. This has encouraged the exploration of natural dietary supplements capable of enhancing growth, metabolic activity and silk gland secretion.

Honey, a complex natural product rich in sugars, amino acids, enzymes, vitamins, minerals and antioxidants has been widely recognized for its nutritional and physiological benefits in insects. Several studies have demonstrated that honey-enriched diets improve larval biomass, silk gland growth and cocoon characteristics in *B. mori* (Thulasi & Siva Prasad, 2015)^[19] reported marked increases in larval weight, silk gland protein content and economic traits when larvae were fed honey-treated leaves. Similar metabolic enhancements including improved glycogen utilization, trehalose turnover and oxidative phosphorylation were observed by (Madhavi & Siva Prasad, 2020)^[7, 9], indicating elevated energetic efficiency under honey supplementation. Studies focusing on productive traits further support honey application in sericulture (Bhatti *et al.*, 2019)^[3] found that *Apis dorsata* honey at 2% significantly enhanced cocoon weight and shell ratio, while (Baci *et al.*, 2021)^[2] reported improved substrate deposition and larval biomass. Additional investigations including (Saad *et al.*, 2014; Shahzadi *et al.*, 2022; Sonone *et al.*, 2024; Yuvanathi *et al.*, 2024)^[11, 15, 17, 21] consistently highlight honey positive influence on larval vigor, cocoon quality and silk

morphology. Research on concentration-specific responses revealed that moderate supplementation often yields superior silk traits compared to higher doses (Madhavi *et al.*, 2018; Thulasi & Siva Prasad, 2014; Alagumanikumar & Prema, 2016)^[1, 8, 18].

Despite extensive evidence supporting honey benefits, several gaps remain. Few studies have evaluated bivoltine hybrids such as FC1 × FC2 and the mechanistic basis for optimal concentration (especially why 2% outperforms 3% in silk traits) remains unexplored. Moreover, integrated analyses combining energetics, cocoon parameters, reeling traits and reproduction within a single experimental framework are limited. Hence, the present work aims to provide a comprehensive, concentration-specific assessment of honey influence on larval energetics and economic traits in *B. mori* FC1 × FC2.

Materials and methods

The experiment was conducted in the Laboratory of Silkworm Physiology and Biochemistry, Department of Studies in Sericulture Science, University of Mysore, Mysuru, Karnataka, during the 2024-2025 rearing season. Disease Free Layings (DFLs) of the bivoltine hybrid *Bombyx mori* (FC1 × FC2) eggs procured from NSSO cold storage. Silkworm rearing was conducted as per the standard method recommended by Krishnaswami (1986). Chawki larvae were fed with freshly harvested S36 mulberry leaves, while later age larvae fed with V1 variety mulberry leaves. Honey solutions of 1%, 2% and 3% were prepared by diluting natural honey in distilled water. Distilled water served as the control. Mulberry leaves were soaked in the respective solutions, shade dried to remove excess moisture and fed once daily at 10:00 AM during the fourth and fifth instars. After the third moult, healthy larvae were randomly divided into four treatments (Control, 1%, 2% and 3% honey concentrations), each with three replications of 100 larvae.

Economic parameters were recorded from 20 randomly selected larvae per replication. Larval, cocoon, pupal, shell weights were recorded using digital balance. Shell percentage was calculated as:

$$\text{Shell (\%)} = \frac{\text{Shell weight}}{\text{Cocoon weight}} \times 100$$

Reeling parameters were assessed using standard methods. Filament length was measured using an eprouvette and calculated as:

$$\text{Filament length (m): } L = R \times 1.125$$

Where, R = Number of revolutions recorded by an eprouvette.

1.125 = Circumference of eprouvette in meter.

Denier was calculated using: It indicates thickness of the filament.

$$\text{Denier} = \frac{\text{Filament weight}}{\text{Filament length}} \times 9000$$

Renditta, indicating the quality of cocoons required to produce 1 kg of raw silk, was computed as:

$$\text{Renditta (kg)} = \frac{\text{Weight of cocoons reeled}}{\text{Weight of raw silk obtained}}$$

$$\text{Effective Rate of Rearing (\%)} = \frac{\text{Total number of larvae brushed}}{\text{Total number of cocoons harvested}} \times 100$$

Reproductive parameters were also assessed. Fecundity (total number of eggs laid per female moth) was recorded and hatching percentage was determined using:

$$\text{Hatching (\%)} = \frac{\text{Number of eggs hatched}}{\text{Total number of eggs laid}} \times 100$$

All the collected data were expressed as mean \pm standard deviation (SD). Statistical analysis was performed using ANOVA test to determine significant differences among treatments at $p \leq 0.05$.

Results and discussion

1. Larval and Pupal Performance

Honey enriched mulberry leaves at all tested concentrations resulted in a distinct concentration-dependent improvement in larval energetics. Wherein both 2% and 3% treatments recorded higher larval and pupal weights compared with the control group. Larvae fed 3% honey reached the maximum larval (4.78 g) and pupal weights (1.78 g), followed by 2% honey (4.65 g; 1.74 g) (Table 1). This trend aligns with previous observations that honey provides readily absorbable sugars, amino acids and micronutrients that enhance digestive metabolism (Thulasi & Siva Prasad, 2015; Madhavi & Siva Prasad, 2020) [7, 9, 19]. The increase in biomass at higher concentrations may be attributed to

intensified trehalase-mediated trehalose hydrolysis and haemolymph glucose availability, which accelerate energy and tissue deposition, as described for insect carbohydrate metabolism (Shukla *et al.*, 2014) [16]. However, the marginal advantage at 3% is accompanied by a metabolic cost: high sugar density on leaf surfaces elevates gut osmotic load, disrupting water and ion homeostasis and impairing nutrient transport across the midgut epithelium effects well documented in insect osmoregulatory physiology (Cohen, E. 2013) [4]. Thus, while 3% honey enhances growth and energy metabolism suggests that 2% honey achieves a more balanced improvement by providing sufficient carbohydrates without inducing osmotic stress, explaining its superior translation into downstream silk parameters.

2. Cocoon and Shell Characteristics

Cocoon traits showed the strongest response at 2% honey, which produced the highest cocoon weight (2.30 g), shell weight (0.500 g) and shell percentage (22.20 %), despite of 3% larvae being heavier (Table 1). This indicates that silk production depends more on protein assimilation efficiency than on total biomass. Several honey feeding studies report that excessive sugar decreases midgut protease activity and increases substrate accumulation in gut tissues, reducing amino-acid availability to the silk gland (Baci *et al.*, 2021; Madhavi *et al.*, 2020) [2, 7, 9]. Molecular studies further show that fibroin gene expression and silk gland capacity decline when nutrient balance is disturbed, with stress-linked pathways (autophagy and proteasomal degradation) becoming activated (Cui *et al.*, 2018; Ye *et al.*, 2021) [5, 20]. Moderate supplementation (2% honey) likely avoids these imbalances, maintaining efficient nitrogen metabolism and stable fibroin secretion, whereas 3% disrupts midgut enzyme activity and silk gland homeostasis. Earlier reports 2% honey from *Apis dorsata* enhances cocoon traits (Bhatti *et al.*, 2019) [3] support the present findings. Thus, 2% honey provides an optimal carbohydrate-protein balance to maximize fibroin deposition, explaining the superior cocoon traits compared to 3% honey.

3. Reeling Parameters

Reeling performance was optimal at 2% honey, which yielded the greatest filament length (1400 m), filament weight (0.470 g) and renditta (5.05 kg), indicating efficient raw silk recovery. Although 3% honey produced the first denier (2.97 d), filament length and silk yield were compromised, reflecting the silk gland sensitivity to nutrient balance. Excessive sugar intake is known to reduce feeding efficiency by making leaf surfaces sticky, altering biting patterns and reducing effective protein intake (Madhavi & Siva Prasad, 2020) [7, 9]. Reduced protease activity under high honey concentration further impacts amino acid flow to the posterior silk gland, where fibroin heavy and light chains are synthesized. Molecular evidence shows that disturbances in silk protein synthesis activate premature autophagy and protein degradation pathways within the silk gland, reducing secretory output (Ye *et al.*, 2021; Cui *et al.*, 2018) [5, 20]. Therefore, 2% honey maintains ideal biochemical conditions, maximizing fibroin secretion and reeling performance, while 3% disrupts this balance despite supporting higher larval biomass.

4. Rearing Efficiency and Reproductive Traits

Rearing efficiency and reproductive traits followed the same optimal pattern at 2% honey, which showed the highest

ERR (83.50 %), fecundity (865 eggs) and hatchability (96.50 %). Honey provides antioxidants, vitamins and organic acids that reduce oxidative stress and improve physiological stability during metamorphosis (Baci *et al.*, 2021) [2]. Earlier studies show that honey supplementation enhances nutrient deposition in midgut tissues and fat body, improving metabolic reserves and adult vigour (Saritha & Siva Prasad, 2022; Madhavi *et al.*, 2020) [7, 9, 12]. The superior reproductive outcomes at 2% honey indicate efficient nutrient partitioning between silk gland function and reproductive tissue development. In contrast, 3% honey, despite improving somatic growth, likely diverts energy disproportionately towards carbohydrate storage and osmotic regulation rather than protein based reproductive development. This interpretation is consistent with energy allocation models and molecular evidence showing that nutrient imbalance activates stress pathways and weakness tissue specific protein synthesis in insects (Ye *et al.*, 2021; Cui *et al.*, 2018) [5, 20]. Thus, 2% honey supports ideal energetic stability, metabolic efficiency and reproductive performance.

Conclusion

The present study demonstrates that honey supplementation significantly enhances larval energetics, cocoon productivity, silk yield and reproductive performance in *Bombyx mori* (FC1 × FC2), but the response is concentration dependent. While 3% honey produced the highest larval and pupal biomass, this increased somatic growth did not translate into superior cocoon or reeling traits, indicating a mismatch between energy intake and amino acid assimilation. In contrast, 2% honey consistently produced the higher cocoon weight, shell percentage,

filament length, filament weight and renditta due to its optimal balance of carbohydrates, proteins and micronutrients. At this moderate level, honey enhances digestive enzyme activity, improve trehalose-glycogen turnover and maintains stable gut osmolality, ensuring the efficient nutrient absorption and silk gland amino acid supply. Molecular evidence from earlier studies suggests that optimal concentrations support fibroin synthesis, whereas excessive sugars at 3% may impair protease activity, reduce nitrogen assimilation and disturb silk gland homeostasis. Thus, 2% honey emerges as the physiological ideal concentration, maximizing fibroin deposition without inducing osmotic or metabolic stress. These findings reaffirm the moderate honey enrichment silk provides a practical, low cost and biological optimal strategy for enhancing silk productivity in bivoltine hybrids under controlled rearing conditions.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this research work.

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Table 1: Effect of honey supplementation on economic traits of *Bombyx mori* L. (FC1 × FC2)

Treatment	Larval weight (g)	Cocoon weight (g)	Shell weight (g)	Pupal weight (g)	Shell percentage (%)	Filament length (m)	Filament weight (g)	Denier (d)	Renditta (kg)	ERR (%)	Fecundity (eggs)	Hatching percentage (%)
Control	4.28 ± 0.07	2.12 ± 0.04	0.460 ± 0.01	1.66 ± 0.03	21.66 ± 0.15	1142.9 ± 30.4	0.383 ± 0.01	3.02 ± 0.03	5.28 ± 0.06	80.13 ± 1.29	757 ± 15.5	94.56 ± 0.98
1% Honey	4.45 ± 0.06	2.18 ± 0.02	0.478 ± 0.01	1.70 ± 0.02	21.90 ± 0.30	1250 ± 18	0.420 ± 0.01	3.01 ± 0.04	5.22 ± 0.08	81.50 ± 1.20	780 ± 16	95.00 ± 0.80
2% Honey	4.65 ± 0.08	2.30 ± 0.03	0.500 ± 0.01	1.74 ± 0.03	22.20 ± 0.90	1400 ± 25	0.470 ± 0.01	2.99 ± 0.02	5.05 ± 0.06	83.50 ± 1.10	865 ± 10	96.50 ± 0.40
3% Honey	4.78 ± 0.09	2.28 ± 0.03	0.495 ± 0.01	1.78 ± 0.04	21.70 ± 1.05	1320 ± 20	0.455 ± 0.01	2.97 ± 0.03	5.12 ± 0.07	82.00 ± 1.30	840 ± 12	96.10 ± 0.50

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