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Evaluation of biological performance and silk production in *Bombyx mori* L. Fed with tryptophan supplementation

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Abstract
The present investigation assessed the influence of tryptophan-fortified mulberry leaves on the economic traits of bivoltine silkworm hybrids. Mulberry leaves were supplemented with tryptophan at concentrations of 250, 500, and 1000 ppm, and their influence on larval weight, cocoon weight, shell weight, shell ratio, filament length, and filament weight was carefully assessed. Supplementation significantly improved all traits compared to the control. The best results for larval weight (4.453 g), cocoon weight (2.34 g), shell weight (0.5336 g), filament length (1298.9 m), and filament weight (0.499 g) were achieved at 1000 ppm, while the highest shell ratio (24.33%) occurred at 500 ppm. These improvements are likely due to enhanced nutrient assimilation, efficient metabolism, and increased silk protein biosynthesis supported by tryptophan. Overall, the findings suggest that fortifying mulberry leaves with tryptophan, particularly at 1000 ppm, can serve as an effective approach to improve growth performance and silk productivity in bivoltine silkworm hybrids.#

Keywords: Tryptophan, silkworm, cocoon weight, economic traits

Introduction

Silkworm rearing is a low-investment yet profitable commercial venture that can enhance the livelihoods of rural communities and contribute to their economic development (Fambayun *et al.,* 2022) [3]. Silk is a naturally occurring, genetically programmed, long, continuous filament made of fibrous protein, produced by several arthropods such as Arachnids and Lepidopteran. Among them, silk from the silkworm (*Bombyx mori* L.) is widely used due to its excellent biocompatibility, biodegradability, and elasticity (Muzalim *et al*., 2023).

Silkworms rely entirely on mulberry leaves to meet their nutritional requirements for proper growth and development. The quality of these leaves has a significant impact on the growth and development of the larvae. Proper nutrition plays a crucial role in improving the economic characteristics of silkworms within sericulture (Laz *et al.,* 2006) [4]. Any notable alteration in nutritional intake, particularly in amino acid levels, has a direct impact on cocoon production (Qayoom *et al.,* 2025) [8]. Several studies have reported that enriching mulberry leaves with supplementary nutrients can improve both silk quality and cocoon yield (Balasundaram, 2013 and Srivastava and Maurya, 2025) [1, 12].

Amino acids are crucial throughout various developmental stages of insects, and their metabolic pathways are generally similar to those found in other animal groups (Murugesh *et al.,* 2021) [5]. Among different supplements such as vitamins, antibiotics, sugars, trace elements, and amino acids, the enrichment of mulberry leaves with amino acids showed the greatest impact, resulting in better leaf quality and a 40–60% rise in silk output (Senguptha *et al.,* 1972). It has been found that silkworms require ten essential amino acids for proper nutrition, including arginine, histidine, isoleucine, leucine, glycine, methionine, phenylalanine, threonine, tryptophan, and valine (Laz *et al.,* 2006) [4]. This study was conducted to improve the commercial and biological characteristics of silkworms through feed supplementation using mulberry leaves enriched with the amino acid tryptophan.

Methodology

The current study employed bivoltine silkworm double hybrids, which were reared according to the standard procedures outlined by Dandin and Giridhar (2010) [2].

Silkworm Dietary Treatment

The study was performed at the 4th and 5th instar stages of silkworm growth, with the larvae allocated into four groups of 100, and each treatment replicated thrice. Tryptophan was administered in three specified concentrations: 250, 500, and 1000 ppm. To prepare the supplements, specific amounts of amino acids were dissolved in distilled water to obtain the desired concentrations. The mulberry leaves were immersed in these solutions and later dried in the shade before feeding. To assess the impact of the amino acid on the experimental silkworms, various parameters were measured and analyzed, including the weight of mature larvae (g), cocoons (g), and shells, the shell ratio (%), shell weight (g), filament length (m) and filament weight (g).

Assessment of Economic Traits

Following the completion of the 5th instar stage, the silkworms began spinning cocoons. Once cocoon spinning was finished across all experimental groups, the cocoons were exposed to bright sunlight to kill the moths inside and remove any remaining moisture. Silk yield from the control group was measured and compared with that of the treated groups. The weight of mature larvae, cocoons, and shells, the shell ratio (%), shell weight, filament length and filament weight were observed.

Shell ratio (%) = (Shell weight ÷ Cocoon weight) * 100

Results

Effects of tryptophan-supplemented mulberry leaves on various economic traits of bivoltine silkworm hybrids are: -

Larval Weight

Silkworms fed on mulberry leaves supplemented with tryptophan showed significant differences in larval weight, with the highest gain recorded at 1000 ppm (4.453g), followed by 500 ppm (4.289g) and 250 ppm (4.159g). The lowest weight was observed in the control group (4.114g) (Table 1). The improvement in larval weight could be attributed to the additional supply of tryptophan along with mulberry leaves.

Shell weight

Shell weight is an important indicator of shell yield and shows a positive correlation with filament length. Larvae fed on mulberry leaves enriched with different concentrations of tryptophan exhibited significant variation in shell weight. The maximum shell weight was recorded at 1000 ppm supplementation (0.5336g), followed by 500 and 250 ppm (0.5333g and 0.4666g). The lowest values were observed in the control batch (0.4333g).

Cocoon Weight

The bivoltine hybrid recorded the highest cocoon weights when reared on mulberry leaves fortified with tryptophan at 1000 ppm (2.34g), followed by 500 and 250 ppm (2.24g and 2.11 g), respectively. In contrast, the lowest cocoon weights were observed in control batch (2.14g). The improvement in cocoon weight in both hybrids may be attributed to enhanced absorption of tryptophan by midgut epithelial cells, its subsequent uptake by body tissues, and its role in cellular growth and development.

Shell Ratio

The bivoltine hybrid achieved the highest shell ratio when fed on mulberry leaves supplemented with tryptophan at 500 ppm (24.33%), followed by 1000 and 250 ppm (23.25% and 22.31%), respectively. The lowest shell ratio was recorded in control and compared to the treated (20.69%) (Table 1). The improvement in shell ratio may be attributed to enhanced silk productivity resulting from additional thiamine supplementation.

Filament Length

Filament length is one of the key quantitative traits determining silk yield in silkworms. Hybrids reared on mulberry leaves enriched with tryptophan showed significant variation in filament length, with the maximum recorded at 1000 ppm (1298.9m), followed by 500 ppm (1288.1m) and 250ppm (1141.8m). In contrast, the lowest values were observed at 250 ppm while the control batches registered lengths of 1172.5m (Table 1). The observed increase in filament length may be attributed to enhanced silk protein synthesis stimulated by additional tryptophan supplementation.

Filament Weight

The bivoltine hybrids recorded higher filament weights when reared on mulberry leaves fortified with tryptophan at 1000 ppm (0.499g), followed by 500 and 250 ppm (0.481g and 0.439g), respectively. In contrast, the lowest filament weights were observed at the control batches (0.414g). The results indicate that supplementation of mulberry leaves with tryptophan at different concentrations had a significant effect on filament weight.

Table1: Effect of tryptophan-enriched mulberry leaves at different concentrations on larval and cocoon traits.

Conc.	Larval Weight (g)	Shell Weight (g)	Cocoon Weight (g)	Shell Ratio (%)	Silk Filament Length (m)	Silk Filament Weight (g)
250 ppm	4.159667	0.466667	2.11	22.31099719	1141.833	0.439
500 ppm	4.289	0.533333	2.243333	24.333991	1288.1	0.481333333
1000 ppm	4.453	0.536667	2.34	23.25612148	1298.9	0.499333333
Controlled	4.114333	0.433333	2.143333	20.6977559	1172.567	0.414
'F' Cal. Value	6.733004293	4.134218289	4.060827251	1.793280688	9.455075117	17.55316184
Probability (p)	0.014009955	0.048141752	0.050149956	0.226243016	0.005230943	0.000704082
'F' Tab. value@0.05	4.0662/7.591	4.0662/7.591	4.0662	4.0662	4.0662/7.591	4.0662/7.591

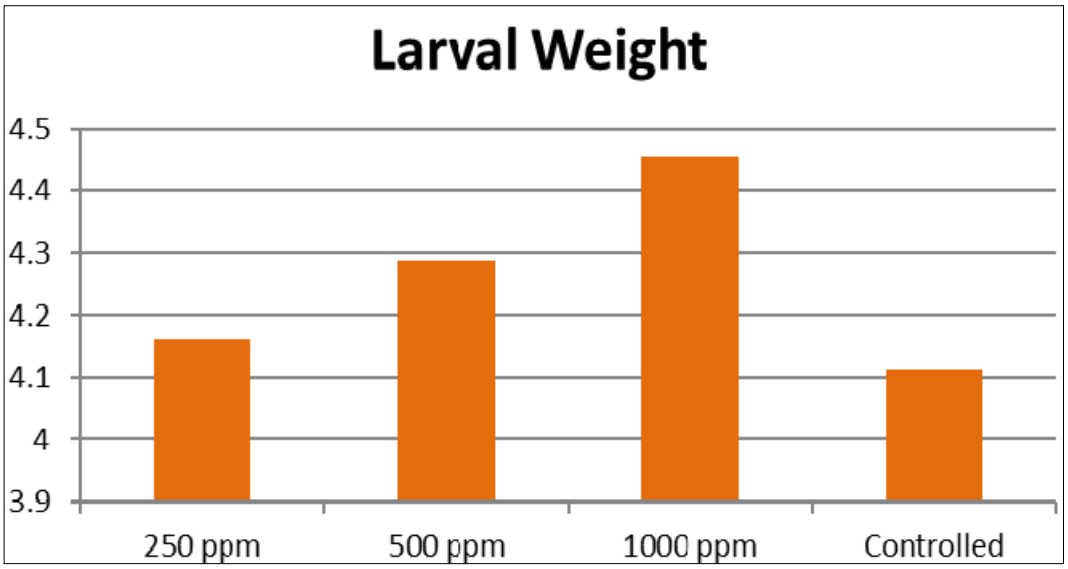

Fig 1. Effect of tryptophan on larval weight of silkworm *Bombyx mori* L.

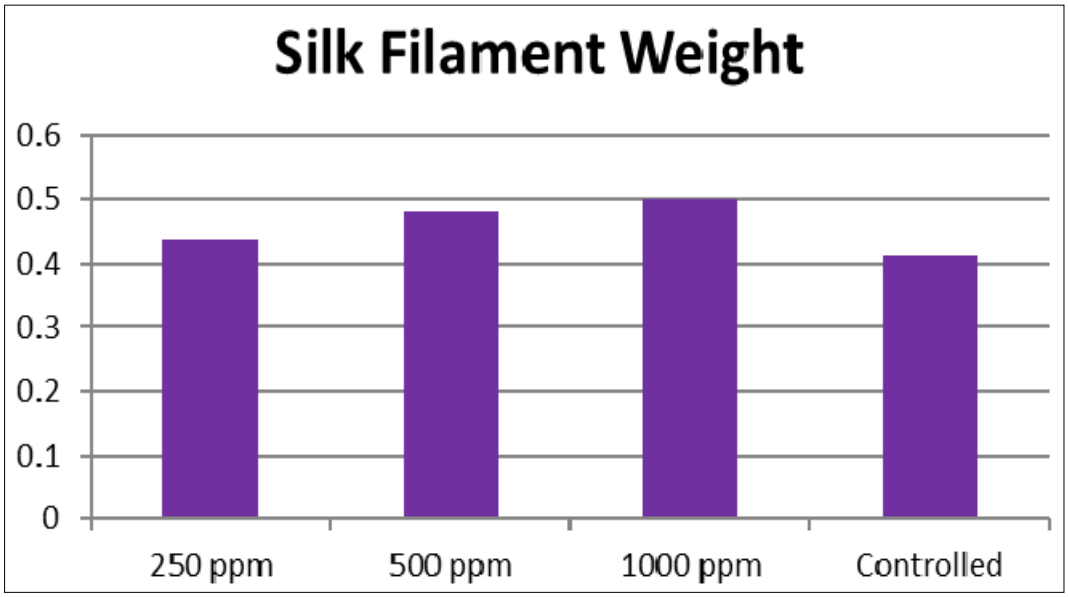

Fig 2. Effect of tryptophan on silk filament weight of silkworm *Bombyx mori* L.

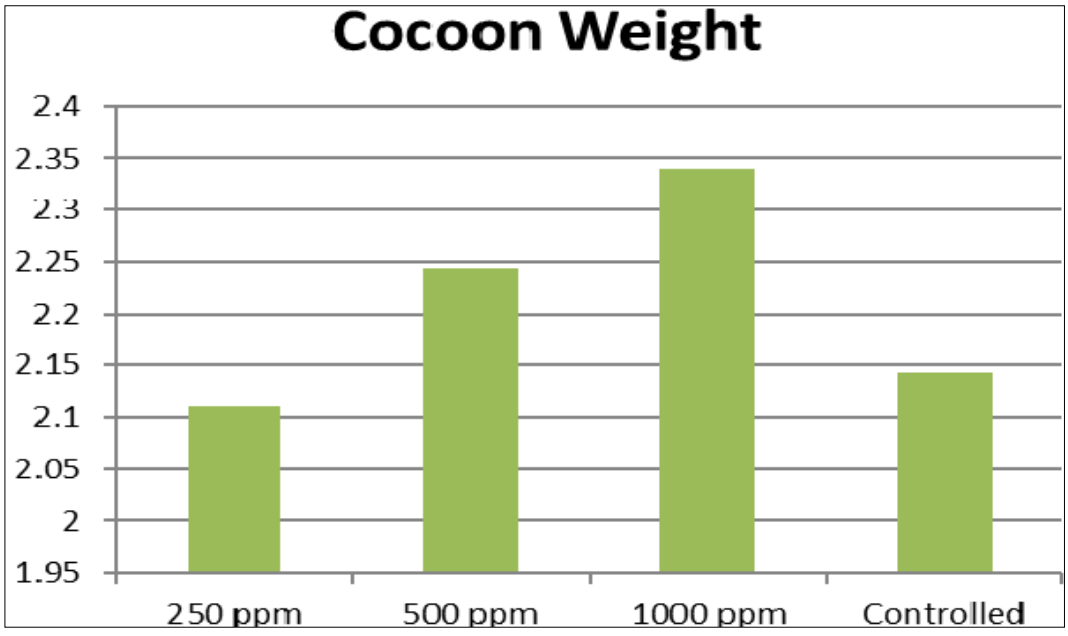

Fig 3. Effect of tryptophan on cocoon weight of silkworm *Bombyx mori* L.

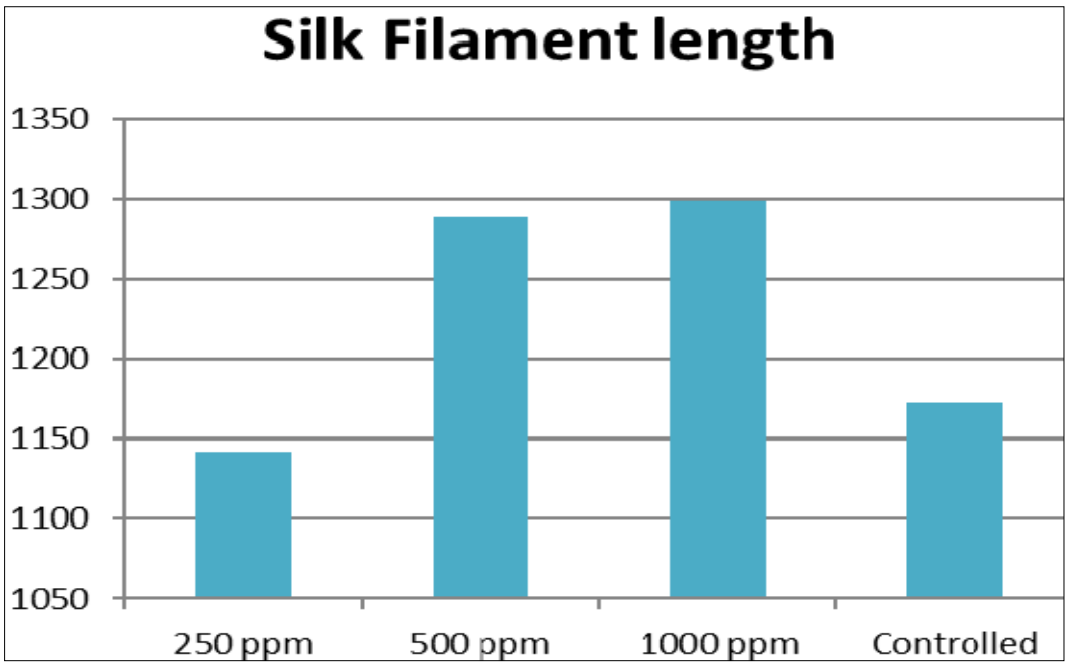

Fig 4. Effect of tryptophan on silk filament length of silkworm *Bombyx mori* L.

Discussion

The results of this study clearly show that adding tryptophan to mulberry leaves has a positive impact on the growth and cocoon traits of bivoltine silkworm hybrids. Traits such as larval weight, cocoon weight, shell weight, filament weight, and filament length all reached their highest values at 1000 ppm, highlighting the importance of tryptophan in boosting protein synthesis and overall metabolic efficiency. Interestingly, the shell ratio was highest at 500 ppm, which suggests that while higher concentrations promote overall growth and silk yield, the proportion of shell weight relative to cocoon weight is optimized at a moderate level.

These findings are in line with earlier studies. Laz *et al.* (2006) [4] and Qayoom *et al.* (2025) [8] reported that methionine and tryptophan supplementation increased raw silk yield, while Radjabi (2010) [9] demonstrated that enriching mulberry leaves with asparagine and alanine improved silk productivity. Similarly, Nicodemo and Olivera (2014) [7] found that silkworms fed on amino acid-enriched leaves showed significant improvements in cocoon and shell weights. Saad (2014) [10] reported that cocoon characteristics and economic traits were significantly improved in larvae treated with 0.1% glycine compared to the control and other treatment levels. Murugesh *et al.* (2021) [5] and Muzamil *et al.* (2023) [6] reported that mulberry leaves fortified with amino acids such as glycine, alanine, and serine during the fifth instar stage has been shown to significantly enhance larval performance, cocoon parameters, and silk reeling characteristics. Together, these consistent results reinforce the role of amino acids particularly tryptophan in improving silk yield and quality.

Conclusion

This study emphasizes the beneficial impact of tryptophan supplementation on improving the economic traits of bivoltine silkworm hybrids. Among the tested concentrations, 1000 ppm proved most effective in improving larval growth, cocoon weight, shell weight, filament length, and filament weight, while 500 ppm was optimal for achieving the highest shell ratio. These improvements are likely due to better nutrient utilization, enhanced metabolic efficiency, and increased silk protein synthesis supported by tryptophan. The fortification of mulberry leaves with tryptophan offers a viable strategy to simultaneously enhance silk productivity and quality. To establish its commercial value, further large-scale field trials and cost benefit analyses are necessary.

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