

# Impact of Methionine and Tryptophan Supplementation on Silk Gland Weight and Tissue Somatic Index in Eri Silkworm (*Samia ricini* Donovan)

**ABSTRACT:** This study evaluated the impact of methionine and tryptophan supplementation on the silk gland weight and Silk Gland Tissue Somatic Index (SGTSI) of eri silkworm, *Samia ricini* Donovan, a key species in vanya silk production. Freshly collected castor leaves were fortified with methionine and tryptophan solutions at various concentrations and fed to silkworm larvae from the third instar until maturity. The treatments significantly enhanced silk gland weight and SGTSI compared to the control. The highest silk gland weight (1.52g) and SGTSI (25.16%) were observed in larvae fed with tryptophan at 500 ppm (T6), followed by methionine at 500 ppm (T3) with 1.47g and 24.61%, respectively. Control larvae exhibited the lowest values (1.07g and 19.43%). Combination treatments of methionine and tryptophan showed less improvement compared to individual treatments. These findings suggest that dietary supplementation with specific amino acids could enhance silk gland development and silk production.

**Keywords:** Castor, eri silkworm, methionine, *Samia ricini* Donovan, silk gland, tryptophan

## 1. Introduction

Eri silkworm, *Samia ricini* Donovan (Lepidoptera: Saturniidae) is a completely domesticated multivoltine species under the non-mulberry (vanya silk) sector (Singh *et al.*, 2003). The silk produced by the eri silkworm is considered economically the third most important silk in the world after mulberry and tasar silks (Sarkar *et al.*, 2016). Like other living organisms, nutrition plays a vital role in sericulture by enhancing the commercial characteristics of silkworms (Rani *et al.*, 2011). Feeding nutritionally enriched leaves provided better growth and development of silkworms as well as gain in their economic cocoon characters (Krishnaswami *et al.*, 1971). The larval weight has a direct impact on silk gland weight (Singh and Kumar, 1996); and the size and weight of the silk gland have a direct impact on cocoon shell size and weight (Muruges *et al.*, 2021). The silk gland is a specialized organ responsible for the synthesis and secretion of silk proteins (Zhang *et al.*, 2006). Amino acid nutrition of the silkworm is closely related to the synthesis of silk protein as well as to the growth of silk glands (Ito, 1972). Silkworm requires essential, non-essential and semi-essential amino acids for its growth and development. It was reported by Chakrabarty and Kaliwal (2012) that oral supplementation of arginine, histidine and their mixture had a significant effect on the larval weight, silk gland weight, survival rate, quantitative cocoon, and reproductive parameters of *Bombyx mori*. Fortification of mulberry leaves with a combination of three amino acids *viz.*, glycine, alanine and serine exhibited increment in silk gland weight and silk gland length; protein content in the silk gland and haemolymph of *B. mori* larvae (Muruges *et al.*, 2021). Muzamil *et al.* (2023) revealed that the percent ratio of silk gland to body weight of *B. mori* larvae was significantly higher in the larvae group fed with amino acid-fortified leaves when compared to the control.

Nevertheless, the utilization of amino acids as a supplementary diet in the eri silkworm, *S. ricini* Donovan remains unexplored so far. Hence, the present investigation has been undertaken to study the

silk gland weight and SGTSI of eri silkworm when fed with methionine and tryptophan-fortified castor leaves, which could help to discover its utilization in preparation of artificial diet and diet supplementation for healthier eri silkworms and thus better quality cocoons for higher productivity.

## 2. Materials and methods

### Study Area and Conditions

The present experiment was carried out in the Department of Sericulture, Assam Agricultural University, Jorhat, during the month of December-February (winter season), 2020-2021. Local red petiole variety (NBR-1) of castor (*R. communis* Linn.) leaves was used in this study. Disease-free layings (DFLs) of eri silkworm were collected from Eri Seed Grainage, Demow. Water-soluble amino acids DL-methionine (GRMO56) and L-tryptophan (GRMO67) of HIMEDIA were used to fortify the castor leaves. Different concentrations of selected amino acids as well as their combinations adopted for the study are given in Table 1.

**Table 1. Concentrations of methionine and tryptophan**

<i>Treatment code</i>	<i>Treatment</i>	<i>Concentration (ppm)</i>
T <sub>1</sub>	Methionine	100
T <sub>2</sub>	Methionine	300
T <sub>3</sub>	Methionine	500
T <sub>4</sub>	Tryptophan	100
T <sub>5</sub>	Tryptophan	300
T <sub>6</sub>	Tryptophan	500
T <sub>7</sub>	Methionine + Tryptophan	100 + 100
T <sub>8</sub>	Methionine + Tryptophan	300 + 300
T <sub>9</sub>	Methionine + Tryptophan	500 + 500
T <sub>10</sub>	Untreated castor leaf (control)	-

### Experimental Design

Three replications were made in each concentration of the amino acids as well as control, each with 100 nos. of larvae. The leaves sprayed with distilled water served as control.

### Amino Acid Treatments and Feeding Methodology

Eri silkworm larvae were reared from the first to fifth instar in the rearing room under natural room temperature and humidity conditions following the rearing method of Chowdhury (1982). They were reared up to second instar on fresh castor leaves. Fortification of castor leaf with methionine and tryptophan was done by the leaf dip method as suggested by Laz (2010) where the fresh castor leaves

were treated by dipping in different concentrations of the amino acid solutions separately and dried up by fanning and then were supplied to the eri silkworm larvae once daily in the morning hours *i.e.* the first feeding from the first day of third instar until maturity. The larvae of a control batch were simultaneously reared on fresh untreated leaves.

### Data collection

1. **Silk gland weight (g):** The silk gland (Fig.1) of ten matured larvae was dissected carefully from each replication of each treatment. Weight of the silk gland was recorded in grams.

2. **Silk Gland Tissue Somatic Index (SGTSI %):** SGTSI % was determined by taking the weight (in grams) of the silk gland and matured larva with the help of digital weighing balance following the method suggested by Rai Reddy and Benchamin (1989).

$$\text{SGTSI (\%)} = \frac{\text{Weight of the silk gland tissue (g)}}{\text{Weight of the larval body (g)}} \times 100$$

### Statistical analysis

The experiment was laid out in Completely Randomized Design (CRD) where each treatment was replicated three times. Statistical analysis for the present investigation was determined by following Panse and Sukhatme (1989).

### 3. Results

The study showed a significant effect on the silk gland weight as well as Silk Gland Tissue Somatic Index (SGTSI) of eri silkworm due to fortification of methionine and tryptophan (table 2). Statistically, both the amino acids enhanced the silk gland weight and SGTSI. Among all the treatments, T<sub>6</sub> (tryptophan@ 500 ppm) exhibited the maximum silk gland weight (1.52g) which was found to be statistically *at par* with T<sub>3</sub> (methionine@ 500 ppm) weighing 1.47g; however, it was found to be least in the control T<sub>10</sub> (1.07g) which was *at par* with T<sub>9</sub> (1.09g). In all the combination batches of methionine and tryptophan, the weight of silk gland was found to be significantly lower than the individual batches except T<sub>7</sub>. However, T<sub>1</sub> and T<sub>7</sub>; T<sub>1</sub> and T<sub>8</sub>; T<sub>8</sub> and T<sub>9</sub> treatments were *at par*.

The highest SGTSI (25.16%) was obtained in T<sub>6</sub> which was *at par* with T<sub>3</sub> (24.61%). Among the combination treatments, T<sub>7</sub> registered significantly higher SGTSI (20.85%) followed by T<sub>8</sub> (19.80%) and T<sub>9</sub> (19.79%) which were *at par* with each other. However, a non-significant difference was observed between T<sub>1</sub> and T<sub>7</sub>; T<sub>2</sub> and T<sub>4</sub>; T<sub>3</sub> and T<sub>5</sub>. T<sub>1</sub> was also statistically *at par* with T<sub>8</sub> and T<sub>9</sub> treatments. The lowest SGTSI was observed in the control T<sub>10</sub> (19.43%) which was *at par* with T<sub>8</sub> (19.80%) and T<sub>9</sub> (19.79%).

**Table 2. Silk gland weight (g) and Silk Gland Tissue Somatic Index (SGTSI%) of eri silkworm fortified with methionine and tryptophan solutions**

Treatment	Silk gland weight (g)	Silk gland tissue somatic index (%)
T <sub>1</sub>	1.17	20.31
T <sub>2</sub>	1.35	22.35
T <sub>3</sub>	1.47	24.61
T <sub>4</sub>	1.29	22.20
T <sub>5</sub>	1.41	24.19
T <sub>6</sub>	1.52	25.16
T <sub>7</sub>	1.21	20.85
T <sub>8</sub>	1.13	19.80
T <sub>9</sub>	1.09	19.79
T <sub>10</sub>	1.07	19.43
S.Ed (±)	0.03	0.33
CD at 5%	0.06	0.68

#### 4. Discussion

Out of all the treatments, 500 ppm tryptophan recorded the highest silk gland weight and SGTSI followed by methionine@ 500 ppm, whereas the lowest was observed in control. Willcock and Hopkins (1906) stated that tryptophan, the first reported amino acid was found to be nutritionally essential. Among various tRNA species in the silk gland cells, methionine tRNA plays an important role in regulating silk protein synthesis (Shimura, 1978). The silk production is directly influenced by the weights of both larvae and glands, with increased larval weights leading to higher silk production (Venugopal Reddy *et al.*, 2015). The increase in silk gland weight and SGTSI in the present study might be due to additional supplementation of tryptophan and methionine along with castor leaves (Kaur and Srivastava, 2022). Present findings conform to Thulasi *et al.* (2015) who reported that the soya protein, which is the chief ingredient of nutilite, has the potential to modulate silk gene expression and silk protein synthesis in *B. mori*. Kamala and Karthikeyan (2019) stated that the larval weight, silk gland and SGTSI of *B. mori* larvae increased as compared to the control due to supplementation of nano-particles of alanine at varied concentrations since alanine increases the efficiency of conversion and metabolic rate. Threonine supplementation in the mulberry silkworm's diet also led to increased silk production (Nicodemo *et al.*, 2014). Combination batches, however, showed less improvement in regard to these parameters. A higher level of non-essential amino acids or acidic amino acids favoured silk production, whereas a higher level of essential amino acids affected it

adversely in *B. mori* (Baruah and Dutta, 2021; Ito *et al.*, 1967). Supplementation of mulberry leaves with amino acids at low concentrations yielded better outcomes in larval weight, percentage of silk gland, crude protein, cocoon and cocoon shells (Mustafa and Elkaraksy, 1990).

## 5. Conclusion

From the present investigation, it can be concluded that fortification of castor leaves with selected amino acids significantly influences the silk gland weight and SGTSI of eri silkworm. The individual treatments of methionine and tryptophan as well as their combinations performed better almost in all aspects compared to untreated larvae. Tryptophan and methionine, especially at 500 ppm concentration are more efficient and exert significant impact over other doses and were identified as promising. The promising treatments (tryptophan and methionine@ 500 ppm) identified in this study can be exploited for better larval growth and development which will lead to better cocoon and silk production in eri silkworm. Generally, spring and autumn seasons are regarded as the optimal seasons for cultivating eri silkworms. Farmers avoid winter rearing due to the unavailability of poor quality and quantity of leaves. From the present investigation, it may be suggested that winter rearing of eri silkworm using castor leaves fortified with methionine and tryptophan at certain concentrations is feasible without compromising cocoon yield and quality.

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## REFERENCES

Baruah, A. and Dutta, P. (2021). Influence of dietary supplementation on the cocoon traits of eri silkworm (*Samia ricini*). *Journal of Insect Science*, 28(3), 210-218.

- Chakrabarty, S. and Kaliwal, B.B. (2012). Application of arginine, histidine and their mixture on economic traits of the silkworm, *Bombyx mori* L. *International Journal of Science*, 1(2), 107-111.
- Chowdhury, S.N. (1982). *Eri Silk Industry*. Directorate of Sericulture and Weaving, Assam, pp. 24-25.
- Ito, T. (1972). Amino Acid Nutrition of the Silkworm, *Bombyx mori*. *Proc. Japan Acad.*, 48(8), 613-618.
- Ito, T., Arai, N. and Inokuchi, T. (1967). Nutrition of the silkworm, *Bombyx mori*. XVII. Effect of dietary levels of amino acids on growth of fifth-instar larvae and on cocoon quality. *Bull. Seric. Exp. Sta.*, 21, 385-400.
- Kamala, M. and Karthikeyan, A. (2019). Effect of the nutritional fortification of nanoparticles of alanine on the growth and development of mulberry silkworm, *Bombyx mori* L. *Journal of Emerging Technologies and Innovative Research*, 6(4), 561-569.
- Kaur, I. and Srivastava, M. (2022). Studies on the effect of amino acids on the growth and development of eri silkworm, *Samia ricini*. *Journal of Sericulture Research*, 61(2), 125-132.
- Krishnaswami, S., Kumararaj, S.S., Vijayaraghavan, K. and Kasiviswanathan, K. (1971). Silkworm feeding trials for evaluating the quality of mulberry leaves as influenced by variety, spacing and nitrogen fertilization. *Indian. J. Seric.*, 10(1), 79-90.
- Laz, R. (2010). Effects of methionine and tryptophan on some quantitative traits of silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). *Univ. j. zool. Rajshahi. Univ.*, 28, 15-19.
- Muruges, K.A., Chozhan, K. and Aruna, R. (2021). Enhancement of larval and cocoon traits of silkworm, *Bombyx mori* L. through the application of amino acids. *Journal of Entomology and Zoological Studies*, 9(1), 2198-2203.
- Mustafa, S.M. and Elkaraksy, I.A. (1990). The effect of certain amino acids on silkworm, *Bombyx mori* L. *Agricultural Research Review*, 66(1), 105-109.
- Muzamil, A., Tahir, H.M., Ali, A., Bhatti, M.F., Munir, F., Ijaz, F., Adnan, M., Khan, H.A. and Qayyum, K.A. (2023). Effect of amino acid fortified mulberry leaves on economic and biological traits of *Bombyx mori* L. *Heliyon*, 9(10), e21053.
- Nicodemo, D., Oliveira, J.E., Sedano, A.A., Marconcini, J.M. and Tonoli, G.H.D. (2014). Impact of different silkworm dietary supplements on its silk performance. *Journal of Materials Science*, 49(18), 6302-6310.
- Panse, V.G. and Sukhatme, P.V. (1989). *Statistical methods for agricultural workers*. Indian Council of Agricultural Research, New Delhi, 5<sup>th</sup> Edition, p. 359.
- Rai Reddy, K.V. and Benchamin, K.V. (1989). Studies on tissue somatic indices (TSI) of silk gland and gonads of silkworm *Bombyx mori* L. during 5<sup>th</sup> instar. *Sericologia*, 29, 453-476.
- Rani, A.G., Padmalatha, C., Raj, R.S. and Singh, R.A.J.A. (2011). Impact of supplementation of amway protein on the economic characters and energy budget of silkworm, *Bombyx mori* L. *Asian Journal of Animal Sciences*, 5(3), 190-195.

- Sarkar, B.N., Sarmah, M.C. and Singh, B.K. (2016). Yield and yield contributing traits in eri silkworm, *Samia ricini* (Donovan) in different combinations. *International Journal of Ecology and Ecosolution*, 3(4), 45-49.
- Shimura, K. (1978). Synthesis of Silk Proteins. In: *The silkworm: an important laboratory tool*, Tazima, Y. Kodansha (Ed.) Ltd., Tokyo, pp. 195-196.
- Singh, B.K., Debaraj, Y., Sarmah, M.C., Das, P.K. and Suryanarayana, N. (2003). Eco-races of eri silkworm. *Indian Silk*, 42(1), 7-10.
- Singh, G.P. and Kumar, V. (1996). Correlation study on the larval silk gland and shell weight in the silkworm *Bombyx mori* L. *Entomon*, 21(3/4), 277-279.
- Thulasi, N., Bhuvanewari, E., Madhavi, R. and Sivaprasad, S. (2015). Larval growth, silk production and economic traits of *Bombyx mori* under the influence of nutilite enriched mulberry leaves. *International Journal of Advances in Pharmacy, Biology and Chemistry*, 4(3), 536-543.
- Venugopal Reddy, B., Divya, P. and Anitha, M. (2015). Quantitative profile analysis of mulberry silkworm *Bombyx mori*. L (CSR<sub>2</sub>XCSR<sub>4</sub>). *International Letters of National Sciences*, 34, 34-41.
- Willcock, E.G. and Hopkins, F.G. (1906). The importance of individual amino acids in metabolism; observations on the effect of adding tryptophan to a diet in which zein is the sole nitrogenous constituent. *J. Physiol.*, (London), 35, 88-102.
- Zhang, P., Aso, Y., Yamamoto, K., Banno, Y., Wang, Y., Tsuchida, K., Kawaguchi, Y. and Fujii, H. (2006). Proteome analysis of silk gland proteins from the silkworm, *Bombyx mori*. *Proteomics*, 6(8), 2586-2599.





Fig. 1. Silk glands of eri silkworm at different treatments