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

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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

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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, *Samiaricini* Donovan (Lepidoptera: Saturniidae) is a completely domesticated multivoltine species under the non-mulberry (*vanya* silk) sector [1]. The silk produced by the eri silkworm is considered economically the third most important silk in the world after mulberry and tasar silks [2]. Like other living organisms, nutrition plays a vital role in sericulture by enhancing the commercial characteristics of silkworms [3]. Feedingnutritionally enriched leaves provided better growth and development of silkworms as well as gain in their economic cocoon characters [4]. The larval weight has a direct impact on silk gland weight [5] and the size and weight of the silk gland have a direct impact on cocoon shell size and weight [6]. The silk gland is a specialized organ responsible for the synthesis and secretion of silk proteins [7]. Amino acid nutrition of the silkworm is closely related to the synthesis of silk protein as well as to the growth of silk glands [8]. Silkworm requires essential, non-essential and semi-essential amino acids for its growth and development. It was reported by Chakrabarty and Kaliwal [9]. 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 [6]. Muzamil et al. [10]. 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

2.1 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-freelayings (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.

2.2 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.

2.3 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 [11]. 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 [12] 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.

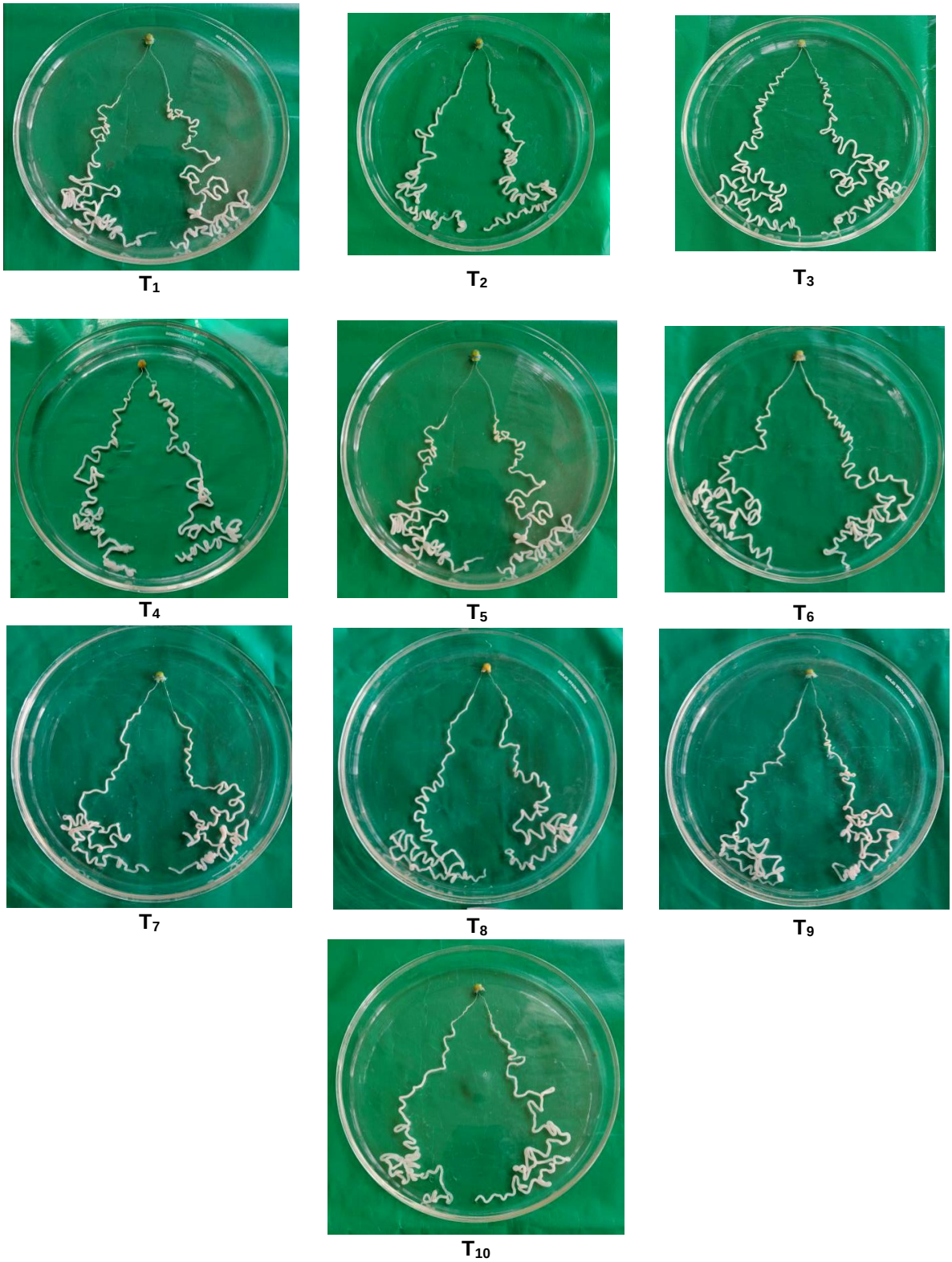

Fig. 1. Silk glands of eri silkworm at different treatments

Table 1. Concentrations of methionine and tryptophan

Treatment code	Treatment	Concentration (ppm)
T_1	Methionine	100
T_2	Methionine	300
T_3	Methionine	500
T_4	Tryptophan	100
T_5	Tryptophan	300
T_6	Tryptophan	500
T_7	Methionine + Tryptophan	100 + 100
T_8	Methionine + Tryptophan	300 + 300
T_9	Methionine + Tryptophan	500 + 500
T_{10}	Untreated castor leaf (control)	-

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_1	1.17	20.31
T_2	1.35	22.35
T_3	1.47	24.61
T_4	1.29	22.20
T_5	1.41	24.19
T_6	1.52	25.16
T_7	1.21	20.85
T_8	1.13	19.80
T_9	1.09	19.79
T_{10}	1.07	19.43
S.Ed (±)	0.03	0.33
CD at 5%	0.06	0.68

2.4 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 [13].

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

2.5 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 [14].

3. RESULTS

The study showed a significant effect on the silk gland weight as well as Silk Gland Tissue SomaticIndex (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_6 (tryptophan@ 500 ppm) exhibited the maximum silk gland weight (1.52g) which was found to be statistically *at par* with T_3 (methionine@ 500 ppm) weighing 1.47g; however, it was found to be least in the control T_{10} (1.07g) which was *at par* with T_9 (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_7. However, T_1 and T_7; T_1 and T_8; T_8 and T_9 treatments were *at par*.

The highest SGTSI (25.16%) was obtained in T_6 which was *at par* with T_3 (24.61%). Among the combination treatments, T_7 registered significantly higher SGTSI (20.85%) followed by T_8 (19.80%) and T_9 (19.79%) which were *at par* with each other. However, a non-significant difference was observed between T_1 and T_7; T_2 and T_4; T_3 and T_5. T_1 was also statistically *at par* with T_8 and T_9 treatments. The lowest SGTSI was observed in the control T_{10} (19.43%) which was *at par* with T_8 (19.80%) and T_9 (19.79%).

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 [15] 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 [16]. The silk production is directly influenced by the weights of both larvae and glands, with increased larval weights leading to higher silk production [17]. 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 [18]. Present findings conform to Thulasi et al. [19] who reported that the soya protein, which is the chief ingredient of nutrilite, has the potential to modulate silk gene expression and silk protein synthesis in *B. mori*. Kamala and Karthikeyan [20] 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 [21]. 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* [22,23]. 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 [24].

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 cultivatingeri 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.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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