**Review Article**

**A review on fortification of mulberry leaf with proteins and amino acid supplements: Impact on larval development and cocoon yield of silkworm, *Bombyx mori.***

**Abstract:** The silkworm, *Bombyx mori* is a monophagous lepidopteran insect which derives required nutrients for its growth and development from mulberry leaf alone. Though nutrients present in the mulberry leaves satisfy nutritional needs of silkworm but the quantity of nutrients present vary depending on environmental conditions, usage of fertilizers in field, mulberry varieties, crop protection measures and other field practices. One of the alternative ways of improving larval nutrition is enrichment of mulberry leaves with supplementary nutrients such as proteins, amino acids, vitamins, minerals, etc. Experimental evidence consistently shows that fortification of mulberry leaf with amino acids (L-Serine and glycine) or protein rich supplements (drone brood, protinex) significantly improves larval growth, silk gland development and cocoon productivity in silkworm, *B. mori.* Optimal gains are observed at moderate supplementation levels during the fifth instar. However, implementation at scale requires careful attention to concentration, timing, cost and farmer acceptance.

**Keywords: Mulberry leaf, Fortification, Silkworm, *Bombyx mori,* Proteins and Amino acids.**

**Introduction:** Nutrition plays an important role in improving the growth and development of the silkworm, *Bombyx mori* L. like other organisms. One of the most important characteristics of the silkworm*, B. mori* is its ability to switch plant proteins from feeding material to silk proteins. The larvae grow 10,000 times in size and 12,000 times in weight from the day of hatching to spinning (Bhatti et al., 2019). The silkworm, *B. mori* is a monophagous lepidopteran insect which derives required nutrients for its growth and development from mulberry leaf alone (Hussain et al., 2011). Therefore, the growth and development of larvae and subsequent cocoon production are greatly influenced by the nutritional quality of mulberry leaves. Mulberry leaves as the only source of nutrition to silkworm, it should contain minimum amount of moisture, carbohydrates, proteins, minerals, vitamins, *etc.,* and at the same time, they must have favourable physical features such as suitable tenderness, thickness and freshness in order to be easily consumed by silkworm (AS, 2024). Significant seasonal variations occur in the nutritional value and composition of mulberry leaves depending on factors *viz.,* weather, insect pests and diseases as well as agriculture practices followed. One of the alternative ways of improving larval nutrition is enrichment of mulberry leaves with supplementary nutrients such as proteins, amino acids, vitamins, minerals, etc. (Etebari et al*.,* 2004; Murugesh et al., 2021). Recent studies also highlights the role of phytoconstituents from flowers of *[Tridax](https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/tirilazad%22%20%5Co%20%22Learn%20more%20about%20Tridax%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages)* trilobata (T. trilobata) in the synthesis of silver [nanoparticles](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/nanoparticle) (AgNPs) and their effects on the [growth and development](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/growth-development-and-aging) of the [silkworm](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/bombyx-mori) Bombyx mori L. besides controlling the occurrence of Flacherie and Sappe microbial diseases (Surendra et al., 2023). So, an attempt has been made to review the effects of enrichment of mulberry leaves with proteins and aminiacids on feeding *Bombyx mori* larvae, evaluating subsequent impacts on larval and cocoon qualtity (Lalfelpuii et al., 2014)*.*

**Importance of nutrient supplementation in silkworm:** Nutrition is the most important factor that influences the growth and development of the silkworm, *B. mori* L. Nutritional components in mulberry leaves have direct impact on the overall growth of silkworm and also increase the larval, pupal and cocoon weight with quality silk production. Cocoon characters both quantitative as well as qualitative, depends on the quality and quantity of mulberry leaves. Feeding nutrition supplement enriched mulberry leaves do positively influence the silkworm growth and development and the silk output (Etebari *et al.,* 2004; Ayandokun & Alamu, 2020).

**Table 1: Nutritional composition of mulberry leaf (Shubhajit *et al.,* 2020).**

|  |  |  |  |
| --- | --- | --- | --- |
| **Sl.****No.** | **Nutritional Compositions and phytochemicals** | **Quantity** | **References** |
| **1** | Total phenols(TP) | 8.76-20.26 mg gallic acid equivalents(GAE) per g dry weight(dw) | (Yu *et al.*2018) |
| **2** | Total flavonoid content(TF) | 21.36-56.41 mg rutin equivalents(RE) per g dry weight(dw) | (Yu *et al.*2018) |
| **3** | Total soluble sugars(TSS) | 58.71-150.31 mg per g dry weight(dw) | (Yu *et al.*2018) |
| **4** | 1. Dexynojirimycin(1-DNJ)
 | 0.20-3.88 mg per g | (Ji *et al*.2016) |
| **5** | Total phenols | 12.81-15.50 mg gallic acid equivalent(GAE) per g dry weight(dw) | (Sanchez *et al.* 2015) |
| **6** | Caffeoylquinic acids | 6.78-8.48 mg per dry weight(dw) | (Sanchez *et al.* 2015) |
| **7** | ABTS | 10.6-13.15mg Trolox per g dry weight(dw) | (Sanchez *et al.* 2015) |
| **8** | DPPH | 10.62-12.64mg Trolox per g dry weight(dw) | (Sanchez *et al.* 2015) |
| **9** | Total soluble carbohydrates | 3.1 g per 100g fresh weight(fw) | (Sanchez *et al.* 2015) |
| **10** | Reducing sugars | 1.5 gper 100g fresh weight(fw) | (Dimitrova *et al.*2015) |
| **11** | Fructose and glucose | 0.3 g per 100g fresh weight(fw) | (Dimitrova *et al.*2015) |
| **12** | Sucrose | 1.1g per 100g fresh weight(fw) | (Dimitrova *et al.*2015) |
| **13** | Crude proteins | 15.31-30.91 per cent  | (Dimitrova *et al.*2015) |
| **14** | Crude fat | 2.07-7.92 per cent  | (Butt *et al.*2008) |
| **15** | Crude fibre | 9.9-13.85 per cent  | (Butt *et al.*2008) |
| **16** | Neutral dietary fibre | 27.6-43.6 per cent  | (Butt *et al.*2008) |
| **17** | Total ash | 11.3-17.24 per cent  | (Butt *et al.*2008) |
| **18** | Ascorbic acid | 100-200 mg per 100g | (Butt *et al.*2008) |
| **19** | Beta-carotene | 8.44-13.13 mg per 100g | (Butt *et al.*2008) |
| **20** | Oxalates | 183 mg per 100g | (Butt *et al.*2008) |
| **21** | Phytaytes | 156 mg per 100g | (Butt *et al.*2008) |
| **22** | Tannic acid | 0.13-0.36 per cent | (Butt *et al.*2008) |
| **23** | Iron(Fe) | 19-50 mg per 100g  | (Butt *et al.*2008) |
| **24** | Zinc(Zn) | 0.72-3.65 mg per 100g | (Butt *et al.*2008) |
| **25** | Calcium(Ca) | 786.66-2,726.66 mg per 100g | (Butt *et al.*2008) |
| **26** | Phosphorus(P) | 970 mg per 100g | (Butt *et al.*2008) |
| **27** | Magnesium(Mg) | 720 mg per 100g | (Butt *et al.*2008) |

**Nutritional requirement of silkworm:** Nutrition is an essential component of life without which it is impossible for any living organism to survive and it is very much important for healthy and disease free life and the same applies to silkworm. Nutritional requirements are the chemical factors of ingested food essential for normal metabolism and development of silkworm. In general, the nutritional requirement of silkworm may vary with developmental stages, sex and physiological stress. Nutrients such as carbohydrates, proteins, lipids, vitamins, amino acids, water etc., are most required essential nutrients which are generally present in mulberry leave.

**1. Carbohydrates**: Carbohydrates are group of organic compounds that includes sugars, starch and cellulose. Carbohydrate utilization depends on the ability of silkworm to hydrolyze polysaccharides. Similar to other species of insects, silkworm does not show any specific requirement for carbohydrates, although several compounds are utilized more readily than others. In general, pentoses are poorly utilized, among hexoses, glucose, fructose, and mannose are well utilized. Disaccharides, especially sucrose, cellulose and maltose are uniformly good. Among sugar alcohols only sorbitol is utilized. Utilization of polysaccharides is entirely dependent on silkworm strains that depend on the presence or absence of amylases in digestive juice. Furthermore, nutritive value of various oligosaccharides are shown to be related with the presence or absence of corresponding glycosidase (Mathur *et a*l 2002; Borah *et al.,* 2020).

**2.Water:** Phytophagous insects normally feed on diets with high water content. However, the amount may vary for the different species. The silkworm body is composed of more than 75% water which is necessary for all kinds of life processes, especially in digestion. Water is the best solvent and only in a solution state can all kinds of nutrients be absorbed into cells. Moreover, water is the only medium for transportation of nutrients and waste matter. It can also adjust the body temperature and keep it relatively stable (Shamsuddin, 2009; Borah *et al*., 2020).

**3. Lipids:** Lipids are the group of organic compounds that are insoluble in water but soluble in organic solvents. Silkworms are able to convert carbohydrates into lipids and can synthesize lipids and accumulate them in body tissue. Fatty acids, phospholipids and sterols are components of cell walls in addition to having other specific functions, silkworm requires polyunsaturated fatty acids such as linoleic and linolenic acids for their normal growth and development. Silk moths deficient in these fatty acids have defects in wing formation and their scales adhere to the pupal case on emergence. Silkworm requires sterols in the diet because they can’t synthesize enough to meet their physiological requirements. Sterol is the precursor for synthesizing the moulting hormone of silkworm. Thus, deficiency of a sterol in the diet results in incapability of the silkworm to moult and they typically die in the early instars (Genc, 2002; Borah *et al*., 2020).

 **4. Protein:** Protein is one of the essential components of the silkworm. It is the most important component of cell molecular structure for survival. Protein can adjust and control the metabolism and physiological function of the silkworms by combining with active substances like enzyme, hormone etc. Adult female silkworm needs protein, as it is crucial for successful secretion of juvenile hormone (JH) which is required for development of ovaries and egg maturity (Vanderzant, E. S., 1974; Vasuki and Basavanna, 1969; Borah *et al*., 2020).

**5. Amino acid:** Amino acids essential for growth and survival of the silkworm are arginine, histidine, leucine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, aspertic acid and glutamic acid. In addition to these twelve essential amino acids, the silkworm requires proline, but proline is considered to be semi-essential since some growth was obtained even in the absence of proline. However, non-essential amino acids, alanine, cystine, glycine, serine and tyrosine are also important, because they motivate growth of the silkworm. Fibroin is derived from four kinds of amino acids viz., alanine, serine, glycine and tyrosine which come from the food material of silkworm. The amino acid alanine plays an important role in metabolism of glucose, tryptophan and organic acid (Vanderzant, E. S., 1974; Vasuki and Basavanna, 1969; Borah *et al*., 2020).

**Table 2: Amino acid content in mulberry leaf and minimum requirement of silkworm (mg/g of dry matter) (Hiroaki *et al.,* 2002)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Amino acid** | **Content** | **(%)** | **S.D.** | **C.V.** | **Minimum requirement\*\*** |
| **Asp** | 20.49 | 10.0 | 3.63 | 17.72 |  |
| **Thr** | 10.52 | 5.2 | 1.75 | 16.63 | 7 |
| **Ser** | 10.12 | 5.0 | 1.60 | 15.79 |  |
| **Glu** | 23.23 | 11.3 | 3.96 | 17.03 |  |
| **Pro** | 10.93 | 5.4 | 3.73 | 34.10 |  |
| **Gly** | 12.02 | 5.9 | 1.95 | 16.22 |  |
| **Ala** | 15.75 | 7.7 | 2.90 | 18.44 |  |
| **Val** | 12.83 | 6.3 | 2.17 | 16.92 | 8 |
| **Cys** | 1.17 | 0.6 | 0.25 | 21.72 |  |
| **Met** | 2.99 | 1.5 | 0.61 | 20.48 | 4 |
| **Ileu** | 10.04 | 4.9 | 1.88 | 18.68 | 8 |
| **Leu** | 19.45 | 9.5 | 3.10 | 15.93 | 8 |
| **Tyr** | 7.40 | 3.6 | 1.39 | 18.74 |  |
| **Phe** | 12.26 | 6.0 | 2.06 | 16.78 | 8 |
| **GABA** | 2.26 | 1.1 | 0.69 | 30.70 |  |
| **NH3** | 2.89 | 1.4 | 0.54 | 18.70 |  |
| **Lys** | 12.33 | 6.0 | 2.58 | 20.91 | 8 |
| **His** | 4.61 | 2.3 | 0.82 | 17.78 | 5 |
| **Arg** | 12.96 | 6.3 | 2.72 | 20.95 | 8 |
| **Total** | 204.25 | 100.0 |  |  |  |

**\*\* Minimum quantity required for silkworm growth (Arai and Ito, 1967).**

**Impact of protein supplements on silkworm rearing parameters and cocoon productivity:** Sharma *et al.* (2023) studied on influence of protein fortification on larval growth parameters and economic parameters of silkworm, *B. mori* L. In this study, the bivoltine silkworm double hybrid FC1 × FC2 was reared on mulberry leaves fortified with proteins namely drone brood, protinex and their combination.

**Table 3: Effect of mulberry leaves fortified with different concentrations of proteins on larval growth parameters of silkworm, *B. mori.* (Sharma *et al.,* 2023).**

|  |  |
| --- | --- |
| **Treatments** | **Growth parameters** |
| **Larval length (cm)** | **OG****R****(%)** | **CPG****R****(%)** | **Larval weight (g)** | **OG****R****(%)** | **CPG****R****(%)** | **Larval perimeter (cm)** | **OG****R****(%)** | **CPG****R****(%)** |
| **Initial** | **Final** | **Initial** | **Final** | **Initial** | **Final** |
| **T1**Drone brood (6%) | 4.92±0.01 | 7.18±0.01bcd | 45. 93 | 6.21 | 1.19±0.00 | 4.62±0.01ef | 288 | 24.26 | 9.93±0.01 | 14.64±0.03de | 47. 43 | 6.41 |
| **T2**Drone brood (2%) | 4.91±0.01 | 7.04±0.02abc | 43. 38 | 5.92 | 1.17±0.01 | 4.52±0.00cd | 286 | 24.03 | 9.92±0.02 | 14.45±0.02bcd | 45. 67 | 6.20 |
| **T3**Protinex (10%) | 4.94±0.01 | 7.24±0.04d | 46. 56 | 6.32 | 1.19±0.02 | 4.70±0.01f | 295 | 24.58 | 9.93±0.00 | 14.75±0.02e | 48. 54 | 6.52 |
| **T4**Protinex (6%) | 4.92±0.00 | 7.07±0.03a bcd | 43. 70 | 5.97 | 1.18±0.01 | 4.56±0.01de | 286 | 24.09 | 9.92±0.00 | 14.49±0.02bcd | 46. 07 | 6.25 |
| **T5**Protinex (2%) | 4.91±0.00 | 7.02±0.01abc | 42. 97 | 5.90 | 1.17±0.01 | 4.45±0.03bc | 280 | 23.81 | 9.90±0.01 | 14.42±0.03bc | 45. 66 | 6.20 |
| **T6**Drone brood (5%) + Protinex (5%) | 4.95±0.02 | 7.20±0.07cd | 45. 45 | 6.16 | 1.19±0.01 | 4.69±0.00f | 294 | 24.54 | 9.94±0.01 | 14.62±0.01cde | 47. 08 | 6.36 |
| **T7**Done brood (3%) + Protinex (3%) | 4.92±0.01 | 7.07±0.01a bcd | 43. 70 | 5.98 | 1.18±0.01 | 4.51±0.02bcd | 282 | 23.93 | 9.91±0.02 | 14.33±0.03b | 44. 60 | 6.06 |
| **T8**Drone brood (1%) + Protinex (1%) | 4.91±0.03 | 7.01±0.00ab | 42. 77 | 5.87 | 1.16±0.00 | 4.43±0.01b | 282 | 23.80 | 9.91±0.02 | 14.11±0.08a | 42. 38 | 5.81 |
| **T9**Distil water | 4.86±0.01 | 6.91±0.04a | 42. 18 | 5.80 | 1.15±0.02 | 4.20±0.01a | 265 | 23.04 | 9.86±0.02 | 13.91±0.04a | 41. 07 | 5.66 |

Note: Each value is mean ± standard error of three replications, Figures followed by same letter in column are non-significant by Tukey’s HSD test, OGR-overall growth rate, CPGR-compound periodical growth rate.

From the above investigation, Sharma *et al*. (2023) reported that the Overall Growth Rate (OGR) of larval body length ranged from 42.18 to 46.56 per cent. Notably, in protinex (10%) concentration a significant elevation in OGR by 46.56 per cent was recorded, representing an additional elevation of 4.38 per cent over control with CPGR of 6.32 per cent. However, the drone brood (6%) and drone brood (5%) + protinex (5%) concentrations recorded OGR for larval body length to the tune of 45.93 and 45.45 with CPGR of 6.21 and 6.16 per cent, respectively. However, drone brood (2%), protinex (6%), protinex (2%), drone brood (3%) + protinex (3%), drone brood (1%) + protinex (1%) and control were found statistically at par with each other with respect to larval body length (Table 3). Later, Sharma *et al*. (2023) stated that the Overall Growth Rate (OGR) of larval body weight ranged from 265 to 295 per cent. The mulberry leaves fortified with protinex (10%) and drone brood (5%) + protinex (5%) concentration exhibited significant influence on body weight of silkworm larvae having OGR of 295 and 294 per cent with CPGR of 24.58 and 24.54 per cent, respectively. However, drone brood (6%), protinex (6%), drone brood (2%), drone brood (3%) + protinex (3%), drone brood (1%) +protinex (1%) and protinex (2%) and concentrations also showed significant increase in larval body weight in terms of OGR and CPGR by 288, 286, 286, 282, 282 and 280; 24.26, 24.09, 24.03, 23.93, 23.80 and 23.81 per cent, respectively. Whereas the larval body weight of the control batch showed an OGR of 265 per cent with CPGR of 23.04 per cent (Table 3). Sharma *et al*. (2023) also reported that the Overall Growth Rate (OGR) for larval body perimeter was recorded maximum (48.54%) at protinex 10 per cent concentration with CPGR of 6.52 per cent. However, in drone brood (6%), drone brood (5%) + protinex (5%), protinex (6%), drone brood (2%), protinex (2%) and drone brood (3%) + protinex (3%) concentrations the OGR for larval body perimeter were recorded to the tune of 47.43, 47.08, 46.07, 45.67, 45.66 and 44.60 per cent with CPGR of 6.41, 6.36, 6.25, 6.20, 6.20 and 6.06 per cent, respectively. Whereas the body perimeter of silkworm larvae fed on mulberry leaves fortified with drone brood (1%) + protinex (1%) and control batch were found statistically at par with each other having OGR of 42.38 and 41.07 per cent, respectively (Table 3).

The possible reason for the increased growth parameters of silkworm, *B. mori* in the present investigation may be due the food additives produced from different sources of protein, raise the protein content of the larvae of silkworm which affects the growth parameters of silkworm (Sharma *et al*., 2023).



**Fig. 1: Effect of protein fortification on gland-body ratio of silkworm, *B. mori* (Sharma *et al*., 2023)**

Sharma *et al*. (2023) analysed that the Gland-Body Ratio of silkworm recorded positive growth trends under the impact of mulberry leaves fortified with different concentrations of proteins. In protinex (10%) and drone brood (6%) concentrations the impact on Gland-Body Ratio was found more pronounced when compared to other concentrations. However, the highest Gland-Body Ratio (32.91%) was recorded for protinex at 10 per cent concentration which was found statistically at par with drone brood (6%) concentration with 32.84 per cent of Gland-Body Ratio. While for drone brood (5%) + protinex (5%), drone brood (2%), protinex (2%), protinex (6%), drone brood (3%) + protinex (3%) and drone brood (1%) + protinex (1%) it was 32.05, 31.83, 30.63, 30.58, 29.65 and 28.65 per cent, respectively. However, the least Gland-Body Ratio of 22.07 per cent was observed in control batch (Fig. 1).

The possible reason for the increased GBR of silkworms in the present investigation was due to availability of optimum proteins in fortified mulberry leaves that were fed to silkworm larvae during 5th instar development period (Sharma *et al*., 2023).

**Impact of amino acid supplements on silkworm rearing parameters and cocoon productivity:** Paital and Kalyani (2011) conducted a study to investigate the effect of dietary glycine (gly) on the growth and production of larva and cocoon of the Nistari breed of silkworm, *B. mori.* Different treatment schedules (2% and 3% gly smeared on the mulberry leaves with once and twice supplementation from 1st and 2nd day of V instar larvae) were imposed with suitable controls.

**List 1 - Treatment details of the above study (Paital and Kalyani, 2011)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sl. No.**   | **Group** **Name**  | **Glycine****(%)**  | **No. of****feeding/day** | **Starting day** **of treatment** |
| **1**  | 0G1I (T1)  | 0  | once  | 1st  |
| **2**  | 0G2I (T2)  | 0  | twice  | 1st  |
| **3**  | 0G2II (T3)  | 0  | twice  | 2nd  |
| **4**  | 2G1I (T4)  | 2  | once  | 1st  |
| **5**  | 2G2I (T5)  | 2  | twice  | 1st  |
| **6**  | 2G1II (T6)  | 2  | once  | 2nd  |
| **7**  | 2G2II (T7)  | 2  | twice  | 2nd  |
| **8**  | 3G1I (T8)  | 3  | once  | 1st  |
| **9**  | 3G2I (T9)  | 3  | twice  | 1st  |
| **10**  | 3G1II (T10)  | 3  | once  | 2nd  |
|  **11**  | 3G2II (T11)  | 3  | twice  | 2nd  |

**Table 4: Effect of glycine on the larval, silk gland and cocoon production in the Silkworm*, B. mori L*. (Paital and Kalyani, 2011)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Group**  | **Mature larval wt. (g)** | **Mature silk gland wt. (g)** | **Cocoon wt. (g)** | **Shell wt. (g)** | **Shell ratio** |
| **T1**  | 1.68 ± 0.26 | 0.18 ± 0.03 | 0.49 ± 0.09 | 0.09 ± 0.03 | 0.19 ± 0.06 |
| **T2**  | 1.7 ± 0.26 | 0.17 ± 0.02 | 0.48 ± 0.04 | 0.09 ± 0.01 | 0.19 ± 0.03 |
| **T3**  | 1.62 ± 0.15 | 0.16 ± 0.02 | 0.46 ± 0.02 | 0.09 ± 0.01 | 0.20 ± 0.03 |
| **T4**  | 1.99 ± 0.16 | 0.18 ± 0.01 | 0.41 ± 0.07 | 0.14 ± 0.01 | 0.20 ± 0.02 |
| **T5**  | 2.08 ± 0.23 | 0.33 ± 0.02 | 0.42 ± 0.12 | 0.15 ± 0.12 | 0.21 ± 0.04 |
| **T6**  | 1.97 ± 0.30 | 0.25 ± 0.07 | 0.63 ± 0.06 | 0.15 ± 0.02 | 0.22 ± 0.03 |
| **T7**  | 2.01 ± 0.23 | 0.33 ± 0.02 | 0.35 ± 0.02 | 0.15 ± 0.07 | 0.26 ± 0.03 |
| **T8**  | 2.09 ± 0.21 | 0.29 ± 0.02 | 0.63 ± 0.04 | 0.16 ± 0.02 | 0.25 ± 0.02 |
| **T9**  | 2.41 ± 0.02 | 0.35 ± 0.02 | 0.66 ± 0.03 | 0.19 ± 0.01 | 0.29 ± 0.03 |
| **T10**  | 2.21 ± 0.19 | 0.29 ± 0.02 | 0.65 ± 0.06 | 0.17 ± 0.01 | 0.25 ± 0.03 |
| **T11**  | 2.40 ± 0.15 | 0.30 ± 0.02 | 0.62 ± 0.05 | 0.17 ± 0.03 | 0.31 ± 0.03 |

From the above study, Paital and Kalyani (2011) reported that gly supplementation with the diets has a remarkable effect on the metabolism of silk worm *B. mori.* Both 2% and 3% gly supplementation was found to enhance the growth of the worm having highest in 3% gly once treated group and was lowest in 2% gly once a day group. It was further noticed that growth of the worms increases from gly treatment in the order of C < 2% once a day < 2% twice a day < 3% once a day < 3% twice a day group irrespective of the treatment started from 1st day or 2nd day of V instar (Table 4).Later, Paital and Kalyani (2011) analysed that growth of the silk gland has also been found to be influenced by dietary gly supplementation in the worms in all treated groups with respect to controls except 2% gly treatment from the 1st day group. Maximum silk gland weight was observed in 2% gly twice a day treatment from the 2nd day of 5th instar group in comparison to other groups. The order of increased silk gland growth was 0G1I = 0G2I = 0G2II = 2G1I < 2G1II < 3G1I = 3G1I < 3G2II < 3G2I < 2G2I < 2G2II (Table 4).Paital and Kalyani (2011) also reported that like growth parameters, cocoon weight was also increased in almost all treated groups except 2G2I group in which it decreased from the control group. The order of the cocoon weight remained as 2G2I < 0G1I = 0G2I = 0G2II < 2G2II < 3G2II ~3G2I ~ 3G1I < 2G1II ~ 3G1II < 2G1I. The shell weight has shown a different pattern than the above three parameters. 3% gly was found to have magnificent role on increased shell production than 2% gly treatment. However, 2% gly had enhanced cocoon weight in comparison to control group. The order of shell weight was 0G1I = 0G2I = 0G2II < 2G1I < 2G1II < 2G2I ~ 2G2II < 3G1I < 3G2I < 3G1II < 3G 2II (Table 4).

The possible reason for the increased silk gland and cocoon production in silkworms supplemented with dietary alpha glycine was due to fibroin is derived from four kinds of amino acids *viz*., alanine, serine, glycine and tyrosine and dietary supplementation of glycine to silkworm increases the availability of the silk gland protein (Paital and Kalyani, 2011).

Ramesh *et al.* (2018) evaluated the nutritional supplementation of amino acid L-serine to silkworm, *Bombyx mori* L. in relation to growth rate and silk production. In this study, first day of III instar silkworms (breed-NB4D2) were fed with normal MR2 mulberry leaves and those fortified with different nutritional supplementary compounds such as L-serine, aspartic acid, arginine, niacin, retinol, calciferol, ascorbic acid and glucose.

**Table 5: Impact of different amino acid supplements on cocoon parameters of *B. mori* L. (Ramesh *et al.*, 2018)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Experimental Groups/ Concentration** | **Cocoon length (cm)** | **Cocoon width (cm)** | **Cocoon weight (g)** |
| Control (C)  | 3.201±0.1547a | 2.183±0.1210a | 1.211±0.1006a |
| L-Serine (T1 - 0.25%)  | 3.764±0.2600c | 2.441±0.2890b | 2.011±0.2631b |
| Aspartic acid (T2 - 0.25%)  | 3.530±0.2464bc | 2.381±0.1983ab | 1.850±0.1963ab |
| Arginine (T3 - 0.25%)  | 3.510±0.2301bc | 2.353±0.1660ab | 1.796±0.1715ab |
| Niacin (T4 - 0.25%)  | 3.496±0.2097bc | 2.349±0.1514ab | 1.624±0.1179ab |
| Retinol (T5 - 0.25%)  | 3.417±0.2012ab | 2.238±0.1425ab | 1.526±0.1605ab |
| Calciferol (T6 - 0.25%)  | 3.389±0.1936ab | 2.229±0.1492ab | 1.403±0.1518ab |
| Ascorbic acid (T7 - 0.25%)  | 3.316±0.1744ab | 2.219±0.1413ab | 1.392±0.1254ab |
| Glucose (T8 - 0.25%)  | 3.300±0.1636a | 2.201±0.1321a | 1.384±0.1127a |

Values are Mean ± Standard Deviation of six observations. Values in the same column with different superscript letters (a, b & c) differs significantly at P.

From the above study, Ramesh *et al.* (2018) evaluated the morphometric data analysis of length, width and weight of cocoon parameters of *B. mori* fed with control MR2 leaves and different nutritional supplementary compounds. The mean length, width and weight of V instar larvae of group ‘C ’were (3.201±0.1547cm, 2.183±0.1210cm and 1.211±0.1006gm), respectively. The mean length, width and weight of V instar larvae of group T1 were (3.764±0.2600cm, 2.441±0.2890cm and 2.011±0.2631gm), respectively. The mean length, width and weight of V instar larvae of group T2 were (3.530±0.2464cm, 2.381±0.1983cm and 1.850±0.1963gm), respectively. The mean length, width and weight of V instar larvae of group T3 were (3.510±0.2301cm, 2.353±0.1660cm and 1.796±0.1715gm), respectively. The mean length, width and weight of V instar larvae of group T4 were (3.496±0.2097cm, 2.349±0.1514cm and 1.624±0.1179gm), respectively. The mean length, width and weight of V instar larvae of group T5 were (3.417±0.2012cm, 2.238±0.1425cm and 1.526±0.1605gm), respectively. The mean length, width and weight of V instar larvae of group T6 were (3.389±0.1936cm, 2.229±0.1492cm and 1.403±0.1518gm), respectively. The mean length, width and weight of V instar larvae of group T7 were (3.316±0.1744 cm, 2.219±0.1413 cm and 1.392±0.1254 gm), respectively. The mean length, width and weight of V instar larvae of group T8 were (3.300 ± 0.1636cm, 2.201±0.1321cm and 1.384±0.1127gm), respectively. In these nine observations, 0.25% amino acid L-Serine (group T1) treated V instar larvae produced cocoon length, width and weight were significantly increased than the other eight groups (‘C’, T2, T3, T4, T5, T6, T7, T8) (Table 5).

**Table 6: Impact of different amino acid supplements on economic parameters of cocoons of *B. mori* L. (Ramesh *et al.*, 2018)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Experimental****Groups /****Concentration** | **Cocooning****Percentage****(%)** | **Shell weight (g)** | **Shell Ratio (%)** | **Silk filament****Length(m)** | **Denier (%)** |
| **Control (C)** | 82.274±0.251a | 0.601±0.141a | 18.070±0.615a | 810.145±11.074a | 2.456±0.080a |
| **L-Serine****(T1 - 0.25%)** | 88.951±1.047c | 0.803±0.805c | 20.020±1.412c | 964.272±12.841c | 3.015±0.198c |
| **Aspartic acid****(T2 - 0.25%)** | 86.428±0.965b | 0.747±0.651bc | 19.453±0.898b | 942.210±11.749b | 2.820±0.189b |
| **Arginine****(T3 - 0.25%)** | 86.207±0.658bc | 0.712±0.620b | 19.340±0.823b | 911.237±11.655bc | 2.763±0.173bc |
| **Niacin****(T4 - 0.25%)** | 85.761±0.492bc | 0.700±0.402b | 19.217±0.788bc | 875.782±11.612bc | 2.701±0.171bc |
| **Retinol****(T5 - 0.25%)** | 85.320±0.476bc | 0.686±0.38z | 19.087±0.754bc | 867.127±11.566ab | 2.684±0.160bc |
| **Calciferol****(T6 - 0.25%)** | 84.490±0.410ab | 0.661±0.318ab | 18.495±0.712ab | 849.576±11.548ab | 2.654±0.148ab |
| **Ascorbic acid****(T7 - 0.25%)** | 84.088±0.381ab | 0.643±0.257ab | 18.370±0.687ab | 840.801±11.484ab | 2.508±0.123ab |
| **Glucose****(T8 - 0.25%)** | 83.828±0.308ab | 0.623±0.204ab | 18.230±0.640ab | 832.785±11.330ab | 2.490±0.110ab |

Values are Mean ± Standard Deviation of six observations. Values in the same column with different superscript letters (a, b & c) differs significantly at P<0.05 (DMRT)

Ramesh *et al.* (2018) reported that the data analysis of control and different nutritional supplementary compounds treated MR2 mulberry leaves fed V instar larvae produced cocoon’s cocooning percentage (CP). The cocooning percentage (%) of group ‘C’ larvae (82.274±0.251%), group T1 larvae (88.951±1.047%), group T2 larvae (86.428±0.965%), group T3 larvae (86.207±0.658%), group T4 larvae (85.761±0.492%), group T5 larvae (85.320±0.476%), group T6 larvae (84.490±0.410%), group T7 larvae (84.088±0.381%) and group T8 larvae (83.828±0.308%) respectively. In these nine observations, 0.25% amino acid L-Serine (group T1) treated V instar larvae produced cocoon’s cocooning percentage (%) was significantly increased than the other eight groups (‘C’, T2, T3, T4, T5, T6, T7, T8). Later, Ramesh *et al.* (2018) stated that the shell weight (gm) of group ‘C’ larvae (0.601±0.141gm), group T1 larvae (0.803±0.805gm), group T2 larvae (0.747±0.651gm), group T3 larvae (0.712±0.620gm), group T4 larvae (0.700±0.402gm), group T5 larvae (0.686±0.380gm), group T6 larvae (0.661±0.318gm), group T7 larvae (0.643±0.257gm) and group T8 larvae (0.623±0.204gm) respectively. In these nine observations, 0.25% amino acid L-Serine (group T1) treated V instar larvae produced cocoon’s shell weight (%) was significantly increased than the other eight groups (‘C’, T2, T3, T4, T5, T6, T7, T8)**.**

Ramesh *et al.* (2018) analysed that the shell ratio (%) of group ‘C’ larvae (18.070±0.615%), group T1 larvae (20.020±1.412%), group T2 larvae (19.453±0.898%), group T3 larvae (19.340±0.823%), group T4 larvae (19.217±0.788%), group T5 larvae (19.087±0.754%), group T6 larvae (18.495±0.712%), group T7 larvae (18.370±0.687%) and group T8 larvae (18.230±0.640%) respectively. In these nine observations, 0.25% amino acid L-Serine (group T1) treated V instar larvae produced cocoon’s shell ratio (%) was significantly increased than the other eight groups (‘C’, T2, T3, T4, T5, T6, T7, T8). Ramesh *et al.* (2018) also reported that the silk filament length (meters) of group ‘C’ larvae (810.145±11.074mts.), group T1 larvae (964.272±12.841mts.), group T2 larvae(942.210±11.749mts.), group T3 larvae (911.237±11.655mts.), group T4 larvae (875.782±11.612mts.), group T5 larvae (867.127±11.566mts.), group T6 larvae (849.576±11.548mts.), group T7 larvae (840.801±11.484mts.) and group T8 larvae (832.785±11.330mts.) respectively .In these nine observations, 0.25% amino acid L-Serine (group T1) treated V instar larvae produced cocoon’s silk filament length(meters) was significantly increased than the other eight groups (‘C’, T2, T3, T4, T5, T6, T7, T8). The silk filament Denier (%) of group ‘C’ larvae (2.456±0.080%), group T1 larvae (3.015±0.198%), group T2 larvae (2.820±0.189%), group T3 larvae (2.763±0.173%), group T4 larvae (2.701±0.171%), group T5 larvae (2.684±0.160%), group T6 larvae (2.654±0.148%), group T7 larvae (2.508±0.123%) and group T8 larvae (2.490±0.110%) respectively. In these nine observations, 0.25% amino acid L-Serine (group T1) treated V instar larvae produced cocoon’s silk filament Denier (%) was significantly increased than the other eight groups (‘C’, T2, T3, T4, T5, T6, T7, T8) (Table 6).

The possible reason for the increased economic parameters of cocoons was due to supplementation of dietary nutrients with the aforementioned promising complementary additives increased content of leaf moisture that might have boosted higher consumption rate, apparent digestibility that in turn has resulted in enhanced digestion, absorption, assimilation and utilization of food energy into larval bio-mass and cocoon. This might have resulted in upgrading economic parameters (Ramesh *et al.*, 2018).

**Conclusion**

Though nutrients present in the mulberry leaves satisfy nutritional needs of silkworm but the quantity of nutrients present vary depending on environmental conditions, usage of fertilizers in field, mulberry varieties, crop protection measures and other field practices. Therefore, certain supplements might be administered to the silkworm along with mulberry leaves to boost silk production. Experimental evidence consistently shows that fortifying mulberry leaf with amino acids (L-Serine and glycine) or protein rich supplements (drone brood, protinex) significantly improves larval growth, silk gland development and cocoon productivity in silkworm, *Bombyx mori.* Optimal gains are observed at moderate supplementation levels during the fifth instar. However, implementation at scale requires careful attention to concentration, timing, cost and farmer acceptance.

**Future perspectives:**

**Systematic field trials** (scaling up the best-performing supplements (e.g. 1% alanine, moderate glycine + serine) and probiotic mixes), **implementation studies** (evaluating cost, ease of use and farmer acceptance under real-world conditions), **genotype-specific trials** (assessing whether particular silkworm strains respond better to certain supplements) and **interdisciplinary collaborations** integrating entomology, molecular biology, biotech and sustainable agriculture.

**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 the writing or editing of this manuscript.

**COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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