***Review Article***

**A review on fortification of mulberry leaf with vitamins and mineral 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. Experimental evidence consistently shows that fortifying mulberry leaf with vitamin C, B-complex vitamins or minerals *viz.,* Zn 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.

**Key words:** Mulberry leaf, Fortification, Silkworm, *Bombyx mori,* vitamins and minerals

**Introduction:** The silkworm, *Bombyx mori* L is the specialist feeder (monophagous), depending exclusively on the mulberry leaf (*Morus* spp.) as its food. Needless to say, it has to derive its nutritional requirements from mulberry leaves for its growth and development. Any variation in the nutritional components of mulberry leaves may have some influence on the growth and development of silkworm (Gautam et al., 2022). Though the availability of silkworm nutrients in mulberry leaf is ensured on most occasions, some time they may not be available in adequate quantities for the larval growth. The quality of the leaf can have profound influence on the superiority of silk produced by *B. mori*. Therefore, the production of good cocoon crop is largely dependent on the quality of leaves. In other words, leaves of superior quality enhance the chances of realizing good cocoon crop (Ravikumar, 1988). Like in other organisms, in the silkworm, *B. mori* too, nutrition plays an important role in improving the growth and development. The intake of nutrient by the silkworm larvae is influenced by the availability of feed (Samami et al., 2019). Therefore, silkworm nutrition is considered as a major area of research in sericulture (Legay, 1958). One of the alternative ways of improving larval nutrition is enrichment of mulberry leaves with supplementary nutrients such as proteins, amino acids, vitamins and minerals (Etebari *et al.,* 2004). So, an attempt has been made to review the effects of enrichment of mulberry leaves with vitamins and minerals on feeding *Bombyx mori* larvae, evaluating subsequent impacts on larval and cocoon qualtity*.*

**Vitamins:** Vitamins are organic molecules that are essential micronutrients which an organism needs in small quantities for normal metabolism (Amrein et al., 2024). Vitamins play a major role, lack of their presence interrupts enzymatic reactions because they often act as a co-enzymes. The silkworm mainly requires vitamin B complex and ascorbic acid for their growth and development. Essential vitamins under vitamin B complex required for silkworm are choline, inositol, nicotinic acid, pantothenic acid, pyridoxine, riboflavin, thiamine, biotin and folic acid (Berger et al., 2022). During V instar of silkworm there is increase in thiamin, riboflavin and pyridoxine vitamins in the haemolymph due to their need at this stage of silkworm (Borah *et al.,* 2020).

**Fig. 1: Classification of vitamins (Kanafi *et al*., 2007)**

a. Water soluble vitamins:

Vitamin B-complex and ascorbic acid are mainly required by the silkworms for their growth and development. The vitamin B complex is traditionally made up of 10 members that differ in their biological actions, although many participate in energy production from carbohydrates and fats.

i. Thiamine (B1): Thiamine is important for energy metabolism (Mrowicka et al., 2023). The thiamine derivative enhanced the growth of larvae, pupae and adults in mulberry silkworm.

ii. Riboflavin (B2): Riboflavin is important in promoting the release of energy from carbohydrates, fats and proteins “i.e. in the metabolic pathway for ATP production”. The enrichment of leaves with riboflavin enhanced certain economic characters of silkworm and improved silk production (Aragão et al., 2024).

 iii. Niacin (B3): Vitamin B3 comes in two forms- nicotinic acid and nicotinamide. It is mandatory for respiration of cells which helps in release of energy and metabolism of proteins, lipids and carbohydrates. Vitamin B3 is essential for silkworm but a high dose of vitamin B3 in silkworm may cause symptoms of “Nicotinamide hypervitaminosis” like immobility, darkening of the skin, interrupts larval feeding and normal growth. High mortality of larvae occurs during moulting and they cannot complete this process normally.

iv. Pantothenic (B5): Vitamin B5 is required for growth and development of the silkworm, which the silkworm is unable to synthesize. Pantothenic acid acts as precursor of coenzyme-A that is vital for metabolism of carbohydrates, synthesis and degradation of fats, synthesis of sterols and resultant steroid hormones (Borah & Boro, 2020).

v. Pyridoxine (B6): Pyridoxine is very much essential for silkworm but required in very low quantity. High dose of pyridoxine in silkworm diet interrupts optimal growth and development of silkworm such as reduction in fecundity, fertility and egg viability of silkworms.

vi. Folic acid (B9): Folic acid plays a major role in cellular metabolism including the synthesis of some of the components of DNA and pigment precursor. It was noticed that the silkworm growth decreased when folic acid was eliminated from artificial diet.

vii. Choline and Inositol: Choline and inositol are required at higher levels compared to other vitamins (Kansakar et al., 2023). They are involved in the production of cell membrane. Inositol is an important part of signaling mechanism that transmits information from outside to the inside of cells.

 Vitamin C (Ascorbic acid): Ascorbic acid has many important functions in silkworm. It is a powerful antioxidant, protecting against oxidative damage to DNA, membrane lipids and proteins. Addtion of ascorbic acid significantly increased the weight of silkworm, since it has gustatory stimulating action. The absence of ascorbic acid in the diet of first and second instar larvae postponed growth and development of silkworm

 b. Fat soluble vitamins. Fat-soluble vitamins consist of the A, D, E and K vitamins. Among these, enrichment of mulberry leaves with vitamin A, D and K have not been studied. Vitamin E: α-tocopherol marginally increases the number of eggs laid by moths and β-carotene is reported to have some growth-promoting effect (Kanafi *et al.,* 2007)

**Table 1: Comparison of quantitative requirement of vitamin B for silkworm with amounts present in mulberry leaves (Kanafi *et al.,* 2007).**

|  |  |  |
| --- | --- | --- |
| **Vitamin** | **Minimal amount required (mg/g of dry diet)** | **Amount in mulberry leaves****(mg/g of dry matter)** |
| Biotin | 1.0 | 0.2- 0.5 |
| Choline | 750 | 930-1350 |
| Inositol | 1000 | 4000 |
| Nicotinic acid | 20 | 60-99 |
| Pantothenic acid | 20 | 16-35 |
| Pyridoxine | 5 | 43-50 |
| Riboflavin | 5 | 13-31 |
| Thiamine | 0.5 | 6.7 |

**Impact of vitamin supplements on silkworm rearing parameters and cocoon productivity:** Balasundaram *et al.* (2013)studied on nutritional supplementation of vitamin C treated MR2 mulberry leaves fed to V instar larvae of silkworm, *B. mori* L.

**Table 2: Impact of vitamin C supplementation on feed efficacy parameters of V instar silkworm (Balasundaram *et al.* 2013).**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Experimental Groups /****Concentration** | **Food****Consumption****Rate (gm)** | **Food****Utilization****Rate (gm)** | **Food****Digestibility (%)** | **Food****Consumption****Index (%)** | **Co-efficient of Food****Utilization (%)** |
| Control (C)  | 47.10±1.15b | 43.34±0.14c | 85.11±0.31b | 37.41±1.04b | 84.39±0.56b |
| Vitamin C (T1) 0.1%  | 41.88±1.09b | 40.09±0.61a | 84.44±0.21ab | 35.94±1.31b | 83.86±0.34b |
| Vitamin C (T2) 0.2%  | 49.61±0.89c | 47.36±1.69d | 89.49±1.11c | 41.52±1.19c | 89.24±0.83c |
| Vitamin C( T3) 0.4%  | 45.86±0.31ab | 40.75±1.61ab | 84.76±0.19ab | 35.19±1.85a | 81.87±0.99a |
| Vitamin C (T4) 0.8%  | 45.51±0.78a | 43.12±1.19bc | 84.24±0.25 a | 33.77±1.48a | 82.73±0.53a |

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 table, Balasundaram *et al.* (2013) found that the food consumption rate (gm), food utilization rate (gm), food consumption index (%), food digestibility rate (%) and co-efficient of food utilization (%) of 0.2% (group T2) Vitamin C treated larvae was 49.61±0.89, 47.36±1.69, 89.49±1.11, 41.52±1.19 and 89.24±0.83,respectively which was significantly higher than the other four groups (‘Control, T1-0.1%, T3-0.4% and T4-0.8%) (Table 2).

 The possible reason for the increased feed efficacy parameters of V instar silkworm was due to antioxidant activity of vitamin C (Ascorbic acid) decreases reactive oxygen species and oxidative pressure and as a result the absorption of nutritious substances in the midgut would increase (Balasundaram *et al.,* 2013).

**Table 3: Impact of vitamin C supplementation on larval parameters of V instar silkworm (Balasundaram *et al.* 2013).**

|  |  |  |  |
| --- | --- | --- | --- |
| **Experimental Groups / Concentrations** | **Larvae length (cm)** | **Larvae width (cm)** | **Larvae weight (gm)** |
| Control (C)  | 6.12±0.21a | 1.04±0.11ab | 2.71±0.07a |
| Vitamin C (T1) 0.1%  | 6.98±0.19bc | 1.01±0.13ab | 3.12±0.51a |
| Vitamin C (T2) 0.2%  | 7.05±0.16c | 1.09±0.09b | 3.45±0.24b |
| Vitamin C( T3) 0.4%  | 6.76±0.13bc | 1.02±0.05ab | 3.16±0.33ab |
| Vitamin C (T4) 0.8%  | 6.54±0.35b | 0.97±0.11a | 2.85±0.38a |

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

Balasundaram *et al.* (2013) analysed that the mean length, width and weight of V instar larvae of group ‘C’ were (6.12 ± 0.21 cm, 1.04 ± 0.11 cm and 2.71 ± 0.07 gm), respectively. The mean length, width and weight of V instar larvae of group T1 were (6.98 ± 0.19 cm, 1.01 ± 0.13 cm and 3.12 ± 0.51 gm), respectively. The mean length, width and weight of V instar larvae of group T2 were (7.05 ± 0.16 cm, 1.09 ± 0.09 cm and 3.45 ± 0.24 gm), respectively. The mean length, width and weight of V instar larvae of group T3 were (6.76 ± 0.13 cm, 1.02 ± 0.05 cm and 3.16 ± 0.33 gm), respectively. The mean length, width and weight of V instar larvae of group T4 were (6.54 ± 0.35 cm, 0.97 ± 0.11 cm and 2.85 ± 0.38gm), respectively. In these five observations, 0.2% (group T2) Vitamin C treated V instar larvae length, width and weight was significantly increased than the other four groups (‘C’, T1, T3 and T4) (Table 3).

 The possible reason for the larval parameters of V instar silkworm was due to the food consumption has a direct relevance to the larval growth rate of silkworm (Balasundaram *et al.*, 2013)

**Table 4: Impact of vitamin C supplementation on cocoon parameters of silkworm (Balasundaram *et al.*, 2013)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Experimental Groups/ Concentration** | **Cocoon length (cm)** | **Cocoon width (cm)** | **Cocoon weight****(g)** |
| Control (C)  | 3.35±0.35ab | 2.10±0.11a | 1.41±0.09a |
| Vitamin C (T1) 0.1%  | 3.36±0.11ab | 2.07±0.08a | 1.49±0.11a |
| Vitamin C (T2) 0.2%  | 3.58±0.17c | 2.38±0.07b | 2.07±0.32b |
| Vitamin C( T3) 0.4%  | 3.28±0.22a | 2.09±0.10a | 1.24±0.17a |
| Vitamin C (T4) 0.8%  | 3.27±0.21a | 2.17±0.14a | 1.68±0.19a |

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).

 Balasundaram *et al.* (2013) reported that the the length, width and weight of the group ‘C’ larvae produced cocoon were found to be about (3.35 ± 0.35 cm, 2.10 ± 0.11 cm and 1.41 ± 0.09 gm), respectively. The length, width and weight of the group T1 larvae produced cocoon were observed to be about (3.36 ± 0.11 cm, 2.07 ± 0.08 cm and 1.49 ± 0.11 gm), respectively. The length, width and weight of the group T2 larvae producing cocoon were observed to be about (3.58 ± 0.17cm, 2.38 ± 0.07 cm and 2.07 ± 0.32gm), respectively. The length, width and weight of the group T3 larvae produced cocoon were observed to be about (3.28 ± 0.22 cm, 2.09 ± 0.10 cm and 1.24 ± 0.17 gm), respectively (Table 4). The length, width and weight of the group T4 larvae produced cocoon were observed to be about (3.27 ± 0.21cm, 2.17 ± 0.14 cm and 1.68 ± 0.19 gm), respectively. In these five observations, the 0.2% (group T1) Vitamin C treated larvae produced cocoon length; width and weight were significantly increased than the other four groups (‘C’, T1, T3 and T4) (Table 4).

 The possible reason for the increased cocoon parameters of V instar silkworm produced cocoons was due to vitamin C exhibits growth stimulant activity and can be used to increase the feed efficacy of silkworm, ultimately resulting in higher yield and better quality cocoons (Balasundaram *et al.*, 2013).

#  Meeramaideen *et al*. (2017) studied on the feed efficacy, growth rate and economic traits of silkworm, *B. mori* L. fed with B complex vitamins treated Kanva-2 mulberry leaves. In this study, the first day of V instar larvae were reared simultaneously both in control and experimental groups separately on mulberry leaves dipped in B-complex vitamins to find out the feed efficacy of vitamins with regard to food utilization by larvae and ultimate impact on the cocoon parameters of silkworm.

# Table 5: Feed efficacy of V instar larvae of *B. mori* fed with B-complex vitamins treated Kanva-2 mulberry leaves (Meeramaideen *et al*., 2017)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Experime ntal****Groups** | **Food Consumptio****n****(g)** | **Food****Utilization****(g)** | **Approximate****Digestibility****(%)** | **Food****Consumptio****n****Index (%)** | **Co-efficient of Food****Utilization****(%)** |
| **Control (C)** | 45.1780±0.16 62b | 44.4076±0.25 11c | 84.7015±0.5684b | 35.4560±1.0 789b | 83.0200±0.4 906b |
| **Riboflavin****0.5%****(T1)** | 54.7617±1.9709c | 50.4733±1.64 97d | 92.3173±1.2137c | 45.5750±2.1 505c | 92.4900±0.9715c |
| **Pantothen ic acid****0.5% (T2)** | 52.8633±0.99 80b | 48.9867±0.90 18a | 87.3981±0.73 89ab | 41.9133±1.3 264b | 88.6300±0.6 762b |
| **Pyridoxal phosphate****0.5% (T3)** | 53.8933±0.88 17ab | 49.9033±0.82 05ab | 89.8357±0.9575ab | 42.1950±1.4 720a | 89.2150±0.7 474a |
| **Biotin 0.5% (T4)** | 52.1500±0.71 67a | 4.0740±0.77017bc | 85.9016±0.81 90a | 40.7583±1.2 203a | 87.3633±0.5 484a |

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).

From the above study, Meeramaideen *et al*. (2017)evaluated for the food consumption (gm) of group ‘C’ larvae (45.1780±0.1662 gm), group T1 larvae (54.7617±1.9709 gm), group T2 (52.8633±0.9980 gm) larvae, group T3 (53.8933±0.8817a gm) and group T4 (52.1500±0.7167 gm), respectively. In these five observations, the 0.5% (group T1) B-complex vitamin (Riboflavin) treated. The food utilization (gm) of group ‘C’ larvae (44.4076±0.2511 gm), group T1 larvae (50.4733±1.6497 gm), group T2 (48.9867±0.9018 gm) larvae, group T3 (49.9033±0.8205 gm) and group T4 (47.0740±0.7701 gm), respectively. In these five observations, the 0.5% (group T1) Bcomplex vitamin (Riboflavin) treated larvae food utilization (gm) was significantly increased than the other four groups (‘C’, T2, T3 and T4). The food consumption index (%) of group ‘C’ larvae (35.4560±1.0789 %), group T1 larvae (45.5750±2.1505 %), group T2 (41.9133±1.3264 %) larvae, group T3 (40.7583±1.2203 %) and group T4 (42.1950±1.4720 %), respectively. In these five observations, the 0.5% (group T1) B-complex vitamin (Riboflavin) treated larvae food consumption (%) was significantly increased than the other four groups (‘C’, T2, T3 and T4). The approximate digestibility (%) of group ‘C’ larvae (84.7015±0.5684 %), group T1 larvae (92.3173±1.2137 %), group T2 (87.3981±0.7389 %) larvae, group T3 (89.8357±0.9575 %) and group T4 (85.9016±0.8190 %), respectively.

In these five observations, the 0.5% (group T1) B-complex vitamin (Riboflavin) treated larvae approximate digestibility (%) was significantly increased than the other four groups (‘C’, T2, T3 and T4). The co-efficient of food utilization (%) of group ‘C’ larvae (83.0200±0.4906 %), group T1 larvae (92.4 900±0.9715 %), group T2 (88.6300±0.6762 %) larvae, group T3 (89.2150±0.7474 %) and group T4 (87.3633±0.5484 %), respectively. In these five observations, the 0.5% (group T1) B-complex vitamin (Riboflavin) treated larvae co-efficient of food utilization (%) was significantly increased than the other four groups (‘C’, T2, T3 and T4) (Table 5).

**Table 6: Influence of B-complex vitamins treated Kanva-2 mulberry leaves on larval parameters of silkworm, *B. mori* L. (Meeramaideen *et al*., 2017)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Experimental Groups** | **Larvae length (cm)** | **Larvae width (cm)** | **Larvae weight (g)** |
| **Control (C)**   | 7.0117±0.19831a | 0.6533±0.07111ab | 3.4350±0.19550a |
| **Riboflavin 0.5% (T1)**   | 9.4560±0.29705c | 1.1233±0.18165b | 4.5283±0.32693b |
| **Pantothenic acid 0.5% (T2)**  | 8.8080±0.28887bc | 0.9700±0.09142ab | 3.9550±0.29302a |
| **Pyridoxal phosphate 0.5% (T3)**   | 8.7863±0.25721bc | 0.8600±0.08325ab | 3.8150±0.25529ab |
| **Biotin 0.5% (T4)**  | 8.5520±0.23340b | 0.7967±0.07663a | 3.7950±0.25609a |

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).

The data in Table 6 showed that the mean length, width and weight of V instar larvae of group ‘C’ were (7.0117±0.19831 cm, 10.6533±0.07111 cm and 3.4350±0.19550 gm), respectively. The mean length, width and weight of V instar larvae of group T1 were (9.4560±0.29705 cm, 11.1233±0.18165 cm and 4.5283±0.32693 gm), respectively. The mean length, width and weight of V instar larvae of group T2 were (8.8080±0.28887 cm, 0.9700±0.09142 cm and 3.9550±0.29302 gm), respectively. The mean length, width and weight of V instar larvae of group T3 were (8.7863±0.25721 cm, 0.8600±0.08325 cm and 3.8150±0.25529 gm), respectively. The mean length, width and weight of V instar larvae of group T4 were (8.5520±0.23340 cm, 0.7967±0.07663 cm and 3.7950±0.25609 gm), respectively. In these five observations, 0.5% (group T1) B-complex vitamin (Riboflavin) treated V instar larvae length, width and weight was significantly increased than the other four groups (‘C’, T2, T3 and T4) (Meeramaideen *et al*., 2017).

**Table 7: Economic traits of B-complex vitamins treated Kanva-2 mulberry leaves fed to V instar larvae produced cocoon (Meeramaideen *et al*., 2017)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Experimental Groups** | **Cocooning percentage****(%)** | **Shell****Weight****(g)** | **Shell****Ratio****(%)** | **Silk filament****Length****(Meters)** | **Denier (%)** |
| **Control (C)** | 85.0750±0.45107c | 0.7033±0.0318ab | 18.9700±0.4958b | 886.9733±12.8734b | 2.1567±0.0906b |
| **Riboflavin****0.5% (T1)** | 90.8067±0.0880a | 1.0283±0.1557c | 20.9200±1.2172c | 974.3250±05.8491 | 3.5450±0.1998c |
| **Pantothenic acid 0.5%****(T2)** | 88.7083±0.86253bc | 0.8967±0.0916a | 19.7583±0.8068ab | 942.7867±11.4419b | 2.9150±0.1749b |
| **Pyridoxal phosphate****0.5% (T3)** | 86.6717±0.75811ab | 0.8067±0.0880a | 19.5583±0.7383ab | 932.5867±15.9575a | 2.8833±0.1503a |
| **Biotin 0.5% (T4)** | 85.4083±0.56839a | 0.7983±0.0756b | 19.3350±0.5198a | 915.0333±40.6181a | 2.6000±0.1397a |

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).

The data in Table 7 showed that the cocooning percentage (%) of group ‘C’ larvae (85.0750±0.45107 %), group T1 larvae (90.1617±1.14792 %), group T2 (88.7083±0.86253 %) larvae, group T3 (86.6717±0.75811 %) and group T4 (85.4083±0.56839 %), respectively. In these five observations, the 0.5% (group T1) B-complex vitamin (Riboflavin) treated larvae cocooning percentage (%) was significantly increased than the other four groups (‘C’, T2, T3 and T4). The shell weight (gm) of group ‘C’ larvae (0.7033±0.0318 gm), group T1 larvae (1.0283±0.1557 gm), group T2 (0.8967±0.0916 gm) larvae, group T3 (0.8067±0.0880 gm) and group T4 (0.7983±0.0756 gm), respectively. In these five observations, the 0.5% (group T1) B-complex vitamin (Riboflavin) treated larvae shell weight (SW) was significantly increased than the other four groups (‘C’, T2, T3 and T4). The shell ratio (%) of group ‘C’ larvae (18.9700±0.4958 %), group T1 larvae (20.9200±1.2172 %), group T2 (19.7583±0.8068 %) larvae, group T3 (19.5583±0.7383 %) and group T4 (19.3350±0.5198 %), respectively.

 In these five observations, the 0.5% (group T1) B-complex vitamin (Riboflavin) treated larvae shell ratio (SR) was significantly increased than the other four groups (‘C’, T2, T3 and T4). The silk filament length (meters) of group ‘C’ larvae (886.9733±12.8734 mts.), group T1 larvae (974.3250±05.8491 mts.), group T2 (942.7867±11.4419 mts.) larvae, group T3 (932.5867±15.9575 mts.) and group T4 (915.0333±40.6181 mts.), respectively. In these five observations, the 0.5% (group T1) Bcomplex vitamin (Riboflavin) treated larvae silk filament length (meters) was significantly increased than the other four groups (‘C’, T2, T3 and T4). The silk filament denier of group ‘C’ larvae (2.1567±0.0906 %), group T1 larvae (3.5450±0.1998 %), group T2 (2.9150±0.1749 %) larvae, group T3 (2.8833±0.1503 %) and group T4 (2.6000±0.1397 %), respectively. In these five observations, the 0.5% (group T1) B-complex vitamins treated larvae silk filament length (meters) was significantly increased than the other four groups (‘C’, T2, T3 and T4). The larvae which were fed with B-complex vitamins showed better performance in terms of feed efficacy, growth rate and economic traits of silkworm. This could be due to B-complex vitamins are important in promoting the release of energy from carbohydrates, fats and proteins (Meeramaideen *et al*., 2017).

**Table 8: Impact of vitamin C and E supplementations on body weight of V instar silkworm (Brahma *et al.,* 2018)**

|  |  |  |
| --- | --- | --- |
| **V Instar Duration** | **Body Weight (g)** |  |
| **CSR2 x CSR4** |  |
| **Vitamin C (1%)** | **Control** | **Vitamin E (1%)** |
| **1st Day** | 0.8924± 0.0026 | 0.7644±0.0012 | 0.7352±0.0027 |
| **2nd Day** | 0.9616±0.0023 | 0.8646±0.0026 | 0.8156±0.0010 |
| **3rd Day** | 1.2888±0.0026 | 1.1284±0.0028 | 1.1144±0.0034 |
| **4th Day** | 2.2250±0.0011 | 2.1948±0.0014 | 2.1814±0.0017 |
| **5th Day** | 2.3506±0.0015 | 2.2672±0.2258 | 2.1090±0.0053 |
| **6th Day** | 2.3516±0.0017 | 2.3310±0.0021 | 2.2894±0.0019 |
| **7th Day** | 2.3354±0.0064 | 2.3080±0.0026 | 2.2702±0.0031 |

The data in Table 8 showed that between vitamin C and vitamin E supplementation, fortification of mulberry leaves with 1% vitamin C showed increased body weight in V instar silkworm. The increased larval body weight of V instar silkworm was due to vitamin C supplementation exhibits growth and gustatory stimulating action (Brahma *et al.,* 2018).

**Table 9: Impact of vitamin C and E supplementations on silk gland weight of V instar silkworm (Brahma *et al.,* 2018).**

|  |  |
| --- | --- |
| **V Instar Duration** | **Silk gland weight (gm)** |
| **CSR2 x CSR4** |
| **Vitamin C** | **Control** | **Vitamin E** |
| **1stDay** | 0.0716±0.0023 | 0.0522±0.0013 | 0.0334±0.0021 |
| **2nd Day** | 0.1150±0.0019 | 0.972±0.0026 | 0.0832±0.0036 |
| **3rd Day** | 0.2094±0.0028 | 0.1646±0.0015 | 0,1588±0.0012 |
| **4th Day** | 0.3962±0.0011 | 0.3230±0.0028 | 0.2908±0.0023 |
| **5th Day** | 0.7088±0.0022 | 0.6806±0.0024 | 0.6230±0.0068 |
| **6th Day** | 0.8760±0.0015 | 0.8568±0.0017 | 0.7816±0.0010 |
| **7th Day** | 0.8796±0.0022 | 0.8712±0.0050 | 0.7896±0.0031 |

The data in Table 9 showed that between vitamin C and vitamin E supplementation, fortification of mulberry leaves with 1% vitamin C showed increased silk gland weight in V instar silkworm. The increased silk gland weight of V instar silkworm was due to vitamin C is a powerful antioxidant, protecting against oxidative damage to DNA, membrane lipids and proteins (Brahma *et al.,* 2018).

**Table 10: Impact of vitamin C and E supplementations on protein concentration in silk (Brahma *et al.,* 2018)**

|  |  |
| --- | --- |
| **5th Instar****Duration** | **Protein Concentration (µg/whole gland)** |
| CSR2 x CSR4 |
| Vitamin C | Control | Vitamin E |
| **1st Day** | 5.5786 ± 0.1828 | 4.686±0.1921 | 4.3614±0.1262 |
| **2nd Day** | 9.5547±0.1195 | 9.0069±0.1094 | 6.5016±0.1596 |
| **3rd Day** | 13.3279±0.1499 | 12.5773±0.0988 | 11.3601±0.1970 |
| **4th Day** | 19.3325±0.1276 | 15.8636±0.15163 | 14.5045±0.13224 |
| **5th Day** | 24.7895±0.1420 | 24.3026±0.2666 | 23.4810±0.1983 |
| **6th Day** | 40.1460±0.1308 | 38.8579±0.1722 | 38.2087±0.2533 |
| **7th Day** | 41.1502±0.2045 | 40.0750±0.1225 | 39.4766±0.2178 |

The data in Table 10 showed that between vitamin C and vitamin E supplementation, fortification of mulberry leaves with 1% vitamin C showed increased protein concentration in silk gland in V instar silkworm. The increased protein concentration in silk gland in V instar silkworm was due to vitamin C is a powerful antioxidant, protecting against oxidative damage to DNA, membrane lipids and proteins (Brahma *et al.,* 2018).

# MINERALS: Minerals are a group of organic compounds which are essential for normal growth and development. Minerals are required in small quantities in the diet because they cannot be synthesized in the silkworm (Mahanta et al., 2023). It has been reported that 28% of the larval structure in different instars include the absorbed minerals. So, the minerals are one of the most important components of the silkworm. Silkworm require essentially at least four minerals namely K, P, Mg and Zn for growth and development (Borah *et al.,* 2020).

**Table 11: Mineral composition of mulberry leaves (Borah *et al.,* 2020)**

|  |  |
| --- | --- |
| **Minerals**  |  **Composition**   |
| Nitrogen | 2.1–3.1 g/100 g |
| Phosphorus | 0.1–0.2 g/100 g |
| Potassium | 1.2–3.9 g/100 g |
| Calcium | 1.7–3.9 g/100 g |
| Sodium | 0.01 g/100 g |
| Magnesium | 0.5–1.4 g/100 g |
| Sulphur | 0.2–0.3 g/100 g |
| Iron | 119.3–241.8 mg/kg |
| Zinc | 23.9–39.5 mg/kg |
| Manganese | 35.8–90.5 mg/kg |
| Boron | 253.5–825.3 mg/kg |
| Copper | 4.2–5.9 mg/kg |
| Molybdenum | 0.8–2.3 mg/kg |
| Nickel | 1.7–5.4 mg/kg |
| Lead | 0.3–0.8 mg/kg |
| Carbon | 37.4–41.4 g/100 g |
| Lithium | 1.9–17.2 mg/kg |
| Titanium | 5.4–10.8 mg/kg |

# Table 12: Amount of minerals present in mulberry leaves and requirement by silkworm (Borah *et al.,* 2020).

|  |  |  |
| --- | --- | --- |
| **Minerals** | **Minimal amount required**  **(mg/g of dry diet)** | **Amount in mulberry leaves**  **(mg/g of dry matter)** |
| Potassium | 9.0 | 25-33 |
| Phosphorus | 2-3 | 1.6-3.4 |
| Magnesium | 1.0 | 2.0-4.9 |
| zinc | 0.02 | 0.021 |

# Impact of mineral supplements on silkworm rearing parameters and cocoon productivity

#  Bhattacharya *et al.* (2005) investigated on the synergetic effect of potassium and magnesium chloride on biochemical contents of the silkworm, *B. mori* L. The present study deals with the oral supplementation with (50, 100 and 150 µg/ml) and synergetic effect of potassium and magnesium chloride on the fat body glycogen, protein, total lipids and haemolymph trehalose protein of V instar larvae of *B. mori.* The fresh mulberry leaves were dipped in each concentration of the above chemicals and then the leaves were dried under shade and fed to the silkworm.



# Fig. 2 The effect of KCl and MgCl2 on the fat Fig. 3 The effect of KCl and MgCl2 on the

#  Body glycogen of the silkworm. haemolymph trehalose of the silkworm.

 From the above investigation, Bhattacharya *et al*. (2005) revealed that the dietary supplementation with 50 µg/ml potassium and magnesium chloride to silkworm larvae resulted in an increase of 26% fat body glycogen (Fig. 2) and 20% haemolymph trehalose (Fig. 3). The dietary supplementation with 100 µg/ml to silkworm larvae resulted in an increase of 33% fat body glycogen and 26% haemolymph trehalose. The supplementation with 150µg/ml potassium and magnesium chloride to silkworm larvae resulted in an increase of 75% fat body glycogen and 44% haemolymph trehalose. The above results indicate that the oral supplementation with potassium and magnesium chloride increased the fat body glycogen and haemolymph trehalose in all the treated.

The increase in fat body glycogen was due to the stimulatory effect ofKCl and MgCl2 on the amylase activity of the midgut resulting in increased production of carbohydrates.The increase in haemolymph trehalose was due to the activating the trehalose synthase activity of the fat body i.e conversion of glycogen into trehalose and its subsequent release into the haemolymph by the fat body (Bhattacharya *et al*., 2005).

**Fig. 4 The effect of KCl and MgCl2 on the fat Fig. 5 The effect of KCl and body total**

 **protein of the silkworm. haemolymph total protein of the**

 **silkworm**

#

From this study, Bhattacharya *et al*. (2005) also found that the dietary supplementation with 50µg/ml potassium and magnesium chloride to silkworm larvae resulted in an increase of 15% fat body protein (Fig. 4) and 78% haemolymph protein (Fig. 5). The dietary supplementation with 100 µg/ml to silkworm larvae resulted in an increase of 19% fat body protein and 84% haemolymph protein. The supplementation with 150µg/ml potassium and magnesium chloride to silkworm larvae resulted in an increase of 22% fat body protein and 160% haemolymph protein. The above results indicate that the oral supplementation with potassium and magnesium chloride increased fat body and haemolymph protein in all the treated groups when compared with those of the corresponding parameters of the carrier control.

The increased protein content of the fat bodies due to the stimulatory effects of the minerals salt of potassium and magnesium chloride on the synthetic activity of the fat body. The increased haemolymph protein content might be due to the release of excess of proteins by the fat body into the haemolymph (Bhattacharya *et al*., 2005).


# Fig. 6 The effect of KCl and MgCl2 on the fat body total lipid of the silkworm (Figures from Fig. 2 to Fig. 6 taken from Bhattacharya *et al.,* 2005).

Bhattacharya *et al*. (2005) reported that the dietary supplementation with 50µg/ml potassium and magnesium chloride to silkworm larvae resulted in an increase of 27% fat body total lipids (Fig. 6). The dietary supplementation with 100 µg/ml to silk-worm larvae resulted in an increase of 13% fat body total lipids. The supplementation with 150µg/ml potassium and magnesium chloride to silkworm larvae resulted in an increase of 5% fat body total lipids. The above results indicate that the oral supplementation with potassium and magnesium chloride increased fat body total lipids in all the treated groups when compared with those of the corresponding parameters of the carrier control.

 The increased total lipids of the fat body might possibly be due to the stimulatory effect of the minerals mixture of potassium and magnesium chloride at a given concentration on the synthetic activity of the fat body (Bhattacharya *et al*., 2005).

Bentea *et al.* (2012) studied on the effect of zinc supplementation on the productivity parameters of *B. mori* L. The study was carried out to evaluate the influence of zinc as food supplement on larval and cocoon parameters of silkworm *B. mori*. The silkworm were randomly distributed in 5 groups, one control group and four experimental groups, each group consisting of 50 larvae. The larvae were fed to fresh mulberry leaf, untreated for the control group and treated by pulverizing the mineral solutions: zinc - 17, 34, 68, 136 mg kg-1 larvae.

# Table 13: The larvae and silk gland weight of V instar silkworm fed on mulberry leaves treated with Zn

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Parameters** | **UM** | **Gr.1** | **Gr.2** | **Gr.3** | **Gr.4** | **Gr.5** |
| **Larvae weight** | X±Sx(g) | 3.930±0.103 | 4.456±0.082\*\*\* | 4.866±0.083\*\*\* | 4.898±0.087\*\*\* | 5.092±0.075\*\*\* |
| Min./Max. | 2.824/5.054 | 3.806/5.265 | 4.137/5.791 | 4.083/5.747 | 4.529/6.178 |
| Relative Value(%) | 100.00 | 113.38 | 123.82 | 124.63 | 129.57 |
| **Silk gland weight** | X±Sx(g) | 1.072±0.012 | 1.173±0.016 | 1.206±0.083 | 1.168±0.038 | 1.289±0.057\* |
| Min./Max. | 1.026/1.106 | 1.097/1.220 | 0.948/1.558 | 1.045/1.304 | 1.155/1.563 |
| Relative Value(%) | 100.00 | 109.42 | 112.50 | 108.96 | 120.24 |

\*\*\* - p< 0.001 very significant differenc, p < 0.05 significant differences (Student test); 5th instar, day 7

From the above study Bentea *et al.,* (2012) revealed that among all the experimental groups, Zn supplemented larvae group (Gr.2 to Gr.5) showed better performance in laraval and silk gland weight compared to control and among Zn supplemented larvae group Gr.5(Zn @ 136 mg/kg larvae) shows better performance for all the parameters (Table 13).

# Table 14: The cocoon weight and cocoon shell weight of the silkworm fed on mulberry leaves treated with Zn (Bentea *et al.,* 2012).

#

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Parameters** | **UM** | **Gr.1** | **Gr.2** | **Gr.3** | **Gr.4** | **Gr.5** |
| **Cocoon weight** | X± Sx(g) | 1.877±0.048 | 2.020±0.048 | 2.042±0.073 | 2.053±0.070 | 2.124±0.060 |
| Min./ Max. | 1.466/2.335 | 1.642/2.438 | 1.488/2.639 | 1.550/2.790 | 1.643/2.555 |
| Relative value(%) | 100.00 | 107.62 | 108.79 | 109.38 | 113.16 |
| **Cocoon shell weight** | X± Sx(g) | 0.392±0.030 | 0.438±0.011 | 0.447±0.018 | 0.465±0.007 | 0.466±0.009 |
| Min./ Max. | 0.338/0.499 | 0.397/0.463 | 0.381/0.481 | 0.453/0.488 | 0.441/0.495 |
| Relative value(%) | 100.00 | 111.73 | 114.03 | 118.62 | 118.88 |

The data in Table 14 showed that among all the experimental groups, Zn supplemented larvae group (Gr.2 to Gr.5) showed better performance in cocoon weight and cocoon shell weightcompared to control and among Zn supplemented larvae group Gr.5(Zn @ 136 mg/kg larvae) shows better performance for all the parameters.This was due to zinc plays a major role in larval growth and development by stimulating metabolism through enhanced enzyme activity (Bentea *et al.,* 2012).

# Devi and Yellamma, (2013) worked on cocoon parameters in the silkworm, *B. mori* on exposure to trace element and nutrients. The study was conducted to investigate the various economic parameters of the silk cocoon when larvae were fed on mulberry leaves fortified with selected trace elements including zinc, vitamin (pyridoxine) and hormone (methoprene). The experimental silkworms were divided into four groups and fed with mulberry leaves soaked in the selected compounds *i.e*., zinc chloride, pyridoxine, methoprene and mixed dose (Zn+B6+H).


# Fig 7: Changes in the qualitative and quantitative economic parameters in the cocoons of control and different experimental groups of silkworms

 The result showed that silkworms which are fed with supplements performed better than control. Among these nutrient supplemented groups, group E4 showed a significantly higher difference in all the qualitative and quantitative economic parameters of cocoons. This could be due to mixed dose of vitamin, mineral and hormone which stimulates silk protein synthesis in the silk glands and enhances silk output, as reflected in higher shell-cocoon ratios, silkbody ratio, raw silk percentage and denier (Devi and Yellamma, 2013).

**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 vitamin C, B-complex vitamins or minerals *viz.,* Zn 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.

 D**ISCLAIMER (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.

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