

Vitamin supplementation in *Bombyx mori* diets: implications for insect biodiversity conservation and cocoon production on *Morus nigra* leaves

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Abstract

The mulberry silkworm (*Bombyx mori*) is a key species in the sericulture industry for silk production. The present study examined the effects of Vitamin C and Vitamin B₁₂ supplementation on growth performance, survival rate, and cocoon production of silkworms fed with *Morus nigra* leaves under semi-controlled environmental conditions. The experiment was conducted at the Sericulture Unit, Department of Wildlife and Ecology, UVAS Ravi Campus, Pakistan, using a randomized block design with three treatment groups: control (*M. nigra*), *M. nigra* supplemented with Vitamin C, and Vitamin B₁₂. Each treatment included one replicate of 250 larvae. Growth performance, cocoon weight, and survival rate were recorded and analyzed through one-way ANOVA and Kaplan–Meier survival analysis. The results showed that vitamin supplementation significantly improved nutritional composition and growth performance. Silkworms fed Vitamin C-fortified leaves achieved the highest body length (4.77 ± 0.04 mm) and weight (893.20 ± 17.39 mg) at the fourth molt, followed by those fed Vitamin B₁₂-supplemented leaves (4.32 ± 0.07 mm; 763.85 ± 17.26 mg), both significantly higher than the control group (4.16 ± 0.06 mm; 763.85 ± 17.26 mg).

mm; 669.75 ± 19.36 mg). Cocoon weights also increased significantly, with Vitamin C-fed larvae producing the heaviest cocoons (2.42 ± 0.079 g) compared to Vitamin B₁₂ (2.14 ± 0.107 g) and control (1.77 ± 0.094 g). Survival analysis revealed the highest cumulative survival probability in the Vitamin C group, indicating enhanced resistance and physiological efficiency. Statistical differences were significant at $p < 0.05$ across most parameters. Future research should focus on optimizing the concentration and combination of vitamins to achieve maximum benefits without causing adverse effects. It is also recommended that future investigations focus on using probiotics alongside vitamin supplements to improve growth performance and cocoon production.

Keywords: Cocoon production, Probiotics, Silkworm, Vitamin supplementation

Introduction

Bombyx mori, commonly known as the silkworm, is a highly valuable member of the Lepidoptera order that produces soft silk fiber, often regarded as the queen of textiles. *Bombyx mori* L. is reared in controlled environments and cultivated for commercial silk production. The leading silk-producing states include West Bengal, Jammu & Kashmir, Karnataka, Tamil Nadu, and Andhra Pradesh (Gautam et al., 2022; Hussain et al., 2025). Sericulture includes the indoor cultivation of domesticated mulberry silkworms, *Bombyx mori* L., for which mulberry (*Morus* sp.) is the primary food source. The *Morus* genus consists of over 70 species, with most of them being native to the Asian continent (Kılınçer et al., 2024). The mulberry silkworm, *B. mori*, holds significant economic value in the silk industry due to its silk-producing capabilities. The production of silk is largely influenced by the larvae's diet, given the nutrient profile of mulberry leaves plays a crucial part in ensuring the healthy development and growth of the silkworm (Ali et al., 2021; Laraib et al., 2022; Murugesh et al., 2021). The genus *Morus* (mulberry) comprises trees that attain a height of 12-15 meters, emerging from China and Japan (white mulberry) and from Persia (black mulberry). Black-reddish (*Morus nigra* L.) fruits, with a sugary, fleeting flavor, whose leaves serve as food for silkworms (Negreanu et al., 2022; Urbanek et al., 2022).

Nutrition is crucial for enhancing the development and growth of the silkworm. Silk production is closely linked to the larvae's diet and the nutritive value of mulberry leaves, which significantly contributes to the production of healthy cocoons. High-quality cocoons can be produced when silkworms are fed on nutritionally supplemented leaves, their results enhanced silk production (Abdelmegeed, 2021). Vitamins played a key part as a biocatalyst in the physiological processes of metabolism, reproduction, and growth. Additionally, supplementing leaves with a 2.5% vitamin complex significantly enhanced larval body weight by 10.2%. However, a 5% supplementation had a negative impact on most biological parameters, except for cocoon weight, which increased by 4.7%. Therefore, this study aims to assess whether the specified concentrations of vitamins, including 0.5% B-complex, 0.5% folic acid, 1.5% ascorbic acid, 1.0% niacin, 0.1% pyridoxine, and 2.5% multivitamins, influence the growth, development, and nutritional parameters of silkworms (Indira et al., 2024).

Sericulture involves two main activities, specifically the rearing of silkworms along with mulberry cultivation. Silkworm exclusively feeds on *Morus* species leaves, and the hereditary basis behind this dietary preference remains unknown. However, researchers have determined that the gene GR66 is the primary component responsible for the silkworm's exclusive consumption of mulberry leaves. Moreover, mutations in this gene can weaken the capacity to consume mulberry foliage and lower the chances of feeding on different plant varieties. Mulberry is a member of the genus *Morus*, consisting of various types such as *Morus nigra*, *Morus alba* var, *Morus alba*, *macrophylla*, *Morus multicaulis*, *Morus australis*, and *Morus bombycis* (Andadari, 2021). The vitamin is referred to as an accessory indispensable food factor required by an organism in small quantities to maintain normal growth and regulation of metabolism. Lack of any vitamins in the diet of young animals causes vitamin deficiency disease and reduces animal growth. These symptoms can be cured or prevented by supplementation with vitamin-rich foods (Soliman, 2024; Bukhari & Sudan, 2021). In silkworm feed, vitamin B1 plays an essential role in maintaining the body's metabolism, which is likely to enhance both the development and yield of the silkworm (Tanjung, 2021; Gupta & Dubey, 2021).

Supplementing mulberry leaves with vitamin C enhanced the commercial qualities of silkworm cocoons. Furthermore, adding vitamin B to the mulberry leaves improved the silkworm larvae's resistance to environmental stress, resulting in a notable increase in body mass in comparison with the control (Mustafa et al., 2021; Ali et al., 2017; Sadaf et al., 2021). Vitamin D3 is crucial in regulating cell metabolism associated with glucose, and its presence may improve the physiology of silkworms, leading to enhanced performance in both economic and biological traits. In recent years, various efforts have been made to enhance both the silk's characteristics and yield through supplementing leaves of mulberry with nutrients along with vitamins, spraying with antibiotics, hormones, and hormone analogues, as well as using extracts or products derived from plants (Akram et al., 2017). A specific dose of Spirulina provides an optimal concentration of essential amino acids and vitamins, which play a crucial role in regulating various metabolic activities in silkworms. The green microalga *Chlorella vulgaris* is a single-celled organism that occurs naturally. It is particularly abundant in chloroplasts and vitamin B₁₂ and contains porphyrin, a compound that stimulates cellular metabolism. Azolla (*Azolla caroliniana*) is a heterosporous aquatic fern that serves as a valuable protein source and is enriched with vitamins (B₁₂, A, and beta-carotene), biopolymers, amino acids, and minerals. The effects of Spirulina, Azolla, and Chlorella on various silkworm traits were evaluated to determine their nutritional potential as supplementary feed for silkworms (Hassan, 2025).

Ascorbic acid plays a crucial role in the silkworm body, serving as a potent antioxidant that protects proteins from oxidative damage. Silkworm cocoon value increased as a result of mulberry leaves being fortified with vitamin C (Abdul & Ayoub, 2023; Ali et al., 2017; Ali et al., 2018; Hussain et al., 2023).

Excessive supplementation of Vitamin C in the silkworm diet has an adverse effect, leading to a reduction in dietary intake and deterioration in cocoon traits due to excessive vitamin levels. Ascorbic acid, Juice from sweet oranges plus Lemon juice enhance cocoon indices and silk filament characteristics, proving to be a beneficial supplementary diet for the silkworm *Bombyx mori* L (Gad & Fathy, 2021; Kabita et al., 2023). Without ascorbic acid in the diet, the growth and development of early-stage larvae were delayed, preventing their progression into the later stages of silkworm development (Aneesha & Kumar, 2022). This study aims to address this gap by evaluating the impact of Vitamin C and Vitamin B₁₂ supplementation on the rearing performance and cocoon production of silkworms. The findings of this research contributed to optimizing sericulture practices and improving silk yield, thereby benefiting both small-scale and commercial silk producers.

Materials and methods

Study area

The research was conducted at the Sericulture Unit, Department of Wildlife and Ecology, UVAS Ravi Campus, to determine the effect of Vitamin C and Vitamin B₁₂ supplementation on the rearing performance, survival, and cocoon production of silkworms fed on *Morus nigra*. The rearing room was whitewashed, and a formaldehyde (2%) solution was used to sanitize all instruments, including the rearing trays, stands, and incubator (Hussain et al., 2025; Shah et al., 2007).

Proximate composition of black mulberry (*Morus nigra*)

The proximate composition of black mulberry (*Morus nigra*) leaves, including dry matter (%), crude fat (%), crude protein (%), and crude fiber (%), was determined according to standard protocol (Khan et al., 2014; Noor et al., 2021; Sree & Vijayalakshmi, 2018).

Estimation of Dry Matter (%)

One gram of the sample was weighed in a petri dish and placed in a drying oven at 105 °C for 12 hours. After cooling for ten minutes, the samples were placed in a desiccator and weighed. The drying process was continued until a constant weight was achieved. The final weight (W₂) was recorded, and dry matter and moisture content were calculated (Sree & Vijayalakshmi, 2018).

$$\text{Moisture \%} = \frac{W_1 - W_2}{\text{weight of sample}} \times 100$$

Dry matter = 100—moisture %

Estimation of Crude Protein (%)

The Kjeldahl method was used to determine crude protein content by measuring nitrogen (N × 6.25) after nitric acid digestion (Azmat et al., 2016; Khan et al., 2023).

Estimation of Crude Fat (%)

Crude fat was measured using the Soxhlet apparatus, following the petroleum ether extraction method (Masood et al., 2021). The crude fat percentage was determined using the following formula:

$$\% \text{ of crude fat} = \frac{(\text{Wt. of the crucible with dry residue} - \text{Wt. of the crucible with ash}) \times 100}{\text{gm of substance taken}}$$

Estimation of Crude Fiber (%)

Crude fiber was determined by digesting fat-free samples with 1.25% sulfuric acid and sodium hydroxide solutions under the required conditions (Khalid et al., 2023).

$$\% \text{ of crude fiber} = \frac{\text{Loss of weight on ignition}}{\text{weight of sample}} \times 100$$

Experimental Design

The study followed a Randomized Block Design (RBD) to assess the effect of vitamin supplementation on silkworm rearing. There were three treatment groups, including a control group (C) in which silkworms were fed with black mulberry (*Morus nigra*) leaves, in 2nd group, silkworms were fed with *M. nigra* leaves supplemented with a 1% Vitamin C (Ascorbic Acid) solution, and in 3rd group, silkworms were fed with *M. nigra* leaves supplemented with a 0.1% Vitamin B₁₂ (Cyanocobalamin) solution. Each treatment group had one replicate with 250 larvae per replicate (Andadari, 2021).

Vitamin Supplementation Protocol

To evaluate the impact of vitamin supplementation on silkworm growth, survival, and cocoon production, vitamin C (Ascorbic Acid) and vitamin B₁₂ (Cyanocobalamin) solutions were prepared and applied to mulberry leaves before feeding.

Preparation of Vitamin C Solution

1% vitamin C solution was prepared by dissolving 1g of ascorbic acid powder in 100 mL of distilled water. The solution was mixed thoroughly and stored in an amber-colored bottle to prevent degradation. Fresh solution was prepared regularly and sprayed evenly onto the mulberry leaves before feeding (Gad & Fathy, 2021).

Preparation of Vitamin B₁₂ Solution

0.1% vitamin B₁₂ solution was prepared by dissolving 100 mg of cyanocobalamin powder in 100 mL of distilled water. The solution was stirred well and stored in a light-protected container. Similar to vitamin C, this solution was freshly prepared and uniformly applied to the mulberry leaves. For 1 kg of mulberry leaves, the typical application was 50 mL of vitamins solution per 200 g of leaves (Kanafi et al., 2007; Mushtaq et al., 2021).

Timeline of Rearing

Silkworms were reared under semi-controlled environmental conditions. The process includes incubation of eggs, hatching, initial feeding, transfer to the grow-out room, observation of molting stages,

maintenance of optimal temperature and humidity, cocoon formation, data collection, and observations (Fig. 1).

Data Collection and Statistical Analysis

Silkworm growth parameters, including weight, length, survival rate, and cocoon weight, were recorded. The data thus obtained was subjected to analysis through the one-way ANOVA technique using PROC GLM in SAS software (version 9.1). For the comparison of significant treatment means, Fisher's least significant difference test was applied (Steel et al., 1996), and the following model was applied:

$$Y_{ij} = \mu + \tau_i + \epsilon_{ij}$$

Kaplan–Meier survival curves were constructed to visualize survival patterns across treatment groups. A p-value of less than 0.05 was considered statistically significant.



Figure 1. Various molting stages of silkworms (*Bombyx mori*) from hatching to cocoon formation during the present study.

Results

The present one-year study was conducted at the Sericulture Unit, Department of Wildlife and Ecology, UVAS Ravi Campus. The experiment was conducted under semi-controlled environmental conditions. A 17g packet of silkworm eggs was obtained from the Punjab Forest Department, Changa Manga Forest, Kasur, Punjab, Pakistan. Silkworm eggs were incubated at 25 °C and 75–80% humidity for 10 days to hatch. Newly hatched larvae were shifted from petri plates to trays, and chopped *Morus nigra* leaves and *Morus nigra* supplemented with vitamin C and B₁₂ were provided to the silkworm's larvae. At the end of 1st molting the silkworm larvae were shifted to rearing room which consists on proper bedding set up to provide enough space to the silkworms and more efficient cleaning process. The amount of food each larva received was uniformed to control the intake of the diet by each treatment. Each treatment group received the same weight of leaves. This design reduced the possible bias of unequal feed access and made the differences in growth and survival due to the type of mulberry leaves, not the amount of feed.

Proximate composition of *Morus nigra*

The fresh *Morus nigra* leaves were collected from Changa Manga Forest, Tehsil Pattoki, Punjab, Pakistan. The leaves were washed to remove any dirt or debris. After those leaves were dried in oven at 60 °C until they reach a constant weight. Once dried, leaves were ground to make fine powder and stored for further analysis. The proximate analysis was done according to standard protocols (AOAC, 2023). The results revealed significant variations in dry matter, crude protein, crude fat and crude fiber contents. Table 1 summarizes the proximate analysis of *Morus nigra*. The dry matter content showed a progressive increase from 90.88 ± 0.349 % in the control (*Morus nigra*) to 91.42 ± 0.828 % in the Vitamin B₁₂ treated group and reached the highest value of 92.78 ± 0.605 % in the Vitamin C-treated group. This indicates that vitamin fortification improved leaf density and reduced moisture content, possibly due to enhanced physiological activity and leaf maturation. Similarly, the crude protein content was significantly higher ($p < 0.041$) in the Vitamin C-supplemented leaves (19.43 ± 0.544 %) compared to the control (18.34 ± 0.556 %) and the Vitamin B₁₂-treated group (19.01 ± 0.130 %). This enhancement in protein percentage may be attributed to the role of vitamins in promoting nitrogen assimilation and protein biosynthesis, which improves the nutritional quality of mulberry leaves and ultimately benefits silkworm growth and cocoon formation. In contrast, the crude fat content decreased with vitamin supplementation, from 4.28 ± 0.324 % in the control to 3.70 ± 0.107 % in Vitamin B₁₂-treated leaves and further to 3.04 ± 0.137 % in Vitamin C-treated leaves. This reduction suggests that vitamin-enriched leaves favor carbohydrate and protein accumulation over fat storage, aligning with the increased metabolic activity observed under vitamin influence. The crude fiber content exhibited a marked rise from 9.88 ± 0.195 % in the control to 11.04 ± 0.150 % and 12.13 ± 0.224 % in Vitamin B₁₂ and Vitamin C treatments, respectively. Higher fiber levels

may reflect strengthened cell wall structure and improved leaf texture, which can influence leaf palatability and digestion efficiency in silkworms. Overall, the data indicate that both Vitamin B₁₂ and Vitamin C supplementation enhanced the nutritional composition of *Morus nigra* leaves, with Vitamin C showing the most pronounced effect. These variations are significant as they influence the growth rate and cocoon yield of silkworms.

Table 1: Proximate composition of *Morus nigra* used during present study (Mean $\pm$ S. D).

Species	Dry Matter (%)	Crude Protein (%)	Crude Fat (%)	Crude Fiber (%)
<i>Morus nigra</i>	90.88 $\pm$ 0.349 ^c	18.34 $\pm$ 0.556 ^c	4.28 $\pm$ 0.324 ^a	9.88 $\pm$ 0.195 ^c
<i>Morus nigra</i> +Vitamin B ₁₂	91.42 $\pm$ 0.828 ^b	19.01 $\pm$ 0.130 ^b	3.70 $\pm$ 0.107 ^b	11.04 $\pm$ 0.150 ^b
<i>Morus nigra</i> + vitamin C	92.78 $\pm$ 0.605 ^a	19.43 $\pm$ 0.544 ^a	3.04 $\pm$ 0.137 ^c	12.13 $\pm$ 0.224 ^a
P value	<0.041	<0.041	<0.038	<0.027

Growth and developmental stages of *Bombyx mori*

Overall, a total of 500 silkworm larvae were kept in each treatment group. At each molting stage randomly 20 specimens were selected for morphometric measurements. The weight (mg) and length (mm) of the silkworms among treatment groups were recorded at each molting stage. The data indicated significant differences in growth patterns among the treatment groups. Table 2 summarizes statistical analysis of body length (mm) of silkworms fed with *M. nigra*, *M. nigra* fortified with vitamin B₁₂ and vitamin C.

The body length of the silkworms in all treatment groups increased progressively from molting 1 to molting 4. At molting 1, the body length of silkworms fed with *M. nigra* was 0.76 $\pm$ 0.02 mm, which was significantly lower compared to those fed with *M. nigra* fortified with vitamin B₁₂ (0.99 $\pm$ 0.03 mm) and *M. nigra* fortified with vitamin C (1.14 $\pm$ 0.04 mm). The body length at molting 2, 3, and 4 also showed similar trends, with silkworms receiving vitamin C showing the maximum body length at each molting stage. At molting 2, the body length of the *M. nigra* group was 1.85 $\pm$ 0.03 mm, while those fed *M. nigra* fortified with vitamin B₁₂ measured 2.02 $\pm$ 0.03 mm, and those fed *M. nigra* fortified with vitamin C reached 2.25 $\pm$ 0.04 mm. At molting 3, the body lengths were 2.81 $\pm$ 0.04 mm for *M. nigra*, 3.03 $\pm$ 0.03 mm for *M. nigra* fortified with vitamin B₁₂, and 3.14 $\pm$ 0.04 mm for *M. nigra* fortified with vitamin C. By molting 4, the body lengths of the silkworms in each group were 4.16 $\pm$ 0.06 mm, 4.32 $\pm$ 0.07 mm, and 4.77 $\pm$ 0.04 mm, respectively. All differences were statistically significant (P-value < 0.0001).

Similarly, significant differences in body weight were observed between the treatment groups. Table 3 summarizes the statistical analysis of body weight (mg) of silkworms fed with *M. nigra*, *M. nigra* fortified

with vitamin B₁₂, and vitamin C. At molting 1, silkworms fed *M. nigra* weighed 12.95 ± 0.50 mg, which was significantly lower than those fed *M. nigra* fortified with vitamin B₁₂ (17.90 ± 0.59 mg) and *M. nigra* fortified with vitamin C (19.75 ± 0.69 mg). At molting 2, the weight of silkworms fed with *M. nigra* was 49.70 ± 2.35 mg, while those receiving *M. nigra* fortified with vitamin B₁₂ weighed 56.55 ± 2.24 mg, and those receiving *M. nigra* fortified with vitamin C weighed 66.05 ± 1.86 mg. By molting 3, silkworms in the *M. nigra* group weighed 235.15 ± 11.59 mg, whereas those fed with *M. nigra* fortified with vitamin B₁₂ weighed 246.95 ± 11.80 mg, and those receiving *M. nigra* fortified with vitamin C weighed 274.75 ± 9.72 mg. The weight difference was most pronounced at the end of molting 4, where the silkworms fed with *M. nigra* had a mean weight of 669.75 ± 19.36 mg, while those fed with *M. nigra* fortified with vitamin B₁₂ weighed 763.85 ± 17.26 mg, and those in the *M. nigra* with vitamin C group weighed 893.20 ± 17.39 mg. The differences were statistically significant at molting 1, 2, and 4 (P-value < 0.0001), while at molting 3 the difference was less significant (P-value = 0.0410).

Table 2: Statistical analysis of body length (mm) of silkworms fed with *M. nigra*, *M. nigra* fortified with vitamin B₁₂, and vitamin C.

Treatment Groups	Molting 1	Molting 2	Molting 3	Molting 4
<i>M. nigra</i>	$0.76^c \pm 0.02$	$1.85^c \pm 0.03$	$2.81^c \pm 0.04$	$4.16^c \pm 0.06$
<i>M. nigra</i> + vitamin B ₁₂	$0.99^b \pm 0.03$	$2.02^b \pm 0.03$	$3.03^b \pm 0.03$	$4.32^b \pm 0.07$
<i>M. nigra</i> + vitamin C	$1.14^a \pm 0.04$	$2.25^a \pm 0.04$	$3.14^a \pm 0.04$	$4.77^a \pm 0.04$
P- value	<0.0001	<0.0001	<0.0001	<0.0001

Superscripts on different means within row differ significantly at $p \leq 0.05$

Table 3: Statistical analysis of body weight (mg) of silkworms fed with *M. nigra*, *M. nigra* fortified with vitamin B₁₂ and vitamin C.

Treatment Groups	Molting 1	Molting 2	Molting 3	Molting 4
<i>M. nigra</i>	$12.95^c \pm 0.50$	$49.70^c \pm 2.35$	$235.15^c \pm 11.59$	$669.75^c \pm 19.36$
<i>M. nigra</i> + vitamin B ₁₂	$17.90^b \pm 0.59$	$56.55^b \pm 2.24$	$246.95^b \pm 11.8$	$763.85^b \pm 17.26$
<i>M. nigra</i> + vitamin C	$19.75^a \pm 0.69$	$66.05^a \pm 1.86$	$274.75^a \pm 9.72$	$893.20^a \pm 17.39$
P- value	<0.0001	<0.0001	0.0410	<0.0001

Superscripts on different means within row differ significantly at $p \leq 0.05$

Cocoon weight analysis

The analysis of cocoon weight revealed significant differences between the treatment groups ($p < 0.0001$). The silkworms fed on *M. nigra* fortified with vitamin C produced the heaviest cocoons, with a mean weight of 2.42 ± 0.079 . In comparison, the silkworms fed on *M. nigra* fortified with vitamin B₁₂ yielded cocoons with a mean weight of 2.14 ± 0.107 g. The control group, which was fed with *M. nigra* alone,

produced the lightest cocoons, with a mean weight of $1.77 \pm 0.094\text{g}$. The data showed that supplementation with vitamins particularly vitamin C positively influenced cocoon weight (Table 4).

Table 4: Average cocoon weight, g produced by silkworms during experiment.

Treatment groups	Cocoon weight	P-value
<i>Morus nigra</i>	1.77 ± 0.094^c	<0.0001
<i>Morus nigra</i> + vitamin B ₁₂	2.14 ± 0.107^b	<0.0001
<i>Morus nigra</i> + vitamin C	2.42 ± 0.079^a	<0.0001

Survival Analysis

Kaplan-Meier survival analysis was performed to estimate the survival probability of silkworms across different treatment groups. Survival time was defined as the number of instars survived by each individual. The event of interest was silkworm mortality, coded as ‘1’ for death and ‘0’ for censored observations (i.e., silkworms that survived until the end of the observation period). Survival curves were compared among treatment groups (*Morus nigra*, Vitamin C, and Vitamin B₁₂) using the log-rank test to assess statistically significant differences. Kaplan-Meier survival analysis demonstrated differences in silkworm survival among treatment groups. The vitamin C group exhibited the highest cumulative survival probability, followed by vitamin B₁₂ and *Morus nigra* (Fig. 2). The survival difference became more apparent at later instars. Censored observations were recorded for all groups. A log-rank test was conducted to determine the statistical significance of these differences ($p = 0.092$).

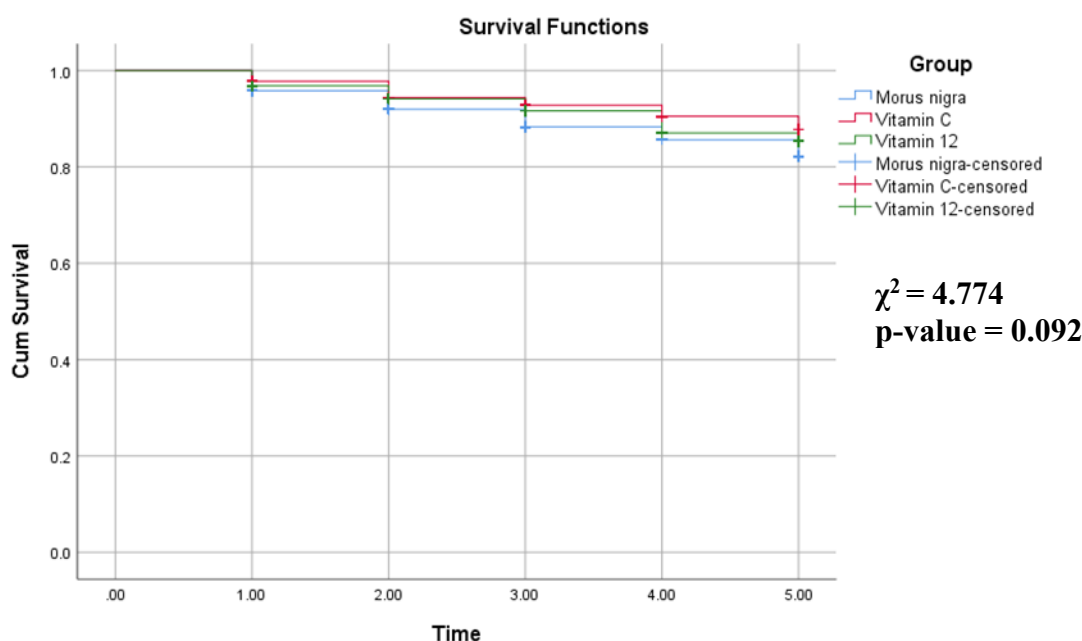


Figure 2. Survival analysis through the Kaplan-Meier test among different treatment groups.

Discussion

The present study aimed to evaluate the effect of vitamin supplementation (vitamin C and vitamin B₁₂) on the growth performance, survival rate, and cocoon production of silkworms (*Bombyx mori*) fed on *Morus nigra* leaves. Our results reveal that both vitamin C and vitamin B₁₂ supplementation had significant positive effects on silkworm growth, survival, and cocoon production, with vitamin C having the most pronounced effect. This section discusses these key findings, compares them with the relevant literature, critically analyzes discrepancies, and explores the broader implications of the study. The results of this study clearly demonstrate that silkworms fed on *Morus nigra* leaves supplemented with Vitamin C exhibited the highest growth in terms of both body length and weight, as well as the heaviest cocoons. At molting stage 4, the silkworms in the Vitamin C-supplemented group had a mean body weight of 893.20 ± 17.39 mg, significantly higher than the 763.85 ± 17.26 mg in the Vitamin B₁₂ group and 669.75 ± 19.36 mg in the control group. This trend continued in earlier stages, with Vitamin C-fed silkworms showing significant increases in body weight and length at each molting stage. Furthermore, the Vitamin C group produced the heaviest cocoons, with a mean weight of 0.38 ± 0.06 g, compared to 0.27 ± 0.05 g in the Vitamin B₁₂ group and 0.23 ± 0.04 g in the control group. The survival analysis, using Kaplan-Meier survival curves, revealed that Vitamin C supplementation resulted in the highest survival probability, particularly in the later instars.

These findings are consistent with several studies that have explored the positive impact of vitamin supplementation on silkworm growth, survival, and cocoon production. Our study shows that Vitamin C supplementation significantly improves growth and cocoon yield, as seen in similar studies by Abdul & Ayoub, (2023), and Udayan & Kumar, (2022), who reported enhanced larval weight, silk gland development, and cocoon weight after vitamin C fortification. In particular, the findings of Abdul & Ayoub, (2023) align with our results, where Vitamin C supplementation boosted both larval growth and cocoon production, likely due to its antioxidant properties that protect silkworms from oxidative stress and enhance overall metabolism. While Vitamin C had the most substantial effect, Vitamin B₁₂ supplementation also led to noticeable improvements in silkworm growth and survival. However, the effects of Vitamin B₁₂ were not as pronounced as those of Vitamin C, supporting findings by Kanafi et al., (2007) and Tanjung, (2021), who observed that Vitamin B₁₂ supplementation positively impacted silkworm growth, although to a lesser extent compared to other vitamins. Our study confirms that Vitamin B₁₂ supplementation can be beneficial, but it is less effective in comparison to Vitamin C, which is likely due to the distinct physiological roles these vitamins play in silkworm metabolism. The results from our study are in line with existing literature that emphasizes the importance of vitamin supplementation for enhancing silkworm growth and productivity. Vitamin C, in particular, has been widely recognized as a crucial factor for improving silkworm development and cocoon quality. Abdul & Ayoub, (2023) highlighted that ascorbic acid supplementation significantly improved larval weight and cocoon

characteristics, which is consistent with our findings. Likewise, Gad & Fathy, (2021) found that Vitamin C-enriched mulberry leaves resulted in a significant improvement in both natural factors (such as larval weight) and commercial characteristics (such as cocoon mass and shell weight), further supporting the role of vitamin C in silkworm rearing. Our findings also align with Udayan & Kumar, (2022), who demonstrated that vitamin C supplementation under thermal stress conditions improved feeding efficiency and larval growth, which is particularly relevant for regions where temperature fluctuations are common. The increased survival observed in the vitamin C group in our study also corroborates the results of Soliman (2024), who noted that vitamin supplementation helped improve survival rates and overall productivity, particularly under stressful environmental conditions.

Vitamin B₁₂ supplementation, while not as effective as vitamin C, also had positive effects on growth and survival, as observed in our study. This supports findings by Kanafi et al. (2007) and Tanjung (2021), who demonstrated that vitamin B₁₂ supplementation improved silkworm development and survival rates, though the magnitude of effect was smaller compared to other vitamins. Although our study showed clear benefits from vitamin C and vitamin B₁₂ supplementation, some unexpected results warrant further exploration. Specifically, at molting stage 3, although a trend toward greater growth in the supplemented groups was observed, the differences in body weight between treatment groups were less pronounced, with a P-value of 0.0410. This discrepancy suggests that while vitamin supplementation enhances growth, the effects may be more evident at later instars. These results are in line with Soliman (2024), who observed that the benefits of vitamin supplementation often accumulate gradually over time. Additionally, variations in growth between stages could be attributed to the complex interactions between nutritional supplementation, environmental conditions, and the silkworm's genetic background (Ali et al., 2020; Hassan, 2025). Another potential explanation for the observed discrepancies could be the different metabolic responses of silkworms at various developmental stages. As noted by Kılınçer et al. (2024), the growth rate of silkworms may vary depending on the timing of nutrient intake, with some vitamins having a more pronounced effect during specific instars. Therefore, future studies should explore the timing and duration of supplementation to determine how different vitamins influence growth across various developmental stages. Moreover, while excessive vitamin supplementation has been reported to have detrimental effects on silkworm growth (Aneesha & Kumar, 2022), no such adverse effects were observed in our study. This highlights the need for further research to determine the optimal concentration of vitamins that maximizes growth and productivity without leading to toxicity. The findings of this study have important practical implications for the sericulture industry. The demonstrated benefits of vitamin supplementation, particularly Vitamin C, suggest that it could serve as a cost-effective strategy for improving silkworm productivity and cocoon yield (Wani et al., 2023). The fact that Vitamin C supplementation significantly enhanced both growth and cocoon weight makes it an attractive option for

sericulture operations, especially in regions with fluctuating environmental conditions that may negatively impact silkworm development. By fortifying mulberry leaves with vitamins, sericulture farmers could increase the efficiency of their operations, leading to higher silk yield and reduced mortality. However, as our study focused on only two vitamins, future research should explore the effects of other vitamins, such as Vitamin D or Vitamin E, on silkworm growth and cocoon production. Additionally, investigating the combined effects of multiple vitamins and other nutrient-rich additives could further optimize silkworm rearing practices. Given the variability in growth rates and cocoon yield between different silkworm breeds, future studies should also investigate how vitamin supplementation affects various silkworm breeds to determine the generalizability of the findings across different strains. Another avenue for future research is the exploration of the biochemical mechanisms through which vitamins influence silkworm growth and cocoon production. By understanding the molecular pathways involved, researchers can identify key metabolic processes that can be targeted to improve silkworm rearing and silk production. Moreover, investigating the long-term effects of vitamin supplementation on silkworm reproduction and overall health could provide valuable insights for optimizing breeding programs.

Conclusion

The present research revealed that vitamin supplementation, particularly Vitamin C, significantly improved the growth, survival, and cocoon production of silkworms (*Bombyx mori*) fed with *Morus nigra* leaves under semi-controlled conditions. The inclusion of Vitamin C and Vitamin B₁₂ enhanced the nutritional composition of mulberry leaves, leading to increased larval length, body weight, and cocoon yield. Among both treatments, Vitamin C showed the most pronounced effects, producing heavier cocoons, higher survival rates, and overall better growth performance. The results confirm that vitamins play a vital role in metabolic regulation, tissue development, and stress resistance in silkworms. Future research should focus on optimizing the concentration and combination of vitamins to achieve maximum benefits without causing adverse effects. It is also recommended that future investigations should focus on using probiotics alongside vitamin supplementation on growth performance and cocoon production.

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