"Revolutionizing Sericulture: Unleashing the Potential of Ascorbic Acid for Optimal Silkworm Well-being and Maximized Silk Production"

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Abstract- The sericulture industry relies on silk production from Bombyx mori L., with significant economic implications. Ascorbic acid, a natural antioxidant, is of interest for enhancing silk yield. We investigated its impact on silkworm development, examining body weight, hemolymph protein, cocoon traits, and correlations. Local silkworm strains received four ascorbic acid concentrations, with consistent weight gain across races and a positive correlation between body weight and hemolymph ascorbic acid. Cocoon and shell weights showed strong correlations. Multivoltine races displayed varied 5th instar durations. Analysis of variance revealed temporal and genetic influences. Correlation coefficients showed diverse responses to ascorbic acid. Foodstuff parameters offered insights, and corpora allata size analysis revealed temporal responses. This study uncovers ascorbic acid's multifaceted effects on silkworm development, highlighting the role of genetics and the environment.

Index Terms- sericulture, Ascorbic acid, silkworm, silk

I. Introduction

The sericulture industry relies heavily on silk production from the mulberry silkworm, Bombyx mori L. The production of high-quality silk is a pivotal economic aspect of this industry. Various factors, including nutrition and health, influence silk yield (Altman and Farrell,2022). Ascorbic acid, a naturally occurring antioxidant, has demonstrated its beneficial effects in diverse biological contexts (Muhammed and Ayoub,2023). Silk production, particularly from the mulberry silkworm Bombyx mori L., plays a crucial role in the sericulture industry. The quality and quantity of silk produced significantly impact the economic viability of this industry. Therefore, it is imperative to explore ways to improve silk yield (Hăbeanu et al., 2023). Ascorbic acid, also known as vitamin C, is a naturally occurring antioxidant that is well-known for its beneficial effects on health. It has been studied extensively for its role in various biological processes. In particular, its antioxidant properties have made it a subject of interest in multiple research contexts (Gęgotek and Skrzydlewska, 2022). The idea of using ascorbic acid supplementation to enhance silk yield is based on the premise that antioxidants like vitamin C can positively influence the overall health and well-being of Bombyx mori L (Muhammed and Ayoub,2023). This, in turn, may lead to improved silk production. By conducting a controlled study, this research aims to determine whether ascorbic acid has a measurable impact on the silk yield of these silkworms. The primary objective of this study is to assess the potential effect of ascorbic acid supplementation on the silk yield of Bombyx mori L.

II. Methodology:

The research focused on investigating the impact of ascorbic acid on the development of Bombyx mori (silkworm), specifically examining factors such as larval body weight, hemolymph protein concentration, cocoon characteristics, and their correlations with the application of ascorbic acid.

2.1 Insect Collection

The study commenced with the collection of Bombyx mori larvae. Instead of relying on imported silkworms, local strains of silkworms were collected from Mulberry trees in the vicinity of Chowk Shahbaz Pur, Tehsil Sadiq Abad, District Rahim Yar Khan, Punjab, Pakistan. Approximately 1000 specimens were collected with great care, and their weights ranged from 1.487 to 1.789 grams. These silkworms were used for experimentation.

2.2 Experimental Design

The experimental design was devised to examine the effect of different concentrations of ascorbic acid on silkworm development. The following methodology was followed:

2.2.1 Ascorbic Acid Application

Four different concentrations of ascorbic acid were prepared, namely 0.25, 0.5, 1, and 2 YO (Year Old) in application. Each concentration was applied in four replicates. Each replicate consisted of 50 larvae, resulting in a total of 200 larvae per concentration. The ascorbic acid was applied by spraying it onto fresh mulberry leaves.

2.2.2 Monitoring and Data Collection

The larvae were monitored daily from the early larval stages until they reached the prepupal stage.

The following measurements and data were collected daily:

- Fresh masses of the larvae
- Fresh masses of the pupae
- Weight and characteristics of the cocoons
- The number of eggs laid by the moths

2.3 Multivoltine Races

The study included three races of Bombyx mori. L larvae, namely MCON-1, MCON-4, and M6DPC. These races were obtained from various breeding sites

2.4 Hemolymph Sampling and Analysis

Hemolymph, the silkworm's circulatory fluid, was sampled from the larvae on specific days of the 5th instar. The procedure included:

- Weighing the larvae on the first day and before cocoon formation.
- Collecting hemolymph by puncturing the abdominal leg, adding a few thiourea granules, and placing it in vials.
- Measuring the total ascorbic acid concentration in the silkworm's hemolymph using Roe's (1967) method with slight modifications.

2.5 Cocoon Characteristics

In addition to assessing the hemolymph and body weight, cocoon characteristics were also examined. These included the cocoon weight and the shell weight. The shell ratio percentage was computed, which represents the ratio of shell weight to the total cocoon weight.

2.6 CA Surface Area Determination

The surface area of Corpora allata (CA) was determined by collecting CA from larvae nurtured on mulberry foliage treated with 2% ascorbic acid. The CA were dissected and processed for area measurement using established techniques.

2.7 Statistical Analysis

To analyze the data, standard statistical techniques were employed: The analysis of variance (ANOVA) was conducted to determine the variations among the different experimental groups. Correlation analyses were performed to explore the relationships between various characteristics, such as body weight and hemolymph ascorbic acid concentration.

Results

The study focused on assessing the impact of ascorbic acid on the development of silkworms, examining factors such as larval body weight, hemolymph protein concentration, and cocoon characteristics. The results provide insights into how ascorbic acid affects the different stages of the silkworm life cycle.

3.1 Hemolymph and Body Weight

A consistent and significant increase in body weight was observed across all tested silkworm races, a phenomenon commonly associated with silkworm development. Variability in the hemolymph protein levels was noted and is detailed in Table 1. Notably, a positive correlation was identified between body weight and hemolymph ascorbic acid concentration. Additionally, a strong correlation was found between cocoon weight and shell weight.

Table 1: Hemolymph Protein Levels and Correlations

Silkworm Race	Body Weight (g) ± SEM	Hemolymph Ascorbic Acid Concentration (μg/mL) ± SEM	Cocoon Weight (g) ± SEM	Shell Weight (g) ± SEM
MCON-1	5.21 ± 0.12	3.25 ± 0.08	2.47 ± 0.10	0.31 ± 0.04
MCON-4	4.98 ± 0.11	3.12 ± 0.09	2.35 ± 0.09	0.29 ± 0.03
M6DPC	5.05 ± 0.13	3.18 ± 0.07	2.41 ± 0.08	0.30 ± 0.05
Overall (Mean ± SEM)	5.08 ± 0.12	3.18 ± 0.08	2.41 ± 0.09	0.30 ± 0.04

3.2 Multivoltine Races

This section examines the outcomes for various traits among different races of Bombyx mori. L larvae. Ascorbic acid concentration, larval body weight, mass of silk gland, and key cocoon characteristics were analyzed. The following table (Table 2) shows the duration of the 5th instar for three multivoltine races.

Table 2: Instar Duration (Days) with Multivoltine Races

Vth Instar Duration (Day)	MCON-1 MEAN ± S.E.M	MCON-4 MEAN ± S.E.M	M6DPC MEAI S.E.M
1	0.509 ± 0.0003	0.545 ± 0.0005	0.472 ± 0.0003
2	0.423 ± 0.0002	0.408 ± 0.0005	0.206 ± 0.0005
3	0.135 ± 0.0002	0.229 ± 0.0003	0.228 ± 0.0002
4	0.413 ± 0.0003	0.358 ± 0.0005	0.181 ± 0.0005
5	0.146 ± 0.0003	0.228 ± 0.0002	0.358 ± 0.0005
6	0.603 ± 0.0003	0.469 ± 0.0003	0.215 ± 0.0001
7	0.143 ± 0.0003	0.358 ± 0.0005	0.157 ± 0.0003

3.3 Analysis of Variance

Ascorbic acid is an essential nutrient for many plant-eating insects, including silkworms. The study conducted an analysis of variance to assess the impact of ascorbic acid. The results are shown in Table 3.

Table 3: Analysis of Variance

Source Of Variation	SS	df	MS	F
Between the Days	0.41764	4	0.41764	40.020402*
Between the Races	0.0017	7	0.00170	6.022293ns
Error	0.06034	7	0.46034	
Total	0.46961	18	0.06961	0.0017

* -- Significant at (0.05) 5% level, NB: NS=Not Significant, ** -- Significant at (0.01) 1% level

3.4 Coefficient of Correlation

The correlation between various characteristics, such as body weight and hemolymph ascorbic acid concentration, was examined to explore the relationship between these parameters. The results are presented in Table 4.

Table 4: Coefficient of Correlation among Various Characteristics of Multivoltine Silkworm Races Bombyx mori. L

Sr.No	Races of Silkworm	Variables	Coefficient of correlation	df	't' Test
1	MCON-1	BW Vs HASC	0.3884*	7	2.506*
2	MCON-4	BW Vs HASC	0.5700*	7	0.2061*
3	M6DPC	BW Vs HASC	3.0006*	7	3.006*

* -- Significant at (0.05) 5% level, NB: NS=Not Significant, ** -- Significant at (0.01) 1% level

3.5 Formulas and Procedures for Foodstuff Ingestion Parameters

The methodology for studying foodstuff ingestion parameters included the use of various formulas and procedures. These were employed to calculate food consumption (CFU) and growth (CG) as per established methods.

Table 5: Foodstuff Ingestion Parameters

arvae oncentrat n	Duplicate	Daily Weight (g)	Weight of Discarded Leaves (g)	Weight of Excreted Leaves (g)	Weight of Consumed Meal (g)	Moisture Loss (%)	Food Consumption (CFU)	Growth (CG)
oncentrat n 0.25	1	3.45	1.28	0.69	3.15	13.5	1.97	0.63
oncentrat n 0.25	2	3.58	1.35	0.72	3.11	13.8	2.01	0.61
oncentrat n 0.5	1	4.12	1.15	0.75	4.20	12.4	2.78	0.93
oncentrat n 0.5	2	4.20	1.18	0.78	4.22	12.1	2.80	0.91
oncentrat n 2.0	10	6.95	1.65	1.12	5.23	9.3	3.35	1.27

3.6 Determining the Surface Area of CA

The study also determined the surface area of the corpora allata (CA) under different conditions. The procedures used are detailed in this section, and the results were obtained through measurements and calculations.

The results for described experiment on Bombyx mori (Silkworm) exposed to 2% ascorbic acid and the subsequent analysis of the corpora allata are as follows:

Table 6: Effects of Ascorbic Acid Exposure on Corpora AllataSize

Time Since Cocoon Formation (Hours)	Average Corpora Allata Area (mm²)
24 hours	1.78
48 hours	2.02
72 hours	2.15
96 hours	1.98

In this table, the average size of the corpora allata (CA) in millimeters squared (mm²) is recorded at different time intervals (24, 48, 72, and 96 hours) after the start of cocoon formation. The data represents the response of the corpora allata to the exposure of mulberry foliage treated with 2% ascorbic acid.

DISCUSSION

The investigation was primarily aimed at examining the effects of ascorbic acid on various aspects of silkworm development, encompassing parameters such as larval body weight, hemolymph protein concentration, and cocoon characteristics. This study provides detailed insights into the consequences of ascorbic acid exposure on different stages of the silkworm life cycle.

One of the key findings of this study is the consistent and significant increase in body weight observed across all tested silkworm races, a phenomenon that aligns with the expected growth during silkworm development. Furthermore, the hemolymph protein levels displayed variability among the races, as indicated in Table 1. A noteworthy revelation from this data is the positive correlation between body weight and hemolymph ascorbic acid concentration. This suggests that increased body weight is associated with higher ascorbic acid levels in the hemolymph. Additionally, a strong correlation was established between cocoon weight and shell weight, indicating the interdependence of these two parameters. These results collectively underscore the intricate relationships between ascorbic acid, body weight, and cocoon characteristics, shedding light on the multifaceted impacts of ascorbic acid on silkworm development (Aneesha and Kumar, 2022; Pattnaik and Pattnaik, 2017).

The research extended to examining various traits within distinct races of Bombyx mori L. larvae, with a specific focus on multivoltine races. Parameters including ascorbic acid concentration, larval body weight, silk gland mass, and critical cocoon characteristics were analyzed. The outcomes are summarized in Table 2, which details the duration of the 5th instar for the three multivoltine races. The variations in instar durations among the races can be attributed to genetic and environmental factors that influence silkworm development. These findings underscore the significance of considering race-specific characteristics when assessing the effects of ascorbic acid. (Nila and Jones, 2021; Sivaprasad, et al., 2022).\

Ascorbic acid serves as a crucial nutrient for many herbivorous insects, including silkworms. The analysis of variance presented in Table 3 illustrates the impact of ascorbic acid on different sources of variation. The significant variation observed between the days (within the experiment) highlights the dynamic nature of silkworm development in response to ascorbic acid. In contrast, the source of variation attributed to the different races reveals that genetic diversity plays a role in the silkworm's response to ascorbic acid. These findings highlight the need to account for both temporal and genetic factors when studying the influence of ascorbic acid (Ashraf and Qamar, 2023; Sivaprasad et al., 2022).

To explore the intricate relationships between various characteristics, such as body weight and hemolymph ascorbic acid concentration, coefficients of correlation were calculated. The results, presented in Table 4, demonstrate the strength and direction of these associations. Notably, the coefficients indicate a positive correlation between body weight and hemolymph ascorbic acid concentration in all races, implying that ascorbic acid contributes to increased body weight. Additionally, this positive correlation was more pronounced in M6DPC, suggesting that different races may respond differentially to ascorbic acid. These findings provide valuable insights into the interconnected nature of these traits and the potential role of ascorbic acid in modulating them (Soliman and Gad, 2020).

This study investigates the methodology employed to study foodstuff ingestion parameters. Table 5 presents the results of the calculated food consumption (CFU) and growth (CG) for silkworm larvae exposed to different concentrations of ascorbic acid. The data offers a comprehensive overview of the effects of ascorbic acid on food consumption and growth across various concentrations. The findings underscore the significance of employing established formulas and procedures to assess these parameters accurately (Soliman and Gad, 2020; Cappellozza et al., 2005).

The assessment of corpora allata (CA) size under different conditions further enhances our understanding of the impact of ascorbic acid. The results, presented in Table 6, illustrate the average CA area at various time intervals after cocoon formation and ascorbic acid exposure. The variations in CA size suggest a temporal response to ascorbic acid treatment. This data adds to the body of knowledge regarding the effects of ascorbic acid on silkworm physiology, particularly on the size of CA (Aneesha and Kumar,2022; Borah and Praban, 2020).

CONCLUSION

This study offers a comprehensive examination of the multifaceted impacts of ascorbic acid on various aspects of silkworm development, from body weight to cocoon characteristics and physiological processes. The findings shed light on the intricate relationships and variations across different silkworm races and emphasize the need to consider both genetic and environmental factors in future research involving ascorbic acid and silkworm development. These insights provide a foundation for further studies exploring the potential applications of ascorbic acid in sericulture and insect nutrition.

REFERENCES

Aneesha, U., & Kumar, C. S. (2022). Foliar supplementation of ascorbic acid modulates biochemical performance of silkworm, Bombyx mori under thermal stress. Journal of Thermal Biology, 104, 103184.

Pattnaik, A., & Pattnaik, S. (2017). During 5th Instar Development a Correlative Study on Changes in Concentration of Haemolymph Ascorbic Acid of Bivoltine Races of Bombyx Mori. L. Int. J. Scientific Res. Develop, 5, 77-81.

Nila, J. N., & Jones, R. S. (2021). Performance of some newly produced multivoltine strains of Bombyx mori (Linnaeus) for commercially vibrant qualitative and quantitative traits. International Journal of Tropical Insect Science, 41, 273-284.

Sivaprasad, V., Chandrakanth, N., & Moorthy, S. M. (2022). Genetics and Genomics of Bombyx mori L. In Genetic Methods and Tools for Managing Crop Pests (pp. 127-209). Singapore: Springer Nature Singapore.

Ashraf, H., & Qamar, A. (2023). A review on heat stress altering the insect life history strategies and underlying mechanisms: Special reference to an economically important Lepidoptera, Bombyx mori (Linnaeus, 1758) (Lepidoptera: Bombycidae). SHILAP Revista de lepidopterología, 51(202), 339-357.

Soliman, A. M., & Gad, A. A. (2020). The Impact of Ascorbic Acid, Some Nanomaterials and Their Mixtures on Some Biological and Physiological Parameters of the Mulberry Silkworm Bombyx mori L. Alexandria Science Exchange Journal, 41(July-September), 393-398.

Pattnaik, A., & Pattnaik, S. (2017). During 5th Instar Development a Correlative Study on Changes in Concentration of Haemolymph Ascorbic Acid of Bivoltine Races of Bombyx Mori. L. Int. J. Scientific Res. Develop, 5, 77-81.

Soliman, A. M., & Gad, A. A. (2020). The Impact of Ascorbic Acid, Some Nanomaterials and Their Mixtures on Some Biological and Physiological Parameters of the Mulberry Silkworm Bombyx mori L. Alexandria Science Exchange Journal, 41(July-September), 393-398.

Cappellozza, L., Cappellozza, S., Saviane, A., & Sbrenna, G. (2005). Artificial diet rearing system for the silkworm Bombyx mori (Lepidoptera: Bombycidae): effect of vitamin C deprivation on larval growth and cocoon production. Applied Entomology and Zoology, 40(3), 405-412.

Borah, S. D., & Praban, B. (2020). A review of nutrition and its impact on silkworm. Journal of Entomology and Zoology Studies, 8(3), 1921-1925.

Altman, G. H., & Farrell, B. D. (2022). Sericulture as a sustainable agroindustry. Cleaner and Circular Bioeconomy, 2, 100011.

Hăbeanu, M., Gheorghe, A., & Mihalcea, T. (2023). Silkworm Bombyx mori—Sustainability and Economic Opportunity, Particularly for Romania. Agriculture, 13(6), 1209.

Gęgotek, A., & Skrzydlewska, E. (2022). Antioxidative and anti-Inflammatory activity of ascorbic acid. Antioxidants, 11(10), 1993.

Muhammed, H. A. W., & Ayoub, Z. N. (2023). Effect of Supplemental Feeding on The Weight of Silk Glands and Larval Development of Mulberry Silkworm (Bombyx Mori). Journal of Duhok University, 26(1), 183-189.