



Estimation of Available Protein Content in Different Tissues of Mulberry Silkworm Larva (*Bombyx mori* L.)

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Authors' contributions

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

Article Information

DOI: <https://doi.org/10.9734/jabb/2024/v27i71081>

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/118935>

Original Research Article

Received: 23/04/2024

Accepted: 27/06/2024

Published: 29/06/2024

ABSTRACT

Mulberry silkworm *Bombyx mori* L. is the only silkworm having monophagous feeding habit with mulberry as the sole food material. Larval body of the silkworm constitutes silk gland as one of the main body organs that accounts for 60% of the body weight during 5th instar and acts as a bioreactor that converts mulberry leaf protein into silk protein composed of sericin and fibroin. The biochemical analysis for detecting total available protein content in different body tissues of the silkworm larvae like silk gland, hemolymph and whole larval body of 5th instar larvae was analyzed. The results showed values varying considerably from one another as 69.2 and 76.5% in silk gland, 51.9 and 47.6% in hemolymph and 50% in whole larval body for the studied hybrid samples namely FC1xFC2 and FC2xFC1. In case of silkworm hybrid FC1xFC2, the studied larval body sample

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Cite as: Chanotra, Suraksha, Avleen Kour, Taahir Shafiq Nazar, and Gurvinder Raj Verma. 2024. "Estimation of Available Protein Content in Different Tissues of Mulberry Silkworm Larva (*Bombyx Mori* L.)". *Journal of Advances in Biology & Biotechnology* 27 (7):1212-19. <https://doi.org/10.9734/jabb/2024/v27i71081>.

revealed the optical density (OD) value in the range of 260 nm, 270 nm and 180 nm for larval body, hemolymph and silk gland sample respectively depicting the presence of fairly pure proteins in sufficient amount in the studied sample. In case of silkworm hybrid FC2xFC1, the studied larval body sample revealed the OD value in the range of 197 nm, 124 nm and 199 nm for larval body, hemolymph and silk gland depicting the presence of fairly pure proteins. The current observations on biochemical profiling could be utilized for future breeding prospects for development of region and season specific hybrids with desirable metric traits.

Keywords: Silkworm; silk; biochemical; hemolymph; protein; cocoon; hybrid.

1. INTRODUCTION

Sericulture is one of the most important economic sectors of the agriculture field. It involves a continuous series of integral activities including moriculture (cultivation of host plant i.e. Mulberry), rearing of lepidopteron larvae i.e. silkworm *Bombyx mori* L. and various industrial aspects (manufacturing of fabric from fibres). Silkworm being monophagous insect feeds only on mulberry leaf because of the presence of morin pigment in it [1]. Under different environment, feeding and nutritional conditions and with ingestion of the same number of mulberry leaves, the silkworm shows significant difference in its ability to digest, absorb and convert food to body matter. Hence, influence of season, temperature, humidity on food intake, assimilation and conversion efficiency of the *Bombyx mori* is significant [2]. On the other hand, environmental factors are also responsible for regulation of physiology and metabolism of silkworm [3,4]. This in turn triggers the synthesis and accumulation of two invariably important proteins namely sericin and fibroin making a single unit of silk fibrils known as silk protein [5].

Silk protein is the ultimate product forming the protective covering around the larvae, known as cocoon which provides shelter to the

metamorphosing pupa and the same cocoon is later reeled in industrial processes to extract silk. The quantity and quality of silk content present in the cocoon shell decides the market value of silk. Silk gland which is predominantly divided into fore, mid and posterior silk gland actually hosts the site of silk synthesis which is the posterior part of the silk gland. Whereas, middle region is the region of storage and accumulation and anterior part is for exclusion through which larvae expels out silk from spinneret and form silk filament that solidified after coming in contact with air [6-9]. Therefore, in order to analyse the content of silk protein present in different tissues of silkworm body an attempt has been made to estimate the available protein content in silkworm silk gland, hemolymph and whole larval body.

2. MATERIALS AND METHODS

The current experiment was carried out by using ruling hybrids of Jammu and Kashmir namely; FC1 x FC2 and its reciprocal cross i.e. FC2 x FC1. For the study different tissues of silkworm larval body were utilized including silk gland, hemolymph and whole larval body of 5th instar larvae for estimation of available protein content Protein content (%) by using slightly modified Lowery et al., [10] method.

2.1 Protocol

0.2 ml of BSA working standard in 5 test tubes and make up to 1ml using distilled water.

↓
The test tube with 1ml distilled water serves as blank.

↓
Add 4.5ml of reagent I and incubate for 10 minutes.

↓
After incubation add 0.5 ml of reagent II and incubate for 30 minutes.

↓
Measures the absorbance at 660 nm and plot the standard graph.

↓
Estimate the amount of protein present in the given sample from the standard graph.

In addition to protein content, following cocoon characters were also studied during the experiment;

1. Green cocoon weight.
2. Cocoon with floss.
3. Deflossed cocoon.
4. Dry cocoon weight.
5. Shell weight.
6. Pupal weight.
7. Cocoon shell ratio.
8. Cocoon grains.
9. Cocoon compactness.
10. Cocoon size.
11. Pupal percentage.

2.2 Statistical Analysis

The raw generated from the current experiment by CRD design have been pooled and subjected to Analysis of Variance (ANOVA) on SPSS software Version; 2021, to determine the significant values for the selected parameters.

3. RESULTS

Protein content (%):

For FC1 x FC2 silkworm hybrid:

- a) **Whole Larval Body Sample:** Total protein content (%) in silkworm body of 5th instar larvae was detected on photo-calorimeter at an optical density (OD) value of 660nm. The studied larval body sample revealed the OD value in the range of 180nm which depicted the presence of fairly pure proteins in sufficient amount in the studied sample (i.e., 50% sericin and 50% fibroin), with the noticeable increase in the total protein content with every subsequent day.
- b) **Hemolymph:** Total protein content percentage in silkworm hemolymph (5th instar larvae) was detected on photo-calorimeter at an optical density (OD) value of 660 nm. The studied hemolymph sample revealed the OD value in the range of 260nm which depicted the presence of fairly pure proteins in sufficient amount in the studied sample i.e., 51.9% of protein

content in silkworm hemolymph, with considerable increase after each day.

- c) **Silk gland:** Total protein content percentage in Silk gland of the silkworm (5th instar larvae) was determined on photo-calorimeter at an optical density (OD) value of 660nm. The studied silk gland sample revealed the OD value in the range of 270nm which depicted the presence of fairly pure proteins in sufficient amount in the studied sample i.e.89.2% of protein content in silk gland of silkworm. Similar to whole larval body and hemolymph a remarkable increase had been noticed with each pairing during in silk gland also.

For FC2 x FC1 silkworm hybrid:

- a) **Whole Larval Body Sample:** The studied larval body sample revealed the OD value in the range of 197nm which depicted the presence of fairly pure proteins in sufficient amount in the studied sample (i.e., 75.7 %protein content in silkworm larvae). Maximum OD value was recorded in silkworm larvae of 5th day.
- b) **Hemolymph:** Total protein content percentage in silkworm hemolymph (5th instar larvae) was detected on photo-calorimeter at an optical density (OD) value of 660nm. The studied hemolymph sample revealed the OD value in the range of 154nm which depicted the presence of fairly pure proteins in sufficient amount in the studied sample i.e., 57.6 percent of protein content in silkworm hemolymph.
- c) **Silk gland:** Total protein content percentage in Silk gland of the silkworm (5th instar larvae) was determined on photo-calorimeter at an optical density (OD) value of 660 nm. The studied silk gland sample revealed the OD value of 199nm which depicted the presence of fairly pure proteins in sufficient amount in the studied sample i.e. 76.5% protein content in silk gland of silkworm. Maximum and minimum values are recorded in case of 5th and 1st day of larval sample respectively (Table 1).



Plate 1. Biochemical profiling of Silkworm larvae

Table 1. Values depicting percentage of protein content on the basis of OD values in studied samples of whole larval body, hemolymph and silk gland of silkworm hybrids viz., FC1 x FC2 and FC2 x FC1

Particulars	FC1 x FC2		FC2 x FC1	
	OD Value (nm)	%	OD Value (nm)	%
Whole larvae body	180 nm	50%	197 nm	75.7%
Hemolymph	260 nm	51.9%	154 nm	57.6%
Silk gland	270 nm	89.2%	199 nm	76.5%

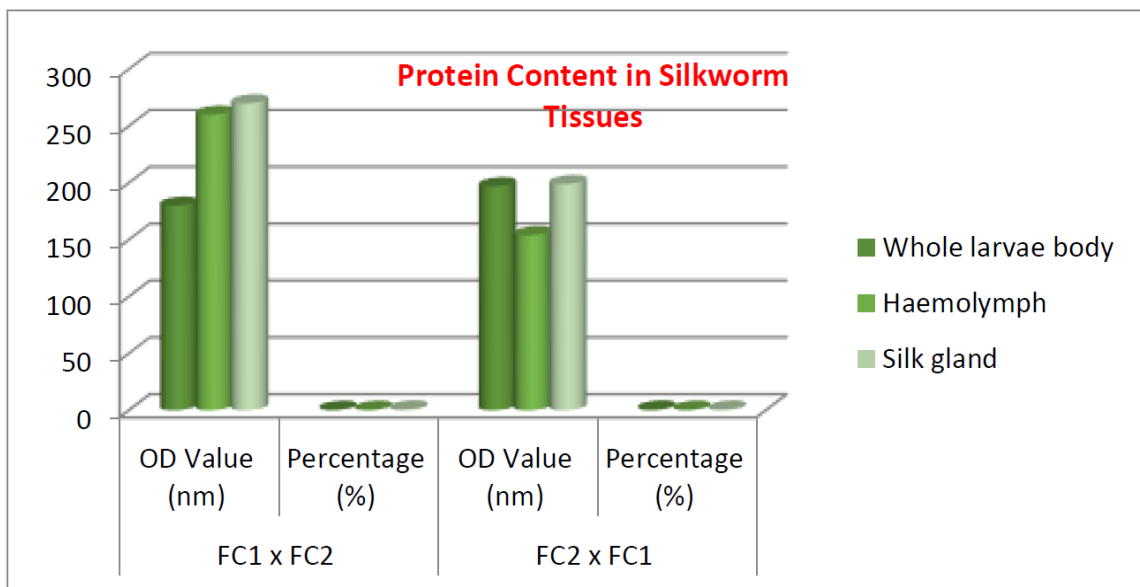


Fig. 1. Protein content in silkworm tissues

Some of the important morphological characters of cocoons recorded includes:

- a) **Green Cocoon Weight:** The cocoon harvested on 6th day of spinning (FC1 x FC2 and FC2 x FC1) were recorded to possess (1.46 gm, 1.30 gm) of single and (14.61 gm, 13.36 gm) for ten cocoons for green cocoon weight.
- b) **Cocoon with Floss:** The same cocoons harvested from FC1 x FC2 and FC2 x FC1 was determined to evaluate cocoon weight with floss and recorded to possess (1.38 gm, 1.19 gm) of single and (15.54 gm, 13.32 gm) for ten cocoons in case of FC1 x FC2 and FC2 x FC1 respectively.
- c) **Deflossed Cocoon:** The cocoons observed on the 6th day of spinning (FC1 x FC2 and FC2 x FC1) were subjected to deflossing by manual deflossing method with help of woollen stick and the studied hybrids were recorded to possess (1.40 gm, 1.08 gm) of single cocoon and (14.98 gm, 10.81 gm) of 10 cocoons in case of FC1 x FC2 and FC2 x FC1, respectively.
- d) **Dry cocoon Weight:** Dry weight of single cocoon was observed as (0.55 gm, 0.44 gm) and (5.94 gm, 4.44 gm) for 10 cocoons on the 6th day of spinning (FC1 x FC2 and FC2 x FC1).
- e) **Shell Weight:** Shell weight of the single cocoon and 10 cocoons was recorded as (0.78 gm, 0.46 gm) and (7.89 gm, 4.63 gm) on the 6th day spinning (FC1xFC2 and FC2xFC1), respectively.
- f) **Pupal Weight:** The cocoon harvested on 6th day of spinning (FC1xFC2 and FC2xFC1) were evaluated for determination of pupal weight and values depicting (1.55 gm, 1.27 gm) of pupal weight for single cocoon and (15.92 gm, 12.73 gm) for ten cocoons, respectively.
- g) **Cocoon Shell Ratio:** The cocoon shell ratio was calculated by the formula = (Weight of the cocoon shell / Weight of cocoon) X 100

And the results showed higher cocoon shell ratio for the hybrid FC1xFC2 as (53.42%, 35.38%) as to that of FC2xFC1 as (54.41%, 36.41%).

- h) **Cocoon Grains:** The cocoon harvested on 6th day of spinning (FC1x FC2 and FC2xFC1) were observed to possess as deep and coarse grains, depicting the comparatively good quality of cocoons.
- i) **Cocoon Compactness:** The same cocoon was observed as hard and compact in

texture showing the superior quality of the cocoons on the basis of visual examination descriptor.

- j) **Cocoon Size:** For both the hybrids the cocoons were recorded to possess oval shaped cocoon with slight constriction at the centre as shown in the plate.
- k) **Pupal Percentage:** Pupal percentage were determined after stifling process and the studied hybrid were recorded to possess high pupal percentage with values as (100%, 100%) and (100%, 90%) for FC1xFC2 and FC2xFC1 respectively.

4. DISCUSSION

The protein content was determined by the presence of fairly pure proteins in sufficient amount in the studied silkworm larval body sample in case of FC1xFC2 hybrid i.e., 50% sericin and 50% fibroin, with the noticeable increase in the total protein content with every subsequent day, 51.9% of protein content in silkworm hemolymph, with considerable increase after each day and 69.2% of protein content in silk gland of silkworm. Similar to whole larval body and hemolymph a remarkable increase had been noticed with each passing during in silk gland too. In case of FC2xFC1 hybrid, 75.7% protein content in silkworm larvae maximum OD value was recorded in silkworm larvae of 5th day, 47.6% of protein content in silkworm hemolymph and 76.5% protein content in silk gland of silkworm. Maximum and minimum values are recorded in case of 5th and 1st day of larval sample respectively which shows close conformity with that of the Devi and Chapman [11], Zhou et al., [12] and Ramchandra et al., [13] observed total proteins in silk gland decreased in early stages and increased in later stages of silkworm larval development. Mondal et al., [14] revealed that protein concentration was found to be in high concentrations in fully matured silk gland of the 5th instar larvae and Reddy et al., [15] who reported maximum weights and lengths of silk gland in 7th day of 5th instar of larval period. Subramaniam et al., [16], Sunderraj et al., [17], Sharma et al., [18] and Viswansath. S. [19], suggested increase in protein content in silk gland and hemolymph of the 5th instar silkworm larvae at an exponential rate thus confirming the results of present investigation.

Morphological characters of cocoon are predominant descriptors for assessment of breeds for distinctiveness, uniformity and stability; but are always influenced by prevailing environment. In present study, the morphological

characters were recorded wide phenotypic variation in cocoon colour, cocoon shape, cocoon shell weight, cocoon grains, cocoon shell ratio, pupal percentage and pupal weight. The observations recorded are in line with earlier workers, Rangeaswami and Govinda [20] who reported the cocoon shape as an extremely relevant variable in commercialization, since automated wiring admits only elliptical cocoons into the machines. However, the breeds evaluated in the present study were recorded with oval, elongated with fair constriction and coarser constricted presenting the superiority of the cocoons. Mallikarjunappa and Etebari [21] observed that cocoon weight, shell ratio and filament length are highly heritable traits determining the quality, quantity and efficiency of the reeling. Present findings are also in conformity with the report of Singh et al., [22] who concluded that environmental factors influence the physiology of the insect and also have deleterious effect on the economic traits. Cocoon shell weight is an important character in determining the silk content. In present study, highly significant and positive correlations were recorded between single cocoon weight and 5th day larva weight (0.864 g) followed by single shell weight and 5th day larval weight (0.816 g), single shell weight and single cocoon weight (0.775 g), malformed cocoon and 5th instar duration (0.730 g), single shell weight and pupation rate (0.624 g), total larval duration and 5th instar duration (0.559 g), single cocoon weight and hatching percentage (0.551 g), larval weight 5th day and hatching percentage (0.545 g), single cocoon weight and pupation rate (0.536 g), pupation rate and larval weight 5th day (0.524 g), single shell weight (0.492 g) and silk percent and single shell weight (0.470 g). These findings are in accordance with the results of Chakarabarty et al., [23], Murthy. Y.N.V. [24], Narayanan et al., [25] and Saratchandra et al., [26]. Whereas, Trivedy et al., [27] reported that the cocoon weight differs significantly when silkworms were fed with leaves harvested from different tree mulberry genotypes. Sharma et al., [28] and Sudo and Chunking [29] also reported highest values of single cocoon weight 1.28 ± 0.04 g when silkworms were fed with mulberry leave harvested from tall trees. The present results are conformity with the findings of Wang and Gilbert [30], who recorded the maximum cocoon weight as 1.74 ± 0.03 g and shell weight 0.39 ± 0.01 g when silkworms were grown on leaves harvested from tree type of mulberry plantation. The present results are on par with the results of earlier workers, Radhakrishna et al., [31] and

Rahmathulla and Suresh [32], recorded the maximum pupal weight 1.36 ± 0.02 g when silkworms were reared on quality mulberry leaves possessing highest mean cocoon shell ratio (17.57%). The results of the present study revealed conformity with the reports cited by Chakarabarty et al., [23], Fang et al., [33] and Kaviraj et al., [34] with the cocoon shell ratio and shell percentage as (53.42%, 35.38%) as to that of FC2xFC1 as (54.41%, 36.41%) and (0.78 gm, 0.46 gm) and (7.89 gm, 4.63 gm), FC1xFC2 and FC2xFC1, respectively. Therefore, current investigation revealed significant and satisfactory results with great validation on the basis of earlier reports of various workers.'

5. CONCLUSIONS

Silk gland in silkworm body accounts for 60% of the body weight during 5th instar and acts as a bioreactor that converts mulberry leaf protein into silk protein composed of sericin and fibroin. The biochemical analysis for detecting total available protein content in different body tissues of the silkworm larvae like silk gland, hemolymph and whole larval body of 5th instar larvae was analyzed and values recorded were found to vary considerably from one another as 69.2 and 76.5% in silk gland, 51.9 and 47.6% in hemolymph and 50% in whole larval body. In case of silkworm hybrid FC1xFC2, the studied larval body sample revealed the optical density (OD) value in the range of 260nm, 270nm and 180nm for larval body, hemolymph and silk gland sample respectively depicting the presence of fairly pure proteins in sufficient amount in the studied sample. In case of silkworm hybrid FC2xFC1, the studied larval body sample revealed the OD value in the range of 197nm, 124nm and 199nm for larval body, hemolymph and silk gland depicting the presence of fairly pure proteins. For estimation of total protein content in the form of cocoon, the weights were recorded for the single cocoon and shell weight. The maximum values of all the studied parameters were recorded as high in case of hybrid FC2xFC1 as compared to its reciprocal one. This short experiment could be utilized to extend further biochemical profiling of different breeds for quantification of all the biochemical and metric traits of silkworm.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image

generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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