

Estimation of available protein content in different tissues of mulberry silkworm larva (*Bombyx mori* L.)

Abstract:

Mulberry silkworm *Bombyx mori* L. is the only silkworm having monophagous feeding habit with mulberry as the sole food material. 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, haemolymph and whole larval body of 5th instar larvae was analysed 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 haemolymph 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, haemolymph 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, haemolymph 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 single cocoon weight was recorded as 1.46g for FC1xFC2 and 1.30g for FC2xFC1, cocoon weight with floss was recorded as 1.19g and 1.38g, deflossed cocoon as 1.40g and 1.08g, floss weight as 0.32g and 0.46g, pupal weight as 1.55g and 1.27g, cocoon shell weight as 0.78g and 2.46g, 100% pupal percentage, dry cocoon weight as 0.55g and 0.44g, cocoon shell ratio as 53.42% and 35.38% for FC1xFC2 and FC2 xFC1 respectively. 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.

Key words: silkworm, silk, biochemical, haemolymph, protein, cocoon, hybrid.

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 (Babu et al., 2009). 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 (Etebari & Matindoost 2004). On the other hand, environmental factors are also responsible for regulation of physiology and metabolism of silkworm (Kerenhap et al., 2005 and Ganie 2022). 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 (Chen et al., 2022).

Silk protein is the ultimate product forming the protective covering around the larvae, known as cocoon which provides shelter to the metamorphosing pupa and is 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. 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, haemolymph and whole larval body.

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, haemolymph and whole larval body of 5th instar larvae for estimation of available protein content Protein content (%) by using slightly modified Lowery *et al.*, (1951) method.

PROTOCOL:

1. 0.2 ml of BSA working standard in 5 test tubes and make up to 1ml using distilled water.
2. The test tube with 1ml distilled water serves as blank.
3. Add 4.5ml of reagent I and incubate for 10 minutes.
4. After incubation add 0.5 ml of reagent II and incubate for 30 minutes.
5. Measures the absorbance at 660 nm and plot the standard graph.
6. 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;

- a) Green cocoon weight.
- b) Cocoon with floss.
- c) Deflossed cocoon.
- d) Dry cocoon weight.
- e) Shell weight.
- f) Pupal weight.
- g) Cocoon shell ratio.
- h) Cocoon grains.
- i) Cocoon compactness.
- j) Cocoon size.
- k) Pupal percentage.

SATISTICAL 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.

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) Haemolymph:

Total protein content percentage in silkworm haemolymph (5th instar larvae) was detected on photo-calorimeter at an optical density (OD) value of 660 nm. The studied haemolymph 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 haemolymph, 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 haemolymph a remarkable increase had been noticed with each pairing during in silk gland also.

II. 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.

a) Haemolymph:

Total protein content percentage in silkworm haemolymph (5th instar larvae) was detected on photo-calorimeter at an optical density (OD) value of 660nm. The studied haemolymph 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 haemolymph.

a) 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 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-2).

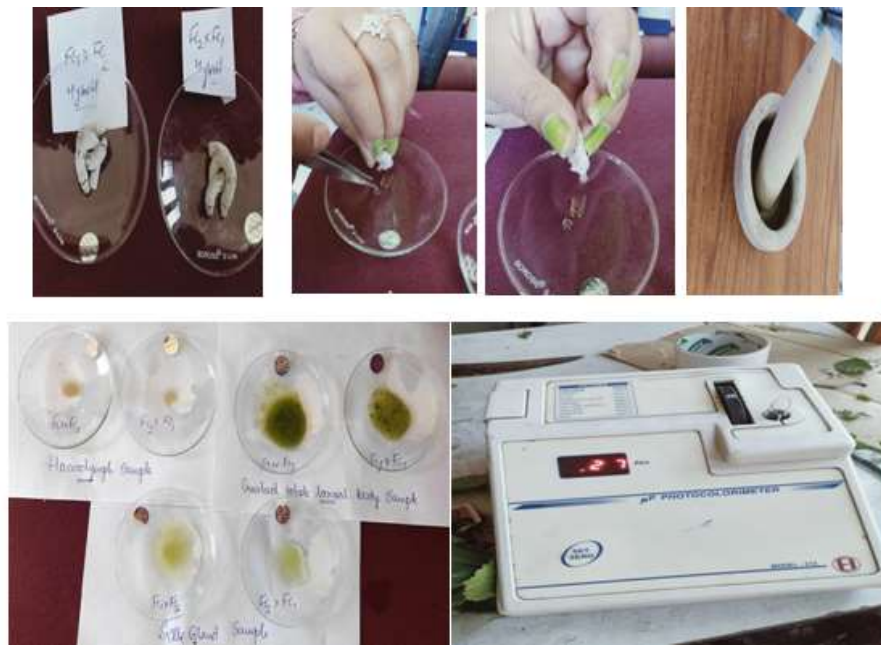


Plate-01: Biochemical profiling of Silkworm larvae.

Table-01: Values depicting Percentage of Protein content on the basis of OD values in studied samples of whole larval body, haemolymph 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	180nm	50%	197nm	75.7%
Haemolymph	260nm	51.9%	154nm	57.6%
Silk gland	270nm	89.2%	199nm	76.5%

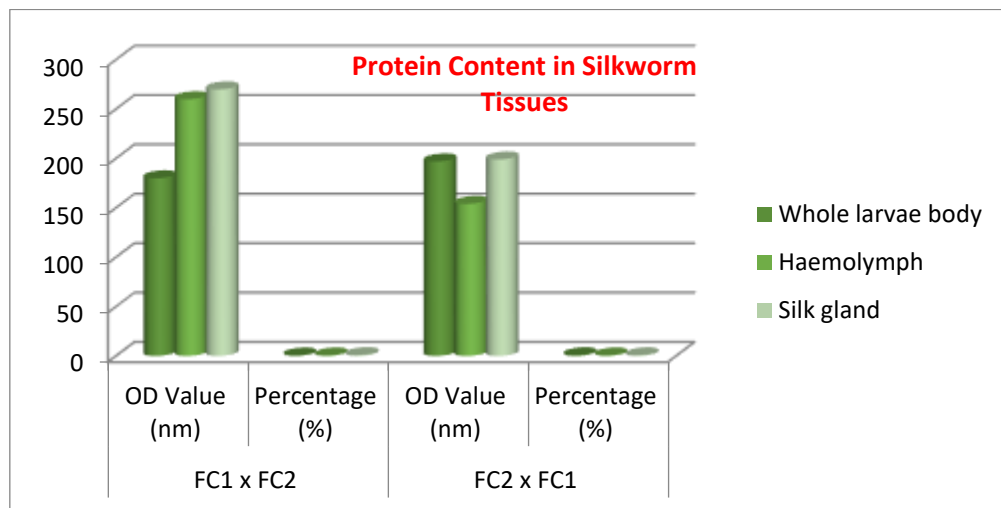


Fig-01: Protein content in silkworm tissues.

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 (1.46gm, 1.30 gm) of single and (14.61gm, 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 (1.38gm, 1.19gm) of single and (15.54gm, 13.32gm) 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 (1.40gm, 1.08gm) of single cocoon and (14.98gm, 10.81gm) 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 (0.55gm, 0.44gm) and (5.94gm, 4.44gm) 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 (0.78gm, 0.46gm) and (7.89gm, 4.63gm) 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 (1.55gm, 1.27 gm) of pupal weight for single cocoon and (15.92gm, 12.73gm) for ten cocoons, respectively.

g) Cocoon shell ratio

The cocoon shell ratio was calculated by the formula

$$= \frac{\text{Weight of the cocoon shell}}{\text{Weight of cocoon}} \times 100$$

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.

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 haemolymph, with considerable increase after each day and 69.2% of protein content in silk gland of silkworm. Similar to whole larval body and haemolymph 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 percent of protein content in silkworm haemolymph 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 (2013), Zhou *et al.*, (2015) and Ramchandra *et al.*, (2020) observed total proteins in silk gland decreased in early stages and increased in later stages of silkworm larval development. Mondal *et al.*, (2011) 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.*, (2015) who reported maximum weights and lengths of silk gland in 7th day of 5th instar of larval period. Subramaniam *et al.*, (2011), Sunderraj *et al.*, (2015), Sharma *et al.*, (2020) and Viswansath. S. (2018), 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 (2017) 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 (2019) 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.*, (2015) 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 (0.864g) followed by single shell weight and 5th day larval weight (0.816g), single shell weight and single cocoon weight (0.775g), malformed cocoon and 5th instar duration (0.730g), single shell weight and pupation rate (0.624g), total larval duration and 5th instar duration (0.559g), single cocoon weight and hatching percentage (0.551g), larval weight 5th day and hatching percentage (0.545g), single cocoon weight and pupation rate (0.536g), pupation rate and larval weight 5th day (0.524g), single shell weight (0.492g) and silk percent and single shell weight (0.470g). These findings are in accordance with the results of Chakaraborty *et al.*, (2020), Murthy. Y.N.V. (2015), Narayanan *et al.*, (2020) and Saratchandra *et al.*, (2011). Whereas, Trivedy *et al.*, (2020)

reported that the cocoon weight differs significantly when silkworms were fed with leaves harvested from different tree mulberry genotypes. Sharma *et al.*, (2017) and Sudo and Chunking (2016) also reported highest values of single cocoon weight 1.28 ± 0.04 g when silkworms were fed with mulberry leaf harvested from tall trees. The present results are conformity with the findings of Wang and Gilbert (2011), 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.*, (2017) and Rahmathulla and Suresh (2012), 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 Chakraborty *et al.*, (2020), Fang *et al.*, (2020) and Kaviraj *et al.*, (2021) with the cocoon shell ratio and shell percentage as (53.42%, 35.38%) as to that of FC2xFC1 as (54.41%, 36.41%) and (0.78gm, 0.46gm) and (7.89gm, 4.63gm), FC1xFC2 and FC2xFC1, respectively. Therefore, current investigation revealed significant and satisfactory results with great validation on the basis of earlier reports of various workers.

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, haemolymph and whole larval body of 5th instar larvae was analysed 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 haemolymph 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, haemolymph 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, haemolymph 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.

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