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Quantitative profile Analysis of Mulberry Silkworm *Bombyx mori. L* (CSR_2XCSR_4)

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ABSTRACT

Bombyx mori. L. (CSR_2XCSR_4) is a bivolitne crossbreed that produces high quantity of silk. The weights of worms and glands are directly related to the yield of silk, higher larval weights leads to higher silk production. In the present study, quantitative parameters of 5^{th} instar and pupal stages of silkworm larvae were observed. The analysis of quantitative estimation showed that the maximum weights and lengths of silk gland and silk worm were recorded on 7^{th} day of 5^{th} instar larval stage. The day dependent variation in protein concentration was observed in total silk gland 5^{th} instar larval and pupal stages.

Keywords: *Bombyx mori*; Growth parameters; silk worm; Quantitative

1. INTRODUCTION

Silk worm is the larvae of insects, which belongs to the order *Lepidoptera* experiencing complete metamorphosis. The mulberry silk worm *Bombyx mori L* (*Lepidoptera: Bombycidae*) is a monophagous insect that feeds exclusively on mulberry foliage for its nutrition and produces the naturally protienous silk [1]. Silkworms go through four stages of development- eggs, larva, pupa and adult. The silk worm larval life cycle is divided into five instars, separated by four molts. Four distinct stages of development completes one generation of the species. The total silk worm life cycle is completed in 56-62 days.

Among all the five instars first three instars are young larva, fourth and fifth instars larvae are grown larvae. Young and grown larvae of silk worms behave differently according to environmental conditions and type of feeding (leaf quality and quantity). Concerning leaf feeding, both quality and quantity are important and different for each instar [2].The silk gland is an organ specialized for the synthesis and secretion of silk proteins [3]. It is the major storage organ of the silk. This silk gland represents one of the most active proteins synthesizing system among the entire organ. Silk glands constitute approximately one quarter of the worms mass and produce liquid silk. This is composed of complex proteins [4]. Specialized cells present at the posterior end of the silk glands devote 85% of their protein synthesis activity to silk production.

Silk fibroin a major protein component of cocoon spun by silk worm, has diverse applications in the biomedical field which can be attributed to its high tensile strength controllable biodegradability, haemostatic properties, non-cytotoxicity, low antigenicity and inflammatory characteristics [5,6,7,8] The gland of silk worm produce an almost pure product and purification of recombinant protein from cocoon seems to be a rather simple process despite of silk fibroin synthesis being maintained in the insect [4]. Several studies indicate that different proteins affect various stages of the insects including the diapause [9,10].

Silk industry is a domestic based industry uniquely suited to the economy and social structure of developing countries because of its minimum investment requirements, high employment and foreign exchange earning potential. It is a holometabolous insect and produces silk of high commercial importance [3]. *Bombyx mori* could become a major source for the production of high value proteins especially in the area of pharmaceutics [4].

2. MATERIALS AND METHODS

2.1. Rearing of silk worm

The eggs of *Bombyx mori* (CSR_2XCSR_4) were obtained from government sericulture center, Vijayawada, Andhra Pradesh, India. The silk worms were reared under standard conditions of 26±2˚c with a relative humidity (RH) 80-85%. These worms are fed with chopped tender and succulent mulberry leaves with high moisture content.

2.2. Quantitative profile analysis

The Quantitative profile analysis of fifth instar larval silk worms was studied by weighing and measuring the worms during 1-11 days of fifth instar and cocoon stages using digital weighing balance and measuring scale. For this five larvae from every replication were randomly selected and their mass was recorded, from which the average larval weights and lengths were derived. Similarly the other physical parameters such as length and weight of the silk gland were studied.

2.3. Protein extraction from silk glands

The silk glands stored at -20˚C are taken and flash flours in liquid nitrogen. The glands were homogenated using motor and pistel. To the homogenate 1X SDS extraction buffer (0.125M tris-HCl (pH6.8), 2% sodium dodecyl sulphate, 10% glycerol, 0.001% bromophenol blue and 5% 2-mercaptoethanol) was added 10 times the net weight of the tissue [11,12]. The samples were then vortexed for one minute at room temperature and then boiled for 10 minutes at 100˚c. The samples were centrifuge at 5500 rpm for 8 mins at 4˚c and the solubilized content (supernatant) was transferred or collected into a labeled eppendorf tube and then the samples were stored at -20˚c.

2.4. Protein concentration determination

Among the several different assays for protein concentration like Absorbance at 280nm, Lowry assay, Bradford assay, Bicinchoninic assay (BCA), Pierce assay, measuring the protein concentration in an SDS extraction buffer requires that the assay is compatible with detergent and reducing agent in the solution. The protein concentration is determined by measuring the absorbance at 280nm in U.V spectrophotometer.

3. RESULTS AND DISCUSSION

3.1. Weights of larvae

The larval growth during the fifth instar showed difference on all days in their weights. The data (table-1) showed that there is logarithmical increase in the larval body weight until day 7 of fifth instar, and there is a sudden decrease in the body weight on day 8 of fifth instar. The maximum value of body weight was recorded on day 7 (2.97gm.) when compared to all the days in the fifth instar. Generally in the fifth instar, larvae increase in their body weight until they reach a stationary state (ready to spinning). In the beginning of fifth instar, larval body weight does not increase much and shows log phase. After the day 3 the larvae start feeding voraciously. The reason behind the excess intake of mulberry was that the silk worm has the ability to digest large amounts of mulberry to accumulate energy, which helps the larvae in its nonfeeding pupal and adult stages [13]. Even though larval body weight increases logarithmically, the % of larval body weight gained from day to day varies. The data (table-1) represents that maximum % of weight gained was observed on day 4 of fifth instar of larva. The percentage of weight gained showed random increase from day1 and showed a gradual decrease from 5^{th} day.

During the process larval spinning (cocoon) it loses weight by ejecting out the silk fiber. Hence there is a decrease in the percentage of weight gained on 8^{th} day (-27.2%). During fifth instar larval period of silk worm, the first four days feeding of the larvae helps in its development and growth and the later stages of feeding helps the larvae to accumulate energy for its utilization during its nonfeeding pupal and adult stages.

3.2. Larval lengths

The larvae during 5^{th} instar (Table-2) showed difference in their length during all the days of fifth instar. The data shows that there is increase in larval length from day 1 to day 7 of 5^{th} instar. The maximum larval length was observed on 7^{th} day (7.43 cm) and a sudden decrease in larval length was noted from day 8. The length of silk worm depends on amount of food it consumes. Length of larvae increases until larvae reaches its spinning state (7^{th} day). When it reaches spinning stage the larvae reduce in size to one third of its normal length, it is the characteristic feature of a silk worm. This reduction in size increases pressure on silk glands to eject silk from the glands. Hence there is a sudden decrease in length on 8^{th} day. This decrease in length continues until pupal stage. The pupa measures 1.33 cm in length.

When day wise % of variation in larval lengths is compared (table-2), the larvae showed random increase in their length variation percentage until 4^{th} day and showed gradual decrease thereafter. The sudden decrease in larval length is due to spinning. The maximum % of larval lengths is observed on 4^{th} day of fifth instar and maximum decreased % of larval lengths was observed on 11^{th} day of instar (pupa).

3.3. Silk gland weight

Data observed on silk gland weights (table-3) during fifth instar showed variation in weight among all days of fifth star. During the fifth instars the weight of silk gland showed logarithmical increase until it reached the spinning state and maximum silk gland weight was observed on 7^{th} day (10.3gm). Even though the growth of gland is triggered suddenly at the beginning of the 5th instar, the weight of gland increased very rapidly due to enlargement of individual cells [14] and various structural elements of cells. Specially, rough endoplasmic reticulum and ribosome are intensively produced only during the fifth instars, hence there is random increase in silk gland weight. The maximum synthesized fibroin is probably accumulated in silk gland hence silk gland shows the maximum weight before spinning (7th day) stage. A decrease in weight of silk gland was observed on 8th day as fibroin and sericine eject out from the silk gland.

Weight of the silk gland was low till day3 as, fibroin synthesis also low till the day3 of fifth instar (table-3). In the first three days of 5th instar larval period the percentage increase of silk gland weight was high probably due to the increase in RNA and lipid level [14].

3.4. Silk gland length

The length of silk gland during fifth instar of silk worm showed (table-4) variation in length among all the days of fifth instar. There is a gradual increase in the gland lengths from day 1 to day 7 and a gradual decline in their lengths from day7. The maximum length of silk gland was observed on day 7 (20.87cm). The development of silk gland during the fifth instar can be categorized into two stages first/ log stage (1-4 days) & second/stationary stage (4-7 days) [14]. In the stationary state gland development is more.

The percentage gain in length from day to day in silk glands showed there is a gradual increase till day 4 and a rapid decrease from day 4 to day 11 .The maximum weight gained percentage was observed on day 4 of fifth instar silk glands.

3.5. Protein estimation

The quantitative estimation of silk gland total proteins (table -5) showed a change in protein concentration among all the days of the 5th instar larva**.** The maximum protein concentration was observed on day 7 (28.99mg) and there is slight decrease in protein concentration on 4th day.

Though there is a change in the protein concentration of silk glands, the total concentration of proteins accumulated increase from the early stages of fifth instar [15]. The data represents that there is increase in protein concentration (table-5) from day 1 to day 7 and later a gradual decrease in protein concentration was observed till day 11. During fifth instar the protein concentration was less in feeding and mature larval stages of fifth instar this is because the silk gland requires more protein for the development and there is a remarkable increase in protein content [16] even though a slight variation was observed on day 4, day 9, day 12.

The maximum protein concentration was observed on day 7 (28.99mg). Increase in protein concentration could be due to specific proteins which are required for the cocoon formation. The slight decrease in protein concentration on day 4 is due to active fibroin synthesis initiation on day 4 and a slight increase on 9th and 12th day was may be due to expression of specific proteins required for the formation of pupal and adult moths. There is continuous increase in total silk gland protein content during fifth instar due to the continuous synthesis and storage of fibroin [14] and hydrophilic protein sericine in middle silk gland *of Bombyx mori* larvae [17].

From the data observed the percentage variation in total protein content (table-5) showed a maximum gain in protein percentage on day 2 probably due to the active protein synthesis in posterior silk glands on 2nd day. And similarly, the maximum gain in protein observed on day 5 may be because of the active fibroin synthesis which starts two days before spinning.

Most of the studies have been limited to one or two days protein quantification of fifth instar larval silk glands, complete comparative and quantitative of fifth instar silk glands were analyzed in the present study. The proteins are the key actors within the cell, known to carry out the studies specified by the information encoded in genes [15], therefore proper changes in the protein profile in different growth stages of the silk worm can be studied. The current analysis contributes to our understanding of the quantitative parameters in different days of fifth in star larvae *Bombyx mori* L.

Table 1. Weights of the larvae.

Days	Weights of silkworms(gm)	% of silk worm weight gained for day to day(gm)
1	0.90±0.02	+00
2	1.10±0.08	+22.2
3	1.45±0.10	+31.8
4	2.05±0.09	+41.3
5	2.46±0.07	+20
6	2.74±0.13	+11.3
7	2.97±0.04	+8.3
8	2.16±0.17	-27.2

Comparison of larval body weights and % of silk worm weight gained during the 5th instar larval and pupal stages of silk worm (1-8 days).

Table 2. Lengths of the larvae/pupal.

Days	Lengths of larvae(cm)	% 0f variation in lengths of lengths of 5th instar larvae(cm)
1	4.07±0.15	+00
2	4.47±0.09	+9.8
3	5.00±0.25	+11.8
4	5.83±0.12	+16.6
5	6.43±0.19	+10.2
6	6.87±0.15	+6.8
7	7.43±0.09	+8.1
8	4.10±0.06	-44.8
9	3.40±0.06	-17
10	3.23±0.12	-5
11	1.33±0.03	-58.8

Comparison of silk worm lengths and % gained on silk worm lengths during the 5th instar larval and pupal stages of silk worm (1-11 days).

Table 3. Weights of larval /pupal silk glands.

Days	Silk gland weights(gm)	%of silk gland weight gained for day to day(gm)
1	0.06 ±0.01	+00
2	0.10 ±0.03	+66.6
3	0.20 ± 0.01	+100
4	0.30 ±0.01	+50
5	0.48 ±0.03	+60
6	0.84 ±0.06	+75
7	1.03 ±0.06	+22.6
8	0.76 ±0.05	-26.2
9	0.35 ±0.01	-53.9
10	0.12 ±0.04	-65.7

Silk gland weights and % gain of silk gland weight gained during the 5th instar larval and pupal stages of silk worm (1-10 days).

Table 4. Lengths Larval/pupal silk glands.

Days	Lengths of silkglands(cm)	% of variation of lengths of silk glands of 5th instar larvae and pupal stages(cm)
1	7.27 ±0.18	+00
2	8.67 ± 0.29	+19.2
3	11.63 ±0.44	+34.1
4	16.63 ±0.26	+42.9
5	19.23 ±0.24	+15.6
6	20.10 ±0.23	+4.5
7	20.87 ±0.52	+3.8
8	18.50 ±0.41	-11.3
9	16.37 ±0.21	-11.5
10	15.20 ±0.12	-7.1
11	7.97 ± 0.29	-47.5

Silk gland lengths during the 5th instar larval and pupal stages of silk worm(1-11 days).larval silk gland lengths and % gained of silk gland lengths (1-11days)

Table 5. Protein concentration of silk glands.

Days	Total protein concentration(mg)	% gain in total protein content (mg)
1	5.67 ±0.87	+00
2	14.39 ±0.11	+153.7
3	16.03 ±0.43	+11.3
4	12.02 ±0.62	-25
5	21.81 ±0.89	+81.4
6	26.96 ±2.27	+23.6

7	28.99 ±1.12	+7.5
8	21.9 ± 0.91	-24.4
9	23.08 ±3.25	+5.3
10	18.96 ±0.37	-17.8
11	12.7 ±0.9	-33.0
12	14.29 ±0.85	+12.5

Total silk gland protein concentration and % gain of total protein conten during the 5th instar and pupal stages of silk glands from day 1 to day 12 (1-12days) .

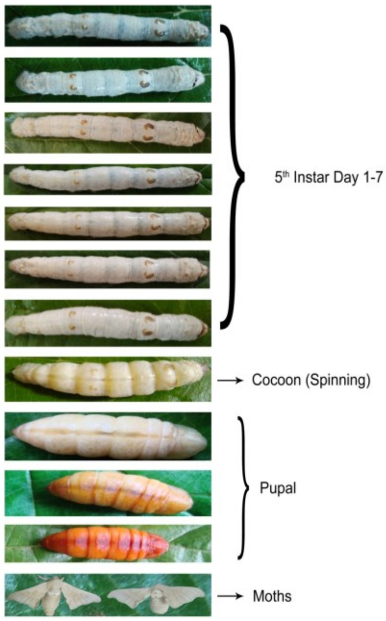

Fig. 1. The different developmental stages of silk worm from larval to adult.

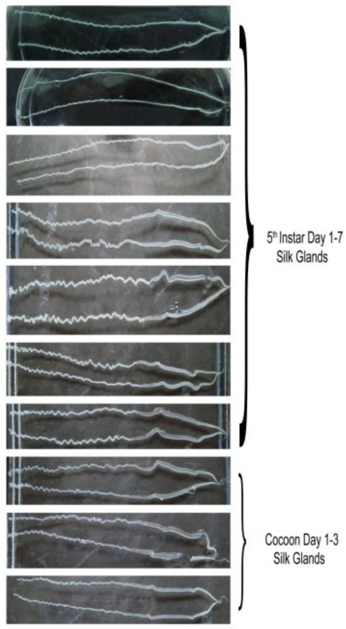

Fig. 2. Silk gland of silk worm larvae of 5th instar (1-7 days) and cocoon (1-3 days).

4. CONCLUSION

B. mori has been used for domestication because of the economic value of silk. It is also a good model system because it is large, easy to rear, low cost, is not involved in ethical issues and does not have any biohazard danger. A successful cocoon crop in sericulture depends mostly on healthy larval growth. Quantitative parameters of fifth instar larval protein concentrations of the total silk gland, weights and lengths showed day to day variations. The present work can be used as standards for further experimental works/designs and can also be used for comparative study.

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