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Haemolymph protein estimation in six different strains of Eri silk worm, *Samia ricini* (Donovan)

Priyanki Sharma and Dr. Jogen Chandra Kalita

Abstract
The present study was conducted to evaluate the haemolymph protein in six different Eri silk worm strains in their 5th instar- in the middle of the instar and at ripening or just before pupation. The result showed that in all the strains the concentration of protein was found highest at ripening when the worm is fully matured. Significant differences were found in protein level in two different study period of fifth instar larva in all six strains. Among all the strains Yellow Spotted (YS) was reported to have highest haemolymph protein concentration followed by Yellow Plain (YP). The protein concentration in ascending order was –GBZ<GBS<GBP<YZ<YP<YS. From this study YS strain was found to have highest protein concentration therefore it may be expected to produce larger cocoon with greater shell ratio and produce more silk. The statistical analysis (t-test) at 5% significance level showed that the differences in protein concentration among the strains were not very significant at ripening, but significant variation was reported between Greenish variety and Yellow variety. Three yellow strains were found to have more protein than the greenish strains in both study period and therefore yellow can be considered better strain in respect to biochemical characterization. Evaluation of variation among these strain may prove to be helpful in breeding programs, to carry out hybridization among better varieties to produce best strain with good cocoon quality.

Keywords: Silk worm, Haemolymph, strains, cocoon, hybridization

1. Introduction
North-East region is endowed with large numbers of sericogenous insects. A total of 47 species of silkworms are recorded from India, out of which 24 reported from North East region [1]. Out of which Four silkwoms are reared commercially in this part of India, they are Muga, Eri, Pat ad Tasar silk and these four silk threads have tremendous demands among these people. The Eri silkworms of the genus *Samia* Hubner contain 19 valid full species extending from tropical to temperate Eastern Asia [2].Of these, three species of genus *Samia* are-*Samia canningi* (Hutton, 1859), *Samia ricini* (Donovan,1798), *Samia fulva* (joran, 1911). *Samia ricini* is a domesticated eri silkworm species dwelling on the leaves of Castor in north eastern India and in many parts of India. This silkworm is polyphagous in nature feeding on wide range of host plants which are abundantly found in natural forests of plains and hilly terrain of the region. This domesticated form does not occur in the wild. Due to domestication many physical and behavioural changes occur between the *samia ricini* and its wild species. The *Samia ricini* species can be reared in indoor. Twenty six eco-races of *samia ricini* have been identified till date. Six strains of *Samia ricini* have been identified-they are *Yellow plain (YP), Yellow spotted (YS), Yellow Zebra (YZ) and Greenish blue plain (GBP), Greenish blue spotted* (GBS), *Greenish blue zebra (GBZ)* [3].Very few works have been done on the diversity of these six strains on the basis of morphology. Studies revealed a degree of morphological differences and also show genetic diversity among these strains. In biochemical level, variation may think to be existing which can be evaluated through analysis of the Haemolymph. Haemolymph, the body fluid of insects circulates freely around the various tissues in the haemocoel of Lepidopteron insects including in the silkworms. It consists of fluid plasma in which haemocytes are suspended. The plasma contains organic and inorganic constituents. The haemolymph plasma contains almost all inorganic constituents like electrolytes or ions, phosphates anions, organic constituents like free amino acids, proteins, lipids, carbohydrate, uric acids [4]. The chemical composition of haemolymph is highly variable among species and at different developmental stage of the same species [5]. Haemolymph, the only extracellular fluid in insects has diverse functions such as transport, growth, metabolism etc. The process of silk production are sequential and interlinked process as food they take is converted into

protein during the course of development and protein into silk fibre**.** During this process the 5th instars haemolymph protein contributes towards silk protein biosynthesis in the silk gland and the final products of silk proteins are fibroin and sericin and form the main components of silk fibre from the total silk produced [6] Protein are the key factors within the cells which is governed by the genes and is evident from the biochemical changes reflected in the Larva [7] Proteins are important biological macromolecules that are required for growth and development of the silkworm and for the synthesis of silk [8]. Silk worm synthesizes silk threads in the form of cocoons are rich in various proteins. At the end of the fifth instar larval stage, the silkworm larvae by to and fro movement of their head produce fibres which finally covers the larva and the larva becomes pupa. Therefore silk fibre synthesis is completely dependent upon the protein content of the larval body. During larval development various proteins are synthesized in large quantities in different stages of development. Synthesis of such proteins is dependent on the nutritional status of the silkworm during the larval development. **[9]** Cocoon is produced by the silk gland present throughout their length of the body and the growth and development of silk gland depends on the healthy silkworm and nutritional status [10]. Therefore the nutritional content of the haemolymph is important for healthy cocoon production and cocoon for Silk production. Insect silk is secreted as a semi crystalline protein. In insect silk alanine, glyine and serine are very common which are the simplest of all other amin acids. The growth and development of muga silkworm and their economic characteristics of cocoon and silk are influenced to a great extent by the nutritional composition of the leaves of their host plants [11]. The survival, growth, development and also the cocoon characteristics of silkworm are greatly influenced by the nutritional content of the food plants [12]. Hamolymph serves as reservoir for nutrients and metabolites during larval growth of insects. The silk is secreted by the silk glands and possess sericin and fibroin proteins. The proteins found in haemolymph act as a storage protein for the synthesis of the silk fibres. The concentration of protein in the haemolymph shows wide interspecific variation among brachyuan crab [13]. Significant variation is found in haemolymph protein content of 5th instar larval stage among eighteen breeds and mutant stock of *Bombyx mori* [14]. Protein quantification reveals that the haemolymph protein concentration increases gradually with the advancement of the larval period from 3rd -5thinstar I muga silkworm grown in som and soalu for both monsoon and autumn [15]. Haemolymph is the store house of many transitory components including proteins, lipids and carbohydrates. Proteins are the important components of silkworm as protein synthesize cocoons from which silk fibres are made. Therefore, the silk fibre is completely dependent on its protein content. During larval development various proteins are synthesized in large amount and the quantity of them varies in different stages also a may vary with different species. Realizing the importance of haemolymph and its nutrient components specially proteins in building cocoon and its fibres this present study was designed to compare the protein concentration among all the six strains of eri silk worm, *Samia ricini*.

2. Materials and Methods

Disease free layings of *Samia ricini* were collected from State Sericulture board Assam. Khanapara. The study was carried out in the department of Zoology at Nowgong College during spring season in the year 2016. The larvae of six strains of *Samia ricini* YP, YS, YZ, GBP, GBS, GBZ were reared separately for several generations with immense care to avoid intermixing of these strains in different trays and protein concentration was measured spectrophotometrically. The six strains named as-

1. Yellow Plain as YP
2. Yellow spotted as YS
3. Yellow zebra as YZ
4. Greenish blue Plain as GBP
5. Greenish blue spotted as GBS
6. Greenish Blue zebra or GBZ

The larvae in the middle of the 5th instar collected from each strain Haemolymph was obtained in the middle of 5th instar and during pre-pupation stage, 5th day after 4th moutling was considered and the haemolymph was collected after 24 hours of feeding. Haemolymph was collected from each strains in a pre chilled test tube by piercing one of the prolegs with a pin gently squeezing the body. 50µl haemolyph was collected in chilled eppendorf tubes containing 1ml of 10% trichloroacetic acid and.95 ml of distilled water to make a diluted volume of 2ml. The protein was precipitated out by centrifugation for 10 minutes at 3000-4000rpm. The precipitate was then treated with pure ethanol and then ethanol and ether (3:1) to remove fat. The precipitate of protein was then dissolved in 1mlof 0.1 N NaoH and Kept in the hot water bath for overnight. Protein estimation was done in by Standard method [16] and using crystalline Bovine Serum Albumin (BSA) as standard and the values were expressed in terms of mg/ml of haemolymph.

3. Results

The levels of protein in the haemolymph were found variable among different strains but the protein concentration were found significantly different in different stages of 5th instar larvae. The protein concentration showed that at ripening larvae had more protein than in the mid of the 5th instar. The protein levels in larval haemolymph in the 5th instar larvae at mid time and at ripening period showed significant variation. Results showed increase in protein concentration in ripening period just prior to pupation.

Table: Data represented as mg/ml. in mean ±SD.

SL. No.	Strains	At Mid Instar	At Ripening
1	GBP	22.6± 1.6	48.60±2.47
h2	GBS	20.8± 1.25	45±1.0
3	GBZ	20.2± 4.1	42.8±1.00
4	YP	26.2± 0.8	48.84±0.95
5	YS	27±2.0	49.62± 0.89
6	YZ	24± 1	47.44±0.89
	Mean	23.47	47.05

Above results showed that at ripening protein concentration increases to its maximum level in each strain. The YS strain larvae showed highest protein concentration in both mid instar and at ripening. Protein concentration in six strains in ascending order-

GBZ<GBS<GBP<YZ<YP< (at mid instar)

GBZ<GBS<YZ<GBP<YP<YS (at ripening)

The statistical analysis showed that there was significant difference in protein concentration between mid-instar and ripening. The difference between them was significant at 5 % level of significance. The t value for this two variables- mid period and ripening showed t=42.995. That is the calculated t value was found greater than the tabulated t value (t_c=2.57) at

5 % significant level. $t_{cal} > t_{c\ (critical\ value).}$ This indicates significant differences between these two variables.

The comparison between greenish and yellow strains at mid period of 5th instar showed that t calculated value was greater than tabulated t value at 5 % level of significance. $t_{cal} > t_{c(.05).}$, $t_{5.42} > t_{4.3.}$ That is the differences between these two variety was found significant. But at ripening there was no significant difference between different strains in protein concentration because the t value was found less than the critical value of t. $t_{cal} < t_{c\ at.05.}$ at 5% significant level.

4. Discussion

In the present study variations were found in protein concentration among different strains. The result showed that during 5th instar when the larvae prepare for cocooning the protein level increases to its highest concentration which may be due to requirement of protein for synthesis of silk fibre for cocoon as well as for pupation as pupa is also regarded a good source of protein. Previous study showed that the protein concentration increases gradually and reaches the maximum level at the ripening period during which larva prepares itself for cocooning [17]. The studies showed that increase in concentration of protein is considered to be related with the larval growth because during growth particularly at metamorphosis an extensive synthesis of protein is known to take place. The present study also shows rise in protein concentration from mid instar of 5th instar larva to ripening. There was highly significant difference between the protein concentration in the middle period and at ripening. The result is supported by the earlier works. From this it can be inferred that the protein synthesis is most active during spinning. The comparison on protein concentration among six different strains showed that the YS strain is reported to have highest protein concentration in both mid instar and at ripening and therefore this strain may be expected to produce larger cocoon with more fibre. Study reported YS and YP as better strain in term of cocoon and shell weight [18]. The present study showed that of protein concentration YS was greater than YP. But there is no significant differences in protein concentration among the six strains during ripening which might be due to their similar food and conversion efficiency as they belong to the same species. The results indicated the correlation between protein concentration and cocoon and Shell weight as haemolymph protein may influence the cocoon character of silkworm. This study reveals YS as better strain in protein concentration as protein is the main constituent of silk fibre. The statistical analysis showed a significant variation.

5. Conclusion

The present study revealed diversity among different silkworm strains of *Samia ricini*. Besides having morphological differences as reported by different authors in their studies, the differences were also found in their biochemical parameter. This present study showed variation in their haemolymph protein concentration. This study also revealed that larval haemolymph reached peak in the ripening stage of 5th instar larva. Therefore this stage of larval development can be considered as crucial for cocoon production. This parameter can be considered very useful for selecting and rearing better strain for commercial rearing.

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7. References

1. Singh KC, Suryanarayan N. Wild silk moth wealth of India, 2005, 419.
2. Peigler RA, Naumann S. Revision of the silk moth genus *Samia*. University of Incarnate Word, *San Antonio, Texas*, 2003, 1-242.
3. Debraj Y, Sarmah MC. Field trail of elite cromsses of eri silkworm, philosamia ricini, Hutt, Indian Silk. 2001; 40(2):15-16.
4. Malik MA, Malik FA. ontogenic changes in haemolymph biochemical composition in the silkworm, /Bombyx mori L,under thermal stress. Academic journal of Entomology. 2009; 2(1):16-21. ISSN 1995-8994.
5. Florkin M, Jeuniax C. Haemolymph composition. The physiology of insecta. (Ed.M.Rockstein). Academic Press, London, 1974.
6. Shivkumar, Subramanya G. Quanititative estimation of Haemolymph protein during different days of 5th intar larvae in Bivoltiness, multivoltines and mutants of the Bombyx mori.2015 Global journal of Bioscience and Biotechnology. 2015; 4(3):239-241.
7. Poonia FS. Consumption, digestion and utilization of castor leaves by larvae of eri silk worm, Philosamia ricini. Ind. Journal of Entomology. 1985; 47(3):253-267.
8. Talukdar K, Rajkhowa RC, Sarma S, Kalita JC, Rahman A. Quantification and electrophoretic profile of haemolymph proteins of muga silkworm (Antheraea assamnsis Ww) larvae reared on two major host plants(Litsea monopetala and Persea Bombycina) for two different crops(season). Jornal of entomology and Zoology studies. 2015; 3(4):473-475.
9. Chen PS. Biochemical aspects of insects development Karger, Basel 1971, 55-56.
10. Kumar R, Gangwar SK. Impact of varietal feeding on *Samia ricini* Donovan in spring and autumn season of Uttar Pradesh. ARPN Journal of Agricultural and Biological Science. 2010; 5(3). ISSN 1990-6145.
11. Unni BG, Kakoty AC, Khanikar D. Lipid and fatty acid composition of muga silkworm, Antheraea assama host plants in relation to silkworm growth, J Lipid Mediators and Cell Signalling. 1996; 13:295
12. Pant R, Unni BG. Free amino acid or haemolymph and silkgland in the developing fifth instar larva of Philosamia ricini. Curr. Sci.1980; 49:538-541.
13. Depledge MH, Bjerregaard P. Haemolymph protein composition and copper levels in decapod crustaceans. Helgol. Meeresunters. 1989; 43:207-223.
14. Shivkumar, Subramanya G. Quanititative estimation of Haemolymph protein during different days of 5th intar larvae in Bivoltiness, multivoltines and mutants of the Bombyx mori.2015 Global journal of Bioscience and Biotechnology. 2015; 4(3):239-241.
15. Talukadar B, Saikia M, Handique PJ, Devi D. Effect of organic solvent on the tensile strength of muga silk produced by Anthaeraea assamensis. International. Journal of pure and applied sciences and technology. ISSN 2229-6107. 2011; 7(1):81.
16. Lowry OH, Rosebrough NJ, Farr AL, Randal RJ. protein measurement with the folin phenol reagent. J. Biochem, 1951; 193:265-275.
17. Goswami B. A study of certain biochemical aspects on Eri silk worm, Philosamiaricini Boisd. in relation to castor plant. *Ricinus communis*. M. Phil desertation. Department of Zoology. Gauhati University, 1990.
18. Chakravorty R, Neog K. Food plants of eri silkworm

Samia ricini (DONOVAN) their rearing performance and prospects for exploitation. Leeds paper of National workshop on eri food plants, Held at Guwahati, 11th - 12th October, 2006.