Changes in the activities of proteins and carbohydrates in haemolymph of silkworm *Bombyx mori* treated with greenleaf

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doi:10.6088/ijes.2012030131013

ABSTRACT

The activity of proteins and carbohydrates in haemolymph of fifth instar in both bivoltine variety and its hybrid, with green leaf treated set and control were assessed. Activity pattern of the proteins and carbohydrates were different in both the races. The activity level of proteins and carbohydrates in bivoltine was higher than that of hybrid in treated set. The results indicate that, the activity of proteins and carbohydrates increased gradually from first day of fifth instar and reached peak towards the end of the spinning stage. The foliar applicant green leaf has played a significant role in increasing the activity of proteins and carbohydrates of haemolymph.

Keywords: Silkworm, Protein, Carbohydrates, Greenleaf, Haemolymph.

1. Introduction

The carbohydrates, proteins and lipids play an important role in the biochemical process underlying growth and development of insects (Ito and Horie, 1959, Wyatt, 1961 and 1967). Haemolymph proteins play an important role in insects for transport functions, as well as for their enzyme action. The synthesis and utilization of haemolymph proteins are controlled by genetic and hormonal factors (Hurliman and Chen, 1974). There is a general agreement that the fat body is one of the sources of haemolymph proteins. In addition to transport functions, the haemolymph of insects performs several physiological functions such as immunity, transport and storage reserve (Mullins, 1985). The concentration of carbohydrates and other biochemical parameters mainly depend on the quality of mulberry leaf. The late age silkworm larvae accumulate higher carbohydrates compared to young age worms. Reducing sugars account for about 5% of the total blood sugars and the fat body. Glycogen serves as the major food reserve in insects (Kilby, 1958). Wyatt and Kalf (1956 and 1957) reported that trehalose is the major blood carbohydrate in insects. Simex and Kodrik (1986) have reported that the glycogen content in the fat body, body wall and silk gland and the free carbohydrates in the haemolymph changed significantly during last larval instar and metamorphosis in silk worms. Carbohydrates are the major components in the food of all the living organisms which either directly or indirectly used as the source of energy for all vital activities.

Since the mulberry leaf serves as the sole food for the silk worm *Bombyx mori*, the quality mulberry leaf influences the growth of the silk worm to a large extent (Benchamin and Jolly, 1986). Foliar application of nutrients is widely employed to improve the yield, quality and to correct the trace elements deficiencies. The response of plants to foliar nutrients is quick,
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foliar fertilization evidently activates plant metabolism and assimilation thus contributing to overcome stress. Further, the use of recent innovation in feeding the plants with nutrients like the use of symbiotic and a-symbiotic nitrogen fixing a-microorganism, green manures, vermicompost, foliar formulations, like vipul, multiplex etc., (Ankalgi and Ansari, 1992) will definitely boost up the mulberry productivity. As there is very little information pertaining to the activity of proteins and carbohydrates in the haemolymph of bivoltine variety (CSR2 X CSR4) and its hybrid with mutivoltine Mysore variety (PM X CSR2) treated with the foliar applicant greenleaf, the present work was undertaken to study the activity in the fifth instar of Bombyx mori.

2. Materials and methods

For the present study two commercially exploited silkworm races namely bivoltine hybrid CSR2 X CSR4 and its hybrid PM X CSR2, were selected and reared in triplicate following the method suggested by Krishna swami (1978). Larvae were fed daily four times with healthy fresh green leaf sprayed leaves (0.25%, 0.5% and 1%). A control batch was maintained separately with out any treatment. A few larvae during fifth instar in the treated sets were collected daily for analyses of proteins and carbohydrates of haemolymph. The larvae so collected were kept at 4-5°C for 5 to 10 minutes to facilitate the free running of haemolymph. The caudal horn of the larvae was punctured and the haemolymph was collected in cleaned, pre cooled vials containing few crystals of phenylthiourea to prevent oxidation of haemolymph. Appropriate dilutions of the haemolymph were made and samples were centrifuged at 3000rpm for 15min. The supernatant was diluted appropriately and used for the assay of proteins and carbohydrates. The quantitative estimation of total soluble proteins in haemolymph was done by the method of Lowry et. al. (1951) by using bovine serum albumin as standard. The quantitative estimation of reducing sugar in haemolymph was estimated by using the 3, 5, dinitro salicylic acid (DNS) method (Burton, 1956) with slight modification and using glucose as standard. The quantitative estimation of total sugar in haemolymph was estimated by anthrone method (Dubois et. al., 1956) using glucose as standard. The results are statistically analyzed and discussed.

3. Results and discussions

3.1. Estimation of total soluble proteins in the haemolymph

The quantitative estimation of total proteins in the haemolymph in bivoltine hybrid increased with an increase in the age from first day till spinning, this trend was observed to be similar for all the sets. However, treatment with 1.0% green leaf treated sets recorded highest value on final day (93.86 mg/ml) followed by 0.5%(93.08 mg/ml), 0.25% (92.16 mg/ml) and control (90.10 mg/ml) (Figure-1). In crossbreed PM X CSR2 the total content of protein in the haemolymph recorded a similar trend as observed in bivoltine hybrid, wherein, it was minimum on first day and increased with an increase in larval age to reach a maximum on the penultimate day of spinning. The protein content was maximum for the set with the treatment 1.0% green leaf treated sets (87.45 mg/ml) followed by 0.5 % (92.84 mg/ml), 0.25% (89.26 mg/ml) and control (87.45 mg/ml) (Figure-2).

In the present study, the increased protein in the haemolymph of the silk worm is due to supplementation of enriched leaves to silk worm. This clearly indicates the influence of dietary protein on the increase in haemolymph protein during fifth instar since fifth instar is considered as prime feeding stage of the silk worm larva where in about 80-85 % of the total leaves is consumed. Krishnaswami et al., (1978) observed that the increase in the protein
concentration in the silkworm body after the fourth moult is due to regular feeding and substantial increase in the body weight by the time the larva attains spinning stage.

![Figure 1: Effect of Green leaf on activity of haemolymph Proteins (Race: CSR$_2$ X CSR$_4$)](image)

![Figure 2: Effect of Green leaf on activity of haemolymph Proteins (Race: PM X CSR$_2$)](image)

3.2. Estimation of carbohydrates in the haemolymph

3.2.1. Reducing sugar

(Figure-3 & 4) shows the level of reducing sugar in bivoltine and crossbreed during fifth instar larval development of *Bombyx mori*, in bivoltine race it gradually increased from first day and attain peak on the last day. Further, the larvae fed with 1.0% (7.40 mg/ml) treated green leaf treated mulberry leaves showed a higher level of carbohydrates followed by 0.5% (6.94 mg/ml), 0.25% (5.78 mg/ml) and control (4.81 mg/ml). In crossbreed the level of reducing sugar gradually increased till sixth day to attain peak value on the last day. Further, the level of reducing sugar in the haemolymph was found to be highest in bivoltine followed by crossbreed. The level of reducing sugar increased from first day and reaches the peak level on the last day in both bivoltine and crossbreed variety. This may be due to the fact that the bivoltine is capable of accumulating the sugars much faster to support its faster growth.
Changes in the activities of proteins and carbohydrates in haemolymph of silkworm Bombyx mori treated with greenleaf (Benchamin and Jolly, 1986) compared to multivoltine which showed delayed accumulation of reducing sugars.

Figure 3: Effect of Green leaf on activity of haemolymph Reducing sugar (Race: CSR2 X CSR4)

Figure 4: Effect of Green leaf on activity of haemolymph Reducing sugar (Race: PM X CSR2)

3.2.2. Total sugar

The total sugar content in the haemolymph increased constantly from first day (4.58 mg/ml) to sixth day (28.18 mg/ml) in bivoltine race when reared with 1.0% Greenleaf treated mulberry leaves followed by 0.5% (1st day 4.42 mg/ml and 6th day 27.32 mg/ml), 0.25% (1st day 4.31 mg/ml and 6th day 27.12 mg/ml) and control (1st day 4.28 mg/ml and 6th day 26.18 mg/ml). In case of crossbreed variety the larvae fed with 1.0% treated green leaf treated mulberry leaves showed a higher level of carbohydrates, from first day (4.67 mg/ml) to sixth day (26.08 mg/ml) of fifth instar. Further the total sugar content was higher when compared to 0.5% (1st day 4.42 mg/ml and 6th day 25.84 mg/ml), 0.25% (1st day 4.06 mg/ml and 6th day
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25.02 mg/ml) and control leaves (1\textsuperscript{st} day 3.87 mg/ml and 6\textsuperscript{th} day 24.88 mg/ml) (Figure-5 & 6). The concentration of total sugar increased from first day to last day. This shows that the capacity to accumulate carbohydrates is more in bivoltine compared to multi-bivoltine hybrid. The accumulation of other sugars might get converted to glucose (Yamamoto & Fujimaki, 1982; Periasamy \textit{et al.}, 1984) later and used as a source of energy for better growth in bivoltine breeds. So the level of carbohydrates during larval development reveals the degree of utilization of carbohydrates, which are the major sources of energy in the body, for growth and development of the larva that might ultimately determine the difference in the quality and quantity of silk production.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5}
\caption{Effect of Green leaf on activity of haemolymph Total sugar Race: CSR\textsubscript{2} X CSR\textsubscript{4}}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6}
\caption{Effect of green leaf on activity of haemolymph total sugar Race: PM X CSR\textsubscript{2}}
\end{figure}
4. Conclusion

The present investigation clearly depicts the role of greenleaf as foliar applicant because of increased activity of proteins and carbohydrates in the haemolymph of CSR hybrid and PM X CSR2. Between the two varieties CSR hybrid has given better results indicating its involvement in the expression of characters genetically when compared with PM X CSR2. Perhaps large scale utilization of greenleaf in the field may bring better characters with regard to the performance of the breeds.

5. References


