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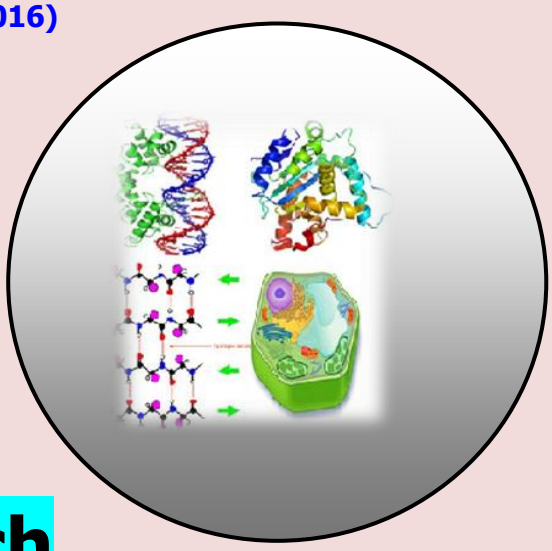

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RESEARCH PAPER

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Biomolecules in Haemolymph and Fat Body Tissues of selected Strains of the Domesticated Vanya Silkworm, *Samia cynthia ricini* Boisduval

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ABSTRACT

The growth, development and performance of an animal (insect) largely governed by the food quality which inturn have marked influence on biomolecules of insects in general and commercial insect like silkworm in particular. In this regard, a study has been undertaken to analyze the quantum of biomolecules in both haemolymph and fat body tissues of selected strains (Strains: Blue-Plain, Blue-Zebra, Blue Greenish-Plain, White-Plain, White-Zebra, Yellow-Plain, Yellow-Spotted and Yellow-Zebra) of eri silkworm. The eri silkworms were reared on leaves of castor (variety: Local Pink), a primary food plant from the day brushing to cocoon formation. Towards estimation of biomolecules (total proteins, carbohydrates, glycogen, total free amino acids as well as alanine and aspartate aminotransferase, succinate dehydrogenase and protease activity) for the current investigation, haemolymph and fat body samples were collected from fifth instar (5th day) larvae. The results of the investigation revealed that, among the selected strains of eri silkworm, White-Plain strain contain significantly higher total protein and carbohydrates with least values being in White-Zebra. Total free amino acid content did not have significant values for haemolymph, while it was glycogen for fat body. Significantly higher total free amino acid content was found with White-Plain and lower with White-Zebra. Alanine and aspartate aminotransferase, succinate dehydrogenase and protease enzymatic activities were statistically more in White-Plain, while White-Zebra registered least enzymatic activities. Results of the current investigation clearly revealed that, White-Plain strain possess significantly higher values of biomolecules which inturn have profound influence on the commercial characters of eri silkworm and thus larvae of White-Plain strain can be exploited for maximization of eri cocoon production.
Key words: Biomolecules, Enzymes, Eri silkworm, Fat body, Haemolymph and Proteins.

INTRODUCTION

Haemolymph in insects is a complex mixture of proteins, lipids, carbohydrates, amino acids, nucleic acids, hormones and their degradation products. It is primarily responsible for supplying nutrients, transferring metabolic wastes to maintain normal growth and development. It serves as a major role in the immune system and in transport of hormones, nutrients, and metabolites. The silkworm has an open circulatory system containing haemolymph, which delivers nutrients and oxygen to all parts of the body. It is also an important repository for nutrition and energy. Major biomolecules such as proteins, carbohydrates and lipids play an important role in biochemical process and responsible for the growth and development of the silkworm (Ito and Horie, 1959).

The chemical composition of haemolymph is highly variable within the same breeds and diversified among the distant breeds at different development stages (Florkin and Jeuniaux, 1974). The fat body synthesizes numerous proteins released in to the haemolymph during active feeding in larval stage (Kumar *et al.,* 1998). In insects, haemolymph is the only extracellular fluid with varied functions and reservoir for the products which are required for every physiological activity that could result in physiological and biochemical transformations in the insect tissues due to changes in the composition of haemolymph (Pawar and Ramakrishnan, 1977).

In insects, as in other organisms, glycogen serves as glucose for utilization at different points of the life cycle. Insect's glycogen is most abundant in fat body, flight muscles and intestine, although there are deposits in other tissues, with the exception of haemolymph (Brown and Nestler, 1985). However, glycogen content in fat body, body wall and silk gland and the free carbohydrates in the haemolymph changes significantly during the last larval instar and metamorphosis in silkworms (Simex and Kodrik, 1986).

Dehydrogeneases are very important tools for the investigation of insect metabolic activities during the course of development. The relative activities of the insect dehydrogenases may be related to the function and energy yielding demands of the tissues (Dickinson and Sullivan, 1975). Keeping these points in view, an attempt has been undertaken to quantify the biomolecules in two tissues of domesticated vanya silkworm (eri silkworm) namely haemolymph and fat body in eight different strains as these have marked influence on economic characters.

MATERIALS AND METHODS

Procedure for rearing of eri silkworm

Among the three types of vanya silkworms (eri, tasar and muga), eri silkworm has been reared completely under indoor conditions, hence at most care must be exercised to rear the eri silkworm like mulberry silkworm. As a prelude to rearing of eri silkworm, disinfection of silkworm rearing house was done with 0.05% of Asthra @ 2.0 l/m^2 to get rid of disease causing germs as a preventive measure. Disease free layings of eight strains of eri silkworm (Blue-Plain, Blue-Zebra, Blue Greenish-Plain, White-Plain, White-Zebra, Yellow-Plain, Yellow-Spotted and Yellow-Zebra) was procured from the Central Sericultural Germplasm Resources Centre, Hosur and incubated at a temperature of 25±1^{o}C and relative humidity of 75±5%. The hatched larvae were offered tender leaves of Local Pink castor variety until the worms start to spin the cocoons.

Collection of haemolymph and fat body

For the current investigation, haemolymph and fat body tissues were collected from fifth instar fifth day in different strains of eri silkworm for the quantification of biomolecules. The haemolymph was collected by scarifying 4-5 larvae from each strain, replication-wise. The hemolymph was collected from the abdominal legs of the larvae by puncturing them and was collected in sterilized 1.5 ml pre-chilled eppendorf tubes containing 1 mg of thiourea to avoid oxidation. Further, from the same larvae fat body samples were collected by dissecting out the larvae. The samples were preserved at -20^{o}C and were utilized for the estimation of biomolecules.

Protocol for estimation of biomolecules

Total protein: Total protein content in haemolymph and fat body of eri larvae was estimated by adopting the procedure of Lowry *et al.* (1951).

Carbohydrates: Carbohydrate content in haemolymph and fat body of eri larvae was estimated by adopting the Anthrone method (Dubios *et al*., 1956).

Total free amino acids: Total free amino acid content in haemolymph and fat body was estimated as per the procedure outlined by Moore and Stein (1968).

Glycogen: Glycogen content was determined in fat body samples in eri larvae as per the procedure of Montgomery (1957).

Alanine (EC: 2.6.1.2) and aspartate (EC: 2.6.1.1) aminotransferase activity: The activity of enzymes *viz*., alanine and aspartate aminotransferase in haemolymph and fat body was estimated as per the procedure of Reitman and Frankel (1957).

Succinate (EC: 1.3.99.1) dehydrogenase activity: The succinate dehydrogenase activity in haemolymph and fat body was estimated as per the procedure of Nachlas *et al.* (1960).

Protease activity: Protease activity in haemolymph and fat body was estimated according to the method of Murata *et al*. (1963).

Statistical analysis of the data

The data obtained in the current investigation was subjected to one-way ANOVA for test of significance ($p \leq 0.05$ and $p \leq 0.01$) as per the methods outlined by Sundarraj *et al.* (1972) through SPSS statistical package (ver. 21.0).

RESULTS

The results on the quantification of biomolecules in selected strains of eri silkworm in two tissues namely haemolymph and fat body were briefed in the following pages.

Total protein

Total protein content in haemolymph among the selected strains of eri silkworm differed significantly (F-value: 6.157; $p \leq 0.01$) with highest value being noticed in White-Plain strain of eri silkworm (39.11 mg/ml) followed by Blue-Zebra (38.66 mg/ml), Yellow-Spotted (36.86 mg/ml), Blue Greenish-Plain (35.46 mg/ml), Yellow-Zebra (35.45 mg/ml), Yellow-Plain (35.41 mg/ml) and Blue-Plain (32.26 mg/ml). Notably, protein content was lowest in White-Zebra (29.41 mg/ml). In fat body too, total protein content was significantly (F-value: 6.440; $p \leq 0.01$) more in White-Plain strain (39.41 mg/g) when compared to Blue-Zebra (38.90 mg/g), Yellow-Spotted (37.42 mg/g), Yellow-Plain (36.24 mg/g), Yellow-Zebra (36.01 mg/g), Blue Greenish-Plain (35.82 mg/g), and Blue-Plain (32.50 mg/g), while total protein content was less with White-Zebra (29.93 mg/g) (Figure 1).

Carbohydrates

Significant variation (F-value: 5.009; $p \leq 0.01$) was noticed with respect to carbohydrate content in haemolymph among different strains of eri silkworm with higher content being in White-Plain (17.13 mg/ml), while Blue-Zebra (16.86 mg/ml), Yellow-Spotted (16.22 mg/ml), Yellow-Plain (15.79 mg/ml), Yellow-Zebra (15.59 mg/ml), Blue Greenish-Plain (15.48 mg/ml), Blue-Plain (14.25 mg/ml) and White-Zebra (13.03 mg/ml) stood next in the order with latter strain registered lower carbohydrate content. Incidentally, in respect of fat body also, White-Plain excelled significantly (F-value: 6.102; $p \leq 0.01$) more carbohydrate content (39.41 mg/g) and next in the order were Blue-Zebra (38.90 mg/g), Yellow-Spotted (37.42 mg/g), Yellow-Plain (36.24 mg/g), Yellow-Zebra (36.01 mg/g), Blue Greenish-Plain (35.82 mg/g) and Blue-Plain (32.50 mg/g). However, carbohydrate content was significantly less with White-Zebra (29.93 mg/g) (Figure 2).

Total free amino acids

Total free amino acid content did not vary statistically (F-value: 0.235) among different strains of eri silkworm, however, the content ranged between 54.99 mg/ml (Yellow-Spotted) to 65.54 mg/ml (White-Plain). On the other hand, fat body could record significant variation (F-value: 5.560; $p \leq 0.01$) with respect to total free amino acid content in selected strains of eri silkworm. Total free amino acid content was highest in White-Plain (22.80 mg/g) over Blue-Zebra (22.64 mg/g), Yellow-Spotted (21.99 mg/g), Yellow-Plain (21.51 mg/g), Yellow-Zebra (21.46 mg/g), Blue Greenish-Plain (21.31 mg/g) and Blue-Plain (19.91 mg/g) strains. Remarkably, total free amino acid content was lowest with White-Zebra strain (18.85 mg/g) (Figure 3).

Glycogen

Glycogen content in fat body among different strains of eri silkworm did not register significant difference (F-value: 0.235) and the values varied between 58.81 mg/g (Yellow-Spotted) to 69.35 mg/g (White-Plain) (Figure. 4).

Alanine aminotransferase activity

Among the selected strains of eri silkworm, significantly (F-value: 7.115; $p \leq 0.01$) highest alanine aminotransferase activity was registered in White-Plain (2010.1 µ moles of pyruvate/g of protein/h) as compared to Blue-Zebra (1985. µ moles), Yellow-Spotted (1905.5 µ moles), Yellow-Plain (1847.1 µ moles), Yellow-Zebra (18.31.8 µ moles), Blue Greenish-Plain (1824.9 µ moles) and Blue-Plain (1642.2 µ moles). However, alanine aminotransferase activity was least with White-Zebra (1499.0 µ moles). In fat body, alanine aminotransferase activity was significantly (F-value: 6.508; $p \leq 0.01$) higher in White-Plain (2075.3 µ moles) and Blue-Zebra (2056.1 µ moles), Yellow-Spotted (1967.6 µ moles), Yellow-Plain (1902.4 µ moles), Blue Greenish-Plain (1888.1 µ moles), Yellow-Zebra (1884.9 µ moles), Blue-Plain (1713.3 µ moles) and White-Zebra (1553.2 µ moles) ranked next in the order with latter strain found to be the least among eight strains (Figure 5).

Aspartate aminotransferase activity

In haemolymph tissue, aspartate aminotransferase activity differed significantly (F-value: 5.767; $p \leq 0.01$) among the selected strains of eri silkworm. Higher aspartate aminotransferase activity was recorded with White-Plain (2759.4 µ moles of oxaloacetate/g of protein/h) and next in the order were Blue-Zebra (2731.6 µ moles), Yellow-Spotted (2603.4 µ moles), Yellow-Plain (2522.1 µ moles), Yellow-Zebra (2513.1 µ moles), Blue Greenish-Plain (2504.6 µ moles) and Blue-Plain (2260.6 µ moles), while it was lower with White-Zebra (2068.6 µ moles). In fat body tissue, aspartate aminotransferase activity exhibit significant variation (F-value: 6.308; $p \leq 0.01$) with highest in White-Plain (2737.1 µ moles) as compared to Blue-Zebra (2716.1 µ moles), Yellow-Spotted (2595.3 µ moles), Yellow-Plain (2517.4 µ moles), Yellow-Zebra (2502.4 µ moles), Blue Greenish-Plain (2474.4 µ moles), Blue-Plain (2247.5 µ moles) and White-Zebra (2036.2 µ moles) with latter being the least among selected strains considered in the investigation (Figure 6).

Succinate dehydrogenase activity

Succinate dehydrogenase activity showed significant variation (F-value: 5.267; $p \leq 0.01$) in haemolymph among eight selected strains of eri silkworm, highest succinate dehydrogenase activity was noticed in White-Plain (14.64 moles of formazon formed/mg of protein/min), while Blue-Zebra (14.52 moles), Yellow-Spotted (14.11 moles), Yellow-Plain (13.80 moles), Yellow-Zebra (13.77 moles), Blue Greenish-Plain (13.67 moles) and Blue-Plain (12.74 moles) stood next in the order. However, succinate dehydrogenase activity was lowest in White-Zebra (12.11 moles). In fat body tissue, succinate dehydrogenase activity was significantly (F-value: 6.008; $p \leq 0.01$) more with White-Plain (14.52 moles of formazon formed/mg of protein/min) followed by Blue-Zebra (14.33 moles), Yellow-Spotted (13.94 moles), Yellow-Plain (13.67 moles), Yellow-Zebra (13.55 moles), Blue Greenish-Plain (13.47 moles) and Blue-Plain (12.59 moles) and it was less with White-Zebra strain (11.97 moles) (Figure 7).

Protease activity

Protease activity in haemolymph of eight selected strains of eri silkworm could vary statistically (F-value: 5.569; $p \leq 0.01$) with higher value being in White-Plain (60.98 units/h) over other strains namely Blue-Zebra (60.46 units/h), Yellow-Spotted (57.25 units/h), Yellow-Plain (55.66 units/h), Yellow-Zebra (55.08 units/h), Blue Greenish-Plain (54.74 units/h) and Blue-Plain (50.06 units/h) and White-Zebra (45.45 units/h). In fat body tissue too, protease activity differed significantly (F-value: 5.915; $p \leq 0.01$) being more in White-Plain (61.84 units/h) followed by Blue-Zebra (61.06 units/h), Yellow-Spotted (58.15 units/h), Yellow-Plain (56.10 units/h), Yellow-Zebra (56.03 units/h), Blue Greenish-Plain (55.73 units/h) and Blue-Plain (50.59 units/h). However, protease activity was less with White-Zebra strain (45.90 units/h) (Figure 8).

DISCUSSION

The biochemical constituents like protein, carbohydrates, amino acids, nucleic acids, etc. largely depend on the quality of food and the degree of their utilization in insects (Horie, 1961). Horie *et al*. (1982) observed that the protein content in mid gut increased from first day to third day of fifth instar. It clearly showed that the digestive activities are high during early part of the fifth instar which results in increased accumulation of protein that are transported to other tissues via haemolymph for ensuing physiological activities in the larva. Nagata and Kobayashi (1990) revealed that increase in protein content of haemolymph and silk gland from beginning to end of the fifth instar may be due to active secretion of proteins in fat body.

The protein content in haemolymph of eri silkworm was gradually increases from first day to last day of fifth instar. But in mid gut, the increase was up to third instar and decreases during fifth instar development. The zebra marked larvae showed highest protein content among the plain and light blue larvae. The free amino acid content in haemolymph increases from first to third instar and decrease significantly in fourth and fifth instars and in mid gut, it gradually increases from first to fifth instar. The plain larvae showed increase in free amino acid content among the strains of light blue and zebra marked eri silkworm (Ravikumar and Sarangi, 2000).

According to Ravikumar and Sarangi (2004), protein content gradually increased from beginning to end of the fifth day of fifth instar and similar trend was also observed in silk gland in all the castor varieties used under investigation. The protein content in haemolymph and silk gland were 53.69 and 70.21 mg/ml in zebra marked larva followed by light blue coloured larva (51.97 and 64.01 mg/ml) and plain larva (49.56 and 61.95 mg/ml), respectively. The mid gut protein increased in the first day (50.59mg/ml) in zebra marked larva and higher in the third day (66.42 mg/ml) in light blue coloured larva and gradually decreases in fifth day (22.72 mg/ml) of light blue coloured larva.

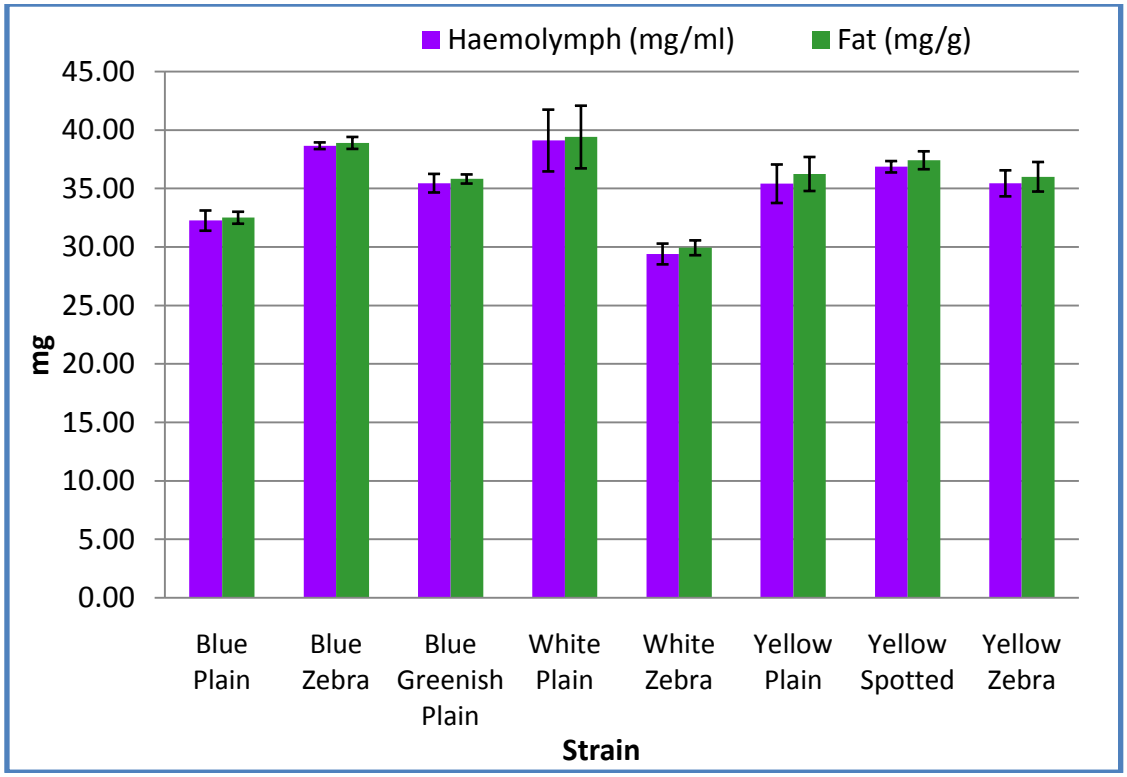

Figure 1. Total protein content in haemolymph and fat body of selected strains of eri silkworm.

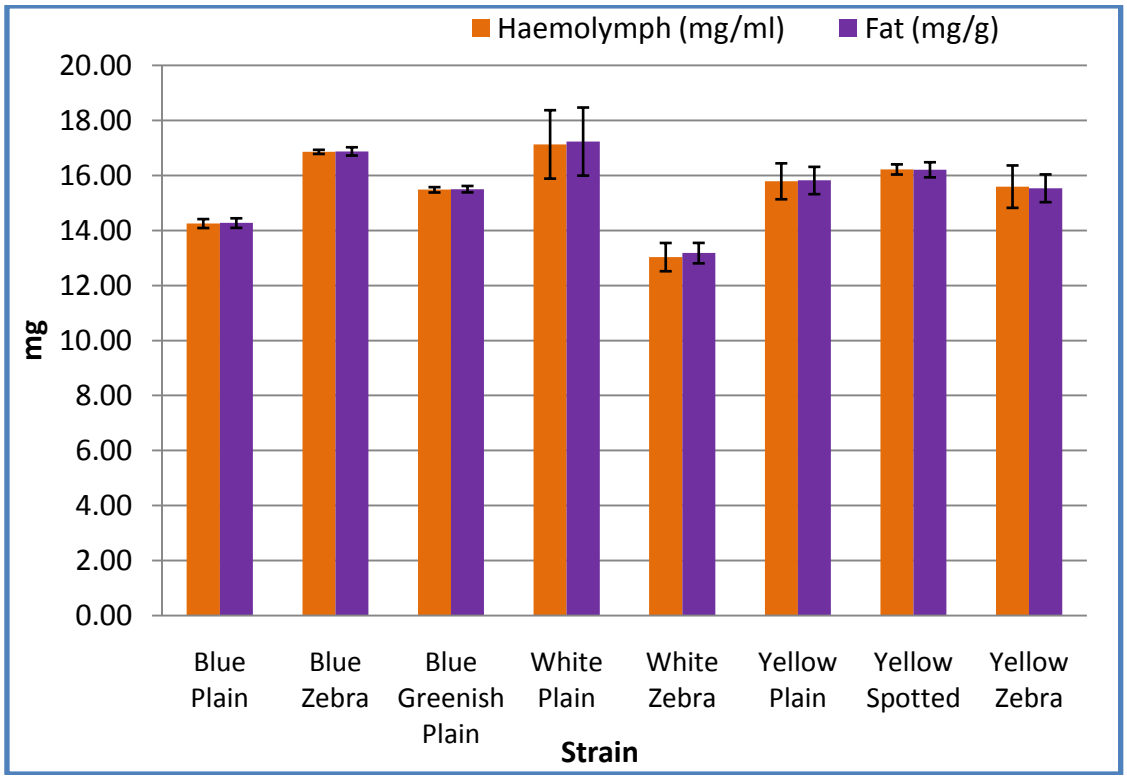

Figure 2. Carbohydrate content in haemolymph and fat body of selected strains of eri silkworm.

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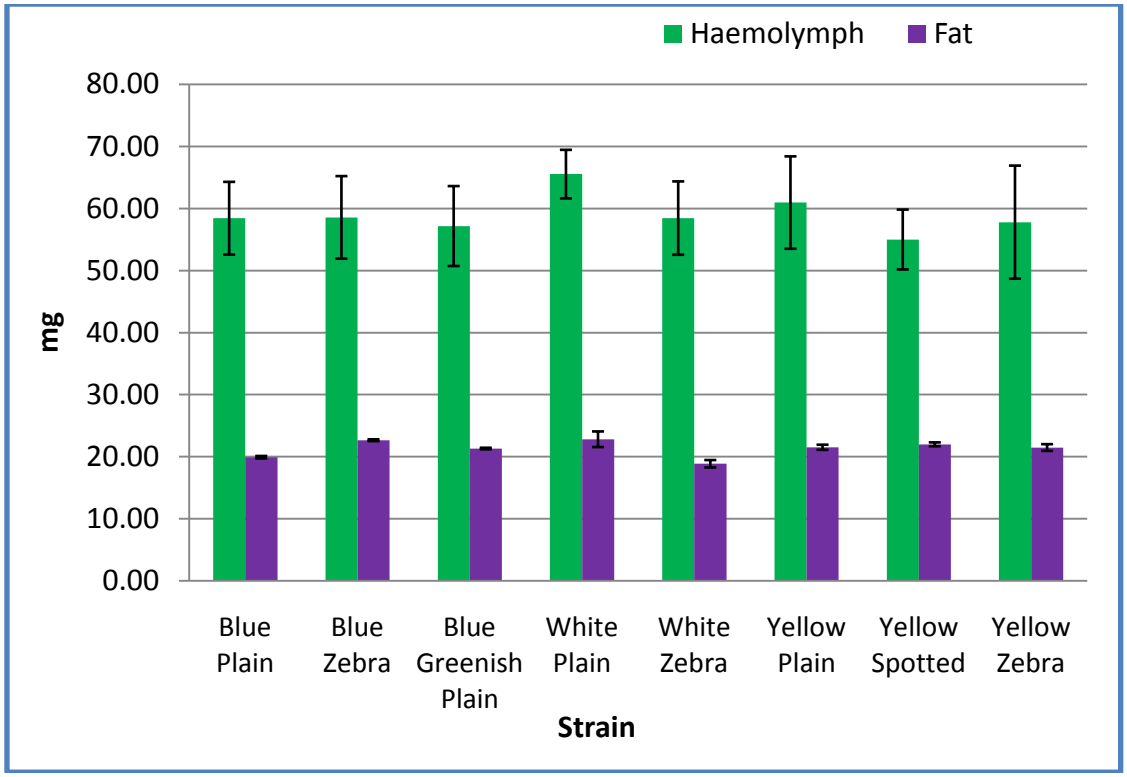

Figure 3. Total free amino acids in haemolymph and fat body of selected strains of eri silkworm.

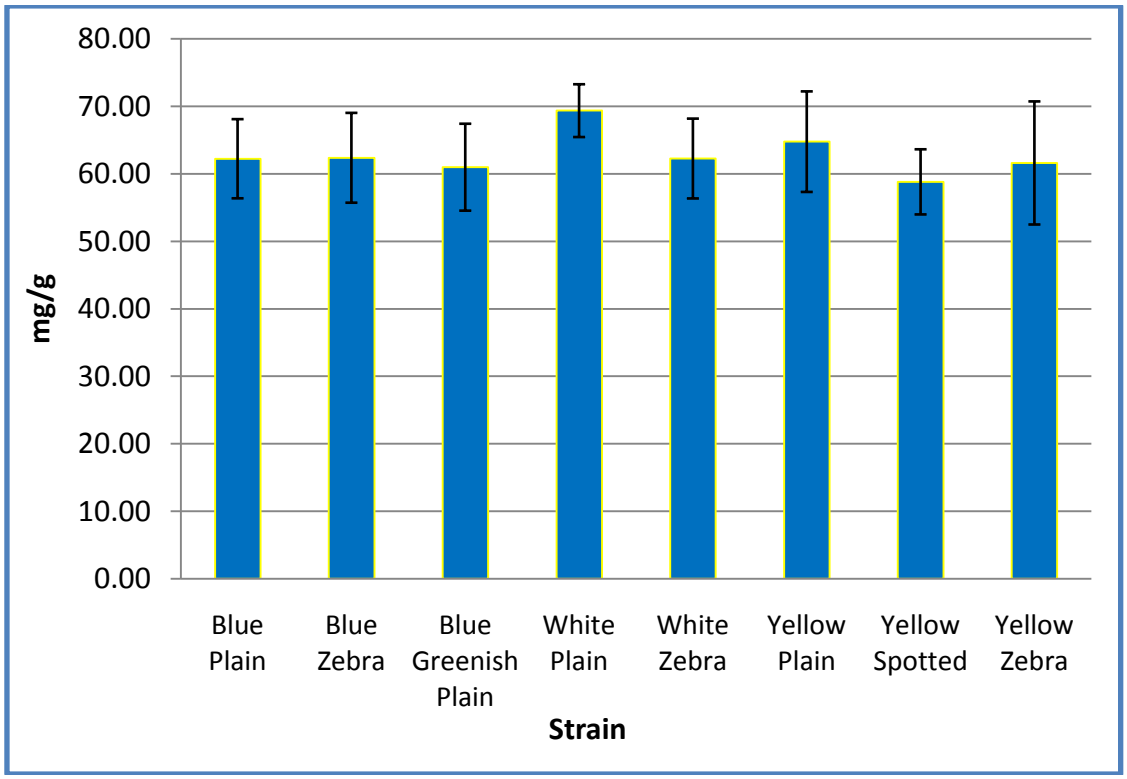

Figure 4. Glycogen content in fat body of selected strains of eri silkworm.

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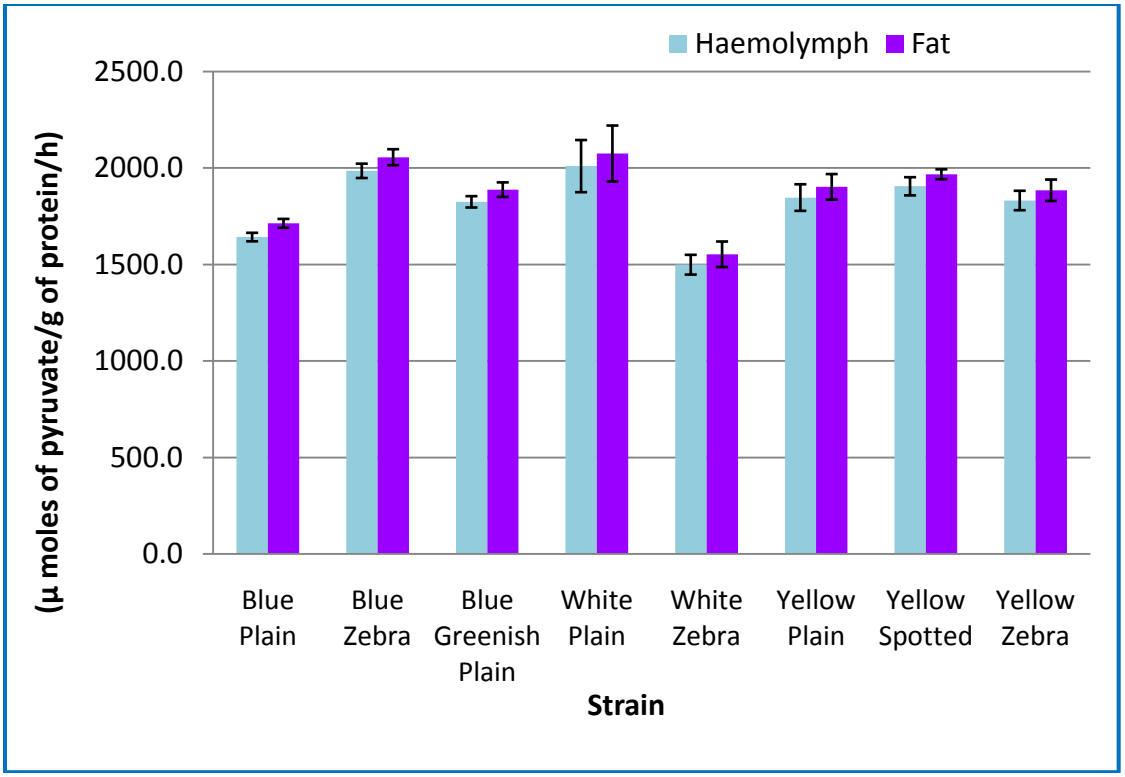

Figure 5. Alanine aminotransferase activity in haemolymph and fat body of selected strains of eri silkworm.

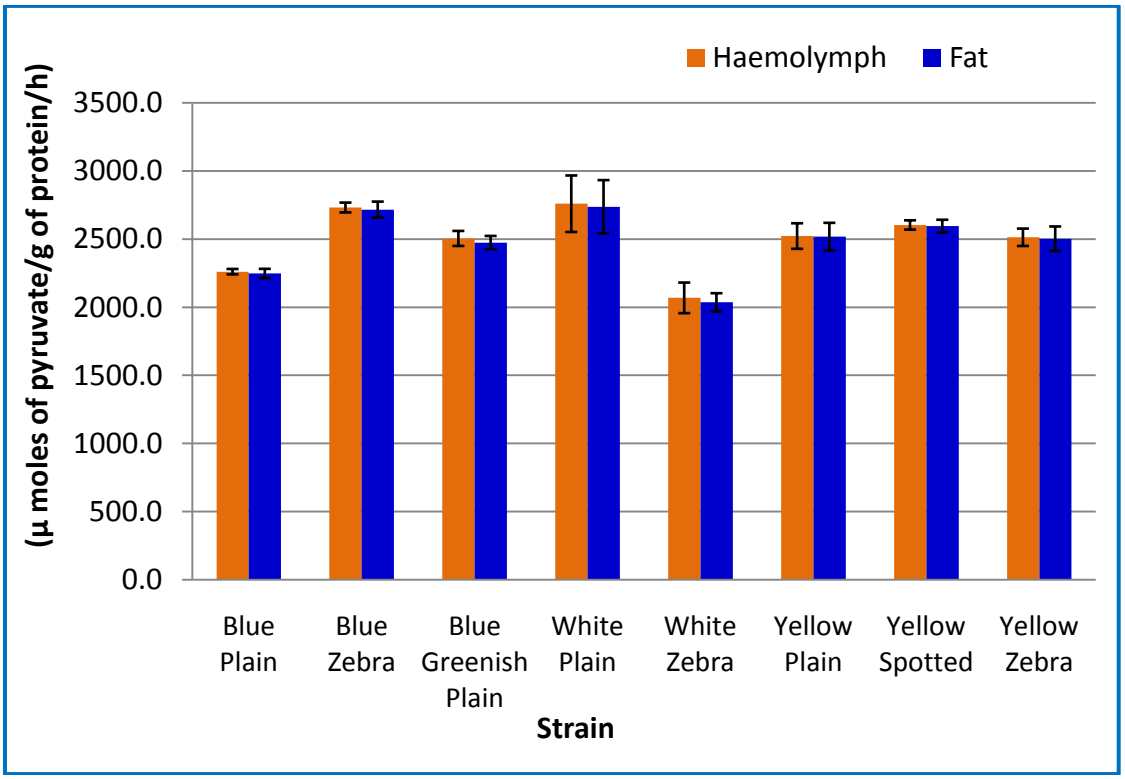

Figure 6. Aspartate aminotransferase activity in haemolymph and fat body of selected strains of eri silkworm.

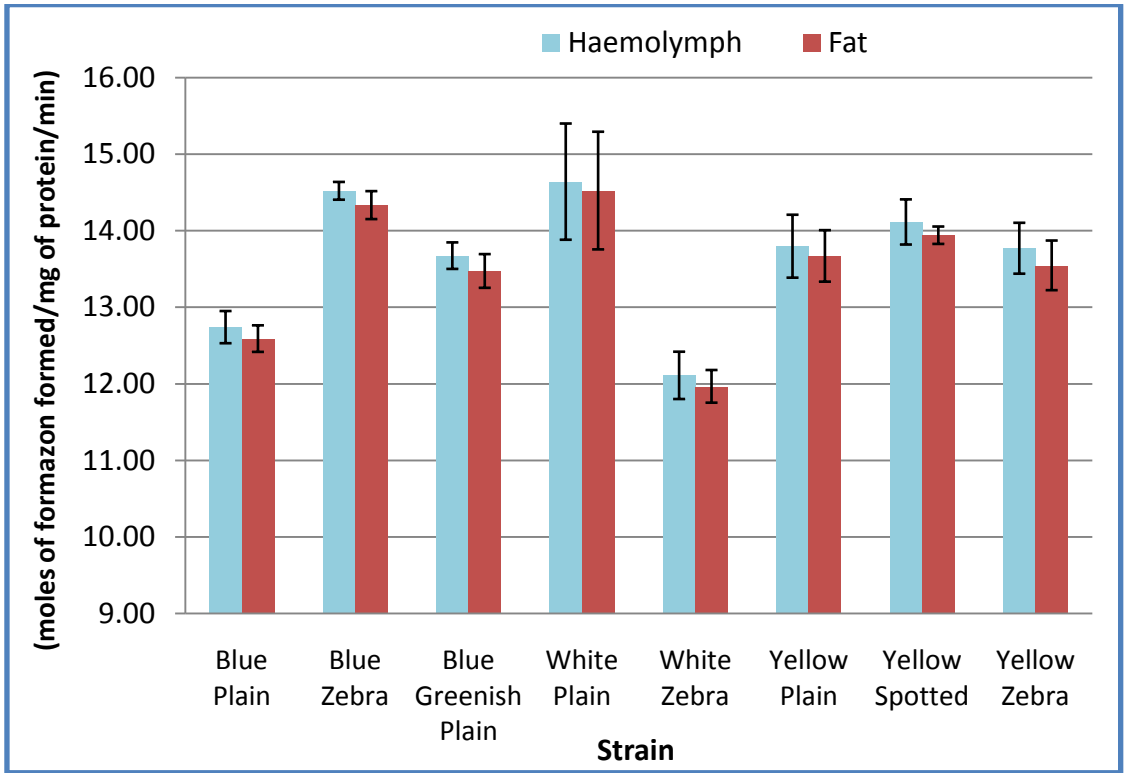

Figure 7. Succinate dehydrogenase activity in haemolymph and fat body of selected strains of eri silkworm.

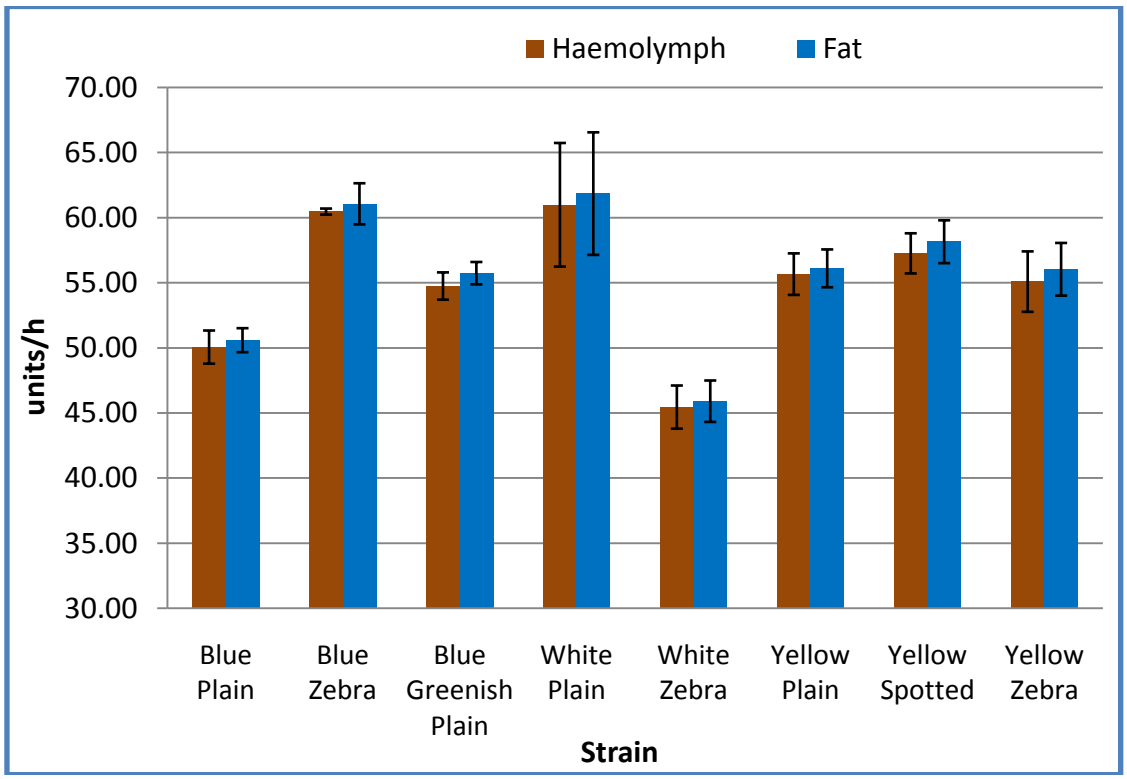

Figure 8. Protease activity in haemolymph and fat body of selected strains of eri silkworm.

The total sugar content in haemolymph was highest (1.99 mg/g) both in zebra marked and plain larva on the first day then decreased in the last day of fifth instar and reaches the lowest level (0.94 mg/g) in light blue coloured larva. Sannappa *et al*. (2015) observed that eri silkworms fed on the leaves of JC-12 castor variety recorded maximum total proteins, carbohydrates, total free amino acids, alanine and aspartate aminotransferase enzymes in haemolymph and minimum was in local green variety. Similarly, for these biochemical components in fat body, DCS-9 variety showed more total proteins, carbohydrates and glycogen and less in local green variety. Further, the activity of alanine and aspartate aminotransferase enzymes were higher in silkworm larvae that were reared on DCH-177 as compared to local green variety.

A study conducted by Manjunath (2015) on quantification of biomolecules in three strains (Blue-Plain, Blue-Zebra and Yellow-Plain) of eri silkworm in first, third and fifth day of fifth instar in haemolymph and fat body tissues. In haemolymph, total protein, total free amino acid, alanine aminotransferase, aspartate aminotransferase, succinate dehydrogenase and protease were significantly more in Yellow-Plain strain compared to Blue-Plain and Blue-Zebra strains of eri larvae reared on castor leaf. Similarly, in fat body too, Yellow–Plain strain had higher amount of total protein, carbohydrates, glycogen, total lipid, alanine aminotransferase, aspartate aminotransferase, succinate dehydrogenase and protease as compared to Blue-Plain and Blue-Zebra strains.

Significant differences were found in protein level during fifth instar larva in all six strains of eri silkworm. Among selected strains Yellow Spotted was reported to have highest haemolymph protein concentration followed by Yellow Plain. The protein concentration in ascending order was Greenish Blue Zebra < Greenish Blue-Spotted < Greenish Blue-Plain < Yellow-Zebra < Yellow-Plain (Priyanki Sharma and Jogen Chandra Kalita, 2017). Biomolecules in both haemolymph and fat body tissues did vary considerably among the selected strains of eri silkworm that were reared only on Local Pink variety of castor leaves which might be due to influence of strain / genotype as evidenced by the results of the previous researchers when eri silkworms were reared on different castor genotypes/varieties/hybrids. Further, literature pertaining to the information with respect to impact of eri silkworm strains on biomolecules was meager and thus limits discussion on these lines in the current investigation.

CONCLUSION

The study on quantification of biomolecules in haemolymph and fat body tissues among eight strains of eri silkworm reared on Local Pink variety of castor inferred that, White-Plain larvae could record higher amount of biomolecules over other strains considered under investigation.

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REFERENCES

Brown, C. and Nestler, C. (1985). Catecholamines and indolalkylamines. In: *Comprehensive Insect Physiology, Biochemistry and Pharmacology* (Eds. G.A. Kerkut and L.I. Gilbert). Pergamon Press, New York, pp. 435-497.

Dickinson, W.J. and Sullivan, D.T. (1975). Gene-enzyme systems in *Drosophila.* Springer, New York, pp.71-78.

Dubios, M., Giles, K.A., Hamilton, T.K., Robeos, R.A. and Smith, R. (1956). Calorimetric determination of sugars and related substances. *Anal. Chem*., **28**: 350-356.

Florkin, M. and Jeuniaux, C. (1974). Haemolymph composition. *The Physiology of Insects* (Eds. M. Rockstein). Academic Press, London.

Horie, Y. (1961). Physiological studies on the alimentary canal of the silkworm, *Bombyx mori* L. III- Absorption and utilization of carbohydrates. *Bull. Seri. Exp. Stn. Jpn.,***16**: 287-309.

Horie, Y., Watanabe, K. and Sakamoto, E. (1982). Evidence of step wise digestion of protein in the digestive system of the silkworm, *Bombyx mori*. *Appl. Ent. Zool*., **17**: 358-363.

Ito, T. and Horie, Y. (1959). Carbohydrate metabolism of the midgut of the silkworm, *Bombyx mori* L. *Arch. Biochem. Biophys.,* 80, 174-176.

Kumar, K.N., Ismail, S.M. and Datta, A. (1998). Differential uptake of storage protein by the fat body of rice moth, *Corcyra cephalonica* during the larval - pupal development. *Entomon*, **23**: 83-90.

Lowry, O.H., Rosebrough, N., Farr, A. and Randall, R. (1951). Protein measurement with Folin phenol reagent. *J. Biol. Chem*., **193**: 265-275.

Manjunath, K.G. (2015). Exploration of castor ecotypes in the Western Ghats of Karnataka and their evaluation using the eri silkworm, *Samia cynthia ricini* Boisduval. *Ph.D. Thesis*, University of Mysore, Mysuru, p. 163.

Montgomery, R. (1957). Determination of glycogen. *Archi. Biochem. Biophy.*, **67**: 378-386.

Moore, S. and Stein, W.H. (1968). A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. *J. Biol. Chem*., **211**: 907-913.

Murata, J., Satake, M. and Suzuki, T. (1963). Studies on snake venom XII. Distribution of proteinase activities among Japanese and formosan snake venoms. *J. Biochem*., **94:** 413-419.

Nachlas, M.M., Magulies, S.I. and Seligman, A.M. (1960). Sites of electron transfer to tetrazolium salts in the succinoxidase system. *J. Biol. Chem.*, **235**: 2739-2743.

Nagata, M. and Kobayashi, M. (1990). Quantitative changes in storages proteins during larval development of silkworm, *Bombyx mori. J. Seri. Sci. Jpn.,* **59**(6): 461-468.

Pawar, V.M. and Ramakrishnan, N. (1977). Biochemical changes in larval haemolymph of *Spodoptera litura* (Fabricius) due to nuclear polyhedrosis virus infection. *Indian J. Experiment. Bio*., **15**:755-758.

Priyanki Sharma, and Jogen Chandra Kalita (2017). Haemolymph protein estimation in six different strains of eri silkworm, *Samia ricini* (Donovan). *Journal of Entomology and Zoology Studies*, **5**(2): 677-680.

Ravikumar, H.N. and Sarangi, S.K. (2000). Changes in the concentration of soluble protein and free amino acid during fifth instar development of eri silkworm, *Philosamia ricini*. In: *Advances in Tropical Sericulture* (Eds. S.B. Dandin, R.K. Mishra, V.P. Gupta and Y.S. Reddy). National Academy of Sericultural Sciences, India, Bangalore, pp. 433-436.

Ravi Kumar, H.N. and Sarangi, S.K. (2004). Changes in protein and total sugars content in eri silkworm, *Philosamia ricini* during fifth instar development. *Bull. Ind. Acad. Seri*., **8** (1):17-22.

Reitman, S. and Frankel, S. (1957). A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. Clin. Pathol*., **28**(1): 56-63.

Sannappa, B., Manjunath, D and Manjunath, K.G. (2015). Bio-chemical composition of haemolymph and fat body of the domesticated vanya silkworm, *Samia cynthia ricini* Boisduval as influenced by castor hybrids / varieties. *National Seminar on Science and Technology for Human Development*, 23-25 February 2015, Kristu Jayanti College, Bangalore, p. 47.

Simex, V. and Kodrik, D. (1986). Changes in the tissue glycogen and free carbohydrates of haemolymph during the last larval instar and metamorphosis of silkworm, *Bombyx mori* L. *Acta Entomol. Bohemaslov*, **83** (2): 92-100.

Sundarraj, N., Nagaraju, S., Venkataramu, M.N. and Jagannath, M.K. (1972). *Design and Analysis of Field Experiments.* Directorate of Research, UAS, Bangalore, p. 419.

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