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Effect of thermal variation on protein contents in the haemolymph of multivoltine mulberry silkworm *Bombyx mori* Linn.

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ABSTRACT: Investigation on different developmental stages of *Bombyx mori* revealed that the total protein content of haemolymph was influenced significantly with the variation in temperature. The maximum level (33.81µg/mg) of total protein content was notice in the haemolymph of fifth instar larvae, reared at 26°C while the minimum level (10.86µg/mg) was recorded in the adults reared at 14°C.

KEY WORDS: *Bombyx mori*, temperature, silkworm, haemolymph protein

INTRODUCTION

The study on the effect of temperature may provide good understanding of various life processes; therefore a possible ideal ecological model with particular reference to temperature may be formulated for the success of sericulture industry. Being poikilotherm the body temperature of *Bombyx mori* Linn. is variable in accordance with the environmental temperature influencing the developmental process, silk producing potential and biochemical constituent (Mortimer *et al*., 2013).The secretion of silk is a complex process which involves a chain of enzymatic and biochemical process as a result the level of various chemical constituent like protein, free amino acid and nucleic acids may also be influenced due to temperature variation. It is well know that temperature plays a major role in their physiological and biochemical behaviour of the insect. The insects will get acclimatized to low temperature by the production of various cryoprotectants like glycerol, trahalose and sorbitol (Sinclair, 2003). There is a direct apart from the above studies it is also reported that correlation among the number of cells size of gland cells and the amount of silk production by amino acids and haemolymph (Shimizu and Horiuchi, 1952). Keeping these views an attempt has been made to investigate the effect of varying temperature on the protein contents in the haemolymph of Nistari race of *B. mori* which affect the rearing and cocoons parameters.

The seed cocoons of multivoltine mulberry silkworm were obtained from the silkworm grainage Bahraich, Directorate of Sericulture, Uttar Pradesh and were maintained in plywood trays (23×20×5cm) under the ideal rearing condition in the laboratory.

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The temperature and RH were maintained at 26±1°C and 80±5% respectively till the emergence of moths from the seed cocoons.

The whole grainage operation was performed as per description given by (Krishnaswami *et al*., 1973). To observe the effect of temperature on the performance of *B. mori* larvae, an experiment was performed at different temperature regimes like 10, 14, 18, 26, 34, and 38°C. At 38°C larvae did not survive after the fourth instar stage. The experiments were conducted in BOD incubator separately one after another. The optimum condition of the experiments like 26±1°C temperature, 80±5% RH and 12 hrs light a day were taken as control for all the experimental designing were similar to Gaur and Upadhyay (2001). After four hours of mating moths were decoupled manually and transferred chronically to BOD incubator maintained 10°C (one of the six experimental temperature regimes), 80±1% RH and 12 hrs light a day. The egg laying moths were covered by open plastic cellules to prevent the intermixing of egg masses deposited by different female moths after 24 hrs of egg laying, the female moths were individually examined for their diseases freeness.

The disease free laying (DFLs) were washed with 2% formalin for 15 minutes to increase the adhesiveness of egg over card on the surface .Thereafter the egg sheets with egg laid on were thoroughly washed with running water to remove the formalin and the egg were dried in shade and transferred chronically transferred to specific experimental condition for further rearing.

To observe the effect of temperature variation on the protein contents present in the haemolymph of experimental *B. mori* were dissected at day 3rdof 4^{th}, 5^{th} instar larvae pupae and adult stage haemolymph was taken out. In the present study the estimation of protein formed in the haemolymph was made according to the method of Lowery *et al*. (1951) as modified by Singh and Agrawal (1989). The values of protein content in haemolymph were analysed statistically by two way ANOVA.

The data clearly demonstrate the changes in the level of the total protein content in the haemolymph of *B. mori* during different developmental stages. The total protein content in the haemolymph of fourth instar larvae was considerably influenced by the variation in rearing temperature regimes. With the gradual increase in temperature from 10 to 26°C, the total protein content increased from 16.40µg/mg at 10°C, to the maximum level of 28.74µg/mg at 26°C while further increase in temperature from 26 to 38°C caused gradual decrease in the total protein content which reached to the lower level of 21.55µg/mg at 38°C. Similarly the protein content in the haemolymph of fifth instar larvae was influenced considerably due to variation in rearing temperature. With the increasing temperature from 10 to 26°C, the total protein content increased from 16.67µg/mg at 10°C, to the maximum level of 33.81µg/mg at°C. But further increase in temperature above 26°C caused gradual decline in the total protein content, which reached to the lower level of 23.64µg/mg at 34°C. At 38°C larvae did not survive after fourth instar stage. The total protein content in the haemolymph of pupae was also influenced due to the variation in rearing temperature. With the variation in temperature from 14 to 26°C the total protein content increased from 12.46µg/mg at 14°C to the maximum level 18.01µg/mg at 26°C while further increase in the temperature from 26 to 34°C caused gradual decreased in the total protein content which reached to the level of 16.62 µg/mg at 34°C. At 10° and 38°C larvae were unable to pupate. Further the protein content in the haemolymph of adult was influenced due to varying temperature regimes. With the increasing temperature from 14 to26°C the total protein content increased considerably from 10.86µg/mg at 14°C to the maximum level of 16.76µg/mg at 26°C. At 10° and 38°C adults were unable to emerge. The total protein content in the haemolymph with the varying temperaturewas almost found to be of increasing trend from 10° to 26°C while decreased above 26°C. The maximum level 33.81µg/mg of total protein was recorded in the haemolymph, obtained from the fifth instar larvae, reared at 26°C. The minimum level 10.86µg/

Table 1 Effect of temperature on protein content (µg/mg) in the haemolymph of different stages of *Bombyx mori*

Stages	Protein content (µg/ mg) in the haemolymph at — Temperature (^{0}C)						$F_{1.}$ ratio n_1=5
	10	14	18	26	34	38	
IV instar	16.40±0.52	18.63±0.53	21.16±0.58	28.74±0.59	22.61±0.84	21.55±0.96	**5.26***
V instar	16.67±0.51	19.52±0.39	21.36±0.42	33.81±0.65	23.64±0.55	N.Sd	
Pupa	N.Sd	12.46±0.41	14.69±0.52	18.01±0.78	16.72±0.53	N.Sd	
Adult	N.Sd	10.86±0.31	12.92±0.13	16.76±0.34	15.10±0.14	N.Sd	

F_2 ratio = 5.67* n 2- 8 N.Sd= Not survived *P<0.025

Each value represents mean ± S.D of six replicates

mg of protein was recorded in the haemolymph, obtained from the adult reared at 14°C. Two way ANOVA indicates that the variation in the temperature and developmental stages have significant (P<0.025) influenced on the total protein content in the haemolymph of *Bombyx mori* (Table 1).

Silk is made up of two protein such as fibroin and sericin. Fibroin forms the core and is surrounded by sericine. These two differs in their characteristic and secreted from different parts of silk gland. A decreased protein content of haemolymph was recorded in *Rhodnius prolixus* at high temperature regimes (Okasha, 1964). Protein synthesis in the haemolymph was suggested to be stimulated at low temperature 15°C by increased neurosecretory activity in *Lucusta migratoria* (Clarke, 1967). In Drosophila melanogaster, protein concentration in the haemolymph seemed to increase marginally in insects reared at 25°C over those maintained at 15°C (Singh and Dass, 1982). By knowing the economic importance and convenience, silkworm has almost become an important tool for several biochemical physiological and genetic studies in insects. Physiological and biochemical studies includes metabolism an morphogenesis in insect, digestion and digestive enzyme, protein synthesis and their metabolism, hormones and their mechanism of action structure and function of chromosomes etc., for better productivity. Major biomolecules such as carbohydrates lipids protein, hormones and chromosomes etc play an important role in biochemical process underlying growth and development of insects (Ito and Horie, 1959). Metabolism and accumulation of these biomolecules in insect tissues during their development in different stages of life cycle was studied by Tanaka and Kusano (1980), Friedman (1985) and Bhattacharya and Kanwal (2004). The concentrations of these biomolcules mainly depend on mulberry leaf quality.

In different developmental stages of Bombyx mori the total protein content of haemolymph was significantly with the variation in temperature. The maximum level 33.81µg/mg of total protein content was noticed in the haemolymph of fifth instar larvae reared at 26°C while the minimum level 10.86µg/mg of that was recorded in the haemolymph of the adult reared at 14°C. Our investigation especially temperature affects the biochemical changes which affects the cocoon morphology as well as its stiffness and strength which we attribute to altered spinning behaviour and sericine curing time. Biochemical change affects cocoon colouration perhaps due to tanning agents. Finally the haemolymph content of cocoon modifies sericin distributed and stiffness without changing toughness. Our findings demonstrate environmentally induced quality parameters that must not be ignored when analyzing and deploying silk cocoon, silk filaments or silk-derived bio-polymers.

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REFERENCES

Bhattacharya A. and Kanwal B.B. (2004) Influence of mineral potassium permanganate on the biochemical constituents in the fat body and haemolymph of silkworm *Bombyx mori* L. International Journal of Industrial Entomology 9(1): 131-135.

Clarke K.U. (1967) Insects and temperature. In thermobiology, ed. Rose, A.H., Acad. Press, New York. p.332.

Friedman S. (1985) Intermediary metabolism. In: Fundamentals of Insect Physiology (Ed. Murry S. Bium). A Wiley – Inter Science Publication, USA. pp: 165-180.

Gaur K.P. and UpadhyayV.B. (2001) Effect of ecological factors on the hatchability of *Bombyx mori* L. egg. Journal of Advanced zoology 22(1): 18-21.

Ito T. and Horie Y. (1959) Carbohydrate metabolism of the midgut tissue of the silkworm *Bombyx mori* L. Archives of Biochemistry and Biophysics 80: 174-186.

Krishnaswamy S,, Narasimhanna M.N,, Suryanaryan S.K. and Kumar Raja S. (1973) Sericulture Manual 2. Silkworm Rearing F.A.O. Agric.Serves Bull., Rome. 15(2): 1-131.

Lowry O.H., Rosebrough N.J., Furr A.C. and Randall R.S. (1951) Cited in Colowick, S.P. and Kaplan, N.O.ed. Methods in enzymology and protein measurements with the follin phenol reagents. Vol.3: 448-450.

Mortimer B., Holland C. and Vollrath F. (2013) The forced reeling of *Bombyx mori* silk: separating behaviour and processing conditions. Biomacromolecules 14(10): 3653–3659

Okasha A.Y.K. (1964) Effect of high temperatures in *Rhodinus prolixus*. Nature, London. 204: 1221-1222.

Shimizu S. and Horiuchi Y. (1952) Relation between the number of silk cell and the amount of silk secretion in the silkworm *Bombyx mori* L. Nippon Sanshigaku Zashi 21 : 37-38.

Sinclair B. J. (2003) Climatic variability and evolution of insect freezing tolerance. Biological Reviews78: 181-195.

Singh D.K. and Agrawal R.A. (1989)Toxicity of Piperonyl butoxide carboryl Synergism on the snail *Lymnae accuminata*. International Review dergesamtem, Hydrobiologic 74: 689-699.

SinghS.P. and DasA.B. (1982)Cytological and cytochemical alternations in fat body due to amylase of cockroach *Periplenata americana* L. Camp. Physiology and Ecology 3(4): 187-194.

Tanaka and Kusano T. (1980) The haemolymph amylase activity during development of the silkworm *Bombyx mori* L. Journal of Sericulture Science Japan 49(2): 95-99.

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