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Influence of temperature on the free amino acids contents in the fat body of multivoltine mulberry silkworm *Bombyx mori* Linn.

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Abstract
Published studies of silks focus on processed fibres or the optimum conditions for their production consequently the effect of temperature on the biochemical properties of cocoon are either poorly understood or kept as closely guarded industrial secrets. Present investigation inferred that the total free amino acids content in the fat body has been influenced significantly by varying temperature. The maximum level 28.44 µg/mg of free amino acids was noticed in the fat body obtained from the pupae reared at 26 °C while minimum 10.12 µg/mg levels was recorded in the fat body of adult Bombyx mori at 14 °C.

Keywords: BOD incubator, Bombyx mori insect etc.

Introduction
Generally silkworm Bombyx mori L. is monophagous insect which character, feeds solely on mulberry leaves [1]. Silkworm larva obtains different amino acids from the mulberry leaves and uses to synthesize silk proteins secreted during spinning [2]. Proteins play an important physiological role in growth and development of silkworm and silk proteins synthesis. Necessary amino acids are derived from the amino acids present in body fluid in a free state and in the posterior silk gland cells [3]. Silk worm requires all the ten essential amino acids for growth and development of Bombyx mori L. Bombyx mori (race: nistari) is a resistant variety of multivoltine mulberry silkworm in the Northern Belt of India [4]. The ultimate aim of developing sericulture industry is heavy production of standard quality of raw silk which can sustain in the world market. Being an animal protein the synthesis of silk in the world market [5]. As animal protein the synthesis of silk in the fat body of Bombyx mori is directly influenced by the amino acids content in the haemolymph and silk gland [6]. Silkworm faces a wide range of temperature variation which may bring certain changes in the biochemical constituent like change in lipid level with the change in temperature [7] and the growth and survival of organisms [8]. The varying concentration of free amino acids has been noticed to influence the protein level in the silk gland, influencing the spinning pattern of silkworm [9-12]. The effect of temperature has been noticed to influence the various life activities in silkworm [13-16]. Thus it is hypothesized that variation in temperature may influence the level of total free amino acids in the fat body of Bombyx mori. Keeping this view an attempt has been made to study the effect of varying temperature regimes on the total free amino acids level in the fat body of Nistari race of Bombyx mori.

Materials and Methods
The seed cocoons of multivoltine mulberry silkworm (Bombyx mori) 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. 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 [17]. To observe the effect of temperature on the performence of *Bombyx mori* larvae, the 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 12hrs light a day were taken as control for all the experimental designing were similar to [18]. 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 24hrs 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 on the free amino acids content, the fat body of experimental Bombyx mori were dissected on the 5th day of fifth instar larvae and fat body was taken out. Estimation of total free amino acids in the fat body was made according to the method of [19] as modified by [20].

Statistical analysis

The values of free amino acids experiment were made the data obtained were analyed statistically by two way ANOVA.

Results

The data presented in (Table -1 and Fig-1) clearly indicates that variation in the rearing temperature influenced the level of total free amino acids in the fat body. The variation in the level of total free amino acids was also noticed with the variation in the developmental stages. The total free amino acids content in the fat body of IVth instar larvae was slightly influenced by the variation in rearing temperature. With the variation in temperature from 10 to 26 °C, the total free amino acids content increased considerably from 16.14 µg/mg at 10 °C to the maximum level of 23.28 µg/mg at 26 °C. But further increase in temperature above 26 °C caused gradual decline in the total free amino acids contents which reached to the level of 21.17 µg/mg at 38 °C. The total free amino acids in the fat body of Vth instar larvae was also influenced by the variation in the temperature. With the increasing temperature from 10 °C to 26 °C, the total free amino acids content increased from 18.59µg/mg at 10 °C to the maximum level of 28.44 µg/mg at26 °C. But further increase in temperature from 26 to 34 °C caused gradual decrease in the total free amino acids content. At 38 °C larvae did not survive after IVth instar stage. The free amino acids content in the fat body of pupae was influenced by the variation in the rearing temperature. With the variation in temperature from 14 to 26 °C the total free amino acids content increase slightly from 16.87 µg/mg at 14 °C to the maximum level 25.04 µg/mg at 26 °C, while above 26 °C rearing temperature gradual decline in the total free amino acids content was noticed which reached to the level of 22.40 µg/mg at 34 °C. At 10 and 38 °C larvae did not pupate. The free amino acids content in the fat body of adult stage was influenced by the variation in temperature. With the increasing temperature from 14 to 26 °C, the total free amino acids content increased slightly from 10.12µg/mg at 14 °C to the maximum level of 12.44 µg/mg at 26 °C. At 10 °C and 38 °C adult did not emerge out.

Table 1: Effect of temperature on free amino acid contents in the fat body of different stages of Bombyx mori.

Developmental stages	Temperature (^{0}C)						F1 ratio n1=5
	10	14	18	26	34	38	
IVth instar larvae	16.14±0.008	16.84±0.021	18.91±0.024	23.28±0.027	21.47±0.021	21.17±0.014	2.33*
Vth instar larvae	18.59±0.014	22.90±0.016	25.66±0.022	28.44±0.028	24.80±0.022	N.Sd	
Pupal stage	N.Sd	16.87±0.018	20.36±0.024	25.04±0.027	22.40±0.021	N.Sd	
Adult stage	N.Sd	10.12±0.008	10.48±0.016	12.44±0.028	10.91±0.021	N.Sd	

F2 ratio=1.84* n2- 3 N.Sd= Not survived * = Non Significant

Each value represent mean ± S.D of six replicate.

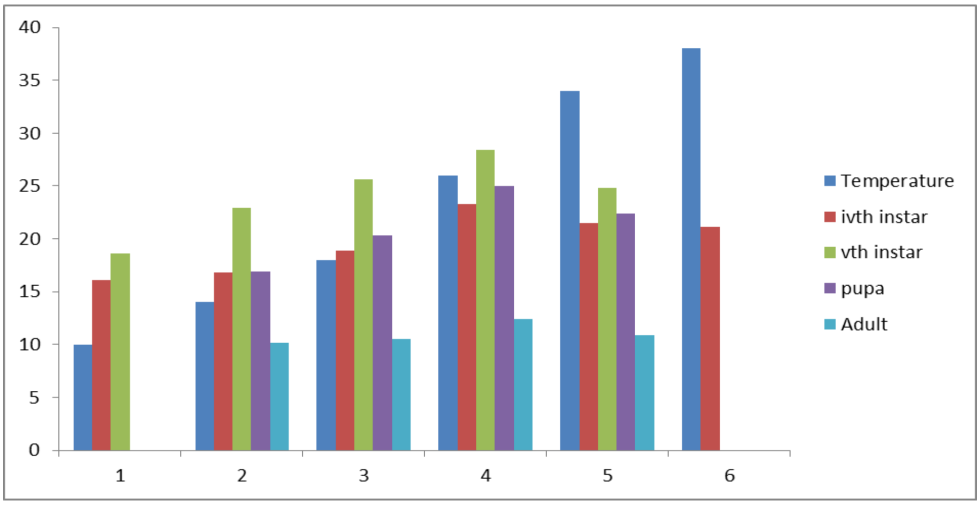

Fig 1: effect of temperature on free amino acid contents (µg/mg) in the fat body of different stages of Bombyx mori.

The trend of variation in the free amino acids content in the fat body with the increasing temperature regimes was of similar fashion in different development stages of Bombyx mori. The maximum level 28.44 µg/mg of free amino acids was in the fat body, obtained from the pupae reared at 26 °C, while minimum (10.12 µg/mg) level was recorded in the fat body of adult Bombyx mori at 14 °C. Two way ANOVA indicates that the variation in rearing temperature and developmental stages has no significant influence on the total free amino acids content in the fat body of Bombyx mori**.**

Discussion

All amino acids, common in protein, occurs in insects eggs in which the concentration of glutamic acids glutamine, alanine and glycine were noticed to be of high level [21]. The free amino acids pool was detected to be increase in the haemolymph and fat body of cockroach, due to thermal adaptation [22]. The thermal adaptation of ectoderms caused an increase in alanine concentration in the diapausing larvae (iiird and ivth instar) of Aractia caja due to its maintenance at low temperature [23]. A strict regulation of the amino acids in the fat body caused significant change due to the thermal acclimation of Periplaneta americana [24]. The decrease in glutamic acids contration was noticed on the basis of the increase transamination through increased glutamic aspartate activity in Drosophila melanogaster during cold adaptation [25]. The extent of 30% diminuation in amino acid /RNA ratio in the fat body and coxal muscle of nymphs and female cockroach was noticed during the acclimation to 15 °C as compared to 35 °C adapted insects [26].On the contrary, a significant increase of 47% was noticed in amino acid/RNA ratio in conjugation with 75% of protein accumulation due to low temperature. Silk is made up of two proteins such as fibroin and sericin. Fibroin forms the core and is surrounded by sericin. These two proteins differ in their characteristics and secreted from different parts of silk gland. Fibroin is secreted from the posterior part and sericin is secreted from middle part of silk gland. Fibroin is formed from the amino acids of posterior silk gland cells. Sericin quality is one of the important features of cocoon. Sericin is classified in various ways, but generally as α-sericin and β-sericin. α-sericin being presents in the inner layer of cocoon and differs from β-sericin present in the outer layer. Amount of sericin in cocoon varies in different strains of Bombyx mori L [27]. 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 general metabolism and morphogenesis in insects, digestion and digestive enzyme, protein synthesis and their metabolism, hormones and their mechanisms of action, structure and function of chromosomes etc., for better productivity. Major biomolecules such as carbohydrates, lipids, proteins, hormones and chromosomes etc., play an important role in biochemical process underlying growth and development of insects [28]**.** Metabolism and accumulation of these biomolecules in insect tissues during their development in different stages of life cycle was studied by many workers [29-34]. The concentrations of these biomolecules mainly depend on mulberry leaf quality. Protein content in haemolymph are at higher concentration during development and are useful in silk protein synthesis. Sericin is a protein produced by the silkworm, Bombyx mori, a holometabolous insect belonging to the Lepidoptera orderand Bombycidae family. Bombyx mori, which produces a great amount of sericin to the end of fifth larval instar and together with the fibroin, form the silk thread used in the production of the cocoon, structure that provides the ideal conditions fourth occurrence of larval metamorphosis to adults [35,36] working on Philosamia ricini have reported the decrease in amino acid content in the fat body of the larvae under cold stress conditions. Decrease in the free amino acid content of fat body may indicate the possibility of active feeding of amino acid in Kreb's cycle and glycolytic pathway to meet the emergent energy needs as well as their utilization in the production of some new proteins synthesized to cope with the low temperature stress.

Conclusion

From the present study it may concluded that the total free amino acids content in the fat body is influenced significantly by varying temperature. The maximum level (28.44µg/mg) of free amino acids was noticed in the fat body obtained from the pupae reared at 26 °C while minimum (10.12µg/mg) level was recorded in the fat body of adult Bombyx mori at 14 °C. Present investigation to rearers that specially temperature affects the biochemical changes which affects the cocoon morphology as well as its stiffness an strength, which we attribute to altered spinning behavior an sericin curing time. Temperature variation affects cocoon colouration, perhaps due to tanning agents. Finally the water content of a cocoon modifies sericin distribution and stiffness without changing toughness. Our results environmentally induced quality parameters that must not be ignored when analyzing and deploying silk cocoons, silk filaments or silk-derive bio-polymers.

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