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INFLUENCE OF TEMPERATURE ON THE TOTAL FREE AMINO ACIDS CONTENT IN THE HAEMOLYMPH OF MULTIVOLTINE MULBERRY SILKWORM, BOMBYX MORI LINN.

Zoology

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ABSTRACT

The present investigation focus on processed silk fibres or optimum condition for their production. Consequently the effect of temperature on the biochemical properties of cocoons are either poorly understood or kept as closely guarded industrial secrets. The variation in the temperature shows the biochemical changes in developmental stages of Bombyx mori, considerable effect on the total free amino acids content in haemolymph. The maximum level of free amino acids (6.96µg/mg) was observed in the haemolymph of Vth instar larval stage at 26°C temperature while minimum (2.12µg/mg) was recorded at 14°C temperature in the haemolymph of the adult silkworm.

KEYWORDS

Introduction:

Thermal acclimation in insects serves as biological clock indicating the temperature changes influencing their life cycle and distribution. The ultimate aim of developing sericulture industry for taking heavy production of standard quality of raw silk and making sustainability in world market. Silkworm require all the ten essential amino acid for growth and development of insect and different amino acid obtained by mulberry leaves and uses to synthesize silk protein secreted during spinning [1]. Bombyx mori is directly influenced by the amino acids contents in the haemolymph, because the animal protein synthesis occurs in their silk gland. The biochemical composition of silk is composed of two kind of protein fibroin and sericin. The fibroin and sericin proteins are the main constituents of the silk fibre. The principal amino acid component of fibroin is analine, glycine, serine and the sericin is composed of aspartic acid, glutamic acid, glysine respectively. The characteristic properties of silk is the low heat conductivity, moderate hygroscopicity high dyeing capacity, tenacity, elasticity, luster soft touch etc. The above character of silk depends on its chemical composition and physical structure. The silk gland is separate into three functionally different divisions as anterior, middle and posterior one. The sericin is synthesize and secreted by the cells of middle region where as fibroin is from posterior part of the gland [2]. The secrete fibroin is then transported and stored in the middle region of gland which is surrounded by sericin in fluid medium, the sericin as secreted by middle region acts as protective covering and fibroin is enclosed inside sericin and release threads of silk. During maturation period the silkworm extrudes the silk through spinnerets. Silk secretion is consider to be the excretion of surplus amino acid and the occlusion of spinnerets which prevent pupation [3]. The cells of the silk gland do not undergo divisions after hatching and growth of the gland is due to increased cell size [4]. The degree of growth shows two peaks. First one is just after hatching where as other one before half of the Vth instar [5]. There is a direct apart from the above studies it is also reported that correlation among the number of cells sizes of gland cells and the amount of silk production by amino acids and haemolymph [6]. Keeping this view, an attempt has been made to investigate the effect of varying temperature variation on the total amino acid contents in the haemolymph of Nistari race of Bombyx mori which affect the rearing and cocoon productivity.

Materials and Method:

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 Relative humidity 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 [7]. To observe the influence of temperature on the performance of Bombyx mori larvae, an experiment was conducted 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 above experiments were conducted in BOD incubator separately at different temperature. For all the experimental designing, a control group were also run at the optimal condition like 26±1°C temperature, 80±5%RH and quantity of light 12hrs [8]. After four hours of incubation the mated moths were decoupled manually and then transferred gently to the BOD incubator adjusted at 10°C (one of the six experimental temperature regimes), 80±1%RH and 12 hours light duration per day. The egg laying moths were covered with the help of the open plastic cellules to prevent the intermingling of egg masses which are deposited by different female moths and after 24hrs of egg laying all the female moths were individually and carefully the diseases freeness condition.

The disease free laying eggs were washed with 2% farmaline for 15 minutes to decrease the adhesiveness of egg over card on the surface. Thereafter the egg sheets along with eggs were thoroughly washed with running water to remove the farmaline. The dried egg were put in shade condition and then transferred carefully to specific experimental condition for further rearing.

To observe the effect of temperature variation on the free amino acids contents present in the haemolymph of Bombyx mori were succeeded at day 3rd, of IVth, Vth instar larvae, pupae and adult stage. Estimation of total free amino acids in the haemolymph was made according to the method of [9] as modified by [10].

Statistical analysis:

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

Result:

The data presented (Table-1 and fig-1) clearly indicates that the variation in rearing temperature influenced the level of total free amino acids in the haemolymph. The level of free amino acids was noticed to be changed slightly with the variation in different developmental stages. The total free amino acid in the haemolymph of IVth instar larvae was influenced notably due to variation in rearing temperature. With the increasing temperature from 10°C and 26°C, the total free amino acids content increased from 4.45µg/mg at 10°C to the maximum level of 6.60µg/mg at 26°C. But above 26 °C an increase in temperature caused considerable decrease in the total free amino acids content and reached to the level of 4.78µg/mg at 34°C.The free amino acids content in the haemolymph of Vth instar larvae was also influenced by the variation in temperature. With the variation in temperature from 10°C to 26, the total free amino acids content increased from the minimum of 5.03µg/mg at 10°C, to the maximum level of 6.96µg/mg at 26°C. But further increase in temperature from 26 to 34°C caused slight decrease in the total free amino acids content.

At 38°C larvae did not survive after IVth instar stage. The total free amino acids content in the haemolymph of pupae was also influenced considerably by the varitation in temperature. With the variation in temperature from 14 to 26°C the total free amino acids content

increased from 4.42 µg/mg at 14°C to the maximum level of 6.76µg/mg at 26°C. But further increase in temperature above 26 caused slight decrease in total free amino acids content which reached to the level of 6.09µg/mg at 34°C. At 10°C and 38 °C larvae did not pupate. The free amino acids content in the haemolymph of adult stage was influenced by the variation in the temperature. With the increasing temperature from 14°C to 26°C, the total free amino acids content increased from 2.12µg/mg at 14°C to the maximum level of 4.44µg/mg at 26°C.

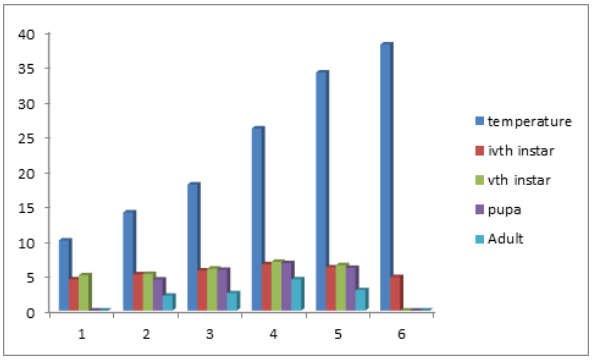
The trend of variation in the total free amino acids content in the haemolymph of all the stages was noticed to be increasing from lower to higher temperature which was maximum at 26°C. At higher temperature 34°C and 38°C the free amino acids content was of lower level.

Table 01: The table showed the effect of temperature on free amino acid contents in the haemolymph of different stages of *Bombyx mori.*

Developmental stages	Temperature (^{0}C)						F1 ratio n1=5
	10	14	18	26	34	38	
IV instar larvae	4.45 ±0.027	5.16 ±0.037	5.71 ±0.052	6.60 ±0.043	6.15 ±0.061	4.78 ±0.029	o.48*
Vthinstar larvae	5.03 ±0.008	5.22 ±0.017	5.97 ±0.025	6.96 ±0.021	6.47 ±0.013	N.Sd	
Pupal stage	N.Sd	4.42 ±0.008	5.81 ±0.014	6.76 ±0.050	6.09 ±0.014	N.Sd	
Adult stage	N.Sd	2.12 ±0.016	2.47 ±0.014	4.44 ±0.021	2.91 ±0.015	N.Sd	

F2 ratio=1.59* n2- 3 N.Sd= Not survived * = Non Significant

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



Free amino acid content (µg/mg) in haemolymph.

Fig 1: Effect of temperature variation on free amino acids in the haemolymph of different developmental stage of Bombyx mori.

Discussion:

At higher temperature the silkworms may be able to extend their bodies further leading to longer cocoons, which may in turn be used to regulate heat dissipation. A long, thin cocoon has a higher surface area to volume ratio than a short, round one hence an ability to vary cocoon shape as a response to different temperature condition during spinning could help maintain a suitable temperature range and by extension also different internal vapor pressure [11]. The changes in the amino acids content in the haemolymph apparently characterized the thermal acclimation in Periplenata Americana [12]. Increase in the amino acids level of the body fluid was noticed in the 4th instar larvae growth in Philosamia cynthia ricini [12, 13]. The total free amino acids content of the body fluid is enhanced during the late 4th instar by the thermal acclimation [14, 15]. The sharp increase in the total free amino acids content in the haemolymph was observed in the early days of spinning due to the hydrolysis of integument and gut protein [16] while decrease in the free amino acids of the haemolymph of Bombyx mori was noticed towards the end of the spinning [17]. Change in the free amino acids level was observed in the pupal haemolymph during the metamorphosis in Philosamia ricini [18]. Some of the amino acids of silk derive from mulberry leaves and some are synthesized in the body [19]says that more than 70% of the main component amino acids is elucidated as follows ; glycine derived from serine [20]. Serine derived from glycine or from 3-phasphohydroxypyruvic acid via phenylalanine, phasphoserine and alanine from pyruvic acid. The formation of glycine from glyoxylic acids take place in the silk gland in the alimentary canal and in fatty tissue while alanine is formed in the silk gland, alimentary canal and fatty tissue and silkworms have been shown to increase their rate of spinning at higher temperature and freed-spun silkworms show an increase in fiber and stiffness with increase reeling rate [21, 22]. During the late spinning process, the decrease in the free amino acids content of haemolymph was influenced by the thermal acclimation. Thus the temperature is the main factor that intensely has role just the stimulus is to induce the biochemical reaction. Temperature affects the biochemical changes, spinning behavior and cocoon crop. Our results demonstrate environmentally induced quality parameters that must not be ignored when analyzing and deploying silk cocoons, silk filaments or silk-derived bio-polymers.

Conclusion

Present investigation concluded that the variation in developmental stages of Bombyx mori has considerable influence in the total content of free amino acids. The maximum level (6.96 µg/mg) of free amino acids was noticed in the haemolymph of Vth instar larvae at 26°C temperature while minimum (2.12 µg/mg) free amino acids was recorded at 14°C in the haemolymph of adult silkworm. At 26ºC is the best temperature for cocoon development.

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