ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2017; 3(6): 1288- 1289
www.allresearchjournal.com
Received: 17-04-2017
Accepted: 20-05-2017

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Role of Photoperiod on temporal changes in free amino acid contents in the fat body of multivoltine silkworm, *Bombyx mori* Linn.

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Abstract
The Present investigation inferred that the total free amino acids content in the fat body has been influenced significantly by varying role of photoperiod. The maximum level 28.37 µg/mg of free amino acids was noticed in the fat body obtained from the fifth instars larvae at 12hrs light a day while minimum 13.25µg/mg was recorded in the fat body of pupae of *Bombyx mori*.

Keywords: *Bombyx mori* Larvae, BOD incubator, Rearing Trays, DFLs of egg, FAA, fat body of silkworm.

Introduction
Sericulture is the science that deals with the production of silk by rearing of silkworm. Silk is called the queen of textiles due to its glittering luster, softness, elegance, durability, and tensile properties and is discovered in China between 2600 and 2700 BC. Silk originating in the spittle of an insect is a natural fibrous substance and is obtained from pupal nests or cocoons spun by larvae known as silkworm. The silk is preferred over all other types of fibres due to its remarkable properties like water absorbency, heat resistance, dyeing efficiency, and luster. Factors mainly influence the physiology of insects are temperature and humidity. Despite wide fluctuations in their surroundings, insects show a remarkable range of adaptations to fluctuating environmental conditions and maintain their internal temperature and water content within tolerable limits. Photoperiod insect's serves as a biological clock indicating the seasonal changes and influencing their life cycle and distribution. The biological responses to photoperiod play and essential role in programming development sequences. Insect have exploited the photoperiod in their evolution of ecological, physiological, morphological and behavioral adaptation. The commercial silkworm L. has been reported as a short day insect by many researchers (Beckk, 1980 & Denlinger, 1985) [1, 2]. Almost all functions of *Bombyx mori* L. are being affected by photoperiod from hatching to cocoon characters. Silkworms are photosensitive and have a tendency to crawl towards dim light. Silkworm larvae reared at 12 or 24 hours light/day ingested significantly more leaves than those reared under continuous darkness (El-Shaaraway *et al.,* 1979a) [3]. Larvae grow faster and more uniformly when reared on artificial diet under 12 or 24 hrs illumination (Yan and Chiang, 1983) [10] has reported acceleration of growth in light condition. Larvae exposed to 0.4, 16 and 20 hrs of daily illumination had relatively heavier silk glands and produced heavier cocoons with high elasticity of silk than those reared in complete darkness (El-Shaaraway *et al.,* 1979b) [4] Rearing either in darkness or bright light leads to irregularity in growth (Krishnaswamy *et al.,* 1973) [7]. Thus, the adaptability to environmental conditions in the silkworm is quite different from those of wild silkworm and other insects. Temperature, humidity, air circulation, gases, light, and so forth, show a significant interaction in their effect on the physiology of silkworm depending upon the combination of factors and developmental stages affecting growth, development, productivity, and quality of silk. However, hardly any work has been published on the impact of photoperiod on free amino acid fat body. This study investigated the developmental changes of fat body free amino acids in *Bombyx mori* different developmental stages under various photoperiods.

Materials & Methods

Disease-free laying eggs of *Bombyx mori* L of multivoltine mulberry silkworm obtained from the silkworm grainage of Behraich Directorate of Sericulture, Uttar Pradesh and were maintained in plywood trays (23x20x5Cm) in the ideal rearing condition in laboratory. The temperature and RH were maintained at 26±1 ºC and 80±5% respectively till the emergence of moths from the seed coons.

Seed Cocoon: The seed cocoon (Pupa enclosed in silken case) of multivoltine mulberry silkworm *Bombyx mori nistari,* a native of West Bengal in India, were obtained from the silkworm grain age Behraich, Directorate of Sericulture, Uttar Pradesh, India and were maintained in play-wood trays (23 X 20 X 5 Cm) under the ideal rearing condition (Krishnaswami, S *et al.,* 1973) [7] in the silkworm laboratory. The temperature, photoperiod and relative humidity were maintained in BOD incubator at 26±1 ºC, 12±1hrs and 80±5% RH respectively till the emergence of moths from the seed cocoons.

Copulation: Moths have a tendency to pairs immediately after the emergence of silken case with releases Bombykol pheromone by female moth thus they allowed with their mates for copulation. A total of 200 pairs each containing one male and one female from newly emerged moths, were allowed to mate at 26±1 ºC, 12±1 hrs light and 80±5% RH condition. After four hours of mating, the paired coupled moths were decoupled manually. The male moths were dies after copulation and female dies after ovipositor of eggs.

Ovipositor: The gravid females laid eggs on the sheet of paper in dark condition at 26±1ºC temperature and 80±5% RH. After 24 hours of egg lying, the female moths were individually in mortar with pestles and blood smears were examined by microscope under magnification of 15X45 for the detection of bacterial, viral, fungal and protozoan pathogens. To the effect of photoperiod on the free amino acids content, the fat body of experimental *Bombyx mori* were dissected on the vth day of Vth instar larvae and fat body was taken out. Estimation of free amino acids in the fat body was made according to the method of (Spice, 1957 as modified by Singh Agraal, 1989) [8].

Result

The data presented in Table-1 and Fig-1 clearly indicates that variation in rearing photoperiod influenced the level of total free amino acids in the fat body. The variation in the level of FAA was also noticed with the variation in the developmental stages. The total FAA content in the fat body of fourth instars larvae was slightly influenced by variation in rearing photoperiod. With the variation in photoperiod from 6hrs to 12hrs, the FAA content increased considerably 12.68µg/mg at 6hrs light to the maximum 22.79 µg/mg at 12hrs light. But further increase in photoperiod above 12 hrs decreases the FAA acid content in fourth development stage. At fifth instars larval development from 6 hrs to 24 hrs the FAA content increased from 18.2568µg/mg at 6hrs to caused gradual increased in total FAA content and maximum 28.37µg/mg at 12 hrs. The FAA content in the fat body of pupae was influenced by the variant in rearing photoperiod. With the variation in photoperiod from 6 hrs to 12 hrs the FAA content increase slightly from 13.25µg/mg at 6 hrs and 19.99µg/mg 12hrs, while above light 12 hrs rearing gradual decline in FAA content was noticed which reached to the level of 15.29µg/mg at 18 hrs and 13.08µg/mg at 24 hrs. The FAA content in the fat body of adult stage was influenced by the variation in photoperiodic light. With the increasing photoperiod from 6 hrs to 12 hrs the FAA content increased slightly 15.25 µg/mg at 6 hrs, 17.10 µg/mg at 12 hrs, 14.55 µg/mg at 18 hrs & 11.97 µg/mg at 24hrs light a days.

Role of Humidity on Growth of Silkworm

Humidity plays a vital role in silkworm rearing and its role is both direct and indirect. The combined effect of both temperature and humidity largely determines the satisfactory growth of the silkworms and production of good-quality cocoons. It directly influences the physiological functions of the silkworm. The young-age silkworms can withstand to high humidity conditions than later-age worms and under such condition, the growth of worm is vigorous. The optimum humidity conditions required for different early-age worms and late-age.

Humidity also indirectly influences the rate of withering of the leaves in the silkworms rearing beds. Under dry conditions especially winter and summer the leaves wither very fast and consumption by larvae will be less. This affects growth of the larvae and results in wastage of leaf in the rearing bed. Retarded growth of young larvae makes them weak and susceptible to diseases. At a humidity of 90 percent or higher, if temperature is maintained at 26 °C–28 °C, they can grow without being greatly affected. Like temperature, humidity also fluctuates widely not only from season to season but also within the day itself. Therefore, it is necessary for the silkworm rearers to regulate it for their successful crop. For this purpose, wax coated (paraffin) paper is used to cover the rearing beds during young-age rearing to raise humidity and to avoid leaf dryness. Otherwise, wet foam rubber pads or paper pads soaked in water can also be used to increase humidity in the rearing beds. Rich famers can use humidifier with humidistat to regulate humidity in the rearing room. However, it is important to lower humidity to 70 percent or below during the moulting time in each instar to facilitates uniform and good moulting. Water forms a large proportion of insect tissues and survival depends on the ability to maintain and to balance water in the body.

Table 1: Role of photoperiod on temporal changes in free amino acid content (µg/mg) in the fat body of different developmental stages of *Bombyx mori.*

Developmental Stages	PHOTOPERIOD (Light a day)				F_1-ratio n¹ .5
	6hrs	12hrs	18hrs	24hrs	
IVth instar	12.68 ±0.031	22.79 ±0.032	18.80 ±0.035	13.92 ±0.038	0.718*
Vth instar	18.25 0.029	28.37 ±0.031	23.98 ±0.028	20.26 ±0.032	
Pupa Stage	13.25 0.029	19.99 0.030	15.29 0.034	13.08 0.036	
Adult Stage	15.25 0.028	17.10 ±0.021	14.55 ±0.027	11.97 ±0.028	

$F_{2\text{- ratio}}$=2.22* n2= 3 *= Significant $P < 0.005$

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

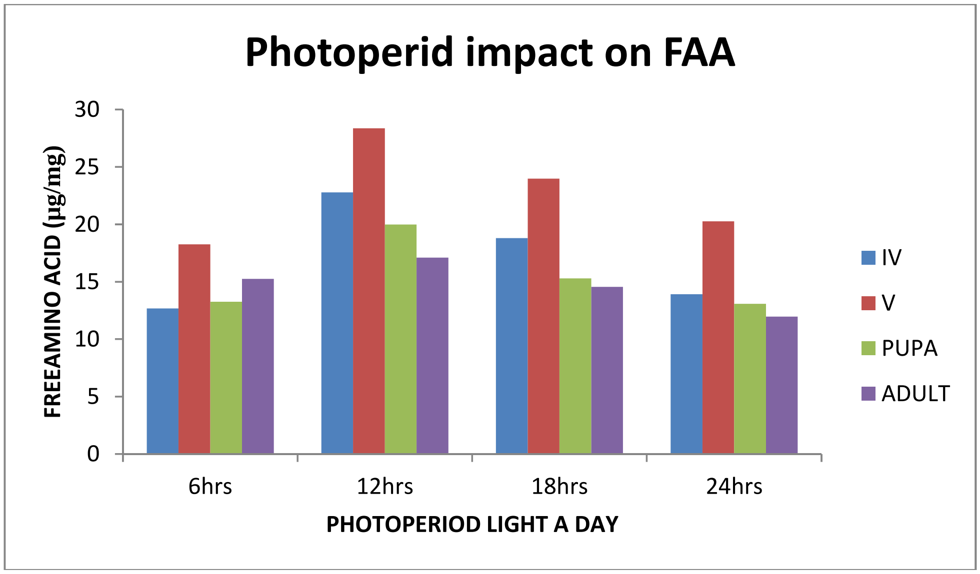

Fig 1: Role of photoperiod on temporal changes in free amino acid content (µg/mg) in the fat body of different developmental stages of *Bombyx mori*.

Discussion

Light has a vital role in silkworm rearing as they are photosensitive. The earlier reports have revealed that rearing of silkworm under different photoperiods influences its growth and cocoon yield. The results obtained from this study revealed that, rearing under complete light has a significant improvement in all the characters studied. 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 silk gland cells. Sericin quality is one of the important features of cocoon. Sericin is classified in various ways, but generally as ᾀ-sericin and β-sericin. ᾀ-ericin being presents in the inner layer of cocoon and differ from β-Sericin present in the outer layer. Amount of sericin in cocoon varies in different strains of *Bombyx mori* L (Ito, T., 1967) [5]. Sericin is a protein produced by silk worm, *Bombyx mori* L, a holometabolous insect belonging to the Lepidoptera order and Bombycidae family, *B.mori,* which produces a great amount of sericin to the end of fifth larval instar and together with the fibroin, formth silk thread used in the production of the cocoon, structure that provides the ideal conditions fourth occurrence of larval metamorphosis to adults (Singh., *et al.* 2010) [9] working on *Philosamia ricini* have reported the decrease in 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 photoperiods. With regard to cocoon characters also, larvae reared under complete light exited a highly significant improvement than all the other treatments. It has been reported by (El-Shaaraway *et al.,* 1979b) [4] that larvae exposed to 4 hrs , 16 hrs and 20 hrs of illumination had heavier silk glands and produced heavier cocoons with good reeling characters. This may be the reason for the improved cocoon characters in 24 hrs light treatment (Kogure., 1933) [6] has revealed that rearing under electric bulb light whole day decreases the disease incidence leading to a better survival rate and good cocoon yield. Though the light intensity is 2-7 lux, all characters are improved significantly in continuous light treatment. This is in agreement with (Denlinger., 1985) [2], who reported that light intensity has no role, just the stimulus is enough to induce the reaction. In the present study, in all characters 24 hours light treatment is closely followed by 6hrs light, 12 hrs light and 18 hrs light treatment. This indicates that a light regimes of 12 hours light temporal changes in free amino acids of fat body of silkworm is required for good cocoon crop yield.

Conclusion

Especially photoperiod affects the biochemical changes and also affects the cocoon morphology as well as its stiffness and strength, which we attribute to altered spinning behavior and sericin curing time. Biochemical changes affect cocoon coloration, perhaps due to tanning agents. Thus the optimum activity of the free amino acid in the fat body of fifth instars stage has a relevance to the process of silk synthesis, silkworm larval growth and its production, particularly during this stage and if the larva is optimum temperature of 26±1 ºC, 80±5% and 12±1 hours light a day there would be a further increase in productivity of silk. 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.

Acknowledgements

The authors thank Prof. Dr. V.B Upadhyay and Prof. C.P.M. Tripathi Ex. Head Department of zoology, University of Gorakhpur for providing laboratory facilities and linguistic improvement of manuscript, respectively.

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