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RHYTHMIC CHANGES IN EMBRYONIC GLYCOGEN LEVEL DURING INCUBATION PERIOD OF DIFFERENT VOLTINE GROUPS OF MULBERRY SILKWORM, *BOMBYX MORI* L

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Abstract : The study investigated the glycogen content in the silkworm eggs of different voltine groups of mulberry silkworm, *Bombyx mori* during the embryonic development and state of diapause. Glycogen, a vital polysaccharide serves as an energy reserve essential for morphogenesis and organogenesis. The research highlights the synthesis of glycogen and its dynamic role in physiological and metabolic processes during embryological development by analyzing five distinct voltine groups haemolymph of MU_{11} (Multivoltine), CSR_4 (Bivoltine), PM x CSR_2 (Crossbreed), FC_1 x FC_2, and FC_2 x FC_1 (Double hybrids). The study reveals significant variations in glycogen levels throughout the incubation period. Initial finding indicates that glycogen content is highest on the first day of incubation with FC_1 x FC_2 silkworm showing highest, followed by the rest of the silkworm. Notably, the MU_{11} silkworm race exhibited a 35.58% decrease in glycogen level compared to silkworm double hybrids. The study also confirms that glycogen levels decrease progressively over the incubation period across all silkworm breeds with double hybrids maintaining higher reserves than others. The findings underscore glycogen's dual role as both an energy source for embryogenesis and a metabolic resource during diapauses development, contributing to our understanding of adaptation strategies and potential implications for sericulture practices.

Keywords: Glycogen, voltine, diapauses and incubation period.

INTRODUCTION

Life existing on earth is naturally exposed to various extraordinary climatic conditions and seasonal variations. Insects are not an exception. Insects are imposed to adapt to vulnerable situations to tide over the unfavourable environment by entering into state of suspended animation referred as ‘diapause’ (Wheeler, 1893). The life cycle of insects includes four different stages of development such as egg, larva, pupa and imago. Each phase must be synchronized with specific seasons, biotic and abiotic factors. To achieve synchronization, insects have developed an ability to accept the seasonal response and to undergo preparatory physiological and behavioural changes. This adoptive anticipation is observed as token of stimuli that leads to diapause development (Fraenkel and Gunn, 1940). The striking feature of terrestrial insect is the moto of physiological and behavioural adaptations to seasons and changing environments. In most regions on earth, the physical and biological conditions suitable for growth, development and reproduction generally prevails only during particular seasons. Therefore, to synchronize metabolic activities to favourable times and to enhance survival during unfavourable conditions, many species undergo a state of dormancy or diapause as reported. (Wheeler,1893). Diapause, which is revealed by a number of morphological, behavioural, physiological and biochemical features (Beck, 1968 and Lees, 1968), is a widespread form of dormancy among insects and acarines. Its onset, maintenance, termination as well the post-diapause development period, the subsequent growth, development and reproduction with distinct phenological episodes in the life episodes of an insect can be observed.

Insect phenology, deals with recurring biological adoptive response in relation to key environmental factors, whose importance is now well recognized and identified for several reasons. The physiological and biochemical processes are not always static, but they progress in a predictable manner. Therefore, any study on ecological, behavioral processes to the natural situation must take into account, the dynamic physiological status and changing response of organisms throughout the phenological strategies. The phenology is fundamental aspect to understand the population growth and behaviour (Morris and Fultan, 1970), insect migration (Dingle, 1972), speciation (Alexander, 1964), species distribution (Messenger, 1959) and species interactions. The distribution of different species population depends on accurate forecasting of seasonal activity. Phenological parameters are a requisite for the efficient control of our biological resources. Modern insect control strategies include a systematic approach to insect pest constrains and a sound knowledge of insect seasonality (Giese *et al.* 1975). The quantitative informations facilitate to predict the correct timing of dormancy, development and reproduction in economically important pests in order to manipulate its genetics for greater good (Klassen *et al.*, 1970) or environmental factors (Hayes *et al.*, 1974) that influences diapause in order to suppress the pest populations. Insect phenology, the seasonal progression of diapause maintenance and termination, as well as the post-diapause developmental period, constitutes a series of vital, interrelated phenological events. It is necessary to re-evaluate current concepts concerning diapause maintenance and termination. Studies should be taken up of environmental stimuli that are important during diapause, dormancy as well the factors that influence its biochemical, neurophysiological or endocrinological attributes occurring during the dormancy and post-dormancy period.

Diapause characters is state of suspended animation are being observed in many groups of insects that are sustainable under unfavourable conditions in certain seasons of the year when the normal life is not possible. The diapause occurrence is stage specific in most of the insects. It's anticipated characteristic differs from cold torpor induced by low temperature. Insects responding to environmental factors during pre programmed life activities are divided into two kinds namely as "short day" and "long day" insects. In short day insect like *Sternocranus minutus* (Muller, 1960) long days induce diapause, whereas in long day insect like *Pieris brassicae* short days induce diapause (Danilevsky, 1965). The silkworm is a short day insect and diapause is induced by long photoperiod in certain races as reported. (Kogure, 1933). The photoperiod and temperature are the two extrinsic physical factors which vary in accordance with seasons of the year, more particularly in temperate regions. In many insects, exposure of early stages of development to short photoperiod and low temperature results in embryonic diapause. But, *Bombyx mori* L is an exception to that, a long photoperiodic regimes and higher temperature induces diapause and low temperatures arrest it. The combination of photoperiod and temperature revealed in a complex way to induce diapause in bivoltine species is maternally inherited (Toyoma, 1902; Kogure, 1933; Sonobe *et al*., 1986). The embryonic development, prior to blastokinesis stage strongly decides the type of silkworm eggs laid (Kogure, 1933). Exposure to long photoperiod and higher temperature of 28ºC leads to production of diapausing eggs. But exposure to short photoperiod and lower temperature of 15ºC leads to production of non-diapausing eggs. In *Bombyx mori*, the type of silkworm egg laid is decided by mother moth while it is in embryonic stage of development to favourable combinations of temperature and photoperiodic regimes and thus sensitivity is retained during early larval stage (Kogure, 1933). In *Antheraea pernyi* the sensitive stages for induction of diapause development are the last larval instar and early pupal stage (Williams and Adkisson, 1964).

Whereas in Aphid, *Megouraviciae* the female individuals are more sensitive when exposed to favourable conditions to produce diapause eggs in third generation (Lees, 1967). But in the case of *Sarcophaga argyroastoma* the complete larval period becomes sensitive leading to pupal diapause development (Denlinger, 1972).

Southern latitude has cold winter for about six months and duration of diapause is limited to 4 to 6 months. Thus, they have two generations in a year restricted by prolonged embryonic stage. The diapause can be altered by providing suitable environmental conditions in sensitive stage in the life cycle. Thus, diapause is observed in bivoltine silkworm breeds is always of facultative type proved and anticipated.

Insects programmed their behaviour of life as per the surrounding environment available to them in all the seasons. The lepidopteran insects survives northern latitude are exposed to persisting cold winters for prolonged periods. Hence, diapause development may be extended to 8 to 9 months and insects exhibits one life cycle per year (Muller, 1970 and Mansingh, 1971). In univoltines, the diapause may be obligatory type and observed at a correct stage irrespective of environmental conditions.

The literature survey showed numerous informations concerning to diapause maintenance and termination. Evidence reflects the current status of knowledge as fundamental aspects to understanding the diapause maintenance and termination. Photoperiod and temperature strongly influenced the rate of diapause development as a external environmental factors. Diapause is adoptive dynamic state, i.e., as the season progresses, diapause depth or intensity decreases a response to diapause-maintaining factors.

Some of the insect species have low temperature optima for diapause development during their autumnal – hibernal diapause (Andrewartha, 1952; Church and Salt, 1952; Danilevsky, 1965; Lees, 1955). The temperature ranges for diapause development in the laboratory may be considerably below or may overlap those for non-diapause development during late summer and early fall to a less or greater degree, slow the rate of diapause development under some circumstances and even reverse it (Church and Salt, 1952; Schneiderman and Horowitz, 1958). Thus diapause is maintained until lower winter temperatures drop below the threshold level for growth. Ultimately, this adaptation precludes untimely post-diapause development and premature mortality during the unfavorable season

There are numerous species in which autumnal hibernal diapause ends more quickly under warm than the under cold conditions in the cricket, *Acheta commodus* (Hogan, 1960), the flesh flies, *Sarcophaga argyrostoma* and *Sarcophaga bullata* (Denlinger, 1972; Force and Messenger, 1968); the Colorado potato beetle, *Leptinotarsa decemlineata* (DeWilde, 1969); *C. carnea* (Tauber and Tauber, 1973c); the south western corn borer, *Diatraea grandiosella* and the mosquito, *Aedes atropalpus* (Kalpage and Brust, 1974). In these species, temperature appears to play either no role or only a very small role in maintaining diapause and photoperiod is probably the primary diapause maintaining factor, at least during late summer and early autumn season. In summer, diapausing species have optimum temperature for diapause development that can be higher than that for non-diapauses development and growth as in case of mites, *Bdellodes lapidaria*

(Wallace, 1971). In contrast, it is seen to be lower than generally occurring summer temperatures in some lepidopterans *Mamestra brassicae* (Masaki and Sakai, 1965).

Keeping in view of the above facts, a experiment has been taken up to investigate the changes in glycogen content of developing embryo during incubation period and comparison of changes in glycogen in the different voltine groups of mulberry silkworm.

MATERIALS AND METHODS

a) Maintenance silkworm Races/Breeds

Silkworm eggs were chosen for the present investigation of comparative analysis of glycogen content during embryonic developmental period under standard room temperature of 25±1°C and relative humidity of 80% maintained till the day of egg hatching on 8^{th} or 9^{th} day.

The silkworm eggs of MU_{11} race represents multivoltine are being collected from Department Of Studies in Sericulture, University of Mysore, Mysuru; CSR_4 breed represents bivoltine are being collected from CSR&TI, Mysuru and PM X CSR_2 represents cross breed are being procured from Ramanagara. FC_1X FC_2 and FC_2 X FC_1 represents double hybrid were obtained from cold storage National Silkworm Seed Organisation, Mysuru for the investigation proposed and were incubated under standard room temperature. The silkworm eggs were maintained under standard laboratory conditions decribed by Tazima (1978) and Krishnaswami (1978) respectively. Plastic trays of 90 x 70 x 10 cm size were cleaned and disinfected with 2% formalin solution and covered with paraffin paper. Ten disease free laying's of each breed were maintained in each plastic trays provided with wet foam rubber pads to regulate required temperature (25±1°C) and relative humidity (80±5%). All the silkworm breeds were maintained with standard procedures till the completion of developmental period and quantification of glycogen content for comparative analysis.

b) Protocol for estimation of glycogen by using Montgomery method (1957)

100 mg of disease free silkworm eggs were homogenated in 10ml of 30% of KOH solution and boiled for 15 minutes in hot water bath. The contents were transferred to centrifuge tubes and centrifuged at 3000 rpm for about 10 minutes. Later, the residues were retained and added 0.1ml phenol and 5ml concentrated H_2SO_4. The contents were cool down to room temperature by leaving it for 30 minutes and developed crimson colour was read against blank at 495 nm in spectrophotometer.

$$\text{Amount of glycogen} = \frac{\text{Optical density of sample x mg of glucose taken}}{\text{Optical density of standard x mg of tissue taken}} \text{X1000}$$

RESULTS & DISCUSSION

Glycogen is a complex polysaccharide that provides energy for transmission, utilization and absorption during the morphogenesis and organogenesis in the egg stage. Hence, glycogen is referred as a dynamic biomolecules included in the physiological and metabolic developmental period of silkworm. It is an important component which is synthesized in the ovary itself from haemolymph trehalose (Yamashita, 1996).

Silkworm eggs provides an ideal material for examining the adaptability of glycogen metabolism and its role in diapause. Chino 1957 stated about the research that studies the changes in the glycogen content in silkworm eggs during diapause. The embryonic development in *B. mori* is interesting because in certain races the embryonic development proceeds uninterrupted wherein in some it is characterized by diapauses as in the case of univoltine and bivoltine silkworm breeds.

Glycogen content in developing diapausing eggs is about 1.5 times higher than that of non-diapause type eggs (Chino,1957; Yamashita and Hasegawa, 1985). Furthermore, the metabolic fate of glycogen in eggs is completely decided by diapause condition. In diapause eggs, Glycogen is converted into sorbitol at the initiation of diapause; but in non-diapause eggs, the glycogen remains unused until histogenesis occurs (Chino, 1958; Yaginuma and Yamashita, 1978). Thus, glycogen in silkworm eggs serves not only as a reserve for metabolic energy for progressive embryogenesis, but also as resource for diapause associated metabolism.

The five different voltine groups of mulberry silkworm were utilised for comparative analysis of the glycogen reserves in yolk of silkworm eggs. The glycogen content was significantly higher on the first day of incubation period of the developing embryo and then slowly decreased with the day to day developmental period in all the voltine groups of silkworm namely MU_{11} race (multivoltine), CSR_4 (bivoltine), PM x CSR_2 (cross breed), FC_1 x FC_2 and FC_2 x FC_1 (double hybrid). Differences in glycogen content were observed in the eggs of different voltine groups of silkworm during incubation period. The data analysis for glycogen content indicated that highest glycogen content was reported on first day in FC_1 x FC_2 at 2.41 g followed by FC_2 x FC_1 at 2.40 g, CSR_4 breed at 1.92 g, PM x CSR_2 egg at 1.74 g and MU_{11} race at 1.56 g. There is a decrease of 35.58% in multivoltine $MU_{11\ in}$ compared to double hybrid FC_1 x FC_2.

The MU_{11} race exhibited a lower glycogen level compared to all the other silkworm breeds, likely due to its geographical isolation within the peninsular Indian region. This finding is consistent with previous studies indicating that environmental factors can influence metabolic reserves in insect populations (Chino, 1957; Yamashita & Hasegawa, 1985).

The cross breed namely PM x CSR_2 also revealed the pattern of changes as MU_{11} race and CSR_4 breed however, the quantum of accumulation is relatively more in CSR_4 followed by PM x CSR_2 and least in MU_{11} race.

The two potential silkworm double hybrid such as FC_1 x FC_2 and FC_2 x FC_1 depicted the highest quantum of glycogen present during the incubation period among all the silkworm breeds. There was a slight difference of 0.45% between both the double hybrid breeds. Thus, FC_1 x FC_2 has the highest glycogen reserve among all the five silkworm breeds of different voltine groups.

These results are in concordance with Chino 1957. It was reported that the glycogen stored in the eggs of silkworm *Bombyx mori* L. was made use when energy required during the course of diapause as well as embryonic development. The level of synthesis of glycogen from day to day basis varies from breed to breed during embryonic incubation period was observed under laboratory conditions. Tribhuwan Singh *et al* (2013) reported the consistency and specificity observed during the diapauses stage within each species. On the other hand, in diapause eggs such as the univoltine and bivoltine, the growth and development are temporarily suspended and glycogen is being broken down and marked decrease in glycogen level also coincides with the onset of diapause. These observations agree with those of Chino (1957) and Yamashita *et al* (1975)

The developing embryo reveals the changes in glycogen content in terms of grams as it decreased day by day of developmental period of silkworm. This reduction indicates that while growth is suspended during diapause, metabolic processes continue to utilize glycogen for essential functions. Thus, throughout the incubation period, glycogen levels exhibited a consistent decline across all the voltine groups of silkworm.

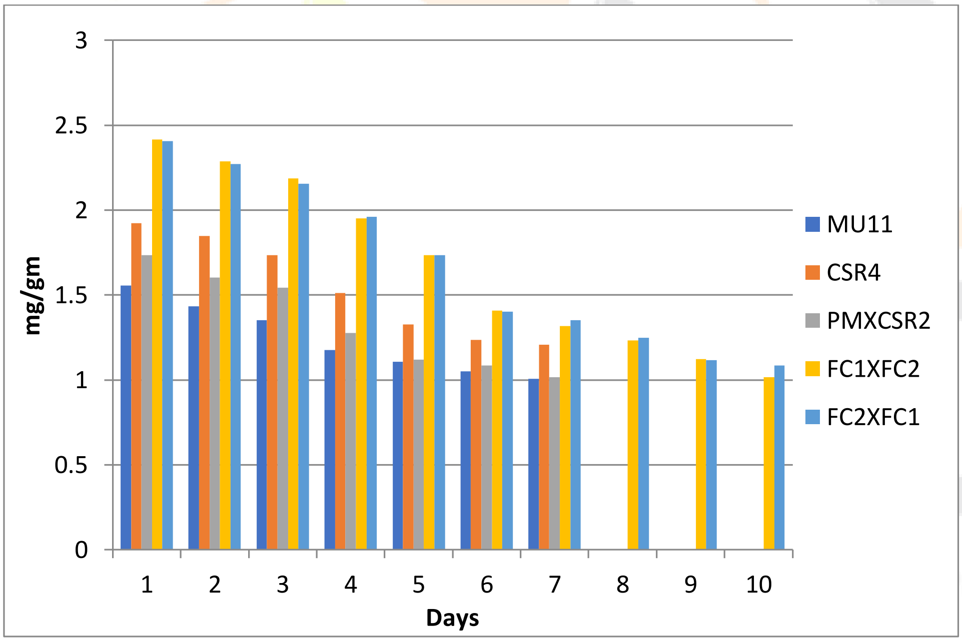
In FC_1 x FC_2 silkworm hybrid, the glycogen synthesized was highest on first day as about 2.42 g .Then there was a significant decrease of about 58.33% in glycogen synthesis over the ten-day period showing that glycogen may be an important source of energy during growth and differentiation in silkworm eggs. It indicates the maximum proportion of synthesis and accumulation from the day-1 of incubation period showing a tendency of a gradual decrease of day by day developmental period until egg hatching. Thus, reflecting utilization and mobilization of glycogen reserve food material for cellular and sub cellular physiological activities as it is required for the organismic changes in insect life. Similar trend of declining curve was noticed in all the voltine races from first to the last day of egg incubation. The results of present findings are on par with Karlson and Sekeris 1964. In many other insects, utilisation of glycogen has been found to exhibit a definite pattern which is indicative of metabolic regulation corresponding to the needs of growth and activity.

Similarly, quantification of glycogen level in FC_2 x FC_1 silkworm revealed highest on first day as about 2.41 g followed by a notable decrease of 58.84% on tenth day. CSR_4, PM x CSR_2, MU_{11}breeds exhibit the highest glycogen content on first day. Following oviposition, the glycogen content started decreasing markedly and it was reduced to 36.84%, 40.59%, and 33.33% respectively of the initial content around the 7th day. Least change of glycogen quantum was observed in the multivoltine MU_{11} among all the breeds. This gradual decrease reflects the mobilization of glycogen reserves to support cellular and sub-cellular physiological activities necessary for embryonic development.

Pant and Nautiyal, 1974 had similar views on the decrease in glycogen level during the incubation period as a general pattern in silkworm being reported in the eggs of *Philosamia ricini*.

BREEDS / Days	MU11	CSR4	PMXCSR2	FC1XFC2	FC2XFC1
1	1.557±0.015	1.924±0.008	1.735±0.018	2.417±0.012	2.406±0.009
2	1.433±0.009	1.847±0.016	1.603±0.010	2.286±0.015	2.271±0.006
3	1.353±0.016	1.736±0.021	1.542±0.007	2.187±0.009	2.154±0.022
4	1.176±0.011	1.513±0.009	1.277±0.006	1.951±0.010	1.960±0.013
5	1.108±0.013	1.328±0.023	1.118±0.070	1.736±0.017	1.737±0.011
6	1.053±0.010	1.236±0.007	1.086±0.022	1.407±0.006	1.402±0.18
7	1.007±0.003	1.209±0.009	1.016±0.011	1.316±0.008	1.351±0.017
8				1.231±0.016	1.247±0.008
9				1.122±0.005	1.116±0.007
10				1.017±0.009	1.085±0.008

Table 1: Change in glycogen contentof developing embryo of different silkworm breeds and hybrids from day 1 to day 10. (mg/gm of wet tissue)



Graph:1 Change in glycogen content of developing embryo of different silkworm breeds and hybrids from day 1 to day 10. (mg/gm of wet tissue)

CONCLUSION

Among all the silkworm voltine groups, the silkworm double hybrids namely FC_1 x FC_2 and FC_2 x FC_1 have the highest reserve of glycogen of 35.58% more than the MU_{11} which has the least glycogen content during the incubation period. Moreover, highest glycogen reserve was observed on the initial day of incubation following a gradual reduction in all the silkworm breeds. These findings underscore the importance of glycogen not only as an energy source for embryogenesis but also in metabolic processes associated with diapause, highlighting its adaptive significance in silkworm development in the present ecological barrier and suitability of silkworm breeds for concurrent organismic changes for the rhythmic adaptations.

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