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RHYTHMIC CHANGES IN LACTIC ACID CONTENT OF ACID TREATED HIBERNATED EGGS OF MULBERRY SILKWORM UNDER PHOTOPHASE AND SCOTOPHASE CYCLE

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ABSTRACT
In the present investigation, the developing embryo of the CSR_4 silkworm breed layings was imposed to longer photoperiodic regimes LD 16:8 under BOD incubator. The changes in lactic acid content of the developing eggs are analyzed in day to day developmental period and found that, a significant changes and positive response in relation light induction compared to the untreated control. The lactic acid content revealed rhythmic changes in CSR_4 breed the persistent however the day first to day 6^{th} and increase in the lactic acid but on 7^{th} day onwards level of decrease lactic acid contents were noticed. The significance of deposition of lactic acid content is an adoptive mechanism during the course of embryonic development under anaerobic conditions to tide over the unfavorable environmental condition. The present programme of the investigation attributes towards concept of photoperiodic implications on the lactic acid content in the developing embryo of the silkworm. During the imposed photoperiodic regimes the lactic acid content slowly disintegrated and activated aerobic respiration due to the increased intake of oxygen in the silkworm eggs proliferates and facilitated the rate of increase morphogenesis and organ differential attributed to the advancement of embryonic age. It has significant role in the adaptive mechanism under extreme environmental situations in hibernated eggs rather than non hibernated eggs of the, mulberry silkworm, *Bombyx mori* L.

KEYWORDS: Lactic acid content, hibernated eggs of the silkworm, Photoperiodic regime, *Bombyx mori*, CSR_4 silkworm breed

INTRODUCTION

The active progression of pre and postembryonic development of mulberry silkworm leads to the attainment of egg stage till the day of circadian rhythmic egg hatching. Hatching is the first behavior of rhythmic adoption manifested in the life cycle of an insect (Saunder, 2002). Among several developmental events, most important in the life cycle of the lepidopteran insect as it freely develops into larvae from constraints of its life process in the egg itself to fulfilling its biological development. Hatching has been referred to as the first developmental marker event in the life cycle of mulberry silkworm (Shanthan Babu, 2014; Srinath 2014) and a crucial aspect in commercial silkworm rearing (Ranjan *et al*; 1996). It is well established that, the daily rhythm in hatching in many insects is a gating event controlled by the circadian system (Beck, 1980; Saunder, 2002). Asin the other insects, hatching in *Bombyx mori* is a gating event regulated by a circadian oscillator (Anantha Narayana *et al*; 1978; Siva Rami Reddy and Sasira Babu, 1990; Siva rami Reddy *et al*; 1998). Reviewing the external signals that regulate the hatching in insects, Saunders (2002) confirmed that, major signals are light and /or temperature cycles, continued in constant temperature and relative humidity, *Bombyx mori* L hatching has been reported to be dependent on the voltinism for certain economic aspects such as hatching duration, hatching magnitude (Shanthan Babu 2014; Srinath 2014) through the basic aspects of hatching rhythmicity did not show any discrepancy. Therefore egg development and hatching rhythmicity and other economic parameters of popular silkworm namely, CSR_4 in the present experimentation under (16:8) photoperiodic schedules viz., Knowledge on the patterns of first developmental marker event in the life cycle. The hatching provides important input for successful economic and commercial hatching. Disease-free laying of extensively exploited commercial mulberry silkworm bivoltine (CSR_4) is introduced into two photoperiodic conditions viz., natural day (LD 12:12), continUous dark (DD) and continUous light(LL) conditions on the oviposition under a constant temperature of, 25 ± 1^0c and relative humidity 80%.The day to day development of till egg hatching. The results on hatching irrevocably proved that the hatching in CSR_4 under LD 12:12 condition treated as control, LL and DD, however, hatching rhythm continued its expression for consecutive days. The lactic acid content was also estimated and recorded on hatching duration in both control and LD (16:08) imposed batches. Embryonic diapause is observed as one of the two cycles in the bivoltine silkworm is characterized by cessation of embryonic development after the blastokinesis stage and sustaining embryonic life for a protracted-time period in a state of suspended animation. The metabolic profile of the embryo changes into characteristics diapause development,

which protects the embryo from vagaries of ambient low-temperature Glycolytic pathway which initiates oxidative metabolism of the glucose is channeled into polyol production at an early stage of glycolysis. The polyols produced in the embryo are 6-carbon sorbitol and 3-carbon glycerol which by virtue of their numerous hydroxyl groups prevent water crystallization in the cellular and intercellular fluids of the embryo. The changes in the preparatory activity in the larvae exposed to different photoperiod and temperature regimes in different selected races/breeds of the silkworm, *B. mori* L

MATERIALS AND METHODS

The popular bivoltine silkworm, *Bombyx mori* L. namely, CSR_4 was used and maintained for utilization of egg stage for the present study.

Maintenance of the silkworm Breed

The Disease-free laying's of the silkworm namely, CSR_4 (Bivoltine) about 450-500 eggs were obtained from NSSO, Mysore. The eggs were incubated at 25 ± 1^0c temperature and 80-85% relative humidity for about 9-10 days but on the 8th-day eggs were hatched, under LD 16:08 in a BOD incubator to study the effect of photoperiod in relation to lactic acid content in the developing embryo of the selected silkworm. On the other hand, control batches were (untreated) were maintained until the completion of the developmental period and quantification of the lactic acid content of the developing embryo for comparative analysis.

Schedule of photoperiod and temperature regimes

The experimental plan was designed and conducted to identify the sensitive stages in the life cycle to environmental stimuli by exposing the egg stage of selected constant temperature and Light (L) and dark (D) conditions. The silkworm eggs were exposed to selected LD cycle and temperature conditions as follows. The first batch of eggs namely CSR_4 (Bivoltine Breeds) were incubated under normal rearing conditions (LD 12:12) as control and second batches were exposed to longer photoperiod and day schedules of LD 16:08 cycle at 80% relative humidity and 25 ± 1^0C temperature was maintained.

Chemical Reagents

20% $CuSO_4$: 20gm of $CuSO_4$ is dissolved in distilled water boiled for some time, cooled, and diluted to up 100ml.

4% $CuSO_4$ solution: 4gm of $CuSO_4$ in 100 ml of distilled water.

$CaOH_2$ and H_2SO_4: 1gm calcium hydroxide

PHDP reagent: 1.5gm of PHDP is dissolved in 10 ml of 5%NaOH solution, warmed, and stirred for some time. It diluted to 100 ml. It is stored in a brown bottle fitted with a stopper.

Standard lactic acid solution: This is prepared from lithium lactate, which is anhydrous for the stock standard 0.384gm of pure lithium lactate is dissolved in 100 ml of water in a 1-liter volumetric flask. About 1 ml of concentrated H_2SO_4 is added and diluted to the mark with distilled water and mixed well. This contains 1mg in 5 ml (lactic acid) and it is stable if kept refrigerated.

Quantification of lactic acid content by Barker and Summerson method (1941)

The sample is de-protinised by the Foiln-Wu method at 1:10 dilution transferred 2 ml of protein-free filtrate, representing 0.1 ml is transferred to a centrifuge graduated to 10 ml. Similarly is a second test tube, 5 ml of standard lactic acid. In the third test tube, 2ml of distilled water is taken to represents the blank. To each test tube,1ml of 20%$CuSO_4$ is solution is added and diluted by adding 1ml of diluted water.1gm of powdered $Ca(OH)_2$ is added to each of the test tubes. Shaken vigorously and allowed to stand for 90 minutes. The test tubes were mixed once in the middle and they are centrifuged.1 ml of supernatant is transferred from each tube to other test tubes. Then 0.05 ml of 4%$CuSO_4$ solution is added followed by adding 6 ml of concentrated H_2SO_4 by a pipetted drop-wise. The contents of the test tube become hot cooling are not necessary. The test tubes are placed in boiling water for 5 minutes and subsequently cooled under running water. To each of the test tubes, 0.1 ml of PHDP reagent added drop by drop. The reagent precipitates and as an entry of H_2SO_4, it is immersed throughout the solution quickly and uniformly by lateral shaking. The tubes test is placed in a beaker containing water at a temperature of 30^0c and allowed to stand for 30 minutes to resistance the precipitating reagent at least once during this period. Finally, the test tubes are placed in boiling water for exactly 90 seconds and cooled at room temperature and optical density is taken at 560 nm.

Statistical methods employed - Analysis of variance

Analysis of variance or ANOVA is a method of testing the null hypothesis that several group means are equal in the population, by comparing the sample variance estimated from the group means to that estimated within the groups. Standard deviation (SD) and ANOVA were conducted. All the values below 5% are designated as significant (*) and those below1% level as highly significant (**) and those values above 5% level as non-significant (NS). All the statistical calculations were performed through SPSS for Windows (Statistical Presentation System Software, 1999, SPSS Inc., New York) version 10.0

RESULTS

The proposed program for the study of the influence of longer photoperiodic regimes on the lactic acid content in the silkworm eggs of CSR4 imposed on the above said physical stimuli. At the same time and untreated control batches were maintained under normal room temperature whereas, the photoperiod LD 16:08 or maintained in a BOD incubator from day 1 to till the day of egg hatching on the 7^{th} day. The quantum of lactic acid content is proportionately lower compared to the untreated batches. In each day of development of the embryonic events are indicated as 2.280 and 1.360 on the first day, 10.00 and 12.10 on the 2^{nd} day, 13.21 and 12.31on 3^{rd} day, 14.07 and 14.01 on the 4^{th} day, 17.28 and 16.47 on the 5^{th} day,19.07 and 17.55 on 6^{th} day,14.27 and 12.09 on 7^{th} day were observed respectively both in control and treated batches. As mentioned in table 1 and figure 1. The lactic acid plays a very important key role in the cessation of embryonic development. Preceding in diapausing eggs during anaerobic respiration. The glycogen content is a source for cellular differentiation, energy budget transformation in presence of oxygen. It is found that when there is a lack of availability of oxygen to the cellular functions, it is provided a static deposition of lactic acid during which the physiological and metabolic activity whole a period of time

in the embryonic development during dark conditions when the photophase is active phase provided the conversion of lactic acid slowly into glucose. It is derived during the entry of the metabolic pathway into the Krebs cycle activated to generate a large number of energy molecules or utilized for the cellular and subcellular functions.

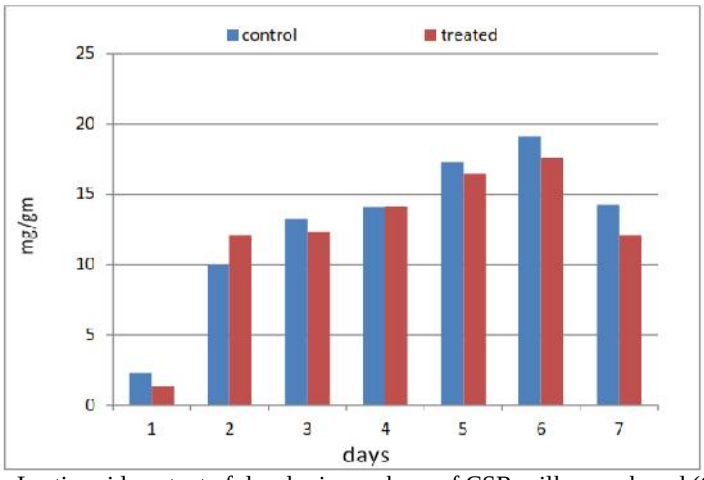

FIGURE.1. Changes in Lactic acid content of developing embryo of CSR_4 silkworm breed (Control) and (Treated)

TABLE 1: Percent change in Lactic acidcontent in developing embryo of CSR_2 silkworm breed.

Days	Control Mean ±SD	LD 16:08 Mean ±SD	Percent Change
1	2.280±0.06	1.36±0.275	-67.64%
2	10.00±0.366	12.10±0.511	17.35%
3	13.21±0.554	12.31±0.083	-7.311%
4	14.07±0.501	14.01±0.004	-0.43%
5	17.28±0.516	16.47±0.045	-4.41%
6	19.07±0.902	17.55±0.111	-8.66%
7	14.27±0.595	12.09±0.57	-18.03%

DISCUSSION

Hasegawa and Shimizu (1987) investigated in-vivo and in-vitro photoperiodic induction of diapause using isolated brain-suboesophageal ganglion complexes of the silkworm. They also reported that a bivoltine race shows a long day photoperiodic response after induction during the last instar. Diapause in relation to photoperiodism was reported by Kostal and Hodek (1997) in Tigermoth, *Cymbalophora pudica*. According to them embryos, larval instars, and pre-pupae were all sensitive to photoperiod and the responses of larvae and pre-pupae to changing photoperiods were similar. Watanabe (1997) studied photoperiodic control of the adult diapause, cold hardiness, and inositol accumulation in a beetle, *Aulacophora nigripennis* (Coleopterans: Chrysomelidae). The adults terminated diapause when transferred from outdoor conditions to a long photoperiod before February but remained in diapause when transferred to a short photoperiod. Effects of photoperiod and temperature in seasonal development and diapause egg oviposition in the bivoltine race (Diazo) analyzed by Tsurumaki *et al.* (1998). They reported that the development of adults is determined by photoperiod and temperature experienced during the embryonic and larval stages. The last instar larvae enter diapause in response to the dark phases longer than 9h. Vinogradova and Reznik (2002) determined the influence of a single change of photoperiod and female age on larval diapause in the blowfly *Calliphora vicina*. Maternally operating photos investigated the influences of the circadian component on the photoperiodic induce period determines the progeny inclination to the diapause, which is manifested depending on temperature. Fantinou *et al.* (2004) reported the reproductive responses to photoperiod and temperature by diapausing and non-diapausing populations of *Sesamia nonagrioides* and also oviposition was suppressed when insects are exposed to long days. Yamaoka and Hirao (1973) and Yamaoka *et al* (1976) studied the circadian rhythm of ovipositional behavior in *B. mori* by means of continuous tracing of egg-laying for 7 to 8 days under the artificial light regimes and under the light/ dark cycles. Fleugel (1978) explained that the oviposition rhythm in *Drosophila melanogaster*. Tsurumaki *et al.* (1999) investigated the effects of photoperiod and temperature on seasonal development and diapause oviposition in a bivoltine race (Diazo) of the silkmoth, *B. mori*.

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CONCLUSIONS

The developing embryo of silkworm breed mainly CSR_4 was selected and imposed on longer photoperiodic regimes of the LD 16:8 under the BOD incubator. The changes in the lactic acid content of the developing eggs were analyzed and found that significant changes and positive response compared to the untreated control. The lactic acid content of the developing embryo revealed rhythmic changes in CSR_4 the persistent change in day-to-day development however the day first today 6^{th} an increase in the lactic acid but the 7^{th} day onwards level of decreasing lactic acid contents. The significance of the deposition of lactic acid content as an adoptive mechanism during the course of embryonic development under the anaerobic condition to tide over the unfavorable environmental condition. The metabolic profiles of active embryo change into characteristics adaptive strategy in cellular and subcellular functions. The glycolytic pathway which initials the oxidative metabolism of the glucose is channelized in polyol production at the early stage of glycolysis. The present program of the investigation attributes the concept of the photoperiodic implications on the lactic acid content in the developing embryo of the silkworm namely CSR_4.The pattern of rhythmic changes of day to day alteration in lactic acid content as an adaptive mechanism to tide over the unfavorable conditions in the extrinsic environmental conditions. The cellular and subcellular active biochemical pathway of carbohydrate and fat metabolism tend to provide energy molecules in the form of ATP could imbibe into cells in order to execute the oxidative process under anaerobic condition in the organismic rhythmicity of the organism.

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