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CHANGES OF CARBOHYDRATE AND PROTEIN IN THE DEVELOPING HEALTHY AND PEBRINE INFECTED EMBRYO OF *ANTHERAEA ASSAMENSIS* HELFER

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ABSTRACT

That the changes of carbohydrates concentration of healthy eggs are found significant to two consecutive days of embryonic development. Gradual decrease from 1st days (34.599±1.11mg/gm) till 8th day (18.146±1.86mg/gm) and a step rise on 6th day (33.411 ±1.72 mg/gm) were noticed. The same trend were observed in total protein concentration of healthy eggs fro 1st day (166.63±1.98 mg/gm) till 8th day (107.058±1.42 mg/gm). However, it was noticed that infestation of Pebrine diseases influences the carbohydrate and protein as lower concentration have been recorded in both the metabolites from 1st day to 8th day. The variation in carbohydrate was found significant, however, only a marginal changes have been recorded in protein concentration. It is indicative that the infestation of Pebrine at embryonic stage is not a fatal agent for embryo. It was observed that although they hatches out as metabolic deficient larvae, lead to death in subsequent larval stages.

Keywords: Carbohydrate, Protein, Pebrine, embryo, *Antheraea assamensis*

INTRODUCTION

The most commonly known protozoan disease of silkworm is Pebrine and it occurs due to transovarian infection of microspore of Nozema species .In some cases the

pathogen is incorporated into the egg or embryo within the female's reproductive tract and the progeny from such infected females are also infected. But in winter moth, Opesopthera brumata (Lepidoptera), the embryo within an egg is not infected, but the larva subsequently becomes infected when the microsporidian spores are infested together with the remains of the yolk as the larva eats its way through the egg shell. In the transovarally transmission, the pathogen pass through the egg to the next generation (Primary Infection) and the larvae hatched from transovarally infected laying exhibit irregular growth and die in the rearing bed before 3rd moult and generally results in total loss of the crop **[1]**. The larva infected at later stages through consumption of infected matter is known as secondary infection. The larvae infected in this manner spin normal cocoon and undergo eclosion but deposit transovarally infected egg. The principal classes of organic compounds in insects are carbohydrate, lipid and protein and they present in considerable amounts in the insect egg. There is a series of well defined structural and biochemical changes within the oocytes leading to genesis of offspring. The newly laid eggs of silkworm are composed of protein (~10%), lipid (~8.5%), glycogen (~1.5%), chorion (18%) and water (60%) **[2]**. The constant energy requirement in insect during embryogenesis for growth, differentiation and maintenance of the cells is met by the stocks of lipids, carbohydrates and to a lesser extent of proteins. More than 95% of the total protein is yolk protein, which deserves special consideration as major constituent in insect eggs. These proteins are used as building materials for the embryos besides providing energy. Therefore optimum condition is required to balance all these components during embryonic development for healthy growth of larva. Infestation of Nosema species in muga silkworm *Antheraea assamensis* Helfer is an important mortality factor threatening the culture as well as seed production. Incidence of micro sporidian parasite infecting this silkworm is reported very high in summer and low in winter **[3]** with a crop loss of 20% / anum.

Extensive study on changes in concentration of protein, carbohydrate, amino acids, total nitrogen and fat in the developing healthy and Pebrine infected embryos of *B. Mori*, *Antheraea mylitta* were reported. Various attempts have been made to suppress the microsporidian infection in insects other than *Antheraea assamensis Helfer*. Being confined to narrow geographical strip of N.E. region of India, the scientific report on *A. assamensis* is very scanty. Therefore, it is attempted to study the changes of total protein and carbohydrate content in the

developing healthy and pebrine infected embryos of *A. assamensis*.

MATERIALS AND METHODS

Disease free cocoon were collected from the Central silk Board, Boko and kept at grainage house of IASST. Fertilized eggs were collected and maintained at 27 ±2°C in Petri dishes. The Pebrine infected eggs have been collected from the infected rearing field of Boko, Kamrup and kept separately under same laboratory conditions. Eggs from both the lots (weighed, cleaned with distilled water and chilled) were homogenized to 10% (w/v) tissue concentration. The homogenates were strained through nylon cloth to remove eggshells and was employed for the estimation. Carbohydrate and total protein concentration were estimated by the methods of **[4, 5]** respectively. The biochemical assays were done throughout the embryogenesis at 24 hours interval.

Statistical Analysis: Data are average of 10 replicas and calculated out the mean ± SD and student't' test has been analyzed for significant values (P>0.05).

RESULTS AND DISCUSSION

Changes of carbohydrate and protein in healthy and Pebrine infected eggs of *A assamensis* have been observed and are presented in the **Figure 1 and 2** respectively .The data were recorded at 24 hours of intervals during the complete period of embryogenesis until hatching. Result of all parameters show a uniform pattern of rise and depression with respect to healthy and infected eggs. Statistical analysis reveals that the changes of carbohydrate concentration are found significant in two consecutive days of embryonic development **(Figure 1)**. Concentration of carbohydrate **(Figure 1)** has decreased from 1st day of embryogenesis (33.948 mg/gm) till 8th day (14.742mg/gm) which is in conformity with the results reported by **[6]** in *Samia cynthia ricini* and **[7]** in *A. mylitta*. However, a steep rise is noticed on day 4th (27.643 mg/gm) as was reported by **[8]**. Blastokinesis is a most important phenomenon of embryonic revolution and it takes place on 6th day of embryo and organ formation starts subsequently. Perhaps for this reasons, embryo acquires more energy. Gradual decline on subsequent days indicates utilization on organ formation. This phase is most sensitive morphologically and physiologically. It has been observed that about 28% has decreased during the early stages (1-3 day) while 40% is utilized from days 5 - 8 and almost same trend has been reported by. It is indicated that the carbohydrate is used as an energy source particularly during early phases of embryogenesis before lipid oxidation prevails. The mobilization of glycogen reserves may again be important during last

stages when chitin biosynthesis required large amount of glucose **[9]**. Gradual depletion of carbohydrate concentration during embryogenesis suggests their utilization for embryonic process, metabolism and chitin synthesis **[6]**. Similar results have been reported during embryogenesis of *Philosamia ricini* and other insects. **[10]** reported initial increase in the level of reducing sugars in *A. myllita*. Significant variation of total protein concentration has been observed from the 1st day to the 8th day with a little rise on 6th day of development. This fluctuation suggests both break down and synthesis of organ specific proteins which is in conformity with the result reported by **[7]**. It is indicated that the depletion on 2nd day may be due to utilization for rapid DNA replication during the nuclear multiplication stage. It has been observed that only 35% of total proteins have been utilized as building materials for organogenesis during embryogenesis. This result suggests that the yolk proteins are less utilized for energy production due to the problems caused by production of nitrogenous waste as reported by **[11]**.

The carbohydrate content (~1.5%) is less than the protein content (10%) in the newly laid eggs **[2]**. In our present study, we have observed that almost all carbohydrate content (70%) has been utilized during embryogenesis, whereas more than 50% of protein remains unutilized and some yolk protein may persist until after hatching the larva. It is assumed that this protein may be utilized for early larval development when food intake is less. **[2]** reported that vitellin protein is utilized only at later stages of embryogenesis and about 40% of the initial amounts remained unutilized in hatched larvae of *Bombyx mori*. Infestation of Pebrine diseases influences the carbohydrate and protein as evident of low concentration recorded in this study **(Figure 1, 2)**. The variation in carbohydrate is found significant, however only a marginal change have been recorded in protein concentration. It is indicative that the infestation of Pebrine at embryonic stage is not a fatal agent for embryo but it hatches out as metabolite deficient larvae which lead to death in subsequent larval stages. **[1]** reported that the larva hatched from transovarially infected laying exhibit irregular growth and die before 3rd moult .Reduction of primary metabolites during embryogenesis due to pebrine infestation as recorded in this study is the main cause of death of the larvae resulting loss of the crop.

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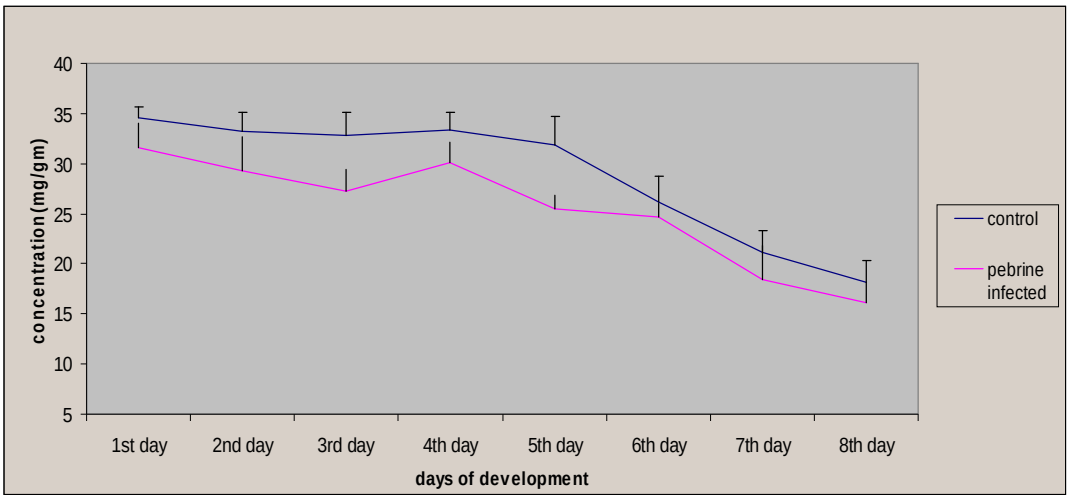

Figure 1: Shows Changes in the Concentration of Total Carbohydrate (mg/gm) During Embryo Genesis; The Data are Average of Mean ± SD (n=10)

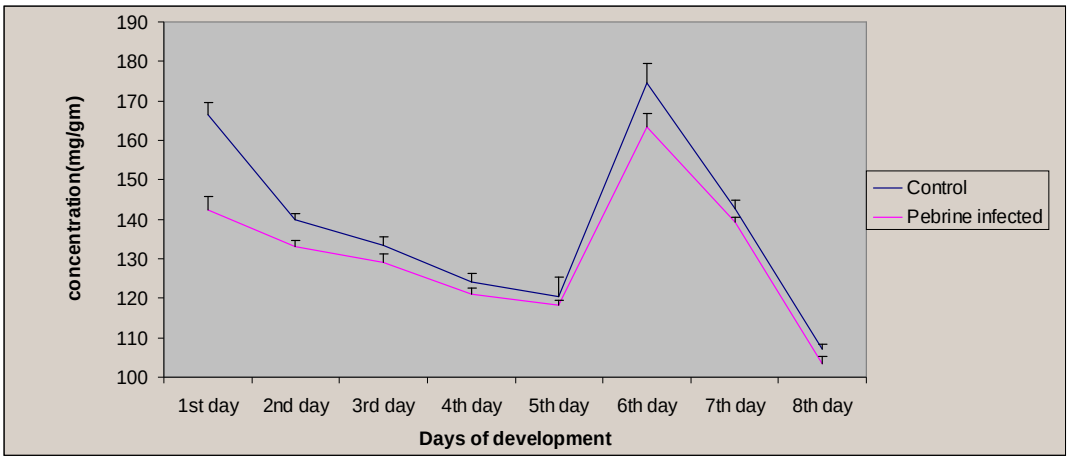

Figure 2: Shows Changes in the Concentration of Total Protein (mg/gm) During Embryogenesis; The Data are Average of Mean ± SD, (n=10)