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Starvation Induced Changes of Some Biomolecules in Eggs and Hatched Larvae of Indigenous Strain of *Bombyx mori* (Lepidoptera : Bombycidae)

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Variations in protein and nucleic acid concentrations were observed in 24 hrs old eggs and hatched larvae of Nistari strain, *Bombyx mori*, exposed to starvation. Three starvation treatments of 24, 48 and 60 hrs were given separately from 0 hr old fifth instar larvae. Biochemical variations were studied in the resultant hatched larvae of one time starved parent, while the eggs obtained from parents receiving starvation in two successive generations were considered for the study. In hatched larvae, protein levels in 24 hrs starvation groups remained significantly higher over control (never starved) while the same was found to be lower in 48 and 60 hrs starvation individuals. The RNA concentration remained significantly higher in all the treated lots. However, DNA content was not found to be significantly altered in hatched larvae after exposure to feeding stress. Protein, RNA and DNA concentration of 24 hrs old eggs produced by all the starved groups of Nistari, which had received two consecutive starvation during parental generations, showed higher concentrations of these biomolecules over control. Hence, starvation induced alterations in protein and nucleic acids in eggs and hatched larvae are indicative of a preparatory phase adopted by the insect to acclimatise itself and its progeny to stress situations.

Key words : Starvation, Protein, Nucleic acids, Egg, Larvae

1. Introduction

Environmental stress causes several behavioural, physiological and biochemical changes in insects (Chen, 1985; Downer, 1985; Scriber and Slansky, 1985). The limited food availability may select for rapid growth to a smaller body size (Robertson, 1965; Opler, 1978) whereas frequently encountered periods of temporary food shortage may select for larger body size (Slobodkin, 1965; Calow, 1977; Derr *et al.*, 1981). In *Bombyx mori* some studies have been made on the effect of food deprivation on quantitative and qualitative traits along with survivability after exposing the silkworm to starvation stress during first and fifth larval instars (Janarthanan *et al.*, 1994; Das *et al.*, 1996).

In addition, starvation during the last instar results in a lowering of silk production as it inhibits both the synthesis of total RNA and the translation of fibroin mRNA. Starvation also induces rapid RNA degradation as evidenced by the decrease in RNA content, as a result, protein synthesis decreases rapidly (Chavancy and Fournier, 1979). However, if starvation does not last beyond 36 - 48 hrs all these modifications may be reversed by food uptake (Prudhomme *et al.*, 1995). Moreover, insufficient feeding in the fifth larval instar causes production of smaller amount of eggs, owing to the sequential determinism of oocyte development along with decrease in the yolk protein content of the oocyte (Kawaguchi *et al.*, 1991; Palii and Klimenko, 1996). Informations concerning the biochemical / metabolic changes in eggs produced by starved larvae are scanty.

Therefore, biochemical studies were made in hatched larvae and eggs produced by the parents which were exposed to starvation for one generation in the former and two generations in the latter case, respectively. Freshly moulted fifth stage larvae were starved for 24, 48 and 60 hrs.

Materials and Methods

Insects and starvation treatment

Bombyx mori L. (race Nistari) were considered for the

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starvation experiment. Freshly moulted fifth instar larvae (0 hr) were subjected to food deprivation for 24, 48 and 60 hrs consecutively for one or two generations.

Assay of protein and nucleic acids

Freshly hatched larvae and eggs were subjected to biochemical estimation. A measured amount of hatched larvae / eggs from each treatment groups were taken separately to extract protein, RNA and DNA. A 5% tissue homogenate was first prepared in cold 0.65% saline using a Potter - Elvehjem all glass homogenizer and then the materials were precipitated with 0.3 N Perchloric acid (PCA). The RNA was extracted in 0.3 N PCA medium after hydrolysis with 0.3 N KOH at 37°C while DNA was extracted in 0.6 N PCA solution after incubating the precipitation at 70°C. The protein pellet was dissolved in 0.3 N NaOH solution after making it free from lipids. Extraction of the biomolecules were made following the method of Chaudhuri and Medda (1987). The protein was estimated by the method of Lowry *et al.* (1951). RNA and DNA were determined by the method of Munro and Fleck (1966) as modified by Abalain *et al.* (1980). The results were statistically analysed using Student t- test.

Results and Discussion

Hatched larvae from the eggs of 24 hr starvation lot of *B. mori* showed significantly higher ($p < 0.001$) amount of total protein over control but it was recorded to be lower ($p < 0.001$) in 48 and 60 hrs starvation exposed groups (Fig. 1). The parental generations of hatched larvae received single starvation during the fifth instar for different durations and then re-fed. Less protein contents in 48 and 60 hrs starved groups indicate that the parents of hatched larvae were not able to withstand the longer periods of food deprivation during the fifth larval age. It reflects in the significantly ($p < 0.01$ to $p < 0.001$) lower freshly hatched larval weight (Fig. 4). It was reported that longer starvation especially during the fifth instar leads to higher mortality (Janarthanan *et al.*, 1996) and production of less number of eggs by the survivors (Kawaguchi *et al.*, 1991; Palii and Klimenko, 1996). In 24 hrs starved lots, the higher protein content may be explained due to more consumption of food when silkworms were re-fed after starvation and thus leading to greater accumulation of energy reserves in the eggs. Protein provides the materials required for the production of mature eggs and stimulates the endocrine mechanisms which control their development (Applin, 1981). This then provides a favourable environment for the development of the larvae.

RNA content was found to be significantly higher ($p < 0.05$ to $p < 0.001$) in hatched larvae in all the treated groups over control (Fig. 2). DNA, however, remained unchanged (Fig. 3). High RNA content in individual hatched larvae is the indication of adaptive strategies developed by all the starved lots for resistance to stress by the next progeny. It was reflected in the yolk protein, RNA and DNA content of the eggs of second generation, where

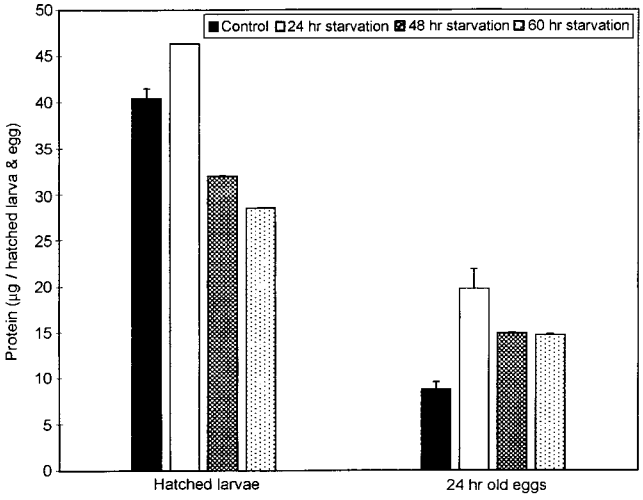

Fig. 1. Effect of starvation on total protein content of freshly hatched larvae and eggs of silkworm *B. mori*. The parental generation of hatched larvae received single starvation during 0 hr of fifth larval instar for different durations, while eggs were obtained from parents receiving two consecutive starvations. Each mean value is the average of five replications and vertical bars represent the standard error of the means.

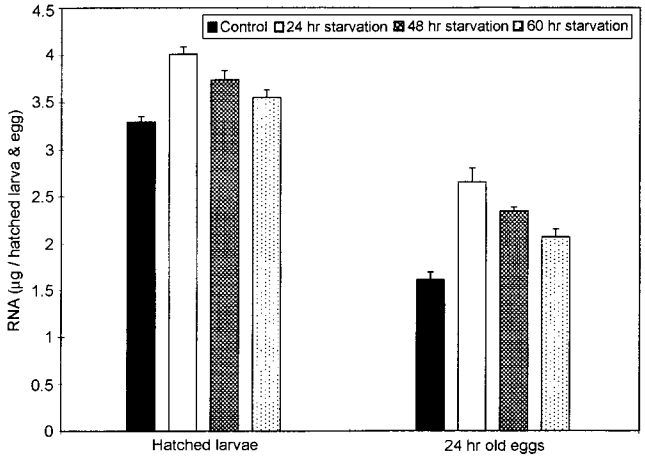

Fig. 2. Effect of starvation on RNA content of freshly hatched larvae and eggs of silkworm *B. mori*. The parental generation of hatched larvae received single starvation during 0 hr of fifth larval instar for different durations, while eggs were obtained from parents receiving two consecutive starvation. Each mean value is the average of five replications and vertical bars represent the standard error of the means.

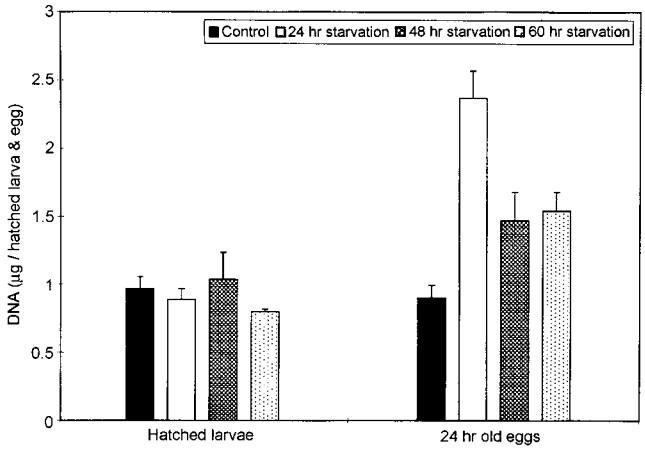

Fig. 3. Effect of starvation on DNA content of freshly hatched larvae and eggs of silkworm *B. mori*. The parental generation of hatched larvae received single starvation during 0 hr of fifth larval instar for different durations, while eggs were obtained from parents receiving two consecutive starvation. Each mean value is the average of five replications and vertical bars represent the standard error of the means.

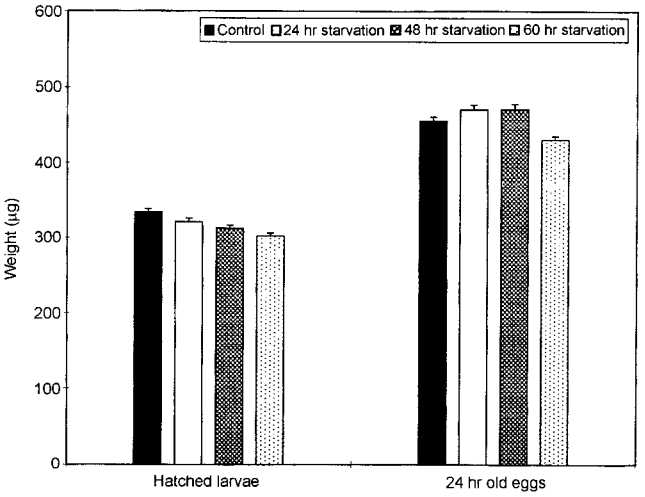

Fig. 4. Effect of starvation on weight of freshly hatched larvae and eggs of silkworm *B. mori*. The parental generation of hatched larvae received single starvation during 0 hr of fifth larval instar for different durations, while eggs were obtained from parents receiving two consecutive starvation. Each mean value is the average of hundred individuals and vertical bars represent the standard error of the means.

the fifth stage larvae were exposed to second consecutive starvation (Fig. 1,2 and 3). In eggs, all the biomolecular components studied were recorded to be significantly higher ($p < 0.01$ to $p < 0.001$). The egg weight was not much affected in starved lots receiving two consecutive feeding stress (Fig. 4). The stress induced alterations in the biochemical constituents studied in the hatched larvae

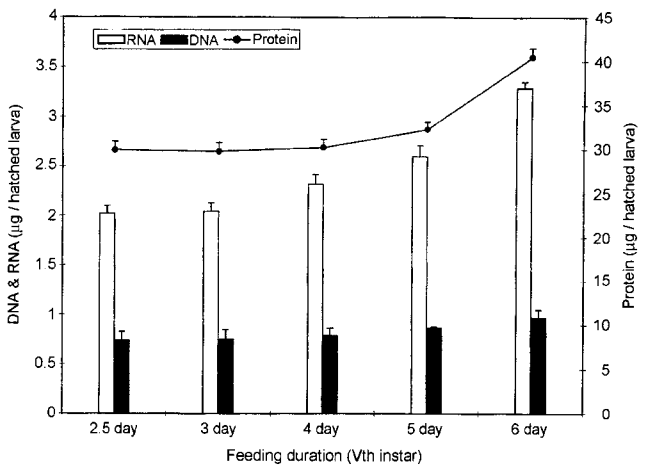

Fig. 5. Effect of starvation on protein, RNA and DNA contents of freshly hatched larvae of silkworm *B. mori*. The silkworms were allowed to feed during fifth instar for 2.5, 3, 4, 5 and 6 days respectively. 2.5 day is the obligatory feeding period during fifth instar for strain Nistari. Each mean value is the average of five replications and vertical bars represent the standard error of the means.

and eggs may therefore be due to the biochemical adaptations adopted by the insect after exposure to feeding stress to acclimatise the progeny to stress conditions in future. There is some indication that starved larvae may increase their feeding duration consumption rate when food again becomes available (Grabstein and Scriber, 1982), but more studies are needed to assess the generality of this behaviour. It should be mentioned here that the yolk precursor protein, vitellogenin is not affected by starvation (Kawaguchi *et al.*, 1991). Hence, fat body cells of the stressed larvae may synthesise more vitellogenin after re-feeding which is transported to mature oocyte to combat stress situations related to food deprivation in the next generation. The synthesis and transport of yolk precursor protein is regulated by the complex interaction of the neuroendocrine events (Yin *et al.*, 1989; 1990; Stoffolano *et al.*, 1992). The long-term effects of diet on the neuroendocrine system and egg development have been reported in a few insect species (Thomsen and Lea, 1968; Lea and Thomsen, 1969; Applin, 1979). However, feeding silkworm parents only for 2.5, 3, 4, 5 and 6 days during fifth instar results in gradual increment in the protein and nucleic acid contents in freshly hatched larvae of the subsequent generation over the 2.5 days fed groups where the least amount of these biomolecules were recorded (Fig. 5). It has also been observed that 5th instar larvae on feeding below 2.5 days for Nistari strain do not survive. Hence 2.5 days was considered as obligatory feeding period for this race.

These observations again strongly established that egg

development and oviposition in *B. mori* occur only when protein is sufficiently available. On the other hand, if starved worms are re-fed after a certain duration of starvation during obligatory feeding phase of fifth instar, then the biometabolic effects may revert.

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