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STUDIES ON METABOLIC ALTERATIONS IN PEBRINISED FIFTH INSTAR LARVAE OF TROPICAL TASAR SILKWORM *Antheraea mylitta drury* (DABA TV)

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ABSTRACT

Pebrine is one of the dreadful diseases seen in Anthereae mylitta drury – a tropical tasar silkworm causes significant yield loss. The present research has been carried out on pebrinised fifth instar larvae of 3rd and fifth day samplings to analyse the effect of pebrine disease on biochemical components like aminoacids, proteins and enzymes like alanine aminotransferase, aspartate aminotransferase of haemolymph, silkgland and fatbody. The results revealed that aminoacids of fifth day sampling have shown 4% increase in haemolymph and decrease in fatbody (15%) and silkgland (18%) whereas third day shown 2% increase (Haemolymph), 9% and 6% reduction in fatbody and silkgland. .Proteins of fifth day and third day samplings have shown a drastic reduction in the range of 17-21% and 13-16% in comparison with respective controls and corresponding tissues. Enzymes like alanine aminotransferase have shown significant increase in its activity in the range of 33.3-36% in fifth day sample whereas third day sample had shown 9-32%.Aspartate aminotransferase activity levels found increased in the range of 25-41% in fifth day sample and 15.4-39% in third day of respective tissues.

Keywords: Pebrine, Anthereae mylitta drury, Aminoacids, Proteins, Alanine aminotransferase, Aspartate aminotransferase, Haemolymph, Fatbody, Silkgland

INTRODUCTION

Anthereae mylitta Drury a lepidopteran insect of the Saturniidae family produces tasar silk of commercial importance. The environment and various diseases of silkworm are major constraints in tasar culture which reduced the crop yield and made the silk industry unreliable [1, 2]. Pebrine is one of the dreadful disease seen in Anthereae mylitta Drury (Daba TV ecorace), caused by intracellular parasite Nosema species. Pebrine can be acquired from the mother moth (primary infection) or through the contaminated environment (secondary infection). The infection accounts for 20-25% yield loss in Anthereae mylitta [3]. The infected larvae of Anthereae mylitta drury show significant changes in the cocoon weight, shell weight, denier, reliability etc. [4]. The growth of silkworm is correlated with the synthesis of carbohydrates, proteins, amino acids and enzymes like proteases, glutamate dehydrogenases and aminotransferases [5]. The infection causes many biochemical changes in the silkworm larvae with a change in metabolic pathways to defend against pathogen invasion [6]. A significant variation in the protein metabolism and nucleic acid metabolism was reported in virus infected silkworm [7]. Flacherie causes a considerable decrease in proteins, lipids and carbohydrates [8]. In the pebrinised embryos of Anthereae mylitta drury a drastic variation in

proteins was reported [9]. Stress also causes a change in biochemical components and transaminases in various tissues of Anthereae mylitta drury [10,11]. Parasitism by usifly in silkworm causes an increase in aminotransferase activity [12].

As biochemical compounds of silkworm under stress conditions used as an appropriate marker, the present work includes the study of proteins, amino acid and aminotransferases in various tissues of pebrinised fifth instar larvae of Antherea mylitta drury (DabaT.V).

MATERIALS AND METHODS

Isolation of Nosema spores and preparation of stock solution:

Pebrinised fifth instar larvae of Anthereae mylitta drury were collected from the forest patches of Jakaram,Andhra Pradesh,and used for isolation of Nosema spores. The larvae were homogenised in 0.6% K2CO3 and filtered. The filterate was centrifused at 3000 rpm for 15 min. The sediment was collected in 1ml distilled water and centrifused at 5000 rpm for 15min after addition of percoll (polyvinyl silica particles). The spores were collected from the sediment, washed in distilled water and stored in 0.85% NaCl as stock at 4^{o}C.Spore count was estimated by Neubaeur haemocytometer. Inoculam dosage of 1x10^{8} spores/ml was obtained by diluting the stock solution with distilled water.

Silkworm rearing:

Daba TV cocoons were collected as per the standard norms such as weight, colour, size of cocoons and length of the peduncle from the forest patches of Jakaram, Warangal District, Andhra Pradesh, India. The cocoons were preserved in the wire mesh cages of size 2 ft x2 ft x2 ft under temperature of 29±1 ºC and humidity 70 % ± 1 %. The emerged moths were tested for pebrine disease by a method derived from that used in sericulture [13]. In this method, the abdomen of an adult is severed with scissors, placed in a small mortar, mixed with water and crushed with pestle. A drop of the smear is placed on a clean slide and examined under a microscope of 600x magnification for Nosema sp., spores. Disease free layings were collected and incubated at 25- 30^{o}C temperature and 70-75% humidity.To study the effect of pebrine disease on biochemical components of 5th instar Anthereae mylitta drury larvae,the newly hatched first instar larvae were grouped as T1(Control)-Larvae reared on freshly cut Terminalia arjuna leaves and T2-,Larvae reared on Terminalia arjuna leaves treated with nosema spores of 1x10^{8} spores/ml.Each group had 100 larvae and reared separately at 28± 2^{o}C temperature and 70 - 75% humidity till fifth instar. Fifth day 5th instar larvae from T1 and T2 groups were used for experiment. Haemolymph was collected in the test tubes by cutting the proleg.1mg of Thiourea was added to the test tubes to prevent melanisation. After centrifugation at 15000 rpm for 15min the supernatant was collected and stored at -20^{o}C for further analysis. The fat body and silk gland was isolated in cold condition by using Bodenstein's Ringer solution. 20% (W/V) homogenates of fat body and silkgland were prepared in 50mM Tris -HCl Buffer (pH- 7.0) and was centrifuged at 10,000 rpm for 20 min. Supernatant was collected for quantitative estimation of biochemical components. Total protein and Aminoacids were estimated according to the standard procedures [14,15]. Enzymes Alanine aminotransferase and Aspartate aminotransferase were measured according to Thomas, 1998 [16].

Statistical Analysis

Each assay was replicated 3 times. Values were expressed as mean ± SE and Student's t-test was applied to locate significant ($P \leq 0.05$) differences between pebrinised and control groups.

RESULTS AND DISCUSSION

The results shown in Table 1 indicate that pebrine affects the levels of aminoacids in haemolymph, fatbody and silkgland of 5th instar larvae of both the sampling days ($p \leq 0.05$).When compared with the control, the level of aminoacids were found increased (2-4%) in the haemolymph of pebrinised larvae and recorded very high on 5th day sampling. Low transaminase activity or high proteolytic activity results in high amino acid content [17]. In comparison with the control the results show that there was a significant decrease in the aminoacid content present in the fatbody(9-15%)and silkgland(6-18%) and recorded very low in the pebrinised

larvae of 5th day. Cold stress causes an increase of aminoacids in haemolymph and decrease in fatbody of Anthereae mylitta drury [10]. It is known that in silkworm, energy deficiency due to stress is compensated by breakdown of proteins to aminoacids and enters the Krebs cycle as Keto-acid [18, 19].

TABLE-1 Effect of pebrine on aminoacids in haemolymph, Fatbody and Silkgland of Fifth instar larvae of Anthereae mylitta drury (Daba T.V)

Source	3rd Day		5th Day	
	T1 (Control)	T2	T1 (Control)	T2
Haemolymph (mg/100ml)	948.6±2.6	966.8±2.8 (2%)	972.4±2.8	992.6±3.2 (4%)
Fatbody (µg/100mg)	2.78±0.18	2.54±0.13 (-9%)	3.12±0.12	2.65±0.14 (-15%)
Silkgland (µg/100mg)	2.85±0.24	2.68±0.17 (-6%)	3.35±0.14	2.76±0.16 (-18%)

TABLE-2 Effect of Pebrine on Proteins in haemolymph, Fatbody and Silkgland of Fifth instar larvae of Anthereae mylitta drury (Daba T.V)

Source	3rd Day		5th Day	
	T1 (Control)	T2	T1 (Control)	T2
Haemolymph (mg/100ml)	19.4±0.8	16.6±0.6 (-14.5%)	24.8±1.2	20.6±0.5 (-17%)
Fatbody (mg/g)	98.8±1.08	85.8±1.2 (-13.1%)	112.4±2.2	88.7±1.2 (-21%)
Silkgland (mg/g)	115.6±1.05	96.6±1.4 (-16.5%)	134.5±1.8	108.5±1.6 (-19.4%)

The biochemical analysis carried out on haemolymph, fat body and silk gland of pebrinised 5th instar larvae showed a remarkable decrease (p ≤0.05) in the level of proteins (14.5-21%) (Table 2). A drastic reduction in the protein content was recorded in the fatbody of the pebrinsed larvae of 5th day sample. In silkworm reduction in protein content in the fat body was reported during NPV infection as it is important site for protein synthesis and sensitive to infection [7]. Philosamia ricini larvae under cold stress conditions have shown the decrease in protein content in the fat body and silk gland [20]. Studies on Chironomus riparius exposed to anoxia had shown a decrease in total protein content due to degradation into aminoacids as they contribute to energy in insect [21]. The decrease in silkgland weight and proteins in Anthereae mylitta larvae infected with Nosema sp. which finally reduces the silk production [22, 11].

Table 3 indicates an increase in Alanine aminotransferase activity in the haemolymph, fatbody and silkgland in 5th day sample of control and pebrinised larvae (p ≤0.05). In comparison with the control of 3rd day the pebrinised larvae of the same day have shown a considerable increase in activity in the range of 9-33% in haemolymph, fatbody and silkgland. A range of 28.5-36% activity increase was recorded in 5th day pebrinised larvae in respective tissues compared to same day control. It has been reported that silkworm larvae under stress conditions like coldstress, parasitism, treatment with pesticides and hormone analogues show variations in the enzyme activities [11,12].

TABLE-3 Effect of Pebrine on Alanine aminotransferase in haemolymph, Fatbody and Silkgland of Fifth instar larvae of Anthereae mylitta drury (Daba T.V)

Source	3rd Day		5th Day	
	T1 (Control)	T2	T1 (Control)	T2
Haemolymh (µmol of pyruvate/hr/ mg protein)	0.24±0.08	0.32± 0.09 (33.3%)	0.28±0.08	0.34±0.04 (36%)
Fatbody (µmol of pyruvate/hr/ mg protein)	0.18±0.04	0.2±0.06 (9%)	0.21±0.06	0.27±0.02 (28.5)
Silkgland (µmol of pyruvate/hr/ mg protein)	0.20±0.04	0.23±0.05 (15%)	0.22±0.06	0.29±0.06 (32%)

TABLE-4 Effect of Pebrine on Aspartate aminotransferase in haemolymph, Fatbody and Silkgland of Fifth instar larvae of Anthereae mylitta drury (Daba T.V)

Source	3rd Day		5th Day	
	T1 (Control)	T2	T1 (Control)	T2
Haemolymph (µmol of pyruvate/hr/ mg protein)	0.18±0.06	0.26±0.08 (39%)	0.22±0.08	0.31±0.04 (41%)
Fatbody (µmol of pyruvate/hr/ mg protein)	0.13±0.04	0.15±0.05 (15.4%)	0.16±0.04	0.2±0.06 (25%)
Silkgland (µmol of pyruvate/hr/ mg protein)	0.14±0.06	0.19±0.07 (36%)	0.18±0.06	0.21±0.08 (39%)

Table 4 indicates an increase in aspartate aminotransferase activity in the haemolymph, fat

body and silk gland in 5th day sample of control and pebrinised larvae (p ≤0.05). Pebrinised larvae of 3rd day have shown an increase in enzyme activity in the range of 15.4-39% and the same pattern was observed in 5th day larvae (25-39%) compared to control. Alanine aminotranferase activity is an index for amino acids breakdown and Aspartate aminotransferase activity is a sign for entrance of amino acids to glucogenesis process [23]. The decrease in amino acids in the fat body and silk gland could be their conversion to pyruvate by enzymes. The increase in activity of aminotransferases in turn increases the supply of precursors to Krebs cycle which finally results in energy production [20].

CONCLUSION

The biochemical components like amino acids were increased in pebrinised fifth instar larvae whereas proteins got reduced. The activity of the enzymes like alanine aminotransferases and aspartate aminotransferases was increased in the pebrinised larvae.

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