ORIGINAL RESEARCH PAPER

Agricultural Science

BIOCHEMICAL CHANGES IN MIDGUT TISSUE OF SILKWORM *BOMBYX MORI*, DURING GRASSERIE INFECTION.

KEY WORDS: Silkworm, midgut, proteins, carbohydrates, Grasserie.

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ABSTRACT

The silkworm, *Bombyx mori* is a quite delicate venture and might be easily susceptible to, Grasserie which is one of the most serious viral diseases of silkworms. During infection, the understanding of quantitative biochemical responses in the body is very important for discussing many biological stresses and proved to be an appropriate system for studying effects of the disease. For the present study, the silkworms were collected from the local sericulture units in Akola district and quantitative changes in midgut tissues biochemical like, total proteins, Carbohydrates, Cholesterol, free amino acids, Urea, and Uric acid, were estimated in 5^{th} instar of silkworm, *Bombyx mori,* infected with Grasserie, at early (2^{nd} day of 5^{th} instar and late infection 5^{th} day of 5^{th} instar. We reported that, in early and late infection with Grasserie the protein content, total free amino acids, glucose, cholesterol content and urea of mid gut tissue in infected silkworm was lowered, as compared to the control healthy non-infected silkworms of the same age. Uric acid however in early infection stage had non-significant changes, but it is too significantly reduced during late infection. The results recorded were discussed in the light of relevant literature.

INTRODUCTION:

The silkworm, *Bombyx mori* is a purely domesticated insect since, 4,500 years but like other domesticated animals it is a quite delicate venture and might be easily susceptible to a number of diseases, most of which develops seasonally (Govindan and Devaiah, 1998 and Prasad, 1999). Grasserie is one of the most serious diseases of silkworms, though occurs throughout the year, its intensity varied with seasons. It is also known as the 'hanging disease'. Caused by Borrelina Bombycis virus, of the family Baculo-viridae causes this disease. The seasonal infection of Grasserie causes patho-physiological changes, at both early and late stages of disease attack. In this infection the virus multiplies and forms polyhedra in the nucleus of infected cells. On incidence of infection the haemolymph as well as the tissues gets affected and shows alterations (Watanabe, 1971). Maratignoni (1964) and Shigematsu and Noguchi (1969) reported that pathogenic infections induce biochemical and physiological alterations in insect and Mahesha *et.al.(*2009 : 2013) explain the same in silkworms. Juliana *et al.,* (2013) suggested that, pathogens for Grasserie released virions into the alimentary system and cross the peritrophic membrane. They combine with midgut epithelial cells and enter into the nuclei, starting the first cycle of viral production and replication. These processes cause many biochemical changes in larvae, which respond to these biological phenomena by changing many of its metabolisms to defend themselves against pathogen invasion (Etebari *et al.,* 2007). The investigation of changes in body fluid and tissues is an appropriate system for studying effects of pathogenic disease. The understanding and identifying these biochemical changes will be very important for discussing many biological stresses. Therefore, it is suggested by Gao *et al.,* (2006) that the determination of the biochemical responses in silkworm against pathogenic diseases could facilitate the control of agricultural pests. Hence, we carried out the present study to understand the specific biochemical responses of midgut tissues during attack of Grasserie infection.

MATERIAL AND METHOD:

The diseased larvae are identified and collected from various sericulture units in the district and were continued to rear on mulberry leaves at 25^{0} C. The quantities analysis was made in the midgut tissues of fifth instar larvae, beginning from the newly molted stage (day one) and continued till the 6^{th} day of the instar. The larval period was divided into two chronologically identified states as early experimental stage on day one and late experimental stage on day five of 5^{th} instar. The midgut was dissected and collected from the control non-infected healthy worms and that from the diseased worms. Constituents like, total proteins, Carbohydrates, Cholesterol, free amino acids, Urea, Uric acid, were estimated in non-infected, healthy silkworms and the silkworm infected with Grasserie. Midgut tissue of the all early and late experimental silkworm were then used to prepare tissue homogenates (20% w/v) in 50 M Tris-HCl buffer (pH 7.0) in a homogenizer. The homogenate was than centrifuged at 10,000 rpm and 4°C for 30 minutes. Supernatant was collected and used for quantification of all the major biomolecules. So was transferred to new tubes and kept at -20°C until the commencement of experiments. An ELICO Clinical chemistry analyzer CI 162 and prescribed assay kits were used for the estimation of all the biochemical constituents.

RESULTS AND DISCUSSION:

Total Proteins: *(Table.1)*

In insects, the most important place for protein synthesis is midgut that is also the most sensitive tissue to Grasserie causing virus in silkworm. In early infection with Grasserie the protein content of mid gut tissue in infected silkworm was 18.71 % gm, as compare to the control healthy non infected silkworms of the same age, which was observed to be 25.28 % gm. As the infection progresses on 5^{th} day of inoculation a significant decrease of the total proteins was reported as compared to the control healthy non-infected silkworms of the same age. As shown in the table the total mid gut tissue proteins in infected silkworm at 5^{th} day of 5instar larva was 25.53 % gm, which is significantly lowered as compared to control was 38.56 % gm. In a similar study quantitative and qualitative changes in protein profiles of various tissues of tropical tasar silkworm *Antheraea mylitta D* was studied by Kumar *et al.,* (2011). They reported decreased protein trend in diseased larvae and suggested that it may be due to drastic degradation of structural proteins. But interestingly there was no increase in amino acid content it means that the proteins degraded might be utilized by pathogen for rapid development. In search of causes of reduction in proteins in infected host Goninan *et al.,* (1998) found that it may be as a consequence of infection and also selective utilization of specific protein fraction by infectious pathogen.

Carbohydrate (Glucose):

According to Chino and Gilbert, (1965), Carbohydrates are known to serve as main source of energy to a number of insect species and in the biological system. This biochemical constituent forms a predominant carbon source of chitin, a participant in energy metabolism and the substrate for protein and lipid synthesis in insects. A breakdown of this according to Manohar Reddy (2004) is mainly essential to meet the energy under stress condition. The quantification of glucose carried out on mid gut tissue of 5th instar larvae at early infection with Grasserie showed no remarkable alteration in the level of glucose as 7.01% mg as compared to control 7 .09 % mg. However, a drastic reduction in the glucose content 4.73 % mg was recorded in the mid gut tissue of the 5^{th} instar larvae at late infection with Grasserie as compared to the control of the same age was 7.53 % mg. The decrease of glucose at the end of larval instar may be due to the decrement of trehalase activity in silkworm haemolymph (Mahesha and Thejaswini, 2013).

Cholesterol:

Along with Triglycerides the cholesterol is known as the compound that takes a part in the structure of total lipids and hence was estimated as the representative of the biochemical constituents, lipids in this study. There were significant differences in mid gut tissue Cholesterol levels analyzed between the infected silkworms at their late infection than from the early infections, as compare to healthy control. In early infection with Grasserie the cholesterol content of mid gut in infected silkworm was 28.11 % mg, as compare to the control healthy non infected silkworms of the same age, which was observed to be 30.36% mg. The results presented in table show a decrease in the activities of cholesterol at late infection was 22.17 mg% as compared to the late healthy control non infected silkworms was 33.24 mg%. The decrease in the cholesterol content as reported during Grasserie infection had been attributed to the altered host lipid metabolism to defend the infections (Komano *et al.,* 1966 : Boctor 1981).

Free Amino acids:

Silkworm and other insects are known to contain usually large amount of free amino acids (Sinha *et al.,* 1990; 1991). Amino acids play an important role in the synthesis of cuticle constituents and in silk production (Pant and Argawal (1964). In the present investigation, significant changes were noticed in the total content of free amino acids in the midgut tissues, during the occurrence of Grasserie disease in silkworm at both early and late infection state. In control non infected silkworm the total free amino acids content of the midgut tissue on the day two of 5th instar larva was slightly lowered 31.32 mg%, where as in the healthy silkworm of the same developmental age was, 34.33 mg%. The control values of free amino acids on day five of 5th instar larva were 31.36 mg%. Where as in silkworms infected with Grasserie, on day five infection was 22.18 mg%. The results of the present study therefore demonstrated that the total free amino acids levels of mid gut tissues at later stages of infection decreased significantly, indicating parasite and host interactions. . In accordance with the present study, Ranjitha *et al.,* (2013) too reported decreased free amino acid levels, pointing faster mobilization of free amino acids to meet the energy requirements through the TCA cycle through transamination. These free amino acids may be utilized in synthesis of structural proteins to meet the defense mechanism in response to viral and bacterial attack in the body during the diseases. Adolkar (1990) too recorded a considerable reduction of amino acid content in silkworm larvae infected with studied diseases Grasserie which was attributed to direct effects of viral lesions or infection in the body tissues of infected larvae.

Urea and Uric acid:

In the silkworm larva, the nitrogenous waste products of metabolism are mainly urea and uric acid excreted as urine, with fecal pellets. Urea is present in small quantities in insects but fluctuated slightly between uninfected and infected larvae. Changes in urea are directly related to nitrogen metabolism and amino acids (Hirayama *et al.,* 1996). Tissues of the infected silkworms showed no significant changes in urea. On the 2nd day of inoculation the amount of urea in infected silkworm tissues was recorded as 6.38 mg % as compared to control healthy 7.34 mg%. While in late Grasserie infection on 5th day of inoculation of the infected silk worm amount of urea was 6.78 mg% as compared to non infected control 7.54 mg %. Similar results were reported by Etebari *et al.,* (2007) in silkworm during Grasserie infections.

Silkworms and humans are similar in purine metabolism, since the end product of purine metabolism of both is uric acid (Hayashi 1960). Midgut tissue of silkworm infected with Grasserie on 2nd day of inoculation showed had non-significant changes, (1.94mg %) as compared to healthy control was (2.72 mg %). While in late Grasserie infection i.e. on 5th day of 5th instar inoculation the amount of uric acids was 1.33mg% and was significant as compared to healthy control in late infection as 2.22mg %. Decrease in uric acid level in infected larvae represents a decrease in some metabolisms, especially protein catabolism activity in them. According to Xiaoli (2012) xanthine oxidase is an enzyme that catalyzes the oxidation of hypoxanthine to xanthine and can further catalyze the oxidation of xanthine to uric acid. The production of uric acid decreases by inhibiting the XOD activity with pathogenic infections. This study, suggest that the activity of XOD in the haemolymph of the diseased silkworms declined, and was significantly lower than that of control non infected silkworms.

Table: Quantification of Major Biochemical constituents in silkworm during Grasserie disease in the Midgut tissue

Biochemical constituents in Mg% in mg%	Control		Grasserie Infection	
	Early	Late	Early	Late
Total protein	25.28 ± 0.28	38.56 ± 0.3	18.71 +0.43 a	25.53 + 0.49 b
Carbohydrate	7.09 ± 0.70	7.53 ± 1.37	7.01 +0.73 a	4.73 + 0.57 b
Lipids (Cholesterol)	30.36 ±1.37	33.24 ± 1.27	28.11 + 1.23 a	22.17 + 0.99 b
Free amino acids	34.33 ±1.27	31.36 ± 0.87	31.32 + 0.43	22.18 + 0.49 a
Urea	7.34 ± 0.39	7.54 ± 0.3	6.38 + 0.76	6.78 + 0.42
Uric acids	2.72 ± 0.14	2.22 ± 0.14	1.94 + 0.13 a	1.33 + 0.14 b

Conc.: Concentration, mean ± SE followed with the same letter (a): is not significantly different (P>0.05) ,(b): significantly different (P<0.05), (c): highly significantly different (P<0.01), (d): very highly significantly different (P<0.001)

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