Quantification of Urea And Uric Acid in Silkworm *Bombyx Mori* During Grasserie Infection

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Abstract

Grasserie is one of the most serious diseases of silkworms, though occurs throughout the year, its intensity varied with seasons. The pathogenic infections induce biochemical alterations including nitrogenous waste like, Urea and Uric acid in larval tissues. Here we estimated urea and uric acid in non-infected, healthy silkworms and the silkworm infected with Grasserie. At early infection with Grasserie the amount of urea in silkworm midgut tissues was recorded as 6.38 mg % as compared to control healthy 7.34 mg%. While in late infection the amount of urea was 6.78 mg% as compared to non infected control 7.54 mg %. Uric acid in Midgut tissue of silkworm infected with Grasserie in early infection showed non-significant changes, (1.94 mg%) as compared to healthy control was (2.72 mg %). While in late Grasserie infection the amount of uric acids was 1.33mg% and was significant as compared to healthy control in late infection as 2.22mg %. The investigation of chemical changes in body tissues is an appropriate system for studying effects of pathogenic disease. The understanding and identifying these tissue biochemical changes will be very important for discussing many biological stresses. The biochemical responses in silkworm against pathogenic diseases could facilitate the control of agricultural pests.

Key words: *Silkworm, midgut, Grasserie, biochemical, alterations.*

Introduction:

Though 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. The pathogenic infections induce biochemical alterations including nitrogenous waste like Urea and Uric acid in larval tissues. Study of changes in levels of bimolecular constituents in the body therefore is very important, to get information on the changes in physiological aspects of the quantification of major biomolecules, specifically proteins, carbohydrates, lipids, free amino acids, urea, and uric acids and of the enzymes in the haemolymph and body tissues of a diseased insect are therefore prerequisite for the understanding of the physio-molecular mechanism behind the host pathogen interaction.

Methodology:

The work involved the study of the silkworm larvae with the pathogens of Grasserie and their physiological effects with reference to quantitative changes in major biomolecules urea and uric acid in healthy and Grasserie infected midgut tissue were studied. The quantification was made in the fifth instar larvae, beginning from the newly molted stage (day one) and continued till the 6th day of the instar. The larval period was divided into two chronologically identified state as early experimental stage on day one and late experimental stage on day six of 5th instar. The 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. For quantification of major biomolecules and enzyme profile in the midgut tissue, the homogenate was centrifuged at 10,000 rpm and 4°C for 30 minutes. Supernatant was collected and used for quantification of all the major biomolecules and enzymes. So was transferred to new tubes and kept at -20°C until the commencement of experiments.

Considering the benefits of automated analyzers with their internal standards and controls in quantification of Urea and Uric acid, an ELICO Clinical chemistry analyzer Cl 162 and prescribed assay kits were used for the quantification. (Hamdah et al., 2010) (Mahesha et al., 2013),

Observations:

Table 1: Quantification of Urea (mg%) and Uric acid (mg%) in Midgut tissue of silkworms during Grasserie:

Biomolecules	Control		Grasserie infection	
	Early	Late	Early	Late
Urea mg%	7.34 ± 0.39	7.54 ± 0.3	6.38 ±0.76	6.78 ± 0.42
Uric acids mg%	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).

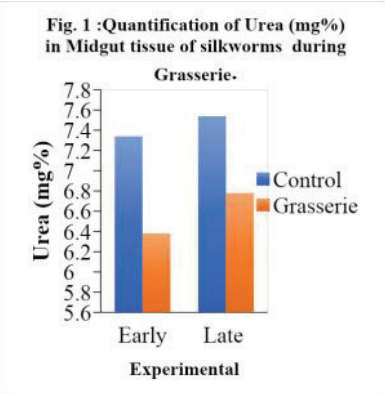

Fig. 1 :Quantification of Urea (mg%) in Midgut tissue of silkworms during Grasserie.

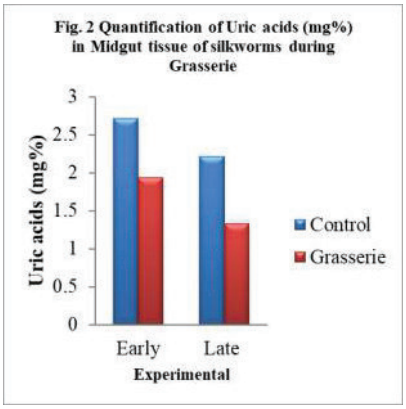

Fig. 2 Quantification of Uric acids (mg%) in Midgut tissue of silkworms during Grasserie

Results and Discussion:

According to Table 1 and fig. 1 the midgut tissues of the infected silkworms showed changes in urea in early and late infection of Grasserie. On the 1st day of infection 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 6th day of the infected silk worm, amount of urea was 6.78 mg% as compared to non infected control 7.54 mg %.

Excretory compounds of silkworm have been investigated under many stress conditions as an appropriate marker. In the silkworm larva, the nitrogenous waste products of metabolism are mainly urea and uric acid excreted as urine, with fecal pellets. The quantity of each nitrogenous compound in urine varied according to the food conditions during the fifth larval instar and also differed between silkworm races. The excretory pattern also depends upon a number of environmental factors such as temperature and humidity (Alexandria and Stanchion, 1981; Dhinaker,1990). Once infected the progressive multiplication of a pathogen in the host system is often reflected by specific metabolic changes coupled with corresponding biochemical changes in the infected body tissues. Pathogenic infections are reported to induce biomolecular and physiological alterations in insect tissues (Martignoni, 1964: Shigematsu, 1969) leading to a new bio-metabolite profile as reported by Taha (2007).

According to table 2 and fig.2 the midgut tissue of silkworm infected with Grasserie on 1st day of infection showed non-significant changes, 1.94mg % as compared to healthy control was 2.72 mg %. While in late Grasserie infection i.e. on 6th day of 5th instar the amount of uric acids was 1.33mg% and was significant as compared to healthy control in late infection as 2.22mg %.

Silkworms and humans are similar in purine metabolism, since the end product of purine metabolism of both is uric acid (Hayashi 1960). Uric acid distributed mainly in the fat body (Tojo 1971). Renuka and Shamitha (2012) documented that, occurrence of uric acid as the main end product of nitrogen metabolism among insects is well established. Moreover, the concentration of UA in midgut tissue also declined with the progress of the diseases. In this study not only no difference was observed between infected and uninfected larvae in the amount of urea in midgut tissue but also no difference was evident in two sampling times. Similar results were reported by Etebari *et al.,* (2007) in silkworm during Grasserie infections. Our results might demonstrate that infection increased the rate of deamination of amino acids and increased the haemolymphatic concentrations of uric acid. In this circumstance, the intense depletion in the carbohydrate's reserves led the

host to use other sources than glucose for energy, such as amino acids (de Souza *et al.,* 2000; Pinheiro *et al.,* 2009).

In the present observation, the nitrogenous excretory products in the fifth instar Grasserie free and Grasserie infected silkworm, urea content is reported to be maximum. It is also reported that there was a heavy loss of worms in the fifth instar. The studies also show an decrease in the uric acid content of Grasserie infected larvae, followed by urea. It was also reported somewhere that the larval span increased in the Grasserie infected larvae without corresponding increase in the body weight and silk gland weight Causes in crop loss and reduced the prospects of silk production. During fifth instar the formation of nitrogenous products lowered due to depleted growth rate while silk synthesis in the glands takes place rapidly. From the present studies it is clear that the content of the major end products viz., uric acid and urea, in the Grasserie infected larvae has been decreased, with a Related decrease in larval and silk gland weight which may be due to poor nutrition coupled with lesser enzyme activity in the malphigian tubule and midgut which are the Major regions involved in throwing away excretory material. The comparatively higher level of nitrogenous products in the uninfected silkworms may be due to higher breakdown of the excretory products which is correlated with the growth rate. As excretion forms a major factor for the balance of nitrogen and water in the body and grasserie infection is found to be one of the major constraints in sericulture which results in heavy loss to the crops , the present study provides an avenue to explore the pathogenic effects of bn infectious diseases and mark way for production of quality silk.

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