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Review

Metabolic alterations and molecular mechanism in silkworm larvae during viral infection: A review

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Silkworm larvae, *Bombyx mori* (L), is an important economic insect and also a tool to convert leaf protein into silk. The industrial and commercial use of silk, the historical and economic importance of production and its application in all over the world finely contributed to the silkworm promotion as a powerful laboratory model for the basic research in biology. The development and economic production of sericulture largely and greatly depends on the metabolic modulations and molecular mechanism of silkworm, besides its genetic composition and immunological resistance. One of the main reasons for decreased cocoon production is the loss due to diseases which account for about 30%. The collection of information on investigations related to disease prevalence in Silkworm in different parts of the country and (or) different seasons is highly helpful to monitor, prevent and control them. However, such collection of data on various diseases of silkworm in general and viral infection in particular in world wide is very limited. Pathological status of the diseased animal can be evaluated in terms of symptoms resulting from the interactions between host and pathogen which in turn depends on the degree of attack, progress of diseases, metabolic modulations, physiological adjustments, molecular mechanisms, and the defence immune system of the silkworm. Such symptoms are manifested in terms of morphological, physiological and biochemical variations in the host body. Hence, there is a dire need to suggest alternative remedial measures for the restoration of sericulture output even during grasserie infection, in order to safe guard the interest of the Seri culturists involved in sericulture practices. An attempt has been made in the present study to provide the relevant information related to the intricate mechanisms involved in metabolic alterations in silkworm larvae on grasserie infection.

Key words: Grasserie infection, silkworm, metabolic modulations, nuclear polyhedrosis.

INTRODUCTION

The silkworm *Bombyx mori* is exploited both as a powerful biological model system and also as a tool to convert leaf protein into silk. Silkworm larvae often suffer from viral infections causing heavy losses to the economy of the silk industry. Insects exhibit both humoral and cellular immune responses in addition to metabolic alterations that are effective against various pathogens like bacteria, fungi, protozoa, etc., but no insect immune response is effective against physiological adjustments during viral infections. To date, no satisfactory reports are available on metabolic alterations and molecular mechanisms during viral infection of the silkworm.

Some efforts have been made by very few workers to identify and characterize the antiviral proteins (Yao et al., 2006), histological changes (Manohar, 2006) and metabolic changes (Rajasekhar et al., 1992; Manohar, 2006) during different diseases in the silkworm. In the present article the mode of viral infection and the activity of certain metabolic alterations involvement in silkworm immunity are studied. The study will be useful in understanding the molecular aspects of metabolic changes role in disease control and may form the basis for potential use of silkworm in other fields such as medicine and virology.

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The silkworm *Bombyx mori* has been used as a silk producer in the silk industry for thousands of years. Recent success on transgenesis of the silkworm has opened new prospects for this insect species (Tamura et al., 2000). The *B. mori* nucleopolyhedrovirus (BmNPV) is the most harmful virus in the sericulture industry, often causing severe economic losses (Ponnuvel et al., 2003). The molecular mechanism by which the insect resists viral infections, recognizes infected cells and recruits metabo-lic alterations in the cell or physiological adjustments in the infected cells is poorly understood (Popham et al., 2004). Insect metabolic changes plays an important role in the interaction between the host and pathogen as a part of survival strategy including physical blockaded such as cuticle and peritrophic matrix, epithetlial barriers, protease cascades leading to coagulation and melanization and also the production of certain metabolic end products (Rajasekhar et al., 1992; Vernick et al., 1995; Lehane, 1997; Lavine and strand, 2002; Ligoxygakis, 2002; Lehane et al., 2004; Manohar, 2006). Studies pertaining to defensive mechanisms in silkworm against various pathogens like bacteria, fungus and protozoa are well documented (Liu, 1981, 1984) but reports on metabolic changes and molecular mechanisms are scanty. In our laboratory several studies on silkworm larvae have been conducted extensively in relation to fortification agents and nutrient impact (Bharathi et al., 1984; Narasimha Murthy et al., 1986; Govindappa et al., 1992) seasonal variation influence (Dhinakar et al., 1991), hormones treatment (Bhaskar et al., 1982, 1983; Bharathi et al., 1983, 1986; Ramesh, 2005; Ramesh and bhaskar, 2006a, 2006b, 2006c; Rama, 2007; Rama et al., 2007; Rama and Bhaskar, 2008) and impact of disease (Rajasekhar et al., 1992; Manohar Reddy, 2006). It is truly surprising to note that among the thousands of pu-blished articles addressing insect baculoviruses as effi-cient recombinant protein expression system, there are virtually no studies dealing with the metabolic changes and molecular mechanism in silkworm host cells. This means that special attention must be focused on this area to determine the relationship between metabolic adjustments and immune response and proteins involved in conferring resistance to modify their expression in silkworms. Proper understanding of physiological changes, metabolic adjustments, molecular mechanisms and histopathological alterations of different tissues of silkworm is essential in addition to host-pathogen interaction, defensive mechanisms evolved in the host body in response to infection, anti-defensive/immunosuppressant molecules released by pathogen to suppress host immunity is necessary before stepping into other aspects like disease control. In this context, it is important to study recent developments in the metabolic adjustments and antimicrobial proteins in silkworm. In this detection, we initiated studies and obtained information on different metabolism and the proteins involved in molecular mechanisms against BmNPV in the silkworm. We hope that this knowledge on viral diseases in the economically important insect like silkworms, but also helps to use silkworm as a bioreactor for the development of antiviral agents which are important in human health and welfare.

DISEASES OF MULBERRY SILKWORM

The term silkworm, while in reality a vague one, has practically come to mean the mulberry silkworm, the caterpillar of various varieties (or) species of the genus of moths *Bombyx.* These are of course silk producing caterpillars of other genera, such as the Eri (*Philosamia ricini*), the Muga (*Antherea assama*) and the Tasar (*Antheraea mylitta*), but the industries annunciated with these worms in India are so small that they are but little known to the world at large, and even in the silk industry they occupy a very unimportant place.

The mulberry silk-worm is a completely domesticate animal which has been rare by man for at least 4,500 years. Like all other domesticated animals and indeed like wild animals too for that matter though in a much less degree it is subjected to certain diseases and pests. Our knowledge of silkworm diseases is however of comparatively up to date. Despite the fact that data back to more than 2,000 years before the Christian era are present (Chitra and Sridhara, 1973), the earliest definite records of disease are to be found in the first European treaties on sericulture. Here the susceptibility of worms to diseases is clearly recognized and made to describe and define the diseases. In this, as indeed in many of the much later works on sericulture, the symptoms of several diseases were confounded together, and, as was to be expected, only the more striking diseases and their most characteristic appearances, such as the white efflorescence on the mummified bodies of worms that had died of Muscardine, were noted. The significant point is, however, that at their early data in the history of Sericulture in Europe diseases was well established among the worms. Subsequent works on sericulture continued to pay attention to the question of diseases in European silkworms and gradually to many different forms of sickness were recognized as different symptoms of the same disease.

The disease of silkworm may be divided into two classes. Those caused by certain easily recognized animal and plant parasites, not bacteria, and those of more indefinite nature in which bacteria may or may not play a part. For want of better names the first group" parasitic diseases" and the second, rot disease" under the parasitic diseases Pebrine, muscardine and fly pest are very important. (1) Pebrine caused by *Nosema bombycis* (2). Muscardine caused by *Beauveria bassiana* and (3) Fly pest caused by *Tricolyga bombycis*. Under rot diseases

Flacherie and Grasserie could be the prominent. The parasitic diseases are reasonably well understood and their diagnosis and control are consequently possible. The rot diseases, in the other hand, are somewhat obscure in their origin and consequently imperfectly understood and difficult to control.

Mulberry silkworm, *B. mori* is affected by a number of diseases caused by viruses, bacteria, fungi and microsporidia. These diseases are known to occur in almost all the silkworm rearing areas of the world causing considerable damage to the silkworm cocoon crop. A number of measures have been suggested for the prevention and control of these diseases, care is also needed to be taken to see that they are not exposed to stress conditions like temperature, humidity, bad ventilation and nutritional deficiency which may make them easily susceptible to viral diseases (Kobayashi et al., 1981). The silkworm, *B. mori* is a delicate venture easily susceptible to a number of diseases due to its continuous domestication. A number of pathogens cause diseases to silkworm larvae. The susceptibility of the silkworm depends upon the hybrids (Chinnaswasmy and Deavaiah, 1984; Baumann et al., 1991).

EFFECT OF DISEASES ON SERICULTURE

The mulberry silkworm *B. mori* (L) is susceptible to different diseases like micospridian, viral, bacterial and fungal. Pathogens were reported to cause several biochemical and physiological alterations in insect tissues (Martignoni, 1964). Stress produced by cow dung, malnutrition other environmental factors had been reported to increase the susceptibility of insects to diseases (Vago and Atger, 1961). All the races of *B. mori* are not equally susceptible or resistant to different types of the diseases. These diseases not only affect the silkworm physiological conditions, but also create the troubles and economic problems to the farmers. Silkworm crop loss is directly attributed to the occurrence of the diseases, rather than unfavourable weather conditions that led to a poor harvest of mulberry leaves (Watanabe, 1987). Of all the silkworm diseases, grasserie is the most serious (Samson et al., 1990; Subba Rao, 1994; Shivaprakasam and Rabindra, 1995), the flacherie was found to have high mortality during rearing (Sivaprasd and Murali, 1990), fungal diseases cause loss of 5 - 10% (Sengupta, 1988) and the pebrine diseases destroyed the sericulture industry in the past. The most common sources of pathogens for infection and stress over the diseases during the rearing are the contaminated rearing trays and seat papers (Ishikawa, 1958; Miyajima, 1978).

VIRAL DISEASES

Viral diseases of silkworm pose a major problem to sericulture as they account for almost 70% of the total loss due to diseases. Viral diseases of silkworm comprises of inclusion and non-inclusion types. The inclusion virus disease form typical inclusion bodies. They are nuclear polyhedrosis and cytoplasmic polyhedrosis, which can be more easily identified through ordinary mi-croscope. The non-inclusion type consists of infections Flacherie and Densonucleiosis, which can be, detected only though electron microscope and serological tests.

Grasserie disease of *B. mori* is caused by a nuclear polyhedrosis virus. In India it is called by various local names such as *"Haul- hula"* in Karnataka, *"Rasa"* in West Bengal, *"Polapurugu"* in Andhra Pradesh and *"Pal Poochhi"* in Tamil Nadu. Grasserie disease accounts for more than 15% loss in yield (Vidya, 1960) and in Karnataka its incidence is 5% (Samson et al., 1990) and 35 - 57% of total incidence. It usually affects fourth and fifth instars silkworms under natural conditions. The infected silkworms appear completely normal and feed as usual till they are close to deaths. About six to seven days after infection, the inter-segmental membranes are swollen and the worms appear to be under stress and exhibit restless behaviour. At this stage, various tissues like epidermis, tracheal matrix, fat bodies, haemocytes, silk glands and pericardial cells are affected and the haemolymph becomes turbid and milky due to the multiplication of polyhedral bodies in large numbers (Chisti and Schaf, 1990). The skin loses its elasticity and becomes fragile and gets ruptured easily, releasing the milky turbid white haemolymph. The outset of deaths from the time of swelling of inter segments is relatively rapid, usually ranging from a few hours to less than a day. The dead worm becomes soft and flabby.

The nuclear polyhedrosis virus multiplies in the nucleus of the infected cells of various tissues and gets crystallized on maturation into a protenaceous material forming the polyhedra (Aruga, 1957). The size and shape of polyhedra vary considerably not only between polyhedra from different insects but often also within polyhedral of some species of insect. They may get crystallized as decahedra, tetrahedral and cubes are as irregular bodies. In the silkworm, *B. mori* the polyhedral are found to be of dodecahedral shape. The nuclear polyhedral are roughly spherical and usually hexagonal in shape and measure 1.64 to 2.11cm with an average of 1.87 cm. The virions are rod shaped and singly enveloped with rounded ends and measure 247 × 96 µm with outer development membrane while the inner core measures 215 × 55 µm. The inner core appears to be electoron dense as per Khosaka et al. (1971), the virions measures 330 × 80 µm. Multiply enveloped virions are also encountered. A total of 33 and 84 structural polypeptides are identified using SDS-PAGE and 2-D gel electrophoresis, Sugimori et al. (1990), Nataraju et al. (1994) has been reported the development of an oral vaccine against NPV in silkworm *B. mori* (L). The efficacy

of some chemicals and their dissolution of cytoplasmic polyhedrosis virus of silkworm B. *mori* (L) were reported by Patil (1994).

BmNPV

The virus belongs the sub group-A of family Baculoviridae containing a single genus Baculovirus. Silkworm NPV though was called previously *Borrelina bomycis* (Paillot, 1930) but in recent nomenclature its name is mentioned as *B. mori* NPV, that is, BmNPV.

Nuclear polyhedrosis

It is one of the most serious viral diseases in tropical countries and occurs though out the year. This disease is other wise known as Grasserie, Jaundice milky disease, fatty degeneration and hanging disease.

Causes of the disease

Borrelina bombycis virus belonging to the sub-group of the family *Baculoviridae* causes this disease. As the name implies, the virus multiplies and forms polyhedral in the nucleus of haemolyph and adipose tissue cell. Infection mostly takes place through wounds and feeding of polyhedral contaminated mulberry leaf. Heat, cold and chemical treatments have also been known to induce their disease factors. Influencing the outbreak are high temperature and humidity, their sudden fluctuation, bad ventilation in the rearing room, in effective disinfection of rearing house and rearing appliances and feeding of tender leaves during late instars, inadequate larval spaces, starvation and excessive moisture in the rearing bed have also been known to contribute toward the out break and spread of disease.

Causative agent

Among the virus microbial factors once attributed to its course were such things as poor nourishment, unsuitable leaves, uneven temperature, damp air, poor ventilation and excessive moisture. Now we know that some of these factors may not be the specific cause of the disease to influence the course of infection and constitute important predisposing factors (Ayuzawa, 1963). Microbial agents were also suspected of being respon-sible for the disease. But based on our present knowledge it is concluded that Grasserie of silkworm is caused by Borrelina virus, which is a parasite principally in the nuclei of certain cells. It initiates the morbid process that ends in the elaboration of polyhedra bodies. The grassier virus is contained in vast quantities in the supernatant haemolymph fluid and in the polyhedral bodies. Polyhedral vary for 0.5 - 15 microns in diameter. The nucleic acid of the nuclear polyhedrosis virus in the silkworm is DNA.

Infection

It is believed that the disease begin with the digestion of infectious material into the alimentary tract of the insect. It has been assumed that the alkaline reaction of the silkworm gut, as well as certain enzymes present there dissolve the polyhedra, releasing the virus which then passes through the susceptible tissues (Harrap, 1970). The polyhedral bodies are formed in the nuclei of adipose tissue, tracheal membranes dermal cells and blood cells, but generally, polyhedra in the middle and posterior portions of the silk glands has been noticed.

There is a close relationship between the atmospheric temperature and the assurance of the disease (Ignoffo, 1966; Inone et al., 1972). The disease may be induce when low temperature or high temperature treatment is applied to the larvae immediately after molting (Kobayaashi and Kawase, 1980)

Symptoms

As the disease advance, appetite decreases and skin tension is lost usually five to seven days after infection, the inter-segmental membranes of the body become swollen and the skin become shiny. The diseased larva in the final stages of attack shows pronounced swelling at the inter segments giving the larva the appearance of the bamboo cane with distinct nodes. The larva becomes restless and impatient causes fatigue (Krishnamohan, 1986). The color changes to light yellow and the normally clear haemolymph become turbid. The integument becomes fragile and when ruptured, a milky haemolymph flows out. In the haemolymph of infected larva numerous polyhedral bodies are observed. The adipose tissue which disintegrates as a result of the attack is released as a milky fluid which mixes with the haemolymph. If the disease occurs just before moulting, the period when it is most likely to occur in the earlier stages, the larva does not go into moult earlier.

Impact of Grasserie diseases on physiology

The grasserie disease causes to affect and changes the normal physiological conditions of the silkworm, *Bombyx mori* (L). Shigematsu (1958) observed fluctuation and hyperproteinemia in the fat body and body fluid of silkworm during early and advanced stages of BmNPV respectively; Mazzone (1985) reviewed the pathophsio-

logy associated with baculovirus infection. The nuclear polymerases virus multiplies in the nucleus of the infected cells of various tissues and gets crystallized on maturation into a portentous material forming the polyhedral (Aruga, 1957). Silkworm infected with grasserie also responds physiologically to the invader and leads to physiological lesions. After five to seven days of infection, the integument membrane gets swollen and the larvae become restless as it under stress and more to the periphery of the rearing tray (Aizawa, 1963).

The skin looses its elasticity and become fragile, gets ruptured with easily releasing of white milky fluid. In case of crystoplasmic polyhedral infected silkworm, midgut may cause functional abnormalities of varying intensities in other tissues and organs as midgut forms the main part of the digestive system and heavily infected one may result in a nutritional deficiency which alters the metabolic activities of the entire silkworm (Govindsan et al., 1998). Pathogens were reported to induce several biochemical and physiological alternations in insect tissues (Begols, 1963; Benz, 1963; Martignoni, 1964).

In comparison to vast literature on various diseases impact on growth and development of silkworm larvae and other biochemical parameters, there have been considerably very less information available on metabolic modulations in various tissues of pre-spinning silkworm larvae during grasserie disease. Hence, as there are several lacunae on the impact of diseases on silkworm with particular reference to grasserie infection on the meta-bolism of silkworm, it is obvious to study the effect of grassier infection on metabolic modulations induced in various tissues of pre-spinning silkworm larvae were to be understood in detail.

Thus the voluminous information has been available on the impact of grasserie infection on physiology of silkworm larvae at large. But comparatively very meagre information is available on the effect of grasserie infection on physiology in general and metabolism in particular of pre-spinning silkworm larvae during grassier infection. Surendranath et al. (1997) studied the toxic impact of organo phosphorus on GCTH activity in the silkworm *B. mori*. Lu and Dum (2000) also reported the effect of methylamine compounds on remission of fluorine toxicosis of silkworm.

Impact of BmNPV on genetics

Zhang et al. (1999) reported protein RNA interaction in cytoplasmic polyhedrosis virus. Yan et al. (1999) characterize the ecdysteroid UDP-Glucosyl transferase gene of *Spodoptera litura* much multinucleocapsid nucleo polyhedrosis virus. Yanase et al. (1999) reported the replication of *Spodoptera exigua* nucelopolyhedrosis virus in permissive and in non permissive Lepidopteran cell lines.

Impact of BmNPV on protein metabolism

Proteins are the derivatives of high molecular weight polypeptides. They play a vital role in the formation of structures in organisms. Like carbohydrates and fats proteins also can be utilized for energy purpose. However, tissue proteins represent the last source of energy which is used only when there are no carbohydrates or fats available, in extreme conditions such as starvation.

The survival ability of an animal to stress majorly depends on its protein synthetic potential. Any stress on an animal invokes compensatory metabolic adjustments in its tissues through modifications or modulations of proteins (Bano et al., 1981; Assem and Hanke, 1983). The total protein content consists of structural and soluble proteins involved in the architectures and metabolism of a cell. They constitute about one fifths of and animal's body on the wet weight basis. Hence, the total protein profile of a tissue may be taken as a diagnostic tool in assessing the physiological status of it or the animal as a whole (Young, 1970; Lehninger et al., 2001). Reports on *B. mori* on the shifts in its protein metabolism during stress are limited. Sinha et al. (1985) studied the changes in protein content in haemolymph of tasar silkworm, *Antheraea mylitta*, and reported that protein content in it increases enormously during larval development.

Ramaiah and Veerabasappa (1970) reported the nuclear polyhedrosis of silkworm causes abnormal increase in the concentration of glutamic aspartate transaminase and of glutamicalanine transaminase activities. Yoshitake (1952) reported high uric acid content in the integument and blood of silkworm. Hayashi (1961) studied the urea formation in various tissues in the silkworm larva, *B. mori.* The infection of fifth instar silkworm larvae with the fungus *Beauveria bassiana* resulting an increase in uric acid content and reduction in ammonia of haemolymph (Raghavaiah et al., 1988).

Ayuzawa et al. (1963) studied the effects of the cytoplasm polyhedrosis virus on silkworm reared at different temperatures of 20, 25 and 30°C and noted increase in occurrence of the diseased larvae with lowering of the temperature. The information on protein metabolism of the silkworm, however, is limited, and no studies are available on comparing the shifts in protein metabolism of the haemolymph and fat bodies in silkworm infected with nuclear polyhedrosis in the fifth instar.

The levels of amino acids of virus infected silkworm haemolymph were investigated by Yoshitake and Aruga (1950), Ishimori and Muto (1951) and Koyanagi and Matsuoka (1954) and reported a decrease of aspartic acid, cystine, glutamic acid glutamine threonine, tyrosine, and valine and increase of histidine in the pupa of silkworm. There are many studies on the change in the electophoretic pattern of haemolymph proteins of larvae infected with the nuclear polyhedrosis virus (Watanabe, 1986; Van der Geest and Craig, 1967). A great reduction

in all the protein fractions was reported in the haemolymph of heavily diseased larvae. The effect of infection by cytoplasmic polyhedrosis virus on the midgut protein metabolism in silkworms was studied by Watanabe (1971) and reported that the active synthesis of midgut proteins, as well as polyhedron proteins, is induced by infection and continues until later stages.

The activity of acid proteases which is involved in the degradation of cellular proteins is also stimulated (Kobayashi et al., 1985). Ramaiah and Veerabasappa (1970) studied the levels of aminotrasferases in silkworms infected with NPV and reported high increase of them in haemolymph. Kobayashi et al. (1990) analysed the proteins by SDS-PAGE and reported that BmNPV infection of the isolated pupal abdomen of silkworms caused elevation of a number of parasitic polypeptides with a concomitant decrease of host cellular polypeptides.

Pathogens are reported to induce several biochemical and physiological alterations in insect tissues (Bergold, 1963; Benz, 1963; Martignoni, 1964). Gururaj et al. (1999) reported that BmNPV infection decreases the activity of digestive enzymes in the midgut of silkworm larvae. In silkworm most of the path physiological conditions associated with viral infection and are concerned primarily with the nucleic acid and protein metabolism (Shigematsu, 1958; Kawase and Hayashi 1965; Horie and Watanabe, 1983). Martignomi (1964) reported that hypoprotenemia occur in the larvae of *Peridroma saecia* during NPV infections. Sarma et al. (1994) observed that significant decrease of total protein content in the haemolymph of BmNPV infected silkworm larvae.

Protein synthesis is not appreciably increased in diseased cells up to a point prior to polyhedra formation, where after an active protein synthesis pronouncedly occurs around the newly developed polyhedral and continues with polyhedral growth. Many pathologists studied on the protein metabolism and changes during the grasserie infection in silkworm, *B. mori* (Sujak et al., 1978; Manohar, 2006). The activity in the haemolymph protein is stimulated by virus infection about 25 h (Govindan et al., 1998) and the amounts of amino acids in both midgut and haemolymph of highly diseased larvae were decreased (Kawase, 1965). Hypoproteinmia occurs in CPV infected silkworm larvae towards advanced staged of infection (Kawase et al., 1974).

In infected midgut epithetlial cells marked difference is seldom seen in DNA, RNA and protein concentration except for a rather high concentration of DNA in infected midgut during last 4 days (Govindan et al., 1998; Watanabe, 1971) has noticed reduction of protein in infected tissue at a latest age of infection and some changes were observed by (Kawase et al., 1974).

Prevention is better than cure is the correct approach that should be adopted in integrated disease management. This means that one should go about actively preventing diseases before they occur and it is only when preventive precautions are in force, we can hope to effectively manage the occurrence and spread of diseases. Dependence on treatment after the disease appearance will always entail losses. In short, the fundamental basis of silkworm disease management is the constant implementation of the policy of prevention first. Under this policy, strict disinfection to eliminate risk of diseases transmission and increasing the vigour of the silkworms to enhance resistance to diseases are given due weight age. Sanitation before, during and after each rearing should be carried out effectively and strictly. One has to possess knowledge to intensify the feeding and management so as to comply with the physiological requirements of the silkworm so as to raise vigorous silkworms. The effect of various diseases on protein metabolism of silkworm larvae has been studied by various investigators. However, the possible effect of the grasserie infection on protein metabolism in various tissues of pre-spinning silkworm larvae has not been worked out. It will be worth while to make an attempt towards understanding the alterations in protein fractions of pre-spinning silkworm larvae during grasserie infection.

Impact on carbohydrate metabolism

The role of glucose in the intermediate metabolism of silkworms has been reviewed by Chitra and Sridhara (1973). In silkworms, blood glucose level can be correlated to their level of metabolism, and is comparable with mammalian blood glucose (Kilby, 1963). Ingalhalli et al. (1995) studied the carbohydrate contents in armyworm infected with nuclear polyhedrosis and reported that the fat body, gut and integument indicated hypoglycemia, whereas the haemolymph demonstrated the hypertrehalosemis and hyperglycemia. Changes in the level of carbohydrates in haemolymph and midgut epithetlium of *B. mori*, during the CPV infection were observed by Kadoya et al. (1984). Jacob (1972) reported that significant decrease in the concentration of glycogen in NPV infected larvae but in the silkworm pupae, decreased glycogen content was observed by Kobayashi and Kawase, (1981). Yaginuma et al. (1990) reported changes in the activity of carbohydrates in the midgut epithelium of the silkworm affected with CPV. It is reported that the levels of glycogen decreases in the fat body during starvation in *B. mori* (Horie, 1961; Saito, 1963). Hypoproteinamea, decreased carbohydrate content unto the 5^{th} day of infection and decreased lipid content are vivid in haemolymph (Sarma et al., 1994). The glycogen content in the tissues of *B. mori* was utilized for viral multiplication along with serving as fuel reserve for the growing energy demand of the host during combating virus infection (Gururaj, 1996).

Sarma et al. (1994) reported that there was a decreased

carbohydrate levels during the early inoculation of BmNPV where they can be increase on the 7th day of infection with the viral bodies to the silkworm larvae similar reports were observed by Kobayashi and Kawase (1981) in the BmNPV infected pupae. Gururaj et al. (1999) reported that there was a significant increase in glycogen content on the 6th day of BmNPV infected larvae. Studies dealing with the carbohydrate metabolism of various tissues of silkworm larvae during infection have been reported extensively however, the effect of grasserie infection on carbohydrate precursors of various tissues of pre-spinning silkworm larvae are very limited.

Impact on lipid metabolism

Lipids play an important role in the biochemical processes derlying growth and development of insects (Ito and Horie, 1959; Wyatt, 1967). Increased lipid content was observed in 5th day of infected larvae in haemolymph by Sarma et al. (1994). Heamocoel volume increase up to 1.5 to 2.0 times and increased in lipid content were reported by Govindan et al. (1998). All these studies emphasize the effect of diseases on metabolic modulating in silkworm larvae but, none of the studies have been focused on the effect of grasserie infection on tissue lipid profiles of pre-spinning silkworm larvae.

Studies on histopathology

A few studies are available on the histopathological changes in larvae of insects infected with NPV (Stairs, 1971; Tinsley 1976). Eliana et al. (2008) reported considerable damage to most of the internal tissues. The BmNPV replication pathways and infectivity has also been studied by some workers (Aizawa, 1959; Bird, 1958; Liu and Shixian, 1984). Mary Flora (1991) studied histopathology of NPV infected silkworms and reported appearance of polyhedra in the nuclei of fat bodies, hypodermis, tracheal matrix, muscles, silk gland, nerve and gonads. Histochemical studies showed a substantial increase in the synthesis of nuclear protein in fat bodies, hypodermal cells and tracheal matrix and silk gland cells following the infection.

The relationship between histopathological changes and disease pattern has been studied extensively. Several studies like morpho-histopathological changes in the larvae of various insects are also observed in large. However, very little work has been reported in histopath logical alternations during grasserie infection in pre spining silkworm larvae.

CONCLUSION

The mulberry silkworm, domesticated and mass reared for several centuries, presumably has weakened inure system which has made the insect highly vulnerable to bacterial and viral infections. Information on the organization and function of the immune system in the silkworm in general and with reference to viral infections in particular is scanty. The present information may pro-vide the underlying mechanisms in altering the metabolic modulations in pre-spinning silkworm larvae during grasserie infection and to protect the commercial characteristics of cocoon yield in addition to suggest suitable measures in regulating the disease.

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