# 2. REVIEW OF THE LITERATURE

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*Antheraea assama* Westwood (Lepidoptera: Saturniidae), the muga silkworm is a multivoltine sericigenous insect reared on two natural and principal host plants Som (*Persea bombycina* Kost) and Soalu (*Litsaea polyantha* Juss.) (Chakravorty, 2004). It is endemic to the North-eastern region of India. A scientific study of this exclusively Indian species has started comparatively recently. Moreover, in the recently introduced non traditional area, such as in and around Coochbehar any study has started only recently for the last three to four years. Hence the published literature on different aspects of this precious silkmoth is very limited. The most of the research literature available are on other sericigenous insects, particularly on *B. mori.*

The environmental factors influence the nutrient contents in the leaves of food plants at different seasons of a year (Hering and Taguchi, 1951), which in turn affect the biochemical constituents of the insect haemolymph. Both morphological and physiological changes are heterogenic during insect growth (Tessier, 1931). Since the larval life of muga silkworm is exposed to all sorts of natural environmental fluctuations, it has been considered worthwhile that the attempted study is to be restricted to the most sensitive stage of development, i.e. larval stages, particularly the fourth and fifth stages which are most susceptible to fatal diseases such as bacteriosis and virosis and which consume most quantity of leaves.

The majority of insect species demonstrated marked biochemical adjustment to the change in environmental temperature. Alteration of tissue biochemical composition in insects has been noted in thermal adjustment (Sing, 1984). Bordoloi and Hazarika (1992) reported the changes in body water content, lipid reserve, blood volume and haemocytes of *A. assama* at different seasons in the traditional geographical area i.e. in own residence of

this species. They have recorded that during summer and spring, the larvae contained low lipid reserves but concomitantly high water content in fifth larval instar of *A. assama*. The effect of environmental temperature on the metabolism of insects is also supported by the findings of Gilbert (1967) and Hoffmann (1984), who has stated that the decrease in rearing temperature, increases the neutral fat content and lipid reserve of insects. Shapiro (1979) has reviewed that the body water content of insects is also affected by fluctuations in the environmental temperature, which in turn directly influences the blood volume and haemocyte population.

**2.1. Diseases**

According to Food and Agriculture Organization (FAO) of the United Nations (UN) that environmental factors including temperature, humidity, air-flow, and quality of leaves as well have influence on the initiation of silkworm diseases (Yup-lian and Fu-an, 1995).

The silkworms are very delicate and susceptible to the infection by a number of pathogens. Though the mulberry silkworm, *B. mori* is domesticated for over centuries with utmost precautions, yet it is susceptible to a number of diseases, resulting in the reduction of cocoon production, leading to substantial loss to silkworm rearers throughout the world. In India the major silkworm diseases are pebrine, grasserie, flacherie and muscardine. Bacterial diseases are common in silkworms and tend to occur in the hot and humid summer and autumn rearing season and these diseases are called flacherie because the corpses of silkworms that die of them soften and rot (Yup-lian and Fu-an, 1995). All the mulberry silkworm diseases are also prevalent in *A. assama*. Rather the incidence of diseases is more frequent in this species because of its almost entirely outdoor rearing. However, very little literature is available on the incidence, causal organisms, and

haemolymph biochemical profiles of the muga silkworms suffering from diseases.

## 2.2. Bacteriosis

In 1763 Abbe'de Sauvages wrote about the flacherie disease of silkworm. In 1870 Pasteur demonstrated the presence of bacteria as the causal organisms of flacherie and in India N. G. Mukerji first reported about the silkworm flacherie disease in the year 1899 (Jameson, 1984).

Once, flacherie alone caused nearly 70 per cent of the total mulberry silk crop loss in Japan (Dasgupta, 1950; Aruga and Tanada, 1971) and also in India (Sidhu and Singh, 1968). Chitra *et al.* (1975) estimated annual crop loss was between 20 and 40 per cent. Microbial flacherie is caused by both bacteria and viruses. Among the various diseases, the bacterial flacherie in *B. mori* is of paramount importance (Krishnaswami, 1978; Samson *et al.,* 1990; Vanitha Rani *et al.,* 1994). Bacteria as etiological agents of silkworm flacherie were first reported by Pasteur (1870). In *Bombyx mori,* according to other reports, the loss in silk production due to bacterial diseases was 30-50 per cent in a year (Vanitha Rani *et al.,* 1994; Dutta, 1995). According to Dutta *et al.* (2007), in Assam the occurrence of bacterial diseases in muga silkworm including the rate of infection and larval loss varies from season to seasons.

In case of *B. mori* Cuboni and Garibini (1890) thought that *Bacillus cubonianus* was the causal agent of flacherie. In Japan, the causal organism of "Sotto" disease was identified as *Bacillus sotto*, which in present nomenclature is called *Bacillus thuringiensis* Var. *sotto* (Ishiwata, 1902). *B. megaterium* and *B. ellenbachi* as pathogens of silkworm were identified by Sawamura (1906). It is reported that a chronic type of flacherie, the 'gattine' of French and 'macilenze' of Italian *B. mori* probably caused by a single

species *Streptococcus bombycis* isolated from intestine of infected worms (Steinhaus, 1949). Metalnikov and Chorine (1928) found *Serratia marcenscens* from flacherie worm. Multibacterial dysentery 'khuto' disease was found in silkworms, which when were fed with chlorophyll deficient leaves (Matsumura, 1930). Chitra *et al.* (1973) isolated 7 bacterial species from 'sappe' worms. Vasantharajan and Munirathnamma (1978) reported the presence of *Serratia marcenscens* in Kenchu diseased of silkworms. Enomoto *et al.* (1987) reported several species of bacteria causing septicemia in silkworms from cocoon.

Vago (1963) reported that the growth of silkworm was retarded due to intestinal bacteria such as *Enterococcus*, *Staphylococcus*, or *Pseudomonas*, which could also cause acute and often fatal dysentery when infected along with the *Bacillus alesti*.

The bacteria belonging to the genera *Bacillus, Streptococcus, Serratia, Staphylococcus and Proteus* cause bacterial flacherie in *Bombyx mori* (Krishnaswami *et al.*, 1973). Vanitha Rani *et al.* (1994) isolated and characterised the bacterial species causing the disease in *B. mori* in Tamil Nadu. Chishti *et al.* (1991) investigated the bacterial flacherie of *B. mori* in Jammu and Kashmir. Nataraju *et al.* (1999) investigated the cause of "Thatte Roga" (diseases) in *B. mori*, which is a mixed infection of bacteria and BmIFV (*Bombyx mori* infectious flacherie virus). An outbreak of flacherie among the muga worms in Titabar of Assam in March 1921 was recorded by the author himself in his book "Report on the Diseases of Silkworms in India" and Bacillus and Micrococcus bacteria were detected from the infected worm (Jameson, 1984).

In Assam, bacteriosis is quite frequent in muga silkworms (Senapati *et al.*, 2001 and Senapati *et al.*, 2002) but no particular bacterium has been isolated and identified for this disease. A preliminary report on the bacterial

strains of diseased muga silkworms is available from the report of Choudhury *et al.* (2002), who worked on the effectiveness of garlic extract in controlling the bacterium causing disease in muga silkworm. Quite a frequent incidence of bacteriosis in *A. assama* has also been noticed in the Coochbehar district of West Bengal. Hence a preliminary attempt has been made in the present study to isolate and identify the bacterial pathogens responsible for the disease. On an average 11 – 15 per cent of mortality per year due to bacteriosis was recorded in the Coochbehar; the highest incidence (24.97%) was recorded in 'Bhodia' (Aug.-Sept.) crop (Das *et al.*, 2006). Bacteria belonging to the Genera *Pseudomonas, Klebsiella, Citrobactor, Proteus, Providentia and Bacillus* were identified from bacteriosis afflicted muga silkworm in this district (Das *et al.*, 2006).

### 2.3. Haemolymph major metabolites

In silkworms, radical metamorphic changes take place from larval to imaginal structures and biochemical profiles within the pupa, as a cleidoic system. The economic output from silkworm is characterized by quality and quantity of silk and viable egg production. As the larvae are the sole feeding stages of silkworm, the raw materials for these two major functions during imaginal development must be available internally, partly from hydrolyzed larval tissues and partly from the resources stored by the larvae in their fat body during their phagoperiod. Haemolymph is the medium of mobility of the resources for the construction of imaginal body.

The haemolymph in insects, which acts as the circulatory medium is similar to that of mammalian circulatory medium. Therefore, haemolymph is considered to be an excellent medium determining the biochemical status of developing insects under stress conditions such as in diseased condition.

### 2.3.1. Proteins

In lepidopteran development of most of the larval organs undergo involution for histolysis during pupal life. The nutrients, thus available, and the abundantly stored nutrients in the fat body and haemolymph are redistributed for imaginal development. Moreover, many of imaginal organs grow from imaginal discs or imaginal cell population or imaginal buds. This involves extensive cell division which again, requires abundant protein synthesis.

Metabolism of protein during holometabolous development has been extensively reviewed by Chen (1985). Protein is synthesized in the larval fat body and released and accumulated in to the haemolymph (Munn, *et al.*, 1969; Kinnear, *et al.*, 1971; Izumi, *et al.*, 1981). At the end of the last larval feeding stage when protein synthesis stops, resorption of accumulated haemolymph protein occurs in to the fat body cells and resequestered as dense protein granules (Martin, *et al.*, 1971; Price, 1973; Thompson, 1975; Tojo, *et al.*, 1980). This storage protein helps in organogenesis in insects and vitellogenin synthesis in the female larvae for egg maturation mostly at the pupal stage (Price, 1973; Tojo, *et al.*, 1980; Sridhara, 1981). The yolk precursor protein, vitellogenin is synthesized in the female larval fat body and is released in to the blood for uptaking by the growing oocyte (Pan, *et al.*, 1969; Bradley, 1983).

The growth, quantity and quality of silk produced by the silkworms and their fecundity depend largely on available nutrients in the leaves and the efficiency of conversion of leave nutrients to biomass (Chaluvaachari and Bongale, 1996; Kar *et al.*, 1994, Sarkar *et al.*, 1992; Sinha *et al.*, 1993 and Ray *et al.*, 2001). The most valuable nutrients are the protein and carbohydrate contents which have significant role on all the metabolic activities such as growth, metamorphosis and fecundity (Kar *et al.*, 1994).

Proteins are among the most complex of all known biomolecules and also the most characteristic of living organism (Chen, 1985). Proteins are also the principal constituents of protoplasm, which forms the material basis of life. In silkworm, proteins are the vital metabolites because of its important role in the determination of chemical characteristics of silk (Shigematsu, 1960), in compensatory mechanism (Bashamohideen and Ameen, 1998), as well as in the development, morphogenesis and almost in all the intermediary metabolic pathway (Kar *et al.*, 1994).

Drilhon (1954) first reported the change in protein concentration of haemolymph during development of holometabolous insects. The changes in the profile of proteins in insect haemolymph in different developmental stages has been reviewed by many authors like Steinhaurer and Stephen (1959), Siakotos (1960), Wyatt (1961), Telfer (1965), Chen and Levenbook (1966), Engelmann (1968), Elliott and Gillott (1979), Kim and Seo (1981), Kim *et al.* (1983), Gherghel and Rosca (1984), Cotton and Austee (1991) and Belgaum *et al.* (1991).

A series of information is available on the haemolymph proteins of *Bombyx mori* (Lauffer, 1943; Wyatt and Pan, 1978; Ogawa and Tojo, 1981; Ray, 2000). Preliminary information related to protein content in larval and pupal haemolymph was reported in the tropical tasar silkmoth, *Antheraea mylitta* (Sinha *et al.*, 1985) and in the muga silkmoth *Antheraea assama* (Sharma *et al.*, 1995; Das and Deb, 2008).

Belgaum *et al.* (1991) reported that in the normal larva of *B. mori*, haemolymph protein content increased in a relatively constant pace from the first up to the fourth stage, in the fifth instar increased nearly two fold on the day four and attained a maximum on day nine. Haemolymph protein level generally increases during interstadial period of each instar but decline during moulting. In general the levels are low in early larval instars but increase

considerably in the late instars of lepidopterous insects (Wyatt and Pan, 1978).

There are evidences that many of the haemolymph proteins may function as enzymes that are crucial for all biochemical reactions. In fact, Laufer (1960a,b) demonstrated that nearly all haemolymph proteins of *Hyalophora cecropia* and *Samia cynthia* acted as specific enzymes. Very little information is available on the changes in the concentration of haemolymph total protein of different larval stages of *A. assama.* Dipali Devi (1993) estimated the protein content of healthy larvae in relation to various environmental factors, Baruah and Handique (1996) investigated on the insecticide induced changes of haemolymph biochemical parameters of *A. assama*, Chetia and Handique (1997) studied the impact of automobile fumes on the protein content and Das and Deb (2005) assayed the protein content of healthy larvae reared on two major host plants and their impact on cocoon characters and of both healthy and bacteriosis afflicted *A. assama* larvae (Das and Deb, 2008).

### 2.3.2. Lipids

Feeding behavior and quantity of consumption of leaves by the larvae depend on environmental temperature, sunlight, humidity and moisture and nutrient contents of leaves. During phagoperiod, silkworm accumulate enough fats not only for maintaining during hibernation or pupal period but also for completing reproduction. Lipids and carbohydrates are very important to silkworm because of their role on diverge physiological and biochemical functions (Mullins, 1985), in energy production as well as their significant impact on economic characters (Saha and Khan, 1996). Research work from Friend (1958), Barlow (1966) and others reveal that diet influences the qualitative composition of body fat of insects. Fatty acids are

accumulated in the fat body as triglycerides to provide reserves of energy, which are obtained from the diet. Essential fatty acids are obtained from the diet because insects are apparently unable to synthesize them.

Mason *et al.* (1990) have suggested that during the winter season, the lipid content may serve as sustaining fuel in many lepidopteran insects and the fall of haemolymph volume during winter is considered as cold temperature withstanding mechanism (Wigglesworth, 1965).

Goel *et al.* (1988) have reported the changes in lipid content during embryonic development of *Antheraea mylitta*. Changes in lipid content during larval period may be due to differential metabolic activity of different larval instars (Sinha *et al.*, 1992). Study of Ziegler (1997) reveals that only a minor portion of the lipid of the egg is synthesized in the oocytes and that most of the oocyte lipids are taken up from the dietary sources.

Unni *et al.* (1996) investigated the lipids and fatty acid composition of the host plants of *A. assama* in relation to larval growth. Bora and Handique (1999) have assessed the impact of photoperiod and some insecticides on the lipid content of muga silkworm in Dibrugarah of Assam. Preliminary information of the lipid contents in *Antheraea assama* is available from the work of Bordoloi and Hazarika (1992) and Hazarika *et al.* (1995). Kataky and Hazarika (1997) assessed the fats and fatty acids in the developmental stages of *A. assama* and in its host plants. But all these works are related to the lean dry weight of lipid of total body, which partially reflects the storage quantities, and not the true picture of current physiological state of the developmental stages. Therefore, the investigation on the haemolymph (which is the ready resource of the raw materials for various physiological activities) total lipid content of late larval instars is necessary.

### 2.3.3. Carbohydrates

Carbohydrates, along with proteins and lipids, form the principal class of organic compound in insects and other organisms. Carbohydrate contributes to the structure and function of all insect tissues and can be found in the muscle, cytoplasm, and membranes of the cells, as well as in the extra cellular haemolymph and supporting tissue.

In general carbohydrate metabolism in insects is similar to that found in vertebrates. However, insects unlike vertebrates possess an exoskeleton that is rich in the aminopolysaccharide, chitin etc. They also contain the disaccharides trehalose, which acts as a storage form of glucose. Trehalose is readily hydrolyzed to glucose, which in turn oxidized to provide energy especially for insect flight. Its highest concentration is normally found in the insect haemolymph where it ranges from 8 – 60 mg/ml depending upon the species, developmental stage and sex (Wyatt, 1967). The carbohydrate content in insects is intimately related to physiological events such as the moulting process, metamorphosis, flight and diapause (Wyatt, 1967). Increasing attention has been paid to tissue interactions and regulatory mechanism in carbohydrate metabolism in insects (Steele, 1963; Murphy and Wyatt, 1965; Friedmann, 1967; Wiens and Gilbert, 1967. Miao *et al.* (1991) has emphasized that the carbohydrate content of silkworm eggs is markedly affected by the strain of the silkworm and maturity of the leaves used as feed. Insect eggs store carbohydrate in the form of glycogen. In silkmoth it has been shown that the source of this store is trehalose from haemolymph (Hasegawa and Yamashita, 1965).

Shimada *et al.* (1984) has noticed that the trehalose content increases in inactive larvae of *Leguminivora glycinivorella* in early winter, attaining a maximum (30 mg/gm) and decreases in spring with a concomitant decrease and increase of glycogen. Sinha *et al.* (1993) reported on the haemolymph

carbohydrate and protein concentration of the *A. mylitta* larva infested by uzi fly, *Blepharipa zebrine* (Walker). In 1961, Horie has recorded that during starvation larvae of *B. mori* decreased their level of fat body glycogen content more quickly than that of their haemolymph sugar (House, 1963).

The energy required by the insect for the various metabolic activities are accelerated or slowed down in relation to the available energy. The haemolymph carbohydrate content has been assessed in *A. assama* by Dutta *et al.* (1997), who have emphasized on the influence of carbohydrate content on high metabolic activity of the larvae. However, no information is available on the profiles of protein, lipid and carbohydrate contents of *A. assama* reared on two major host plants and their impact on the cocoon characters as well as on disease susceptibility, if any, in an area outside the original residence of the species.

An attempt has therefore been made to investigate the protein, carbohydrate and lipid contents of haemolymph and water content of larval body of the fourth and fifth larval instar larvae of *A. assama* reared on the two major host plants in the recently introduced non-traditional region in the district of Coochbehar, West Bengal. Moreover, only the information on the lean dry weight of lipid and proteins of total body are available from the previous investigations on *A. assama* Ww, which partially reflects the storage quantities, and not the true picture of current physiological state of the developmental stages. Therefore, the present study on the haemolymph (which is the ready medium of the raw materials for various physiological activities) protein, lipid and carbohydrate content of fourth and fifth instar larvae has been undertaken with a view to get an idea about the balance of these three components in developmental stages.

The deficiencies of any metabolites in the haemolymph are encountered in the larval development and silk synthesis. The balanced

haemolymph metabolites will keep the larvae healthy, uniform in size and will also enable them to produce better cocoons.

### 2.4. Free Amino acids

The amino acids utilized for silk protein biosynthesis are derived from the diets and from other tissues utilized for various purposes. The amino acids are stored in the fat bodies or haemolymph and transported to the silk gland at the time of spinning. In general, insects require common ten essential amino acids for their growth and development (House, 1963).

The principal amino acid constituents of silk proteins are glycine and alanine, with some serine and tyrosine. Hence, the quality of silk fibre mainly depends upon the protein content during the larval life of silkworm.

Wyatt (1961) pointed out the high titer and wide variety of free amino acids (FAA) in haemolymph, which was a special characteristic of insects. Supporting this view, there are several literature in this line such as Chen (1958a & b, 1962, 1966, 1971, 1985), Gilmour (1961), Florkin (1958), Joly *et al.* (1972), Pant and Gupta (1980) and Lazar and Mohamed (1988). The presence of high titre of amino acids in the haemolymph can be used to evaluate the dynamic metabolism of protein.

Wyatt (1961), Florkin and Jeuniaux (1974) reviewed and elaborated the functional significance of free amino acids in the maintenance of osmotic pressure of insects. Buck (1953) suggested that the high titre of amino acids in the haemolymph is correlated with active protein degradation and synthesis that occurs during growth, moulting and metamorphosis. Wyatt (1961) attempted to relate the high titre of free amino acid in insect haemolymph to that of cocoon formation in *B. mori*, but could not generalize for insects not spinning a cocoon.

Characteristically insects have high levels of free amino acid, the quantities in the haemolymph are about 100 to 300 times higher than that in human body (Chen, 1985). Apart from protein synthesis, the excess amount is needed for various metabolic functions such as osmoregulation in aquatic insects, neurotransmitter, detoxication of certain metabolites, synthesis of phospholipids, protein as energy source during flight, tyrosin in the cuticle sclerotization and tryptophan in the eye pigment formation (Chen, 1985). Again, the composition of free amino acids is species specific and even sex specific.

The higher free amino acid concentration in the haemolymph is associated with the healthy growth of the larvae (Ito and Arai, 1967; Lazar and Mohamed, 1988). A series of information is available on the haemolymph free amino acid of *B. mori* larvae (Chen, 1962; Alieva and Filippovich, 1968; Aruttyunyan and Davtyan 1972; Inokuchi, 1972; Bose *et al.,* 1989). Parenti *et al.* (1985) observed that only two neutral amino acids along with threonine, aspartate, glutamate and their amides are taken up by the silk gland directly from the haemolymph for the biosynthesis of protein, whereas the basic amino acids are involved in the regulation of haemolymph osmotic pressure (Florkin and Jeuniaux, 1974).

Sinha *et al.* (1988a) reported the profiles of free amino acids in haemolymph of the larvae of *Antheraea mylitta.* Sharma *et al.* (1995) recorded a seasonal variation of haemolymph free amino acid of *A. assama* reared on two host plants. Sinha *et al.* (1988b) showed the changes of free amino acid of pebrine infected *A. mylitta.* Sinha *et al.* (1997) assessed the haemolymph for free amino acid profile of tasar silkworm affected by uzi fly *Blepharipa zebrina.* A sharp depletion of major free amino acid contents in the haemolymph of muga silkworm having bacteriosis has also observed by Das and Deb (2007). So far there is no detail report on the free amino acid

profiles of the larvae of *A. assama* and any relation of the amino acids to the susceptibility to diseases. An attempt was therefore made to investigate the haemolymph free amino acid profiles of the fourth and fifth instar healthy and bacteriosis afflicted larvae of *A. assama* reared on the two major host plants, som and soalu, in recently introduced non-traditional region in the district of Coochbehar, West Bengal, India. Furthermore, the amino acid contents of the diseased larvae were studied only in the worms reared on som plant, as it is popularly used during commercial rearing by the farmers.

Considering the points mentioned above, the present investigation have been designed to evaluate the effect of seasons (temp., humidity etc.) and host plants (som and soalu) on the haemolymph metabolite status in the fifth stage larvae of *A. assama* and their relation to bacteriosis if any.

### 2.5. Differential protein and peptides by HPLC and Electrophoresis

Results obtained using modern devices like electrophoresis, tracer and immunological methods, facilitate the general view that proteins in haemolymph undergo unique change during development of insects. No sexual difference in electrophoretic pattern of proteins observed at different larval stages of two lepidopteran insects *Mocis letipes* and *Empyreuma pugione* by Castaneda *et al.* (1985). The alteration in protein concentration is known to be due to histolysis and histogenesis of various tissues during metamorphosis of insects (Laufer, 1960a; Chippendale and Beck, 1966; Chippendale, 1970; Kim and Seo, 1981).

Cotton and Austee (1991) reported eight haemolymph protein bands by gel electrophoresis working on *Locusta migratoria*. They opined that blood protein concentration in the insect rose during fifth larval stadium, fell after the final molt and increased slightly by the 8$^{th}$ (eighth) day of adult life. Supporting results were obtained by Telfer and Williams (1953). They

reported that in *Hyalophora cecropia,* high total protein content was present. Among the seven proteins, five occurred in all stages, while a 6$^{th}$ (sixth) one appeared first in the late fifth instar. All the six proteins increased in concentration in the last larval stage but decreased at pupal-adult transformation. Seven proteins specific for females appeared first in the pre-pupa. The investigation was carried out by using PAGE (Poly Acrylamide Gel Electrophoresis) in this species.

Miller and Silhacek (1982) analyzed the haemolymph protein of *G. mellonella* at various stages of development using one-dimensional SDS-PAGE (Sodium dodecyl sulfate-PAGE) and identified the storage proteins. Seo *et al.* (1985) separated fifteen protein bands in the haemolymph of final instar stage of *B. mori* and noted high concentration of protein during the final larval instar, which declined after pupation.

In *A assama*, a group of researchers isolated a lysozyme like anti-bacterial protein from its pupal stage (Unni *et al.*, 2006). So far there was no report of any natural anti-bacterial protein from the larval stages of muga silkworm. Das and Deb (2008) have recorded a low molecular weight (22-23 Kda) protein band from the bacteriosis afflicted *A. assama* which is similar (in its weight) to the band identified by Unni *et al.* (2006) from pupal stage.

In the holometabolous insects, the so-called storage proteins are the most important class of proteins in larval haemolymph (Thomtion, 1975). Thomas (1979) isolated three lipoproteins from the haemolymph of *G. mellonella* larvae by preparative ultra-centrifugal floatation. The storage proteins are characteristically the most abundant class of polypeptides found in larval and pupal tissues of insects belonging to this group (Miller and Silhacek, 1982). They reported that these proteins initially accumulated in haemolymph of final instar larvae and utilized in later development. Sinha *et al.* (1991) also observed a decrease in protein content in the pebrine infected

larvae of *A mylitta* and suggested that the haemolymph protein might be an index of growth. The same group of authors recorded the gradual decrease in protein concentration from the first day of embryogenesis to the hatching of the larvae, with a sharp increase in the fourth day of embryogenesis in both healthy and pebrine infected embryos of *A mylitta*.

Alimentary (nutritional) factors do not have much influence on cryptogamic infections, but they are often connected with bacterial diseases such as flacherie (Vago, 1963). Abnormal food in the form of an unusual plant may lead to digestive troubles followed by the increase of intestinal bacteria, which could act as "potential pathogen" of septicemia (Vago, 1963). Vago (1963) also stated that silkworm flaccidity is preceded by alterations in the functioning of the malpighian tubes, which were gradually followed by reduced feeding and later by bacterial dysentery.

**Antibacterial peptides and polypeptides:** Bacterial attack induces rapid appearance of peptides / polypeptides in the haemolymph. These have antibacterial activity against both Gram-positive and Gram-negative bacteria. The molecules are synthesized in the fat body and in some haemocyte types and then are released in the haemolymph. Hetru *et al.* (1998) grouped all the antibacterial peptides in to four families. Cecropins and defensins are the two families, relatively homogeneous and are well defined. Cecropins have no cysteines. The other two families are heterogeneous and less known. These are proline-rich and glycine-rich inducible antibacterial peptides / polypeptides.

Cecropins were the first antibacterial peptides isolated from *Hyalophora cecropia* and fully characterized by Steiner *et al.* (1981). Subsequently many cecropins have been isolated from several species belonging to the orders Lepidoptera and Diptera. Lepidopteran cecropins

have considerable variation in the amino acid sequences. Cecropins can kill both Gram-positive and Gram-negative bacteria. These have 31 – 39 amino acid residues.

Defensins are mainly known to be active against Gram-positive bacteria, but rarely also against the Gram-negative bacteria. Other than Lepidoptera, these have been recorded in species belonging to several order of Insects. Defensin have 34 – 43 amino acid residues. These are rich in strong cationic amino acids such as arginine and lysine.

Small proline-rich peptides are known from Hymenoptera, Diptera and Hemiptera. These are active against Gram-negative bacteria (Casteels, 1998). These consist of 16 – 20 amino acid residues. The well known peptide is Apidaecin.

Several large glycine-rich polypeptides are known as antibacterial in nature against the Gram-negative bacteria, sometimes against the Gram-positive ones (Hetru *et al.*, 1998).

Another group of polypeptides has been isolated first from *H. cecropia* by Kockum *et al.* (1984). These polypeptides, known as attacins, have molecular weights higher than 20 kDa and are known to function as bacteriostatic agents. These are both basic and acidic in nature.