CHAPTER V

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Muga silkworm is a prerogative of India and cultured across the Brahmaputra valley of Assam. India occupies a prominent status in the silk map of the world for its commercial production of natural silk thread extracted from four varieties of sericigenous insects, viz. eri, muga, tassar and mulberry. Out of these four varieties, the muga silkworm has a unique status among all due to its golden coloured silk thread. The muga silk thread is a protein in nature. Its quality is mainly dependent on the nutrient intake during the larval stages of the muga silkworm. The nutritional quality of the host plant leaves of silkworm and their balanced proportion play a vital role in the proper growth and development of silkworm larvae and also the cocoon characteristics of silkworm. Muga silkworm is a multivoltine species, i.e., it can be reared for six broods in a year. Moreover, the muga silkworm, i.e., *A. assamensis* is a polyphagous insect. It feeds on various food plants, some of which are primary and some are secondary host plants.

In the present investigation the effect of primary and secondary food plants in six different broods of *A. assamensis* Helfer viz, bhodia (August-September), kotia (October-November), jarua (December-January), chotua (February-March), jethua (May-June) and aherua (June-July) in larval growth, rearing performance, cocoon and silk filament characters including certain biochemical parameters such as protein, lipid and carbohydrate in the haemolymph and tissue of different larval stages of muga silkworm was carried out.
5.1. Larval growth:

The larval features such as larval length, breadth, weight of *A. assamensis* Helfer greatly varies in different crops/broods reared in different seasons in a year indicating relationship between larval growth and cocoon yield. The study on the larval period of various instars of *A. assamensis* Helfer revealed (Table.4.1.1) variable larval durations for the larvae grown in different rearing crops and reared on different host plants. Larval breadth and weight showed a positive correlation with all the commercially important cocoon characters and also yield when the worms were reared on the two primary host plants i.e., som (*P. bombycina*) and soalu (*L. monopetala*). From this, *P. bombycina* has been found to be the best food plant for kotia crop whereas *L. monopetala* for jethua. Kotia and jethua are the two commercial crops aimed at production of cocoons for silk production. Dighloti (*L. salicifolia*), a secondary food plant of muga silkworm used under the present study during the kotia and jarua crops showed better Effective Rate of Rearing (ERR) and cocoon characters during the jarua crop which is attributed to higher nutrient content of the foliage at all maturity level.

In the present study the larval period observed during different crops in different time of the seasons reared on two primary and one secondary host plants under the prevailing temperature and relative humidity conditions (Table: 3.4b & Fig. 4.1.1) clearly indicated the impact of lower and higher temperature on the larval development resulting the longer or the shorter larval durations. The longest larval period was recorded in the jarua crop being 43, 46 and 48 days respectively in *L. monopetala*, *P. bombycina* and *L. salicifolia* with corresponding temperature and relative humidity ranging from a minimum of 9°C to a maximum of 30°C and 37-100% respectively. Whereas, the shortest larval periods being 21 days for the larvae reared on *L. monopetala* and 23 days on *P. bombycina* were recorded in
the aherua crop with temperature ranging from a minimum of $23^\circ$C to a maximum of $36^\circ$C and 59-100% relative humidity. On the basis of the results it has been confirmed that temperature greatly influenced the larval development during different crops reared in different seasons and different host plants with resultant lower and higher larval periods. Similar results were reported by Sahu et al. (2000) and Singh et al. (2012).

Out of the three different host plants of *A. assamensis* selected for the present study, the silkworm larvae reared on *L. monopetala* showed a shorter larval period than on the other two host plants *P. bombycina* and *L. salicifolia*. From the findings of the present work it was shown that reared on specific host plant, the shorter larval period resulting better larval quality followed by better cocoon quality. Similar findings were also reported by Ahmad et al. (2007) that lesser larval developmental period, more larval weight gain, and higher percentage of adult emergence are the major criteria to determine the advantage of a host plant on other. Mishra and Upadhyay (2002); Morohoshi (1969); Ramathulla (2012) and Kamili (2004) indicated that changes in temperature along with relative humidity has a profound effect on their moulting period and also decreasing temperature enhances the moulting period. Similar results were also reported by Datta et al. (2001) and Pandey and Tripathi (2008) that according to silkworm breeders, the low temperature is always better than higher temperature for productivity of the silkworm and larval duration in various instars. Robinson (1941); Chowdhury (1992); Bharali (1971, 72); Gogoi (1977); SubhaRao (1998); Sahu et al. (2000) mentioned that the complete life cycle of muga silkworm lasted for about 54 days in summer season and 81 days in the winter season. Chowdhury (1982b) reported that silkworm larvae complete their larval period within a period of 24-70 days with variation in the different rearing crops within the range. Watt (1893) also mentioned that the range of minimum and maximum larval periods was 26-40 days during larval development. Kakati and Chutia, (2009) reported that muga silkworm is multivoltine in nature and under
normal climatic conditions the rearing of the larvae is completed within 20-25 days in
summer and 45-55 days in winter. Tribhuvan and Singh (1998) reported that the temperature
and relative humidity were among the various environmental factors that influence the
growth, behaviour and larval periods in silkworm.

5.2. Rearing performance:

The findings of the present study on the rearing performance of *A. assamensis* showed
(Table.4.1) that there was a strong positive correlation with the rearing characters like larval
length and breadth in the bhodia crop reared on *L. monopetala* and in aherua crop reared on
*P. bombycina*. While a negative correlation was observed in aherua crop reared on *L.
monopetala* with no positive correlation when reared on *L. salicifolia*. Larval characters like
length, breadth and weight have paramount effect on the cocoon characters such as cocoon
weight, shell weight and shell ratio of *A. assamensis*.

The present study firmly revealed that the differences in cocoon characters have
strong positive correlation with the larval features in all the crops when reared on *P.
bombycina*. The same was observed when reared on *L. monopetala* in the jethua crop and on
*L. salicifolia* in the jarua crop. The present result therefore is an indicative of selecting of host
plant for rearing of *A. assamensis* for the best quality muga silk.

Host plants play an important role in better commercially important characters such as
effective rate of rearing (ERR), larval growth, cocoon quality etc. In the present investigation
it has been found that *P. bombycina* was the best food plant for kotia crop whereas *L.
monopetala* for jethua; and *L. salicifolia* for jarua with respect to ERR, cocoon weight, shell
weight which is attributed to the superiority of plant leaves. Further, the silkworms fed with
L. monopetala leaves exhibited better larval weight and cocoon weight which might be due to higher moisture and nutrient content of the leaves.

In an earlier study Siddiqui et al. (2000), Thangavelu and Sahu (1986) reported that L. monopetala is the most suitable host plant for muga silkworm rearing during different crops for improvement of ERR whereas the female cocoon weight and fecundity were found to be significantly higher on P. bombycina as against in L. monopetala. Bora (2006) reported that temperature has impact on the photophase during the developmental period. He also stated that the high temperature and constant light as well as low temperature and short photophase were detrimental with respect to utilization of food and growth of the silkworm. Similar results were also reported by Barah et al. (1988), Raja Ram and Samson (1991); Singh et al. (2004); Chakravorty (2004); Chakravorty et al. (2004).

In the present study it was observed that the host plants have influence on silk production profoundly affecting the survival behaviour, rate of quantity of food intake, digestion and assimilation. These directly influence the growth and development of the silkworm (Krishnaswami et al., 1970; Sinha et al., 2000; Ray et al., 1998; Rahman et al., 1999 and 2004).

Pant and Unni (1980) reported that the growth, development and economic characteristics of silkworm are influenced to a great extent by nutritional content of food plants that the rate of consumption of quality leaf has significant influence on the larval growth, weight and survivality was studied by Murugan and George (1992). Sarmah et al. (2012) reported that the larval weight was the highest in the kotia crop (7.32g) followed by jethua (7.02g) and bhodia (6.66g). This shows that there is no significant difference in weight for the larvae reared in jethua and kotia. The jethua crop is also not significantly different from bhodia. The shell weight of cocoons reared in August-September (bhodia) and
October-November (kotia) season showed almost the same results with 0.53g and 0.52g respectively. Cocoon harvested from November-January (jarua) crop however yielded cocoons with lesser shell weight of 0.45g. The abiotic factor temperature plays a chief role on the larval growth and productivity of the silkworm (Ueda et al., 1975; Benchamin and Jolly, 1986). They also reported that the silkworm larvae prefer to consume the host plant leaves containing high moisture and less dry matter. Periaswamy (1994) reported that the moisture content of host plant leaves plays a significant role on food utilization and growth in phytophagous insects.

High temperature affects the biochemical and physiological changes that ultimately affects the larval weight (Hazel, 1995). The effect of high humidity on weight of larvae of silkworm was studied by Pandey et al. (2006). Humidity has indirect effect on growth and development of silkworm larvae. Under too dry conditions, the leaves become unfit for insect consumption resulting in retarded growth of the larvae which makes them weak and easily susceptible to diseases and other adverse conditions (Singh et al., 2009). The environmental factors in particular the temperature and the humidity at the time of rearing and moisture content of mulberry leaf affects the growth of the silkworm (Rapusa and Gabrieal, 1975; Rahmathulla et al., 2003). Similar results were found in the present study which indicates that the silkworms fed with L. monopetala leaves exhibited heavier larval weight and cocoon weight which might be due to higher moisture content of the plants leaves.

The availability of essential nutrients in food plant is vital for successful life cycle, cocoon quality, from metamorphosis to moth stage and reproductive activity of the silkworm (Pattanayak and Dash, 2000; Rath et al., 2006; Reddy et al., 2009). Similar results have been reported by Reddy et al. (2010), according to whom the larval span, ERR and cocoon percentage of silkworm are significantly different among the larva fed on their different host
plants. There is also a significant difference between the different host plants with regard to single shell weight, shell ratio and silk yield. Thangavelu et al. (1988) recorded the cocoon weight as 4.1gm, 5.2gm, 4.5gm, 4.5gm, and 5.8gm and shell weights as 0.28gm, 0.48gm, 0.35gm, 0.35gm and 0.57gm in ‘chatua’ ‘jethua’, ‘aherua’, ‘bhodia’ and ‘kotia’ respectively. Low nutrition levels or less succulent foliage results in inferior growth and maturity to spin the cocoons for further advancement of its life cycle.

This also was concomitant with the present rearing performances including the larval duration, effective rate of rearing and the cocoon characters such as single cocoon weight, shell weight, shell ratio and reeling parameters. The lesser increase in both cocoon and pupal weight resulted in significant elevation in shell ratio but the absolute silk yield again changed negatively due to less ERR and cocoon yield. The cocoon traits like cocoon weight, shell weight, shell ratio are the positive cocoon characters to attain the commercial advantage of silk productivity. Chowdhury et al. (1998) also reported a range of cocoon weight from 4.6 to 6.5gm, shell weight 0.42 to 0.67gm while the shell ratio from 7.80 to 11.4 per cent in *A. assamensis*. Higher single cocoon weight, single shell weight and SR% at 23°C was reported by Tanaka (1964) who opined that the spinning temperature about 23°C and 70% humidity improves the cocoons and silk quality. Shell ratio (%) is the character to determine the cocoon quality of the silk. Cocoon traits, i.e., shell ratio, single shell weight and also cocoon weight show the significant difference depending on the type of host plant. Similar result was reported by Barah et al. (1988). Further, Neog et al. (2013) reported that muga silkworm reared on different host plants in different crops indicated *P. bombycina* to be the best host plant in respect of the various cocoon characteristics like ERR, cocoon weight, shell weight and shell ratio. Srivastava (1998) reported that the environmental conditions are the main causes of variability in the cocoon weight, shell weight, absolute shell ratio, filament length, denier, and sericin percentage. The present findings of shell weight and shell ratio are
supported by the results. Further, these characters are highly influenced by the type of food plants and also the environmental factors during silkworms rearing (Rathi and Mathur, 1988; Siddiqui et al., 2006).

5.3. **Biochemical parameters:**

In the present study the protein concentration in haemolymph and tissue showed a relationship with the silk synthesis. *L. monopetala* is the best host plant for the specified rearing crops regarding quality of the silk thread. In the present study it has been revealed that various biochemical parameters of the larvae of *A. assamensis* grown in different crops are found to influence the tensile properties of the silk thread is relevant to the different biochemical parameters of different rearing crops. In jethua crop, the haemolymph protein of silkworm larvae correlated with the tensile properties of silk thread of *A. assamensis* (Table.5) reared on the host plant *P. bombycina*; whereas, *L. monopetala* is the best host plant for the rearing crops bhodia, chotua, jethua and aherua related to silk quality. No relationship of tensile character was found in the silk thread obtained from the cocoons resulted from the larvae reared in any crop on *L. salicifolia*. Regarding strain properties no such correlation character was observed. However, the toughness of the silk thread showed that not only the haemolymph protein correlated with jarua crop on the secondary host plant but also the other biochemical parameters, such as have a correlation with the toughness of the silk thread in different crops. Therefore, the present results show that, different properties of silk thread differ based on the host plants used for rearing. Silk thread is chemically made up of two proteins, i.e., fibroin and sericin synthesized from haemolymph protein. The good quantity haemolymph protein is correlated with the better tensile properties of the silk thread which is concomitant with Hurliman and Chen (1974) who found that the important biological organic macromolecules like protein are used for the growth and development of the silkworm larvae.
as well as their silk biosynthesis. It was also reported that the protein contents present in silkworm tissue may be converted to the haemolymph protein for silk synthesis which is derived from the feeding habit of the silkworm larvae. The present result also supported by Sabhat et al. (2011). Nagata and Kobayashi (1990) also stated that in silkworm Bombyx mori, there is an increase amount in the synthesis of protein during feeding stage. Another findings corroborated by Loughton and West (1965) and Roma et al. (2010) observed that the total concentration of protein in haemolymph and fat body of A. myllita changes from pre instars to shortly before pupation.

The carbohydrate and lipid content of both haemolymph and tissue of the muga silkworm larvae have a positive correlation with tensile properties of muga silk thread (Table.5) in jethua crop which is the better rearing crop for the host plant P. bombycina. These two biochemical parameters of both haemolymph and tissue of the larvae and the tissue protein has also an indirect correlation with silk quality of A. assamensis in jarua crop reared on P. bombycina. L. monopetala is the best host plant for the rearing bhodia, chotua, jethua and aherua crops related to silk quality for carbohydrate and lipid content also. In L. salicifolia also revealed that there is no any relationship of tensile character in any crop on different host plants in the parameters considered during the present study. In strain properties of muga silk thread, a strong positive correlation was recorded in aherua crop with haemolymph carbohydrate. So, the different biochemical properties like tissue protein, carbohydrate and lipid have an indirect effect on silk synthesis. There are also reports that the carbohydrate and lipid content of larval haemolymph and tissue is converted into protein due to the absence of haemolymph protein. In support of the present result stated by Seong et al. (1985) the proteins are synthesized by the major biochemical process during morphogenesis and the storage proteins are most likely to be synthesized by the larval fat body and secreted into the haemolymph. So, there is also an indirect relationship with silk synthesis by the
biochemical parameters carbohydrate and lipid of both haemolymph and tissue which is totally dependent on the nutritive content during larval development in the present study.

The present observation is in agreement with Kumar and Michael (2012) who reported enriched amount of protein in the silkworm haemolymph by the supplementation of enriched leaves. It clearly indicates that the influence of the dietary protein on the increase in haemolymph protein during ultimate larval stages, i.e., fifth instar larvae which considered as the prime feeding stage of the silkworm larva where in about 80-85% of the total leaves is consumed. Further, the concentration of haemolymph protein increased progressively during the post larval development and reaches the maturity in the larvae, i.e., late fifth instar larvae. The increase in protein content is also attributed to the development of reproductive organs of silkworm insect (Sinha and Sinha, 1994). Haemolymph performs various physiological functions during insect development. It serves as the transport milieu for the exchange of numerous essential materials. Krishnaswasmi(1978b); Tazima (1978) reported that the growth and development of silkworm vary depending upon the environmental condition and quality of the leaf feed. The overall results of protein concentration in the developmental stages of the larvae reared on both the host plants indicated that the protein content increases with the ascending larval stages. The results also corroborated the findings of Saikia et al. (1993) and Roma et al. (2010). The stage and age specific changes in protein content of different tissue during post-embryonic development of silkworm might be used for adequate growth, development, maintenance and repair of tissues. There is an either positive or negative correlation between haemolymph and tissue protein indicated by quantitative estimation analysis by Kasmaei and Mahesha (2012). The feeding habit of the silkworm larvae ultimately increases their physiological parameters of haemolymph and tissue resulting better quality of cocoon and silk. There is also a gradual increase in various biochemical parameters according to increasing larval maturity. The similar results reported by
Krishnaswami et al. (1978a) stated that due to regular feeding in larval period there is increase protein concentration in the silkworm body after the fourth moult and substantial increase in the body weight as the larva attains the spinning stage. The higher protein content is an indication of a greater metabolic activity in the tissues. It was also reported that the protein content of the muga silkworm larvae reared on *P. bombycina* exhibited a fluctuating trend in its gradual levels of development, while the larvae reared on *L. monopetala* showed a uniform increasing trend towards the matured larval development. Generally, the tissue protein of sericigenous insects is responsible for the formation of silk proteins in the silk glands. Obviously, there is conversion of the majority of haemolymph proteins into silk substances as advocated by Lokesh and Narayana (2011).

It may be due to the rate of transforming amino acids of digested food protein into tissue protein is lessened which can be attributed to intracellular transformation of few protein molecules to some other compounds. It can also be interpreted that due to high rate of consumption of food at this stage, tissue proteins may transform into digestive enzymes, which remains undetected in the present investigation due to the removal of the gut of the larval instars before homogenization. This is supported by the higher protein content found in the fifth instar larval tissue. This higher protein content must have resulted owing to absorption of higher amount of amino acids from the gut due to increased enzymatic action of protein. Moreover, the carbohydrate and lipid content of both haemolymph and protein of muga silkworm is utilised in their different physiological activities like moulting spinning, flight, silk synthesis etc. Further, the lipid is the storing energy of the muga silkworm and utilized in silk synthesis in absence of protein. Therefore, the present investigation reveals a reverse result that there is maximum lipid content in jarua whereas protein content was maximum in jethua. The present result are concomitant with Kumar and Michael, (2012) who indicated that the carbohydrate is the main components in the food of all living organisms.
which are either directly or indirectly used as the source of energy for all vital activities as like growth and development of the larvae that might ultimately determine the difference in the quality and quantity of the silk production. It is also suggested that carbohydrates, particularly the reducing sugars are very important for growth and development of silkworm. Carbohydrates are utilized by the silkworms for their source of energy and synthesis of both lipid and amino acids. Some sugars possess a gustatory stimulation effect on the larval feeding of the silkworm (Ito, 1960). The carbohydrate content in insect is intimately related to the various physiological events like moulting process, metamorphosis, flight and diapause (Wyatt, 1967) and the increasing attention has been paid to the regulatory mechanism of carbohydrate metabolism in insects (Stele, 1963; Murphy and Wyatt, 1965; Friedman, 1967; Wiens and Gilbert, 1967; Goldsworthy, 1970; Yamashita et al., 1972). Poonia and Mishra (1975) found an increasing trend in carbohydrate in the first instar larvae of *A. mylitta* from 0.325-1.425mg/ml. Studies carried out by the Malik and Reddy (2007) indicate that the enrichment in carbohydrate level during feeding larval stages correlate with the amount of silk synthesized through their silk glands. Malik and Reddy (2008) stated that the haemolymph carbohydrate level increased significantly during feeding and spinning larvae and an acute drop in haemolymph carbohydrate level was observed in prepupal stages which are similar with the present results. The levels of haemolymph glucose in feeding larvae are relatively low as excess glucose is utilized for glycogen synthesis in the fat body. Low glucose levels in haemolymph in feeding larvae provide a suitable gradient for the absorption of glucose from the lumen of the midgut.

The present study carried out on the concentration of carbohydrates and the other biochemical parameters is primarily dependent on the quality of host plant leaves. The late age silkworm larvae accumulate higher amount of carbohydrate content than the young age
worns. Glycogen plays as the source of major food reserve in insects (Kilby, 1958). Wyatt and Kalf (1956 and 1957) states that trehalose is the major blood carbohydrate in insects. Simex and Kodrik (1986) have reported that the glycogen content in the fat body, body wall and silk gland and the free carbohydrates in the haemolymph changed significantly during last larval instar and metamorphosis in silk worms. Carbohydrates are the chief components in the food of all the living organisms which are either directly or indirectly used as the energy source for all vital activities of silkworm insect (Unni et al., 1995b). Similar results also reported that in the mature fifth instar larvae there is enormous amount of lipid contents (Gupta and Pathak, 1984). Gilbert (1967); Hoffmann (1984) found that the lipid content of insects are affected qualitatively and quantitatively by the fat in the diet and the decrease as rearing temperature increases the neutral fat content and also the lipid reserve of insects. Similar results were also reported by Hiremath et al. (2009) who found that the lipid content showed variation within the sexes with higher lipid in male (7.23% and 8.10%) as compared to female (6.34% and 7.10). It was established that the environmental factors, i.e., temperature has a pronounced effect on both the general lipid level metabolism and the composition of ectothermic lipid and inverse relationship between the temperature and degree of saturation of the total fatty acid complement (Hoffmann, 1985). It is also stated that the saturated and polyunsaturated fatty acids and lipid of silkworm haemolymph along with other essential biochemical constituents are the important requirements for growth and other functional properties like spinning of A. assamensis (Unni et al., 1995a). The results of the present study has a conformity with Mason et al. (1990) who found that the lipid content is high during the winter crop (jarua) which serves as a sustaining fuel in many lepidopteron insects.

It has assumed a considerable functional significance during the evolutionary history of the class Lepidoptera. It provides a rich source of metabolic energy for periods of
sustained energy demand. Chowdhuri et al. (2000) found that total concentration of unsaturated fatty acids remains higher in the haemolymph serum than the fat body tissue in each sex. The tissue lipid content of silkworm larvae has an increasing trend with increasing larval stages. These results owe to the difference in pattern of lipid metabolism in both types of the larvae. Overall, the data on lipid content represent an increasing rate of lipid content in the increasing larval instars. The increasing lipid content enhances larval growth by providing energy which aids the larvae in silk synthesis. Thus, lipid also forms a major component for determining the quality of the silk produced. Similar study have been reported by Unni et al. (1995b) who found that the secondary host plant reared muga silkworm contain a low level of lipids as compared to the larvae reared in primary host plants. The declined level of fatty acid causes delayed larval growth or reproductive decline and sometime it requires more than one generation of deprivation to become apparent. Beneficial effects of some lipids in addition to a sterol on growth of other insects (Scoggin and Tauber, 1950) have been reported. It also stated that insects from nutrient reserve may carry out net conversion of fat into carbohydrate (Deuel & Morehouse, 1946; Buck, 1953; Chauvin, 1956). Similar observation was made by Dhinkar et al. (1991), who studied seasonal changes in the composition of silk glands in Bombyx mori.

5.4. Profile of haemolymph protein:

In the present investigation, several prominent protein bands corresponding to the molecular markers were detected by using SDS-PAGE analysis from the haemolymph sample collected from the larvae of bhodia crop. Electrophoresis has been the powerful tool to analyze the separation of number of protein bands found in three tissues, i.e., haemolymph, silk gland and midgut (Singh et al., 2011a). In addition to these prominent protein bands, one
additional protein band was recorded in the haemolymph protein of the larvae of *A. assamensis* reared on *L. monopetala* in between mol.wt.35 and 25 kDa with relative mobility 0.384 (Fig 4.5.1 and Table 4.5.1). The present result was concomitant with that of Dash *et al.* (2007) who detected protein bands within the range of molecular weight between 45-35 kDa indicating the low molecular weight sericin in the cocoons of *A. assamensis*. In favour of the present results, Kumar *et al.* (2011) reported that the high molecular weight sericin increases the strength of the silk fibre while the low molecular weight sericin protects the pupa from various environmental stresses. Therefore, from the present study it may be concluded that the high and low molecular weight protein correspond to the high and low molecular weight sericin protein.

In kotia crop, the SDS-PAGE analysis revealed the separation of different prominent protein bands with additional bands corresponding to molecular markers 116, 95, 66 and 47kDa with relative mobility 0.166, 0.188, 0.348, 0.424 respectively and three additional bands, one just after the molecular band close to the molecular marker 35kDa with rel.mob. 0.545 and two just after 25kDa with rel.mob. 0.727 and 0.803 (Fig 4.5.2 and Table 4.5.2) have been recorded. Besides these bands, three additional protein bands just after mol.wt.14kDa with rel. mob.0.984, 1.045 and 1.06 and one just after mol.wt.10kDa with rel.mob. 1.106 have been recorded. From the results, it may therefore be opined that the 66kDa protein corresponds to the sericin protein of haemolymph. Similar results was reported by Ahmed *et al.* (2004) indicating the prominent band at 66KDa molecular weight for sericin from *Antheraea assamensis* and *Philosamia ricini*.

In jarua crop, the analysis of protein band through SDS-PAGE revealed that there were several prominent protein bands of haemolymph protein of the larvae of *A. assamensis* along with one additional band in between mol.wt. 95 and 66kDa with rel. mob.0.213 and many additional bands in between mol.wt.47 and 35kDa with rel. mob.0.311, 0.360, 0.409
and 0.475; and in between mol.wt.35 and 25kDa with rel.mob. 0.524 and 0.537. Two additional protein bands were also recorded in between mol.wt.20 and 14kDa with rel.mob.0.754 and 0.803 and one in between mol.wt.14 and 10kDa with rel. mob. 0.950 along with two bands below mol.wt.10kDa with rel. mob.1.016 and1.065 (Fig 4.5.3 and Table. 4.5.3) were recorded. Kumar *et al.* (2011) also reported that the stage and age dependent variation in SDS-PAGE observed in different tissue of the larvae, pupae, adults and eggs revealed that the mol.wt.36 and 64kDa protein bands in different tissues during post embryonic development has a concomitant with the present observation and may be concluded that the proteins with different molecular weight may be used in different developmental site during post embryonic development.

In chotua crop, additional prominent protein bands were recorded in between mol.wt.95kDa and 66kDa with relative mobility 0.239 and 0.267 along with another band in between mol.wt.66kDa and 47kDa with rel. mob.0.309. One more protein band was recorded in between 47kDa and 35kDa with rel. mob. 0.422. Besides these, a protein band just below the mol.wt. 35kDa close to the molecular marker with rel. mob.0.508, 0.526 and 0.536 was also recorded. Additional bands with relative mobility 0.595 in between mol.wt.25kDa and 20kDa, between mol.wt. 20kDa and 14kDa with relative mobility 0.667 in third, fourth and fifth instar larval haemolymph protein reared on three different host plants, *i.e.*, *P. bombycina, L. monopetala* and *L. salicifolia* were also detected (Fig. 4.5.4 and Table. 4.5.4). These findings are also in concomitant with the statement that, identification of 36 and 64kDa protein bands found in different tissue like haemolymph, fat body, midgut and reproductive organ with different intensity of protein band reveals their occurrence in different tissues with stage and age specific patterns crucial for development and reproduction (*Rai* *et al.*, 2010).
In jethua crop, two additional protein bands in between mol.wt. 95kDa and 66kDa with rel. mob. 0.239 and 0.267 and one between mol.wt. 66kDa and 47kDa with rel. mob. 0.309 have been recorded. One more protein band was recorded in between mol.wt. 47kDa and 35kDa having rel. mob. 0.422 and three bands between mol.wt. 35kDa and 25kDa with rel. mob. 0.508, 0.526 and 0.536 were also recorded. Two more additional bands were recorded one each in between mol.wt. 25kDa and 20kDa with rel. mob. 0.595 and between mol.wt. 14kDa and 10kDa with rel. mob. 0.931 in the haemolymph of all the three larval instars reared on three different host plants *P. bombycina, L. monopetala* and *L. salicifolia* (Fig. 4.5.5 and Table. 4.5.5). The SDS-PAGE analysis revealed prominent band at mol.wt. 66kDa for sericin on *A. assamensis* and *P. ricini* as reported by Prasanna *et al.* (2013). The sericin in the cocoons of *A. assamensis* reported by Prasanna *et al.* is an indicative of the protein band between 45-35kDa.

In aherua crop also many prominent protein bands corresponding to molecular marker used were recorded along with some additional bands. The additional prominent protein bands appearing between mol.wt. 66kDa and 47kDa with relative mobility 0.323 and in between mol.wt. 35kDa and 25kDa with relative mobility 0.430 were recorded. More protein bands in between mol.wt. 25kDa and 20kDa with the relative mobility 0.615, 0.661, 0.723 and 0.753 have also been detected. Protein bands in between mol.wt. 20kDa and 14kDa with relative mobility 0.784 and in between mol.wt. 14kDa and 10kDa were also detected in the haemolymph of third, fourth and fifth instar larvae with relative mobility 0.953, 0.969 and 1.00 reared on the three different host plants *P. bombycina, L. monopetala* and *L. salicifolia* (Fig. 4.5.6 and Table. 4.5.6).

The protein fraction study stated above in different larval instars indicated that changes of the protein banding pattern into the intensity of the protein bands, i.e., either more or less in between different instars, presence of some bands or their absence indicated the
production or non-production, utilization or degradation of the haemolymph protein. Similar findings were made by Mahesha et al. (2000). The present results are also supported by the works of Kasmaei and Mahesha, (2012). Similarly, the impact of mutagenic agent on the total protein in both midgut and haemolymph of silkworm revealed that the protein content was higher at the end of the fifth instar and it is mainly due to the maximum amount of food (leaves) consumed by the larvae during that period (Lokesh and Narayana, 2011). It is also reported that the high protein concentration is an indication for greater metabolic activity of the silkworm tissue. The haemolymph, as a carrier of all nutrient substances distributes to each and every part of the body for cellular metabolism, whereas in the micromolecules get converted into complex macromolecules like proteins and carbohydrates (Tazima, 1978). Further, there is increased protein content in the midgut and haemolymph of the silkworm due to the supplementation of enriched leaves to silkworm.

The SDS-PAGE analysis of haemolymph protein in different crops of *A. assamensis* showed different prominent protein bands corresponding to the different molecular marker with different molecular weight. Out of the different protein bands, the prominent protein band corresponding to the molecular marker molecular weight 66kDa indicating the homology of the cell lines of the larvae reared in different crops and also the influence on sericin protein of the cocoon by this specific protein band.