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Quantification and Electrophoretic Profile of Haemolymph Proteins of Muga Silkworm (*Antheraea Assamensis* Ww) Larvae Reared On Two Major Host Plants (*Litsea Monopetala* and *Persea Bombycina*) For Two Different Crops (Seasons)

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Abstract
In the present study the total haemolymph protein content and its electrophoretic profile of 3rd, 4th and 5th instar larval stages of *Muga* silkworm (*Antheraea assamensis*) grown on two different primary host plants - *Som* (*Persea bombycina*) and *Soalu* (*Litsea monopetala*) for two different crops, namely, *Bhodia* (monsoon crop) and *Kotia* (autumn crop) was determined. The study showed that the protein content increases from 3rd to 5th instar larvae grown in both *L. monopetala* and *P. bombycina* for both the crops. The patterns of protein bands observed in the haemolymph using 12% of Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) indicated distinct nine bands with various intensities for the larvae grown on both the host plants for both the crops with an exception of an additional band of poor resolution in the haemolymph of larvae reared on *L. monopetala* for monsoon crop and the absence of the second band with molecular weight 116kDa in third instar larvae of the autumn crop. But the same was present in fourth and fifth instar larvae. On the other hand, the protein bands with molecular weight 66kDa and 47kDa were observed in all the three larval stages grown on both the host plants in autumn crop. Interestingly, in the 5th instar larvae of the same crop a band of high intensity was observed which was not found in the larvae of monsoon crop. However, the protein bands in all the larval stages grown on both the host plants with molecular weight 25 kDa, 20 kDa and 14kDa were found to be identical. The electrophoretogram evidences revealed that all the three haemolymph protein patterns with molecular weight 25kDa, 20kDa and 14kDa showed homology with minor variations in all the three larval stages reared on both the host plants for both the crops.

Keywords: Haemolymph, Protein, Host plants, Muga silk worm.

1. Introduction
Muga silkworm (*Antheraea assamensis* Ww), is a polyphagous, semi domesticated and multivoltine insect which is indigenous to Northeast India and reared only under outdoor condition unlike other silkworms, which are reared under indoor condition. It feeds on primary and secondary host plants. The primary host plants are those which are the most preferred and the rest where the silkworm can sustain its life, but are generally not preferred are the secondary host plants [1]. *P. bombycina* and *L. monopetalaIt* are the primary host plants of *A. assamensis*. It also feeds on some other secondary host plants namely, *Mezankari* (*Litsea citrata)*, *Dighloti* (*Litsea salicifolia)*, Bogori (*Ziziphus mauritiana)* etc. [2, 3]. The growth and development of the Muga silkworm and their economic characteristics of cocoon and silk are influenced to a great extent by the nutritional composition of the leaves of their host plants [4]. Phytophagous insects show a varying degree of association with host plants, particularly the plant species or group of plants on which they feed [5]. The survival, growth, development and also the cocoon characteristics of the silkworms are greatly influenced by the nutritional content of the food plants [6]. Haemolymph serves as a reservoir for nutrients and metabolites during larval growth of insects. The silk is secreted by the silk glands and possesses sericin and fibroin proteins [7]. The haemolymph proteins serve as storage protein for the synthesis of silk. Haemolymph proteins undergo radical changes both in quality and Quantity during the development in lepidopteran insects [8]. It is observed that the protein profile changes gradually during larval, pupal and adult development [9]. Protein quantification studies by SDS-PAGE reveal the molecular weight of the proteins in the tissues as well as haemolymph. SDS-PAGE protein profile studies on Tasar silkworm suggested that haemolymph proteins influence the

growth and development of silkworm larvae [10]. For increasing the raw silk production, it is essential to study the effect of different food plants on haemolymph protein quantity and protein pattern with their variations in the larvae of different crops.

In the light of above consideration, an attempt was made to carry out a comparative study about the protein profile of haemolymph protein of Muga silkrworm larvae grown on their two primary host plants in different crops by using SDS-PAGE.

2. Materials and Methods

2.1. Collection of Larvae

The muga silkworm larvae of 3rd, 4th and 5th stages reared on Som and Soalu during monsoon season (Bhodia crop) and autumn season (Kotia crop) were collected from Assam Sericulture Farm, Khanapara and immediately after collection of the larvae, the haemolymph was extracted as quickly as possible.

2.2. Haemolymph Collection

Haemolymph was collected in pre-chilled test tube after adding a few crystals of thiourea by cutting the prolegs of the larvae. It was then immediately centrifuged at 6200rpm for 10 min at 4^0C and the supernatant was then stored at 4^0C for further estimation.

2.3. Protein Quantification

For the estimation of haemolymph protein of the silkworm larvae, the protein was precipitated by adding TCA (Protein: TCA ratio of 1:4).The protein precipitate was then extracted by centrifugation and dissolved in 0.1N NaOH solution and then its concentration was estimated by Lowry *et al*. (1957) [11] method.

2.4. Experimental Procedure of SDS-PAGE for Protein Profile

The qualitative analysis of total protein was carried out according to Laemmli (1970) [12] by using 12% separating gel with 5% stacking gel in sodium dodecyl sulphate polyacrylamide gel electrophoresis with slight modification. A uniform quantity of protein from each batch was loaded to each slot of the gel. Molecular weight markers from SRL were also used in a slot to compare the molecular weight of the proteins separated from that of the sample. After appropriate destaining, the gels were scanned, analysed and photographed in a gel scanner.

3. Results

3.1. Haemolymph Protein Concentration

The results of protein estimation revealed gradual increase in haemolymph protein concentration from 3rd to 5th instar larvae grown on *Litsea monopetala* and *Persea bombycina* with a slight increase in the haemolymph of *Litsea monopetala* grown larvae. The haemolymph protein concentrations of *P. bombycina* grown larvae for monsoon crop were recorded as 327.68±0.94mg/dl in 3rd instar larvae, 333.72±0.86mg/dl in 4th instar larvae and 355.25±0.21mg/dl in 5th instar larvae as compared to 393.70 ±0.08mg/dl in 3rd, 432.09±0.14mg/dl in 4th and 451.22±0.46mg/dl in 5th instar larvae grown on *Litsea monopetala*. However, the protein concentrations in the *P. bombycina* grown larvae of autumn crop were recorded as 447.42±1.47mg/dl in 3rd, 546.83±1.16mg/dl in 4th and 621.26±0.81mg/dl in 5th instar larvae as compared to 362.67±1.25mg/dl, 537.52±0.24mg/dl and 556.16±0.20mg/dl in 3rd, 4th and 5th instar larvae respectively in *L. monopetala* grown larvae (Table 1).

Table 1: Concentration of haemolymph protein (Average ± SD) of Muga silkworm (*Antheraea assamensis*) reared on two primary host plants (Som and Soalu) in two different crops (monsoon and autumn)

Biochemical parameter	Concentration in Som grown larval stages (mg/dl)			Concentration in Soalu grown larval Stages (mg/dl)		
	3rd	4th	5th	3rd	4th	5th
Protein/ monsoon	327.68±0.94	333.72±0.86	355.25±0.21	393.70±0.08	432.09±0.14	451.2451.4 51.22±0.46
Protein/autumn	447.42±1.47	546.83±1.16	621.26±0.81	362.67±1.25	537.52±0.24	556.16±0.20

Values of haemolymph protein of muga silkworm are the Average ± SD of monsoon and autumn season**.**

3.2. Haemolymph Protein Profile

The protein profile of haemolymph protein of muga silkworm (*Antheraea assamensis*) grown on two host plants (*Litsea monopetala* and *Persea bombycina*) studied by single dimension SDS-PAGE for monsoon crop showed nine different bands (Fig.1). The protein bands were more intense in *L. monopetala* grown larvae than *P. bombycina* grown larvae in monsoon crop with variations in molecular weight. There were nine bands with molecular weight of 14, 20, 22, 25, 35, 47, 66, 95 and 220kDa based on molecular markers. In *L. monopetala* reared larvae of *A. assamensis*, the haemolymph protein shows another band with 35 to 20 kDa which was not observed in *P. bombycina* grown larvae.

On the other hand, in autumn crop, the protein profile of haemolymph protein of *A. assamensis* larvae showed same nine major bands grown on both the host plants for both the crops but with the absence of the second band with molecular weight 116kDa in third instar larvae (Fig.2). Further, it has been revealed that the protein band with molecular weight of 66 and 47 kDa of 5th instar larvae for autumn crop was of very high resolution of monsoon crop. However, the protein bands with molecular weight 25, 20 and 14kDa were found to be identical in all the three larval stages grown on both the host plants for both the crops.

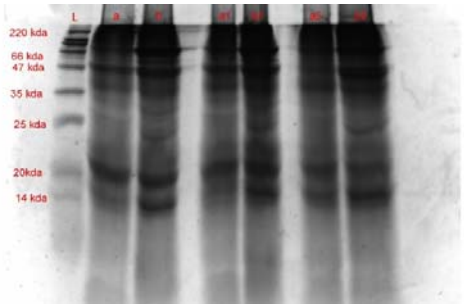

Fig 1: SDS-PAGE (Laemmle's method) Protein profile of haemolymph of Muga silkworm with Commassie Brilliant Blue
A-3rd stage larval haemolymph protein reared in som plant
B-3rd stage larval haemolymph protein reared in soalu plant
A1-4th stage larval haemolymph protein reared in som plant
B1-4th stage larval haemolymph protein reared in soalu plant
A2- 5th stage larval haemolymph protein reared in som plant
B2-5th stage larval haemolymph protein reared in soalu plant
L-molecular marker

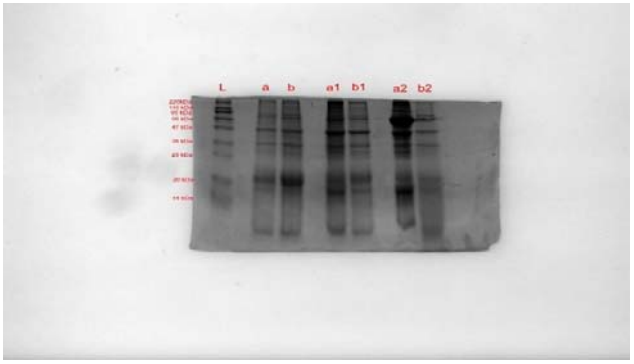

Fig 2: SDS-PAGE (Laemmle's method) Protein profile of haemolymph of Muga silkworm with Commassie Brilliant Blue.
A-3rd stage larval haemolymph protein reared in som plant
B-3rd stage larval haemolymph protein reared in soalu plant
A1-4th stage larval haemolymph protein reared in som plant
B1-4th stage larval haemolymph protein reared in soalu plant
A2- 5th stage larval haemolymph protein reared in som plant
B2-5th stage larval haemolymph protein reared in soalu plant
L-molecular marker

4. Discussion

Proteins are important biological macromolecules that are required for growth and development of the silkworm as well as biosynthesis of silk. They are the major source of nitrogen and essential amino acids, which are to be obtained only through leaves of host plants. The result of the present investigation of protein quantification reveals that the haemolymph protein concentration increases gradually with the advancement of larval period from 3rd to 5th instar stage grown in both the host plants, namely, *Persea bombycina* (Som) and *Litsea monopetala* (Soalu) for both the monsoon (Bhodia) and autumn crop (Kotia). The increase in the protein content of haemolymph and silk gland from the beginning to the end of the fifth instar may be due to active secretion of proteins by other tissues like fat bodies [13]. The present investigation also reveals that the haemolymph protein concentration is always high in *L. monopetala* (Soalu) grown larvae than those grown in *P. bombycina* for monsoon crop whereas the concentration of protein is higher in *P. bombycina* than those grown in *L. monopetala* for autumn crop. This might be due to moisture content in the leaves of host plants due to seasonal variation.

As evidenced by the SDS-PAGE analysis, the qualitative comparison of haemolymph protein of Muga silkworm reared on the two different host plants reveals that there were total nine protein bands in all the larval stages grown on both the crops with a slight variation in the intensity and with an additional band of poor intensity in all three larval stage grown in *L. monopetala* for monsoon crop with molecular weight between 35-20 kDa and the absence of the second protein band with 116kDa in 3rd instar larvae reared on both the host plants for autumn crop. The qualitative haemolymph proteins may be considered as a marker molecule for cocoon weight, shell weight, filament length and denier [14]. SDS-PAGE analysed protein band at 66kda was a sericin protein from *A. assamensis* and *Philosomia ricini* [15]. Another smear was found in the region of 45-35 kda which indicates low molecular weight sericin in the cocoons of *A. assamensis*, reveals that high molecular weight sericin increases the strength of the fibre while the low molecular weight sericin protects the pupa from the various environmental stress condition [16]. Sericin protein is generally a waste product during degumming the silk thread, i.e removal of sericin by boiling in hot alkaline solution to improve the fiber quality [17].

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