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# QUANTITATIVE ESTIMATION OF HAEMOLYMPH PROTEIN DURING DIFFERENT DAYS OF V INSTAR LARVAE IN BIVOLTINES, MULTIVOLTINES AND MUTANTS OF THE *BOMBYX MORI*

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**ABSTRACT**
An experiment was carried out utilizing two different voltine groups comprising indigenous and exotic races/breeds of mulberry silkworm *Bombyx mori viz.*, six bivoltines; $C_{108}$, Kalimpong-A (KA), $NB_4D_2$, $CSR_2$, $P_{31}$, $NB_{18}$, six multivoltines; Pure Mysore (PM), Nistari, C.nichi, $MU_1$, *npnd*, Hosa Mysore (HM) and six mutants namely, zebra, ursa, knobbed, *pere (*pink eyed red eggs*)* lemon and *pre* (Precocity). The haemolymph was extracted from 2nd, 4th and 5th days of V instar larvae by puncturing abdominal legs and the haemolymph was collected in pre-chilled eppendorf tubes containing 1m*M* thiourea-crystals to prevent melanization. The different samples for the protein assay along with standard were prepared and the quantitative protein estimation was made utilizing Lowry's method and obtained data was subjected to standard statistical analysis. The mean value of haemolymph protein content recorded at V instar 2nd day showed, a highest of 45.01±1.110 (mg/ml) in $NB_{18}$ race, whereas a lowest of 29.26±0.079 (mg/ml) in *npnd* race. During V instar 4th day, the quantitation of protein content highest in $CSR_2$ breed (47.90±1.335 mg/ml), whereas a lowest total protein content was recorded in C.nichi race (30.99±0.510 mg/ml). Further, the total of protein content was a maximum in $CSR_2$ breed (45.19±1.301mg/ml), whereas a lowest of 25.99±0.666 (mg/ml) haemolymph protein content was exhibited by *npnd* race during V instar 5th day. Perusal of average mean data from the eighteen races/breeds clearly indicates that, the total of protein content significantly increased during 2nd and 4th day of V instar with declining trend on 5th day of V instar. The general trend of variation in the haemolymph proteins among the eighteen genotypes is a noteworthy investigation useful for silkworm breeding and genetics.

**KEYWORDS:** Haemolymph protein, Quantitative estimation, Bivoltines, Multivoltines, Mutants, *Bombyx mori*.

## INTRODUCTION

Haemolymph of the insects are very commonly utilized in understanding the genetic differences existing between the population and such differences in understanding the population structure of different species of Drosophila is well documented (Hubby and Lewontin, 1966; Lewontin and Hubby, 1966). In fact, Silkworm, *Bombyx mori* is one of the popular beneficial insects for the production of sleek, sensuous silk fibre, which is considered as "Queen of Textiles". The silkworm belongs to family Bombycidae, which produces two types of proteins that get converted into a delicate twin thread of silk fibroin, coated by a protective cover of sericin (Mondal *et al.,* 2007). The process of silk production involves sequential interlinked mechanism of silk production from mulberry as food to protein and protein into silk fibre. During this process haemolymph of V instar mainly contributes towards silk protein biosynthesis in the different compartments of the silk gland and final products of silk proteins are fibroin and sericin and form the main component of silk fibre from the total silk produced. The biochemical characterization through protein profiling is very important in the lepidopteran insects, since proteins may change with physiological state, age, race and sex (Wigglesworth, 1965 and Chapman, 1998). It is important to note that protein variation occurs at every stages of Development, such type of variations in the proteins of the domesticated silkworm *Bombyx mori* is quite significant in understanding the genetic constitution of a race/breed. Proteins are the key factors within the cells, known to be governed by genes and it is evident from the biochemical changes reflected in the larva (Singh and Singh, 1987; Poonia, 1985 and Eid *et al.,* 1989). Keeping the above concept in the mind, we have subjected eighteen silkworm genotypes belonging to bivoltines, multivoltines and mutants to understand the variability of haemolymph protein content at 2nd, 4th and 5th days during V instar larval stage of the silkworm *Bombyx mori*.

## MATERIALS & METHODS

Parental seed cocoons of the above said eighteen races/breeds and mutants were collected from the Germplasm Bank of Department of Studies in Sericulture, Manasagangotri, Mysore and layings were prepared and reared by adopting the methods described by Tazima (1978) and Krishnaswami (1978) respectively. The haemolymph was extracted from 2nd, 4th and 5th days of V instar larvae by puncturing abdominal legs and the haemolymph was collected in pre-chilled eppendorf tubes containing 1m*M* thiourea- crystals to prevent melanization. The samples for the protein assay were prepared by using above mentioned different days of V instar larvae haemolymph and all the eighteen races/breeds

and mutants were selected for the biological assay. The protein was estimated through standard procedure suggested by Lowry *et al.* (1951). For accurate quantification, the sample protein is compared with a known amount of a standard protein used Bovine Serum Albumin (BSA).

## RESULTS

The observation obtained from the quantitative estimation of protein is presented in the table-1, which represents the total haemolymph protein content with standard errors (±SE) in the crude extracted haemolymph protein among the eighteen races/breeds. The perusal of the data clearly indicates, significant variation of protein content according to different days ($2^{nd}$, $4^{th}$ & $5^{th}$) of V instar larval developmental stage among the eighteen races/breeds and mutant stocks selected for the study. The mean value of haemolymph protein content recorded at V instar $2^{nd}$ day showed a highest of 45.01±1.110(mg/ml) in $NB_{18}$ race, whereas a lowest of 29.26±0.079(mg/ml) mean value protein content was found in *npnd* race. Similarly, the total content of protein is highest in $CSR_2$ breed (47.90±1.335mg/ml), whereas lowest was recorded in C.nichi race (30.99±0.510mg/ml) during V instar $4^{th}$ day and remaining races/breed showed intermediate protein content. Further, the protein is highest in $CSR_2$ (45.19±1.301 mg/ml), whereas a lowest of 25.99±0.666 (mg/ml) haemolymph protein content was exhibited by *npnd* race and remaining races have exhibited inbetween during V instar $5^{th}$ day. Furthermore, the average mean values were drawn from over all the eighteen races/breeds, the average haemolymph protein content of V instar during $2^{nd}$, $4^{th}$ and $5^{th}$ days were exhibited 38.60±0.724 (mg/ml), 41.32±0.887 (mg/ml) and 38.032±0.828(mg/ml) respectively.

**TABLE 1:** Mean values (±SE) of haemolymph protein content at V instar $2^{nd}$, $4^{th}$ and $5^{th}$ day in eighteen races/breeds of the silkworm, *Bombyx mori*

| Sl.no. | Races/breeds/ mutants | Haemolymph protein content & ± SE (mg/ml) during V instar | | |
|---|---|---|---|---|
| | | $2^{nd}$ day | $4^{th}$ day | $5^{th}$ day |
| 1 | $C_{108}$ | 42.45 ± 1.025 | 46.89 ± 1.259 | 41.95 ± 1.010 |
| 2 | KA | 44.40 ± 0.620 | 47.78 ± 1.251 | 43.35 ± 0.845 |
| 3 | $NB_4D_2$ | 40.55 ± 0.411 | 43.40 ± 1.199 | 40.11 ± 1.091 |
| 4 | $CSR_2$ | 44.95 ± 1.291 | 47.90 ± 1.335 | 45.19 ± 1.301 |
| 5 | $P_{31}$ | 41.09 ±1.502 | 43.00 ± 1.091 | 41.10 ± 0.655 |
| 6 | $NB_{18}$ | 45.01 ± 1.110 | 46.99 ± 1.141 | 43.05 ± 0.900 |
| 7 | Pure Mysore | 37.99 ± 0.092 | 39.51 ± 0.230 | 40.59 ± 0.841 |
| 8 | Nistari | 36.76 ± 0.915 | 38.59 ± 0.951 | 32.01 ± 1.005 |
| 9 | C.nichi | 30.00 ± 0.411 | 30.99 ± 0.510 | 28.90 ± 0.088 |
| 10 | $MU_1$ | 32.11 ± 0.715 | 37.33 ± 0.912 | 34.56 ± 0.750 |
| 11 | *npnd* | 29.26 ± 0.079 | 31.05 ± 0.059 | 25.99 ± 0.666 |
| 12 | Hosa Mysore | 33.30 ± 0.511 | 40.66 ± 0.750 | 35.50 ± 0.856 |
| 13 | zebra | 39.51 ± 0.550 | 38.90 ± 1.009 | 36.65 ± 0.991 |
| 14 | ursa | 37.99 ± 0.612 | 35.89 ± 1.105 | 33.00 ± 0.852 |
| 15 | knobbed | 40.02 ± 0.705 | 43.59 ± 1.045 | 41.10 ± 1.009 |
| 16 | pere | 41.25 ± 0.205 | 46.88 ± 0.491 | 44.40 ± 0.852 |
| 17 | lemon | 40.11 ± 0.099 | 45.55 ± 0.735 | 40.55 ± 0.711 |
| 18 | *pre* | 38.06 ± 0.080 | 38.00 ± 0.901 | 36.59 ± 0.493 |
| Average | | 38.60±0.724 | 41.321±0.887 | 38.032±0.828 |
| Range | | 29.26±0.079 – 45.01±0.110 | 30.99±0.510 – 47.90±1.335 | 25.99±0.666 – 45.19±0.301 |

## DISCUSSION

In the present investigation, an attempt has been made utilizing haemolymph protein to understand the quantitation of haemolymph protein content during $2^{nd}$, $4^{th}$ and $5^{th}$ days of V instar larval developmental stage in the eighteen races/breeds and mutants of the silkworm *Bombyx mori*. The haemolymph protein was estimated quantitatively (mg/ml) following the method suggested by Lowry *et al.* (1951). Based on the results, it is clearly indicated that, the quantity of soluble protein content varies in the eighteen races/breeds during $2^{nd}$, $4^{th}$ and $5^{th}$ days of V instar. It is an evident from the results shown in Table-1, $CSR_2$ on the $4^{th}$ day recorded highest amount protein content of 47.90±1.335 mg/ml, whereas the lowest of 25.99±0.666 mg/ml was observed in *npnd* on the $5^{th}$ day, while all the other observations were intermediary between them.

From the obtained results, it is clear that, gradual increase from $2^{nd}$ day to $4^{th}$ day, it is due to the fact that the silkworm is an voracious eater especially at this period, but during $5^{th}$ day the haemolymph protein content gradually decreases. This type of phenomenon was observed by Sarangi, (1985), he clearly shown that, It is possibly because of the soluble protein in silkworm gets converted into the silk fibre during this period. The present findings also corroborates with the results of Muralimohan (2005), who, while studying the day-to-day biochemical studies in the V instar larvae, demonstrated that the

soluble protein concentration is variable among the races and was more conspicuous in the V instar 5th day. It is in this context the author in the present investigation has utilized 2nd, 4th and 5th days of V instar larval haemolymph protein as the material for the study, since the quantification of protein may be considered as non-ambiguous indicators of pure races (Gamo, 1977). Further, Murray *et al.,* (1972) in their investigation pointed out that, the variation of the protein content changes in terms of increases protein content as the growth rate proceeds till some extents of the porcine uterine. Moreover, also few observations suggested by Hill and Breidenbach, (1974) in soybean plants, fresh weight and dry weight as well as quantitative protein changes in the developing soybean (Glycinemax) seed were described from 12 days after flowering until maturity. Apart from this, several research investigations were carried out on haemolymph protein concentration utilizing haemolymph are Sarangi, (1985); Nagata and Yoshitake, (1989) and Ravikumar and Sarangi, (2000), *etc*, who have reported that variation in total protein depends on the larval developmental stages of the silkworm *Bombyx mori*. The result also clearly demonstrated a general trend of increase in total protein content (with few exceptions) during 4th day of V instar then decreasing the protein during late 5th day of V instar. The present findings from quantitative estimation have clearly showed protein variability (genetic differences) between silkworm races/breeds, which will be one of the important yardstick in selecting parents for silkworm genetics and breeding programmes.

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