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Comparative Study of the Changes in Haemogram of *Antheraea assama* Ww reared on two Host Plants, Som (*Machilus bombycina* King) and Soalu (*Litsea polyantha* Juss)

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ABSTRACT

The present study was conducted to compare the influence of two primary host plants, Som (Machilus bombycina King) and Soalu (Litsea polyantha Juss) on the haemogram of Antheraea assama Westwood. The Total Haemocytes Count (THC), Differential Haemocytes Count (DHC) and Blood volume (BV) of different developmental stages (larval instars and pupae) of A. assama reared on the two selected host plants, Som and Soalu was studied in the laboratory. Quantitative analysis of haemocytes in both the groups reared on different host plants have shown that the THCs increased continuously from 1st to 5th instar larvae and recorded a steep decline in the pupa of both the groups. Though THC was not significantly different between the early instars (i.e, 1st, 2nd and 3rd instar larvae) reared on the two host plants, there was a significant difference in total count in the matured instars and pupae suggesting the impact of host plant. Comparison of the different haemocyte types (PR, PL, GR, SP and OE) between the som and soalu reared groups of A. assama did not show any significant variation between them. However, a slight difference was recorded in PR at the 2nd and 3rd instars along with pupa, being significantly higher in M. bombycina reared insects. Studies showed that the pattern of changes in blood volume in som and soalu reared groups were similar to that of THC. Significant difference in blood volume between the two groups was recorded only in the 5th instar larva of A. assama, BV recording higher in those reared on L. polyantha.

Key words: *Antheraea assama, som, soalu, haemogram, haemocytes*

INTRODUCTION

Haemogram is a statement of the haemocyte population picture in an insect at a given time. It is a quantitative and qualitative expression of the haemolymph and its constituent inclusions[25,15]. Haemogram represent the total number of haemocytes (THC) together with an estimate of the relative number of haemocytes in different categories[16,2].

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The study of haemogram in insects, assumes importance as haemocytes play an important role in the physiology of the organism. The haemocytes are responsible for coagulating the haemolymph as and when necessary[7,8], connective tissue synthesis[28,27,26], wound healing, self recognition, general and specific immune response and opsonisation[9,29]. Furthermore, haemocytes produce and store substances which may be discharged after infection, viz. Lectins and haemolysins and elements of the phenoloxidase system[29]. In some arthropods haemocytes are also responsible for production and storage of the respiratory pigments[29]. That is why the quantitative (Total haemocytes count) and qualitative (Differential haemocytes count) estimation of haemocytes is considered as an important measure of overall health of an organism. Since the number and concentration of haemocytes is affected by volume of haemolymph in which they float, the estimation of the total haemogram constitutes determination of blood volume (BV) too; together they represent the survival potential of the insect.

Essentially to test this hypothesis the present study is designed to compare the influence of two primary host plants *Machilus bombycina* King and *Litsea polyantha* Juss on the haemogram of *Antheraea assama* Westwood a native sericigenous insect of NE India[5]. The study is aimed at determining the efficiency of rearing performances of the silkworm on these two host plants in terms of changes in their haemogram picture.

MATERIALS AND METHODS

Insects: Muga silkworm (*A. assama)* larvae, 1st instar through 5th instars along with pupae reared on *M. bombycina* (Som) and *L. polyantha* (Soalu) of same age and planted in adjacent plots were obtained from Regional Muga Research station, Boko, Assam.

Total haemocytes count (THC): The total haemocytes count (THC/mm) of 48 hours post moult, 1st through 5th instars along with the pupae reared on both host plants were determined by the method of Hazarika and Gupta[12], after heat fixing the larvae in 55°C for 1 minute following Rosenberger and Jones[23]. 48 hours post moult is the preferred time as the full compliment of haemocytes are usually present during this time[1]. However, since 1st and 2nd instars were too small to be bled individually, haemolymph from 10 larvae in each of the two stages was pooled and THC was determined[12,6].

Differential haemocyte count (DHC): Unfixed haemolymph drops were directly collected on clean slides from the different instars (48 hours post moult) reared on the two host plants. Air dried haemolymph smear was fixed in methanol and stained by Giemsa stain. The stained films were mounted in DPX. A minimum of 200 cells were classified per insect and were replicated in a minimum of three insects of a given stage and physiological strains. The percentage of different haemocyte types were calculated on the basis of the total number of all the haemocyte which had been obtained in a number of haemolymph smears[18].

Determination of blood volume (BV): Blood volume (BV) of the 48 hours post moult larvae of all the instars and the pupae, from the two host plants were determined following the methods of Richardson *et al*.[22] and substantiated by Hazarika *et al.*[11] in *A. assama.*

Data Analysis:

Statistical analysis for all data was performed using MS Office Excel, 2007 and SPSS 21. The data were subjected to a complete randomized design analysis of variance (ANOVA). Means of THCs were compared by the least significant difference (LSD) procedure ($P < 0.05$). Likewise, means of blood volume (BVs) and means of different haemocytes of the larval and pupal stages were compared by Student's t-test to find out if there was any significant difference between the two sets reared on the two host plants. The results were expressed as Mean ± Standard Deviation ($X \pm SD$).

RESULTS

Total Haemocyte Count (THC) of different larval and pupal stages of *A. assama* reared

on *M. bombycina* and *L. polyantha*: Figure-1 and Figure-2 shows the THC (quantitative changes in haemocyte number) of the 1st through 5th instar larvae and pupae of *A. assama* reared on *L. polyantha* and *M. bombycina*. It is evident from the figures that instar conditioned the haemocytes to change in both the groups, irrespective of host plants. However during the early instars *i.e*, 1st and 2nd instars in both the groups there was no significant variation in THC and similarly the 2nd and 3rd instars too showed no significant difference in haemocyte number (Fig.1 and Fig.2). But the number of cells changed significantly during the later instars- 3rd, 4th and 5th in both the groups of larvae reared on two different host plants (Fig.1 and Fig.2). However, there was a steep decline recorded in the pupae of both the groups in comparison to the late instar larvae.

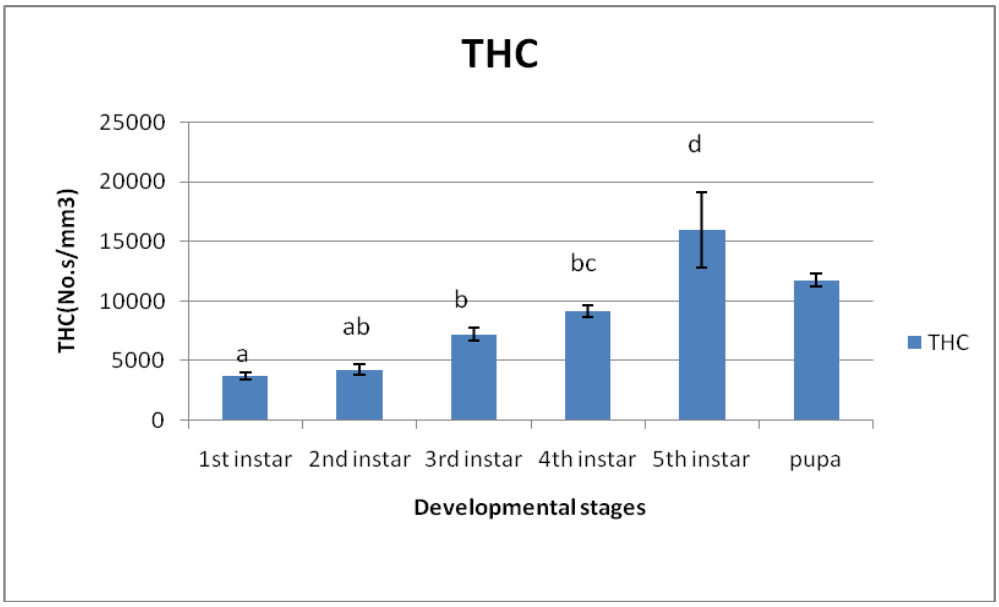

Fig. 1: THCs in the different larval instars of *A. assama* reared on *L. polyantha*
(Means with the same alphabet are not significantly different from each other)

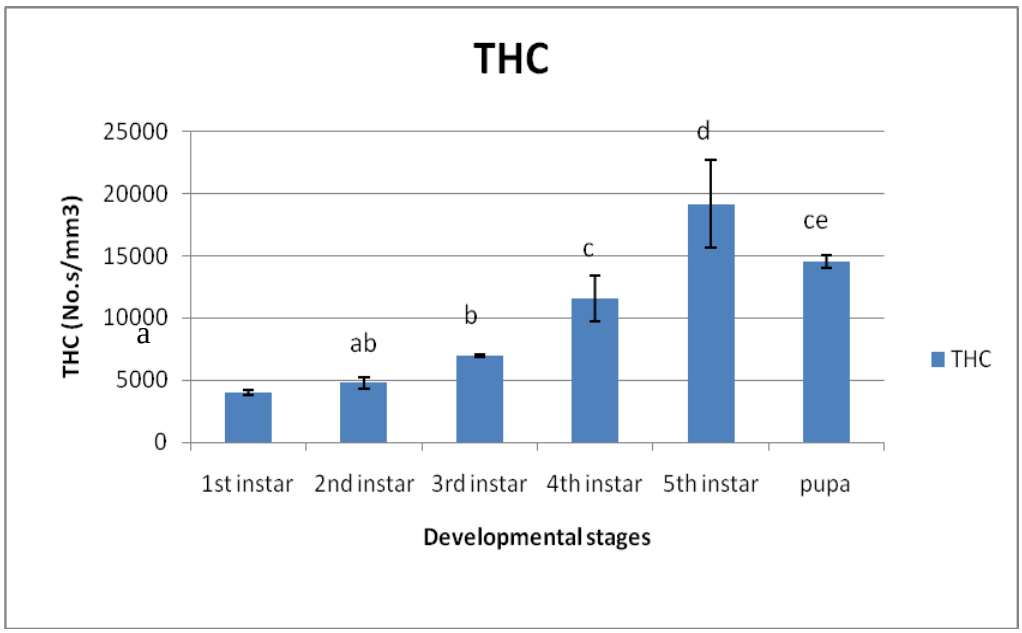

Fig. 2: THCs in the different larval instars of *A. assama* reared on *M. bombycina*
(Means with the same alphabet are not significantly different from each other)

Table-1 compares the differences in the total haemocyte count (THC) of both the larvae and pupae reared on the two host plants. From the figures it is evident that in the early stages (1st, 2nd and 3rd instars) there seems to be no significant differences between THC of both the groups, nullifying any host plant effect on quantitative haemocyte population. However in the matured instars as well as in the pupae the change in THC is found to be significant suggesting the impact of host plants on physiology of the insects. THC of the 4th instar larvae reared on *M. bombycina* (11546.20 ± 1845.10) is significantly higher than the larvae reared on *L. polyantha* (9187.36 ± 473.39). Similarly 5th instar larvae reared on *M. bombycina* recorded significantly higher haemocytes (19192.59 ± 3540.06) than those reared on *L. polyantha* (15976.43± 3176.39). The pupae reared on *M. bombycina* too revealed higher THC (14541.18 ± 477.57) than those reared on *L. polyantha* (11800.00 ± 542.49). So it is evident from our results that in the first three initial instars the different host plants did not induce any significant change in the total number of haemocytes between the two different groups. But towards the later instars (4th, 5th) and pupal stages, *M. bombycina* reared larvae generated a significantly higher number of haemocytes than *L. polyantha* reared larvae.

Table 1: Comparison of THCs of *A. assama* reared on *M. bombycina* and *L. polyantha*

Developmental Stages	Mean THC (Nos/mm^3)		t-value
	Som	Soalu	
1st instar	3981.81 ± 205.24	3707.69 ± 277.97	t=1.374, p=0.241, df=4
2nd instar	4784.61 ± 457.65	4270.59 ± 459.92	t=1.372, p=0.242, df=4
3rd instar	6979.95 ± 117.03	7242.02 ± 560.19	t=0.793, p=0.472, df=4
4th instar	11546.20 ± 1845.10	9187.36 ± 473.39	t=4.763, p=0.008, df=4 *
5th instar	19192.59 ± 3540.06	15976.43 ± 3176.39	t=3.468, p=0.025, df=4 *
Pupa	14541.18 ± 477.57	11800.00 ± 542.49	t=6.569, p=0.002, df=4 *

(*=The mean difference is significant at the 0.05 level; df=degree of freedom)

DHC of different larval instars and pupae reared on *M. bombycina* and *L. polyantha:*

A cytological approach to evaluate the variation in the type of haemocytes present in the different developmental stages of *A. assama* reared on the two selected host plants *M. bombycina* and *L. polyantha* reveals the presence of Prohaemocytes (PR), Plasmatocytes (PL), Granulocytes (GR), Spherulocytes (SP) and Oenocytes (OE) in different numbers in different stages. PR being the most abundant shows highest numbers in the 1st instars (Table-2) gradually decreasing with maturation and recording the least numbers in the pupae in both the groups. PL recorded the highest numbers in the 5th instars in *M. bombycina* and in the 2nd instar in *L. polyantha* (Table-2) GR, the second most abundant group of cells too showed similar trend, being highest in the 4th and 5th instars and lowest in the 1st instars. OE are poorly represented in the various stages of *A. assama* reared on both the host plants and did not show much variation between developmental stages. Likewise SP count too showed poor representation in different stages with no significant variation among them (Table-2).

Comparison of the different haemocyte types between the *M. bombycina* and *L. polyantha* reared groups of *A. assama* exhibited certain supple changes in numbers. PR% showed significant differences in the 2nd and 3rd instars, being significantly higher in *M. bombycina* reared larvae. The same trend was seen in case of the pupal PR% too. PL concentration between the two groups did not vary significantly in the larval instars although somewhat higher variation was observed in the pupae of *M. bombycina* reared larvae. GR exhibited no marked variation between the two sets of larvae, so were OE and SP (with an exception in 4th instar of *L. polyantha*).

Table 2: Comparison of PR, PL, GR, OE and SP % between *M. bombycina* and *L. polyantha* reared groups of *A. assama*

Type of haemocyte	Developmental stages	Som	Soalu	t-value
PR	1st instar	70.940 ± 9.516	84.075 ± 2.793	t=0.942, p=0.445,df=2
	2nd instar	69.170 ± 2.658	45.815 ± 4.1931	t=6.65, p=0.02,df=2 *
	3rd instar	58.145 ± 0.361	35.225 ± 2.679	t=11.986, p=0.006,df=2 *
	4th instar	38.483 ± 4.779	37.646 ± 4.325	t=0.224, p=0.83,df=4
	5thinstar	37.770 ± 9.430	15.005 ± 7.68	t=2.64, p=0.118,df=2
	Pupa	32.235 ± 1.29	13.030 ± 0.028	t=20.983 ,p=0.0023,df=2 *
PL	1st instar	14.870 ± 2.643	6.860 ± 0.933	t=0.893, p=0.46,df=2
	2nd instar	13.470 ± 2.177	24.215 ± 2.050	t=1.068, p=0.39,df=2
	3rd instar	16.420 ± 0.594	11.785 ± 1.546	t=3.95, p=0.05,df=2
	4th instar	15.776 ± 2.923	13.586 ± 2.275	t=0.69, p=0.52,df=4
	5thinstar	24.280 ± 1.762	21.780 ± 2.826	t=0.21, p=0.85,df=2
	Pupa	19.069 ± 0.767	11.635 ± 1.535	t=6.213, p=0.025,df=2 *
GR	1st instar	7.650 ± 2.644	4.445 ± 1.393	t=1.516, p=0.26,df=2
	2nd instar	15.465 ± 3.387	24.310 ± 6.122	t=0.759, p=0.52,df=2
	3rd instar	16.170 ± 2.022	37.035 ± 3.660	t=2.493, p=0.130,df=2
	4th instar	34.186 ± 6.274	27.746 ± 1.753	t=1.712, p=0.162,df=4
	5thinstar	25.885 ± 1.251	45.350 ± 6.970	t=3.88, p=0.06,df=2
	Pupa	11.153 ± 2.018	13.578 ± 1.362	t=1.408, p=0.294,df=2
OE	1st instar	3.310 ± 0.551	0.385 ± 0.149	t=1.949, p=0.19,df=2
	2nd instar	0.430 ± 0.208	2.975 ± 0.207	t=0.846, p=0.48,df=2
	3rd instar	3.830 ± 2.022	6.875 ± 1.282	t=0.761, p=0.52,df=2
	4th instar	7.203 ± 0.899	6.633 ± 0.961	t=0.457, p=0.67,df=4
	5thinstar	5.725 ± 0.247	8.650 ± 1.30	t=3.213, p=0.08,df=2
	Pupa	4.914 ± 0.263	4.061 ± 0.211	t=3.568, p=0.239,df=2
SP	1st instar	3.225 ± 1.171	0.775 ± 0.296	t=1.424, p=0.29,df=2
	2nd instar	1.460 ± 0.264	3.035 ± 0.609	t=0.669, p=0.57,df=2
	3rd instar	5.430 ± 0.240	9.080 ± 1.164	t=2.371, p=0.14,df=2
	4th instar	4.346 ± 0.866	14.383 ± 2.190	t=4.703, p=0.009,df=4 *
	5thinstar	6.330 ± 1.828	9.205 ± 0.516	t=1.414, p=0.292,df=2
	Pupa	5.365 ± 0.346	3.945 ± 1.153	t=1.668, p=0.237,df=2

(* The mean difference is significant at the 0.05 level)

BLOOD VOLUME IN THE TWO GROUPS:

Fig-3 and 4 depicts the pattern of BV in the *L. polyantha* reared group and *M. bombycina* reared group respectively. Similar pattern of instar conditioning of BV is observed, being lowest in the 1st instars (0.010 ± 0.01 and 0.030 ± 0.010) and the highest in the 5th instars (1.690 ± 0.355 and 0.673 ± 0.407) dipping exceptionally in the pupae (0.030 ± 0.020 and 0.020 ± 0.01).

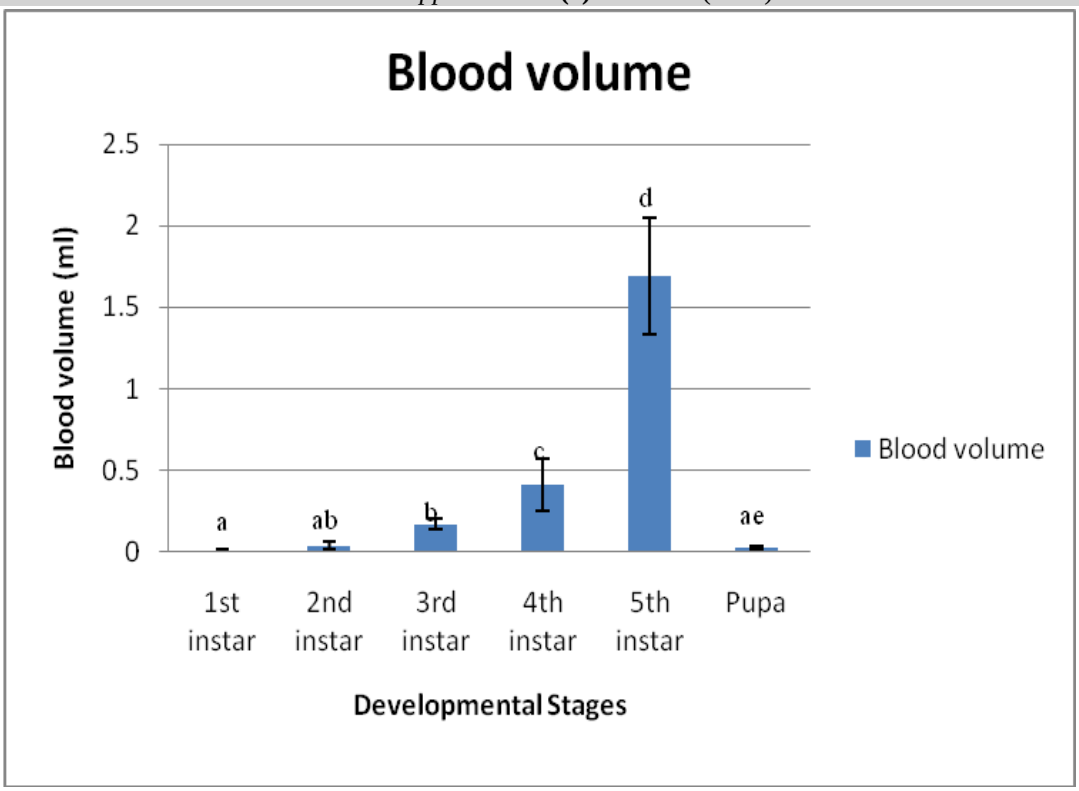

Fig. 3: Blood volume of *A. assama* reared on *L. polyantha.*

(Means with the same alphabet are not significantly different from each other)

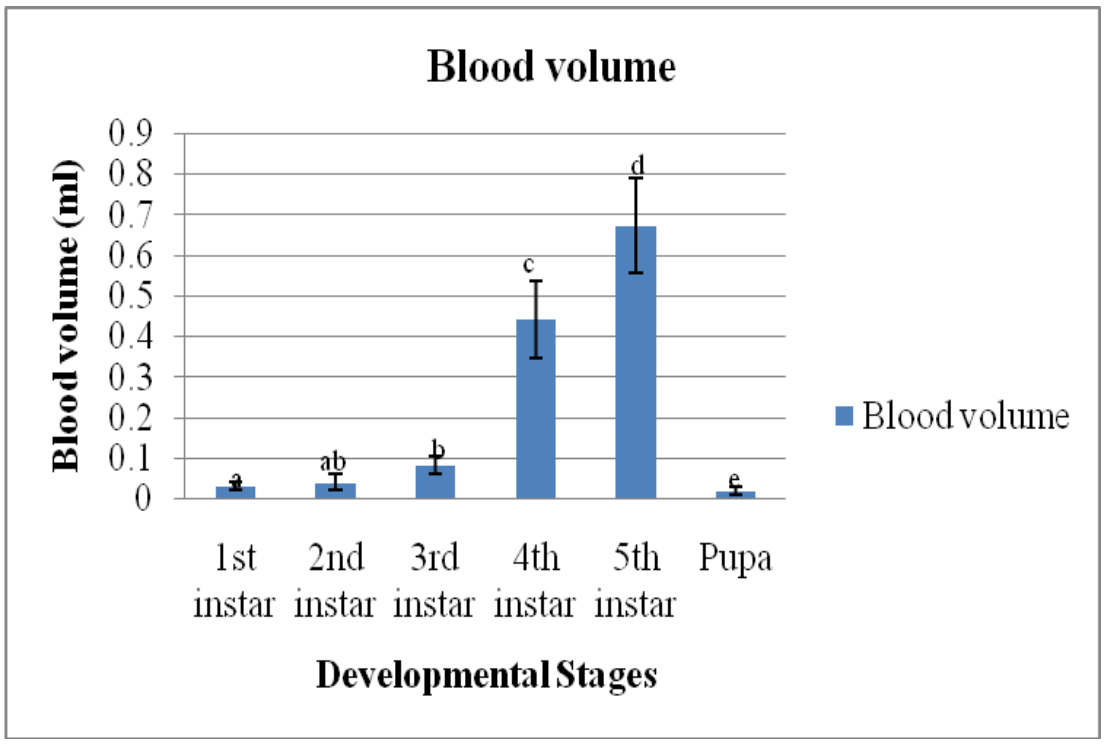

Fig. 4: Blood volume of *A. assama* reared on *M. bombycina.*

(Means with the same alphabet are not significantly different from each other)

Comparison of the instar wise blood volume of the two groups (Table-3) reveals a significant difference between the two groups only in the 5th instars. *A. assama* reared on *M. bombycina* recorded significantly lower BV compared to those reared on *L. polyantha*.

Table 3: Comparison of BV of *A. assama* reared on *M. bombycina* and *L. polyantha*

Developmental stages	Mean BV		t-value
	SOM	SOALU	
1st instar	0.030 ± 0.010	0.010 ± 0.010	t=2.449, p=0.07, df=4
2nd instar	0.040 ± 0.020	0.040 ± 0.020	t=0.000, p=1.00, df=4
3rd instar	0.083 ± 0.075	0.170 ± 0.034	t=1.80, p=0.146, df=4
4th instar	0.443 ± 0.095	0.410 ± 0.161	t=0.308, p=0.772, df=4
5th instar	0.673 ± 0.407	1.690 ± 0.355	t=3.259, p=0.031, df=4 *
Pupa	0.020 ± 0.01	0.030 ± 0.020	t=0.774, p=0.482, df=4

(*The mean difference is significant at the 0.05 level.)

DISCUSSION

The present investigation revealed that the THCs of different larval and pupal stages of *A. assama* reared on two different host plants showed more or less similar trends. A gradual increase in the different progressive stages was evident in both the groups. The continuous increase in THC during the different larval stages of *A. assama* feeding on any one of the host plant is a widespread phenomenon commonly observed in insects. This has previously been observed in *A. assama*[11], in *Bombyx mori*[20], *Periplanata americana*[25], *Halys dentate*[3], *Galleria mellonella*[24], *Blatella germanica*[12]. Kunkel[17] suggested that a series of allometric changes occur at both biochemical and morphological level during development with an increasing demand for nutrient supply. The observed increased haemocyte load at different stages of development perhaps serves this purpose together with supplying tyrosine and phenoloxidase for sclerotization and recognition of non-self tissues[9,6]. Thus, it is obvious that as the larva metamorphoses from one instar to another, the body size and consequently the physiological demand also increase and to address this demand, the cell numbers/ml of haemolymph also needs to increase[6]. Some authors further reported that ecdyson too contribute in increasing haemocyte numbers by stimulating the rate of mitosis in haemocytes[14,13,21]. Since the ecdyson titre is high towards the later part of larval life, the sudden rise in the haemocyte count observed therein could be an effect of the hormone.

However in case of pupa, a steep decline in haemocytes number was observed as compared to that of 5th instar larvae. This may be due to the fact that the pupal stage is an inactive phase. As such most of its physiological activities are cut down and accordingly, the total cell count also decreases as there is no demand for supplying nutrient by the circulating haemocytes.

The observed changes in BV may also be assigned to the same explanation as that of changes in THC. However, the difference in BV, particularly in the 5th instar, might have influenced the THCs in the two sets of larvae (*L. polyantha* fed and *M. bombycina* fed). *L. polyantha* reared larvae having higher BV (in 5th instars) contained lower THCs, whereas *M. bombycina* reared larvae with lower BV contained higher THCs. Similar relationships have also been reported by Bardoloi and Hazarika [5] in *A. assama*, Wheeler [25] in *P. americana* and by Nittono [20] in *B. mori.* So it can be assumed that the difference in THC in the two groups (in late instars) is actually due to the amount of dilution of haemocytes by haemolymph rather than any difference in metabolism or nutrient level in the two host plants; the apparent increase or decrease in blood cell count might be due to increase or decrease in blood volume [25]. The difference in BV between the two set of larvae reared on *M. bombycina* and *L. polyantha* however has been reported by Hazarika *et al.* [11] to be an effect of leaf moisture content of the two host plants. The higher moisture content of *L. polyantha* influences higher BV in larvae reared on it as haemolymph volume (BV) serves as a reservoir of water[19]. Similarly lower moisture

content of *M. bombycina* leaves is reported to be the observed reason for lower blood volume of larvae reared on it[5].

So we can tentatively assume that the observed differences in THC and BV in the two sets of larvae in the two different host plants is more of an effect of dietary water than any other nutrient[11] available in the host plants in question.

The observed higher percentages of prohaemocytes (PRs) in the 1st intar of both the groups of larvae reared on *M. bombycina* and *L. polyantha*, suggests that the PRs are the stem cells[18] which undergoes transformation into PLs and other cell types in the later instars. The transformation process probably completes in the 3rd, 4th instars, as suggested by no further significant differences in PR counts in the later instars[6]. Similar suggestions were offered by Hazarika and Gupta[12] for different percentage of PR in different developmental stages of *Blatella germanica.*

The progressive increase in plasmatocytes (PLs), which constitute one of the immunocytes[10,4], through the different developmental stages, probably corresponds to the growing demand for cellular immunity[10]. The same trend shown by the GRs, which are also recognised immunocytes[10,4] can be substantiated by the same explanation.

Comparison of the different haemocytes between the two sets of larvae reared on two different host plants (Table-2) failed to show any significant variation between them except a slight difference in PR at 2nd and 3rd instars, being significantly highest in *M. bombycina* reared larvae. The only possible explanation may be the haemodilution being less in *M. bombycina* due to lower blood volume as suggested earlier. However conclusive explanation needs further investigation.

So from the above discussion we can tentatively conclude that haemograms of larvae reared on the two host plants do not exhibit much difference suggesting that both host plants are equally efficient in supporting the muga silkworms. Since, haemocytes play an important role in the physiology of the insect and moreover, as they are the vital components of immune system, the above discussion concludes that the two host plants do not show much difference on their impact on the immune system as well as on the physiology of the larvae and pupae reared on them.

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REFERENCES

1. Arnold, J.W. & Hinks, C.F., Biosystematics of the genus *Euxoa* (Sepidoptera : Noctuidae) III Haemocytological distinctions between two closely related species, *E. Campestris* and *E. Declarata.*, *Can. Entomol*., **107:** 1095-1100 (1975).
2. Arnold, J.W., A comparative study of the hemocytes (blood cells) of cockroaches (Insecta: Dictyoptera: Blattaria), with a view of their significance in taxonomy, *Can. Entomol*., **104:** 309-348 (1972).
3. Bahadur, J. & Pathak, J.P.N., Changes in the total haemocyte counts of the bug, *Halys dentate*, under certain conditions, *J. Insect Physiol*., **17:** 329-334 (1971).
4. Baishya, B.P., Bardaloi, S. & Bharali, R., Ultrastructure of the hemocytes of Muga silkworm larva *Antheraea assama* Westwood (Lepidoptera; Saturniidae): a phase-contrast and electron microscopic study, *Int. J. Pure App. Biosci*., **3(3):** 234-240 (2015).
5. Bardoloi, S. & Hazarika, L.K., Seasonal variation of body weight, lipid reserves, blood volumes and haemocyte population of *Antheraea assama*, *Environ. Entomol*., **21:** 1398-1404 (1992).
6. Bardoloi, S. & Hazarika, L.K., Variation in haemocyte population during different larval instars of *Antheraea assama* (Lepidoptera: Saturniidae) Westwood and their roles in the defence mechanism of the insect, *J. Assam Science Society*, **37(2):** 96-102 (1995).

7. Gregoire, C., Blood coagulation in arthropods V. Studies on hemolymph coagulation on 420 species of insects, *Archs. Biol*., **66:** 104-148 (1995).
8. Gregoire, C., Studies by phase-contrast microscopy on distribution patterns of hemolymph coagulation in insects, *Smithson. Misc. Collns*., **134:** 1-35 (1957).
9. Gupta, A.P., Arthropod immunocytes: their identification, structure, function, and functional analogies with those of vertebrate B- and T- lymphocytes. In Haemocytic and Humoral Immunity in Arthropods. Ed. A. P. Gupta. Wiley & Sons, New York, (1986).
10. Gupta, A.P., Cellular elements in the hemolymph. In Comprehensive Insect Physiology, Biochemistry and Pharmacology. Ed. G.A. Kerkut and L.I. Gilbert. Pergamon Press, Oxford, 402-451 (1985).
11. Hazarika, L.K. & Gupta, A.P., Variation in haemocyte populations during various developmental stages of *Blattella germanica* (L.), *Zool. Sci*., **4:** 307-313 (1987).
12. Hazarika, L.K., Bardoloi, S. & Kataky, A., Effects of host plants on haemocyte populatios and blood volumes of *Antheraea assama* Westwood (Lepidoptera: Saturniidae), *Sericologia*., **34(2):** 301-306 (1994).
13. Hinks, C.F. & Arnold, J.W., Hemopoiesis in Lepidoptera II. The role of the hemopoietic organs, *Can. J. Zool*., **55:** 1740-1755 (1977).
14. Hoffman, J.A., Regulations endocrine de la production et la differentiation des hemocytes chez un insect *Locusta migratoroides*, *Gen. Comp. Endoicr*., **15:** 198-219 (1970).
15. Jones, J.C., Changes in the haemocyte picture of *Galleria mellonella* (L.), *Biol. Bull*., **132:** 211-221 (1967a).
16. Jones, J.C., Current concepts concerning insect haemocytes, *Am. J. Zool*., **2:** 209-246 (1962).
17. Kunkel, J.G., A minimal model of metamorphosis: Body fat competence to respond to juvenile hormone. In Current Topics in Insect Endocrinology and Nutrition. Ed. G. Bhaskaran, S. Friedman and J. Rodrigues, 102-129 (1981).
18. Mall, S.B. & Gupta, G.S., Haemocyte picture during metamorphosis of *Atteva fabriciella* (Swed.), Ind. J. Entomol., **44:** 101-112 (1982).
19. Mellanby, L., The function of insect blood, *Biol. Rev*., **14:** 243-260 (1939).
20. Nittono, Y., Studies on the blood cells in the silkworm *Bombyx mori* (L.), Bull. *Seric. Exp. Stn. Tokyo*., **16:** 171-266 (1960).
21. Prasada Rao, C.G., Ray, A. & Ramamurty, P.S., Effect of ligation and ecdysone on total haemocyte count in the tobacco caterpillar, *Spodoptera litura* (Noctuidae: Lepidoptera), *Canadian Journal of Zoology*., **62:** 1461-1463 (1984).
22. Richardson, C.H., Burdethae, R.C. & Eagleson, C.W., The determination of blood volume of insect larvae, *Ann. Entomol. Soc. Am*., **24:** 503-507 (1931).
23. Rosenberger, C.R. & Jones, J.C., Studies on total blood cell counts of the Southern armyworm larva *Prodemia eridania.*, *Ann. Entomol. Soc. Amer*., **53:** 351-355 (1960).
24. Shapiro, M., Changes in hemocyte populations. In Insect hemocytes, development, forms, functions and techniques. Ed. A.P. Gupta. Cambridge University Press, Cambridge, 475-523 (1979).
25. Wheeler, R.E., Studies on the total haemocyte count and haemolymph volume in *Periplaneta americana* (L.) with special reference to the last moulting cycle, *J. Insect Physiol*., **9:** 223-225 (1963).
26. Wigglesworth, V.B., Haemocytes and basement membrane formation in Rhodnius. *J. Insect. Physiol*., **19:** 831-844 (1973).
27. Wigglesworth, V.B., The haemocytes and connective tissue formation in an insect *Rhodnius prolixus* (Hemiptera), *Q.J. Microsoc. Sci.*, **97:** 89-98 (1956).
28. Wigglesworth, V.B., The role of hemocytes in the growth and moulting of an insect- Rhodnius prolixus (Hemiptera), *J. Exp. Biol*., **32:** 649-663 (1955).
29. Xylander, W.E.R., Hemocytes in Myriapoda (Arthropoda): a review, *Int. Survival J*., **6:** 114-124 (2009).