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During 5th Instar Development Changes In Haemolymph Protein Concentration In Certain Bivoltine Silkworm Races Of Bombyx Mori.L

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Abstract: ***Haemolymph protein of the fifth instar larvae of four Bivoltine races of Bombyx mori. L was studied. GEN-3 race showed best performance in haemolymph protein content as well as certain economic characters like cocoon weight (CW), Shell weight (SW) and Shell Ratio Percentage (SR%). Basing upon the above characters the efficiency of the silkworm races was considered. The present study showed that there is a direct correlationship established between haemolymph protein with body weight, cocoon weight, shell weight (SW) and shell ratio percentage (SR %). The above characters were important for the selection for hybridization as well as commercial rearing.***

Keywords: ***Haemolymph protein, Bivoltine, Cocoon weight, Shell weight, Shell ratio percentage (SR%), Rearing performance.***

I. INTRODUCTION

Haemolymph protein is an important component for the metamorphosis of silkworm. The chemical composition of haemolymph is highly variable among the diverse species and at different developmental stages of the some species (Florkin and Jeuniaux, 1974). It constitutes a variety of nitrogenous substances such as amino acids, proteins, amines, ammonia and peptides. Proteins are among the most complex of all known chemical compounds and also the most characteristic of living organisms. Major changes in protein content occur during development (Agosin, M. 1978; Wyatt, G. 1960). A characteristics feature of insect haemolymph is its high amino acid content. Sometimes it is considered as a taxonomic character for the insect (Florkin, M. 1959).

Some important proteins, growth hormone such as juvenile hormones are transported through haemolymph lipoprotein in silkworm and locusts (Chino *et. al*, 1977, 1981 and 1982). Plasma proteins are synthesized in the fat body cell and then secreted into the haemolymph periodically during post embryonic development and metamorphosis. (Tojo *et al*, 1980, Tomino, 1985, Sakai *et.al***.** 1988, Mori *et. al.* 1991a.b, Kishimoto *et.al* 1999). The haemolymph protein plays an important role in the production of silk in *Bombyx mori* (Denuce, 1958 and Fukuda *et. al.* 1958). The significant increment of protein in the 5th instar larvae might be due to synthesis of fibroin protein (Maekawa H and Suzuki Y.1980) as well as certain stage specific proteins. Considering the above importance of haemolymph in *Bombyx mori.L* the present work was undertaken to understand the variability among races for future rearing programmes.

II. MATERIAL AND METHODS

Four Bivoltine races of Bombyx mori.L namely GEN-3, BCON-1, DUN-22 and BCON-4 races were collected from different breeding stations of Central Silk Board. For the present work silkworms have been collected from Central Sericulture Research and Training Institute (CSRTI), Berhampore (wb). The races were reared and maintained by adopting the Krishnaswami (1978) method. Fifth instar larvae were weighted and sacrificed from the 1st day onwards till

cocoon formation. The haemolymph was collected in a clean vile containing few thiourea grannules by pricking the abdominal leg just before the last leg. For the estimation of total protein in the haemolymph of silkworm Lowry's method (1951) was adopted with a little modification. Besides the cocoon weight, the shell weight and shell ratio percentage were calculated. All the data were subjected to ANOVA using standard statistical procedure and the relationships between two continuous variables was computed. The data's were also subjected to correlation coefficient.

III. OBSERVATION

The protein content in the haemolymph increased significantly to its peak on 6th day in all the races (Table-1). Beside haemolymph protein progressive significant increments in the body weight were also observed in all the races is a common feature. The present data's showed a significant variation in the quantity of the haemolymph protein was also recorded as in Table-1. The observed data showed strong degrees of positive Correlationship between the haemolymph protein and the body weight. However a significant correlationship was also exists between the cocoon weight and shell weight.

Vth Instar Duration	BIVOLTINE RACES			
	GEN-3 MEAN ± S.E.M	BCON-1 MEAN ± S.E.M	DUN-22 MEAN ± S.E.M	BCON-4 MEAN ± S.E.M
1st Day	9.027 ± 0.005	8.215 ±0.004	7.911 ±0.043	7.201 ±0.008
2nd Day	9.23 ± 0.006	9.027 ±0.012	8.317 ±0.018	8.012 ±0.009
3rd Day	20.083 ±0.046	15.113 ±0.015	15.52 ±0.018	14.808 ±0.034
4th Day	25.053 ±0.022	29.617 ±0.013	29.211 ±0.014	28.229 ±0.024
5th Day	27.284 ±0.004	32.356 ±0.043	31.139 ±0.005	30.53 ±0.016
6th Day	39.76 ±0.007	38.137 ±0.032	37.427 ±0.008	36.514 ±0.025
7th Day	24.038 ±0.034	23.126 ±0.008	21.503 ±0.009	20.387 ±0.008

Table 1: Comparison in the Concentration of Protein (mg/ml.) in the Haemolymph of some Bivoltine Silkworm Races, Bombyx mori L during 5th instar development

IV. DISCUSSION

In the present study the amount of the haemolymph protein in fifth instar period from four bivoltine races were recorded to establish a profile of silkworm haemolymph protein. The present data also gave an insight into a comprehensive understanding of silkworm metamorphosis.

The significant increment in a haemolymph proteins in GEN-3 of the fifth instar period was due to active synthesis of proteins by the fat body (Shigemasthu,1958). Protein storage in the haemolymph and fat body appears to be a common phenomenon during growth and development. Generally a high rate of protein synthesis occurs during the larval feeding stage. The mature larvae contain high protein level in haemolymph. The storage proteins synthesized during the feeding stage of last instar larvae are utilized as an energy and amino acid source for protein synthesis during metamorphosis. (Thompson, J. 1975: Wyatt H. G. and Pan, M., 1978). During metamorphosis the haemolymph protein of insects indicates a quantitative differences (Laufer, 1960; Buckmann *et al*., 1966; Chippendale and Beck, 1966; Pantelouris and downer, 1969; Colin, 1969). The specific haemolymph protein are sequestered in the fat body (Chippendale, G., 1973b, Thomposon, J. 1975; R. Dean *et al*., 1985).

Such type of *Bombyx mori.L* races may be said as better nutrigenetic trait in silkworm. The silkworm *Bombyx mori.L* feeds exclusively on the mulberry foliage for its nutrition and the larvae obtain its nutrients from mulberry leaves to build up body, sustain life, spin cocoons and egg production. Such nutritional requirement in food consumption has direct impact on the overall genetic traits such as larval and cocoon weight, amount of silk production pupation and reproductive trait.

The present data on Haemolymph protein as in Table-1, showed a significant variations among different bivoltine (Table-2) races of silk worm and different days also.

Such variation in the quantity of Haemolymph protein among the races might be due to their nutritional requirement in different races of *Bombyx mori.*L. Such variation probably reflect the balance between the synthesis, storage, transport and degradation of structural and functional protein during ontogeny as well as a response to particular ecological and physiological condition.(Florkin and Jeuniuax, 1974).

The highest recorded value in the concentration of haemolymph protein in GEN-3, may bedue to active synthesis of protein and their release in to the haemolymph (Nuguta and Kobayashi, 1990) have shown an increase in the protein structure during feeding phase.The increased value of protein content in bivoltine and multivoltine races might be due to higher feeding activity of bivoltine races (Ramadevi *et. al* 1992) than multivoltine races.

The present results clearly indicated that the total haemolymph protein has positive correlation with certain selected commercial characters like cocoon weight and shell weight.

Haemolymph protein increased to its peak on the 6th day of 5th instar period as in the Table: - 1, is due to accumulation of many stage specific proteins. It has been reported in *Bombyx mori.L* that the 30K protein (Izumi et.al. 1981) and the major haemolymph protein (Planteivin et. al.1987) appeared during the last instar development. In *Bombyx mori.L* a group of structurally related proteins, termed 30K accumulated in the haemolymph of the last instar larvae. The 30K proteins are mainly synthesized in fat bodies after juvenile hormone disappearing from the third (3rd) day of the 5th instar, and then mainly discharging to haemolymph. It is presumed, therefore that the 30K proteins may be the source of amino acids for the synthesis of other functional proteins during embryogenesis (Zhong, B. X.,1999). According to few authors metabolism of 30K proteins in a pre-requisite for the embryos normal development (Zhong B.X. et.al. 2005). These proteins also have properties like anti apoptotic and defence beside embryogenesis.

Correlation studies (Table: 2) performed to establish the relationship between haemolymph protein, body weight, shell weight and SR%.

The degree of association or relationship between two variables is measured by correlation coefficient (r). The correlation coefficient may be positive or negative. Positive correlation indicates that the two variables are varying in the same direction. If one variable increases, the other variable is also increase. In negative correlation the two variables vary in opposite direction. If one variable increases other variable decreases. Correlation between different pairs of quantitative characters has been studied as in Table: 2.

The data revealed high significant ($p<0.001$ level) positive value between haemolymph protein concentration and body weight and also between haemolymph protein, Cocoon weight, Shell Weight and SR% as in Fig: 1.A up to 4.D.

Considering the positive and negative correlation between many characters due consideration are given at the appropriate developmental stage. This indicates that selection by haemolymph protein correlation_lead to an increased body weight and cocoon weight, shell weight and ultimately SR%.

Hence by studying the silkworm haemolymph protein with commercial characters like Cocoon weight and Shell weight, it is possible to have a clean picture of correlation between them. An understanding of such relationship will help us to identify the marker molecule during evolution of new races of silkworm *Bombyx mori*.L.

ANOVA OF BIVOLTINE RACES (HAEMOLYMPH PROTEIN)

ANALYSIS OF VARIANCE				
Source Of Variation	**SS**	**df**	**MS**	**F**
Between the Days	3053.323	6	508.8872	171.4684**
Between the Races	8.383169	3	2.79439	0.941564^{NS}
Error	53.42073	18	2.967819	
Total	3115.127	27		

*NB: NS=Not Significant, *-- Significant at (0.05) 5% level, ** --Significant at (0.01)1% level*

Table 2: Coefficient of Correlation of Haemolymph protein with body wt. of Bivoltine Silkworm races, Bombyx mori L

Sl. No.	Races of Silkworm	Variables	Coefficient of Correlation "r"	df	"t" Test	df
1	GEN-3	BW Vs HP	0.9604***	6	2.447*	6
		CW Vs SW	0.3346*	06	2.447*	6
2	BCON-1	BW Vs HP	0.964***	6	2.447*	6
		CW Vs SW	0.3343*	06	2.179*	12
3	DUN-22	BW Vs HP	0.921***	6	2.447*	6
		CW Vs SW	0.4317*	06	2.447*	6
4	BCON-4	BW Vs HP	0.950***	6	2.447*	6
		CW Vs SW	0.2101*	06	2.365*	7

BW-Body Weight * *Significant at 0.05 level*
GW-Gland Weight ** *Significant at 0.01 level*
TSI-Tissue Somatic Index *** *Significant at 0.001 level*
HP-Haemolymph protein
HASC-Haemolymph Ascorbic acid
HFAA-Haemolymph Free Amino acid
CW-Cocoon Weight
SW-Shell Weight
GP-Gland Protein

Table 3

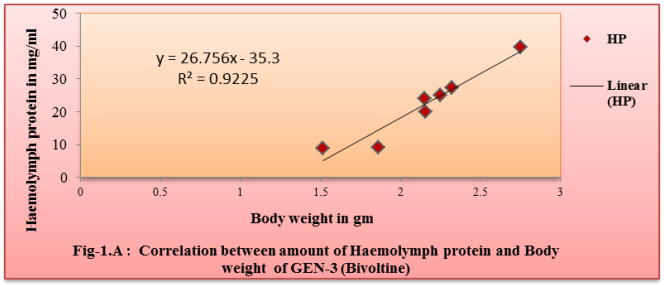

Fig-1.A : Correlation between amount of Haemolymph protein and Body weight of GEN-3 (Bivoltine)

Figure 1

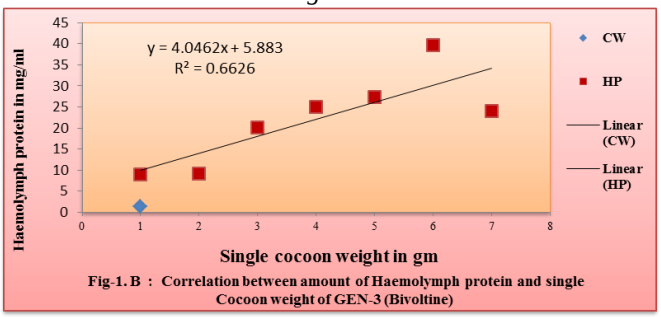

Fig-1. B : Correlation between amount of Haemolymph protein and single Cocoon weight of GEN-3 (Bivoltine)

Figure 2

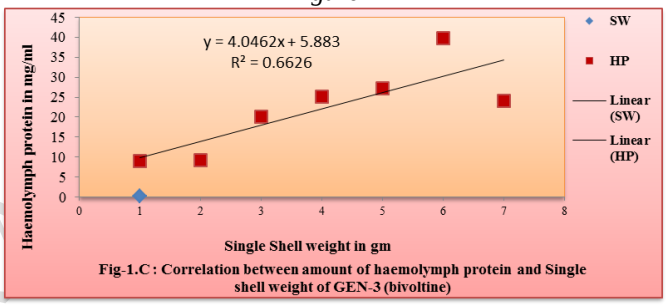

Fig-1.C : Correlation between amount of haemolymph protein and Single shell weight of GEN-3 (bivoltine)

Figure 3

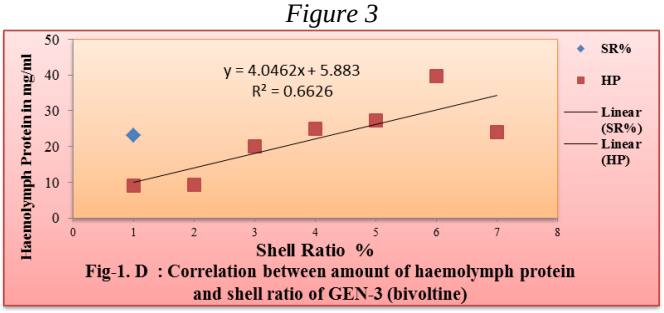

Fig-1. D : Correlation between amount of haemolymph protein and shell ratio of GEN-3 (bivoltine)

Figure 4

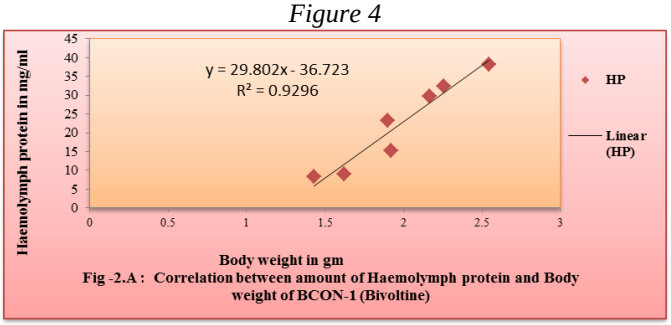

Fig -2.A : Correlation between amount of Haemolymph protein and Body weight of BCON-1 (Bivoltine)

Figure 5

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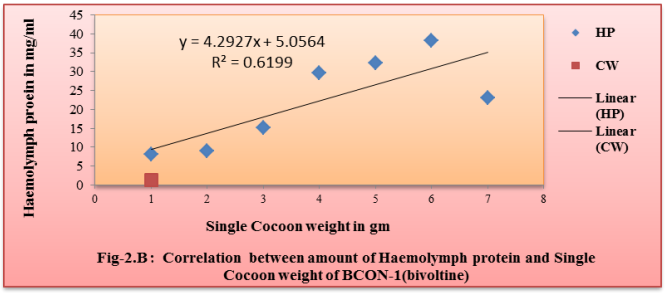

Fig-2.B: Correlation between amount of Haemolymph protein and Single Cocoon weight of BCON-1(bivoltine)

Figure 6

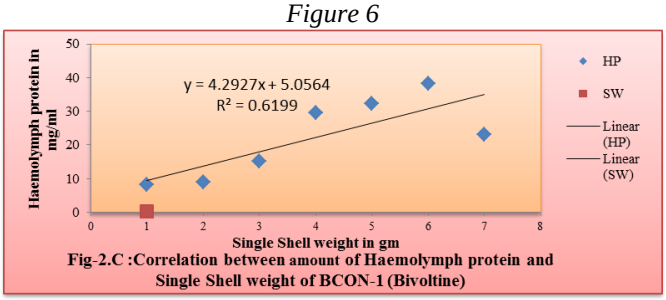

Fig-2.C :Correlation between amount of Haemolymph protein and Single Shell weight of BCON-1 (Bivoltine)

Figure 7

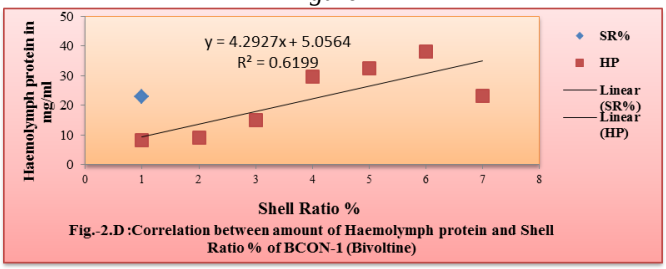

Fig.-2.D :Correlation between amount of Haemolymph protein and Shell Ratio % of BCON-1 (Bivoltine)

Figure 8

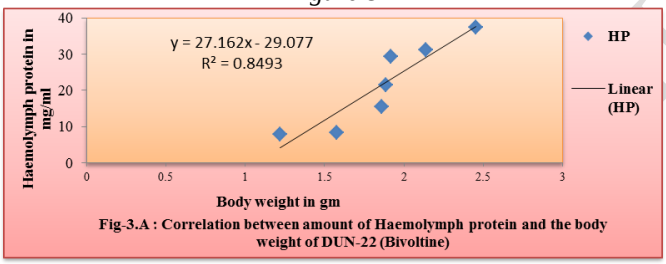

Fig-3.A : Correlation between amount of Haemolymph protein and the body weight of DUN-22 (Bivoltine)

Figure 9

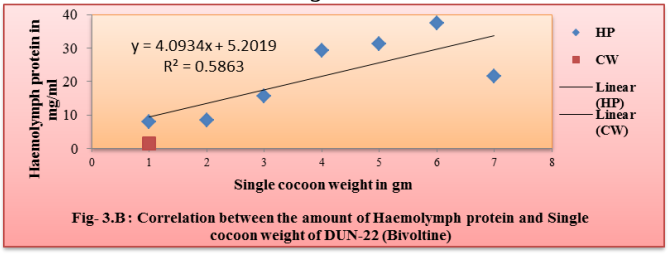

Fig- 3.B: Correlation between the amount of Haemolymph protein and Single cocoon weight of DUN-22 (Bivoltine)

Figure 10

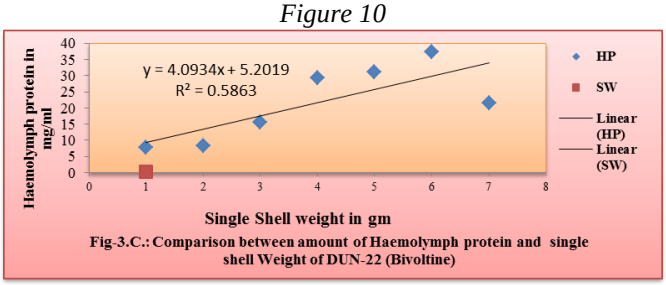

Fig-3.C.: Comparison between amount of Haemolymph protein and single shell Weight of DUN-22 (Bivoltine)

Figure 11

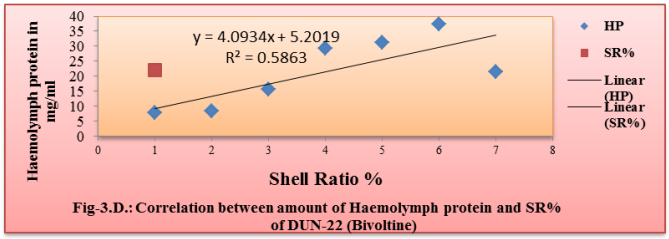

Fig-3.D.: Correlation between amount of Haemolymph protein and SR% of DUN-22 (Bivoltine)

Figure 12

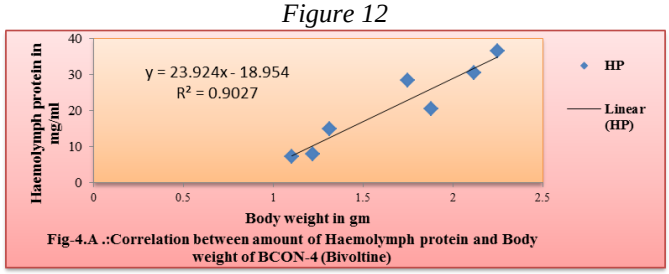

Fig-4.A .:Correlation between amount of Haemolymph protein and Body weight of BCON-4 (Bivoltine)

Figure 13

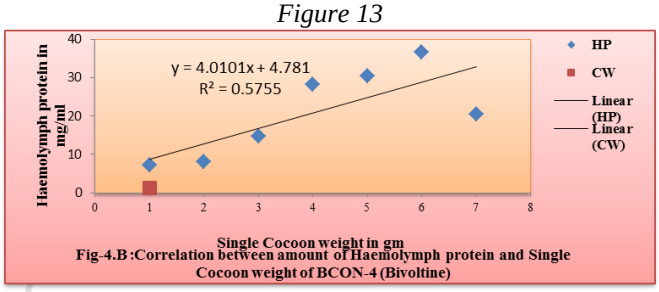

Fig-4.B :Correlation between amount of Haemolymph protein and Single Cocoon weight of BCON-4 (Bivoltine)

Figure 14

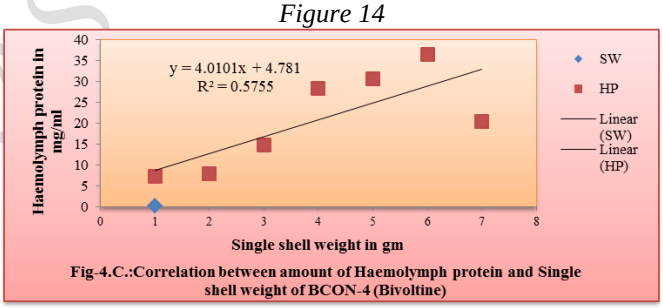

Fig-4.C.:Correlation between amount of Haemolymph protein and Single shell weight of BCON-4 (Bivoltine)

Figure 15

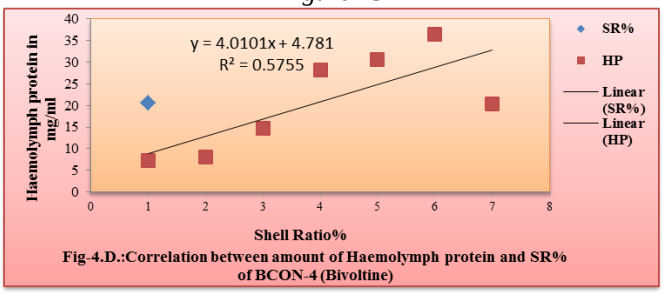

Fig-4.D.:Correlation between amount of Haemolymph protein and SR% of BCON-4 (Bivoltine)

Figure 16

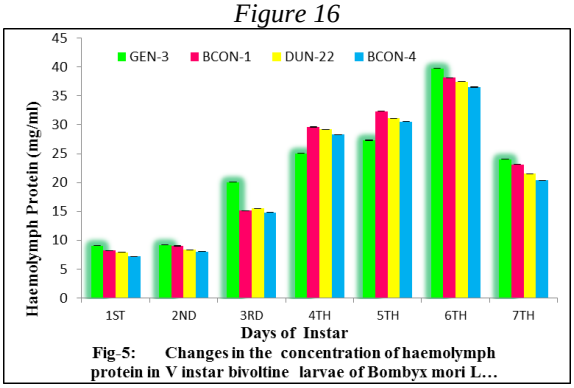

Fig-5: Changes in the concentration of haemolymph protein in V instar bivoltine larvae of Bombyx mori L…

Figure 17

V. CONCLUSION

The present work on haemolymph protein showed an understanding of larval development during fifth instar larval period. The work is aimed to assess variations among bivoltine races basing on the amount of haemolymph protein through some light to know the genetic variability among races. It also helpful to setup hybridization for selection processes and breed the best.

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