

# **A Correlational Study in Changes of Haemolymph Amino Acid Concentration in Certain Bivoltine Silkworm Races of *Bombyx Mori*. L During 5<sup>th</sup> Instar Development**

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**ABSTRACT:** Haemolymph amino acid of the fifth instar larvae of four Bivoltine races namely GEN-3, BCON-1, DUN-22, BCON-4 of *Bombyx mori*. L was studied. Out of four different varieties of Bivoltine races, GEN-3 race showed best performance in haemolymph amino acid 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. GEN-3 is showing the best performance and highly significant by ANOVA. The correlational study indicates that GEN-3, BCON-1, DUN-22 are highly significant than BCON-4. Their changes in concentration of haemolymph amino acid is gradually increased from 1<sup>st</sup> day to the 6<sup>th</sup> day as well and it started declining from the 7<sup>th</sup> day. The present study showed that there is a direct relationship established between haemolymph amino acid 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 amino acid, Bivoltine, Cocoon weight, Shell weight, Shell ratio percentage (SR%), Rearing performance.

## **I. INTRODUCTION**

Amino acids are the basic structural and molecular building blocks of proteins. Amino acids contain a basic amino group (NH<sub>2</sub>) and an acidic carboxyl group (COOH), both attached to the same carbon atom. Since the carboxyl group has a proton available for binding with the electrons of another atom, and the amino group has electrons available for binding with a proton from another atom. The amino acid behaves as an acid and a base simultaneously. Twenty of the naturally occurring amino acids are the building blocks of proteins, which they form by being connected to each other in chains. Amino acids form short polymer chains called peptides or longer chains called either polypeptides or proteins. The process of such formation from an m-RNA template is known as translation, which is part of protein biosynthesis. Twenty amino acids are encoded by the standard genetic code and are called proteinogenic or standard amino acids. Other amino acids contained in proteins are usually formed by post-translational modification, which is modification after translation in protein synthesis.

## **II. RELATED WORK**

Amino acids as precursors of proteins are essential to all organisms including the silkworm. Literature on nutritional significance of amino acids in silkworm *Bombyx mori* reveals the quantitative and qualitative requirement of the amino acids (Arai and Ito, 1964, Ito and Arai, 1967, Bose *et al.* 1989 and Nair and Kumar, 2004). Amino acids which bind together as long chains in proteins will be broken down to amino acids in the process of digestion mediated by the digestive enzymes in the midgut. The amino acids thus separated are released into the bloodstream and will be

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utilized by individual cells to assemble new and different proteins required for specific functions. Silkworms do not have enzymes required to synthesize all the amino acids. The six amino acids which silkworm can produce are proline, alanine, glycine, serine, tyrosine and cystine. Essential amino acids required for synthesis of silk are obtained only through food and they are Arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (Group-1) and Aspartic acid and glutamic acid (Group-2). These amino acids are required for the growth of the larva.

With this novel idea the present investigation was undertaken to know the content of free amino acid in the haemolymph of the 5<sup>th</sup> instar larval period. The quality of diet can be made to improve the free amino acid on the basis of its amino-acid content.

Basing on this piece of work many work has been done on the basis of the amino acid content of silkgland in this context, but my piece of work is a bit different that is a correlational study on the concentration of the haemolymph amino acid of certain bivoltine larvae of the silkworm *Bombyx mori*. L during 5<sup>th</sup> instar development. It also includes which factors are able to do the concentration change in subsequent varieties of bi and multivoltine also.

### III. 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 1<sup>st</sup> day onwards till cocoon formation. The haemolymph was collected in a clean vile containing few thiourea granules by pricking the abdominal leg just before the last leg. For the estimation of total amino acid content in haemolymph of silkworm the method of **Moore and Stein (1957)** with a little modification was followed. 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.

### IV. OBSERVATION

The free amino acid content in the haemolymph increased significantly to its peak from the 1<sup>st</sup> day upto the 6<sup>th</sup> day of 5<sup>th</sup> instar period all the races and it starts declining from the 7<sup>th</sup> day up to the last day. (Table-1). Beside haemolymph amino acid 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 amino acid was also recorded as in Table-1. The observed data showed strong degrees of positive Correlationship between the haemolymph ascorbic acid and the body weight. However a significant correlationship was also exists between the cocoon weight and shell weight.

**Table-1 : Comparison in the Concentration of Amino acid (mg/ml.) in the Haemolymph of some Bivoltine Silkworm Races, *Bombyx mori* L during 5<sup>th</sup> instar development**

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
1 <sup>st</sup> Day	112.77 ± 0.043	103.373 ± 0.005	99.036 ± 0.008	95.42 ± 0.024
2 <sup>nd</sup> Day	151.084 ± 0.123	135.18 ± 0.067	129.397 ± 0.004	123.614 ± 0.035
3 <sup>rd</sup> Day	229.156 ± 0.143	217.59 ± 0.097	211.084 ± 0.003	204.578 ± 0.047
4 <sup>th</sup> Day	360.722 ± 0.049	349.156 ± 0.076	344.819 ± 0.024	339.036 ± 0.098
5 <sup>th</sup> Day	456.265 ± 0.007	454.578 ± 0.034	448.674 ± 0.027	444.457 ± 0.056
6 <sup>th</sup> Day	585.301 ± 0.009	581.084 ± 0.005	574.337 ± 0.067	571.807 ± 0.008
7 <sup>th</sup> Day	199.036 ± 0.036	195.662 ± 0.008	192.289 ± 0.063	188.915 ± 0.017

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## Anova of Bivoltine Races (Haemolymph Amino acid)

ANALYSIS OF VARIANCE				
Source Of Variation	SS	df	MS	F
Between the Days	745146	6	124191	13486.35**
Between the Races	1264.823	3	421.6075	45.78389**
Error	165.7556	18	9.208644	
Total	746576.5	27		

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

Table-2 : Coefficient of correlation between the haemolymph free amino acid with body weight in bivoltine races of silk worm *Bombyx mori*.L during 5<sup>th</sup> instar development

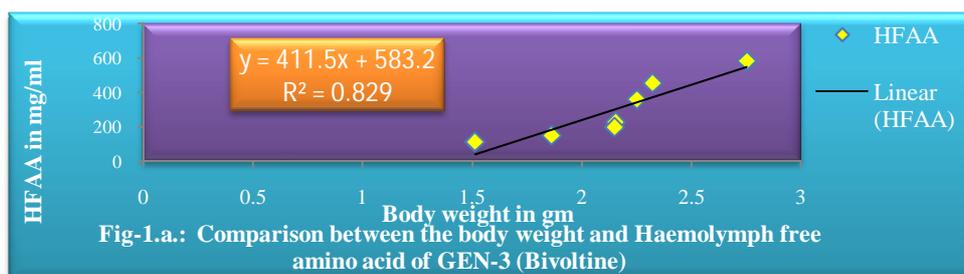
Sl. no.	Races of silkworm	Variables	Coefficient of correlation 'r'	df	't' Test	df
1	GEN-3	BW Vs HFAA	0.9109***	06	2.447*	6
2	BCON-1	BW Vs HFAA	0.9660***	06	2.447*	6
3	DUN-22	BW Vs HFAA	0.9192***	06	2.447*	6
4	BCON-4	BW Vs HFAA	0.8916**	06	2.447*	6

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

## V. RESULT AND DISCUSSION

In the present study the amount of haemolymph free amino acid from different days in fifth instar larval period were studied to establish a profile of silkworm haemolymph free amino acid to understand certain development as well as taxonomic characters. Haemolymph free amino acid in different races of *Bombyx mori* also gives an insight into a comprehensive understanding of silkworm metamorphosis.

The Haemolymph free amino acids in four races of Bivoltine is studied.. The concentration of free amino acid was increased progressively from the first day up to the last larval day as in the Table-1 . The data were subjected to ANOVA test. A significant increment in the amount of free amino acid was observed in each day of all the races. Analysis of variance showed that there was a significant variation in the concentration of free amino acid among four races as in Table-1 .GEN-3 showed a higher value in the concentration of free amino acid in comparison to other *Bombyx mori* races as in Table- 1 & 2 . However, the above races showed a common pattern in the concentration of the free amino acid as in Fig-1 . At the 7<sup>th</sup> days or the day just before pupation all the races showed decrement in the concentration of free amino acid as in Fig-2..

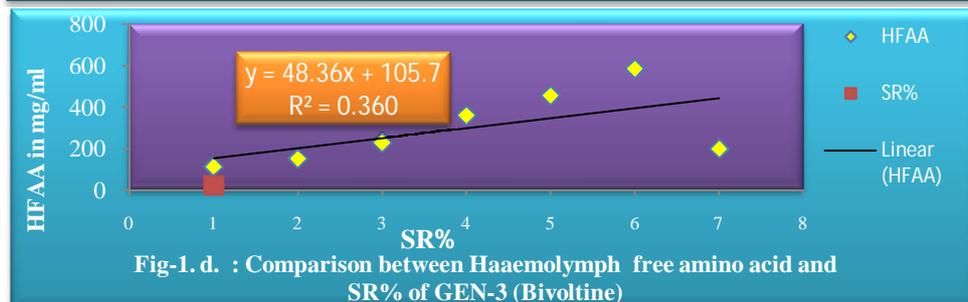
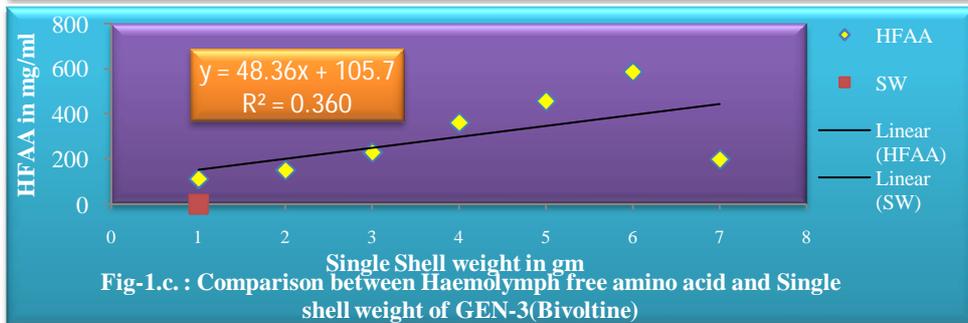
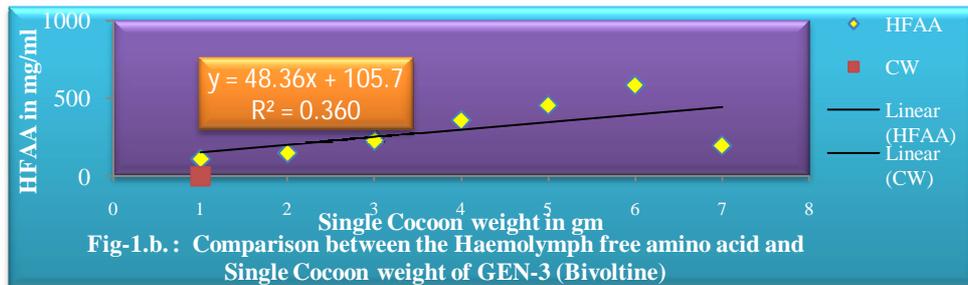


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The correlation between the Body weight and Haemolymph Free amino acid were established. The data of Body weight and Haemolymph Free amino acid of four races were subjected to co-efficient of correlation. Table-1 & 2 indicates a positive correlation between body weight and Haemolymph free amino acid. Correlation was further confirmed by the 't' test analysis. It was found that there was significant correlation between body weight and Haemolymph free amino acid as in Table:-1 & 2.

The morphological characteristics of an organism are basically the results of the metabolic processes and the synthesis of proteins. The free amino acids (FAAs) are the building blocks of their proteins. These are used in their synthesis as coded by the genetic constitution of the organism. The free amino acid shows characteristic pattern for a particular insect. Thus, the free amino acid pattern exhibited by seven races of silkworm has been studied with a view to establish certain similarities and variations among them. The similarities and variations in free amino acid patterns at species level have been considered as a tool in taxonomy (Hardon and Mitchell 1951, Micks and Ellis 1951, Ball 1952, Micks 1954, Fox 1956, Kirk *et.al.*, 1954 and Micks 1956).

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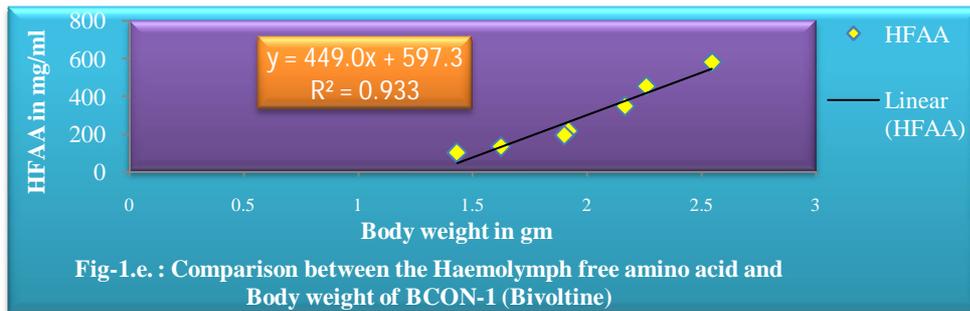


Fig-1.e. : Comparison between the Haemolymph free amino acid and Body weight of BCON-1 (Bivoltine)

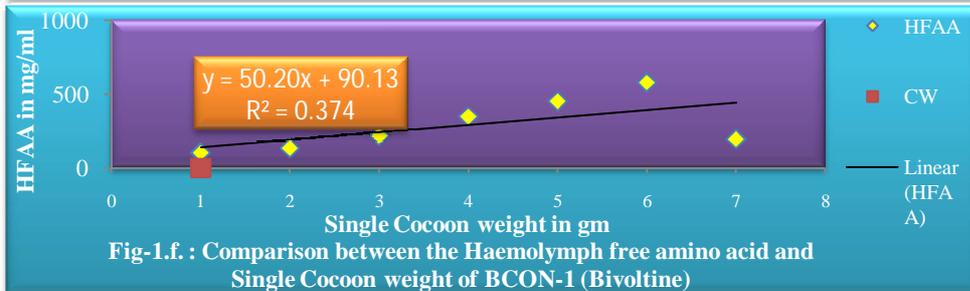


Fig-1.f. : Comparison between the Haemolymph free amino acid and Single Cocoon weight of BCON-1 (Bivoltine)

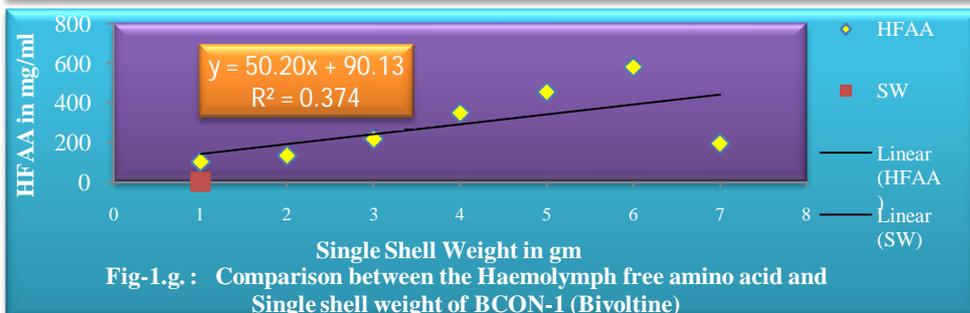


Fig-1.g. : Comparison between the Haemolymph free amino acid and Single shell weight of BCON-1 (Bivoltine)

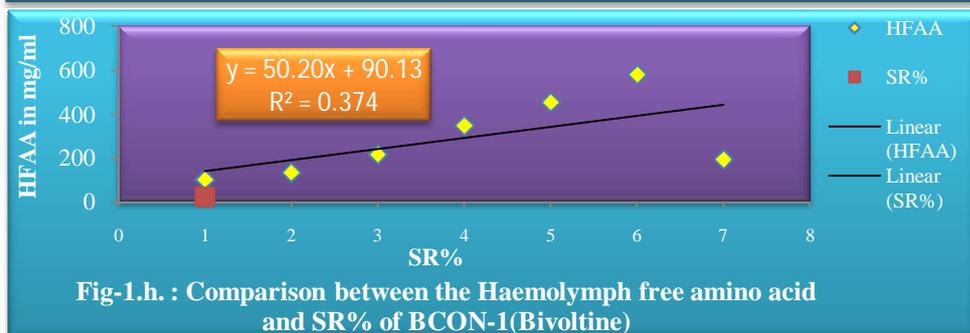


Fig-1.h. : Comparison between the Haemolymph free amino acid and SR% of BCON-1(Bivoltine)

Insects have a very high free amino acid content in the Haemolymph and this fact was appropriately been proposed as a biochemical characteristic of the class (Florkin, 1959). The function of the high free amino acid content in the Haemolymph is not clear (Chen, 1966; Corrigan, 1970). Some function have however been attributed to the free amino acid of the haemolymph and according to Chen (1966) they are precursor for protein synthesis, participation in detoxification mechanisms, buffering and osmoregulation. Most of the work on the free amino acids of blood has been concerned with holometabolous insects.

As in Table-1 and Fig-2 the result shows that there is a significant increment in the free amino acid level as the larvae progresses from the 1<sup>st</sup> day to the 6<sup>th</sup> day during 5<sup>th</sup> instar larval period. The analysis of the available data

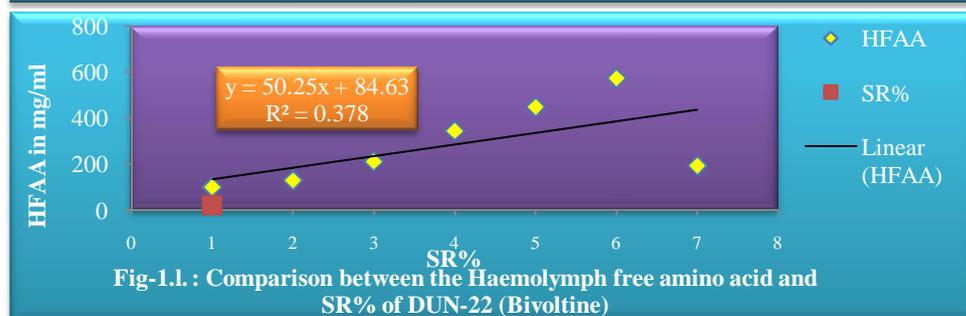
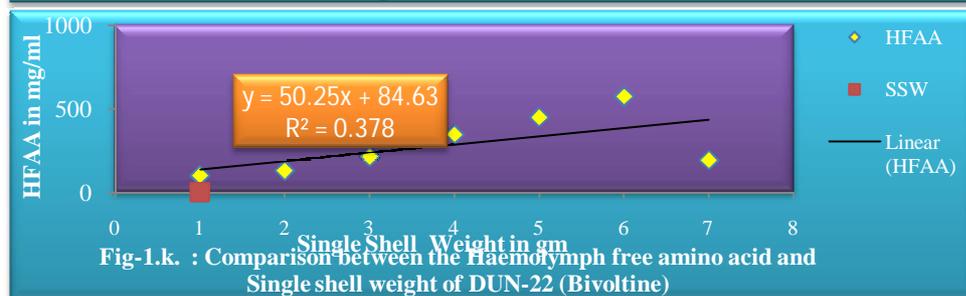
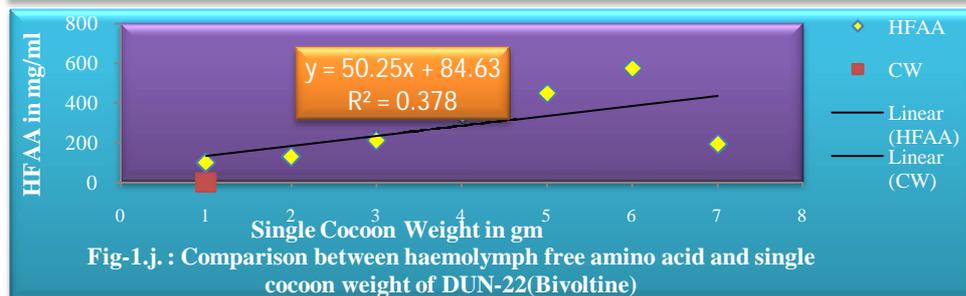
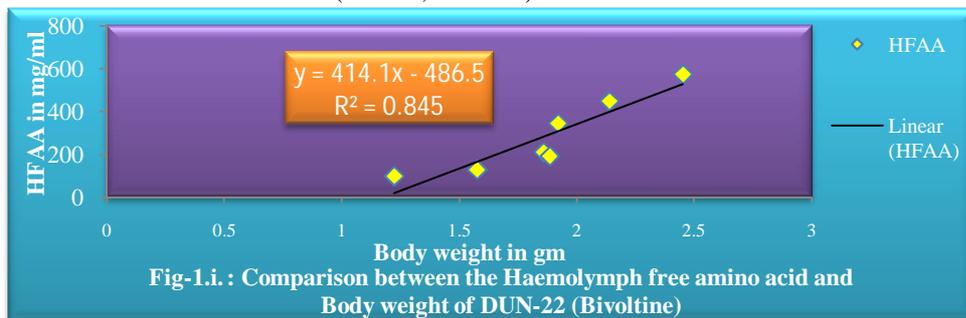
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showed a variation in the free amino acid content of the haemolymph. Similar type of variation in amino acids are also found in many insects species (Chen, 1971). Species specific variations in the ontogenic pattern of haemolymph free amino acid have been found during the larval development of the number of insect species. Such variations probably reflects the balance between synthesis, storage, transport and degradation of structural and functional proteins during ontogeny (Florkin and Jeuniaux, 1974). At one time it was proposed that variation in free amino acid be considered as an important taxonomic characters for the insects (Florkin, M. 1959)



Changes in amino acid patterns are influenced by several factors which include diet, insect activities, disease and exposure to toxin. Developmental changes in amino acid concentration as in Table-1 may be associated with molting (Florkin and Jeuniaux, 1974) metamorphosis (Wirtz, F and Hopkins, T. 1974) and diapauses (Boctor, I, 1981).

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In developing *Melanoplus sanguinipes* various Haemolymph amino acid concentration fluctuate from the egg to adult stage with large increase in asparagines, proline, alanine, glycine, glutamine and valine occurring in the third instar nymph.(Roberts,R. and Smith, H, 1971a). Free Haemolymph amino acids increased from 982 (2<sup>nd</sup> instar) to 1190 (6<sup>th</sup> instar) in developing *Spodoptera littoralis*, with a decline in glutamine and tyrosine, but an increase in histidine, citruline and Gamma amino butyric acid (GABA) (Boctore, I and Salem, S, 1973). Total Haemolymph amino acid decline in aging *Schistocerca gregaria* females and males ( Kulkarni, A and Mehrota, K., 1970). The effects of diet on Haemolymph levels have been demonstrated. Differences in the amino acid spectra from laboratory-fed and field collected adult melanoplus have been reported (Roberts, R. and Smith, H. 1971b). Starvation results in a slight increase in Haemolymph amino acids in *Philosoma Cynthia* (Boswquet, G., 1977), but in *Spodopetera exigua* they decrease (Cohen, A. and Patana, R., 1982). Disease and parasitism may result in reduced amino acid levels in some species (Mack, S. et al., 1979a: Wang, D. and Moeller, F., 1970) but increases in others (Rutherford, T., and Webster, J., 1978). Exposure of insects to certain insecticides results in significant reduction of some amino acids including depletion of Haemolymph proline (Corigan, J. and Kearns,G, 1963 : Kulkarni, A. and Mehrota, K., 1973).

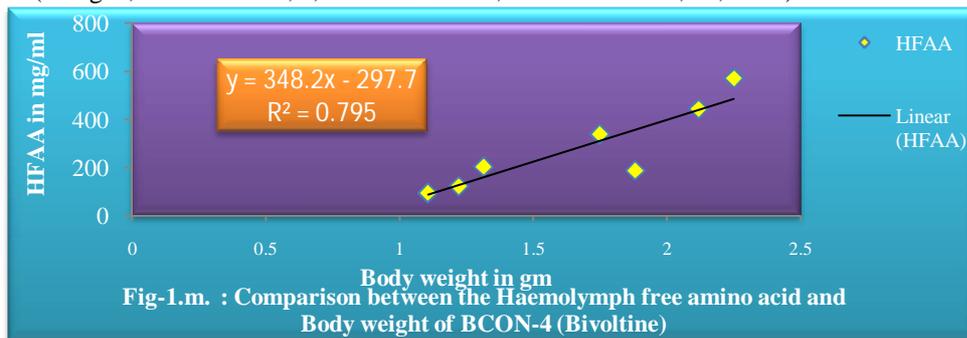


Fig-1.m. : Comparison between the Haemolymph free amino acid and Body weight of BCON-4 (Bivoltine)

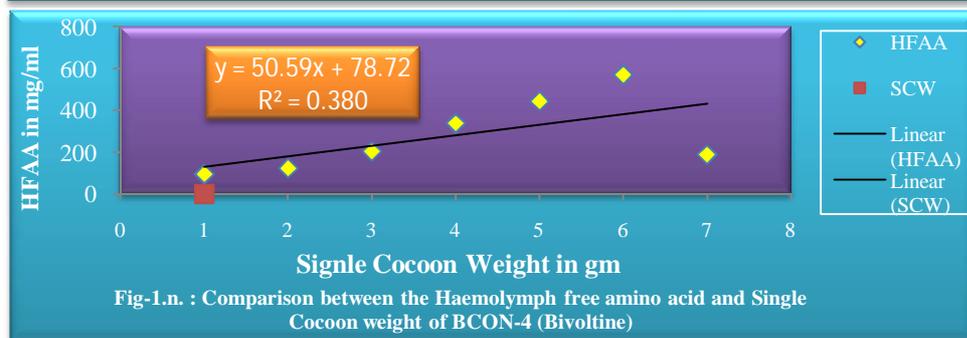


Fig-1.n. : Comparison between the Haemolymph free amino acid and Single Cocoon weight of BCON-4 (Bivoltine)



Fig-1.o. : Comparison between the Haemolymph free amino acid and Single Shell weight of BCON-4 (Bivoltine)

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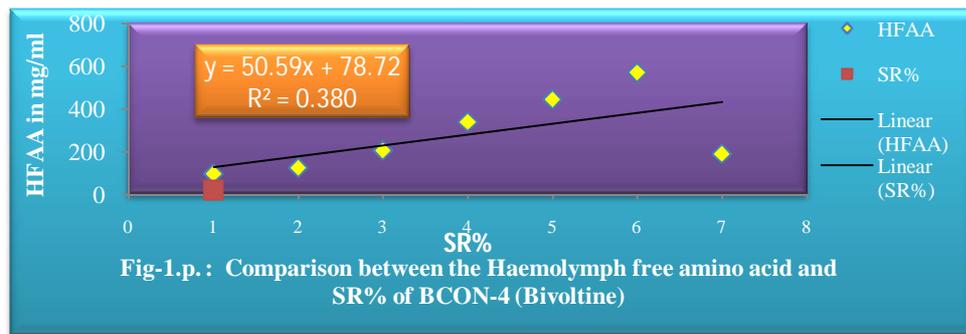


Fig-1.p.: Comparison between the Haemolymph free amino acid and SR% of BCON-4 (Bivoltine)

However, the concentration of free amino acid in the Haemolymph declines significantly just before pupation as in Fig-1 and Fig-2 in all the races. The reason might be the transfer of free amino acid to the silk gland for the utilization of silk synthesis during spinning period. During intermoult stage, most of the amino acids resulting from digestion are transported directly to the silk gland via the Haemolymph (Amanieu. M. *et al.*, 1965).

Analysis of amino acids and their ratio in mulberry leaf protein and cocoon silk protein revealed no such significant difference. Requirement of either glutamate or aspartate and non-essential amino acids for full growth, and partially replacement of dietary alanine, glycine and serine by glutamate and aspartate was studied by Ito and Arai, 1966 & 1967. Their study indicated that growth of silkworm greatly depends not only on the level of amino acids in the diet but also on the mutual balance of amino acids. Horie *et al.*, 1970 studied the effect of balance of three amino acid groups' viz. essential, non-essential and acid amino acids on larval growth and cocoon quality and concluded that tryptophan and phenylalanine could be synthesized by their precursors. By the use of artificial diets, nutritional requirement of silkworm has been elucidated.

The available data were subjected to coefficient of correlation between haemolymph free amino acid with body mass, cocoon weight, shell weight and SR% as in Fig:- 1 .a up to Fig:-1. p. Coefficient of determination ( $R^2$ ) indicated a positive but, high correlation exists between haemolymph free amino acid and body weight with the regression line obtained  $Y = 411.5X + 583.2$ . The result signifies that 82.9% relationship exists between the above two variables. So, the relationship of haemolymph free amino acid with cocoon weight, shell weight and SR% were also found as in Table:-1 & 2 and Fig:-1 .a ...1 .p. However, with the  $R^2$  value, it was indicated a positive moderate correlation  $R = 0.360$  (Cocoon weight) with line of regression  $Y = 48.36X + 105.7$ . From the above data, it was evident that the growth of the body depends upon the free amino acid pool in haemolymph.

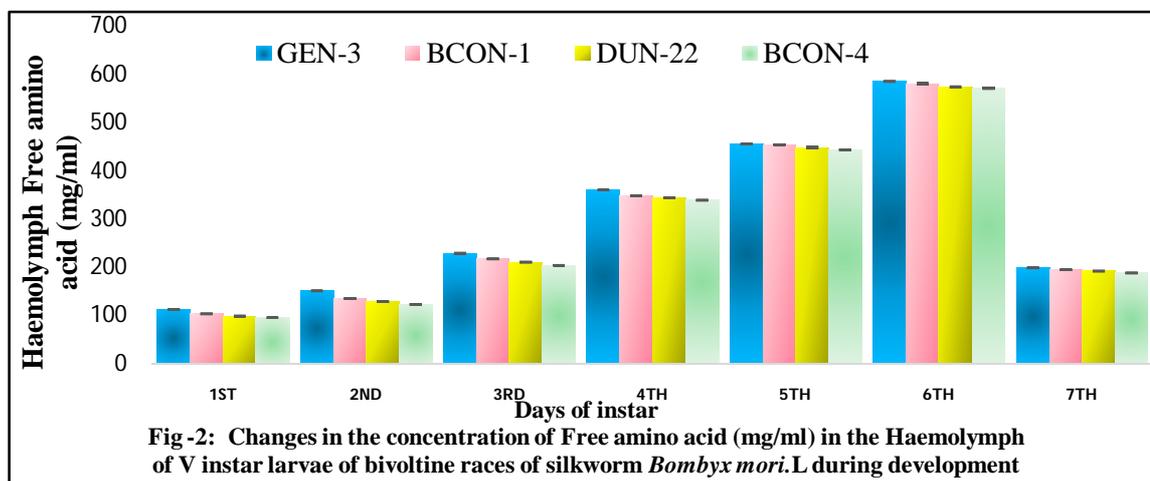


Fig -2: Changes in the concentration of Free amino acid (mg/ml) in the Haemolymph of V instar larvae of bivoltine races of silkworm *Bombyx mori* L during development

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## VI. CONCLUSION

In the present study the amount of the haemolymph free amino acid in fifth (5<sup>th</sup>) instar period from four bivoltine races were recorded to establish a profile of silkworm haemolymph Amino acid. The present data also gave an insight into a comprehensive understanding of silkworm metamorphosis.

## VII. ACKNOWLEDGEMENT

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