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Estimation of haemolymph biomolecules in some bivoltine silkworm (*Bombyx mori* L.) breeds

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Abstract

While estimating the biomolecular profile of different silkworm, *Bombyx mori* L. breeds, it was found that NB4D2 silkworm breed excelled in both the biochemical parameters viz., Protein and amino acid content. NB4D2 recorded the highest haemolymph total protein content of 47.36 mg/ml and highest haemolymph total free amino acid content of 3.17 mg/ml. APS4 breed recorded lowest values for both the estimated parameters.

Keywords: Silkworm, haemolymph, biomolecule protein, amino acids

Introduction

The silkworm (*Bombyx mori* L.) is an important economic insect that feeds mainly on mulberry leaves and converts the leaf protein into silk protein (Babu *et al.*, 2009) ^[1]. The cocoon and silk production are dependent on nutritive value of mulberry leaves (Etebari, 2004) ^[2]. Silkworm requires nutrients such as sugars, amino acids, proteins and vitamins for its optimal growth and development (Khedr *et al.*, 2013) ^[4].

Insect blood or haemolymph is best described as the intracellular circulating fluid that fills the body cavity or haemocoel. The hemolymph circulates freely within the body of the insects bathing different tissues. It is physically isolated from direct contact with the body tissues by a thin permeable membrane, which lines the haemocoel. In insects, the haemolymph, like the blood of higher animals comprises of two main components, the plasma and the corpuscles or haemocytes (Kerenhap *et al.*, 2005) ^[3]. Haemolymph is the only extracellular fluid in insects with diverse functions such as transportation of nutrients and hormones to cells and tissues, maintenance of correct moisture ratio, body shape and optimal body temperature and protection against insect and pathogens. The organic constituents of haemolymph (proteins, carbohydrates, free amino acids, lipids, enzymes etc.) play an important role in biochemical processes underlying growth and development of insects.

Considering, the importance of biochemistry for the vital physiological processes of silkworm, an attempt was made to study the biochemical aspects in some prolific bivoltine breeds reared under temperate climatic conditions so as to identify a superior silkworm breed for future breeding programmes.

Materials and Methods

Disease free layings of different breeds of the mulberry silkworm, *Bombyx mori* L. were obtained and incubated under laboratory conditions at 25 °C and relative humidity of 75 percent and then allowed to hatch. Rearing of all the silkworm breeds under study was carried out as per the standard package of practices (Raja, 2000) ^[5]. The experiment was laid out in a completely randomized block design with four replications for each treatment. Each replication comprised of 200 silkworms of uniform age and size retained after third moult.

The haemolymph of different silkworm (*Bombyx mori* L.) races was collected on fourth, fifth, sixth and seventh days of fifth instar. Haemolymph was obtained by puncturing the abdominal legs with sterilized needle/blade. The haemolymph thus bled was collected in pre-cooled tubes containing a few crystals of phenyl thiourea @ 1mg/sample. Phenyl thiourea was used to avoid the activity of prophenol oxidase followed by melanization of the haemolymph samples (Takeda *et al.*, 1996) ^[8]. The samples were stored at -20 °C till further use.

Results and Discussion

During the course of the study it was found that highest mean haemolymph protein content of

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47.36 mg/ml was recorded in the silkworm breed NB4D2, which was statistically higher than rest of the genotypes under study. The protein content recorded in other breeds include: CSR4 (38.63 mg/ml), CSR2 (36.43 mg/ml), DUN 6 (35.6 mg/ml) and APS4 (23.23 mg/ml). The least value of haemolymph total protein content of 23.22 mg/ml was recorded in the silkworm breed APS4 (Table 1). During the study, it was also reported that highest peak of haemolymph total protein content of 40.00 mg/ml was achieved on seventh

day of fifth instar which was statistically higher than rest of the days. The least value of haemolymph total protein content of 33.44 mg/ml was recorded on fourth day of the fifth instar. Rajannan *et al.* (1994) [6] while working on the biochemical aspects of various silkworm breeds has reported that total protein content differed among silkworm breeds and there is a profound influence of breed and feed on the expression of proteins. Therefore, these results are in line with the present findings.

Table 1: Protein content recorded in the haemolymph of different silkworm (*Bombyx mori* L.) breeds

Season	Protein content (mg/ml)				
Days	4 th Day	5 th Day	6 th Day	7 th Day	Mean
Genotypes					
NB4D2	44.21	45.29	48.69	51.26	47.36
CSR4	35.26	36.87	37.85	44.54	38.63
CSR2	34.28	35.45	36.54	39.45	36.43
Dun6	33.25	34.21	36.41	38.54	35.6
APS4	20.22	22.14	24.32	26.25	23.23
Mean	33.44	34.79	36.76	40.00	
CD ($p \leq 0.05$)	Day: 0.064				
	Genotype: 0.100				
	Day x Genotype: 0.201				

During the course of the study it was found that highest mean haemolymph amino acid content of 3.17 mg/ml was recorded in the silkworm breed NB4D2, which was statistically higher than rest of the genotypes under study. The amino acid content recorded in other breeds include: CSR4 (2.63 mg/ml), CSR2 (2.60 mg/ml), DUN 6 (2.49 mg/ml) and APS4 (1.68 mg/ml). The least value of haemolymph amino acid content of 1.68 mg/ml was recorded in the silkworm breed APS4 (Table

2). During the study, it was also reported that highest peak of amino acid content of 3.19 mg/ml was achieved on 4th day of fifth instar whileas the least value of 1.69 mg/ml was recorded on seventh day of the fifth instar. Significant variations in the free amino acid levels of some breeds of silkworm breeds have been reported by Taha *et al.* (2017) [7] which support the present findings.

Table 2: Amino acid content recorded in the haemolymph of different silkworm (*Bombyx mori* L.) breeds

Season	Carbohydrate content(mg/ml)				
Days	4 th Day	5 th Day	6 th Day	7 th Day	Mean
Genotypes					
NB4D2	4.23	3.82	2.72	1.92	3.17
CSR4	3.23	2.95	2.63	1.72	2.63
CSR2	3.42	2.83	2.42	1.73	2.60
Dun6	2.94	2.55	2.52	1.95	2.49
APS4	2.14	1.93	1.53	1.13	1.68
Mean	3.19	2.81	2.36	1.69	
CD ($p \leq 0.05$)	Day: 0.048				
	Genotype: 0.076				
	Day x Genotype: 0.153				

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