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CHANGES IN BIOCHEMICAL COMPOSITION IN THE HAEMOLYMPH OF FIFTH INSTAR LARVAE OF *PHILOSAMIA RICINI* DURING THERMAL STRESS

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ABSTRACT

Haemolymph of *Philosamia ricini* is a water reservoir which maintains homeostasis. When the insect is exposed to stresses naturally then it passes through various changes in biochemical composition. Fifth instar larvae of eri silk worm Philosamia ricini were kept under thermal stress of low and high temperature to observe the changes in carbohydrates, proteins and free amino acids. At high temperature carbohydrates showed a significant increase and a significant decrease at low temperature. Proteins and amino acids showed a significant decrease at high temperature and increase at low temperature.

Key Words: Homeostasis, Stress, Haemolymph, Temperature

INTRODUCTION

It is evident that amino acids, carbohydrates and proteins play an important role to maintain the internal environment of haemolymph in different stages of insect life. They largely affect various metabolic pathways as well as physiological conditions of insect (Edwards, 1982) Three different reviews of Chen (1962, 1966, 1971) provide comprehensive information on the concentration of free amino acids, in the haemolymph of insects. The studies related to the occurrence of proteins and amino acids during the unusual conditions *i.e.* during the high temperature, desiccation, and low temperature are still very scanty. Recently Malik and Malik (2009) studied the fluctuations in the biochemical composition of silk worm *Bombyx mori* under the thermal stress. In 2010, Singh *et. al.* also reported the changes in the certain biochemical composition of fifth instar larvae of *Philosamia ricini* when they were exposed to low temperature.

In general pattern the haemolymph is the water reservoir, when water is removed from haemolymph, then the osmotic conditions are disturbed to replace cellular water. The osmotic steady state is maintained by removal of solutes. The various stresses such as deficiency of food, temperature and desiccation may decrease the hemolymph volume, Albers and Bradley (2004) proposed that in the dehydrated *Drosophila melanogaster*, osmotic concentration was maintained by reducing the haemolymph volume up to 60% when the dehydration was prolonged. The osmoregulation continued during the period of rehydration and recovery from desiccation. On this basis they concluded that this insect does not require any external source of osmolytes or energy to regulate the osmotic concentration of haemolymph. According to Denlinger and Lee (2010) low temperature affects insects differently based on the severity of cold and duration of exposure. In insects, ions, sugars, proteins, amino acids and other dissolved solutes in haemolymph colligatively depress the melting point by 1.86ºC per osmol of solute and protect the insect. Cohen and Patana (1982) reported that in cold stressed larvae of *Spoddoptera exigua*, haemolymph osmotic pressure is highest as compared to the normal control larvae. There is little change in sodium ion concentration at low temperature but potassium concentration increased significantly. There was a decrease in calcium and magnesium ion concentration in comparison to the control. Delinger and Lee (2010) mentioned that magnitude of chilling injury is closely associated with the loss of ions homeostasis, particularly increase in potassium concentration and decrease in sodium and magnesium concentration in the haemolymph. Similar conclusions were made by Kostal *et al* (2007) in a bug *Pyrrochorus*.

Wyatt *et al.* (1955) studied the concentration of sugar, proteins and free amino acids in the silk worm *Bombyx mori* and

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other species of insects. The effect of high temperature on haemolymph sugar levels in the three selected races of silk worm was reported by Malik and Reddy (2008). They reared the fifth instar larvae and pupae at two selected temperatures *i.e*. 31ºC and 36ºC. At higher temperatures, an increase in blood sugar level and trehalase acitivity was observed in the spinning larvae (Malik and Malik, 2009).They exposed the larvae and pupae to selected temperatures *i e*. 31ºC and 36ºC the glucose and trehalose levels increased significantly, and increase was higher at 36ºC than 31ºC. The protein level of haemolymph was found to be significantly lower in pupal stage as compared to the larval stage. At high temperatures the protein level decreased significantly in larvae as well as in pupae, which was more at 36ºC than 31ºC. Free amino acids in pupae were higher as compared to the larval haemolymph. At high temperatures it increased significantly, and this increase was higher at 36ºC than 31ºC.

Thus to maintain osmotic balance during stresses, the insect passes through many changes in solute composition. In the present study we have observed the changes in three important components of haemolymph *i.e*. carbohydrates, proteins and free amino acids.

MATERIAL AND METHODS

2.1 Control

The worms of *Philosamia ricini*, were reared under normal conditions *i. e*. at 29ºC ±2ºC, R.H. 90%±5% (Pant and Agarwal, 1965). The larvae of *Philosamia ricini* are voracious feeder, specially fifth instar larvae, and were provided full diet as per recommendation of Sericulture Department.

2.2 Thermal stresses

Fifth instar larvae were kept at 36ºC±2ºC, and relative humidity was 90%±5% for three days. Haemolymph was withdrawn on the fifth day for analysis (*i.e*. they were exposed to high temperature stress for three days). Worms for low temperature stress were kept at 10ºC±2ºC, R.H. 90%±5%, for three days. Thus in both cases, haemolymph was withdrawn on the fifth day for analysis.

Estimation of total carbohydrates

The estimation of carbohydrates was performed by the method of Dubois *et al.* (1956).

Estimation of total Proteins

The total proteins were estimated by the method of Lowry *et al.* (1951).

Estimation of total free amino acids

The free amino acids were analyzed by colorimetric method *(Lee and Takahashi; 1966*).

RESULTS

As shown in graph and table (1) the concentration of carbohydrates increased in the fifth instar worms which were reared at high temperature and decreased at low temperature (graph and table 2). The variation was significant.

The concentration of proteins decreased at high temperature The decrease in concentration was statistically significant. The worms which were reared at low temperature showed an increase in the concentration of proteins significantly.

The free amino acids in the haemolymph of fifth instar larvae decreased at high temperature and increased significantly at low temperature.

DISCUSSION

Many workers have performed experiments on different insects to observe the effect of temperature. Cohen and Patana (1982) observed the stress related changes in the beet army worm *Spodoptera exigua*. They were of the opinion that the concentration of carbohydrates decreased due to stress of heat. Malik and Reddy (2009) studied the impact of high temperature in the *Bombyx mori* and concluded a significant increase in the concentration of glucose and trehalose in the worms reared at high temperature and the increase was higher at 36º C as compared to 31ºC.

Pant and Gupta (1979) studied the impact of cold stress in the larvae of *Philosamia ricini* and concluded that the total soluble carbohydrates decrease in the worms when exposed to low temperature. Singh *et al*. (2010) investigated the impact of low temperature on the fifth instar larvae of *Philosamia ricini* and concluded that the concentration of total carbohydrates decrease in the haemolymph of worms exposed to low temperature.

Thus our observations in *Philosamia ricini* under the stress of high and low temperature, supports the view of above mentioned workers.

Malik and Malik (2009) studied the impact of high temperature on the concentration of proteins in the haemolymph of fifth instar larvae of *Bombyx mori*. They kept the worms at two different temperatures i.e. 31ºC and 36ºC. In their studies, they found a significant decrease in protein concentration of haemolymph. They further reported that the order of decrease was found to be more at 36°C then at 31°C. The concentration of proteins in the haemolymph of fourth instar larvae *Spodoptera exigua* was higher when exposed to low temperature (Cohen and Patana, 1982). Pant and Gupta (1979) also studied the impact of cold stress in *Philosamia ricini* and suggested that the increase in concentration of proteins takes place at low temperature. Singh *et al.* (2010) reported that in *Philosamia ricini* the concentration of proteins increased when fifth instar larvae were exposed to low temperature.

When worms were reared at high temperature (36°C±2°C), the total protein concentration decreased significantly while at low temperature the protein concentration increased significantly. Therefore, we conclude that due to high temperature the quantity of storage protein decreases while in the worms which were reared at low temperature, the concentration of storage proteins increases.

In present study, when the fifth instar worms were reared at high temperature, there was a significant decrease in the amino acid concentration, while the worms which were exposed to low temperature showed a significant increase in concentration amino acids.

Cohen and Patana (1982) suggested that there was a dramatic increase in the concentration of the total free amino acids in the haemolymph of fourth instar larvae of *Spodoptera exigua* when exposed to high temperature (40°C) for 20 hours. They also suggested that at low temperature the concentration of total free amino acids decreased significantly when larvae were exposed to 10°C for 20 hours. Pant and Gupta (1979) reported that the concentration of free amino acids increased at low temperature in fifth instar larvae of *Philosamia ricini* when these larvae were exposed to 2°C. Malik and Malik (2009) reported that the concentration of total free amino acids increased at high temperature which was higher at 36°C than that of 31°C. Singh *et al*. (2010) suggested that at low temperature the concentration of free amino acids increased significantly when the fifth instar worms of *Philosamia ricini* were reared at low temperature *i e.* 10°C. The increase in concentration of total amino acid was about 11 to 29% as compared to the control. Our observations of amino acids in the *Philosamia ricini* at low temperature were same as reported by Singh *et al.* (2010). The increase may be due to less consumption of proteins at low temperature.

CONCLUSION

The change in concentration of carbohydrate, protein and amino acid was observed at high and low temperature. The concentration of carbohydrate increased at high temperature and decreased at low temperature which may be due to the more and less activity of insect. The protein concentration decreased at high temperature and increased at low temperature due to decrease in storage protein and breakdown of proteins to fight against stresses. Total free amino acids increased at low temperature may be due to less consumption of proteins and decrease at high temperature is due to more excretion through malpighian tubules.

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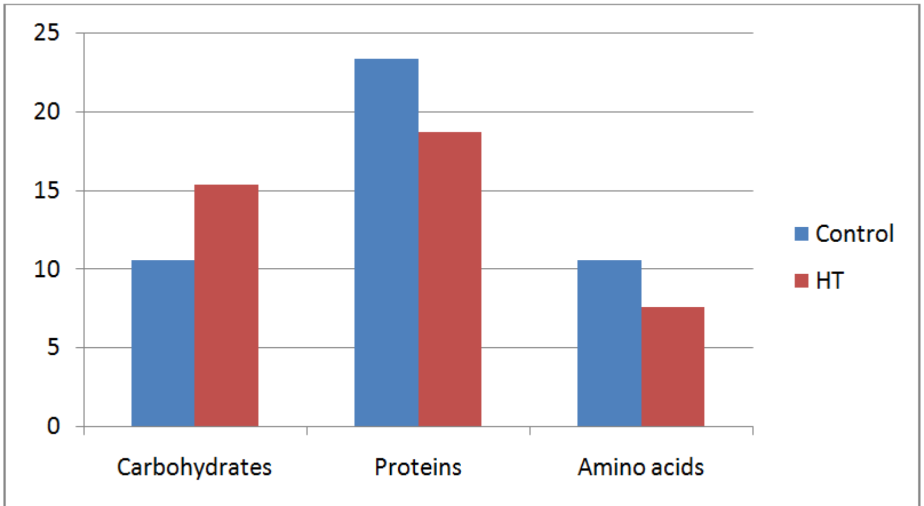
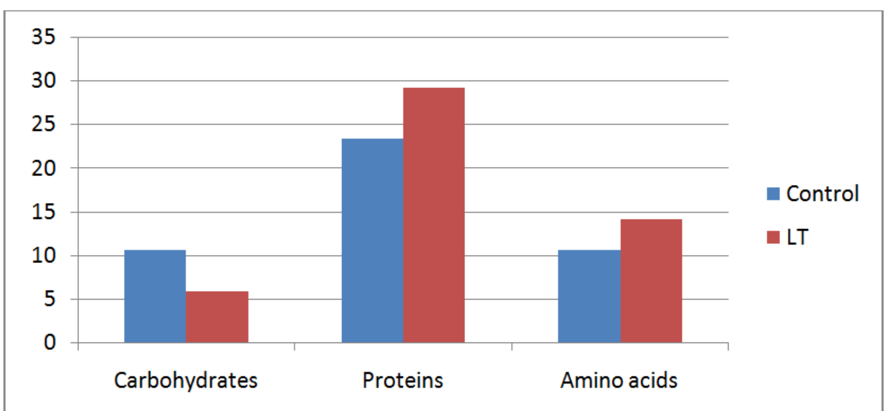


	carbohydrates	proteins	Amino acids
control	10.6±1.1	23.33±1.3	10.54±0.67
HT	15.4±0.58	18.7±0.35	7.6±0.27

Graph and table 1: Concentration of carbohydrates, proteins and amino acids (mg/ml) in the fifth instar larvae of *Philosamia ricini* during high temperature stress (36°C±2°C).



	carbohydrates	proteins	Amino acids
control	10.6±1.1	23.33±1.1	10.54±0.67
LT	5.8±0.18	29.2±0.36	14.1±0.27

Graph and table 2: Concentration of carbohydrates, proteins and amino acids (mg/ml) in the fifth instar larvae of *Philosamia ricini* during low temperature stress (10°C±2°C).