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CERTIFICATE

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IMPACT OF TEMPERATURE ON THE PROTEIN CONTENT IN DIFFERENT STRAINS OF *BOMBYX MORI*. L

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Abstract

As silkworms are cold-blooded animals, temperature will have a direct effect on various physiological activities. Biomolecules such as carbohydrates, lipids, proteins, hormones and chromosomes etc., play an important role in biochemical process underlying growth and development of insects. The concentrations of these biomolecules mainly depend on the impact of temperature. Proteins in haemolymph, silkgland, fatbody and muscle are at higher concentration during development and are useful in silk proteins synthesis. Keeping this in view, in the present experiment an attempt has been made to study the impact of temperature on the protein content in different silkworm races viz., pure Mysore (PM), bivoltine (CSR_2) and crossbreed (CSR_2xPM). On the basis of the experiment, the study revealed that proteins in haemolymph, silkgland, fat body and muscle of cross breed are at higher concentration when compared to multivoltine and bivoltine breeds. The cross breed (CSR_2xPM) has thermotolerant capacity than other two breeds PM and CSR_2 at 28°C.

Keywords: Temperature, Bombyx mori, protein, haemolymph, silkgland, fat body, muscle.

Introduction

Temperature is one of the key environmental factors that influence the physiology of insects. The silkworm is sensitive to environmental fluctuations and unable to survive naturally due to continuous domestication since the dawn of sericulture (Bhattacharjee, 2008). Fluctuation in environmental conditions is maintained by maintaining the internal temperature and water content, but it has a limit of tolerance (Singh et al., 2009). Low temperature is always lighter than high temperature with reference to productivity of silkworm for different instars (Datta et al., 2001). Majority of the economically important genetic traits of silkworms are qualitative in nature and that phenotypic expression is greatly influenced by environmental factors such as temperature, relative humidity, light and nutrition (Wu and Hou, 1993). Temperature has a direct correlation with the physiology of silkworm.

Proteins play an important physiological role in growth and development of silkworm and silk proteins synthesis. Necessary amino acids are derived from the amino acids present in body fluid in a free state and from the amino acids formed in the posterior silk gland cells. Silkworm requires all the ten essential amino acids for growth and development (Ito, 1978). Silk is made up of two proteins such as fibroin and sericin. Silkworm has almost become an important tool for several biochemical, physiological and genetic studies in insects. Physiological and biochemical studies include general metabolism and morphogenesis in insects, digestion and digestive enzyme, protein synthesis and their metabolism, hormones and their mechanisms of action, structure and function of chromosomes etc., for better productivity (Bhattacharya and Kaliwal, 2004). In the present experiment an attempt has been made to study the proteins level during silkworm larval development in different silkworm races at different temperature.

Materials and methods

The disease free layings of silkworm *B.mori* commercial cross breed PM (multivoltine) and CSR_2 (Bivoltine) was obtained from the State Government Sericulture Centre at Thenkasi and were incubated at 27°C in ant proof racks at 70-80% humidity. Since the experiments required continuous maintenance of the test species, silkworms were reared in the laboratory itself in accordance with the procedure adopted by Krishnaswami (1988). Three strains cross breed (CSR_2xPM), bivoltine (PM×PM) and multivoltine (CSR_2 ×CSR_2) are collected for experiment. Each strains are divided into 4 groups in which 3 groups as experiment and one as control. Each group consist of 50

larvae. The experimental group larvae are exposed to different temperature as 22°C, 25°C and 28°C separately. These treatments are followed to other strains also. Every strain has separate control group. During the fifth instar the haemolymph, silkgland, fatbody and muscle (4th day) are collected and analysed for protein estimation (Lowry et al., 1951).

Results

The present study observed the protein content of different strains of silkworm, *B.mori*, with the influence of temperature. Effect of temperature on the Protein content of fifth instar *B.mori* exposed to I[st] instar are shown in Table 1. The maximum concentration of protein in haemolymph (49.60 ± 4.48 mg/ml), silkgland (33.17 ± 3.09 mg/g), fatbody (8.06 ± 0.75 mg/g) and muscle (22.01 ± 1.99 mg/g) of cross breed (CSR_2×PM) are higher when compared to bivoltine (PM×PM) and multivoltine (CSR_2×CSR_2) breeds.

Table 2 shows the maximum concentration of protein in haemolymph (49.60 ± 4.48 mg/ml), silkgland (38.06 ± 3.47 mg/g), fatbody (38.06 ± 3.47 mg/g) and muscle (20.23 ± 1.80 mg/g) of cross breed (CSR_2×PM) are higher when compared to bivoltine (PM×PM) and multivoltine (CSR_2×CSR_2) breeds. At the same time, protein concentration was recorded the maximum at 28°C in cross breed (CSR_2×PM).

Table 1

Effect of temperature on the Protein Content of fifth instar *B.mori* larvaeexposed to I[st] instar

N = 50

Per cent deviation over control values in parentheses

Races	Parameter / Temperature	Haemolymph (mg/ml)	Silk gland (mg/g)	Fat body (mg/g)	Muscle (mg/g)
♂PM × PM♀ (Bivoltine)	Control	27.14±1.75	26.22±1.81	5.56±0.43	15.37±1.37
	22°C	23.04±1.60	24.85±2.15	6.38±0.51	16.09±1.23
	25 °C	21.92±2.07	27.05±1.99	7.90±0.56	19.37±1.85
	28°C	23.06±1.93	30.16±2.35	7.65±0.60	21.40±1.93
♂CSR_2× CSR_2♀ (Multivoltine)	Control	37.11±2.95	30.14±2.30	5.87±0.37	17.09±1.52
	22°C	35.09±3.06	27.24±1.87	5.80±0.41	16.35±1.47
	25 °C	32.40±2.83	33.15±3.01	4.93±0.38	21.29±1.95
	28°C	36.59±2.91	31.65±2.75	5.39±0.49	20.97±1.38
♂CSR_2× PM♀ (Crossbreed)	Control	41.06±3.85	37.20±3.15	6.25±0.54	19.38±1.50
	22°C	42.35±4.01	31.10±2.81	9.38±0.75	19.70±1.71
	25 °C	47.92±3.81	35.63±3.21	9.95±0.81	21.83±1.85
	28°C	49.18±3.28	33.17±3.09	8.06±0.75	22.01±1.99

* not significant

All other deviations significant at P≤ 0.05 (t-test)

Table 2

Effect of temperature on the Protein Content of fifth instar *B.mori* larvae exposed to II[nd] instar

Races	Parameter / Temperature	Haemolymph (mg/ml)	Silk gland (mg/g)	Fat body (mg/g)	Muscle (mg/g)
♂PM × PM♀ (Multivoltine)	Control	27.14±1.91	26.22±2.15	5.56±0.38	15.37±1.29
	22°C	21.70±2.06	22.09±2.09	5.27±0.42	11.60±0.92
	25 °C	25.71±2.14	28.36±2.70	7.72±0.57	16.82±1.33
	28°C	32.42±2.39	28.19±2.51	8.14 ±2.51	13.91±1.05
♂CSR_2× CSR_2♀ (Bivoltine)	Control	37.11±2.90	30.14±2.98	30.14±2.98	17.09±1.28
	22°C	33.60±3.07	31.76±3.01	31.76±3.01	11.15 ±1.05
	25 °C	39.73±3.31	34.35±3.09	34.35± 3.09	14.21±1.19
	28°C	43.30±3.99	33.09±3.00	33.09±3.09	17.37±1.20
♂CSR_2× PM♀ (Cross breed)	Control	41.06±3.80	37.20±3.53	37.20±3.53	19.38±1.38
	22°C	46.52±4.07	38.37±3.85	38.37±3.85	12.43±1.14
	25 °C	42.37±4.01	40.80±3.91	40.80±3.91	18.21±1.39
	28°C	49.60±4.48	38.06±3.47	38.06±3.47	20.23±1.80

N = 50

Per cent deviation over control values in parentheses

* not significant All other deviations significant at P≤ 0.05 (t-test)

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Discussion

The purpose of this study is to obtain recent data to get an idea of performing different trials of experiment on various larval stages of silkworm *B.mori* under various temperatures. Protein concentration increased rapidly from 1st instar and reaches maximum level at the end of 4th instar (Banno et al., 1993). Haemolymph, silkgland, fatbody and muscle brings about functional homeostasis of insect organs by transporting chemical substances into and out of the cells of the tissues and thus serves as a medium of chemical communication of distant and distinct organ systems of insect body. Haemolymph, silkgland, fatbody and muscle proteins undergo radical changes both in quality and quantity during development. In the present study the concentration of protein in haemolymph, silkgland, fatbody and muscle increased progressively during the larval development and reached maximum in the late fifth instar larvae of crossbreed (PM×CSR2) when compared to multivoltine (Pure Mysore PM), and bivoltine (CSR2). The cross breed breed was found to have higher protein level in the haemolymph, silkgland, fatbody and muscle of the fifth instar at 28°C when compared to 22°C and 25°C.

Conclusion

The differences in the multivoltine, bivoltine and cross breeds of silkworm in terms of major biochemical composition in the haemolymph, silkgland, fatbody and muscle are breed specific and are attributed to their genetic characters. In this study it is concluded that the protein concentration of silkworm got significant changes depend on temperature. The current study opens up avenues for breeding temperature tolerant silkworm strains for sustainable tropical sericulture industry for quality silk production.

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