Caspian J. Env. Sci. 2006, Vol. 4 No.1 pp. 17~24

CJES
Caspian Journal of Environmental Sciences

[Article]

The Effects of Potassium Bromide on Biochemical Contents of the Fat Body and Haemolymph of Crossbreed Races of the Silkworm, *Bombyx mori* L.

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ABSTRACT
Oral supplementation with potassium bromide (10, 20 and 40 µg/ml) to fifth instar larvae of CSR2, CSR4 and CSR2xCSR4 crossbreed races of the silkworm, *Bombyx mori* resulted in a significant increase in the fat body glycogen in all the treated groups and in all the three races of the silkworm, *B. mori*. The fat body protein content was increased with 20 and 40 µg/ml treated groups in CSR2 and CSR4 races and in all the treated groups in CSR2xCSR4 cdrossbreed race of the silkworm, *B. mori*. However, there was a decrease in fat body protein with 10 µg/ml treated groups in CSR2 and CSR4 races. There was a significant increase in the haemolymph trehalose with 10 µg/ml in CSR2 race, in all the treated groups in CSR4 race and with 20 and 40 µg/ml treated groups in CSR2xCSR4 crossbreed race when compared over the respective carrier controls. There was also a significant increase in the haemolymph protein treated with 20 and 40 µg/ml in CSR2 race, with 10 and 20 µg/ml in CSR4 race and in all the treated groups in CSR2xCSR4 crossbreed race when compared over the respective carrier controls. There was a significant increase in the fat body total lipids with all the treated groups and in all the three races of the silkworm, but there was a significant decrease with 40 µg/ml treated group in CSR2xCSR4 race of the silkworm, *B. mori* when compared over the respective carrier controls. These results indicated that the biochemical contents of the fat body and haemolymph to potassium bromide showed good response in all the three races of the silkworm, *Bombyx mori* L.

Keywords: *Bombyx mori*. Fat body, Glycogen, Haemolymph, Potassium bromide, Trehalose

INTRODUCTION

Nutritional status and environmental conditions play a vital role and affects growth, metabolism and development of silkworm, *Bombyx mori* L. The main source of metabolic fuels are the carbohydrates, the building blocks are the proteins and energy reserves are the carbohydrates in the form of glycogen and lipids which are essential for the development of pupa and adult that are derived from the food material. The metabolic fuels are stored in the fat body and haemolymph during fifth instar. Metals are essential for the activity of several enzymes where the metals can directly bind to proteins or may attach through an organic legend such as haem. House (1974) has suggested requirements of minerals in various insects but the indispensability of metallic ions for the growth promotion of the insects are yet to be established. It has been reported that mineral salts enhances the growth and development of the silkworm, *B. mori* (Dasmahapatra, 1989; Hugar *et al.*, 1998). The requirement of different minerals in various insects has been investigated (Chapman, 1998; Ito, 1978).

The metals and their salts play a key role as some of them are acting as a catalyst important to biological system. It has been reported that magnesium is essential for complete activity of trehalose synthesis (Murphy, and Wyatt, 1965). Dasmahapatra *et al.* (1989) have suggested that supplemen-tation with minerals increase the biochemical contents of the silkgland in the nistari race of the silkworm, *B. mori*. It has also been reported that supplementation with salts increase the biochemical contents of the silkworm, *B. mori* (Etebari and Fazilati, 2003). It has also been suggested that the supplementation with minerals increase the biochemical contents of the silkworm, *B. mori* (Nirwani and Kaliwal, 1996; Hugar *et al.*,

1998; Goudar and Kaliwal, 2001; Bhattacharya and Kaliwal, 2004). Hence, the present investigation was undertaken to find out and compare the effect of potassium bromide on the fat body glycogen, protein and total lipids and haemolymph trehalose and protein of CSR2, CSR4 and CSR2×CSR4 crossbreed races of the silkworm, *B. mori* L.

Materials and Methods

Rearing technique and treatment

The disease free laying (DFLS) of the bivoltine crossbreeds silkworm, *Bombyx mori* L., were obtained from Rayapur, Dharwad, Karnataka and reared in the laboratory by the improved method of rearing technique (Krishnaswami, 1978). Eggs were incubated at 250C with 80% R.H. The hatched larvae were reared in the rearing room and fed with fresh mulberry leaves (K2 variety) four times a day by maintaining optimum humidity, spacing and ad libitum mulberry leaves. The fifth instar larvae weighing an average of 2.5 g were selected randomly as they consume 80-90% of mulberry leaves and grouped into different batches for the experiments. Each group consisted of five replications each with 20 larvae.

potassium bromide was procured from British Drug House (India) Limited, Worli, Mumbai was dissolved in distilled water and diluted in three concentrations (10, 20 and 40 µg/ml). The potassium bromide was uniformly sprayed on fresh mulberry leaves out of four feedings per day feeding of treated leaves were altered with the feeding of untreated leaves. The controls were fed with the leaves sprayed with distilled water and normal leaves. In each supplementation, 500 ml of solution was used to treat 100 larvae (50 µl/larva). The parameters were recorded 10 larvae two from each replication. The normal controls, carrier controls and the treated larvae were used for the estimation of glycogen, protein and total lipids from the fat body and protein and trehalose from the haemolymph.

Tissue preparation

To prepare the tissue for the fat body glycogen and protein estimation the larvae were dissected in 0.9% saline at pH 6.5 on 6th day. The haemolymph collected from 2-3 larvae by amputating one of the thoracic legs of the larvae in the prehilled centrifuge tube and used immediately for the protein and trehalose estimation.

Glycogen Estimation

Anthrone method of Sciefter *et al.* (1950) was used to determine the fat body glycogen. A known quantity of fat body was homogenized with 2 ml of 20% potassium hydroxide. The glycogen was precipitated by adding equal volume of 80% ethanol and the mixture was kept overnight at room temperature for digestion. It was then centrifuged at 3000 rpm for 15 min and the supernatant was discarded. The residue was dissolved in a known volume of distilled water. Glycogen content was estimated with known aliquots in triplicate by the anthrone method. Glucose was used as the reference standard and the intensity of the colour was read on the spectrophotometer at 620 nm.

Trehalose Estimation

The estimation of haemolymph trehalose was carried out according to the method of Roe (1955). Known quantity of haemolymph was collected in each test tube and added 0.5 ml of 2% of sodium hydroxide to each test tube. After shaking, the tubes were kept in boiling water for 10 min and then the tubes were cooled in the ice box. Then 5 ml of anthrone reagent (0.05% anthrone in 70% sulphuric acid) was added to the tubes, and they were again kept in boiling water for 15 min for the development of colour. Then the tubes were cooled to room temperature. Then the colour intensity was read on spectrophotometer at 620 nm. For the reference standard the trehalose (Sigma, USA) was used. Anthrone positive carboh-ydrate in the haemolymph is considered as trehalose.

Total protein estimation

The method of Lowry *et al.* (1951) was used for the estimation of total protein in the fat body and haemolymph. The tissue protein was precipitated by the addition of 1 ml of 30% trichloroacetic acid (TCA) solution followed by centrifugation at 3000 rpm for 30 min. It was repeated twice, and then the precipitate was dissolved in 1 ml of 0.1 N sodium hydroxide. A known aliquot of this solution was then mixed with 5 ml of alkaline copper reagent (20% sodium carbonate prepared in 0.1 N sodium hydroxide containing sodium potassium

tartarate and 1% copper sulphate). After 10 min 0.5 ml of Folin Ciocalteu's reagent was added to the tubes and the tubes were shaken thoroughly. Then the tubes were kept for 20 min for colour development. The readings were taken on the UV spectrophotometer at 650nm.

The estimation of total haemolymph protein was also carried out. A known quantity of haemolymph was diluted with 0.5 ml of distilled water. A known aliquot of this solution was added with 5 ml of alkaline copper reagent. After 10 min 0.5 ml of Folin Ciocalteu's reagent was added and were mixed thoroughly and kept for 20 min until the colour develops. The readings were taken on the UV spectrophotometer at 650 nm. Bovine Serum Albumin (BSA) (Fatty acid free) was used as the reference standard.

Extraction and estimation of lipids

The method of Folch et al (1957) was used for the lipid estimation, using chloroform: methanol mixture (2:1 V/V). First all the fat body was homogenized with appropriate volume of chloroform: methanol mixture (1:10). The homogenate was then quantitatively transferred to a 50 ml separating funnel and then similar volume of chloroform was added. The two solvents were partitioned by the addition of 0.2 volume of water. After the funnel was shaken, the mixture was allowed to stand overnight. The lower chloroform layer containing lipids was drawn off. The lipids sample was kept in a vacuum desiccators until constant weight was obtained.

Statical Analysis

The experiment were designed by the complete randomized block design (CRBD) method and the data collected were subjected to the statistical analysis of variance (ANOVA) test to determine the significant difference between the treatment and control groups (Raghava Rao, 1983).

RESULTS

The results of the oral supplementation of potassium bromide on biochemical contents in the fat body and haemolymph of the silkworm, *B. mori* are presented in Table 1 and 2.

Carbohydrates

The dietary Supplementation with 10 µg/ml potassium bromide to silkworm larvae resulted in a significant increase in glycogen of the fat body of 207, 73 and 40 percent, with 20 µg/ml showed significant increase of 279, 152 and 48 percent and with 40 µg/ml showed an increase 87, 172 and 91 percent in CSR2, CSR4, and CSR2×CSR4 crossbreed races of the silkworm respect-tively when compared over the respective carrier controls. However, there was a significant decrease in the fat body glycogen in all the three races of the normal control silkworms except in CSR2 race where it was shown to increase when compared over the respective carrier controls. These results indicate that all the three races of the silkworms showed good response to potassium bromide to increase the glycogen

Table 1. Effect of potassium bromide on fat body glycogen, protein and total lipids content in crossbreed races of *B. mori*.

Treatment (Dose in µg/ml)	Glycogen (µg/mg)			Protein (µg/mg)			Total lipids (µg/100mg)		
	CSR_2	CSR_4	$CSR_2 \times CSR_4$	CSR_2	CSR_4	$CSR_2 \times CSR_4$	CSR_2	CSR_4	$CSR_2 \times CSR_4$
10	16.35* (207)	9.03* (73)	22.91* (40)	40 (-17)	56 (-1)	180* (52)	245* (77)	180* (147)	298* (16)
20	20.17* (279)	13.22* (152)	24.24* (48)	90* (87)	76* (31)	210* (77)	281* (77)	257* (210)	473* (84)
40	9.939* (87)	14.25* (172)	31.25* (91)	78* (62)	100* (72)	240* (103)	378* (173)	329* (269)	236* (-8)
Carrier control (distilled water)	5.31 (100)	5.23 (100)	16.36 (100)	48 (100)	58 (100)	118 (100)	138 (100)	122 (100)	257 (100)
Normal control	7.03 (32)	4.51 (-14)	11.22 (-31)	30 (-38)	38 (-34)	42 (-54)	130 (-6)	100 (82)	140 (-46)
±SEM	1.04	0.58	0.74	4.44	1.98	5.70	2.64	3.583	2.70
C. D. at 5%	2.92	2.02	2.33	18.63	6.22	18.84	8.21	12.571	8.27

*-Significant increase/decrease (-) at 5% ±SEM: Standard Error C. D.: Critical difference
Values in parenthesis indicate percent change over carrier control

content in the fat body (Table 1).

The dietary supplementation with 10µg/ml potassium bromide to silkworm larvae resulted in a significant increase in trehalose of haemolymph of 72, 27 and 10 percent in CSR2, CSR4 and CSR2×CSR4 crossbreed races, with 20 µg/ml showed an increase of 3%, significant increase of 46% and 78%, with 40 µg/ml showed an increase of 11% and significant increase of 63% and 42% in CSR2, CSR4 and CSR2×CSR4 crossbreed races of the silkworm respectively when compared over the respective carrier control. The results also indicated that there was a significant decrease in the haemo-lymph trehalose in all the three races of the normal controls when compared over the respective carrier controls. These results indicate that CSR4 race showed good response to potassium bromide in increasing haemolymph trehalose as compared to CSR2 and CSR2×CSR4 races of the silkworm, *B. mori* depending on dose employed (Table 2).

Total protein

The oral supplementation with 10 µg/ml potassium bromide to silkworm larvae resulted in decrease in fat body protein of 17%, 1% and a significant increase of 52%, with 20 µg/ml showed a significant increase of 87%, 31%, 77% percent, with 40 µg/ml showed a significant increase of 62%, 72% and increase of 2% in CSR2, CSR4 and CSR2×CSR4 crossbreed races of the silkworm respectively when compared over the respective carrier controls. The results also showed that there was a decrease in fat body protein in all the three races of the normal control when compared over the respective carrier controls. (Table1). These results indicated that all the three races showed good response to higher doses of potassium bromide to increase fat body protein when compared to carrier controls.

The oral supplementation with 10 µg/ml potassium bromide to silkworm larvae resulted in no change in haemolymph protein, increase of 2% and a significant increase of 77% , with 20 µg/ml showed an increase of 1%, 1% and a significant increase of 84%, with 40 µg/ml showed a significant increase of 49% and a decrease of 1% and a increase of 1% haemolymph protein in CSR2, CSR4 and CSR2×CSR4 crossbreed races of the silkworm respectively when compared over the respective carrier controls. The results also showed that there was no significant change in the haemolymph protein in all the three races of the normal control when compared over the respective carrier controls (Table 2). These results indicated that CSR2×CSR4 race showed good response to potassium bromide to increase the haemolymph protein when compared to CSR2 and CSR4 races of the silkworm, *B. mori*.

Total lipids

The dietary Supplementation with 10 µg/ml potassium bromide to silkworm larvae resulted in a significant increase in fat body total lipids of 77, 47 and 16 percent, with 20 µg/ml showed in a significant increase of 77, 110 and 84 percent, with 40 µg/ml showed in a significant increase of 173, 169 and a significant decrease of 8 percent in

Table 2. Effect of potassium bromide on haemolymph trehalose and protein content in crossbreed races of *B.mori*.

Treatment (Dose in µg/ml)	Trehalose (µg/ml)			Protein (µg/ml)		
	CSR_2	CSR_4	$CSR_2 \times CSR_4$	CSR_2	CSR_4	$CSR_2 \times CSR_4$
10	559* (72)	390* (27)	331.2 (10)	2124 (00)	2140* (2)	2326* (77)
20	336 (03)	446* (46)	534* (78)	2150* (01)	2122* (01)	2412* (84)
40	360 (11)	498* (63)	426* (42)	3164* (49)	2107 (-1)	3400* (160)
Carrier control (distilled water)	324 (100)	306 (100)	300 (100)	2120 (100)	2108 (100)	1308 (100)
Normal control	294 (-9)	242 (-21)	270 (-10)	2142 (01)	1165 (-44)	1280 (-2)
SEM ±	16.16	9.85	14.53	5.74	2.46	5.65
C. D. at 5%	49.96	41.30	46.05	25.83	7.46	133.45

*-Significant increase/decrease (-) at 5% ±SEM: Standard Error C. D.: Critical difference
Values in parenthesis indicate percent change over carrier control

CSR2, CSR4, and CSR2×CSR4 crossbreed races of the silkworm, *B. mori* respectively when compared over the respective carrier controls (Tables 1 and 2). The above results indicated that the oral supplementation with potassium bromide increased fat body total lipids with 10 and 20 µg/ml in CSR2 and CSR4 races respectively where as high dose of 40 µg/ml potassium bromide decreased the total lipids of the fat body in CSR2×CSR4 crossbreed race of the silkworm, *B. mori*.

The results also showed that there was a decrease in total lipids of the fat body in all the three races of the normal control when compared over the respective carrier controls. These results indicated that CSR2 and CSR4 showed good response to all the doses of potassium bromide to increase the fat body total lipids as compared with CSR2×CSR4 crossbreed race of the silkworm, *B. mori* as it was shown good response to only lower doses of potassium bromide.

Discussion

In the present study oral supplementation with each of the three concentrations of potassium bromide to CSR2, CSR4 and CSR2×CSR4 crossbreed races of the silkworm, significantly increased the fat body glycogen in all the treated groups in all the three races of the silkworm, *B. mori*. The CSR2 race showed significant increase in haemolymph trehalose with 10 µg/ml, CSR2×CSR4 crossbreed with 20 and 40 µg/ml but in CSR4 race with all the potassium bromide treated groups. These results indicate that CSR4 race showed good response to potassium bromide as compared to CSR2 and CSR2×CSR4 races of the silkworm, *B. mori* depending on dose employed.

It has been reported that increase in glycogen content in the fat body during feeding period in Philosamia ricini may be due to the increased amylase activity, glycogenesis and metabolic shift from lipogenesis to glycogenesis occurs in the fat body in the middle of the last instar (Inagaki and Yamashita, 1986). The increase in the amylase activity of the midgut and the increased production of carbohydrates has been reported after supplementing the feed with mineral samples in the beetle, Leptinotarsa decemlineata (Izhevskiy, 1976). In the present study the increased fat body glycogen after supplementing the feed with potassium bromide may possibly be due to the stimulatory effect on the amylase activity of the midgut and glycogenesis resulting in an increased production of glycogen as suggested by earlier workers (Pant and Morris, 1969; Izhevskiy, 1976). Goudar and Kaliwal (2001) have reported that supplementation with potassium nitrate significantly increased the fat body glycogen, haemolymph trehalose, protein and lipids in the silkworm, *B. mori*. Oral supplementation with potassium permanganate to fifth instar larvae of the silkworm, *B. mori* resulted in a significant increase in the glycogen content of the fat body and haemolymph trehalose.

Dietary carbohydrate is the principal source of sugar for trehalose synthesis. Upon formation, trehalose is released in to the blood and typically occurs at high concentration. The concentration of blood trehalose is highly variable depending on physiological and nutritional status. Blood trehalose level reflects the nutritional status of the insect and affects the feeding behaviour to ensure a balanced consumption of nutrients for optimal growth (Raubenheimer and Simpson, 1999; Thomson and Redak, 2000; Thompson *et al*., 2003). The results of the present study indicate that haemolymph trehalose was significantly increased after supplementing the feed with low and higher doses of potassium bromide in CSR2, CSR4 and CSR2×CSR4 crossbreed races of the silkworm respectively. The amount of trehalose present in the haemolymph is directly related to the glycogen content of the fat body, which is influenced by a number of endogenous organic and inorganic factors and also has been reported that calcium ions enhance the production of trehalose by the fat body in the insect Periplaneta americana (Downer, 1979). Murphy and Wyatt (1965) have reported that magnesium is essential for complete activation of trehalose synthesis. Ito and Tanaka (1959) have reported that the level of total sugar is changed by hydrolytic enzymes in the gut and haemolymph and is due to various intermediary metabolic pathways in phosphorylation. Therefore, in the present study the results suggest that the supplementation with potassium bromide may have a role similar to that of magnesium and calcium in activating the trehalose syntheses activity of the fat body resulting in the increased production and release of

trehalose into the haemolymph by the fat body (Murphy and Wyatt, 1965; Downer, 1979).

In the present study, the dietary supplementation with 20 and 40 µg/ml higher doses and with all doses of potassium bromide to CSR2, CSR4 and CSR2×CSR4 crossbreed races of the silkworm resulted in a significant increase in the fat body and haemolymph protein respectively. Wigglesworth (1977) has stated that the fat body in insects is the main site for protein synthesis as well as the intermediating metabolism of amino acids. It has been reported that the dietary supplementation with minerals influences the protein content of the fat body and haemolymph of the silkworm, *B. mori* (Dasmahapatra *et al*., 1989; Hugar *et al*., 1998; Goudar and Kaliwal, 2001). Recently, it has been reported that the oral supplementation with potassium permanganate, potassium and magnesium chloride and their synergetic effects resulted in a significant increase in the fat body and haemolymph protein and at the same time the weight of the silkgland, cocoon weight and shell weight were significantly increased in the silkworm, *B. mori* (Bhattacharaya and Kaliwal, 2004, 2005 a,b,c,d). In the present study the increased protein content of the fat body and haemolymph might possibly be due to the stimulatory effect of the mineral potassium bromide at the given concentrations on the synthetic activity of the fat body and the increased haemolymph protein content might be due to the release of excess of protein by the fat body into the haemolymph.

The lipids are the important component of the body used for synthesis of chitin and are the energy reservoir which can be mobilized rapidly during starvation, oogenesis, embryogenesis, moulting and sustain continuous muscular activity (Wyatt, 1967; Gilbert and Chino, 1974). It has been reported that the oral supplementation with minerals increases the lipids in the eri silkworm, Philosomia ricini and *Bombyx mori* (Padaki, 1991; Shah and Khan, 1995).

The result of the present study showed that significant increase in the fat body total lipids with 10 and 20 µg/ml, in CSR2×CSR4 crossbreed race in all the three concentrations treated with potassium bromide. in CSR2 and CSR4 races of the silkworm, *B. mori*. It has been reported that the oral supplementation with potassium nitrate significantly increased the total lipids, phospholipids and neutral lipids of the fat body in the silkworm, *B. mori* (Goudar and Kaliwal, 2001). Recently it has been reported that oral supplementation with potassium permanganate, potassium and magnesium chloride and their synergetic effects significantly increased the total lipids of the fat body in the silkworm, *B. mori*. (Bhattacharya and Kaliwal, 2004, 2005 a, b, c, d). From the present study it is inferred that potassium bromide has stimulatory effect on the fat body synthetic activity. However, further investigation is essential to study the mechanism of action of potassium bromide on the lipogenesis in the fat body of the silkworm, *B. mori*.

In conclusion, the results of the present study showed that the mineral salt of potassium bromide significantly increased the fat body glycogen, protein, trehalose and total lipids of the silkworm, *B. mori*.

ACKNOWLEDGEMENTS

The authors express their sincere thanks to Chairman, P. G. Department of Studies in Zoology, Karnatak University, Dharwad for providing financial assistance and necessary facilities.

REFERENCES

Bhattacharya, A. and Kaliwal, B. B.(2004) Influence of the mineral potassium permanganate on the biochemical constitutents in the fat body and haemolymph of the silkworm, *Bombyx mori* L. *Int. J. Indust. Entomol.* **9** , 131-135.

Bhattacharya, A. and Kaliwal, B.B. (2005 a) The biochemical effects of potassium chloride on the silkworm, *Bombyx mori* L. *Insect Science*, **12**, 95- 100.

Bhattacharya, A. and Kaliwal, B. B. (2005 b) Biochemical content of the fat body and haemolymph of silkworm (*Bombyx mori* L.) larvae fed with mulberry leaves fortified with mineral magnesium chloride (MgCl2). *The Philippine Agricultural Scientist.* **88**, 337-340.

Bhattacharya, A. and Kaliwal, B. B. (2005 c) Synergetic effects of potassium and magnesium chloride on biochemical contents of the silkworm, *Bombyx mori* L. *Caspian J. Env. Sci.* **3**, 1- 7.

Bhattacharya, A. and Kaliwal, B. B. (2005 d) Influence of themineral potassium

permanganate on the economic parameters of the silkworm, *Bombyx mori* L. *Proc. National Conference on Sericulture for Global Competitiveness*, 338- 340.

Chapman, R. F. (1998) The insect structure and function, Cambridge University Press, Cambridge.

Dasmahapatra, A. K., Chakraborthi, m. k. and Medda, A. K. (1989) Effect of potassium iodide, cobalt chloride, calcium chloride and potassium nitrate on protein, DNA and RNA content of silkgland of silkworm (*Bombyx mori* L) *Nistari race. Sericologia.* **29**, 355- 359.

Downer, R. G. H. (1979) Trehalose production in isolated fat body of the American cockroach, *Periplaneta americana. Comp. Biochem. Physiol*. **62**, 31- 34.

Etebari, K. and Fazilati, M. (2003) Effect of feeding of mulbery's supplementary leaves with NP and K in some biological and biochemical characteristics of silkworm, *Bombyx mori*. *J. Sci Technol Agric. and Natur. Resour.* **7**, 233- 244.

Folch, J., Less, M. and Sloane Stanley, G. H. (1957) A simple method for isolation and purification of total lipids from animal tissue *J. Biol. Chem.* **226**, 497- 509.

Gilbert, L. J. and Chino, P. (1974) Transport of lipids in insects *J. Lipid Res.* **15**, 439-456.

Goudar, K. S. and Kaliwal, B. B. (2001) Effect of potassiu m nitrate on the biochemical parameters of the silworm, *Bombyx mori* L. *Int. J. Indust. Entomol.* **3** , 93- 96.

House, H. L. (1974) The physiology of Insecta 5, 2nd Edn. (Ed Rockstein M.) Academic press, New York, 63- 117.

Hugar, I. I., Nirwani, R. B. and Kaliwal, B. B. (1998) Effect of zinc chloride on the biochemical changes in the fat body and haemolymph of the bivoltine silkworm, *B. mori* L. *Sericologia.* **38**, 299- 303.

Inagaki, S. and Yamashita, O. (1986) Metabolic shift from lipogenesis to glycogenesis in the last instar larval fat body of the silkworm, *Bombyx mori*. *Insect Biochem.* **16**, 327- 331.

Ito, T. and Tanaka, M. (1959) Administration of the nutrients to the silkworm larvae. 1. Effect of the administration of glucose. *Sanshi Shikenjo Hokuku.* **15**, 353- 364.

Ito, T. (1978) *Silkworm Nutrition*; in The silkworm an important Laboratory Tool, Tazima, Y (ed). pp. 121- 157, Kodansha Ltd., Tokyo.

Izhevskiy, S. S. (1976) The physiological effects of mineral salts on *Leptinotarsa decemlineata*(Coleoptrera, Chrysomelidae). *Ecologiya.* **4**, 90 - 92.

Krishnaswami, S. (1978) New technology of silkworm rearing, Bulletin No.**2**, CSRTI Mysore, 1- 24.

Lowry, H., Rosebrough, N. I., Far, A.L. and Randall, R. J. (1951) Protein measurement with Folin phenol reagent, *J. Biol. Chem.* **193**, 265- 275.

Murphy, T. A. and Wyatt, G. R. (1965) Theenzymes of glycogen and trehalose synthesis in silk moth fat body. *J. Bio. Chem.* **240**, 1500– 1508.

Nirwani, R. B. and Kaliwal B. B. (1996) Increase of silk production and quantitative changesof carbohydrates and protein in fat body andhaemolymph after feeding potassium sulphate to bivoltine silkworm, *B. mori* L. *Sericologia.* **36**, 523-530.

Padaki, P. R. (1991) Some aspects of physiology of the Eri silkworm, Philosamia ricini. Ph.D. thesis, Karnatak University, Dharwad, India. 48- 62.

Pant, R. and Morris I. D. (1969) Changes inactive phosphorylase activity and glycogen content during larval and pupal development of P*hilosamia ricini*. *J. Biochem.* (Tokyo) **66**, 29- 31.

Raghava Rao, D. (1983) Statistical Techniques in Agricultural and Biological Research, Oxford Publishing Co, New Delhi.

Raubenheimer, D. and Simpson, S. J. (1999) Integrating nutrition: a geometrical approach. *Entomol. Exp. Appl.* **91**, 67- 82.

Roe, J. H. (1955) The determination of sugar in blood and spinal cord fluid with anthrone reagent. *J. Biol. Chem.* **242**, 424- 428.

Sciefter, S. S. Dayton, B. Novic and Myntiyer E. (1950) The estimation of glycogen with the anthrone reagent. *Arch. Biochem.* **25**, 191.

Thompson, S. N., Borchardt, D. B. and Wang, L. W. (2003) Dietarynutrient levels regulate protein and carbohydrate intale, gluconeogenic/glycolytic flux and blood trehalose level in the insect Manduca sexta L. *J. Comp. Physiol.* B **173**, 149- 163.

Thompson, S. N. and Redak, R. A. (2000) Integration of dietary protein and carbohydrate determine blood sugar level and regulate nutrient selection in the insect *Manduca sexta*. L. *Biochim Biophys*.

Acta **1523**, 91- 102.
Wigglesworth, N. B. (1977) The principles of insect physiology 7th ed., Chapman and Hall, London.
Wyatt, G. R. (1967) The biochemistry of sugars and polysc chlorides in insects. *Adv. Insect physiol.* **4**, 287- 360.

(Received: May. 4, Accepted Nov. 5, 2005)