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Effect of Potassium Nitrate on the Biochemical Parameters of the Silkworm, *Bombyx mori* L.

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The effect of supplementation with potassium nitrate on biochemical constituents was analyzed following treatment of last larval stadium. The fat body glycogen and haemolymph trehalosre significantly increased in all the treated groups. The fat body and haemolymph protein also increased significantly in all the treated groups except 500 μg/ml where increased fat body protein and decreased haemolymph protein were not significant. The total lipids, phospholipids and neutral lipids of the fat body increased significantly in all the groups except 50 and 300 μg/ml where the fat body phospholipids increased but the increase was not significant. However the total lipids, phospholipids and neutral lipids are significantly decreased in 500 μg/ml treated group when compared with that of the carrier control.

Key words : Potassium nitrate, Biochemical constituents, Silkworm

Introduction

The silkworm, *Bombyx mori* is a monophagous and holometabalous insect. The nutritional status and environmental conditions play a major role in the silkworm growth, development and metabolism. The growth represents an interaction of a wide range of physiological processes.

Silkworm larvae accumulates a large quantity of fuel reserves in various tissues and it is endowed with a unique biochemical adaptation to conserve nutritional resources available during the active larval stage.

Metals and their salts, play a key role by acting as catalyst, which are indispensable for life. It has been reported that magnesium is essential for complete activity of trehalose synthesis (Murphy and Wyatt, 1965). It has been reported that calcium, iron, magnesium, phosphorus, potassium, manganese and zinc are essential for the growth and development of the silkworm, *B. mori* (Tobias, 1948; Ito, 1967). Dasmahapatra *et al.* (1989) have reported that supplementation with cobalt, iodide, potassium, calcium chloride and potassium nitrate increases the protein, RNA and DNA content of the silkgland in the nistari race of the silkworm, *B. mori.* Feeding with zinc and cupric chlorides were shown to increase the trehalose and glycogen constituents in the eri silkworm, *Philosomia ricini* (Padaki, 1991). Recently, it has been reported that the supplementation with potassium sulphate increases the fat body glycogen and haemolymph trehalose and zinc chloride increases the fat body glycogen, haemolymph trehalose and protein in the bivoltine silkworm, *B. mori* (Nirwani and Kaliwal, 1996; Hugar *et al.*, 1998).

Hence, the present investigation was undertaken to find out the effect of potassium nitrate on the fat body glycogen, protein, total lipids, phospholipids, neutral lipids and haemolymph trehalose and protein of the silkworm, *B. mori*

Materials and Methods

The eggs of the silkworm, *B. mori* were obtained from the Rayapur, Dharwad, and Karnataka and reared in the laboratory by the improved method of rearing technique (Krishnaswami, 1978). The fifth instar larvae were divided into six experimental groups including control groups. Each group consisting of five replications of 20 worms each. The potassium nitrate was procured from M/S British Drug House (India) Ltd. Bombay and was dissolved completely in distilled water. The potassium

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nitrate was diluted to form 50, 100, 300 and 500 μg/ml dilutions. The treated, carrier control and normal control larvae were utilized for the estimation of glycogen, protein, total lipids, phospholipids and neutral lipids from the fat body and protein and trehalose from the haemolymph.

The silkworm larvae were dissected in *Bombyx* saline at pH 6.5 on the 6th day of the fifth stadium. The fat body was immediately collected and used for the glycogen (Shiefter *et al.*, 1950), protein (Lowry *et al.*, 1951), total lipids, phospholipids and neutral lipids (Folch *et al.*, 1957) estimations. The haemolymph was collected by amputating one of the thoracic legs in a prechilled centrifuse tube and was used for the estimation of trehalose (Roe, 1955) and protein (Lowry, 1951). Anthrone positive carbohydrate in the haemolymph is considered as trehalose, statistical analysis.

The experiments 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 (Raghav Rao, 1983).

Results and Discussion

The requirement of minerals in various insects has been investigated (House, 1974), but the information regarding the indispensability of metal ions for proper nutrition or growth promotion of the insects are not adequately established. Metals are essential for the activity of several enzymes. The metals can directly bind to proteins or may attach through an organic ligand such as haem. It has been reported that a number of mineral salts are essential for growth and development of the silkworm, *B. mori* (Tobias, 1948; Ito, 1967; Dasmahapatra *et al.*, 1989; Padaki, 1991; Nirwani and Kaliwal, 1996; Hugar *et al.*, 1998).

The results of the present study in general indicates that, potassium nitrate have some effect on biochemical parameters of the silkworm, *B. mori* (Table 1).

Effect of potassium nitrate on the fat body glycogen and haemolymph trehalose

The oral supplementation of each of the three concentrations of potassium nitrate significantly increased the fat

Table 1. Effect of potassium nitrate on the biochemical constituents of the silkworm, *B. mori*

Treatment	Dose μg/ml	Fat body glycogen μg/mg	Haemolymph trehalose μg/ml	Fat body protein μg/mg	Haemolymph protein μg/ml	Fat body total lipids μg/100 mg	Fat body phospholipids μg/100 mg	Fat body neutral lipids μg/100 mg
Potassium nitrate	50	14.583*	766.50*	43.332*	3705.00*	313.3*	150.0	163.3*
		(291)	(214)	(130)	(123)	(104)	(102)	(106)
Potassium nitrate	100	16.666*	695.62*	45.999*	6521.25*	323.3*	153.3	166.6*
		(333)	(194)	(138)	(217)	(107)	(104)	(108)
Potassium nitrate	300	21.916*	548.37*	40.999*	3847.50*	330.0*	156.6*	176.6*
		(438)	(153)	(123)	(128)	(110)	(106)	(115)
Potassium nitrate	500	17.166*	580.12*	36.166	2752.50	276.6*	136.6*	140.0*
		(343)	(162)	(109)	(91)	(92)	(93)	(91)
Carrier control	Distilled water	4.999	357.00	33.166	3000.00	300.0	146.6	153.3
		(100)	(100)	(100)	(100)	(100)	(100)	(100)
Normal control	-	4.666	372.75	33.164	4050.00*	293.3	150.0	143.3
		(93)	(104)	(104)	(135)	(97)	(102)	(93)
		S	S	S	S	S	S	S
S.Em±		1.967	75.309	3.251	159.90	4.172	4.268	4.172
CD at 5%		4.191	160.409	6.925	340.59	9.304	9.519	9.304

* - Significant increase/decrease at 5%
** - Angular transformed values
S - Significant
S.Em± - Standard error mean
CD - Criticle difference
Percent increase/decrease over that of the carrier control in parenthesis.

body glycogen in all the treated groups. Similar increase in the fat body glycogen has been reported after supplementing the feed with manganese, zinc and cupric chloride and copper sulphate in the eri silkworm, *Philosomia ricini* (Padaki, 1991), potassium sulphate (Nirwani and Kaliwal, 1996) and zinc chloride in the bivoltine silkworm, *B. mori* (Hugar *et al.,* 1998). Accumulation of glycogen during the feeding period in *P. ricini* was shown to be due to the increased amylase activity and glycogenesis (Pant and Morris, 1969). 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 nitrate may possible be due to the stimulatory effect on the amylase activity of the midgut and glycogenesis resulting in an increased production of carbohydrates as suggested by Pant and Morris (1969), and Izhevskiy (1976).

The results of the present study indicate that the haemolymph trehalose was significantly increased with each of the four concentrations of the potassium nitrate. It has been stated that the absolute amount of trehalose present in the fat body is directly related to the glycogen content of the tissue and trehalose production in insect fat body 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 of the insect *Periplaneta americana* (Downer, 1979). It has also been stated that magnesium is essential for complete activation of trehalose synthesis (Murphy and Wyatt, 1965). 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 according to Ito and Tanaka (1959). It is therefore, likely that the mineral salts used by Padaki (1991), Nirwani and Kaliwal (1996), Hugar *et al.* (1998), and potassium nitrate in the present study may have a role similar to that of calcium (Downer, 1979) and magnesium (Murphy and Wyatt, 1965) in activating the trehalose synthase activity of the fat body resulting in the increased production and release of trehalose into the haemolymph by the fat body. On the contrary feeding with each of the three concentrations of the potassium nitrate significantly decreased the trehalose content of the haemolymph. This may suggest that potassium nitrate may inhibit the synthesis and release of trehalose by the fat body.

Effect of potassium nitrate on the fat body protein and haemolymph protein

It has been stated that the fat body in insects is the main site for protein synthesis as well as the intermediating metabolism of amino acids (Wigglesworth, 1977). There are few reports on the effect of mineral salts on the protein content of the fat body in *B. mori.* Feeding of mulberry leaves supplemented with potassium iodide or cobalt chloride or calcium chloride increased silkgland protein in nistari race of *B. mori* (Dasmahapatra *et al.*, 1989), cobalt sulphate increase the rate of protein synthesis in early stages and it is decreased in V stadium larvae of *P. ricini* (Padaki, 1991). It has been reported that the feeding of potassium sulphate decreases the fat body protein and haemolymph protein of the silkworm, *B. mori* (Nirwani and Kaliwal, 1996) but zinc chloride significantly decreased the fat body protein and significantly increased the haemolymph protein (Hugar *et al.,* 1998).

Protein content of the fat body and haemolymph in potassium nitrate fed groups

The fat body protein and haemolymph protein in 50, 100 and 300 μg potassium nitrate fed groups are significantly increased. The increased protein content of the fat body and haemolymph might possibly be due to the stimulatory effect of the mineral salt 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 and at the same time the weight of the silkgland is also significantly increased (Goudar and Kaliwal, 2000). This also coincides with the subsequent increase in the cocoon weight and shell weight (Goudar and Kaliwal, 2000). However, increased fat body protein and decreased haemolymph protein were not significant in 500 μg/ml potassium nitrate treated groups.

The significant increase in the silkgland weight may possible suggest that the silkgland has not sequestered the protein from the haemolymph and fat bodies in excess of its requirement, hence, the accumulation of protein in the fat body and haemolymph. However, the protein content from the fat body and haemolymph might have been utilised for the synthesis of silk, which is evidenced by a significant increase in the cocoon weight, shell weight filament length, filament weight and denier in all the potassium nitrate fed groups (Goudar and Kaliwal, 2000).

Effect of potassium nitrate on total lipids, phospholipids and neutral lipids content of the fat body

Lipids are important constituents of cuticle and help in acylation of glucose-6-phosphate during chitin synthesis (Wyatt, 1967). The lipid in the fat body is an energy reserves which can be mobilized rapidly during starvation, oogenesis, embryogenesis and moulting and is used to sustain continuous muscular activity (Gilbert and

Chino, 1974). There are no reports on the effect of mineral salts on the fat body total lipids, phoshpolipids and neutral lipids of the fat body in *B. mori* except that feeding mulberry leaves supplemented with manganese, zinc and cupric chloride and copper sulphate increased total lipids, phospholipids and neutral lipids of the fat body in the eri silkworm, *Philosomia ricini* (Padaki, 1991) and nickel chloride in the bivoltine silkworm, *B. mori* (Saha and Khan, 1995).

The results of the present study indicate that the oral supplementation of each of the four concentrations of potassium nitrate significantly increased the total lipids, phospholipids and neutral lipids of the fat body except 500 μg treated group where the total lipids, phospholipids and neutral lipids are significantly decreased whereas there was no significant change in the phospholipids at 50 and 100 μg potassium nitrate fed groups.

From the present study it is inferred that potassium nitrate has stimulatory effect on the fat body synthetic activity. The significant increase in the total lipids, phospholipids and neutral lipids may possibly suggest that the ovariole has not sequestered the lipids from the fat body. Hence, the fecundity decreased significantly.

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