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Synergetic Effects of Potassium and Magnesium Chloride on Biochemical Contents of the Silkworm, *Bombyx mori* L.

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ABSTRACT
The present study deals with the oral supplementation with (50, 100 and 150 µg/ml) synergetic effect of potassium and magnesium chloride on the fat body glycogen, protein, total lipids and haemolymph trehalose, protein of fifth instar larvae of *Bombyx mori* L (Lepidoptera: Bombycidae). The observed parameters significantly increased in all the treated groups when compared with those of the corresponding parameters of the carrier control. These results may suggest that the minerals may stimulate the enzyme activity which influences the metabolic process thereby increasing the biochemical contents of the fat body and haemolymph of the silkworm, *B. mori* L.

Key words: Biochemical Contents, *Bombyx mori* L, Magnesium Chloride, Potassium Chloride, Silkworm.

INTRODUCTION

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. It has been reported that the mulberry silkworms, *Bombyx mori* require calcium, iron, magnesium, manganese, phosphorus, potassium and zinc for their growth and development (Ito, 1967). Search is being continued to explore the different exogenous agents, such as minerals, hormones or vitamins that can effectively augment the various life processes of silkworms and ultimately aggravate the production of silk and eggs. In insects, absorption of nutrients depends on 3 important factors, namely composition of the diet, functioning of nutritive enzymes and membrane permeability of intestinal epithelium (Primor and Zlotkin, 1980; Harvey, 1982). Composition of mulberry leaf and the activity of digested enzymes in *Bombyx mori* have been studied (Ito, 1978; Sarangi, 1986). Earlier studies on nutrient absorption in silkworm, *B. mori* have confirmed the presence of carrier mediated transport and also active transport mechanisms (Sacchi and Giordana, 1980; Sacchi *et al.*, 1981). Despite the overall similarities, major differences in nutritional requirements do occur. These may be the result of evolutionary changes associated with feeding on substrates with quantitatively and sometimes qualitatively, different balances of nutrient chemicals. Therefore, the present investigation was undertaken to study the synergetic effect of potassium and magnesium chloride on the biochemical contents of the fat body and haemolymph in the silkworm, *Bombyx mori* L.

MATERIALS AND METHODS

Rearing technique and treatment

Disease free layings (DFLs) of the bivoltine crossbreed silkworm, (CSR_2 x CSR_4) *Bombyx mori* L used in the present investigation were obtained from the grainage centre Rayapur, Dharwad District, Karnataka State. The hatched larvae were reared in the rearing room and fed with fresh mulberry leaves (S_{36} variety) four times a day by maintaining optimum humidity (85-95 %) and temperature (27°C) providing optimum spacing and mulberry leaves ad *libitum* raised by improved methods of rearing techniques (Krishnaswami, 1978). The fifth instar larvae were selected randomly and grouped into different batches for the experiment. Each group consisted of five replications each with 20 silkworms.

The potassium and magnesium chloride was procured from Fisher Inorganics and Aromatics Limited, Chennai– 600 029. The chemical was individually dissolved completely in lukewarm distilled water and diluted to form 50, 100 and 150 µg/ml dilutions. The fresh mulberry leaves were dipped in each concentration of the said chemical and then the leaves were dried under shade and fed to the silkworms. In each supplementation, 500 ml of solution was used to treat 100 larvae. Amongst the four feedings per day, feeding of treated leaves was given one time with the feeding of untreated leaves. Treatment was given alternate day. The carrier control was fed with distilled water dipped mulberry leaves, while the normal control was fed with untreated leaves. The treated carrier control and normal control larvae were utilized for the estimation of glycogen, total protein and total lipids from the fat body and total protein, and trehalose from the haemolymph.

Biochemical parameters

Tissue preparation, 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 some biochemical compounds estimations. The haemolymph was collected by amputing one of the thoracic legs in a pre chilled centrifuge tube and was used for the estimation of trehalose and total protein.

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 2ml 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 mins and the supernatant was discarded. The precipitate was dissolved in a known volume of distilled water. Glycogen content was estimated with known aliquots in triplicate by the anthrone method. Glucose-D was used as the reference standard and the intensity of the colour was read on the spectrophotometer at 620nm.

Trehalose estimation, The estimation of haemolymph trehalose was carried out according to the method of Roe (1955). In each test tube 1 ml haemolymph was collected and added 0.5 ml of 2% of sodium hydroxide to each test tube. After shaking, the tubes were kept in boiling water for 10min and then the tubes were cooled in an ice box. Then 5ml of anthrone reagent (0.05% anthrone in 70% sulphuric acid) was added to the tubes, and they were again kept in boiling water for 15min for the development of colour. Then the tubes were cooled to room temperature. Then the colour intensity was read on the spectrophotometer at 620nm. For the reference standard the trehalose (Sigma, USA) was used. Anthrone positive carbohydrate in the haemolynmph 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 1ml of 30% trichloroacetic acid (TCA) solution followed by centrifugation at 3000 rpm for 30 min. It was repeated twice, then the precipitate was dissolved in 1ml of 0.1 N sodium hydroxide. A known aliquot of this solution was then mixed with 5ml of alkaline copper reagent (20% sodium carbonate prepared in 0.1 N sodium hydroxide containing sodium potassium tartarate and 1% copper sulphate). After 10min 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 20mins for colour development. The readings were taken on the spectrophotometer at 650 nm. 1ml haemolymph was diluted with 0.5ml of distilled water and the total protein of haemolymph was measured as mentioned above. For the reference standard Bovine Serum Albumen (BSA) (Fatty acid free) was used.

Extraction and estimation of total 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). Then homogenate was quantitatively transferred to a 50 ml separating funnel and then added similar volume of chloroform. 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 vacuum desiccators until constant weight was obtained.

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 among the treatment groups, the least significant difference test (Lsd) was carried out at level 0.05 (Raghava Rao, 1983).

RESULTS

The effect of oral supplementation with different concentrations of potassium and magnesium chloride on the some biochemical macromolecules in fat body and haemolymph have been estimated in the crossbreed silkworm larvae of *Bombyx mori* and the results are presented in figures 1-5.

Carbohydrates

The dietary supplementation with 50 µg/ml potassium and magnesium chloride to silkworm larvae resulted in an increase of 26% fat body glycogen (Fig. 1) and 20% haemolymph trehalose (Fig. 2). The dietary supplementation with 100 µg/ml to silkworm larvae resulted in an increase of 33% fat body glycogen and 26% haemolymph trehalose. The supplementation with 150µg/ml potassium and magnesium chloride to silkworm larvae resulted in an increase of 75% fat body glycogen and 44% haemolymph trehalose. The above results indicate that the oral supplementation with potassium and magnesium chloride increased the fat body glycogen and haemolymph trehalose in all the treated groups when compared with those of the corresponding parameters of the carrier control.

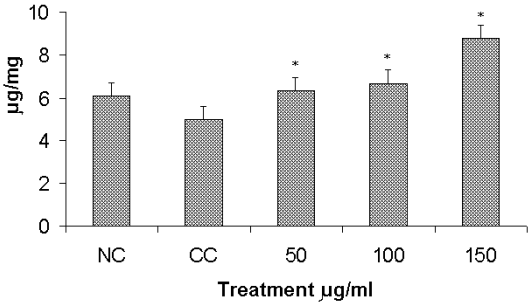

Fig. 1 The effect of KCl and $MgCl_2$ on the fat body glycogen of the silkworm.

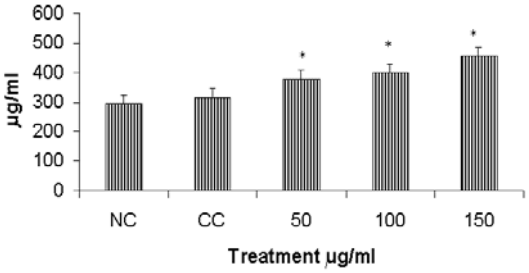

Fig. 2 The effect of KCl and MgCl2 on the haemolymph trehalose of the silkworm.

Total Protein

The dietary supplementation with 50µg/ml potassium and magnesium chloride to silkworm larvae resulted in an increase of 15% fat body protein (Fig. 3) and 78% haemolymph protein (Fig. 4). The dietary supplementation with 100 µg/ml to silkworm larvae resulted in an increase of 19% fat body protein and 84% haemolymph protein. The supplementation with 150µg/ml potassium and magnesium chloride to silkworm larvae resulted in an increase of 22% fat body protein and 160% haemolymph protein. The above results indicate that the oral supplementation with potassium and magnesium chloride increased fat body and haemolymph protein in all the treated groups when compared with those of the corresponding parameters of the carrier control.

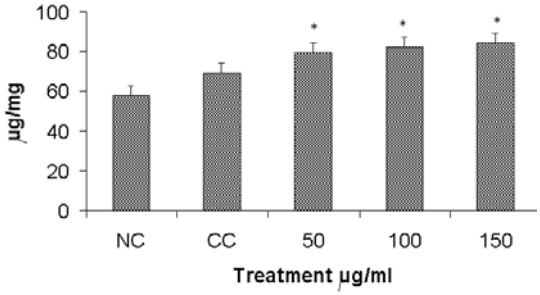

Fig. 3 The effect of KCl and $MgCl_2$ on the fat body total protein of the silkworm.

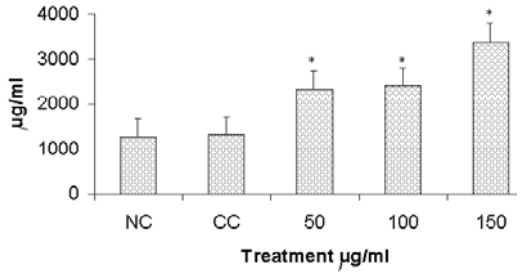

Fig. 4 The effect of KCl and MgCl2 on the haemolymph total protein of the silkworm.

Total Lipids

The dietary supplementation with 50µg/ml potassium and magnesium chloride to silkworm larvae resulted in an increase of 27% fat body total lipids (Fig. 5). The dietary supplementation with 100 µg/ml to silk-

worm larvae resulted in an increase of 13% fat body total lipids.

The supplementation with 150µg/ml potassium and magnesium chloride to silkworm larvae resulted in an increase of 5% fat body total lipids. The above results indicate that the oral supplementation with potassium and magnesium chloride increased fat body total lipids in all the treated groups when compared with those of the corresponding parameters of the carrier control.

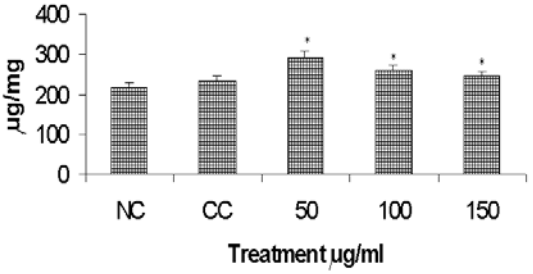

Fig. 5 The effect of KCl and $MgCl_2$ on the fat body total lipid of the silkworm.

DISCUSSION

Carbohydrates are the major components in the food of all the living organisms, which are either directly or indirectly used as the source of energy for all vital activities. The energy requirement of the larva serves as a determinant factor for the normal growth and development of the larva, which ultimately determines the quality of the silk produced. The administration of carbohydrates such as glucose, fructose, sucrose and maltose to the starved larva increased both blood trehalose and glycogen content. Simex and Kodrik (1986) have reported that the glycogen content in the fat body, body wall and silkgland and the free carbohydrates in the haemolymph changed significantly during last larval instar and metamorphosis in silkworms. The glycogen content in the fat body reached its maximum level before spinning. In the present study the oral supplementation of each of three concentrations potassium and magnesium chloride significantly increased the fat body glycogen in all treated groups. Similar increase in the fat body glycogen content has been reported after supplementing the feed with potassium and magnesium sulphates and potassium permanganate in the bivoltine silkworm, *B. mori* (Nirwani and Kaliwal, 1995; Bhattacharya and Kaliwal, 2004). 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 increase in the fat body glycogen may possibly be due to the stimulatory effect on the amylase activity of the midgut resulting in increased production of carbohydrates as suggested by Pant and Morris (1969).

Trehalose is the major and metabolically active, non reducing disaccharide in the insect blood (Wyatt and Kalf, 1957) which is synthesized in the fat body (Candy and Kilby, 1959) and utilized during spinning, flight and starvation of insects (Saito, 1960; Horie, 1961). It is well known that haemolymph, the only extra cellular fluid in insect is having divers functions. It is the reservoir for most of the biochemicals that are required for nearly every physiological activity of the insect (Pawar and Ramakrishnan, 1977). Thus the change in the composition of haemolymph reflects the morphogenic and biochemical changes taking place in insect tissues. 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). Magnesium is also essential for complete activation of trehalose synthesis (Murphy and Wyatt, 1965). Similar results have been administrated by (Padaki, 1991; Nirwani and Kaliwal, 1996; Hugar *et al.*, 1998; Goudar and Kaliwal, 2001). The increase in the haemolymph trehalose content has also been revealed after feeding with magnesium and potassium sulphate and potassium permanganate in the bivoltine NB_4D_2, CSR_2 x CSR_4 race of the silkworm, *B. mori* (Nirwani and Kaliwal, 1995; Bhattacharya and Kaliwal, 2004). Chatterjee and Datta (1992) have also reported that the production of silk cocoon and other production parameters of silkworm are very much dependent to the metabolism of carbohydrates. Satake *et al.*, (2000) showed that the quality of the food

taken by larvae would have considerable effect on the haemolymph glucose.

In the present study the significant increase in the haemolymph trehalose might possibly be due to the conversion of glycogen into trehalose and its subsequent release into the haemolymph by the fat body. Synergetic effect of potassium and magnesium chloride 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. Therefore, in the present study, it may be inferred that the increased fat body glycogen and haemolymph trehalose in all the groups treated with potassium and magnesium chloride may be utilized as additional sources of fuel or energy required during the pupal and adult transformation (Kagiyama, 1976).

Wigglesworth (1972) has stated that the fat body in insect is the main site of protein synthesis as well as the intermediating metabolisms of amino acids. Haemolymph of insects, a reservoir of a number nutrients and metabolites, undergo physiological fluctuations in its composition at different developmental stages. Nutrition of proteins is particularly of importance for the silkworm larvae because of their active utilization of nitrogen substances involving the synthesis of silk protein. In silkworm, the protein content of the fat body increased gradually during the fifth instar and early pupal period and decreased at the time of adult emergence. 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 and magnesium iodide or cobalt chloride or calcium chloride increased silk gland 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). Similar results have been observed by using other mineral salts in the silkworm, *B. mori* (Nirwani and Kaliwal, 1996; Hugar *et al*., 1998; Goudar and Kaliwal, 2001). The increased protein content of the fat body and haemolymph in the present study might possibly be due the stimulatory effects of the minerals salt of potassium and magnesium chloride on the synthetic activity of the fat body and the increased haemolymph protein content might be due to the release of excess of proteins by the fat body into the haemolymph and at the same time the weight of the silk gland is also significantly increased.

Feeding mulberry leaves supplemented with manganese, zinc and cupric chloride and copper sulphate increased total lipids of the fat body in the *eri* silkworm, Philosamia ricini (Padaki, 1991) and nickel chloride in the bivoltine silkworm, *B. mori* (Saha and Khan, 1995). In the present study, treatment with potassium and magnesium chloride has resulted in a significant increase in the fat body total lipids. The increased total lipids of the fat body might possibly be due to the stimulatory effect of the minerals mixture of potassium and magnesium chloride at a given concentration on the synthetic activity of the fat body. The possible mechanism of action of potassium and magnesium chloride on the biochemical contents of the silkworm, *B. mori* was may be due to their influence on nervous system or hormones or the stimulation or inhibition of enzyme activity, or the induction or suppression of enzyme synthesis is not known.

In conclusion, the results of the present study showed that the minerals salts of potassium and magnesium chloride significantly increased the fat body glycogen, protein, total lipids and haemolymph protein and trehalose in all the treated groups of the silkworm, *Bombyx mori* L. However, it was inferred that there was a significant increase in the total lipids but it was a gradual decrease in the total lipids with gradual increase in the dose. Further investigation is essential to understand the mechanism of action of potassium and magnesium chloride on the biochemical contents in the fat body and haemolymph of the silkworm.

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