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Effect of Testosterone Propionate and Estradiol -17β on the Biochemical Changes in the Fat Body and Haemolymph of the Bivoltine Silkworm *Bombyx mori* L.

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Effect of topical application with 10, 20 and 30 μg/ml testosterone propionate and estradiol -17β on the fourth and fifth instar bivoltine NB_{18} silkworm larvae *Bombyx mori*, on the glycogen and protein contents of the fat body and trehalose and protein contents of the haemolymph has been studied. Glycogen content of the fat body was significantly decreased in both testosterone propionate and estradiol -17β treatment groups except in the group treated with 30 μg testosterone propionate where the increase was not significant when compared with those of carrier controls. The increase/decrease in haemolymph trehalose content did not show any significant difference in all the treated groups. Protein content of the fat body significantly increased in 10 and 20 mg testosterone propionate and estradiol -17β treated groups but in 30 mg treated groups the increase was not significant when compared with those of carrier controls. There was no significant change in the haemolymph protein content in all the testosterone propionate and estradiol -17β treated groups except in group treated with 10 μg estradiol -17β where it showed a significant decrease when compared with that of carrier control.

Key words : *Bombyx mori*, Glycogen, Trehalose, Protein, Fat body, Haemolymph

Introduction

Karmer (1983), De Loof (1987) and Lafont (1991) have reported that hormone like substances, similar to those of vertebrates are found in the tissues of insects and some of them are chemically as immunologically similar to those of vertebrates. The presence of vertebrate steroid hormones pragnenolone, progesterone and dehydroepiandrosterone has been reported in the tissues of the larvae *of Tribolium confusum* (Smissman *et al.*, 1964) testosterone, progesterone and estradiol -17β like immunoreactive substances in the haemolymph of the silkworm *B. mori* and in the mulberry leaves (Venkataramireddy *et al.*, 1994) has been reported. Ogiso and Ohnishi (1986) have reported that estradiol at higher concentration has considerable effect on oviposition whereas testosterone decreased the rate of oviposition and hatchability of eggs in the silkworm. Magadum and Magadum (1992, 1993) have reported that the topical application with estradiol -17β and testosterone propionate at different concentrations in different larval instars has significant effect on some of the economic parameters.

However, there are no reports on the effect of testosterone propionate and estradiol -17β on the protein and glycogen contents of the fat body and protein and trehalose content of the haemolymph of the silkworm *B. mori*. Hence, the present investigation was undertaken to know the effect of testosterone propionate and estradiol -17β on biochemical parameters of the bivoltine (NB_{18}) silkworm, *B. mori*.

Materials and Methods

Insect and hormone treatment

The eggs of bivoltine NB_{18} silkworms were obtained from Grainage Centre, Rayapur, Dharwad, Karnataka and reared in the laboratory by improved method of rearing techniques (Krishnaswami, 1978). The larvae were maintained on fresh mulberry leaves (K2). The fourth and fifth instar

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larvae were grouped in various experimental groups, each group consisted of five replications each with 20 worms. The hormones testosterone propionate and estradiol -17β were procured from Biological Laboratories Division of B.D. Lab. Inc. Baltimore, Maryland, USA and Sigma Chemical Company, USA respectively.

The known quantity of testosterone propionate and estradiol -17β were dissolved separately in small quantity of acetone and then diluted to form 10, 20 and 30 μg/ml solution by adding acetone. The topical application was made on the dorsal side of the larvae. Each larva in its group was topically applied with one of the three concentrations of the testosterone propionate and estradiol -17β on alternate day after the third ecdysis till the maturation of the larvae. In each application 5 ml of solution was used to treat 100 larvae. A total of 6 replications were made. The larvae of the carrier controls were topically applied with distilled water, while the normal controls were not treated. The treated larvae, carrier controls and the normal controls were utilized for the estimation of glycogen and total protein from the fat body and total protein and trehalose for the haemolymph.

Analysis of glycogen, trehalose and protein

The silkworm larvae were dissected in *Bombyx* saline at pH 6.5 on 6th day of fifth instar. The fat body was immediately collected and used for glycogen (Shiefter *et al.*, 1950) and protein (Lowry *et al.*, 1951) estimation. The haemolymph was collected by amputing one of the thoracic legs in pre chilled centrifuge tube and was used for the estimation of trehalose (Roe, 1955) and protein. Anthrone positive carbhydrate in the haemolymph is considered as trehalose.

Statistical analysis

The experiments were designed complete Randomised Block Design (CRBD) method and the data collected were subjected to statistical analysis of variance (ANOVA) test to determine the significance between the parameters of treated and control groups (Raghav Rao, 1983).

Results and Discussion

Effect of testosterone propionate on the glycogen and protein contents of the fat body and the trehalose and protein contents of the haemolymph was described in Table 1. The results of the present study indicate that the treatment with 10 and 20 μg testosterone propionate significantly decreased the glycogen content and significantly increased the protein content of the fat body, whereas trehalose and protein contents of the haemolymph are not significantly changed. The treatment with 30 μg testosterone propionate has no significant effect on these parameters of the fat body and haemolymph. These results possibly suggest the stimulatory effect of 10 and

Table 1. Effect of testosterone propionate and estradiol-17β on glycogen and protein contents of the fat body and trehalose and protein contents of the haemolymph of the silkworm, *B. mori*

Treatment	Concentration (μg/ml)	Fat body glycogen (μg/ml)	Haemolymph trehalose (μg/ml)	Fat body protein (μg/ml)	Haemolymph protein (μg/ml)
Testosterone propionate	10	10.66(71)*	1986(116)	20.79(163)*	2976(105)
Testosterone propionate	20	11.79(79)*	1652(97)	23.99(188)*	2858(101)
Testosterone propionate	30	16.73(112)	1522(89)	13.86(108)	3288(116)
Estradiol-17β	10	11.73(78)*	1766(103)	18.73(147)*	2020(71)*
Estradiol-17β	20	11.80(79)*	1562(91)	22.66(178)*	3084(109)
Estradiol-17β	30	11.26(75)*	1990(117)	13.33(104)	2502(88)
Carrier control	Acetone	11.90(100)	1700(100)	12.72(100)	2828(100)
Normal control	--	12.94(86)	1730(101)	12.57(98)	2595(91)
		S	NS	S	S
S. Em +	--	1.084	160.50	1.884	176.54
CD at 5%	--	2.002	418.90	3.478	460.77

S – Significant
* – Significant increase/decrease at 5%
S. Em± – Standard error mean.
CD – Critical difference
Percent increase/decrease over that of the carrier control in parentheses.

20 μg testosterone propionate on the protein synthetic activity of the fat body and these concentrations seem to adversely affect the glycogen synthesis of the fat body. The decreased glycogen content of the fat body may also likely to be due to its release as trehalose into the haemolymph and its immediate utilization by the silkgland in 20 μg treated group and in 10 μg treated group it is accumulated in haemolymph as trehalose which might be in excess of that utilized by the silk gland. The treatment with 30 μg testosterone propionate seems to stimulate glycogen synthesis of the fat body since there is a increase in the glycogen content of the fat body in this group whereas its effect on fat body protein synthesis is not as much as those of 10 and 20 μg concentrations. The increased glycogen content of the fat body may be due to non release of the glycogen by the fat body, since the trehalose content of the haemolymph is decreased in this group which might have been utilized by the silk gland for its synthetic activity as it is increased in this group (Hugar and Kaliwal, 1996).

The increased protein content of the fat body in all the groups seems to have been due to excess of that utilized by the silkgland for its growth prior to the commencement of its spinning acitivity, since the silkgland weight is significantly increased in all the three groups and later for the production of silk in males and females in general as there is overall increase in the cocoon shell weight in these groups (Hugar and Kaliwal, 1996).

Effect of estradiol -17β on the glycogen and protein contents of the fat body and trehalose and protein contents of the haemolymph was also described in Table 1. The results of the present study indicate that the treatment with estradiol -17β of all the three concentrations significantly decreased the glycogen content of the fat body and significantly increased the protein content of the fat body in 10 and 20 μg estradiol -17β treated groups but the increase/decrease in the trehalose and protein contents of the haemolymph is not significant except for the significant decrease in protein content of the haemolymph in 30 μ estradiol -17β treated group. These results possibly suggest that 10 and 20 μg estradiol -17β has stimulatory effect on the protein synthesis of the fat body and adverse effect on the glycogen synthesis of the fat body, which is similar to that of 10 and 20 μg testosterone propionate treatment. The decreased glycogen content of the fat body in 10 μg and 20 μg estradiol -17β treated groups may also likely to be due to its immediate release as trehalose into the haemolymph which is immediately utilized by the silkgland for its synthetic activity, since the silkgland weight is significantly increased in these groups (Hugar, 1996). The significant decrease in the glycogen content of the fat body in 30 μg estradiol -17β treated group might be due to its release into the haemolymph as trehalose since the haemolymph trehalose content is increased in this group.

The increased protein content of the fat body in all the 3 groups might have been due to excess of that utilized by the silkgland for its growth prior to the commencement of its spinning activity, since the silkgland weight is significantly increased in all the groups and later for the production of silk yield in males and females in general as there is overall increase in the cocoon shell weight in these groups (Hugar, 1996).

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