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Effect of Androstenedione and Methyltestosterone on the Biochemical Constituents of the Fat Body and Haemolymph of the Silkworm, *Bombyx mori* L.

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The effect of topical application with 5, 10 and 15 μg/ml androstenedione and methyltesterone to the fifth stadium larvae of the silkworm, *B. mori* on fat body glycogen, protein, total lipids, phospholipids, neutral lipids and haemolymph trehalose and protein has been studied. The fat body glycogen increased significantly in all the treated groups except in the 15 μg/ml androstenedione treated group. However the fat body glycogen increased significantly in 10 μg/ml methyltesterone treated group. The androstenedione treated groups showed increased haemolymph trehalose but the increase was not significant. However the haemolymph trehalose increased significantly in all the methyltestosterone treated groups. The fat body protein decreased in all the androstenedione and methyltesterone treated groups except 15 μg/ml methyltesterone where the decrease in fat body protein was significant. Whereas that of haemolymph protein significantly decreased in all the groups except in the group treated with 5 μg/ml androstenedione where the decrease was not significant. The total lipids, phospholipids and neutral lipids of the fat body decreased significantly in all the groups treated with androstenedione and methyltestosterone when compared with those of carrier control.

Key words : Androstenedione, Methyltestosterone, Biochemical parameters, *Bombyx mori*

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Introduction

In insects, hormones control metamorphosis and reproduction. For a long time, the endocrine system of insects was considered to be much less complicated than that of vertebrates (Swevers *et al.*, 1991). In recent years, it has become clear that the endocrine system of insect is much more complicated than previously assumed and in addition, it shares many common features with that of vertebrates (De Clerck *et al.*, 1983, 1984, 1987, 1988; Bradbrook *et al.*, 1990).

The presence and activity of various vertebrate steroid hormones have been demonstrated in life system of many insects. Vertebrate steroid hormones and hormone like compounds have been detected in a wide variety of insect species (De loof and De Clerck, 1986; Denlinger *et al.*, 1987; Novak and Lambert, 1989; Bradbrook *et al.*, 1990). The presence of testosterone like substances has been demonstrated in the haemolymph of the silkworm, *B.mori* (Nagashima *et al.*, 1983), androstenedione in the haemolymph extract from the larvae of *Leptinotursa decemlineata* (De clerck *et al.*, 1988). These findings may indicate a wider potential use of vertebrate steroid hormones outside the mammalian system specifically in the insects. It has been reported that topical application of testosterone propionate influences economic parameters of the silkworm, *B. mori*. Therefore, the present investigation is undertaken to find out the effect of androstenedione and methyltestosterone on the fat body glycogen, haemo- lymph trehalose, fat body protein, haemolymph protein, fat body total lipids, phospholipids and neutral lipids 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 eight experimental groups including control groups. Each group consisting of five replications of 20 worms each. The hormones, androstenedione and methyltestosterone were procured from M/S Sigma Laboratories Pvt. Ltd. Bombay and were dissolved separately in small quantity of acetone. The cortisone and hydrocortisone were diluted to form 5, 10 and 15 μg/ml solutions by adding acetone. The topical application was made on dorsal side of the larvae. Each larva in its group was topically applied with one of the three concentrations of androstenedione and methyltestosterone on alternate day in the V stadium up to the spinning stage. In each application 5 ml of solution was used to treat 100 larvae. The larvae of carrier control was topially applied with acetone and served as carrier control for cortisone and hydrocortisone treated groups, while the normal controls did not receive any treatment. 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 amputing 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 randomised 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).

Table 1. Effect of androstenedione and methyltestosterone 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
Androstanedione	5	21.888*	763	22.666	4960	302.3*	124.3*	178.0*
		(191)	(104)	(94)	(85)	(95)	(93)	(97)
Androstanedione	10	20.332*	889	23.110	4300*	291.3*	118.6*	172.6*
		(177)	(122)	(96)	(74)	(91)	(89)	(94)
Androstanedione	15	10.444	910	20.444	3685*	287.3*	116.0*	171.3*
		(91)	(125)	(85)	(63)	(90)	(87)	(93)
Methyltestosterone	5	15.555	1078*	18.444	4185*	298.6*	122.0*	176.6*
		(135)	(148)	(76)	(72)	(94)	(91)	(96)
Methyltestosterone	10	18.222*	994*	15.333	4510*	282.0*	115.0*	167.0*
		(159)	(136)	(63)	(78)	(88)	(86)	(91)
Methyltestosterone	15	14.999	945*	14.222*	3980*	272.0*	111.0*	161.0*
		(131)	(129)	(59)	(68)	(85)	(83)	(87)
Carrier control	Acetone	11.444	728	23.999	5770	317.0	133.0	183.3
		(100)	(100)	(100)	(100)	(100)	(100)	(100)
Normal control	-	9.000	553	15.777	5555	311.3	131.6	179.6*
		(78)	(75)	(65)	(96)	(98)	(98)	(97)
		S	S	S	S	S	S	S
S.Em±		2.066	100.329	4.503	492.938	1.490	0.768	0.786
CD at 5%		4.443	215.708	9.681	1059.817	3.203	1.653	1.691

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

Results and Discussion

Effect of androstenedione and methyltestosterone on the fat body glycogen and haemolymph trehalose

Effect of androstenedione and methyltestosterone on the fat body glycogen and haemolymph trehalose is described in Table 1. In the present study, the treatment with androstenedione showed a significant increase in the fatbody glycogen in all the groups except in the group treated with 15 mg androstenedione. However, the haemolymph trehalose was increased in all the treated groups though the increase was not significant. Similarly treatment with methyltestosterone has resulted in a significant increase in the fat body glycogen and haemolymph trehalose in all the groups except in the group treated with 5 and 15 μg methyltestosterone, where the increase in the fat body glycogen is not significant.

The significant increase in the fat body glycogen might be due to the stimulatory effect of these steroids on the fat body glycogen synthesis and storage and the increased trehalose content in the haemolymph might be due to the excess of glycogen synthesized by the fat body released into the haemolymph as trehalose. From the present study it may be inferred that the increased fat body glycogen and haemolymph trehalose may be utilized as additional source of fuel or energy required during the pupal and adult transformation.

Effect of androstenedione and methyltestosterone on the fat body and haemolymph protein

Effect of androstenedione and methyltestosterone on the fat body and haemolymph protein is described in Table 1. The treatment with all the doses of androstanedione and methyltestosterone has resulted in decreased fat body protein but the fat body protein decreased significantly at 15 μg of methyltestosterone treated group. The haemolymph protein was also decreased significantly in all the groups except in the group treated with 5 μg androstanedione where the decrease was not significant. In fact the silkgland weight was increased significantly in the groups treated with higher doses of androstanedione and methyltestosterone.

The decrease in the fat body protein and haemolymph protein might be due to the uptake of protein for the growth of the silkgland.

Effect of androstenedione and methyltestosterone on the fat body total lipids, phospholipids and neutral lipids

Effect of androstenedione and methyltestosterone on the fat body total lipids, phospholipids and neutral lipids is described in Table 1. The fat body total lipids, phospholipids and neutral lipids decreased significantly in all the groups treated with androstanedione and methyltestosterone. The decreased total lipids, phospholipids and neutral lipids of the fat body might possibly be due to the inhibitory effect of the androstanedione and methyltestosterone at the given concentrations on the synthetic activity of the fat body and at the same time the fecundity decreased significantly in all the groups.

The probable mechanism of action of androstenedione and methyltestosterone on the biochemical constitutents of the silkworm, *B. mori* is due to their influence on nervous system, or hormones, or the stimulation or inhibition of enzymes activity, or the induction or suppression of enzyme synthesis is not known. However, further investigation is essential to know the exact mechanism of action of vertebrate steroids on the biochemical constituents in the fat body and haemolymph of the silkworm, *B. mori*.

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