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Effect of Hydrocortisone on the Economic Parameters of the Domestic Silkworm, *Bombyx mori* L.

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The effect of topical application with hydrocortisone on economic parameters was analysed following treatment of last larval stadium. The treated larvae showed a significant increase in larval weight at higher concentrations along with other enhanced larval, cocoon and adult parameters. This suggests that hydrocortisone can be used effectively for commercial silkworm rearing. The larval period was significantly increased in all the treated groups with increased female cocoon weights, its shell weights and male cocoon shell weight at 20 µg/ml treated group. Filament length, weight and denier were increased significantly in all the treated groups. Moth emergence percentage, length of the ovariole, eggs per ovariole, fecundity and and hatching percentage were unaffected when compared with that of the carrier control group. This suggests that hydrocortisone in addition to affecting larval growth, also affect silk crop.

Key words : Hydrocortisone, Economic parameters, *Bombyx mori*

Introduction

Since the initial study of Mordue (1967) the effect of mammalian corticosteroids on insect growth, development and puffing pattern has been extensively investigated (Sang, 1968; Smith *et al.*, 1968; Rosinski *et al.*, 1978; Gawienowski *et al.*, 1987). In the recent article, the physiological effects of several glucocorticoids on mung bean seeding (*Phaseolus aureus*) was demonstrated to enhance the plant growth (Genus, 1980). Therefore the treatment with glucocorticoids is not unusual in insects and their relatives are phylogenetically closer to mammals than are plants. In fact, cortisol therapy has been shown to increase the mite (*D. brevis*) population in human skin (Soto *et al.*, 1965). The precise mechanism of action of exogenous glucocoids in insects is still obscure, although numbers of earlier studies have shown that glucocorticoids affect growth, development, protein catabolism, free amino acids as well as production of larger amounts of uric acid (Mordue, 1967; Rosinski *et al.*, 1978; Gawienowski *et al.*, 1987). Rosinski *et al.* (1978) have been reported that the early age larvae shows more response than that of late age larvae. Recently it has been reported that cortisone significantly increases the larval duration, larval weight, filament length, filament weight and denier of the silkworm, *Bombyx mori* (Goudar and Kaliwal, 1999). Thus, it seems that the mechanism of action of corticosteroids on insects may be dependent on the time of its administration, age and also on developmental status of experimental animal. However, there are no reports on the effect of hydrocortisones on the economic parameters of he silkworm, *B. mori* L. Hence, perusal of these literatures and sporadic reports prompted this examination of the possible effects to explain the physiological function of hydrocortisone on the domestic silkworm, *B. mori,* studying its effects on the economic parameters.

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Materials and Methods

The eggs of multiivoltine cross breed (PM × NB_{18}) silkworms were obtained from grainage center, Rayapur, Dharwad, Karnataka and reared in the laboratory by the improved methods of silkworm rearing (Krishnaswami, 1978). The larvae were maintained on fresh mulberry leaves (K_2). The V stadium larvae were divided into five experimental groups including controls and every group consists of uniformly weighed larvae in five replications each with 20 silkworms. The hydrocortisone was procured from M/s Sigma Laboratory Pvt. Ltd., Bombay. It

was dissolved in small quantity of acetone and diluted to form 10, 20 and 30 µg/ml by adding acetone. At each application 5 ml of solutions was used to treat 100 larvae.

The larval, cocoon and adult parameters were recorded separately. The larval and silkgland weights were measured before commencement of cocooning. The larval duration was recorded from the day of hatching till the completion of cocooning. The cocooning parameters such as cocoon weights and cocoon shell weights were measured on the 5th day after the completion of cocooning. The filament length was measured with epprovette by reeling a single cocoon. The reeled silk was dried in hot-air oven and weight was taken in an electrical balance. The cocoon shell ratio and denier of the filament was calculated. The adult parameters such as moth emergence percentage, length of the ovariole, eggs per ovariole, fecundity and hatching percentage were recorded in the adult after mating. The cocooning percentage, moth emergence percentage and hatching percentage were also calculated by the formulas shown in the Tables.

Each mean value, calculated from ten silkworms is shown in Tables 1, 2 and 3. The data collected were subjected to analysis of variance tests to find out the significance between the treated and control group by following Raghava Rao (1983). The percent values of cocoon shell ratio, cocooning percentage, moth emergence percentage and hatching percentage were transformed to sine angular values for statistical analysis.

Results and Discussion

The results of the present study on the effect of hydrocortisone on larval, cocoon and adult parameters of the silkworm *B. mori* are presented in Tables 1, 2 and 3.

Larval weight

The larval body weight was significantly increased in all the treated group except for the group treated with 10 µg/ml of hydrocortisone, However, maximum increase of 30% was observed in the group treated with 20 µg/ml when compared with that of the carrier control (Table 1).

Table 1. Effect of hydrocortisone on the larval parameters of the silkworm, *B. mori*

Treatment	Dose µg/ml	Larval Weight (g)	Silkgland Weight (g)	Larval Duration (h)	Cocooning Percentage (%)
		3.079	1.294	650*	93.0
Hydrocortisone	10				74.66**
		(104)	(103)	(103)	(99)
		3.820*	1.490*	660*	94.0
Hydrocortisone	20				75.82**
		(130)	(118)	(104)	(100)
		3.267*	1.314	670*	94.0
Hydrocortisone	30				75.82**
		(111)	(104)	(106)	(100)
		2.935	1.256	630	93.8
Carrier Control	Acetone				75.58**
		(100)	(100)	(100)	(100)
		2.886	1.238	628.6	93.0
Normal Control	-				74.66**
		(98)	(98)	(99)	(99)
		S	S	S	NS
S.Em ±		0.131	0.046	3.017	1.479
C.D.at 5%		0.258	0.092	6.185	3.032

* -Significant increase/decrease at 5%
** -Angular transformed values
S.Em± -Standard error mean
CD -Critical difference
NS -Non significant
S -Significant
Percentage increase/decrease over that of the carrier controls in paranthesis.

$$\text{Cocooning percentage} = \frac{\text{Number of cocoons formed}}{\text{Total number of cocoons kept}} \times 100$$

Table 2. Effect of hydrocortisone on cocoon parameters of the silkworm, *B. mori*

Treatment	Dose µg/ml	Female			Male			Felament Length (mts)	Filament Weight (g)	Denier
		Cocoon Weight (g)	Cocoon Shell weight (g)	Cocoon Shell ratio (g)	Cocoon Weight (g)	Cocoon Shell weight (g)	Cocoon Shell ratio (%)			
Hydrocortisone	10	1.747	0.311	18.02 25.10**	1.538	0.272	17.74 24.88**	721.66*	0.282*	3.520*
		(99)	(107)	(108)	(96)	(101)	(104)	(105)	(122)	(116)
Hydrocortisone	20	2.174*	0.342*	15.74 23.34**	1.713	0.307*	18.12 25.18**	740.00*	0.309*	3.757*
		(124)	(118)	(94)	(107)	(114)	(107)	(108)	(134)	(124)
Hydrocortisone	30	1.867	0.304	16.27 23.73**	1.666	0.285	17.38 24.58**	710.33*	0.300*	3.804*
		(106)	(105)	(98)	(104)	(106)	(102)	(103)	(130)	(125)
Carrier control	Ace-tone	1.749	0.289	16.58 23.97**	1.600	0.268	16.93 24.27**	685.00	0.230	3.021
		(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)
NormalControl	-	1.770	0.285	16.29 23.73**	1.548	0.273	17.77 24.88**	660.00*	0.213*	2.907*
		(101)	(98)	(98)	(96)	(101)	(104)	(96)	(92)	(96)
		S	S	NS	NS	S	NS	S	S	S
S.Em±		0.105	0.014	0.810	0.140	0.018	0.880	6.190	0.004	0.027
C.D.at 5%		0.207	0.027	1.589	0.275	0.036	1.725	13.310	0.008	0.059

* -Significant increase/decrease at 5%
** -Angular transfer values
S.Em± -Standard error mean
CD -Critical difference
NS -Non significant
S -Significant
Percentage increase/decrease over that of the carrier controls in paranthesis

$$\text{Female/male cocooning shell ratio} = \frac{\text{Cocoon shell weight (g)}}{\text{Cocoon weight (g)}} \times 100$$

$$\text{Denier} = \frac{\text{Single cocoon shell weight (g)}}{\text{Single cocoon filament length (mt)}} \times 100$$

The significant increase in the larval weight might be due to the prolongation of larval feeding period. The significant increase in larval body weight was also observed in *Oncopeltus fascitus, D. brevis* and *B. mori* by administration of glucocorticoids (Gawienowski *et al.*, 1987; Soto *et al.*, 1965; Goudar and Kaliwal, 1999). Therefore similar stimulatory effect could also have taken place in *B. mori* by the hydrocortisone treatment.

Silkgland weight

The wet weight of the silkgland was significantly increased in 20 and 30 µg/ml of hydrocortisone. However, the increase of 18% was observed in 20 µg/ml treated group when compared with that of carrier control (Table 1). Similar results have been reported after the treatment of cortisone in silkworm, *B. mori* (Goudar and Kaliwal, 1999). The significant increase in silkgland weight might be due to the response of the hormone since the corticosteroids regulates the puffing pattern of the salivary gland chromosomes in *Drosophila melanogaster* (Smith *et al.*, 1968; Sang, 1968).

Larval duration

The larval duration was significantly increased in all the treated groups of hydrocortisone when compared with that of carrier control (Table 1). However, the increase in larval period was dose dependent. Similar results have been reported in *D. melanogaster* and *B. mori* where larval duration was increased to high cortisone concentrations (Sang, 1968; Goudar and Kaliwal, 1999). These results suggest that the concentrations applied to the larvae, may have some effect on the hormonal level, juvenile hormone and moulting hormone which control moulting and metamorphosis in the silkworm.

Cocooning percentage

The cocooning percentage did not show any significant change in all the treated groups of hydrocortisone when compared with that of the carrier control (Table 1). These results suggest that the larvae did not show any

Table 3. Effect of hydrocortisone on the adult parameters of the silkworm, *B. mori*

Treatment	Dose µg/ml	Moth emergence percentage(%)	Length of the ovariole (mm)	Eggs per ovariole (No)	Fecundity (No)	Hatching percentage (%)
		88.4	130	90.0	725.0	89.2
Hydrocortisone	10	70.09**				70.81**
		(100)	(100)	(98)	(100)	(98)
		88.2	135	90.8	730.4	90.8
Hydrocortisone	20	69.91**				72.34**
		(99)	(103)	(99)	(100)	(100)
		89.0	140	92.3	732.2	90.6
Hydrocortisone	30	70.63**				72.15**
		(100)	(107)	(101)	(101)	(100)
		88.4	130	91.2	724.0	90.2
Carrier Control	Acetone	70.09**				71.76**
		(100)	(100)	(100)	(100)	(100)
		88.0	130	90.2	721.4	89.4
Normal Control	-	69.73**				71.00**
		(99)	(100)	(98)	(99)	(99)
		NS	NS	NS	NS	NS
S.Em±		1.449	2.087	1.754	6.625	0.507
C.D.at 5%		2.971	4.092	3.439	13.582	1.040

* -Significant increase/decrease at 5%
** -Angular transformed values
S.Em± -Standard error mean
CD -Critical difference
NS -Non significant
S -Significant
Percentage increase/decrease over that of the carrier controls in paranthesis.

$$\text{Moth emergence percentage} = \frac{\text{Number of moths emerged}}{\text{Number of cocoons kept}} \times 100$$

$$\text{Hatching percentage} = \frac{\text{Total number of eggs hatched}}{\text{Total number of eggs laid}} \times 100$$

mortality at their larval stage and used concentrations are safe and have not adversely affected the cocooning percentage.

Cocoon weight and its shell weight

The cocoon weight in female increased significantly following the treatment of 20 µg/ml of hydrocortisone up to 24%. The shell weight of both male and females increased significantly with percent increase of 18 and 14 respectively in 20 µg/ml of hydrocortisone treatment when compared with that of carrier control (Table 2). The increase in the cocoon weight and its shell weight might be due to the stimulatory effect of hydrocortisone on the silkgland protein synthesis since the silkgland weight was significantly increased in 20 µg/ml of hydrocortisone treated group (Table 1). However, there was no significant change in the cocoon shell ratio in both sexes of silkworm when compared with that of carrier control.

Silk filament length, weight and denier

The silk filament length, silk filament weight and denier of a single cocoon was significantly increased in all the treated group when compared with that of the carrier control (Table 2). Similar results have been reported with cortisone treatment in the silkworm, *B. mori* (Goudar and Kaliwal, 1999).

Moth emergence percentage

There was a significant change in the moth emergence percentage in all the treated groups of hydrocortisone when compared with that of carrier control (Table 3). This indicates that these concentrations do not have toxic effect on the cocoon crop.

Ovariole length, eggs per ovariole and fecundity

There was no significant increase in ovariole length, eggs per ovariole and fecundity in all the treated groups when

compared with that of the carrier control (Table 3). Similar results were obtained after the treatment with cortisone in *D. melanogaster* (Smith *et al.*,1968).

Hatching percentage

There was no significant change in hatching percentage in all the treated groups when compared with that of the carrier control (Table 3). This indicates that these concentrations of hydrocortisone has no toxic effect on the embryo. Similar results were obtained after the treatment with cortisone in *D. melanogaster*, where egg viability was unaffected (Smith *et al.*, 1968).

Conclusion or consideration of the action of corticosteroids

The effect of corticosteroids (either added to the diet, injected or topically applied) has been investigated in insects, but the results were different depending on the study. Corticosteroid inhibited growth of *S. gregaria* (Mordue, 1967) and *Tenebrio molitor* (Mordue, 1967; Rosinski *et al.*, 1978). However, these experiments were repeated by Jones and Reynolds, (1980), and observed no effect in *S. gregaria* or *Mandura sexta.* Finally, it was recently shown that cortisol significantly increased the growth rate in *O. fasciatus* (Gawienowski *et al.*, 1987). Of course, it is difficult to draw clear conclusion from these data, but it seems of great interest to mention here in each case the results agree the four working hypotheses by the different authors viz. (1) Corticosteroids inhibit insect growth, probably as a result of increased protein catabolism and reduced protease production by midgut (Mordue, 1967). (2) Corticosteroids prolong the moulting cycle of the larvae, probably as a result of hormone action on the neurosecretory systems of insects (Rosinski *et al.*, 1978). (3) Corticosteroids probably have no effect in insects, since insects probably lack receptors for steroids and they do not produce themselves (Jones and Reynolds, 1980). (4) Corticosteroids stimulate plant growth, why not insect growth (Gawienowski *et al.*, 1987). In our study it is interesting to note that the corticosteroid enhance the silk yield like larval weight, silk gland weight, filament length, filament weight and denier. However, larval duration was significantly increased. Additional studies using other races of silkworm and variety of exercise paradigms will be necessary to determine the physiological significance and generalization of the present results.

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