Int. J. Indust. Entomol. Vol. 2, No. 1, 2001, pp. 69~74

International Journal of
Industrial Entomology

Effect of Androstenedione on the Economic Parameters of the Silkworm, *Bombyx mori*

K. S. Goudar and B. B. Kaliwal*

Post-Graduate Department of Studies in Zoology, Karnatak University, Dharwad 580 003, India.

(Received 27 December 2000; Accepted 10 February 2001)

The effect of topical application with androstenedione on economic parameters was analyzed following treatment of last larval stadium. The treated larvae showed significantly increased larval weight along with other enhanced larval, cocoon and adult parameters. The larval period was significantly shortened in all the treated groups with increased cocooning percentage, cocoon weight and its shell weight in female and filament length, weight and denier in all the treated groups. Hatching percentage increased significantly in dose dependent manner in all the treated groups when compared with the corresponding parameters of the carrier control group. This suggests that androstenedione can be used effectively for commercial silkworm rearing.

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

Introduction

The presence and activity of various mammalian 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 species (De Loof and De Clerck, 1986; Denlinger, 1987; Novak and Lambert, 1989; Bradbrook *et al.*, 1990). There are only few reports to explain whether vertebrate steroid hormones have any effect on insects, and how the insect steroid hormones act on vertebrates. Although a recent review by Slama and Lafont (1995), throws some light on the ecdysteroid effects on vertebrates.The study on the effect of vertebrate steroids in insects is still in its infancy. It has been reported that androstenedine was identified in the haemolymph extract from larvae of *Leptinotarsa decemlineata* (De Clerck, *et al.*, 1988). Treatment of different vertebrate steroids influences the economic parameters in different races of the silkworm, *Bombyx mori* (Ogiso and Onishi, 1986; Magadum and Magadum, 1993; Hugar *et al.*, 1997; Goudar and Kaliwal, 2000). The metabolic significance of vertebrate type insects steroids in the silkworm, *B. mori* L. has been reviewed (Venkataramireddy *et al.*, 1994). This suggests that androstenedione can be used effectively for commercial silkworm rearing by the treatment at late stage of silkworm rearing. These findings and studies may indicate a wider potential use of vertebrate steroid hormones outside the mammalian systems specifically in the insects. However, there is no report on the effect of steroid hormone, androstenedione on the economic parameters of the silkworms. Perusal of this literature and sporadic reports prompted this examination of the possible effects to explain the physiological function of androstenedione on the multivoltine silkworm, *B. mori* by studying its effect on the economic parameters.

Materials and Methods

The disease free layings of multivoltine cross breed (PM× NB_{18}) silkworm were obtained from grainage centre Rayapur, Dharwad, Karnataka and reared in the laboratory by improved methods of silkworm rearing (Krishnaswami, 1978). The larvae were maintained on fresh mulberry leaves (K_2). The fifth stadium larvae were divided into five experimental groups including controls and every group consists of uniformly weighed larvae in five replications of 20 worms. The androstenedione procured from M/s. Sigma Laboratories Pvt. Ltd., Bombay. It was dissolved in small quantity of acetone and diluted to form 5, 10 and 30 μg/ml by adding acetone. Each larva was topically applied with one of the three

*To whom correspondence should be addressed.
Post-Graduate Department of Studies in Zoology, Karnatak University, Dharwad 580 003, India. Tel: +92-0836-747121; Fax: +92-0836-747887; E-mail: karuni@bgl.vsnl.net.in

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

Treatment	Dose (μg/ml)	Larval weight (g)	Silkgland weight (g)	Larval duration (hrs)	Cocooning percentage (%)
Androstenedion	5	2.915	1.248	686.2*	98.16* 82.08**
		(104)	(105)	(98)	(101)
Androstenedion	10	3.067*	1.301*	679.8*	98.64* 83.20**
		(110)	(110)	(97)	(101)
Androstenedion	15	3.067*	1.341*	672.5*	98.83* 83.71**
		(110)	(113)	(96)	(102)
Carrier Control	Acetone	2.779	1.180	695.6	96.77 79.53**
		(100)	(100)	(100)	(100)
Normal Control	-	2.693	1.190	689.8*	95.45* 77.61**
		(96)	(100)	(99)	(98)
		(S)	(S)	(S)	(S)
SEM±		0.128	0.052	1.072	0.051
C.D. at 5%		0.252	0.102	2.199	0.105

* : Significant,
** : Angular transformed values
SEM± : Standard error mean
C.D. : Critical difference
NS : Non-Significant
S : Significant
Percent increase/decrease over that of the carrier controls in parenthesis.

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

doses of androstenedione at alternate day. In each application 5 ml of solution was used to treat 100 larvae.

The larval, cocoon and adult parameters were recorded separately. The larval and silkgland weights were recorded before commencement of spinning activity. The larval duration was recorded from the day of hatching till the completion of spinning activity. The cocoon parameters such as female and male cocoon weight and their shell weights were recorded on the 5^{th} day after the completion of the spinning activity. The filament length was recorded with epprovette by reeling a single cocoon. The reeled silk was dried in an 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, moth emergence percentage and hatching percentage were also calculated by the formulas shown in the tables.

Each mean value, a record of ten worms is shown in Table 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 (Raghawa Rao, 1983). The percent value of cocoon shell ratio, cocooning percentage, moth emergence percentage and hatching percentage was transformed to sine angular value for statistical analysis.

Results and Discussion

The data on the effect of androstenedione on larval, cocoon and adult parameters of the silkworm, *B. mori* are summarized in Table 1, 2 and 3.

Larval weight

The larval body weight was significantly increased except in

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

Treatment	Dose (μg/ml)	Female				Male		Filament shell ratio (mts)	Filament length (g)	Denier weight
		Cocoon (g)	Cocoon weight (g)	Cocoon shell weight (%)	Cocoon shell ratio (g)	Cocoon weight (g)	Cocoon shell weight (%)			
Androstenedione	5	1.571	0.241*	15.52 23.11**	1.569	0.246	15.88 23.42**	658.33*	0.253*	3.458*
		(104)	(106)	(101)	(107)	(105)	(99)	(105)	(111)	(105)
Androstenedione	10	1.673*	0.256*	15.41 23.42**	1.570	0.249	16.27 23.73**	700.00*	0.296*	3.813
		(111)	(112)	(101)	(107)	(106)	(101)	(112)	(130)	(116)
Androstenedione	15	1.710*	0.269*	15.84 23.19**	1.621*	0.256*	16.06 23.58**	726.66*	0.318*	3.942*
		(114)	(118)	(103)	(110)	(109)	(100)	(116)	(140)	(120)
Carrier control	acetone	1.497	0.227	15.24 22.95**	1.463	0.233	16.03 23.58**	625.00	0.226	3.263
		(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)
Normal Control	-	1.561	0.213	13.64 21.64**	1.505	0.224*	15.14* 22.87**	623.33	0.225	3.245
		(104)	(93)	(89)	(102)	(96)	(94)	(99)	(99)	(99)
		(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)
SEm±		0.089	0.014	0.909	0.078	0.011	1.089	4.114	0.003	0.031
C.D. at 5%		0.176	0.027	1.782	0.152	0.022	2.134	8.845	0.008	0.067

* : Significant
** : Angular transormed values
SEm± : Standard error mean
C.D. : Critical difference
S : Significant
Percent increase/decrease over that of the carrier controls in parenthesis.

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

$$\text{Denier} = \frac{\text{Single cocoon silk filament weight(g)}}{\text{Single cocoon silk filament length (mts)}} \times 9000$$

the group treated with 5 µg/ml when compared with corresponding parameters of the carrier control (Table 1). Similar results are obtained by treatment with testosterone propionate (Magadum and Magadum, 1993; Hugar *et al.,* 1997) and methyltesterone (Goudar and Kaliwal, 2000) in the silkworm, *B. mori.* There was a significant increase in larval weight at high concentrations, which might be due to the stimulatory effect of androstenedione on the body weight, since testosterone propionate (Hugar *et al.,* 1997) and methyltesterone (Goudar and Kaliwal, 2000) are suggested to stimulate the larval body weight in the bivoltine silkworm, *B. mori.*

Silkgland weight

The wet weight of the Silkgland was significantly increased except in the group treated with 5 µg/ml of androstenedione. However, maximum increase of 13% was observed in 15 µg/ml treated group when compared with that of carrier control (Table 1). The significant increase in wet weight of silkgland is dose dependent. The significant increase in silkgland weight might be due to the response of the androstenedione at high concentrations. Similar results were obtained in the silkworm, *B. mori.*treated with testosterone proprionate (Hugar *et al.,* 1997) and methyltesterone (Goudar and Kaliwal, 2000).

Larval duration

The larval duration was significantly shortened in all the treated groups when compared with that of the carrier

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

Treatment	Dose (μg/ml)	Moth emergence (%)	Length the of ovariole (mm)	Eggs per ovariole (No.)	Fecandity percentage (No.)	Hatching percentage (%)
Androstene	5	96.72* 79.53**	129.5*	67.62*	540.0*	99.12 84.62**
		(103)	(97)	(67)	(93)	(100)
Androstene	10	97.29* 80.37**	127.5*	65.75*	526.0*	99.23 84.97**
		(104)	(95)	(91)	(91)	(100)
Androstene	15	97.67* 81.09**	125.2*	63.12*	504.0*	99.23 84.97**
		(104)	(93)	(87)	(87) .	(100)
Carrier control	Acetone	93.54 75.23**	133.3	72.25	578.0	98.27 82.29**
		(100)	(100)	(100)	(100)	(100)
Normal control	-	93.18 74.77**	137.8*	80.50*	675.0*	97.80* 81.47**
		(99)	(103)	(111)	(116)	(99)
		(S)	(S)	(S)	(S)	(S)
S.EM±		0.041	0.728	0.580	18.936	0.047
C.D at 5%		0.085	1.426	1.137	37.115	0.101

* : Significant
** : Angular transformed values
SEM± : Standard error mean
C.D. : Critical difference
NS : Non Significant
S : Significant
Percent increase/decrease over that of the carrier controls in parenthesis.

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

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

control (Table 1). It could be suggested that the concentration applied to the larvae, may have some effect on the hormonal lavel, juvenile hormone and moulting hormone which control moulting and metamorphosis in insects. Recently similar reslts were pbtained in the silkworm, *B.mori* treated with methyltestosterone (Goudar and Kaliwal, 2000). However, it has been reported that the testosterone propionate did not show any significant change in the larval duration in the silkworm *B. mori.* (Magadum and Magadum, 1993; Hugar *et al.,* 1997). Hence, variety of exercise paradigms will be necessary to determine the physiological significance of the above results.

Cocooning percentage

The cocooning percentage was significantly increased in all the treated groups of androstenedione when compared with that of carrier control (Table 1). This shows the larvae did not show any mortality at their larval stage and the used concentrations are allowance limits and have not adversely affected the cocooning percentage. Similar results are obtained by teratment with testosterone propionate (Hugar *et al.*, 1997) and methyletestosterone (Goudar and Kaliwal, 2000) in the silkworm, *B. mori.*

Cocoon weights and its shell weights

The cocoon weight in female increased significantly, following the treatment of 5 μg/ml and 10 μg/ml of androstenedione with percent increase of 11 and 14 respectively. The shell weight of female increased significantly in all the treated group of androstenedione with maximum percent increase of 12 and 18 at 5 μg/ml and 10 μg/ml respectively when compared with that of carrier control (Table 2). The significant increase in the female cocoon weights and its shell weights are preceded by increase in silk gland weight. There was no significant change in male cocoon weights and its shell weights in all the treated group when compared with that of carrier control (Table 2). The response of the both sexes to the androstenedione is not known in case of cocoon and its shell weights. Hence, these results are need to be conformed by using variety of exercised paradigms. The increase/decrease in female and male cocoon shell ratio is given in Table 2.

Filment length, weight and denier

The filament length, weight and denier was significantly increased in all the treated groups of androstenedione when compared with that of carrier control (Table 2). A maximum increase was observed in 15 μg/ml treated group.

Moth emergency percentage

The moth emergence percentage increase significantly in all the concentrations after the treatment of androstenedione. However, maximum increase of 4% was observed in 10 μg/ml and 15 μg/ml when compared with that of the carrier control (Table 3). The increased in moth emergence may be attributed to non increased mortality in pupal/moth stage. Similar results were reported after the treatment with testosterone propionate in bivoltine silkworm, *B. mori* (Hugar *et al.,* 1997) and methyl testosterome in multivoltine silkworm *B. mori* (Goudar and Kaliwal, 2000).

Ovariole length, eggs per ovariole and fecundity

There was significant decrease in ovariole length, eggs per ovariole and fecundity when compared with that of the carrier control (Table 3). These parameters are in dose dependent. Similar results have been obtained by teratment with testosterone propionate (Hugar *et al.*, 1997) and methyletestosterone (Goudar and Kaliwal, 2000) in the silkworm, *B. mori.* However, testosterone propionate increases the fecundity in both polyvoltine pure Mysore breed and bivoltine of the silkworm, *B. mori* (Magadum and Magadum, 1993; Hugar *et al.,* 1997). Hence, it needs further investigation.

Hatching percentage

There was no significant change in the percentage of egg hatching percentage in all the treated groups when compared with that of the carrier control (Table 3). This indicates that the used concentrations had no adverse effect on the embryo.

There was significant increase/decrease in larval duration,cocooning percentage, male cocoon weight, male cocoon shell weight, length of the ovariole, eggs per overiole, fecundity and hatching percentage of the normal controls when compared with the corresponding parameters of the acetone treated controls. This significant difference might be due to the effects of acetone since the acetone has consideable effect on the larwal body weight and food utilization in silkworm, *B. mori* (Padaki, 1991; Radhakrishna and Delvi, 1992).

Hormones are molecules that carry messages and their structures have been quite conserved during evolution, this means that identical or at least very similar molecules (both for massages and their receptors) can be found in vertebrates and invertebrates. But this does not mean that the massages that they carry are equally conserved, either between vertebrates and invertebrates or even within invertebrates (Lafont, 1991). The effects of steroids (either added to the diet, injected or topically applied) have been investigated in silkworm, but this lead to conflicting reports. Testosterone decrease the rate of oviposition, egg hatchability in *B. mori* (Ogiso and Onishi, 1986). Magadum and Magadum (1993) have reported that testosterone propionate improve the fecundity and decrease the cocooning percentage and moth emergence in pure Mysore breed of *B. mori.* Recently it has been reported that testosterone propionate increase larval weight, silkgland weight, denier, moth emergence percentage fecundity and hatching percentage in bivoltine silkworm, *B. mori* (Hugar *et al.,* 1997). But a paradoxial thing is that the results due to the effect of these steroids on physiological process and economic parameters do not reveal similar in silkworm, *B. mori.* Since the physiological processes and economic parameters are dependent on the dose, way of administration duration of treatment and race of the silkworm.

In conclusion, the results of the present study shows that the androstenedione enhance the silk yield like larval weight, silkgland weight, cocooning percentage, female cocoon weights and its shell weights. However, androstenedione has toxic effect on the reproductive performance like length of the ovariole, eggs per ovariole and fecundity. Additional studies using other races of silkworm and variety of exercise paradigms will be necessary to determine the physiological significance and generalizability of the present results.

Acknowledgments

The authors express their sincere thanks to Karnatak University, Dharwad for providing financial assistance and Prof. H. S. Patil, Chairman and late Prof. M. A. Hooli, Postgraduate Department of Studies in Zoology, Karnatak University, Dharwad for providing necessary facilities.

References

Bradbrook, D. A., C. Y. Clement, B. Cook and L. Dinan (1990) The occurrence of vertebrate-type steroids in insects and a comparison with ecdysteroid levels. *Comp. Biochem. Physiol.* **95B**, 365-374.

De Clerck, D., H. Diederik, G. Paesen and A. De Loof (1988) Identification and quantification of C21 and C19 steroids in the haemolymphof *Leptinotarsa decemlineata* a phytophagous insect. *Insect Biochem.* **18**, 93-99.

De Loof, A. and D. De Clerck (1986) Vertebrate-type steroids in arthropods : identification, concentrations and possible functions. in *Advances in Invertebrate Reproduction.* Porchet, M., J.C. Andries and A. Dhainaut (eds.), pp. 123-177, Elsevier, Science Publishers.

Denlinger, D. L., R. W. Brueggemeier, R. Mechoulam, N. Katlic, L. B. Yacum, G. D. Yacum (1987) Estrogens and androgens in insects; in *Molecular Entomology.* Law J. H. and A. R. Liss (eds.) pp.189-199.

Goudar, K. S. and B. B. Kaliwal (2000) Effect of methyltestosterone on the economic parameters of the silkworm, *B.mori* L. *Int. J. Indust. Entomol.* **1**, 35-40.

Hugar, I. I., R. B. Nirwani and B. B. Kaliwal (1997) Effect of testosterone propionate on the larval, cocoon and adult parameters of the bivoltine silkworm, *B. mori* L. *Sericologia* **37**, 695-702.

Krishnaswami, S (1978) New technology of silkworm rearing. *Central Sericulture Research and Training Institute, Mysore* **2**, 1-24.

Lafont, R (1991) Reverse endocrinology or "Hormones" seeking functions. *Insect Biochem.* **21**, 697-721.

Magadum, V. B. and S. B. Magadum (1993) Effect of testosterone propionate on the economic traits of the silkworm, *B. mori* L. *Korean J. Seric. Sci.* **35**, 69-72.

Novak F. J. S. and J. G. D. Lambert (1989) Pregnenolone, testosterone and estradiol in the migratory locust, *Locusta migratoria* : a gas chromatographical-mass spectrometrical study. *Gen. Comp. Endocr.* **76**, 73-78.

Ogiso, M. and E. Ohnishi (1986) Does estradiol play a role in ovarian maturation or embryonic development of the silkworm! *Gen. Comp. Endocrinol.* **61**, 82-86.

Raghava Rao, D. (1983) Statistical techniques in agricultural and biological research. Oxford and IBH publishing Co., New Delhi.

Venkatarami Reddy, K., S. B. Magadum, V. Meera and R. K. Datta (1994) Metabolic significance of vertebrate type steroids in silkworm *B. mori* L. *Indian J. Seric.* **33**, 6-11.