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[Research]

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Pattern of activity levels of amino trnasferases in the gonads of silkworm, *Bombyx mori* L.on exposure to prostaglandin F2α

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ABSTRACT
The impact of PGF2α on total proteins, free amino acids and the amino transferases in the gonads of silkworm, Bombyx mori L. were investigated in the present experiment. The effect of PGF2α resulted in increase in the total protein and free amino acid contents in the gonads of silkworm larvae indicating improved structural and dynamic levels of organization. The enhanced activity levels of amino transferases and glutamate dehydrogenases (GDH) in the gonads suggest the mobilization of amino acids for glycogen synthesis or protein synthesis or both.

Keywords: *Bombyx moi L.*, PGF2α, gonads, Proteins, Free amino acids.

INTRODUCTION

Prostaglandins are powerful hormone like molecules produced in most mammalian tissues and are arachidonic acid derivatives. Prostaglandins are of two types: 1. E-series and 2. F-series. Prostaglandins with particular reference to PGF2α had been known to participate actively in reprod-uction, digestion and other physiological activities of vertebra-tes (Pharris and Wyngarden, 1969 and Mc Kee and Mc Kee 2000). The active principles such as prostaglandins have been of late, found to induce significant growth and reproductive potential of silkworm (Bharathi and Govindappa, 1987). These are not only the precursors for protein synthesis but also for keto acids (Swami *et al*. 1983 and Harper, 1985).

Effect of PGF2αon the organic constituents of haemolymph of silkworm larvae, Bombyx mori L has been studied (Bharathi, 1993). The administration of PGF2α on the silk quality and biosynt-hesis of silk proteins of silkworm, *Bombyx mori* L. has been investigated *(* Bharathi *et al*. 2002*)*.

Comparative studies of PGE1 and PGF2α on some enzyme activities and metabolism of silkworm was studied (Shan-Zhang *et al*. 2005). The effects of different fortification agents of nutrition on growth and economics of sericulture was studied (Narasimha Murthy *et al*.1986).Effect of thyroxine on growth and activities of dehydrogenases of silkworm, *Bombyx mori* L. during development has been reported (Hemavathi, 2001 and Hemavathi, *et al*. 2004). The organic composition of silk gland, testes and ovaries of silkworm, *Bombyx mori* L.on exposure to thyroxine was reported (Pushpa Rani. and Bharathi, 1999). Thyroxine induced changes in the ovarian protein and ecdysteroid levels in silkworm (Thyagaraja *et al*. 1993). The administration of vertebrate hormones resulted in increase in the tissue proteins in silk gland and gonads (Venkatarami Reddy *et al*. 1992). The vertebrate hormones have been known to improve various economic traits in silkworm

(Magadum and Hooli, 1988 and 1989).

The transaminase activity in *Bombyx mori* L. (Siva Prasad and Murali Mohan, 1990 and Venkatarami Reddy *et al*. 1992), in Antherea

mylitta (Pant and Jaiswal., 1981 and Sinha *et al*. 1996) and in Philosamia ricini (Pant and Morris, 1972 and Baishiang Tsou, 1979) has been reported. The balance between total keto acid and total free amino acids is also regulated by amino transferases (Pant and Kumar, 1979). The AlAT reveals higher activity than AAT all through the embryonic development of silkworm (Pant and Kumar, 1979).

Prostaglandins with particular reference to PGF2α had been known to participate actively in reproduction, digestion and other physiological activities of vertebrates. Hence an attempt has been made to study the impact of PGF2α on the protein metabolism of gonads of silkworm, *Bombyx mori* L.

MATERIAL AND METHODS

Disease free layings of multivoltine silkworm of the race pure Mysore was used in the present study. The silkworm larvae were reared under standard conditions of temperature 24-28 degree centigrade, relative humidity, 70-85% and 12hr light: 12hr dark photoperiod.

Prostaglandin F2α was procured from UpJohn company, Calamazoa, Michigan, USA. Topical application of PGF2α (0.0025µg/ml saline/50 larvae) was given to the first and second instar larvae. The topical application is more effective, as PGF2α easily absrbed through the pores of the integument of silkworm larva. Hence the topical method has been chosen for the present study. The control larvae were sprayed with 0.9% of saline because of its physiological significance. 0.9% saline is isomolar with the body fluids. So the saline was used for control larvae.

In the present investigation, the fifth instar larvae were sorted and maintained as groups, with 500 worms in each group and a sum of five replications were maintained. For each biochemical estimation, 50 larvae each of control and experimental were used.

Isolation of silkworm tissues:

The testes and ovaries of control and experimental 5th instar larva before spinning were dissected in ice cold insect ringer and weighed and used for biochemical estimations.

Biochemical analysis:

The total proteins (Lowry *et al*. 1951), free amino acids (Moore and Stein, 1954), AlAT and AAT (Reitman and Frankel, 1957 and Bergmeyer, 1965), and IlAT, LAT and VAT (Taylor and Jenkins, 1966) and GDH activity (Lee and Lardy, 1965) were estimated in the testes and ovaries of control and experimental larva.

Statistical analysis*:*

Standard deviation and student's 't' test was conducted.

RESULTS AND DISCUSSION

The data presented in tables1 - 6 reveal the changes in protein and amino acid metabolism in gonads of silkworm, Bombyx mori L. treated with PGF2α.

Total proteins: The per cent increase in the total protein content in the testes and ovaries was 26.39 and 29.62 respectively over control.

Free amino acids: The observed per cent increase in the free amino acid content was 31.77 and 29.97in the testes and ovaries of experimental larvae.

AlAT: AlAT activity was significantly increased ($P<0.001$) and the per cent increase was 25.0 and 26.32 in the testes and ovaries respectively over control.

AAT: AAT activity was significantly increased ($P<0.001$) and the per cent increase was 18.18 and 33.33 respectively in the testes and ovaries of experimental larvae.

LAT: The per cent increase in LAT activity was 23.08 and 23.53in the testes and ovaries over control.

IlAT : The per cent increase noticed in the IlAT activity was 27.27 and 31.03 in the testes and ovaries over control.

VAT: The per cent increase observed in the VAT activity was 26.92 and 25.71 in the testes and ovaries of experimental larvae.

GDH: The per cent increase in the GDH activity was 20.89 and 19.51 in the testes and ovaries over control.

The elevated protein content of experimental larvae indicates the possibility of extensive protein buildup / protein synthesis in the testes and ovaries of silkworm (Thyagaraja *et al*. 1985). The total protein content of the gonads was significantly enhanced suggesting an increase in structural and dynamic levels of

organization in the body of larvae exposed to PGF2α.

The increase in the total protein content of testes may be due to increase synthesis of macro molecules and / or decreased degradation (Sailaja, 1999). The enhanced protein content in ovaries indicates the increased synthesis of egg specific proteins in oocytes, follicular cells and nurse cells (Sailaja, 1999).

The increase in free amino acid content will always be associated with increased amino acid catabolism (Hemavathi, 2001).The free amino acid content was markedly elevated indicating building up of positive nitrogen balance that may be the characteristic feature of growth phase (Bharathi and Govindappa, 1987).The free amino acids are considered to be effective osmotic factors (Bhaskar, 1982) and thereby increased proteolytic activity, oriented towards increasing the free amino acid content of gonads.

The activity levels of AlAT, AAT, IlAT, LAT, VAT and GDH were significantly increased in the gonads of treated silkworm larvae. The activity levels of AlAT and AAT were significantly enhanced in the experimental larvae. This observation was in consonance with the reported enhanced amino transferases (Pant *et al*.1982). Since these enzymes mark the operation of gluconeogenesis, the formation of carbohydrates from amino acids can be envisaged (Bharathi, 1984). Elevation in the AlAT and AAT strongly suggests the activation of gluconeogene-sis. The rise in the AlAT and AAT might be due to increased concentration of alanine and aspartate and active involvement in protein synthesis. The AlAT and AAT activity activity levels reflect on the general index of mobilization of free amino acids into gluconeogenesis and oxidation of amino acids (Venkatarami Reddy *et al*. 1992 and Sinha *et al*. 1996).

The increased LAT and IlAT may be attributed to increased levels of leucine and iso leucine in the gonads. VAT was significantly enhanced which was suggestive of mobilization of valine towards metabolic activities, probably due to its accumulation in the gonads.

The increased GDH content might be due to increased oxidation of glutamate. GDH is an enzyme of great importance in the intermediary metabolism of amino acids. GDH activity acts as a general marker of amino acid oxidations in the tissues (Harper, 1985).Glutamate and GDH have a unique role in amino group transfer. It is through this enzyme that the α – keto glutarate is made available for the citric acid cycle, at the same time from ATP to release ammonia (Lehninger, 1978). The balance between total keto acid and total free amino acids is also regulated by the amino transferases (Pant and Kumar, 1979).

In the citric acid cycle, it is through the α – keto glutarate path way that some amino acid metabolites could be mediated in which case glutamate could be a precursor or a successor of α – keto glutarate (Lehninger, 1978). Higher the – keto glutarate, higher the mobilizati-on of spermatiozoa to facilitate higher choice to fertilize more number of eggs and thus higher fecundity and viability (Pushpa Rani, 1997).

It can be concluded that PGF2α induce the metabolic events of gonads towards enhanced reproductive potential of silkworm, *Bombyx mori* L. The gonads of experimental larvae showed that the amino acids were concerned with gluconeogenesis in the gonads. Hence it is essential to utilize PGF2α towards the improvement of Sericulture industry.

Table 1: Levels of total proteins and free amino acids (mg/gm wet wt) in the testes and ovaries of control and experimental (PGF2αtreated) silkworm, *Bombyx mori* L. Values are the mean of 50 individual observations. Mean ± S.D; '+' indicate per cent increase over control. 'P' denotes the statistical significance.

S. No.	Component	Control Testes	Ovaries	Exptl (PGF2α) Testes	Ovaries
1	Total proteins	58.64±4.93	76.27±5.89	74.12 ±6.74 +26.39 P<0.001	98.86 ±8.41 +29.62 P<0.001
2	Free amino acids	10.23±0.92	12.48±1.19	13.48 ±1.17 +31.77 P<0.001	16.22 ±1.48 +29.97 P<0.00

Table 2: Changes in the activity levels of AlAT, AAT (µmol of sodium pyruvate formed /mg protein/hr) and LAT (µmol of keto acid formed /mg protein/hr) in the testes of control and experimental (PGF2αtreated) silkworm, *Bombyx mori* L. Values are the mean of 50 individual

observations. Mean ± S.D; ' + ' indicate per cent increase over control.'P'denotes the statistical significance.

S. No.	Component	Control	Exptl(PGF2α)
1	AlAT	0.16±0.014	0.20 ±0.018 +25.0 P<0.001
2	AAT	0.11 ±0.010	0.13 ±0.012 +18.18 P<0.001
3	LAT	0.13 ±0.012	0.16 ±0.014 +23.08 P<0.001

Table 3: Changes in the activity levels of AlAT, AAT (µmol of sodium pyruvate formed /mg protein/hr) and LAT (µmol of keto acid formed /mg protein/hr) in the ovaries of control and experimental (PGF2αtreated) silkworm, Bombyx mori L. Values are the mean of 50 individual observations. Mean ± S.D; ' + ' indicate per cent increase over control.'P'denotes the statistical significance.

S. No.	Component	Control	Exptl(PGF2α)
1	AlAT	0.19 ±0.016	0.24 ±0.022 +26.32 P<0.001
2	AAT	0.15 ±0.011	0.20 ±0.016 +33.33 P<0.001
3	LAT	0.17 ±0.014	0.21 ±0.018 +23.53 P<0.001

Table 4: Changes in the activity levels of IlAT, VAT (µmol of keto acid formed /mg protein/hr) and GDH (µmol tyrosine equivalents/mg protein/hr) in the testes of control and experimental (PGF2αtreated) silkworm, Bombyx mori L. Values are the mean of 50 individual observations. Mean ± S.D; ' + ' indicate per cent increase over control.'P'denotes the statistical significance.

S. No.	Component	Control	Exptl(PGF2α)
1	IlAT	0.22 ±0.017	0.28 ±0.024 +27.27 P<0.001
2	VAT	0.26±0.019	0.33 ±0.028 +26.92 P<0.001
3	GDH	0.067± 0.0053	0.081 ±0.0062 +20.59 P<0.001

Table 5: Changes in the activity levels of IlAT, VAT (µmol of keto acid formed /mg protein/hr) and GDH (µmol tyrosine equivalents/mg protein/hr) in the ovaries of control and experimental (PGF2αtreated) silkworm, Bombyx mori L. Values are the mean of 50 individual observations. Mean ± S.D; ' + ' indicate per cent increase over control.'P'denotes the statistical significance.

S. No.	Component	Control	Exptl($PGF_{2\alpha}$)
1	IlAT	0.29 ± 0.024	0.38 ±0.0 35 +31.03 P<0.001
2	VAT	0. 35 ±0.032	0.44 ±0.039 +25.71 P<0.001
3	GDH	0.082 ±0.0068	0.098 ±0.0083 +19.51 P<0.001

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