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EFFECT OF MELATONIN ON THE PRODUCTION OF SILK

***G.SIRISHA AND N.ANITHA**

Department of Zoology, Sri Venkateswara University, Tirupati – 517 502, India.

ABSTRACT

The silkworm (*Bombyx mori* L) hybrid (PM X NB_4D_2) were sprayed with different concentrations of melatonin (i.e.10^{-9}, 10^{-10}, 10^{-11} g/g) on alternate days from the beginning of IV instar to the end of V instar. Melatonin treatment at the concentration of 10^{-9} g/g showed significant increase in the levels of fibronin in the cocoon and quality of silk when compared to control. Further melatonin treatment at the concentration of 10^{-9} g/g significantly increased size, weight of silk gland, net weight of the cocoon and ultimately resulted in production of finer quality of silk. From the present data, it can be suggested that topical application of 10^{-9}g/g melatonin resulted in increased quality and quantity of silk from the silkworm.

KEY WORDS: ***Bombyx mori***, **fibronin, cocoon, silk, instar**

***G.SIRISHA**
Department of Zoology, Sri Venkateswara University, Tirupati – 517 502, India.

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INTRODUCTION

The conventional breeding programmes have contributed substantially by the introduction of improved silkworm breeds and more than 2000 races of silkworm are maintained in the germ plasma bank of several countries.[1] The metabolic modulation, cellular and molecular mechanisms of silk worm is directly proportional to the development and economic production of silkworm industry. The decrease of cocoon weight depends upon the disease state, beside the genetic and immunological resistance.[2] The Meta cellular and molecular mechanisms of silk worm are directly proportional to the development and economic production of silkworm industry. Melatonin was first identified in the bovine pineal gland extract by Lerner et al.[3]. Since then, several researchers studied its biosynthesis, metabolism, physiological, and patho-physiological functions. Until a decade ago, melatonin was thought to be only produced only in the animal kingdom. Later it has been identified in the edible plants, such as bananas, cherries, white sprouts, cucumbers[4], walnuts[5] and in olive oil[6]. Melatonin is an almost ubiquitous molecule in the animal and plant kingdom. Melatonin is involved in the regulation of circadian rhythms[7], seasonal reproduction, sleep, again[8], mood, thermoregulation, and behaviour performance[9] immune functions[10], locomotor activity, osmoregulation, food intake[11] and most importantly scavenges free radicals[11]in vertebrates. Thus, it seems apparent that melatonin is a potential biological cue in the synchronization of environmental signals and integrating those signals with an animal's physiology and behaviour. Melatonin (N-acetyl-5 methoxytryptamine) was originally found in the vertebrate pineal gland, where its synthesis and release exhibit diurnal rhythms with peak activity during the dark period of light/dark cycles and a circadian rhythm under constant darkness[12]. The occurrence of melatonin is not only restricted to vertebrates but also detected in several invertebrates. Furthermore, exogenously administrated melatonin entrains other circadian rhythms such as metabolic activity of the suprachiasmatic nucleus and locomotor activity[13]. Itoh et al.[14] reported the presence of melatonin in head and haemolymph of silkworm and suggested that melatonin may function as regulatory chemical mediating developmental events such as molting, eclosion and diapause via acting as neurochemical mediator of photoperiodic rythms. Ritcher et al.[15] reported that melatonin induces the release of photoracicotropic hormone a neuropeptide which stimulates the production of the moulting hormone ecdysone in the moulting gland in cockroach (*Perplaneta americana).* In view of the importance of melatonin in the grown of invertebrates the present study was undertaken to test the effect of melatonin on silk production in *Bombyx mori* using economical parameters.

MATERIALS AND METHODS

Bi-voltine x multi-voltine hybrid race (PM X NB4D2) of silkworm used in the present study were obtained from silkworm seed production. Centre, central silk board, Chittoor Dist, Andhra Pradesh, ministry of textiles, Govt. of India. A standard procedure was used for the rearing the silkworm larvae as given by Pandey et al.[16]. Larvae feeding healthy mulberry leaves were used in the present study Temperature and humidity were maintained at 23°Cto 25and 70°C to 75% RH respectively. The late age worms were reared in bamboo trays. Three feedings were given on alternate days and large feeding was given at night times. Proper care was taken during the moulting periods.

Experimental design

Melatonin was dissolved in 1% ethanol and required concentrations 10^{-9} to 10^{-11} g/g tissue were obtained by diluting the stock solution with sterile water. The experimental design consists of four treatments i.e., Control (without melatonin) and three different concentrations of melatonin i.e 10^{-9}, 10^{-10}, 10^{-11} g/g applied on two development stages of silk worm i.e., IV+V instarand V instar. Every group consists of uniformly weighted larvae in 4 replicates of 30 worms for each treatment. The period of group IV instar is 4 days, melatonin was treated on alternate days. In each group and in each treatment 1/3rd of worms were used recording cocoon weight shell weight, pupa weight etc. Other 1/3rdof the worms were used for evaluating the quality and quantity of silk. Remaining 1/3rd of the worms were used to evaluation of fibroin and sericin levels.

Cocoon weight, pupa weight, shell weight, shell ratio and shell percentage measurements in different treatments

In the present experiment 10 worms were taken from each treatment of a particular group to measure weight of the cocoon, shell weight, shell ratio and pupa weight. The shell weight was obtained after cut opening the cocoons by removing the pupa body and exuviate and weight were taken in gram units. Shell percentage = weight of the cocoon shell/weight of the entire cocoon X 100.

Filament length

In the present study, the length of the silk filament was measured by subjecting the single cocoon to reeling in a mono-cocoon reeling machine called Eupprouvette. The total filament length was calculated by using the following equation[17].

$$\text{Total filament length in mts} = \text{no. of rotations} \times \text{circumference of the reel}$$

Filament weight

The total filament was weighed accurately. The weight of the total filament was calculated in grams.

Denier

The denier can be calculated by using the following formula

Dinier = filament weight in gms/filament length in mts X 9000

Estimation of Fibroin and Sericin

The Fibroin and sericin contents of control and experimental cocoons were measured following the procedure as given by Sonwalker [17] (degumming by soap and soda method).

RESULTS

Cocoon weight, pupa weight, shell weight, shell ratio and shell percentage in different treatments

Cocoon weight, pupa weight, shell weight, shell ratio and shell percentage were significantly higher in 10^{-9}g/g melatonin treatment group compared to all other groups in both IV+V instar an V instars (Table 1 and 2). When compared to V instar the percentage increases of these parameters were higher in IV+V instar (Table 1 and 2).

Quality and quantity of silk in different treatments

Average filament length, weight were significantly higher in 10^{-9}g/g melatonin treatment group compared to all other groups in both IV+V instar an V instars (Table 1 and 2). When compared to V instar the percentage increases of these parameters were higher in IV+V instar (Table 1 and 2). Filament denier was significantly lower in 10^{-9}g/g melatonin treatment group compared to all other groups in both IV+V instar and V instars (Table 1 and 2). When compared to V instar the percentage increases of denier were lower in IV+V instar (Table 1 and 2).

Fibroin and serecin percentages in different treatments

Fibroin and sericin percentages were significantly higher in 10^{-9}g/g melatonin treatment group compared to all other groups in both IV+V instar and V instars (Table 1 and 2). When compared to V instar the percentage increases of these parameters were higher in IV+V instar (Table 1 and 2).

Table 1

Effect of different concentrations of Melatonin on Economical parameters of V-Instar

S.No.	Name of the Parameter	Control	10^{-9} gram of Melatonin/ gram tissue	10^{-10} gram of Melatonin/ gram tissue	10^{-11} gram of Melatonin/ gram tissue
1	Cocoon Wt.	1.643 ± 0.013^{a}	2.297± 0.017^{b} (+28.471)	2.037±0.025^{c} (+19.342)	1.812^{d} ±0.016 (+9.326)
2	Pupa Wt.	1.365^{a} ± 0.011	1.853^{d} ±0.012 (+26.335)	1.671^{c} ±0.015 (+18.312)	1.535^{b} ±0.026 (+11.074)
3	Shell Wt.	0.223^{a} ± 0.069	0.422^{d} ± 0.066 (+47.156)	0.343^{c} ±0.069 (+34.985)	0.277^{b} ±0.06 (+19.494)
4	Shell ratio %	13.53^{a} ± 0.412	18.43^{d} ± 0.262 (+26.587)	16.71^{c} ±0.297 (+19.030)	15.30^{b} ±0.307 (+11.568)
5	Silk Thread (mts)	647^{a} ±16.084	974^{d} ±40.867 (+33.572)	861^{c} ±30.053 (+24.85)	767^{b} ±25.44 (+15.645)
6	Silk Thread (wt.)	201.25^{a} ±3.031	255.5^{d} ± 2.828 (+21.232)	246.45^{c} ±3.012 (+18.340)	229.75^{b} ±3.028 (+12.404)
7	Denier	3.1^{a} ± 0.18	2.41^{d} ±0.014 (-28.630)	2.65^{c} ±0.126 (16.9)	2.99^{b} ±0.16 (-6.8)
8	Fibroin %	59.33^{a} ± 2.96	71.81^{d} ±2.98 (+17.379)	66.23^{c} ±2.09 (+10.418)	62.20^{b} ±2.28 (+4.614)
9	Sericin %	33.16^{a} ±1.35	25.83^{d} ± 1.99 (-28.377)	28.16^{c} ±1.61 (-17.75)	30.52^{b} ±1.58 (-8.65)

Values are mean ±S.D. of 8 individuals
Values in the parentheses are percent change from that of control
Mean values with same superscript do not differ significantly from each other P<0.05.

Table 10
IV to V

S.No.	Name of the Parameter	Control	10^{-9} gram of Melatonin/ gram tissue	10^{-10} gram of Melatonin/ gram tissue
1	Cocoon Wt.	1.631^{a} ± 0.04	2.535^{d} ± 0.021 (+35.660)	2.261^{c} ±0.022 (+27.863)
2	Pupa Wt.	1.342^{a} ± 0.017	1.897^{d} ±0.029 (+29.256)	1.742^{c} ±0.028 (+22.962)
3	Shell Wt.	0.24^{a} ± 0.012	0.617^{b} ±0.0119 (+61.102)	0.477^{d} ±0.096 (+49.685)
4	Shell %	14.89^{a} ± 0.822	24.30^{d} ± 0.920 (+38.724)	21.11^{c} ±0.898 (+29.464)
5	Average filament length	640^{a} ±10.07	1184^{d} ±52.54 (+45.945)	985^{c} ±40.04 (+35.025)
6	Filament Wt.	201.25^{a} ±3.041	254.75^{d} ± 6.26 (+21.001)	245.12^{c} ±5.59 (+17.897)
7	Filament Denier	3.1^{a} ± 0.10	2.15^{d} ±0.21 (-44.186)	2.48^{c} ±0.18 (-25)
8	Fibroin %	57.83^{a} ± 2.96	78.16^{d} ±2.13 (+26.010)	71.56^{c} ±2.21 (+19.186)
9	Sericin %	32.5^{a} ±1.64	20.14^{d} ± 1.14 (-61.370)	25.83^{c} ±1.31 (-25.822)

Values are mean ±S.D. of 8 individuals Values in the parentheses are percent change from that of control
Mean values with same superscript do not differ significantly from each other P<0.05.

DISCUSSION

In the present study, effect of melatonin on silk production in *Bombyx mori* was evaluated using some economical parameters like weight of cocoon, shell weight and ratio, deiner, emergence of eggs, hatching percentage, pupa weight, filament length, filament weight, moth weight, fibroin and sericin (Table 1 and Table 2). Different hormones and factors such as thyroxine and juvenile hormone are reported to increase the growth and silk production.[18,19] There was significant increase in above mentioned economical parameters in melatonin treated group when compared to the controls. Among, melatonin treated groups 10^{-9}g/g tissue showed significant increase when compared to other melatonin treated groups. It is well known that melatonin has positive effect on the growth in insects.[14,15] It is also known that melatonin is potent antioxidant, scavenges free radicals produced during metabolism which in turn results in better growth rates in insects.[5, 20] In the same way, silk production is energy demanding process during this process increased production of ATP (Adenosine Tri Phosphate) leads to generation of free radicals by A TP synthase. Melatonin can combat effectively against free radicals generated by A TP synthase due to high metabolic demand.[21, 22] It is also observed that 10^{-9} g/g tissue melatonin concentration is effective when compared to other concentrations i.e. 10^{-10}, 10^{-11}.

CONCLUSION

Thus from the present study, it was concluded that topical application of 10^{-9}g/g melatonin resulted in increased growth, filament length, filament weight, denier fibroin and sericin levels resulted in the increased quality and quantity of silk.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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