

Full Length Research Paper

The effect of thyroxine on silk gland and the effect of two thyroxine-treated mulberry species feeding on silk quality in the silkworm *Bombyx mori* (Lepidoptera: Bombycidae)

Intan Ahmad^{1*}, Sinta Patrakomala¹, Susi Dwiyantri¹ and Ramadhani Putra²

¹School of Life Sciences and Technology, Bandung Institute of Technology, Jalan Ganesha 10 Bandung, 40132, Indonesia.

²Graduate School of Natural Science and Technology, Kanazawa University, Ishikawa, Japan.

Accepted 13 January, 2009

The influence of feeding mulberry leaves treated with thyroxine to the growth of the silk gland, and the effect of two different mulberry species, that is, *Morus nigra* and *Morus multicaulis* treated with thyroxine on silk quality in the silkworm were studied. The silk glands from thyroxine treated *Bombyx mori* larvae weighed heavier than control. The weight of the posterior silk gland, where fibroin is synthesized, increased significantly compared to the anterior and median silk glands. No difference between thyroxine treatment on the second instar and fourth instar larvae in terms of silk gland weight was observed. Different mulberry species treated with thyroxine fed to silkworm larvae, affected silk quality. Higher silk tenacity and elongation were observed when silkworm larvae were fed thyroxine-treated *M. nigra*, while silkworm larvae fed on thyroxine treated *M. multicaulis* produced longer unbreakable filament.

Key words: *Bombyx mori*, thyroxine, silk gland, silk quality, mulberry species.

INTRODUCTION

It has been known that application of hormones to *Bombyx mori* could be used to improve the quality of silk (Akai et al., 1985; Ahmad et al., 2007; Mamatha et al., 2006). For example, Miranda et al. (2002) reported that topical application of methoprene (a Juvenile Hormone Analog) prolonged larval period and caused an increase in the weight of silk gland and cocoon of *B. mori*. Another example: Thyagaraja et al. (1991), reported that thyroxine fed to *B. mori* larvae resulted in increased cocoon shell weight up to 150% in some cases with no loss in silk quality. Ahmad et al. (2007) reported that the application of thyroxine to *B. mori* larvae increased the ecdysteroid titer 33.34% higher than control; higher titer of ecdysteroid presumably would promote larval growth, as well

as sericin and fibroin protein synthesis (Thagaraja et al., 1991). In addition, it is known that nutrition plays a major role in improving the growth and development of *B. mori*, and the nutritive values of mulberry leaves vary by mulberry varieties (Kanafi et al., 2007). The present investigation was developed to observe the effect of thyroxine on the silk gland growth and cocoon characters as well as the effect of different mulberry species on silk quality.

MATERIALS AND METHODS

Experimental insects

B. mori type Polyhybrid bivoltine C.301, obtained from Silkworm Rearing Center, Candirotto Temanggung, Central Java, Indonesia and were reared under laboratory conditions in the School of Life Sciences and Technology, Bandung Institute of Technology, Bandung, Indonesia. The animals were reared on mulberry leaves at 22 -27°C, 85% RH, and 12 h light: 12 h dark cycle.

*Corresponding author. E-mail: intan@itb.ac.id. Tel: (+62) 816607082. Fax: (+62) 22 253 4107.

Table 1. Effect of thyroxine (T) feeding on silk gland growth.

Larval groups	Wet weight (mg)			
	Anterior	Middle	Posterior	Total
2 nd instar + T	21.05 ^b	1073.48 ^b	381.23 ^c	1475.76 ^c
3 rd instar + T	18.20 ^{ab}	1043.87 ^b	312.33 ^b	1374.40 ^b
4 th instar + T	19.62 ^b	1064.65 ^b	347.45 ^{bc}	1431.72 ^{bc}
Control	15.16 ^a	703.37 ^a	214.11 ^a	928.13 ^a

N = 20 per treatment.

All values are means. Means within a column followed by the same superscripts are not significantly different (ANOVA followed by LSD, $p < 0.05$)

Experiment I

Preparation of thyroxine solution

Thyroxine was obtained from thyroid powder Grade II (Sigma Ltd. USA). 150 mg thyroxine was diluted in 5 ml sodium hydroxide 0.1 N (4 g NaOH + 1000 mL distilled water). The solution then was added with 95 mL distilled water to obtain a 1.5 mg/mL (1500 ppm) thyroxine solution (Thyagaraja et al., 1991). This solution was mixed with mulberry leaves and given to the larvae.

Thyroxine treatment

Each group consisted of 50 larvae with the following treatments:

Group I: Mulberry leaves (*Morus nigra*) with thyroxine were given to the third instar larvae

Group II: Mulberry leaves (*M. nigra*) with thyroxine were given to the fourth instar larvae

Group III: Mulberry leaves (*M. nigra*) without thyroxine were given from second to fourth instar larvae (control)

Weight measurement of the silk gland

At the end of the fifth instars, when the larvae stopped eating and emptied their gut, 20 larvae were randomly selected, anesthetized with ether, and dissected to remove the silk gland. The anterior, middle, and posterior silk glands of both sides were dissected and washed briefly with Ringer Solution (NaCl 1.54 mM, KCl 2.68 mM, CaCl₂ 1.8 mM, NaHCO₃ 0.7 mM, and glucose 11.1 mM). The weight of the anterior, middle and posterior silk glands was measured.

Experiment II

In the second experiment, we observed the effect of thyroxine addition on leaves of two mulberry species on the quality of silk. Two species used in this study were *M. nigra* and *M. multicaulis*. Both species are the common mulberry species used in Indonesia silk industry and have some nutritive differences. Previous research by Ahmad et al. (1995) found that *M. nigra* had higher protein content than *M. multicaulis* (9.0 and 5.0%, respectively) and also better ECD (efficiency of conversion of digested food to biomass) and ECI (efficiency of ingested food to biomass).

For this purpose, we made three concentrations of thyroxine, that is, 1.0, 1.5, and 2.0 mg/mL were prepared in experiment I. These solutions were mixed with leaves from two different species of mulberry and fed to newly molted fourth instars larvae *ad libitum*.

We used mulberry leaves mixed with sodium hydroxide solution as the control. This procedure was carried out until the larvae molt into fifth instars when only mulberry leaves without thyroxine were given up to the time the larvae pupate.

Cocoon harvest and qualitative determination of the silk character

Five days after spinning, the cocoons were harvested. All cocoons were then sent to the Institute for Research and Development of the Textile Industry in Bandung where non-breakable filament length, tenacity, and elongation were measured.

Statistical analysis

Where appropriate ANOVA was conducted and followed by LSD test at the 5% level (Zar, 1984)

RESULTS AND DISCUSSION

In general, as expected, the silk glands from thyroxine treated *B. mori* larvae showed heavier silk glands compared to the control group (Table 1), either in total weight or in weight of each part of the gland (anterior, middle, and posterior). There is one possible explanation for this finding, that is, application of thyroxine would increase the ecdysteroid titre in haemolymph, stimulates protein synthesis, including sericin and fibroin synthesis in the silk gland (Thyagaraja et al., 1991; Ahmad et al., 2007).

The difference in weight of each silk gland part between treatments as compared to control shows that the posterior part of the silk gland gained more weight after thyroxine treatment as compared to control (Table 2). A similar finding was reported by Horie and Watanabe (1980); they observed the wet weight of posterior silk gland increased significantly as compared to the anterior and median silk gland after the *B. mori* larvae were injected by ecdysteroid. It might be because of cells in the posterior silk gland had better sensitivity to the ecdysteroid than the cells in the anterior and middle part of the silk gland. In this case, we are particularly interested in the growth of the posterior part of the silk gland where fibroin, the most important silk protein, was synthesized.

Table 2. Silk gland weight differences (percent) between larvae feeding on thyroxine (T)-treated leaves and control.

Larval group	Difference (%) in silk gland weight as compared to the control		
	Anterior	Middle	Posterior
2 nd instar + T	27.98	34.48	43.83
3 rd instar + T	16.07	32.62	31.45
4 th instar + T	22.73	33.93	38.37

Table 3. Effect of feeding on thyroxine-treated *M. nigra* on silk parameters quality.

Treatment groups (mg/mL)	Silk parameters quality		
	Filament length (m)	Tenacity (g/Denier)	Elongation (%)
1.0	880.00 ^a	3.89 ^a	17.57 ^a
1.5	885.71 ^a	3.36 ^a	19.72 ^a
2.0	950.91 ^a	1.60 ^b	8.52 ^c
Control	800.73 ^a	1.38 ^b	15.5 ^b

N = 50 per treatment.

All values are means. Means within a column followed by the same superscripts are not significantly different (ANOVA followed by LSD, $p < 0.05$)

Table 4. Effect of feeding on thyroxine-treated *Morus multicaulis* on silk parameters quality.

Treatment groups (mg/mL)	Silk parameters quality		
	Filament length (m)	Tenacity (g/Denier)	Elongation (%)
1.0	726.84 ^c	3.07 ^b	13.92 ^b
1.5	1598.2 ^b	2.48 ^c	15.38 ^a
2.0	1850.3 ^a	1.62 ^d	12.35 ^a
Control	507.27 ^d	3.45 ^a	13.87 ^b

N = 50 per treatment.

All values are means. Means within a column followed by the same superscripts are not significantly different (ANOVA followed by LSD, $p < 0.05$).

Our finding shows that there is no difference between thyroxine treatment on second instar larvae and fourth instar larvae in terms of silk gland weight (Table 1). The results in fact support the finding by Thyagaraja et al. (1991), who showed that thyroxine treatment at either second or fourth instar larvae was more effective than treatment at other stages of development in improving silk output. However, our previous experiment (unpublished data) shows that treatment of thyroxine during fourth instar produced the highest total protein concentration as compared to the second instar, which led us to believe that thyroxine treatment during the fourth instar is a better choice to improve the quality of the cocoon. This was the reason why we conducted the experiment II with fourth instar using two different mulberry leaves, that is, *M. nigra* and *M. multicaulis* that were known to have different protein content (Ahmad et al., 1995).

From Experiment II, our study found that different mulberry species have different results, which suggest that nutrition may have important role for improving silk

quality. In general, silkworm fed with *M. multicaulis* + thyroxine produced longer unbreakable filament while silkworm fed with *M. nigra* + thyroxine produced higher silk tenacity and elongation (Tables 3 and 4). This is indeed an interesting finding, as *M. multicaulis* + thyroxine had significant effect on the length of filament, while *M. nigra* + thyroxine did not. Our previous experiments (Ahmad et al., 1995) showed that the protein content of *M. nigra* is higher than *M. multicaulis*, that is, 9.0 and 5.9%, respectively. Nutrition indices (ECI and ECD) of larvae fed with *M. nigra* was also in general better than larvae fed with *M. multicaulis*, which suggest that *M. nigra* is a better food for *B. mori*. So far we have not been able to find a plausible explanation (why *M. multicaulis* + thyroxine produced longer filament than those fed with *M. nigra* + thyroxine). Definitely more work is required to answer this phenomenon.

The increase in silk filament length (Table 4) on larvae treated with 1.5 and 2.0 mg/mL thyroxine showed the benefit of supplementary hormone to increase one of the

most important commercial characters in silk quality and yield (Kamimura and Kiuchi, 1998). Both treatments resulted in filament length of 1598.22 and 1850.30 m, respectively, which is better than the average length of filament produced by the Indonesian silk industry (1000 m) (Moerdoko, 1991) and by larvae treated with fenoxycarb (a Juvenile Hormone Analogue) (890.50 m) (Mamatha et al., 2006). However, it remains unclear why larvae fed with *M. multicaulis* + thyroxine produced longer filament than those fed with *M. nigra* + thyroxine (Tables 3 and 4). Furthermore, larvae fed with *M. multicaulis* produced significantly longer filament length with increasing thyroxine treatments while it was less significant in larvae fed with *M. nigra*. This finding showed the possibility of synergy effect between thyroxine and some compound in *M. multicaulis* which was crucial to the production of longer and unbreakable filament as found on larvae fed with *M. multicaulis* with no thyroxine addition; and addition of less than 1.5 mg/mL thyroxine did not produce longer filament than larvae fed with *M. nigra*.

However, silk tenacity and elongation, other economically important characters of silk quality, of silk produced by silkworm fed on *M. nigra* treated with 1.0 and 1.5 mg/mL thyroxine generally better than the ones that fed on *M. multicaulis* (Table 3). Ashfaq et al. (2001) mentioned that silkworm fed with *M. nigra* showed high food consumption, coefficient of nutrition utilization, larval size, larval weight and cocoon weight that may provide important factors for increasing silk tenacity and elongation.

We also found that higher dose of thyroxine (2.0 mg/mL) caused poor silk elongation on both larvae that fed on *M. multicaulis* and *M. nigra*. This finding suggested a threshold for thyroxine application for improving silk quality in our study.

Based on all of the results we concluded that improvement of the silk quality through thyroxine supplement is not only dependent on the dose of the hormone but also on the species of mulberry. This study also confirms previous findings by other researchers (Mamatha et al., 2006) which suggested that matching economic traits (silk quality) with biological parameters after hormone treatment on *B. mori* does not always provide expected results. We also would like to highlight the need for more comprehensive studies on this subject to find better silkworm management for the tropical region.

ACKNOWLEDGEMENT

This work was partly supported by a competitive grant from the Directorate General of Higher Education Republic of Indonesia given to Intan Ahmad

REFERENCES

- Ahmad I, Lubis A, Sastrodihardjo S (1995). Food utilization parameters could be used to indicate food suitability in the silkworm, *Bombyx mori*. *J. Biosains*, 1(1): 5-7.
- Ahmad I, Rahayu R, Permana AD, Astari S (2007). Alteration of Ecdysteroid titre by Thyroxine and Juvenile Hormone Analogue (Methoprene) in *Bombyx mori* (Lepidoptera: Bombycidae). *Biota* 12(2): 116-121.
- Akai H, Kimura K, Kiguchi M, Shibukawa K (1985). An increase of silk production by repeated treatment with a juvenile hormone analogue. *J. Seric. Sci. Jpn.* 54: 297-299.
- Ashfaq M, Nadeem MK, Nadeem S (2001). Impact of mulberry varieties and various alternate hosts on the development of *Bombyx mori* L. and silk yield. *South Pacific Study* 21(2): 61-64.
- Horie Y, Watanabe H (1980). Recent Advances in Sericulture. *Ann. Rev. Entomol.* 25: 49-71.
- Kamimura M, Kiuchi M (1998). Effect of Juvenile Hormone Analog Fenoxycarb on 5th stadium larvae of the silkworm, *Bombyx mori*. *Appl. Entomol. Zool.* 33(2): 333-338.
- Kanafi RR, Ebadi R, Mirhosseini SZ, Seidavi RR, Zolfaghari, Etebari K (2007). A review on nutritive effect of mulberry leaves enrichment with vitamins on economic traits and biological parameters of silkworm *Bombyx mori* L. *Inv. Sur. J.* 4: 86-91.
- Mamatha DM, Cohly HPP, Raju AHH, Rao RM (2006). Studies on the qualitative and quantitative characters of cocoons and silk from methoprene and fenoxycarb treated *Bombyx mori* larvae. *Afr. J. Biotechnol.* 5(13): 1422-1426.
- Miranda JE, de Bortoli SA, Takahashi R (2002). Development and Silk Production by Silkworm Larvae after Topical Application of Methoprene. *Sci. Agric.* 59(3): 585-588
- Moerdoko W (1991). *Serat-serat ALAM*, Balai Besar Penelitian Tekstil, Bandung.
- Thyagaraja BS, Kelly TJ, Masler EP, Borkovec AB (1991). Thyroid Induced Haemolymph Protein and Ecdysteroid Increases in the Silkworm, *Bombyx mori* L.: Effect of Larva Growth and Silk Production. *J. Insect. Physiol.* 37: 153-159.
- Zar JH (1984). *Biostatistical Analysis*, 2nd ed. Prentice Hall, Englewood Cliff, New Jersey.