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Effect of lactic acid on the growth and production of high-quality cocoons in *Bombyx mori* (Lepidoptera: Bombycidae) silkworm larvae by adding it to their diet

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Abstract

The silkworm *Bombyx mori* L. (Lepidoptera: Bombycidae) was used to investigate the impact of lactic acid on the growth of silkworm larvae and cocoon quality. In this study, larvae from the first day of the 1st until the end of the 5th instar were fed on mulberry leaves treated with lactic acid as a nutritional supplement with different concentrations (0.1% - 1% - 10%). Results showed that the larvae fed with nutritional supplements of lactic acid (0.1%, 1%) were positively affected in larval weight and length; also, results recorded good cocoon quality in low concentrations of lactic acid (0.1% - 1%). In contrast, lactic acid at high concentration (10%) had a toxic effect, leading to the death of most of the larvae, and negatively affecting cocoon quality. All concentrations have no significant effect on the number of egg/female and the fertility rate%.

Introduction

Sericulture is a method for obtaining silk fiber production. A large number of by-products from sericulture can also be utilized, from silkworm formation to post-cocoon technologies, albeit it is primarily used in the textile industry (Jaiswal *et al*., 2021). *Bombyx mori* L. (Lepidoptera: Bombycidae) is a monophagous insect that feeds on mulberry leaves only during the larval stage (Singhal *et al*., 1999). Also considered of special importance for biochemical, physiological, and developmental biology research due to its short life cycle (Jaing *et al*., 2020). One of the most important biological properties of the silkworm is its ability to convert plant protein (Mulberry leaves) into silk protein (Ude *et al*., 2014). Silkworm larvae must be fed with good quality mulberry leaves for high-quality cocoons (Vijaya *et al*., 2009). Larval growth and production of high-quality silk depend on the different nutrients and quality of mulberry leaves presented (Smitha and Rao 2010). It is necessary to pay attention to large-scale rearing of silkworm larvae and provide the necessary labor to promote the sericulture industry offered (Saviane *et al*., 2014 and Shifa, 2016). The artificial diet of silkworm larvae generally includes mulberry leaf powder, soybean, corn powder, cellulose, vitamins, and proteins, which aims to improve the nutritional composition of larvae and works to provide their nutritional needs for growth (Saviane *et al.,* 2014 and Bhatti *et al*., 2019). Lactic acid, a naturally occurring (3-hydroxy carboxylic acid), is in almost all living things. Commercially, it is a colorless to slightly yellow, hygroscopic liquid that comes in solutions of 50 to 90 weight percent (Jem *et al*., 2010). Lactic acid is a common occurrence in nature and is

created by a variety of organisms, including bacteria and human cells. pharmaceuticaled in 1780 as a component of sour milk and has since been employed in a variety of products for the food, beverage, pharmaceutical, and cosmetic industries to preserve the microbial stability of lower (PH) products. (Beales, 2004 and Dang *et al*., 2009).

This study was conducted to investigate the impact of lactic acid on silk products. To know the appropriate concentrations of lactic acid in the diet provided to silkworm larvae, treat mulberry leaves with various concentrations of lactic acid and measure the [Fifth instar larval weight, length (cm), cocoon weight (g), cocoon shell weight (g), silk ratio (%), eggs/ female, and fertility rate (%)] of silkworms.

Materials and methods

The current research was conducted in spring in the Sericulture Lab in the Plant Protection Research Institute, Mansoura, Dakahlia.

1. Silkworm resource:

Hybrid silkworm eggs (*B. mori*) were obtained from the Silk Research Department, Plant Protection Research Institute, Agricultural Research Center, Giza, in the spring. Fresh mulberry leaves of *M. alba* var. *Canava*.

2. Preparation of different concentration solutions:

Distilled water was used to dilute lactic acid to obtain different concentrations. (0.1%, 1%, 10%) solution. Then, 15 liters of supplementation were put in several clean, airtight bottles for use during the experiment (Jiang *et al*., 2020).

3. Rearing technique:

The rearing laboratory and equipment used in rearing were disinfected with a 5% formaldehyde solution. Newly hatched *B. mori* larvae were placed in plastic trays (60 × 90 × 7 cm). The room temperature was 24.9°C ± 0.118°C, and the relative humidity was 72.02% ± 5.693, according to techniques (Krishnaswami, 1978).

4. Experimental design:

Silkworm larvae (*B. mori*) were fed the experiential diet from the 1st day of the first instar until the end of the 5th instar. Larvae were distributed into 3 groups after hatching. Every group consisted of three replicates, each containing 150 larvae in addition to a control. Mulberry leaves should be collected daily in the early morning and immersed in the pre-prepared solution for 15 minutes, and then the silkworm (*B. mori*) larvae are fed on the treated leaves after they are air-dried four times daily. The control group was fed on clean mulberry leaves soaked in the distilled water.

5. Biology and economy characteristics:

Biological criteria of the larvae of *B. mori* feeding on mulberry leaves treated with different concentrations of the nutritional supplements used in the experiment were recorded. Weight and length of larvae are measured, and collected larvae at the maturity stage are manually transported to montages for spinning cocoon. After a week, collect cocoons and divide them into groups. The first group was dried, and the weight of cocoon (g), the weight of the cocoon shell (g), and the silk ratio (%) were recorded.

$$\text{Silk ratio \%} = \frac{\text{Cocoon shell weight(g)}}{\text{Cocoon weight(g)}} \times 100$$

The second group, the cocoon left fresh without drying until the butterflies emerge and mate, to record the number of eggs and measure the fertilization ratio.

6. Statistical analysis:

Data was obtained for statistical analysis to identify low and significant differences between treatment and control groups. CoHort Software (2004) was used in all tests by ANOVA

appropriate to Duncan's Multiple Range Test.

Results and discussion:

1. Effect of lactic acid on *Bombyx mori* 5 th larval weight and length:

The larval stage of the silkworm, *B. mori*, is one of the most important periods for determining growth and development, as the weight of the larvae and length reflect their growth and development. This research measured the weight and length of the 5th instar after adding lactic acid as nutritional supplements in different concentrations (0.1%, 1%, and 10%). Low concentration significantly increased the average larval weight and length compared with other groups.

Although concentration (10%) increased larvae weight and length. However, the average weight recorded was the lowest compared to other concentrations (Table1). The larvae feeding on mulberry leaves supplemented with high concentrations remained in the larval stage and were only able to spin cocoons in small numbers. These results agreed with Nishida *et al*., 2016; Li *et al*., 2017; Zhen *et al*., 2021 and Deepali *et al*., 2025. Who reported that one possible reason for these results is that adding lactic acid to mulberry leaves prevents the growth of microorganisms that cause damage to these leaves. Another reason is that lactic acid lowers the larvae's intestinal PH to prevent the production of pathogenic microorganisms. However, preserve a balanced environment, which supports the growth of silkworm larvae.

Table (1): Effect of Lactic acid with different concentrations on larval weight and length on the fifth instar of the silkworm *Bombyx mori.*

Lactic acid concentration	Larval weight (g) Fifth instar	Larval length (cm) Fifth instar
0.1 %	a 5.45	a 8.15
1 %	a5.40	a 8.07
10 %	b5.15	b 7.35
Control (Distilled water)	c 4.8	c 7.05

2. Effects of lactic acid on *Bombyx mori* silk cocoon properties:

In silk production, the weight of cocoons (g), cocoon shells (g), and silk ratios %, are considered important factors that can clarify the economic value of cocoons. The results indicated that adding lactic acid significantly affects the quality of the cocoons' production at concentrations (0.1% -1 %) compared to other groups (Table 2). However, a concentration of 10% had a significant negative effect on cocoons' production; also, the control group gives better results than this concentration. Results are supported by the observation of Huang *et al*., 2013; Sekar *et al.,* 2016; Tassoni *et al.,* 2022 and Deepali *et al.,* 2025. Noticing that the cocoon yield was better than another group's, which shows that lactic acid can affect the silk gland's growth and increase the quality of silk proteins in silkworm larvae at low concentrations (0.1%-1%) conversely, high concentrations (10%).

Table (2): Effect of lactic acid with different concentrations on the properties of cocoons on silkworm *Bombyx mori.*

Lactic acid concentration	Cocoon weight (g)	Cocoon shell weight (gm)	Silk ratio %
0.1 %	a 1.85	a 0.370	a 20.00
1 %	a 1.82	a 0.362	a 19.95
10 %	c 1.65	0.30 c	c 18.20
Control (Distilled water)	b 1.72	b 0.330	b 19.50

3. Effects of lactic acid on the egg/female and fertility percentage of *Bombyx mori*:

The results showed that nutrition supplementation had no effect on the number eggs/female and fertility% in all concentrations. No significant difference was observed in the number of eggs laid between the test and control groups. The same in the fertilization (Table 3). Results showed that all various concentrations do not cause any change in regulating female fertility. The current study showed results consistent with Zhen *et al*., 2021; Niharika *et al*., 2022 and Iman *et al.,* 2025

Table (3): Effect of lactic acid with different concentrations on the number of egg/female and fertility% of the silkworm *Bombyx mori.*

Lactic acid concentration	Egg/ female	Fertility %
0.1 %	612a	a 98.92
1 %	615a	a 98.90
10 %	608a	a 98.85
Control (Distilled water)	a 600	a 98.88

The mulberry silkworm *B. mori* is extremely sensitive to environmental change and additives in mulberry leaves for nutrition. One of the most important stages of silk production is the larval stage, as the weight of the larvae indicates the growth of the larvae and their ability to produce high-quality cocoons. In the present study, we inspected the effect of lower concentration on increasing weight, length of larvae *B. mori* weight of cocoons, cocoon shell weight, and silk ratio. Although a high concentration of 10% negatively affects the cocoon characters. Subsequently, lower concentrations of lactic acid help larvae grow and evolve and produce better quality cocoons, unlike higher concentrations.

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