LED Lighting as a Modulator for *Bombyx mori* L. Egg Hatching and Biochemistry

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ABSTRACT

*Bombyx mori* L. rearsers usually face the problem of non-synchronized egg hatching and subsequent low hatchability rate as they do not have equipment to control optimum temperature, humidity and light intensity. The experiment depends on focusing LED lighting on eggs with adjustment of temperature and humidity till hatching. The effect of continuous LED lighting on *B. mori*, on two hybrids (Egyptian and Bulgarian) embryos, for different periods (24, 72, 120, 168, 216 hrs.) was studied. The changes in total proteins, carbohydrates and lipids (mg/g egg weight) titters were estimated colorimetrically. The results showed that a gradual improvement in egg hatchibility percentages with shortage in incubation period for treated eggs compared with control. The best results were achieved under the continuous LED lighting till hatching (216 hrs.). The embryos respond to LED exposure by modulating metabolism to adapt lighting stress for both the two hybrids. Also, response of embryos depends on the hybrid origin. It may be recommended to use LED light at the last stage of incubation period for treated eggs compared with control. The best results were achieved under the continuous LED lighting till hatching (216 hrs.). The embryos respond to LED exposure by modulating metabolism to adapt lighting stress for both the two hybrids.

Keywords: *Bombyx mori* L., eggs, LED (Light Emitting Diode), total protein, total carbohydrates, total lipids, metabolism.

INTRODUCTION

Intense efforts have been made on *B. mori* egg storage programs to find-out the appropriate techniques for achieving highest number of hatchable eggs. One of the used techniques is exposing eggs to different temperature and light regimes. Egg hatching is under endogenous control (Lazzari, 1991), whereas in others it is triggered by environmental factors (Saunders, 1982). The embryonic stage of an insect is a period of higher sensitivity to external stimuli (Tilton and Brower, 1983). LED (Light Emitting Diode) is a solid-state unit that converts electricity to light with minimal heat production therefore, it is a very efficient source of light. Light-emitting diode color can range from ultraviolet (350 nm) to infrared (700 nm) depending on the chemical composition of the LED (Humphreys, 2008). Damage effect of visible light with short wavelengths have been reported for spider mites. Whereas, irradiation with UV-A, blue, and green lights caused photo-reactivation of mites damaged by UV-B (Murata and Osakabe, 2014). Hori, et al. (2014) suggested that, the toxicity of visible light with short wavelengths is species-specific in insects, and that shorter wavelengths are not always be toxic. Diapause in *B. mori* eggs is determined by genetic characters and endocrinological mechanisms, mediated by environmental factors such as temperature and photoperiod (Singh and Saratchandra, 2002). Consequently, there were physiological, biochemical and metabolic changes associated with the initiation, maintenance and termination of diapause (Yaginuma et al., 1990).

The present study aims to investigate the effect of using a LED lamp as a cheap and non-emitting heat source of light for improving *B. mori* egg hatchability.

MATERIALS AND METHODS

*Bombyx mori* eggs of two different hybrids (Egyptian and Bulgarian) were used in this experiment. Egyptian hybrid was obtained from the Sericulture Research Department (SRD) of Plant Protection Research Institute (PPRI). The Bulgarian hybrid was imported from Bulgaria. *B. mori* eggs incubation period was realized using the gradual raising of temperature as followed by the SRD routine work which is used especially for incubating eggs kept in the refrigerator and proceed for the second rearing season as follows; after removing the eggs from the refrigerator 5 °C, they kept for three days at 15°C then balance between 27°C and 28°C till hatching.

Two wooden boxes with dimensions (100 X 100 X 100 cm) were used; one was provided with a commercial LED Lamp, Tirivina LED 15 W 6500 k 220 V 50/60 Hz as a non-emitting heat light source. The other one was kept in dark for control egg groups. The two boxes were provided with Hygro-thermometers for adjusting temperature (27–28°C) and humidity (75–85 %) after removing from 15°C. Egg patches were exposed to LED lighting for different periods (24, 72, 120, 168, 192 and 216 hrs.) then transferred to dark box till hatching. Every 48 hrs. 80 eggs from both hybrids (4 replicates of 20 eggs) were transferred from the LED lighting box to the dark one. Another half gram of eggs was taken every 48 hrs. from each egg group for biochemical analysis. Daily follow-up and registration were done till complete egg hatching.

Biochemical Analysis:

Egg samples were homogenized in distilled water then centrifugated at 500 rpm. Homogenates were collected in cold tubes with crystals of phenylthiourea to prevent melanization, then centrifuged at 6000 rpm for 10 min. Supernatant fluid was divided into small aliquots and stored at −20 °C until analysis of main component (total proteins, total carbohydrates and total lipids).

1. Determination of total proteins content

Total proteins were determined by the method of Bradford (1976) using standard of Bovine serum albumin. The absorbance was measured spectro-photometrically at 595 nm. The optical densities is plotted against concentrations, thus a curve can be constructed .

2. Determination of total lipid content

Total lipid was estimated according to Knight, et al. (1972) spectro-photometrically at 620 nm using phosphovanillin reagent. The content of lipid was expressed as mg / gram body weight.

3. Determination of total carbohydrates content

Total carbohydrates were determined according to Singh and Sinha (1977) using Anthron reagent. The absorbance was recorded at 620 nm. The content of carbohydrates was expressed as mg/g body weight.

Statistical analysis

Collected data were recorded and analyzed using statistical analyzing system version 9.1 program proc. GLM (SAS Institute, 2003).

RESULTS AND DISCUSSION

The present study is introducing a simple technique by using wooden box containing a LED lamp and tested...
exposure egg of two hybrids to different light periods (24, 72, 120, 168, 216 hrs);

**Egyptian hybrid:**

As presented in Figure (1), Egyptian hybrid eggs control took about fifteen days till fully hatched. While, under the influence of LED lighting, a gradual improvement in hatchability percentages and reducing the incubation period to fourteen days. Hatching percentages at 216 hrs. LED lighting group were (44, 34, 4%) during (12, 13 and 14th day of incubation, respectively).

![Figure 1](image1.png)

**Figure 1.** Hatching percentages in the Egyptian hybrid eggs under the effect of LED lighting for different periods

**Bulgarian hybrid:**

Control group of Bulgarian eggs took about eleven days to complete hatching while kept at 27 °C. Under LED lighting for 24 hrs, there was a reduction in egg hatchability comparing with control (65 and 75.5 %, respectively). Then, a gradual improvement in hatchability with increasing LED exposure was detected. As well as, egg hatching started at 168hrs.

After nine days by 25 % then increased to 100 % at 216hrs. It could be concluded that, continuous exposure to the LED lighting increases the hatching rate with shortage of incubation period.

The hatchability improvement may be related to the effect of short wavelengths that penetrate egg shell to DNA of the embryo (Longcore et al., 2015).

Embryonic diapause in *B. mori* is attributed to metabolic adjustment which serves to bring about a new physiological state (Singh and Saratchandra, 2002), which encourage the importance of studying the changes in metabolic parameters; proteins, carbohydrates and lipids under the effect of LED lighting. Insect eggs need to be provisioned with nutrients for successful embryonic development, lipids and proteins comprising the main components as ascertained by Diss, *et al.* (1996). Short wavelengths indirectly affect lipids, proteins, and DNA by enhancing the production of reactive oxygen species (ROS) in microbial cells (Santos et al., 2013). The present data clarified that Egyptian eggs are more sensitive and respond to LED lighting than Bulgarian ones. These findings suggest that light absorption by certain inner tissues of the embryo is wavelength-specific. The species-specific photo-sensitizers in insect tissues absorb specific wavelengths of light, thereby generating free radicals and subsequently die from tissue damage caused by free-radical formation as suggested by Hori, *et al.* (2014). Faruki, *et al.* (2007) found that young embryos of *Cadra cautella* (Lepidoptera) were more sensitive to UV rays than older ones. This is support our findings in the present study as the embryos at late stage did not negatively affected under the effect of LED waves.

![Figure 2](image2.png)

**Figure 2.** Hatching percentages of the Bulgarian hybrid eggs under the effect of LED lighting for different periods

**Total protein:**

Egg proteins consist mainly of vitellins, which are mostly transformed into structural elements during embryonic development, while rarely being used as energy sources (Gillot, 2005).

Table (1), represented the statistical analysis between the four tested groups after 24 hrs. following transferring from 15 °C to 27 °C. Bulgarian LED lighting group significantly recorded the highest protein concentration (216.93±2.04 mg / g) comparing with the other tested groups. Observing the protein titer in all tested periods revealed that, the concentration increased gradually till 168hrs. then decreased significantly to (179.78±2.22 mg/g) at 216 hrs.

<table>
<thead>
<tr>
<th>Lighting periods</th>
<th>Egyptian</th>
<th>Bulgarian</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hrs</td>
<td>164.06 ± 1.78 b**</td>
<td>159.67 ± 1.62 b**</td>
</tr>
<tr>
<td>72 hrs</td>
<td>199.26 ± 2.48 b**</td>
<td>151.62 ± 2.69 d*</td>
</tr>
<tr>
<td>120 hrs</td>
<td>195 ± 3.09 b*</td>
<td>159.88 ± 2.94 c*</td>
</tr>
<tr>
<td>168 hrs</td>
<td>203.74 ± 3.16 b*</td>
<td>160.83 ± 3.41 c*</td>
</tr>
<tr>
<td>216 hrs</td>
<td>176.22 ± 3.45 a**</td>
<td>185.83 ± 3.87 c*</td>
</tr>
</tbody>
</table>

Letters in same raw represent the significancy at P < 0.01 between the Egyptian and Bulgarian egg groups. Stars in the same column represent the significancy at P < 0.01 of different periods in the same egg group.

Table 1. Protein levels (mg/g egg weight) in Egyptian and Bulgarian hybrid *B. mori* eggs under LED lighting for different periods (mean ± SE).

After 24 hrs. of LED exposure, Egyptian LED lighting group showed significantly lower protein concentration (164.06±1.78 mg/g) than Bulgarian LED group (216.93±2.04 mg/g). The protein titer increased gradually in all tested periods then decreased significantly after 216hrs. to (176.22±3.45 mg/g and 179.78±2.22, respectively).

From the obtained data, it may be concluded that, LED lighting stimulate the production of proteins in embryos as a kind of modulation to the continuous LED
exposure till 168 hrs, then the concentration remain stable without significant changes at 216 hrs. While, in control groups, protein titer mostly remain stable during all incubation period except Bulgarian control group showed slight increase.

The main egg protein of B. mori was defined by Irie and Yamashita (1983) is glycol-lipoprotein with a mol. wt of 125,000 Da. This molecule contained about 2 % carbohydrate and 4 % lipid, which may explain the high protein concentration to carbohydrates and lipids in the present study. Zhu, et al. (1986) discovered that more than 95 % of the total protein consist of yolk proteins: vitellin (~ 40%), 30 KDa protein (~35%) and egg-specific protein (~20%). Egg-specific protein and 30K protein were gradually disappeared, which may explain the decrease in the protein concentration near egg hatching in the present study.

**Total Carbohydrates**

Changes in total carbohydrates levels (mg / g egg weight) in the tested egg groups are presented in Table (2). In all tested periods, total carbohydrate concentrations of both Egyptian and Bulgarian egg groups did not exceed 45 mg / g egg weight in LED exposure for 24 hrs. resulting in increment in carbohydrate concentrations of Egyptian and Bulgarian egg groups (37.31±0.44 and 39.16±0.52 mg/g) comparing with their controls (32.52±0.43 and 32.78±0.19 mg/g). respectively. The same trend was recorded under continuous exposure for 72 hrs. (45.67±0.62 and 45.17±0.50), and their controls (41.87±0.72 and 45.17±0.50), respectively. After 168 hrs. carbohydrate concentration significantly decreased in LED lighting groups then increased after 216 hrs. of continuous exposure. Total carbohydrate and lipid concentrations increased at hatching time to enhance egg hatchability (Jaronik et al., 2004). Miura and Shimizu (1987) suggested that, during embryogenesis the glycogen content gradually decreased for 5-6 days after oviposition, then there was a rapid decrease until the day of hatching.

**Total Lipids**

Total lipids (mg/g egg weight) concentrations among studied egg groups were showed in Table (3). Generally, continuous LED exposure stimulate the production of lipids in both Egyptian and Bulgarian egg groups and were significantly higher than their controls in all tested periods. A significant increase in lipid titers in control groups of both hybrids egg groups till 120 hrs. then non-significant decrease were recorded at hatching time (216 hrs.).

**Table 2. Total carbohydrate levels (mg / g egg weight) in Egyptian and Bulgarian hybrid B. mori eggs under LED lighting for different periods (mean ± SE).**

<table>
<thead>
<tr>
<th>Periods</th>
<th>LED</th>
<th>Egyptian</th>
<th>LSD</th>
<th>Control</th>
<th>Bulgarian</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hrs</td>
<td>37.31±0.44 a***</td>
<td>32.52±0.43 b***</td>
<td>39.16±0.52 a**</td>
<td>32.78±0.19 b***</td>
<td>1.88</td>
</tr>
<tr>
<td>72 hrs</td>
<td>45.67±0.62 a*</td>
<td>44.80±0.71 a**</td>
<td>45.17±0.50 a*</td>
<td>43.75±0.50 b*</td>
<td>2.68</td>
</tr>
<tr>
<td>120 hrs</td>
<td>46.95±0.81 a*</td>
<td>45.71±0.65 a**</td>
<td>45.73±0.78 a*</td>
<td>44.38±0.47 a</td>
<td>2.88</td>
</tr>
<tr>
<td>168 hrs</td>
<td>42.04±0.47 ab**</td>
<td>43.99±0.63 a*</td>
<td>40.74±0.43 b**</td>
<td>44.41±0.64 a*</td>
<td>2.49</td>
</tr>
<tr>
<td>216 hrs</td>
<td>44.06±0.71 a**</td>
<td>42.55±0.47 a*</td>
<td>44.57±0.61 a*</td>
<td>43.73±0.64 a*</td>
<td>2.77</td>
</tr>
</tbody>
</table>

Letters in same row represent the significance at P < 0.01 between the Egyptian and Bulgarian egg groups. Stars in the same column represent the significance at P < 0.01 of different periods in the same egg group.

**Table 3. Total lipids levels (mg/ g egg weight) in Egyptian and Bulgarian hybrid B. mori eggs under LED lighting for different periods (mean ± SE).**

<table>
<thead>
<tr>
<th>Light period</th>
<th>LED</th>
<th>Egyptian</th>
<th>LSD</th>
<th>Control</th>
<th>Bulgarian</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hrs</td>
<td>20.07±0.17 b***</td>
<td>21.65±0.26 b***</td>
<td>21.93±0.16 b***</td>
<td>4.59</td>
<td></td>
</tr>
<tr>
<td>72 hrs</td>
<td>44.30±0.66 a**</td>
<td>31.75±0.65 b**</td>
<td>38.81±0.38 b**</td>
<td>4.48</td>
<td></td>
</tr>
<tr>
<td>120 hrs</td>
<td>45.10±0.81 a**</td>
<td>46.47±1.42 a**</td>
<td>38.81±0.38 b**</td>
<td>4.48</td>
<td></td>
</tr>
<tr>
<td>168 hrs</td>
<td>43.75±0.89 ab**</td>
<td>43.92±0.64 bc*</td>
<td>36.96±1.97 c*</td>
<td>7.64</td>
<td></td>
</tr>
<tr>
<td>216 hrs</td>
<td>48.49±0.37 a*</td>
<td>32.51±0.61 b*</td>
<td>34.53±0.50 b*</td>
<td>2.82</td>
<td></td>
</tr>
</tbody>
</table>

Letters in same row represent the significance at P < 0.01 between the Egyptian and Bulgarian egg groups. Stars in the same column represent the significance at P < 0.01 of different periods in the same egg group.

Glycogen content decreased with the onset of diapause in B. mori eggs and reached a minimum level then increased again when the diapause was terminated (Miura and Shimizu, 1987). These observations showed that early utilization of carbohydrates and following concomitant consumption of triacylglycerol (TG) in late stage. The second major component of eggs is lipid, which is composed of triacylglycerol (~80%) and phospholipids (~20%). Most of the metabolic energy (approximately 70% of total energy) utilized during embryogenesis is derived from the oxidation of triacylglycerol (Yamashita and Yaginuma 1991).

Since ATP is the key energetic molecule in all cellular states, the effect of visible light with short wave lengths ranged from 655nm to 830 nm on ATP may be of biological significance when cells and tissues are irradiated (Aam et al., 2005). Irradiation increases enzymatic and hexokinase reactions (phosphorylation of glucose), that initiates the metabolic pathway for glycolysis outside the mitochondria (Storey 1980). Glycolysis, where one molecule of glucose is metabolized into two molecules of pyruvate and two molecules of ATP, begins with the hexokinase reaction (Pastorino and Hoek, 2003). Pyruvate can be further processed anaerobically to lactate by lactic acid fermentation (Robers et al., 2004). This reaction is a source of ATP when mitochondria are unable to synthesize ATP and when the cell has a low oxygen concentration (Westerblad and Allen 2003).

**Recommendation**

It may be recommended to use LED (Light Emitting Diode) lighting on *Bombyx mori* L. embryos at the last stage of diapause (after transferring egg boxes from 15 °C to 27 °C) to enhance hatchability and shorten incubation period.

**REFERENCES**
