

MANAGING THE HATCHING OF NON-MULBERRY ERI SILKWORM (*Samia cynthia ricini*) EGGS USING PHOTOPERIOD METHODS

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ABSTRACT

One of the non-mulberry silkworm species that has been widely cultivated is the eri silkworm (*Samia cynthia ricini*). The eggs of the eri silkworm are non-diapause and polyvoltine, allowing the life cycle to continue uninterrupted 8 days after oviposition. This condition challenge eri silkworm farming, as the maintenance schedule cannot yet be regulated. Photoperiod is one method that influences the embryonic development of eggs. This study aimed to evaluate the effect of different photoperiod treatments at a light intensity of 30–40 lux on the hatching regulation of eri silkworm eggs (*S. c. ricini*). The experiment consisted of five treatments with three replications: P0 (12L:12D), P1 (0L:24D), P2 (24L:0D), P3 (8L:16D), and P4 (16L:8D), where L represents light and D represents dark. The observed variables included the temperature and humidity of the treatment chamber, hatching percentage, hatching delay duration, embryonic development, and larval survival. In general, photoperiod treatments did not significantly affect the hatching delay duration across all treatments but successfully delayed hatching by 3.38 ± 1.04 days. The 16 hour light and 8 hour dark photoperiod treatment (16L:8D) resulted in the lowest hatchability, at $84.00 \pm 6.92\%$, which is suspected to be due to the treatment temperature (29.00 ± 0.50 °C) exceeding the optimal hatching temperature range (24–28 °C). The embryonic development pattern and larval viability did not differ significantly

across treatments. Therefore, further research is needed to determine the optimal light intensity (within the range of 30 to 50 lux), so that photoperiod treatments can effectively influence hatching outcomes.

Keyword: Egg; hatching; photoperiod; *Samia cynthia ricini*; eri silkworm

ABSTRAK

Salah satu spesies ulat sutera bukan mulberi yang telah banyak ditenak ialah ulat sutera eri (*Samia cynthia ricini*). Telur ulat sutera eri bersifat tidak diapause dan polivoltin, membolehkan kitaran hidup berterusan tanpa gangguan 8 hari selepas oviposisi. Keadaan ini memberikan cabaran dalam penternakan ulat sutera eri kerana jadual penyelenggaraan belum dapat diatur. Fotoperiod ialah salah satu kaedah yang mempengaruhi perkembangan embrio telur. Tujuan kajian ini adalah untuk menilai kesan rawatan fotoperiod yang berbeza pada intensiti cahaya 30–40 lux terhadap pengaturan penetasan telur ulat sutera eri (*S. c. ricini*). Eksperimen ini terdiri daripada lima rawatan dengan tiga ulangan: P0 (12L:12D), P1 (0L:24D), P2 (24L:0D), P3 (8L:16D), dan P4 (16L:8D), di mana L mewakili cahaya dan D mewakili gelap. Pemboleh ubah yang diperhatikan termasuk suhu dan kelembapan ruang rawatan, peratusan penetasan, tempoh kelewatan penetasan, perkembangan embrio, dan kelangsungan hidup larva. Secara amnya, rawatan fotoperiod tidak memberikan kesan yang signifikan terhadap tempoh kelewatan penetasan bagi semua rawatan tetapi berjaya menunda penetasan selama 3.38 ± 1.04 hari. Rawatan dengan 16 jam cahaya dan 8 jam kegelapan (16L:8D) menghasilkan kebolehpentetasan paling rendah iaitu $84.00 \pm 6.92\%$, kemungkinan disebabkan suhu rawatan melebihi optimum ($29.00 \pm 0.50^\circ\text{C}$). Corak perkembangan embrio tidak menunjukkan perbezaan yang signifikan antara rawatan, begitu juga dengan kelangsungan hidup larva. Oleh itu, kajian lanjut diperlukan untuk menentukan intensiti cahaya yang optimum (dalam julat 30 hingga 50 lux), supaya rawatan fotoperiod dapat mempengaruhi kadar penetasan dengan berkesan.

Kata kunci: Telur; penetasan; fotoperiod; *Samia cynthia ricini*; ulat sutera eri

INTRODUCTION

One of the non-mulberry silkworms domesticated for economically viable cultivation is the eri silkworm, scientifically known as *Samia cynthia ricini* (Lepidoptera: Saturniidae). The eri silkworm is a polyvoltine species, meaning it produces multiple generations within a year, and its eggs do not undergo diapause (Ibrahim et al. 2017). This results in the life cycle continuing 8-10 days after the eggs are oviposited. During the larval phase (from the 1st to the 5th instar), eri silkworms require about 5.045 kg of feed per 100 larvae (Ibrahim et al. 2017). An eri silkworm moth can produce 300-500 eggs per female (Das & Das 2018). As a result, a method for regulating egg hatching is crucial to facilitate the scheduling of rearing times based on feed availability. This practical application of our research can significantly improve the efficiency and productivity of eri silkworm farming, ensuring that the nutritional needs of the silkworms are met for optimal growth and development. Our findings have the potential to inspire new methods and approaches in the field of sericulture, offering hope for improved practices and increased productivity.

One method of regulating silkworm egg hatching is by inducing non-diapause eggs to enter diapause. By inducing diapause in the eggs, their development can be temporarily halted, allowing the hatching time to be controlled. Diapause in insects can occur when the

environment falls outside their comfort zone (extraordinarily high or low conditions) (Caporale et al. 2017; Liu et al. 2016). In such conditions, the eggs respond by suppressing their metabolism to conserve energy. The main factors causing diapause are temperature, humidity, photoperiod, and light intensity (Gill et al. 2017; Rahmathulla 2012). Photoperiod refers to the daily cycle of light and dark periods (Nisak et al. 2017). According to Narasimhulu et al. (2020), differences in day and night lengths (photoperiod) can control hatching time by influencing insects' diapause and circadian rhythm. Photoperiod regulation can result in varying diapause effects in different insects. For example, *Meleoma signoretti* will enter diapause with 16-18 hours of light, while diapause terminates at 12-14 hours of light (Gill et al. 2017). The tasar silkworm *Antheraea pernyi* has a similar photoperiod induction and termination at 12 hours of light and 12 hours of darkness. The intensity of light falling on a surface also affects the diapause condition of eggs, with light intensity measured in lux. Silkworm eggs cannot tolerate light intensities above 50 lux (Narasimhulu 2020; Reddy & Babu 1990). The successful regulation of photoperiod for the hatching of several insect eggs, particularly in silkworms offers a promising opportunity for eri silkworms to control their hatching time by temporarily halting embryonic development. Until now, eri silkworm eggs typically hatch 8–10 days after oviposition, as they are classified as non-diapause eggs. Therefore, the objective of this study was to evaluate the influence of various photoperiod regulation approaches on the hatching performance of eri silkworm eggs (*S. c. ricini*) under controlled light intensities of 30–40 lux.

MATERIALS AND METHODS

Eggs Selection

The eri silkworm eggs (*S. c. ricini*) used in the study were sourced from the Natural Silk Laboratory, Department of Animal Production and Technology, Faculty of Animal Science, IPB University, Indonesia. Eggs sample were collected 48 hours after oviposition. We meticulously selected the eggs to ensure they were fertile by examining and weighing their physical characteristics. The physical attributes of fertile silkworm eggs are perfectly oval, not wrinkled, creamy white, and adhere to one another (Das & Das 2018). The average weight of eri silkworm eggs on the second day after oviposition is approximately 1.7 mg per egg (Naika et al. 2006). A total of 1500 eggs were selected for the study.

Egg Photoperiod Treatments

The selected eggs were placed in a treatment box set up with controlled photoperiod, light intensity (30-40 lux) (Narasimhulu 2020; Reddy & Babu 1990), temperature (24–26 °C), and humidity (75%) (Sarkar & Borpuzari 2022). The photoperiod was regulated using a 3-watt lamp controlled automatically by a Digital Timer Switch Programmable AL-06G, light intensity was measured with a Mini Light Meter UNI-T UT383, and temperature and humidity were monitored using a Digital Thermohygrometer TFA 30-5002. A rigorous pre-lab test, which ensured the photoperiod and light intensity settings met the target conditions, was designed to instill confidence in the reliability of our research. The photoperiod treatments were adapted from Wibowo et al. (2004) for a different type of silkworm and included adjustments for light intensity. Five photoperiod treatments were applied, as shown in Table 1. Each treatment used a sample of 100 eggs with three replications, resulting in a total of 1500 eggs. The experiment was conducted until the eggs hatched or 25 days after oviposition. If no eggs hatched within this period or physical changes such as flattening or wrinkling occurred, the observation was terminated, assuming the eggs were dead and had not developed. The treatment conditions used are displayed in Table 1.

Table 1. Photoperiod treatment on eri silkworm eggs (*Samia cynthia ricini*)

Treatments	Description
P0 (12L:12D)	Photoperiod 12 hours light:12 hours dark
P1 (0L:24D)	Photoperiod 0 hours light:24 hours dark
P2 (24L:0D)	Photoperiod 24 hours light:0 hours dark
P3 (8L:16D)	Photoperiod 8 hours light:16 hours dark
P4 (16L:8D)	Photoperiod 16 hours light:8 hours dark

Evaluation of Egg and Larval Development Parameters

The average delay in hatching time was calculated as the difference between the time it took for the treated eggs to hatch and the normal hatching time for eri silkworms, which is typically 8 days. The hatching time for the treated eggs was determined by multiplying the number of days until hatching by the number of eggs that hatched on day n , then dividing by the total number of sample eggs used (Wibowo et al. 2004). The percentage of eggs hatched is calculated by dividing the treated eggs by the total number of treated eggs and multiplying by 100% (Mishra 2018).

All treatments included embryo observation. The eggs were examined under an Olympus CX33 microscope after being soaked for 3 days in a 5 M sodium hydroxide (NaOH) solution. The NaOH solution dissolved the chorion layer (eggshell), making the embryo transparent and facilitating its development to be more easily observed (Andriani 2009). The larvae of eri silkworms hatched in each treatment were observed for their development up to the 5th instar. Larval survival was calculated by dividing the number of surviving larvae by the total number of hatched larvae during the observation period and multiplying by 100%.

Data Analysis

The data on egg weight, percentage of eggs hatched, and delay in hatching time were analyzed using a Completely Randomized Design (CRD) in R Studio. Meanwhile, data on temperature, humidity, and larval survival were summarized using descriptive statistics (Montgomery 2013).

RESULTS AND DISCUSSION

Temperature and Humidity of The Photoperiod Treatments

In this study, the temperature conditions were at the upper limit (28.74 ± 0.44 °C), while the humidity was at the lower limit ($69.71 \pm 3.39\%$) of the optimal range for eri silkworm egg embryo development (24–28 °C; 70–85%). The temperature and humidity data during the study are presented in Table 2. Silkworms are sensitive to temperature, humidity, and climate changes, affecting embryo development, larval growth, and cocoon production (Ashraf & Qamar 2023). Changes in temperature and humidity can influence fecundity and hatching percentages. High temperatures above 30°C can reduce the metabolic function of the eggs, leading to lower hatching rates (Joan et al. 2022; Rahmathulla 2012). According to Joan et al. (2022), at temperatures between 24–28°C and humidity levels of 70–85%, eri silkworm eggs can achieve hatching rates of up to 96.50%. The hatching trend varies within a 15–35°C temperature range, with maximum hatching percentages occurring at 25°C. The hatching rate declines when temperatures fall below or exceed 25°C (Mishra 2018).

Table 2. Average temperature and humidity in the photoperiod treatment of eri silkworm eggs (*Samia cynthia ricini*)

Treatments	Temperature (°C)	Humidity (%)
P0 (12L:12D)	28.51±0.41	70.89±2.81
P1 (0L:24D)	28.61±0.40	70.28±3.26
P2 (24L:0D)	28.73±0.43	69.42±3.16
P3 (8L:16D)	28.84±0.46	69.17±3.71
P4 (16L:8D)	29.00±0.50	68.81±4.04

P0 = photoperiod 12 hours light:12 hours dark; P1 = photoperiod 0 hours light:24 hours dark; P2 = photoperiod 24 hours light:0 hours dark; P3 = photoperiod 8 hours light:16 hours dark; P4 = photoperiod 16 hours light:8 hours dark

Selected Sample Eggs

The sample eggs used in the study are shown in Figure 1. The sample eggs are perfectly oval, not wrinkled, yellowish-white in colour, and adhere to one another. These conditions are consistent with the findings of Das and Das (2018), which state that fertile eggs are characterized by an oval shape, lack of wrinkles, and the tendency to stick together with a creamy white colour. Wrinkled eggs indicate embryo death, as there is no growth of the embryo (Punyavathi et al. 2022). Fertile eggs can also be detected through their weight. The egg weight data is presented in Table 3. The egg samples used did not show any significant differences ($P>0.05$) among the treatments, aligning with the findings of Naika et al. (2006), which indicate that the weight of eri silkworm eggs is 1.9 mg/egg on day 1, 1.7 mg/egg on day 2, 1.2 mg/egg on day 3, 1.0 mg/egg on day 4, and 1.1 mg/egg on day 5 of oviposition. This study utilized egg samples 2 days old or 48 hours post-oviposition. Therefore, based on the physical characteristics and weight of the eggs, the samples used are confirmed to be fertile.

Figure 1. Fertilized eggs of eri silkworm (*Samia cynthia ricini*)Table 3. Average egg weight for the photoperiod treatment of eri silkworms (*Samia cynthia ricini*)

Treatments	Weight of Egg (mg)
P0 (12L:12D)	1.72±0.07
P1 (0L:24D)	1.70±0.00
P2 (24L:0D)	1.64±0.07
P3 (8L:16D)	1.72±0.06
P4 (16L:8D)	1.59±0.14

P0 = photoperiod 12 hours light:12 hours dark; P1 = photoperiod 0 hours light:24 hours dark; P2 = photoperiod 24 hours light:0 hours dark; P3 = photoperiod 8 hours light:16 hours dark; P4 = photoperiod 16 hours light:8 hours dark

Egg Hatchability

The statistical analysis results that the treatment significantly affected the percentage of hatched eggs ($P < 0.05$), with the lowest hatching percentage observed in the treatment P4 (16L:8D). However, the treatment did not have a significant effect on the hatching time and the delay in hatching time among the treatments ($P > 0.05$). The data analysis results are presented in Table 4.

The photoperiod treatment did not influence the hatching time, likely due to insufficient light intensity (30–40 lux) to affect embryonic development. This phenomenon is also evident from the images of embryo development in the eggs, which appeared relatively similar (Figure 2). Eri silkworm (*Samia ricini*) eggs exhibit a thicker chorion and larger size than *Bombyx mori* eggs. This suggests that a 30–40 lux light intensity has differential effects between eri silkworm eggs and *B. mori* eggs. A 30–40 lux light intensity is optimal for manipulating *B. mori* eggs. The chorion thickness of eri silkworm eggs ranges between 20–25 μm , while that of *B. mori* ranges between 12–20 μm (Kawaguchi et al. 2000; Papanikolaou et al. 1986). The chorion functions as a pathway for sperm entry, a protective barrier for the embryo against environmental conditions such as temperature fluctuations, excessive humidity, desiccation, and bacterial attacks, and facilitates the exchange of carbon dioxide and oxygen during respiration. When photoperiod does not impose stress, the biological cycle of the embryo in the egg progresses normally (Wibowo et al. 2004).

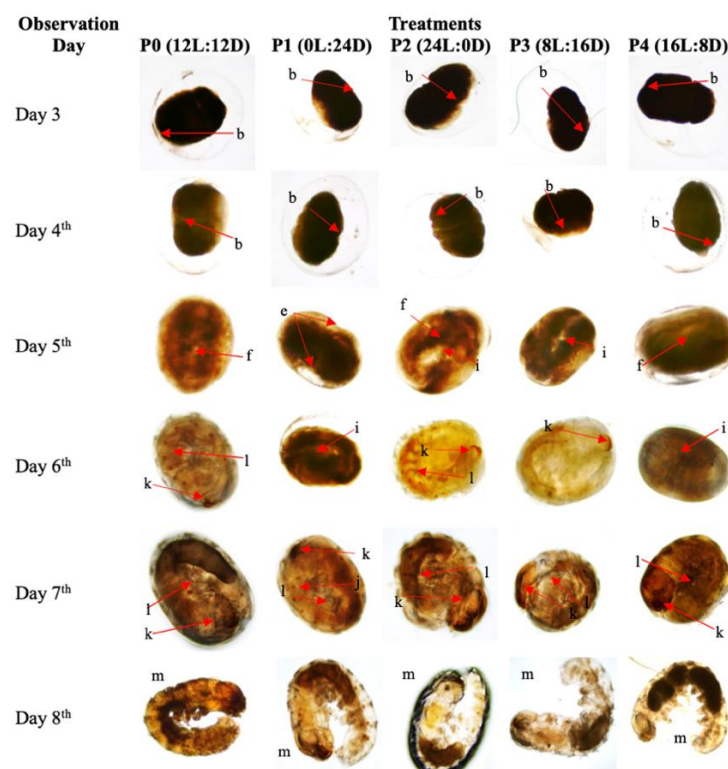


Figure 2. The developmental egg of eri silkworm embryos (*Samia cynthia ricini*) under the photoperiod treatment. Magnification 40x. (a) germinal stage (*germband I*), (b) separation of the protocorm and protocephalon (*germband II*), (c) pyriform shape (pear-shaped), (d) germband resembling a spoon, (e) S-shaped embryo, (f) embryo resembling a circular shape, (g) blastokinesis, (h) setae (bristle-like structures), (i) limbs, (j) spiracles, (k) head pigmentation, (l) body pigmentation, (m) complete pigmentation

Table 4. Average hatching rates for the photoperiod treatment of eri silkworm eggs (*Samia cynthia ricini*)

Treatment	Hatchability (%)	Hatching Time (Day)	Hatching Time Delay (Day)
P0 (12L:12D)	94.67±0.58 ^a	12.17±1.73	4.17±1.73
P1 (0L:24D)	95.33±1.15 ^a	11.01±0.55	4.17±1.73
P2 (24L:0D)	98.00±2.00 ^a	10.80±1.35	2.80±1.35
P3 (8L:16D)	96.67±1.15 ^a	11.74±0.46	2.80±1.35
P4 (16L:8D)	84.00±6.92 ^b	11.17±0.59	3.17±0.59

P0 = photoperiod 12 hours light:12 hours dark; P1 = photoperiod 0 hours light:24 hours dark; P2 = photoperiod 24 hours light:0 hours dark; P3 = photoperiod 8 hours light:16 hours dark; P4 = photoperiod 16 hours light:8 hours dark. Means with the same letters in the same row are not significantly different ($P>0.05$)

The lack of significant differences in hatching time across treatments also resulted in no significant differences in the range of hatching delays due to the hatching delay was calculated as the difference between the hatching time observed in this study and the typical hatching time for eri silkworm eggs, which is 8 days. Hatchability in this study is hypothesized to be more affected by temperature than photoperiod. The temperature during the 16L:8D treatment was higher than other treatments, recorded at 29.00±0.50°C. Temperature influences not only larval development but also embryonic development, which can reduce the egg-hatching percentage. Both temperature and humidity affect fecundity, hatching percentage, and cocoon production (Ashraf & Qamar 2022). The hatching percentage declined at temperatures below 25°C or above 25°C (Mishra 2018).

Embryo Development

Embryo development was observed starting from day 3 until the eggs hatched. Generally, all treatments had eggs hatching by day 8, so the photos displayed are limited to day 8 (Figure 2), even though the average hatching time ranged from 11 to 14 days. The delay is because the hatching time is calculated by multiplying the duration until hatching by the number of eggs hatched on day n , then dividing by the total number of sample eggs used (Wibowo et al. 2004). Embryo observation only began on day 3 (the day the photoperiod treatment started) because the eggs were collected 36-48 hours after oviposition to ensure they were mature. Using eri silkworm eggs (*S. c. ricini*) that were 36-40 hours post-oviposition resulted in the best hatching rate of 72% (Sarkar et al. 2012).

The photoperiod treatments resulted in nearly identical embryo development across all treatments (Figure 2). This result aligns with the hatching times, which showed no significant differences among treatments ($P>0.05$). On days 3 to 4, the eggs entered the germband II phase, which involves the separation of the protocephalon and protocorm. By day 5, the embryos took on a circular shape in all treatments except for P1 (0L:24D), where the embryos still exhibited an S-shape. On day 6, the embryos in treatment P1 (0L:24D) developed legs, while the other treatments began to show head pigmentation. By day 7, almost all embryos had spiracles and exhibited head and body pigmentation. On day 8, the body pigmentation of the larvae was complete, and the eggs were ready to hatch. These results are consistent with the findings of Yokoyama et al. (2021), which indicate that continuous exposure to darkness at a temperature of 25°C can inhibit egg development and even lead to diapause. In this study, treatment P1 (0L:24D) showed delayed embryo development until day 6 compared to the other treatments. The development of silkworm embryos consists of several stages, starting from fertilization and culminating in larvae formation (Miya 2003; Sarkar et al. 2012). A zygote is formed after

fusion during fertilization, followed by cell division occurring approximately 10 hours post-oviposition (cell nucleus division and cell membrane formation). The eggs then enter germband I (separation of the germanlage from the surface of the egg), germband II (separation of the protocephalon and protocorm), germband III (more apparent distinction of the protocephalon and protocorm), and germband IV (formation of the digestive tract). Subsequently, the embryo enlarges, and the mesoderm divides into right and left portions, forming the nervous system and organs. The following stages include the formation of the abdomen, labrum, and spiracles, segmentation of the cephalothorax, development of trichogen cells producing setae and taenidia, and pigmentation of the head and body, concluding with hatching.

Larva Survivability

The larval survivability data shown in Figure 3 indicate that larval survivability remains relatively stable from 1st to 5th instar stage across all treatments, with a slight decrease observed in instar 5. The survivability percentages are approximately $96.89 \pm 2.03\%$ for 1st instar, $94.89 \pm 3.62\%$ for 2nd instar, $93.11 \pm 3.89\%$ for 3rd instar, $91.33 \pm 4.79\%$ for 4th instar, and $91.33 \pm 4.79\%$ for 5th instar. The photoperiod treatments did not significantly affect larval survivability at each instar ($P > 0.05$). This stability is likely because the three photoperiod treatments, P1 (0L:24D), P2 (24L:0D), and P3 (6L:18D), produced similar hatchability, hatching time, and hatching time delays (Table 4). This delay suggests that the photoperiod treatments did not impact egg quality, leading to comparable metabolism and embryonic development. These findings are further supported by the similar embryo development observed in the eggs for treatments P1, P2, and P3 (Figure 2).

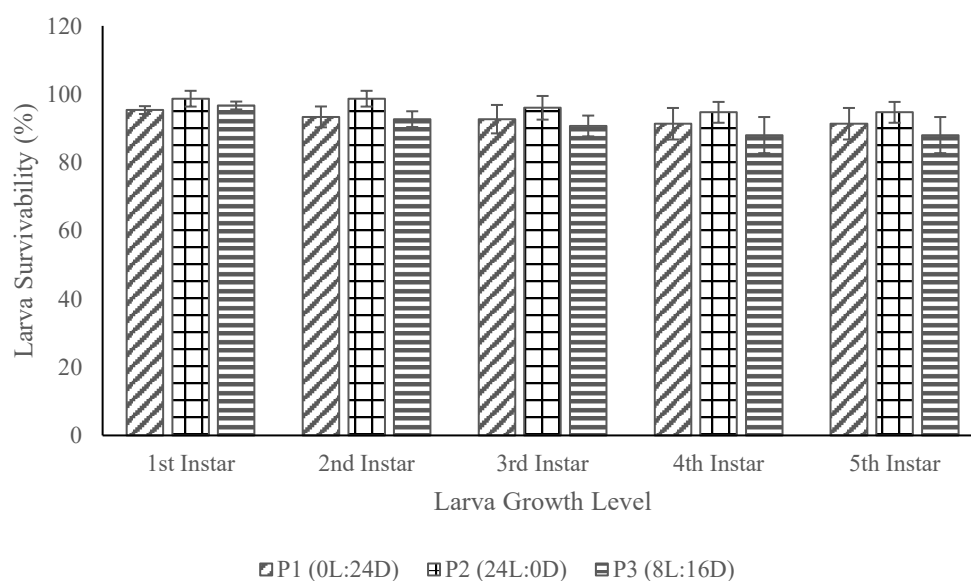


Figure 3. Larva survivability of eri silkworm (*Samia cynthia ricini*), P1 = photoperiod 0 hours light:24 hours dark (0L:24D); P2 = photoperiod 24 hours light:0 hours dark (24L:0D); P3 = photoperiod 8 hours light:16 hours dark (8L:16D)

The survivability of larvae is influenced by environmental conditions such as temperature and humidity, egg quality, and the type of feed (Setiyawan & Fitasari 2018). During the larval rearing phase, all treatments were maintained under identical environmental conditions and fed the same diet, which included a temperature range of $28.69 \pm 0.72^\circ\text{C}$ and humidity of $72.22 \pm 4.57\%$, along with cassava leaves (*Manihot utilissima*). According to

Swathiga et al. (2019), the ideal temperature range for rearing larvae of *S. c. ricini* for optimal development is between 20-35°C. Trisuji et al. (2024) indicated that suitable conditions for the growth of *S. c. ricini* larvae are at a temperature of 27.9°C and humidity of 85.7%. Teronpi et al. (2020) stated that rearing temperature affects the physiological conditions of silkworms in nutrient absorption, digestion, transportation, and circulation, which subsequently impact larval duration and survivability.

In this study, all larvae were provided with the same type and quality of feed, specifically cassava leaves (*Manihot utilissima*). Cassava leaves serve as a secondary feed for *S. c. ricini*, containing 22.23% protein, 2.38% fat, 7.98% ash, and 13.77% crude fiber (Putra et al. 2023). The survivability of the final instar larvae in this study was higher than that of Putra et al. (2023), who used the same feed (*M. utilissima*) but did not apply photoperiod treatments. This study achieved a larval survivability rate of $91.33 \pm 4.79\%$, compared to $82.78 \pm 6.73\%$ in Putra et al. (2023). The survivability of the final instar is crucial as it determines the productivity or the number of cocoons produced, highlighting the significance of these findings in silkworm rearing.

CONCLUSION

In general, photoperiod treatments did not significantly affect the duration of hatching delay across all treatments but successfully delayed hatching by 3.38 ± 1.04 days. Photoperiod treatment at a light intensity of 30–40 lux could not influence the egg-hatching of eri silkworm (*Samia ricini*). This lack of significance is likely due to the thicker chorion of eri silkworm eggs compared to *Bombyx mori*, resulting in different impacts of light intensity on embryonic development. The result is also supported by the embryonic development patterns, which did not show significant differences across treatments, and the larval viability, which remained unaffected. The treatment with 16 hours of light and 8 hours of darkness (16L:8D) produced the lowest hatchability, at $84.00 \pm 6.92\%$, presumed to be due to the treatment temperature exceeding the optimum level ($29.00 \pm 0.50^\circ\text{C}$). Temperatures above 25°C can affect embryonic development, reducing eggs hatching percentage. Further studies are needed to determine the optimal light intensity for eri silkworm eggs so that photoperiod treatments can effectively influence hatching outcomes.

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AUTHORS DECLARATION

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Conflict of Interest

The authors declare no conflict of interest.

Ethics Declarations

No ethical issue required for this research.

Data Availability Statement

The original data is available on request from the author.

Authors' Contributions

Yuni Cahya Endrawati: research design; preparation and execution of the research; data collection; data analysis; writing of the original manuscript; manuscript review and editing. Ronny Rachman Noor: research design; data analysis; manuscript review. Andwi Russpita and Sonia Maretha: preparation and execution of the research; data collection; article administration. Tri Atmowidi: research design; data analysis; manuscript review and editing; article administration.

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