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Effect of Diet Type and Population Density on the Biology of *Samia cynthia ricini* Boisduval (Lepidoptera: Saturniidae)

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ABSTRACT

The silkworm is an insect with high economic value due to its ability to produce cocoons, which are processed into silk fibers. *Samia cynthia ricini* is a type of silkworm relatively new to Indonesia. This insect can utilize various plants as feed, with castor leaves as the primary host and cassava leaves as the secondary host. However, information regarding the impact of diet type and population density on biological parameters, such as survival rate, development time, cocoon weight, and fecundity, remains limited. This study aims to evaluate the effects of diet type (castor and cassava leaves) and population density (20, 30, 40, and 50 individuals per container) on the biology of *S. c. ricini*. The research used a factorial randomized complete block design (RCBD) with two factors. Results indicated that diet type and population density significantly influenced the biological parameters of *S. c. ricini*. Silkworms fed castor leaves exhibited higher survival rates and heavier cocoon weights than those fed cassava leaves. Increased population density reduced survival rates, cocoon weights, and fecundity. The optimal population density for rearing was 30 individuals per container. These findings provide valuable insights for improving *S. c. ricini* rearing practices. Using castor leaves as the primary diet and maintaining an optimal population density of 30 individuals per container can enhance survival and cocoon quality. This information is particularly useful for small-scale and laboratory rearing efforts, contributing to the sustainable development of *S. c. ricini* cultivation and silk production.

Keywords: Eri silkworm, *Manihot glaziovii, Ricinis communis*, survival rate, fecundity.

INTRODUCTION

The silkworm is one type of insect from the order Lepidoptera. It holds significant economic value due to its ability to form cocoons at the end of its larval stage, which can be processed into silk fibers (Hani & Das, 2019). Typically, silk fabrics are produced from *Bombyx mori*, a monophagous insect that feeds on fresh mulberry leaves (Zhang et al., 2019). Meanwhile, *Samia cynthia ricini* is a relatively new silkworm in Indonesia (Trisnawati & Nurkomar, 2020). Unlike *B. mori*, *S. c. ricini* is oligophagous, primarily using castor plants as its primary host and cassava as a secondary host (Nangia et al., 2000; Nurkomar, Trisnawati, & Tedy, 2022). Additionally, it has been reported that *S. c. ricini* can thrive on *Terminalia catappa* plants (Kavane, 2014).

Various factors influence the life of insects, and diet is a primary factor affecting their survival and development, including *S. c. ricini*. For instance, the intrinsic rate of increase, finite rate of increase, gross reproductive rate, and net reproductive rate of *Pectinophora gossypiella* are better when fed an artificial diet than natural food sources (Saeed et al., 2023). Similarly, *Spodoptera frugiperda* reared on a standard artificial diet produces healthy larvae and pupae that emerge into healthy adults with egg-laying capacity comparable to those reared on natural food, such as corn (Navasero et al., 2021).

In addition to diet, space competition (population density) can influence insect life. Previous studies have shown that survival rates and development times can vary significantly with population density. For example, *Coleomegilla maculata* exhibited better survival at a density of 20 individuals compared to higher densities (Riddick & Wu, 2015), while *Lymantria dispar* had longer development times at higher densities (Lazarevic et al., 2004). *Diabrotica virgifera virgifera* female adults originating from larvae reared at high densities exhibited more extraordinary dispersal ability than those reared at low densities (Yu et al., 2019). Similarly, density-dependent morphological effects have been observed in migratory locusts *Locusta migratoria* (Nijhout & Wheeler, 1982), pea aphids *Acyrthosiphon pisum* (Tsumuki et al., 1990), and the migratory moth *Anticarsia gemmatalis* (Fescemyer & Hammond, 1986).

While diet and population density have each been studied independently, their combined effects on insect survival, development, and reproduction remain largely unexplored. Given that both factors interact in natural and artificial rearing conditions, understanding their combined influence is crucial for optimizing the mass rearing of *S. c. ricini* in captivity. Although previous studies (Nurkomar, Trisnawati, & Wening, 2023) have examined some aspects of *S. c. ricini* biology, no research has systematically investigated the interaction between diet type and population density on survival, development time, cocoon weight, and fecundity. Addressing this gap, the present study aims to evaluate how diet type and population density interact to influence these key life history traits in *S. c. ricini*. The findings will contribute to improving rearing strategies and enhancing the economic viability of silk production from this species.

METHODS

The study used a factorial randomized complete block design (RCBD) with two factors. The treatments included diet type (A), castor and cassava leaves, and population density (B) of 20, 30, 40, and 50 individuals per container. The chosen density levels were based on previous research (Nurkomar, Trisnawati, & Wening, 2023), ensuring a range from low to high crowding conditions to assess their impact on *S. c. ricini.* Each treatment was replicated five times.

Diet Source

The diet used is castor (*Ricinis communis*) and cassava (*Manihot glaziovii*) leaves. Leaves were collected from areas around Universitas Muhammadiyah Yogyakarta, Yogyakarta, Indonesia. Freshly picked leaves were washed under tap water and dried using cloth towels to remove dirt and potential pathogens. Leaves were collected daily in the morning and provided to larvae by ad libitum method, starting from the first to the fifth instar.

Insect Source

Samia cynthia ricini's Eggs were obtained from CV. Kupu Sutra, Pasuruan, East Java, Indonesia. The eggs originated from multiple generations reared on castor leaves. For treatments using cassava leaves, larvae were initially reared for one generation on cassava leaves before the experimental generation was used.

Experimental Procedure

The insects were reared starting from the egg stage. The research began with the preparation of eggs based on the studied population density treatments, specifically 20, 30, 40, and 50 eggs, which determined by referring to previous research where the minimum density for *S. c. ricini* propagation was 20, and based on the availability of insects in the lab

Eggs were incubated in a petri dish (86 x 13 mm) until hatching. Hatchlings were transferred to rearing trays (30 x 11.5 x 3.5 cm) containing the designated diet. Larvae were reared in enclosed containers until the fourth instar. Afterward, they were transferred to open trays placed in insect cages (37 x 30 x 33 cm) to prevent overcrowding, reduce disease transmission, and maintain cocoon cleanliness. Feeding schedules varied by instar stage, with more frequent feeding as larvae matured.

After pupation, cocoons were hung with their heads upward to facilitate moth emergence and minimize wing deformities. Adults were maintained in the same cage until death.

Data Analysis

The observed parameters included survival rates, development times for each stage, cocoon shell weight, pupal weight, and adult fecundity. Survival rate was analyzed descriptively, while other data were analyzed using Analysis of Variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) at a 5% significance level using SAS version 9.0.

RESULTS AND DISCUSSION

In previous studies, population density was reported to influence the biology of *S. c. ricini* reared on castor leaves (Nurkomar, Trisnawati, & Wening, 2023). Since *S. c. ricini* also uses cassava leaves as a secondary feed (Nurkomar, Trisnawati, & Arrasyid, 2022), this research was conducted to examine the effects of diet type and population density on survival rate, development time, cocoon shell weight, and adult fecundity of *S. c. ricini*. Which factor has the most significant impact on these parameters? Is it diet type, population density, or their interaction?

The research results indicate a difference in the survival of *S. c. ricini* when reared at different population densities and fed various diets (Figure 1). Under both feeding conditions, survival rates remained high during the early developmental stages across all population densities. However, differences became more evident in the later stages. When fed castor leaves (Figure 1A), survival of *S. c. ricini* began to decline at larval instar five and dropped more significantly at the pupal stage. *Samia cynthia ricini* reared at a population density of 30 individuals showed the highest survival rate at the adult stage (92%) compared to those at other densities. Conversely, when fed cassava leaves (Figure 1B), survival declined sharply at the pupal stage. *Samia cynthia ricini* reared at a population density of 50 had the highest survival rate at the adult stage (90%) compared to other densities. The lowest survival rate was observed in *S. c. ricini* reared at a density of 20, regardless of whether they were fed castor or cassava leaves. Previous studies also reported that *S. c. ricini* reared on castor leaves had better survival than those fed cassava leaves (Nurkomar, Trisnawati, & Tedy, 2022; Ramadhan, 2019).

The larval stage is the most crucial developmental stage for silkworms. The nutrition

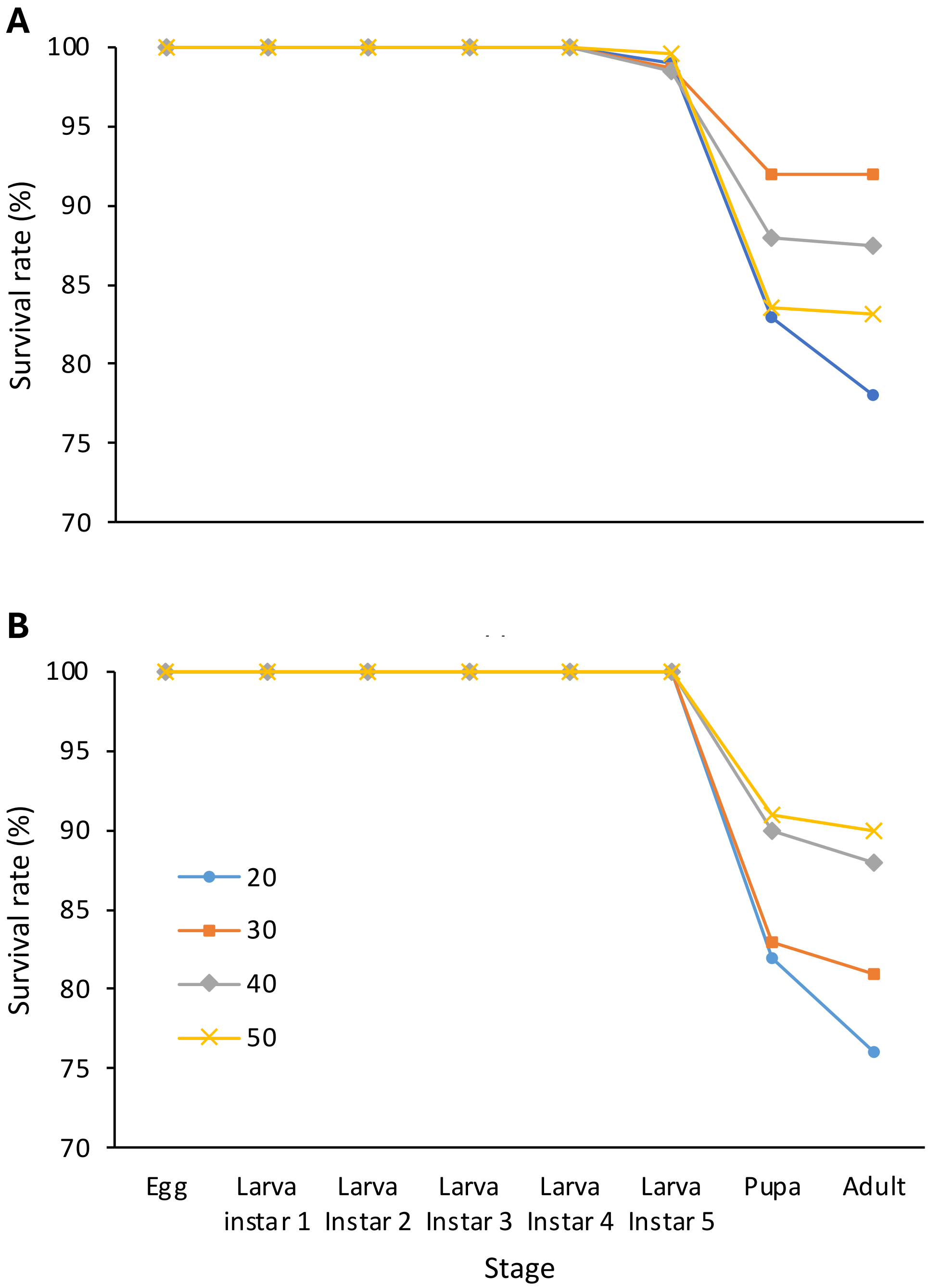

Figure 1. Survival of *Samia cynthia ricini* reared at different population densities and types of diet. A. Castor, B. Cassava

consumed during this stage determines the quality of the resulting cocoons. Castor leave have been reported as the primary feed for *S. c. ricini*, while cassava is considered secondary (Nangia et al., 2000; Nurkomar, Trisnawati, & Arrasyid, 2022; Nurkomar, Trisnawati, & Tedy, 2022; Ramadhan, 2019).

Population density and diet type interact to determine *S. c. ricini's* survival rate. Our previous study found that *S. c. ricini* larvae exhibit gregarious behaviour (Nurkomar, Trisnawati, & Wening, 2023). However, excessively high population density can negatively affect insect growth and development (Hooper et al., 2003). The decline in survival of *S. c. ricini* -fed castor leaves began at larval instar 5. In contrast, the decline in survival of those fed cassava leaves began at the pupal stage, likely due to differences in feed characteristics. Larvae-fed castor leaves produced wet frass, which could disrupt larval development. Meanwhile, larvae

Table 1. Results of analysis of variance (ANOVA)

Parameter	Source	F	dF	P Value
Development time of egg	Type of diet (A)	322.05	1	< 0.0001
	Population density (B)	114.06	3	< 0.0001
	A*B	54.35	3	< 0.0001
Development time of larva	Type of diet (A)	8.91	1	0.0029
	Population density (B)	43.98	3	< 0.0001
	A*B	3.91	3	0.0086
Development time of pupa	Type of diet (A)	3.85	1	0.0499
	Population density (B)	1.59	3	0.1909
	A*B	3.15	3	0.0241
Development time of adult	Type of diet (A)	20.51	1	< 0.0001
	Population density (B)	17.14	3	< 0.0001
	A*B	3.68	3	0.0117
Total development time	Type of diet (A)	0.26	1	0.6196
	Population density (B)	17.07	3	0.0001
	A*B	8.65	3	0.0025
Cocoon shell weight	Type of diet (A)	8865.08	1	< 0.0001
	Population density (B)	1643.80	3	< 0.0001
	A*B	396.82	3	< 0.0001
Fecundity	Type of diet (A)	3.31	1	0.0699
	Population density (B)	2.63	3	0.0504
	A*B	0.40	3	0.7549

fed cassava leaves produced dry frass, better-maintaining survival during the larval stage, which also affected cocoon formation, as indicated by the decrease in survival rate at this stage.

Analysis results showed that egg development time was significantly influenced by diet type, population density, and their interaction (Table 1). The development time of eggs-fed castor leaves remained stable (around 7 days) across various population densities, except at 30 individuals (6.49 ± 0.50 days). In contrast, the development time of eggs-fed cassava leaves increased with higher population density, from 6.12 ± 0.32 days (20 individuals) to 6.83 ± 0.37 days (50 individuals). These findings indicate a strong interaction between diet type and population density (Table 2). Previous studies also reported that *S. c. ricini a* eggs typically develop within 6-7 days (Nurkomar, Trisnawati, & Arrasyid, 2022; Nurkomar, Trisnawati, & Tedy, 2022), and refrigeration can influence egg development time (Nurkomar, Trisnawati, Piyasaengthong, et al., 2023).

Results also showed that larval development time was significantly influenced by diet type, population density, and their interaction (Table 1). Larvae reared on castor and cassava leaves at a density of 30 individuals had longer development times than those at 20 individuals. Larvae reared at densities of 40-50 individuals also had longer development times than those at 30. In other words, larval development time increased with higher population density, regardless of whether the diet was castor or cassava leaves (Table 2). The larval stage is susceptible to population density. This stage is critical for obtaining nutrition from the diet. High population density increases intraspecific competition, limiting resources for each individual, which may prolong development time (Klepsatel et al., 2018).

Pupal development time was influenced only by diet type, while population density did not

Table 2. Development time of *Samia cynthia ricini* reared on different diet and population density

Stadia	Development time (mean ± SD)			
	20	30	40	50
Castor				
Egg	7.00 ± 0.00a	6.49 ± 0.50d	7.00 ± 0.00a	6.96 ± 0.19a
Larva	19.46 ± 1.52d	19.97 ± 1.23c	20.36 ± 1.38b	20.35 ± 1.41b
Pupa	16.52 ± 0.63a	16.30 ± 0.98c	16.33 ± 0.91c	16.37 ±0.96bc
Adult	4.32 ± 0.63d	4.58 ± 0.70bc	4.41 ± 0.63cd	4.38 ± 0.69d
Cassava				
Egg	6.12 ± 0.32f	6.36 ±0.48e	6.58 ± 0.49c	6.83 ± 0.37b
Larva	19.44 ± 0.49d	20.36 ± 0.97b	20.37 ± 0.56a	20.78 ± 0.89a
Pupa	16.43 ± 0.49 abc	16.58 ± 0.49a	16.40 ± 0.49bc	16.48 ± 0.50abc
Adult	4.38 ± 0.77d	5.00 ± 0.86a	4.48 ± 0.69bcd	4.64 ± 0.86b

Means with different letters in the same column are significantly different based on the Duncan test at the 5% level

have a significant effect (Table 1). The interaction between the two also showed no significant impact, making pupal development time relatively stable across all treatments (Table 2). Pupal development time was relatively the same as previous research (Nurkomar, Trisnawati, & Arrasyid, 2022; Nurkomar, Trisnawati, & Tedy, 2022). Refrigeration also affects the cooling time of the pupa (Nurkomar et al., 2024). However, diet type, population density, and their interaction significantly affected the cocoon shell weight of *S. c. ricini* reared on different diets (Table 1). Overall, *S. c. ricini* cocoons reared on castor leaves were heavier than those on cassava leaves. However, both treatments' cocoon weight decreased with increasing population density (Table 3).

These findings highlight the importance of population density management in *S. c. ricini* cultivation to maximize cocoon weight and quality, key success parameters in the silk industry (Winardi, 2021). Cocoon weight is influenced by the larval stage's ability to store proteins for silk fiber production during instars and 5 (Cholifah et al., 2012). High population density may inhibit protein processing into amino acids, resulting in lower cocoon weights (Putro et al., 2016).

Lastly, diet type, population density, and their interaction significantly affected adult development time (Table 1). Adult *S. c. ricini* lived longer when reared at a population density of 30, regardless of whether they were fed castor

Table 3. Cocoon shell weight and adult female fecundity of *Samia cynthia ricini* reared on different diet and population density

Diet	Population density			
	20	30	40	50
Cocoon shell weight (mean ± SD)				
Cassava	0.264 ± 0.001e	0.257 ± 0.002f	0.244 ± 0.005g	0.212± 0.032h
Castor	0.420 ± 0.066a	0.324 ± 0.008b	0.303 ± 0.006c	0.281± 0.005d
Fecundity (mean ± SD)				
Cassava	172.79 ± 103.65b	192.98± 166.90ab	231.41± 211.56ab	232.86± 182.84ab
Castor	175.65 ± 103.65b	263.81± 213.68ab	285.19± 261.48a	269.31± 221.40ab

Means with different letters in the same column are significantly different based on the Duncan test at the 5% level or cassava leaves (Table 2). Diet type, population density, and their interaction also influenced adult female fecundity (Table 1). Females reared at a density of 20 on both diets produced the fewest eggs. There was no significant difference in egg production among females reared on cassava leaves at densities of 30-50. The highest number of eggs was produced by females reared on castor leaves at a density of 40 (Table 3). Endrawati et al. (2006) stated that egg production is more influenced by maternal effects, such as body size, age, and physical condition, which affect egg quality, growth, and embryonic characteristics post-fertilization.

This study has significant implications for the development of *S. c. ricini* cultivation. Castor leaves are recommended as the primary diet, with a population density not exceeding 30 individuals per container, to achieve optimal survival rates and cocoon productivity. Proper diet and population density management can also help reduce disease transmission risks associated with wet frass accumulation at high densities. However, the availability of castor leaves in nature should be considered, given their limited supply, to avoid overexploitation.

CONCLUSIONS

Castor leaves resulted in higher survival rates and heavier cocoon weights than cassava leaves. Increasing population density reduced survival rates, cocoon weights, and fecundity, with an optimal rearing density of 30 individuals per container. These findings provide essential insights for optimizing *S. c. ricini* cultivation on a small scale, such as in laboratory settings, and can serve as a foundation for sustainable silkworm farming. By selecting the most suitable host plants and maintaining optimal rearing densities, farmers and breeders can enhance cocoon production efficiency, improve silkworm health, and support the long-term development of *S. c. ricini* as an alternative silk-producing species.

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