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LIFE CYCLE AND NUTRITIONAL CONTENT OF PUPAE OF *Samia cynthia ricini* (LEPIDOPTERA: SATURNIIDAE) FEEDS ON CASTOR AND CASSAVA

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ABSTRACT

Utilization of *Samia cynthia ricini* is still limited to cocoons. Meanwhile, silkworm pupae can be used as an alternative protein source. This research aims to study the effect of diet on the life cycle of *S. cynthia ricini* fed different diets and analyze the nutritional content of the pupae. The research was conducted by rearing *S. cynthia ricini* using castor and cassava leaves. The observed variables included survival, life duration, fecundity, and nutritional content of pupae. The results showed that the life duration of *S. cynthia ricini* fed with castor leaves and those fed with cassava leaves were not significantly different. 85 percent of the *S. cynthia ricini* population lived until adulthood when fed with castor compared to cassava leaves (65%). *Samia cynthia ricini* fed with castor leaves also has a 0.318 g cocoon shell weight, heavier than *S. cynthia ricini* fed with cassava leaves (0.231 g). Adults raised from larvae fed with castor produced 686.6 eggs, more than the cassava leaves treatment (321.6 eggs). *Samia cytnhia ricini* pupae derived from larvae fed on cassava leaves had higher protein content (73.65%) than castor treatments (55.73%). However, the fat (20.31%), crude fiber (6.64%), carbohydrate (18.35%), and energy (415.55 kcal) contents of pupae derived from larvae fed on castor leaves were higher than those derived from larvae fed on cassava leaves with fat, crude fiber, carbohydrate and energy contents of 14.62%, 5.27%, 5.34%, 374.27 kcal respectively. Using castor leaves in *S. cynthia ricini* cultivation can enhance production quality and provide pupae with better nutrition than those reared using cassava leaves.

Keywords: Life duration; eri silkworm; *Manihot glaziovii; Ricinus communis*; survival rate

ABSTRAK

Penggunaan *Samia cynthia ricini* masih terhad kepada kepompong, sedangkan pupa ulat sutera ini berpotensi digunakan sebagai sumber protein alternatif. Kajian ini bertujuan untuk mengkaji kesan diet terhadap kitaran hidup *S. cynthia ricini* yang diberi makan diet yang berbeza dan

menganalisis kandungan nutrisi pupa. Kajian ini dijalankan dengan menternak *S. cynthia ricini* menggunakan daun kastor dan ubi kayu. Pembolehubah yang diperhatikan termasuk kemandirian, masa perkembangan, kesuburan, dan kandungan nutrisi pupa. Hasil kajian menunjukkan bahawa masa perkembangan *S. cynthia ricini* yang diberi makan daun kastor dan makan daun ubi kayu tidak perbezaan yang signifikan. Sebanyak 85 % daripada populasi *S. cynthia ricini* hidup sehingga dewasa apabila diberi makan kastor berbanding hanya 65% pada daun ubi kayu. *Samia cynthia ricini* yang diberi makan daun kastor juga mempunyai berat sarung kepompong 0.318 g, lebih berat daripada *S. cynthia ricini* diberi makan daun ubi kayu (0.231 g). Individu dewasa yang dibesarkan daripada larva yang diberi makan kastor menghasilkan 686.6 biji telur, lebih tinggi daripada rawatan daun ubi kayu (321.6 biji). Walaupun begitu, pupa *Samia cytnhia ricini* yang diperoleh daripada larva yang diberi makan daun ubi kayu mempunyai kandungan protein yang lebih tinggi (73.65%) berbanding rawatan kastor (55.73%). Namun, kandungan lemak (20.31%), serat kasar (6.64%), karbohidrat (18.35%), dan tenaga (415.55 kcal) pupa yang diperoleh daripada larva yang diberi makan pada daun kastor adalah lebih tinggi daripada yang diperoleh daripada larva yang diberi makan pada daun ubi kayu dengan kandungan lemak, serat kasar, karbohidrat dan tenaga sebanyak 14.62%, 5.27%, 5.34%, 374.27 kcal masing-masing. Penggunaan daun kastor dalam pemeliharaan *S. cynthia ricini* dapat meningkatkan kualiti pengeluaran dan menyediakan pupa dengan nutrisi yang lebih baik daripada yang diternak menggunakan daun ubi kayu.

Kata kunci: Tempoh hayat; ulat sutera eri; *Manihot glaziovii; Ricinus communis*; kadar kemandirian

INTRODUCTION

Indonesia is one of the potential silk-producing countries. So far, two types of silkworms can be cultivated as commercial silkworms, such as mulberry silkworm *Bombyx mori* and non-mulberry silkworm, including Tasar silkworm *Antheraea mylitta*, Muga silkworm *A. assamensis*, and Eri silkworm *Samia cynthia ricini* (Reddy & Yang 2014). *Samia cynthia ricini* is a natural non-mulberry silkworm relatively newly developed in Indonesia. *Samia cynthia ricini* is a commercial silk-producing insect that is thought to have originated in India (Jolly 1981) and has now spread to several countries such as China, Japan (Peigler 1993), Korea (Kim et al. 2012), Kenya (Oduor et al. 2021), Ethiopia (Pallabi & Sharma 2017), Thailand (Tungjitwitayakul & Tatun 2017), and Indonesia (Nurkomar et al. 2022a). *Samia cynthia ricini* is known as peace silk because the pupa can be removed from the cocoon to continue its life cycle when harvesting cocoons as silk thread material. Silk produced from *S. cynthia ricini* is very typical. The texture is slightly rough with a slightly dull color and is easy to combine with other materials such as cotton (Capinera 2008).

So far, the use of *S. cynthia ricini* is still limited to cocoons as a primary material for silk thread. Other utilization efforts are needed to carry out cultivation activities more efficiently and provide more economic value. Ramos-Elorduy et al. (1997) said that insects contain protein, nutrients, fat, minerals, vitamins, and energy suitable for humans and animals. Agbidye et al. (2009) said that 100 g of caterpillars could fulfil 76% of the required protein requirements and almost 100% of the recommended daily amount of human vitamins. Sirimungkararat et al. (2008) also said that the average energy content of insects is comparable to meat based on fresh weight. Therefore, using *S. cynthia ricini* as an alternative protein source is another way to maximize the cultivation processes.

Samia cynthia ricini is an oligophagous insect that can feed on several types of plants, such as castor, cassava (Nangia et al. 2000), and tropical almonds (Trisuji et al. 2024). So far, research has been conducted on the effect of diet type on survival, development, life cycle, fecundity, and cocoon quality (Devaiah et al. 1988; Kedir et al. 2014; Nangia et al. 2000; Nurkomar et al. 2022a, 2022b). Nevertheless, the nutritional composition of *S. cynthia ricini* pupae reared on various diet sources remains unknown. Understanding the dietary profile of *S. cynthia ricini* pupae is essential as a preliminary step in evaluating their potential as an alternative protein source. We hypothesize that differences in diet sources significantly influence the life cycle parameters and nutritional composition of *S. cynthia ricini* pupae. To test this hypothesis, the study aims to: (1) assess the impact of different diet sources on the survival rate, life duration, and reproductive performance of *S. cynthia ricini*; (2) determine the nutritional composition, including protein, fat, and essential micronutrients, of *S. cynthia ricini* pupae reared on different host plants; and (3) evaluate the potential of *S. cynthia ricini* pupae as a sustainable alternative protein source based on dietary variation. By achieving these objectives, this study seeks to provide a scientific basis for optimizing *S. cynthia ricini* cultivation and expanding its utilization beyond silk production.

MATERIALS AND METHODS

Samia cynthia ricini

Eggs were obtained from CV. Kupu Sutra, Pasuruan, East Java. These eggs result from multiplication over several generations and are reared using castor leaves. After hatching, larvae are reared using castor and cassava leaves in different populations for one generation. The 3rd generation was used in the experiments. Eggs were reared until adult in the Plant Protection Laboratory, Faculty of Agriculture, Universitas Muhammadiyah Yogyakarta, at 25±1°C, 80±10% humidity, and 16:8 of a light and dark photoperiod. Rearing activity is carried out for two purposes: to observe the life cycle and to analyze the nutritional content of the pupae.

Effect of Diet on the Life Cycle of *Samia cynthia ricini*

The diet used in this experiment was castor leaves (*Ricinus communis*) and cassava leaves (*Manihot glaziovii*), each with distinct nutritional compositions Table 1 (Purnamasari 2021). These plants were chosen as the primary and secondary diet source for *S. cynthia ricini* (Nangia et al. 2000). The leaves were obtained from areas around Universitas Muhammadiyah Yogyakarta campus, Kasihan, Bantul, Yogyakarta. Before use, the leaves are washed with tap water and then air-dried at room temperature.

The experiment was conducted by rearing *S. cynthia ricini* in the leaves diet tested as a treatment. Each treatment was repeated three times. Each treatment was tested using 30 *S. cynthia ricini*. In total, six experimental units and 180 silkworm larvae were used in this research.

Table 1. Nutritional content of castor and cassava leaves

Component	Castor	Cassava
Water (%)	67.79	68.58
Ash (%)	3.62	4.13
Fat (%)	1.00	2.43
Protein (%)	5.55	7.53
Carbohydrate (%)	12.03	17.33
Crude fiber (%)	9.21	9.09

Eggs were incubated in an 83 mm petri dish until hatching. Larvae instar 1-3 were reared in plastic boxes (17 x 11.5 x 7 cm) as rearing containers. The 4th instar larvae were reared in larger plastic boxes (32 x 25 x 5 cm) until cocooned. The rearing container is lined with damp paper to maintain the moisture and freshness of the leaves' diet. First-stage larvae (instar 1 to 3) are fed small pieces of chopped leaves, while fourth and fifth-instar larvae are fed intact leaves. First instar larvae are fed once per day in the morning, second instar larvae are fed twice daily in the morning and evening, and third to fifth instar larvae are fed thrice daily in the morning, afternoon, and evening. One to two leaves are provided during each feeding. After cocooning, pupae are reared in a moth cage (37 x 30 x 33 cm) until the moth emerges. The moths are kept in these cages until they die.

During rearing, survival, life duration (eggs, larvae, pupae, and adults/moths), and adult fecundity were observed every day for one generation. The weight of the cocoons produced was also weighed using an analytical balance.

Nutrient Content of *Samia cynthia ricini* Pupae Reared on Different Diets

The rearing of *S. cynthia ricini* follows the same procedure as described earlier. When *S. cynthia ricini* enters the pupal stage, the pupa is separated from the cocoon. The pupae were then dried using an oven for 4 hours at 100°C. After the pupae were wholly dried, they were homogenized into a fine powder using a blender. Nutrient content was analyzed, including ash, water, fat, protein, crude fiber, and calories. This analysis refers to the Association of Official Analytical Chemists International - AOAC method (Horwitz & Latimer 2012) with three replications. Water content (oven) and ash content are determined using a gravimetric approach. Fat content was determined using the Soxhlet method, the protein content was quantified using the Micro Kjeldahl technique, crude fiber was analyzed using the acid-base hydrolysis method, and carbohydrates were calculated using the by-difference method. The calorific value per 100 grams of sample is determined by the Atwater factor conversion, namely 4 kcal per gram of protein, 9 kcal per gram of fat, and 4 kcal per gram of carbohydrates. The energy value of total carbohydrates was corrected for the fiber content of both groups of pupae.

Statistical Analysis

The survival of *S. cynthia ricini* was analyzed using Kaplan Meier's survival analysis. Differences in life duration (eggs, larvae, pupae, and imago), cocoon weight, pupal weight, and pupae nutritional content (water content, ash content, fat, protein, crude fiber, carbohydrates, and calories) *S. cynthia ricini* reared using castor and cassava leaves were analyzed using an independent *t* test (Student's *t* test). Meanwhile, the adult's daily fecundity was analyzed using an analysis of variance with repeated observations. The result was subsequently subjected to Duncan's Multiple Range Test at $\alpha = 5\%$ for further testing. Data analysis was performed by using R Statistic ver. 4.2.2 (R Core Team 2015).

RESULT AND DISCUSSION

Diet is the main factor in the life of insects, including *S. cynthia ricini* because it can affect their growth and development. Several studies regarding the effect of diet on the growth and development of *S. cynthia ricini* has been carried out (Devaiah et al. 1988; Kedir et al. 2014; Nangia et al. 2000; Nurkomar et al. 2022a, 2022b). However, how diet affects the life cycle and nutritional content of *S. cynthia ricini* pupae remain unknown. It is essential to determine the nutritional composition of *S. cynthia ricini* pupae before using it as an alternative protein source.

The results showed that *S. cynthia ricini* fed on castor leaves had better survival than those fed on cassava leaves (*P<0.001*, Figure 1). Survival of *S. cynthia ricini* decreased to 98.8% during the 1st instar larvae when fed on castor leaves and again reduced to 92.2% during the 4th instar larvae. Survival decreased to 85.5% from the 5th instar larvae to adulthood. Meanwhile, the survival of *S. cynthia ricini* decreased to 80% during the 1st instar larvae when fed on cassava leaves and continued to decline to 77.78, 75.56, and 65.56%, respectively, during the 3rd, 4th, and 5th instar larvae until adulthood.

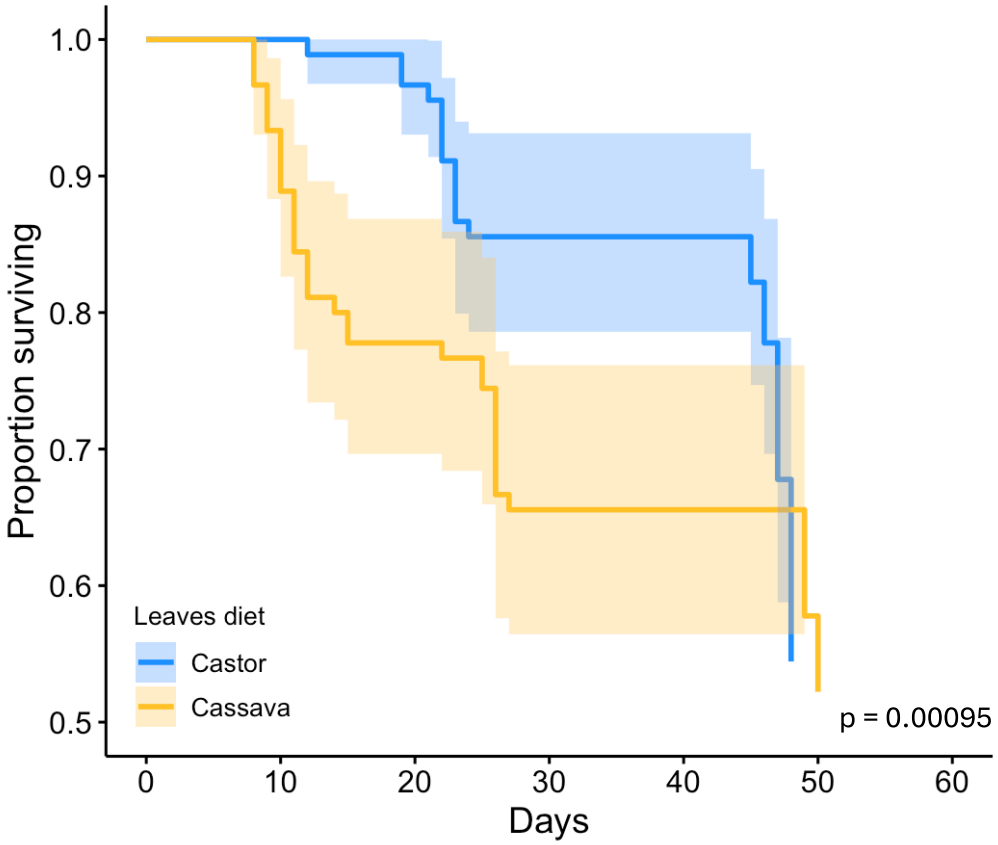

Figure 1. The survival rate of *Samia cynthia ricini* fed on castor and cassava leaves

Previous research also revealed that the survival of *S. cynthia ricini* fed on castor leaves differs from those feds on cassava leaves (Nurkomar et al. 2022a; Ramadhan 2019). Similarly, *S. cynthia ricini* exhibited varying survival rates when fed on different varieties of cassava leaves (Nurkomar et al. 2022b).

Meanwhile, there was no difference in the life duration of eggs, larvae, pupae, or adults of *S. cynthia ricini* when fed on castor or cassava leaves. Eggs *S. cynthia ricini* originating from adults whose larvae were fed both types of feed developed for six days. Larvae developed for 21.8 and 20.8 days when *S. cynthia ricini* was fed on castor and cassava leaves, respectively. Meanwhile, the pupae developed for 16.08 and 16.38 days, while the adults lived for 4.62 and 4.54 days when the larvae were fed on castor and cassava leaves, respectively (Figure 2). In contrast, previous studies also reported that the life duration of *S. cynthia ricini* fed on castor leaves differed from those feds on cassava leaves, especially in the life duration of larvae, pupae, and adults (Nurkomar et al. 2022a). The different results may occur because plant

genotypic characteristics can influence egg hatchability, larval life duration, and survival of *S. cynthia ricini* (Kedir et al. 2014).

Figure 2. Life cycle of *Samia cynthia ricini*. Life duration of *Samia cynthia ricini* when fed on castor (A) and cassava leaves (B). A number indicates the life duration of each stadia

However, pupae from larvae that were fed on castor leaves had either heavier pupae (Independent t test, $P = 0.005$) or cocoon weights (Independent t test, $P = 0.01$) (Table 2). Cocoons are critical in silk production because the heavier the cocoon, the more thread can be produced.

Table 2. The cocoon's shell weight and fecundity of *Samia cynthia ricini* fed on castor and cassava leaves

Diet Source	Pupal Weight (G)	Cocoon Weight (G)	Total Fecundity
Castor	2.095±0.345	0.318±0.089	686.0±19.79
Cassava	1.669±0.348	0.231±0.068	321.63±5.61

Devaiah et al. (1988) also said that *S. cynthia ricini* reared on castor leaves had heavier larvae and cocoon weights. Hartati & Umar (2012) added that the weight of the cocoon shell is influenced by the ability to produce fiber, caterpillar species, feed quality, temperature, and humidity levels during the transition to the pupal stage.

Another critical aspect is adult fecundity. Adult females raised from castor treatments could also produce eggs twice as much as those from cassava treatments (dF = 1; F = 13.55; $P<0.01$). Furthermore, the number of eggs laid by females was also influenced by female age (dF = 8; F = 8.46; $P<0.001$). Generally, females lay the most eggs on the second and third days. As the female ages, the number of eggs will decrease (Figure 3). Gowda et al. (1989) said that cocoon and pupal weights had a positive relationship with egg production. Additionally, Jayaswal et al. (1993) said that as the pupal weight increases, the number of eggs it produces increases, and vice versa. Although the relationship between pupal weight and the number of

eggs laid was not observed in this study, *S. cynthia ricini* larvae fed on castor leaves had heavier pupal weight and higher fecundity than *S. cynthia ricini* larvae fed on cassava leaves.

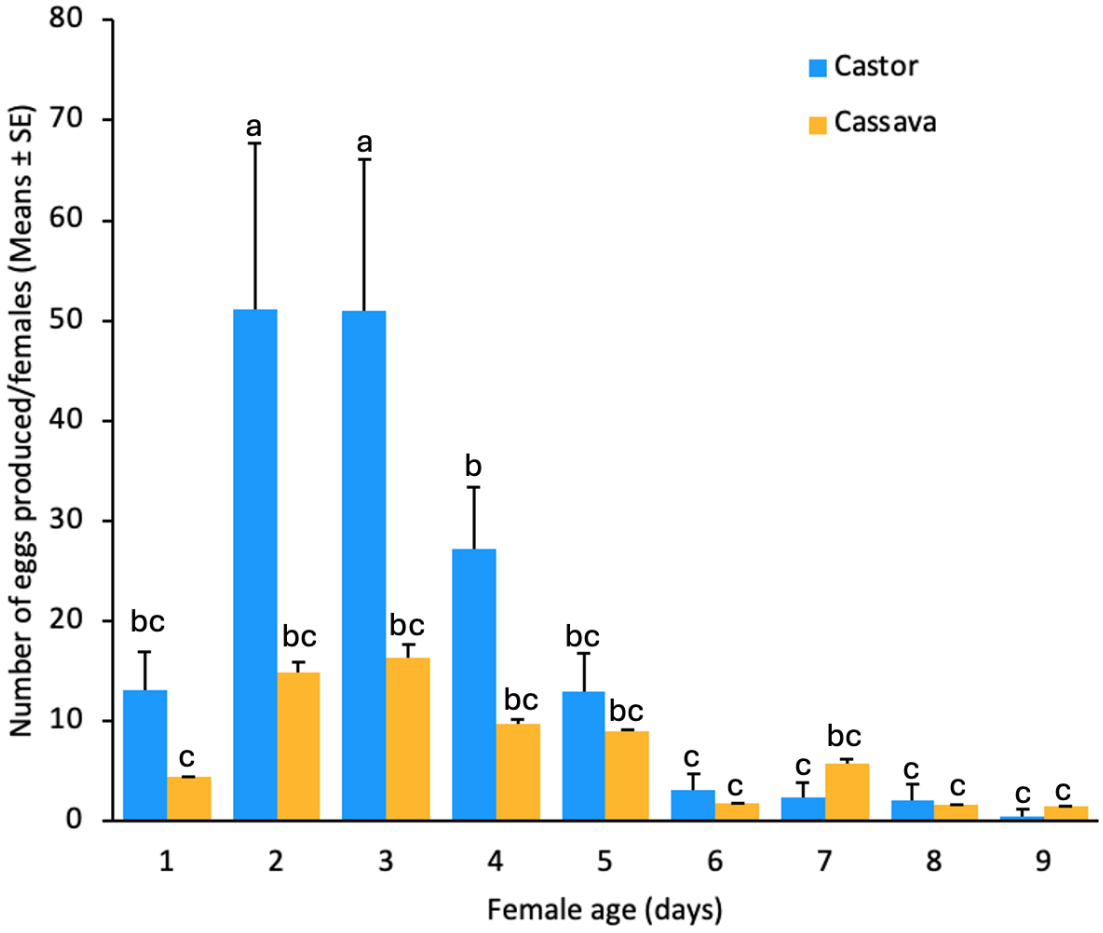

Figure 3. Adult fecundity of *Samia cynthia ricini* fed on castor and cassava leaves. Means followed by different letters indicate significant differences based on Duncan's Multiple Range Test. SE: Standard Error

The proximate analysis showed that the pupae originating from larvae fed on cassava leaves contain higher ash and protein (Independent t test, $P<0.05$) than those fed on castor leaves. However, the fat, crude fiber, carbohydrate, and energy levels of pupae originating from larvae fed on castor leaves were higher (Independent t test, $P<0.05$) than those fed on cassava leaves (Table 3). The protein and fat content of pupae from the two treatment groups ranged between 55.73-73.65% and 14.62-20.31%, respectively. Other studies consolidated by Hăbeanu et al. (2023) also reported that several silkworm pupae contain around 48-75.1% level protein, followed by fat content ranging from 12.1-35.7%. Pupae of *S. cynthia ricini* reported in this study has a higher protein content than other insects such as grasshoppers, black soldier flies, house flies, mealworms (Shah et al. 2022), and even *Bombyx mori* (Pereira et al. 2003). The fat content of *S. cynthia ricini* pupae was higher in the castor leaves treatment group than in the cassava leaves treatment group. This finding implies that the castor leaves treatment group pupae have a higher total energy content per 100 grams.

Table 3. Nutritional content of *Samia cynthia ricini* pupae fed on castor and cassava leaves

Component	Diet Source (Mean±SE)	
	Castor	**Cassava**
Water (%)	8.21±0.03[a]	12.24±0.02[b]
Ash (%)	5.60±0.04[a]	6.39±0.05[b]
Fat (%)	20.31±0.11[b]	14.62±0.04[a]
Protein (%)	55.73±0.31[a]	73.65±0.04[b]
Crude fiber (%)	6.64±0.24[b]	5.27±0.03[a]
Carbohydrates *by difference* (%)	18.35±0.34[b]	5.34±0.05[a]
Calories (kcal/100 g)	415.44±1.37[b]	374.27±0.13[a]

Means followed by different letters indicate significant differences based on the independent Student's *t*-test. SE: Standard Error

This study's *S. cynthia ricini* pupae fiber content ranged from 5.27-6.64%. The fiber content of pupae for several types of silkworms has been reported to vary between 3.5-14%. The protein in various silkworm pupa comprises 18 kinds of amino acids with a superior essential amino acid composition. In addition, the protein in various silkworm pupa is also known to have high digestibility. Several studies report that the fat of various silkworm pupae is dominated by unsaturated fatty acids (60-70%), especially the essential fatty acids omega-3 (linolenic acid) and omega-6 (linoleic acid). The carbohydrates in various silkworm pupa are composed of chitin and chitosan, which have various biological activities. Furthermore, the pupae of various silkworms are also known to be rich in phosphorus, magnesium, and calcium, along with various types of water-soluble vitamins (B1, B2, B3, B5, B7, B9, B2, and C) and fat-soluble vitamins (A and E) (Mahanta et al. 2023). This fact can support the potential of silkworm pupa as a nutritious food/feed ingredient. Differences in nutrient content in silkworm pupae can be influenced by various factors, such as the chemical composition of the diet used, diet intake, digestibility coefficient, availability (bioavailability) of nutrients, analysis methods, sampling techniques, etc. (Hăbeanu et al. 2023).

In this study, protein levels in pupae of *S. cynthia ricini* from the cassava leaves treatment group were higher compared to the castor leaves treatment group. Although testing for the nutritional content of both types of leaves was not carried out, a study conducted by Purnamasari (2021) reported that cassava leaves had higher protein content (23.9%) compared to castor leaves (17.2%). The ash, fat, and carbohydrate content of cassava leaves are also higher than that of castor leaves, which can support protein and biomass synthesis in *S. cynthia ricini* larvae and pupae. The availability of minerals in the diet can also influence the accumulation of minerals (ash) in pupae. A positive correlation between diet/plant protein levels and insect protein levels was reported by Gou et al. (2020). The correlation between protein levels in the leaf diet and *S. cynthia ricini* pupae is an additional intriguing component that warrants further investigation. This condition can be associated with anti-nutritional substances such as tannins and HCN (cyanic acid) found in cassava leaves. Tannins have been known to reduce the digestibility and availability of protein and minerals in the diet. Tannin levels in fresh cassava leaves can reach 20-50 g/kg depending on the level of maturity and cassava variant. HCN is known to have quite significant toxic effects on cells. Normal levels of HCN in cassava leaves are around 200-800 mg/kg (Hue et al. 2012). Sakthivel (2018) reported that the levels of tannin and HCN in cassava leaves of various variants were negatively

correlated with the energy efficiency ratio, cocoon weight, survival, and fecundity of the silkworm *S. cynthia ricini*.

CONCLUSION

There is no difference in the life duration of *S. cynthia ricini* fed on castor leaves and those fed on cassava leaves. However, *S. cynthia ricini* fed on castor leaves exhibited higher survival rates, cocoon weight, pupal weight, and fecundity. On the other hand, pupae of *S. cynthia ricini* fed on cassava leaves had higher protein levels, whereas those fed on castor leaves showed higher fat, crude fiber, carbohydrate, and energy content. These findings have significant implications for sustainable agriculture and commercial insect farming. Farmers cultivating *S. cynthia ricini* for silk production may prefer castor leaves to maximize survival rates and silk yield. However, if the goal is to produce *S. cynthia ricini* as an alternative protein source, cassava leaves could be a viable option due to their ability to enhance protein content in pupae. Additionally, integrating *S. cynthia ricini* cultivation with existing agricultural systems could promote circular farming practices, where castor and cassava plants serve as dual-purpose crops providing food for insects while maintaining soil health and biodiversity. This approach could enhance the economic viability of silk farming and edible insect production, offering farmers diversified income streams and more efficient resource utilization.

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AUTHORS DECLARATIONS

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Conflict of Interest

Not applicable

Ethics Declarations

Not applicable

Data Availability Statements

The data supporting this study's findings are available on request from the author.

Author's Contributions

Ihsan Nurkomar (IN) supervised the overall research project and contributed to the initial draft, data analysis, and data curation. Dina Wahyu Trisnawati (DWT) managed project administration and participated in data analysis. Saraswati (S) conducted the proximate analysis and contributed to data interpretation. Rahmadhyta Fitria Fadilah Riali (RFFR) performed the experiments and co-wrote the initial draft. All authors contributed to the revision process and approved the final version of the manuscript.

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