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Impact of glycine fortification of cassava leaves on the late instar larvae of eri silkworm (*Samia cynthia ricini* D.)

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ABSTRACT

The fortification of cassava leaves by adding nutrition is a recent technique in the ericulture study. Glycine is one of the amino acids necessary for the growth of silkworms. This study aimed to analyse the effect of glycine fortification of cassava leaves on the growth and development of eri silkworms. The parameters of this study were cocoon parameters, the wingspan of imago, development time and mortality, and egg parameters of *Samia cynthia ricini* D. This study had four treatments: control group, P1 group (adding glycine 0.5% on cassava leaves), P2 group (adding glycine 1% on cassava leaves), and P3 (adding glycine 1.5% on cassava leaves). The early instar of Eri silkworm larvae was fed on castor leaves, and glycine fortification was fed on the late instar of Eri silkworm larvae (fourth and fifth instars). Based on the results, all parameters showed significant differences from the control group. Glycine fortification increased cocoon weight (2.03 ± 0.62 g), extended the wingspan of the imago (12.15 ± 1.12 cm), shortened the development time (44.03 ± 0.84 days), had no mortality, and increased the fecundity of eggs (134.80 ± 28.78 eggs). The most effective concentration of glycine fortification on cassava leaves was 1.5%. Conclusively, cassava leaves with glycine fortification affect the growth and development of eri silkworm.

Keywords: glycine, eri silkworm, cassava leaves, cocoons, eggs

INTRODUCTION

Silkworm rearing aims for economic empowerment and boosts the country's textile industry (Oduor et al., 2016). Ericulture is the traditional eri silkworm rearing based on industrial agriculture, producing protein-rich cocoons for silk fabrics (Sakthivel and Qadri, 2013). Eri silkworm (*Samia cynthia ricini*) is in the non-mulberry sector that is commercially exploited to produce the textile industry's natural silk (Subramanianan et al., 2013). Due to the increased price of eri silk at the marketplace, there was a growing demand to produce Eri cocoons. The demand for eri cocoon production leads to the massive growing of eri silkworm food plants on a large scale as crops to supply ericulture (Sakthivel, 2018).

Eri silkworm (*Samia cynthia ricini* D.) is a polyphagous species that feeds on Euphorbiaceae, Araliaceae, Apocynaceae, and Simaroubiceae family leaves. Castor (*Ricinus communis*) leaf is the primary host plant of eri silkworms, and cassava leaf (*Manihot esculenta*) is a secondary host plant (Chutia et al., 2014). Based on Deuri et al. (2017), the biomass of fresh castor leaves contains crude protein (17.43%), lipid (10.95%), and crude fiber (5.53%), and the biomass of fresh cassava leaf contains lower crude protein (10.83%). Moreover, another study about the nutrition of cassava leaf showed that cassava leaf composite, especially the variety MVD1, contains more crude protein (28.18%) than variety CO_2 cassava leaves (19.75%) (Sakthivel, 2016).

In Indonesia, castor and cassava are abundances of crops. Cassava (*Manihot esculenta*) has a high yield potential of 100 tons of fresh roots/ha (Saleh et al., 2001). The abundance of cassava crops could support the rearing Eri silkworm commercially and add income for the country by using a portion of the foliage without affecting the primary income generated from the main produce of the host plant.

Rearing Eri silkworm not only feeds primary and secondary food plants but also involves supplementation for the food plants. Fortification is a technique used in advanced sericulture research and development to increase cocoon yield and silk content by supplementing amino acids on food plants for their growth and development (Shivkumar et al., 2020). Furthermore, Murugesh et al. (2021) revealed that glycine fortification on the late instar (4^{th} and 5^{th}) of *Bombyx mori* larvae increased the diameter of the silk cocoon.

Many studies on the fortification of amino acids in silkworm food plants are limited to the silkworm *Bombyx mori* fed on mulberry leaves. However, the investigations on Amino acid fortification in Eri silkworm (*Samia cynthia ricini* D.) feed during rearing are extremely limited. Therefore, this study aimed to determine the effect of glycine fortification on cassava leaves in late-instar silkworms on growth and development.

MATERIALS AND METHODS

This study used experimental design and randomized block design. There were four treatment groups with 30 larvae per group. One hundred twenty eggs of eri silkworm (*Samia cynthia ricini* D.) were collected from the silkworm farm Kupu Sutra Pasuruan, East Java, Indonesia. The study was conducted from July 2021 to January 2022 at Faculty Teacher Training and Education, University of Jember

Rearing the early instar of larvae

The early larvae instar is Eri silkworm larvae's 1^{st} – 3^{rd} instar. These larvae were fed with young castor leaves (3 x 3 cm^2). Feeding was a little at first and later used much feed gradually. Humidity was 70% during the molting process for complete skin replacement. The rearing methods were adopted from Murugesh et al. (2021)

Glycine fortification for the late instar of larvae

Glycine fortification in cassava leaves was administered to late instar larvae of eri silkworms with three concentrations (P1 for 0.5%; P2 for 1%; P3 for 1.5%). Five grams of glycine powder were dissolved in 1000 ml of distilled water for a concentration of 0.5% (5000 ppm). Likewise, making a concentration of 1% (10000 ppm) required 10 grams in 1000 ml of distilled water and 15 grams in 1000 ml for a concentration of 1.5% (15000 ppm). The fresh, full-bloomed cassava leaves from the seventh shoot were sprayed separately with glycine solutions using a hand automizer. The leaves were air-dried for a minute and fed to the late instar (4^{th} – 5^{th}) of larvae three times a day until the larva became transparent, indicating that it would start the cocoon process. One day of feeding required 450 ml of fortification solution for 12 leaves at instar 4th and 18 leaves for instar 5^{th}.

Parameters observation

Cocoon parameters

The cocoon parameters involved cocoon weight, cocoon shell weight, and shell ratio. Cocoon weight was measured five days after cocoon formation. The estimated weight was the accumulation of pupa weight, which contained the prospective imago (pupa), fluid, exuvium, and cocoon shell. Cocoon shell weight was measured after the pupa was removed from the cocoon. The shell ratio was calculated using Evans' formula (1939).

Wingspan imago

The wingspan of an adult moth was measured with a vernier caliper. The wingspan was calculated from the right to the widest side's left end.

Development time and mortality

Development time was taken from the time (days) for the silkworm to develop from egg hatching to larvae cocooning. Mortality was the number of dead larvae per larvae reared.

Egg parameters

The egg parameters involved fecundity – the number of eggs produced by female imago—and egg hatchability. Hatchability was measured from the number of hatched larvae per disease-free laying on percentage (see Nagadevara, 2004).

Statistical analysis

All data were measured by mean ± standard deviation (SD) and were analysed by SPSS 24.0 for Windows. The data were subjected to one-way analysis of variance (ANOVA) following the LSD test as a post hoc analysis. The significance of variance was $P<0.05$.

RESULTS AND DISCUSSION

Cocoon parameters

The cocoon parameters included cocoon weight, cocoon shell weight, and shell ratio. The results revealed that fortified 1% and 1.5% glycine (P2 and P3 groups) significantly enlarged the cocoon and shell weights (Table 1). The shell ratio increased dramatically in the P3 group with 1.5% glycine fortification. Recent studies showed that glycine is a vital amino acid to regulate silk synthesis and significantly enhanced the growth of silkworms (*Bombyx mori*) (Paital and Bohidar, 2011; Saad et al., 2019). Glycine fortification improves the glycine content within the larvae body. Once the quantity of glycine in the larval body exceeds the metabolic needs, it converts into acetyl-CoA into amphibolic intermediates as a substrate for carbohydrate and lipid synthesis to produce energy (Murray et al., 2003). The shell ratio results of this study significantly increased in the P3 group 20.87. The shell ratio data were directly proportional to the increase in cocoon weight and cocoon shell weight of a 1.5% glycine concentration. These results are better than Deka's (2011) study that a shell ratio of 12.66% on 1% glycine concentration increased the percentage by 10.4% compared to the control.

Wingspan imago

These study results showed that glycine fortification significantly enlarges the wingspan on 1% and 1.5% concentrations (Table 1). The higher concentration of glycine, the wider wingspan. Glycine fortification helps cocoon formation supply the nutrition of larvae. Glycine synthesis in the cocoon forms high pupae weight and forms a perfect body structure in the moth (imago) (Deka et al., 2011). Imago of *Samia cynthia ricini* D. shows the existence of sexual dimorphism in morphological size. The female imago has a larger size than the male imago. The wingspan in this study was more significant than that in the study on the development of the silkworm without glycine fortification conducted by Brahma et al. (2015). Imago, which has an enormous wingspan, is influenced by larval activity before entering the cocoon. When the larvae are larger and heavier, they will produce cocoons of a larger size and weight. Cocoons with a larger size will provide more space to form moth organs (Sõber et al., 2019). The wingspan in male moths affects mating

Table 1. Glycine fortification of cassava leaves on growth parameters (cocoon parameters and wingspan)

Treatment groups	Cocoon parameters			Wingspan (cm)
	Cocoon weight (g)	Cocoon shell weight (g)	Shell ratio (%)	
K	1.32±0.09^{a}	0.19±0.05^{a}	15.62±5.15^{a}	10.89±0.49^{a}
P1	1.59±0.42^{ab}	0.22±0.08^{a}	15.84±3.28^{a}	11.22±0.95^{a}
P2	1.66±0.42^{b}	0.28±0.07^{b}	16.79±3.20^{a}	12.09±0.11^{b}
P3	2.03±0.62^{c}	0.32±0.10^{b}	20.87±6.05^{b}	12.51±1.12^{c}

Note: Different letters in each data label show significant differences ($P<0.05$). K for control group; P1 for 0.5% glycine fortification; P2 for 1% glycine fortification; P3 for 1.5% glycine fortification

success because males with broad wings find it easier to mate. After all, it is easier to fly to female moths (Teronpi et al., 2020).

Development time and mortality

Development time is significant when raising eri silkworms because the faster the development time, the more efficient it is. The results of this study (Table 2) explained that all the treatment groups have significant differences from the control group. The higher the glycine concentration, the shorter the development time. Glycine fortification increases protein concentration in the larval body because it can be a protein precursor and purine unit that plays a role in protein synthesis. Dong et al. (2017) reported that glycine fortification indirectly increases hormones in the larva's body from the TCA cycle or amino acid metabolism, one of which is the hormone for molting. The molting process is influenced by ecdysone and juvenile hormones, which require protein and sufficient energy to synthesize them. The higher glycine concentration in cassava leaves provides higher energy for silkworms. This energy will be used for the molting process and the formation of ecdysone and juvenile hormones to improve their growth and development (Sheng et al., 2008).

Table 2. Glycine fortification of cassava leaves on development parameters (egg parameters and development time)

Treatment groups	Egg parameters		Development time (days)
	Fecundity (eggs)	Hatchability (%)	
K	88.00±6.96^{a}	78.12±10.71^{a}	50.37±0.49^{d}
P1	99.80±7.56^{a}	85.80±9.65^{a}	46.23±0.95^{c}
P2	107.55±10.54^{ab}	87.38±15.90^{a}	45.47±0.11^{b}
P3	134.80±28.78^{b}	95.76±4.60^{a}	44.00±1.12^{a}

Note: Different letters in each data label show significant differences ($P<0.05$). K for control group; P1 for 0.5% glycine fortification; P2 for 1% glycine fortification; P3 for 1.5% glycine fortification

The mortality results revealed that no larvae died from the first instar to the fifth instar in all treatments. No mortality data indicated that the environmental conditions in the study area were suitable for the larvae of the Eri silkworm. Glycine fortification prevents oxidative stress in Eri silkworms (Dutta et al., 2018). It is because glycine can form glutathione (GSH). The formation of glutathione is assisted by GSH synthetase and ATP. Glutathione is an antioxidant that maintains silkworms' immune system (Litwack, 2018).

Egg parameters

This study showed that the fecundity significantly increased on the 1.5% glycine fortification (134.8± 28.78 eggs) compared to the control group. Unfortunately, for the results of data from the egg hatchability of Eri silkworms (Table 2), there was no significant difference between the treatment and control groups. According to Uranli et al. (2011), amino acid, including glycine, is vital for egg production. Glycine provides more energy for juvenile hormone production. The juvenile hormone plays a role in synthesizing vitellogenin (egg yolk). Thus, the more vitellogenin is produced, the more fecundity. In hatching eggs, it is enough to give glycine, glycerol, and sorbitol (Chino, 1961). In addition, laying eggs before hatching requires a lower temperature (25 °C) and 70-80% humidity (Hussain et al., 2011).

CONCLUSIONS

Based on the results and discussion, glycine fortification significantly affects almost all growth and development parameters compared to the control group, except for the hatchability. The concentration of glycine that had the most influence on the overall growth and development of the silkworm of *Samia cynthia ricini* D. was 1.5% concentration on the use of late instar of cassava leaves. Thus, glycine fortification on cassava leave affects the growth and development of eri silkworms.

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