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# Artificial diet for the cultivation of eri silkworm (*Samia ricini* Drury 1773) (Lepidoptera: Saturniidae)

Siti Shofa Assyifa'ul Qulbi Barid[a,e], Fathur Syahrian Ramadhani[a], Hari Purwanto[a], Hendry T.S.S.G. Saragih[b], Abdulrahman Saad Aldawood[c], Tri Rini Nuringtyas[d], Sukirno Sukirno[a,*]

[a]*Entomology Laboratory, Gadjah Mada University, Yogyakarta 55281, Indonesia*
[b]*Laboratory of Animal Structure and Development, Universitas Gadjah Mada, Yogyakarta, Indonesia*
[c]*Plant Protection Department, King Saud University, Riyadh 11451, Saudi Arabia*
[d]*Laboratory of Biochemistry, Gadjah Mada University, Yogyakarta 55281, Indonesia*
[e]*Department of Sciences Education, Jember University, Jember 68121, Indonesia*

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**Abstract**

The objective of this present study was to identify a suitable artificial diet formulation to support the development of eri silkworms, with castor leaves (*Ricinus communis*) constituting the primary ingredient. The quality of the artificial diet was evaluated using neonate larvae, comparing it to fresh castor leaves. The nutritional value was assessed by analyzing the protein content in the hemolymph of fifth-instar larvae using the Folin-Ciocalteu method and proximate analysis. The findings demonstrated that an artificial diet containing castor leaf powder during early instars, with fresh leaves being incorporated into the diet later instars, resulted in higher larval protein content. The weight of cocoon, empty cocoon, and pupa was 1.59 ± 0.05 g, 0.23 ± 0.02 g, and 1.37 ± 0.05 g, respectively. The shell ratio, female wingspan, and egg fertility were found to be 15.31 ± 0.11%, 2.42 ± 0.20 cm, and 79.2 ± 5.83 eggs, respectively. Formulation P2 exhibited the lowest larval mortality (4.23 ± 0.58%) and hemolymph protein content of 27.51 μg/mL These findings are of imperative for the cultivation of eri silk worm using artificial diet to avoid pathogen contamination and controllable nutrient content considering the early larval instar that is highly sensitive to microbes and nutrient deficiencies.

*Keywords:* Artificial diet; castor; ericulture; hemolymph; wild silkworm

## 1. Introduction

The eri silkworm, *Samia ricini*, a domesticated wild silkworm species that can be readily reared indoors, is capable of producing robust, durable, non-lustrous, and fine 'peace silk' [1,2]. It extensively cultivated in tropical climates such as India, Thailand, and Indonesia [3]. These polyphagous silkworms primarily feed on castor leaves (*Ricinus communis L.*), with cassava leaves (*Manihot utilissima Phol.)* and kesseru (*Heteropanax fragans Seem*.) as secondary diets [4].

In Indonesia, silkworm cultivation demonstrates considerable potential as a natural commodity in view of its high potential, ease of development, cost-effectiveness, and household accessibility. The demand for natural silk in the textile industry has been increasing, reaching 174 tons per year in 1999 [5]. Sericulture offers employment and income generation [6], particularly for rural women [7,8]. Furthermore, eri silk cocoons have been demonstrated to possess potential as a protective biomaterial against ultraviolet rays [9,10].

*Ricinus communis* L. has been identified as a highly suitable silkworm feed [11] owing to its rich biochemical composition when compared to other secondary host plants [12]. The nutrient content of the feed has been demonstrated to have a direct impact on cocoon weight; silkworms fed on *R. communis* produce heavier cocoons (2.221 g) than those fed on cassava (2.183 g), thereby indicating its nutritional superiority for eri silkworms. For this, effective feed management is deemed imperative for optimizing silkworm productivity, facilitating the efficient progression of their life cycle, and ensuring the production of high-quality silk [13,14].

The utilization of artificial diets has emerged as a promising strategy for the rearing of silkworms, with the potential to mitigate the risks of feed contamination. The advantages of this method include the ability to prepare the environment in a sterile manner, thereby minimizing pathogen introduction and facilitating precise nutrient control for optimal health and silk production [15]. A further benefit is the ability to rear silkworms year-round, thus eliminating reliance on seasonal host plants [16]. These diets also facilitate research into silkworm disease by enabling precise dietary control [17]. However, the successful development of artificial diet necessitates thorough consideration of factors such as palatability, digestibility, and the provision of all essential nutrients for growth, development, and silk production.

Research on artificial diets for silkworms includes studies on

* Corresponding author. Tel.: +628156572593
Email: sukirnobiougm@ugm.ac.id
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*Bombyx mori*, where supplementation of 1% pollen yielded 1.26 ± 0.17 g cocoons [18]. *Attacus atlas* larvae, reared on diets comprising barringtonia leaves, produced the highest cocoon weights at 7.81 ± 0.16 g [19]. Though eri silkworms have also been reared in an artificial context, one study reported the cocoon weight of 0.655 ± 1.075 g for fifth instar larvae [20], though other treatments resulted in high larval mortality, thereby preventing cocoon formation.

The rearing of eri silkworms on artificial diets frequently yields suboptimal results, largely due to the nutritional value of the diet impacting larval survival. It is therefore critical that continuous formulation and evaluation processes are in place for enhancing the quality of artificial diet. An ideal diet must contain appropriate nutrients, be easily digestible, absorbable, and metabolizable [21]. The objective of this study is to develop an artificial diet that optimally supports eri silkworm growth and development.

## 2. Materials and Methods

This research on the formulation of artificial diets for eri silkworms was conducted from November 2023 to April 2024 at the Entomology Laboratory, Faculty of Biology, Universitas Gadjah Mada, Indonesia. Eri silkworm eggs were obtained from Jantra Mas Sejahtera, a micro, small, and medium enterprise located in Kulon Progo, Yogyakarta. The parent eri silkworms had been reared at JAMTRA for six successive generations using fresh castor leaves. During the study, the eri silkworms were reared at a room temperature of 27-30°C and an average relative humidity of 57-60%.

### *2.1. Preparation of artificial diet*

The artificial diet formula employed in this study constitutes a modification of the formula developed by [19]. The primary plant component utilized was castor (*Ricinus communis* L.) leaves. Fresh castor leaves, specifically the second to third leaves from the apex for instars I-II and the fourth to seventh leaves for instars III-V, were cautiously selected, ensuring they were free from pests and pathogens. Prior to use, the leaves were washed with tap water, cut into 3 mm widths, then dried in an oven at 50°C for 24 hours, and finally ground into a powder using a commercial grinder.

Table 1. Ingredients for *Samia ricini per* 1,000 g of artificial diet

| Ingredients | Dried Castor Leaves-based Artificial Diet | Fresh Castor Leaves-based Artificial Diet |
|---|---|---|
| Castor leaves (g) | 48.93 | 139.82 |
| Cellulose powder (g) | 6.99 | 6.99 |
| Corn bee pollen (g) | 41.94 | 41.94 |
| Low fat soya bean powder (g) | 6.99 | 6.99 |
| Vitamin C (g) | 2.09 | 2.09 |
| $K_2HPO_4^{2-}$ (g) | 1.12 | 1.12 |
| $CaCO_3$ (g) | 1.34 | 1.34 |
| Chloramphenicol (g) | 0.11 | 0.11 |
| Acrylic acid (g) | 1.12 | 1.12 |
| Vitamin B complex (g) | 0.08 | 0.08 |
| Sorbic acid (g) | 1.40 | 1.40 |
| Agar (g) | 48.93 | 48.93 |
| Distilled water (ml) | 839 | 559 |

To prepare the diet, all ingredients (as listed in Table 1), except ascorbic acid, were homogenized using a blender, after which the mixture was boiled. Following the boiling stage, the mixture was allowed to cool to room temperature for approximately 15 minutes. Once the mixture's temperature reached 50°C, ascorbic acid was added and thoroughly stirred. Subsequently, the mixture was allowed to solidify at room temperature. The resulting artificial diet was then cut into small pieces (30mm x 10mm x 10mm) and placed on wax paper in disposable Petri dishes (90mm x 15mm) (see in Fig. 1). For the purpose of comparison, an additional artificial diet formula was prepared using fresh leaves, alongside the formula incorporating dried powdered leaves.

Fig. 1. Second larval instar of eri silkworm reared in artificial diet

### *2.2. Artificial diets effects on the growth and development of eri silkworms*

The treatments were divided into seven groups: C, $P_1$, $P_2$, $P_3$, $P_4$, $P_5$, and $P_6$.

a. C: Eri silkworms were fed castor leaves from the first larval instar to the pre-pupal stage.
b. $P_1$: Eri silkworms were fed fresh castor leaves from the first to the second larval instar, prior to be transferred to a fresh-leaf-based artificial diet from the third instar to the pre-pupal stage.
c. $P_2$: Eri silkworms were fed a fresh-leaf-based artificial diet from the first to the second larval instar. Thereafter, they were transferred to fresh castor leaves from the third instar to the pre-pupal stage.
d. $P_3$: Eri silkworms were fed fresh castor leaves from the first to the second larval instar, and transferred to a dried-castor-leaves-based artificial diet from the third instar to the pre-pupal stage.
e. $P_4$: Eri silkworms were fed a dried-castor-leaves-based artificial diet from the first to the second larval instar and then fed fresh castor leaves from the third instar to the pre-pupal stage.
f. $P_5$: Eri silkworms were fed a fresh-castor-leaves-based artificial diet from the first instar to the pre-pupal stage.
g. $P_6$: Eri silkworms were fed a dried-castor-leaves-based artificial diet from the first instar to the pre-pupal stage.

Each treatment was replicated five times, with each replicate starting with 20 neonate larvae. Initially, larvae were reared in disposable petri dishes (90 mm diameter, 15 mm height) per treatment. After molting to the third instar, the larvae were transferred to perforated rearing boxes (200mm x 200mm x 100mm) and reared until the fifth instar. Subsequently, the matured fifth instar larvae were individually transferred to wooded seriframe boxes (300mm x 300mm x 50mm) until the process of pupation was complete. The frass was removed on a daily basis, and the wax paper located at the bottom of the rearing boxes was also replaced on a daily basis. The artificial diet was modified once a week or when consumed.

Five days after pupation in the seriframe boxes, cocoons were collected and weighed. The cocoons were then subjected to incubation in cages (500mm x 500mm x 750mm) with mesh walls until adult emergence. The parameters observed in this study included larval development time, cocooning percentage, cocoon weight, shell ratio, adult emergence, adult wingspan, and fecundity. For the purpose of comparison, 100 neonates were also reared in the laboratory using fresh castor leaves as a control.

### *2.3. Nutritional value evaluation of the diets based on protein hemolymph and proximate assay*

The protein content of eri silkworm hemolymph was quantitatively determined using the Folin-Ciocalteu method [22]. This method can also be adapted to evaluate the nutritional content of artificial diet formulations. For hemolymph collection, late fifth instar larvae were euthanized at a temperature of -20°C for a duration of 20 minutes and then surface-sterilized using 70% ethanol. The collection of hemolymph was achieved by severing the abdominal prolegs, which was then deposited into a 1.5 ml test tube. Subsequently, the collected hemolymph was subjected to centrifugation at 10,000 rpm for 5 minutes. Saturated ammonium sulphate was added to the supernatant at a volume ratio of 1:1, and the solution was incubated for 24 hours at a temperature of -4°C. Subsequent to the process of incubation, the solution was subjected to a second round of centrifugation at a speed of 12,000 rpm for 5 minutes.

The resulting pellet was resuspended in 1 ml of demineralized water and homogenized using a vortex mixer. Subsequently, 10 µl of this sample was added to 0.4ml of alkaline solution (2% $Na_2CO_3$ in 0.1 N NaOH and 0.5% $CuSO_4$ in 1% NaK), homogenized, and incubated at room temperature for 10 minutes. Subsequently, 1.2 ml of Folin-Ciocalteu reagent was added, and the solution was re-homogenized and incubated for 30 minutes at room temperature. Furthermore, the protein content of the solution was measured spectrophotometrically at a wavelength of 595nm, with bovine serum albumin solution serving used the standard.

The nutritional values of the artificial diets (both dried-castor-leaves-based and fresh-castor-leaves-based), as well as fresh castor leaves, were evaluated through both proximate analysis and bomb calorimetry. These analyses were performed in two repetitions at the Integrated Research and Testing Laboratory, Universitas Gadjah Mada, and the data obtained from the facility were provided as averages.

### *2.4. Research design and statistical analysis procedure*

The present study employed a completely randomized design. Data analysis was conducted using analysis of variance to compare treatments, followed by Duncan's multiple range test for mean separation. Regression analysis, performed using SPSS 26, estimated the potential interaction between hemolymph protein content and the growth and development of eri silkworms. Furthermore, curve fitting and equation estimation using Curve Expert version 1.3 were utilized to determine correlation coefficients and $P$-values.

## 3. Results and Discussion

### *3.1. Eri silkworm growth after fed with different artificial diets and different rearing method*

The present study observed the growth and development of *Samia ricini.* The growth parameters included larval weight, cocoon weight, cocoon yield, shell ratio, pupa weight, adult emergence, male wingspan, and female wingspan (see Table 2 for details). Eri silkworm larval weight observations and calculations were performed daily following each molt. For the first instar, weight measurements were conducted one day after hatching to avoid disturbing the molting process.

The utilization of artificial diets derived from fresh castor leaves resulted in lower yield parameters, specifically cocoon weight, shell ratio, and adult emergence, in comparison to diets using castor leaf powder. For instance, treatments P1, P2, and P5 exhibited significantly higher values for these parameters. Fresh castor leaves contain secondary metabolites such as phenolic acids and ricinine alkaloids [23]. Although castor leaves have a relatively high protein content (17.43%) compared to other silkworm feed plants [24], they contain various secondary metabolites, including phenolic compounds. Nevertheless, it is important to note that processing them into powder may potentially cause damage some compounds. Such compounds have been observed to decrease the nutritional value of plant tissues through binding to amino acids and proteins, with the potential to interfere with digestive enzymes in the insect gut [25]. Furthermore, the utilization of fresh leaves in the formulation of artificial diets has been observed to be susceptible to mold contamination, likely due to their high-water content.

Table 2. Effects of artificial diets and different rearing methods on the development of *Samia ricini* (mean±SE)

| Parameters | C | $P_1$ | $P_2$ | $P_3$ | $P_4$ | $P_5$ | $P_6$ |
|---|---|---|---|---|---|---|---|
| Cocoon yields (%) | 65±4.74b | 59±5.78b | 62±2.54b | 59±2.91b | 41±7.96a | 34±5.33a | 25±5.70a |
| Cocoon weight (g) | 1.61±0.04c | 1.59±0.50c | 1.42±0.06b | 1.43±0.04b | 1.31±0.03ab | 1.38±0.03ab | 1.25±0.03a |
| Shell ratio (%) | 15.30±0.10c | 14.84±1.13bc | 13.53±1.18ab | 12.24±0.30a | 13.25±0.76a | 12.37±0.49a | 11.51±0.52a |
| Adult emergence (%) | 60±0.00e | 54.24±0.00c | 48.39±0.00a | 52.24±0.00b | 68.29±0.00f | 75.53±0.00g | 56±0.00d |
| ♀ Wingspan (cm) | 12.04±0.18c | 12.42±0.20c | 10.77±0.26b | 10.97±0.23b | 9.90±0.26a | 10.42±0.18ab | 10±0.44a |
| ♂ Wingspan (cm) | 10.77±0.14c | 10.25±0.20bc | 9.86±0.13b | 10.42±0.22bc | 10.45±0.18bc | 9.93±0.19b | 9.08±0.21a |

Note: Means in the same row followed by the same notation indicate the results that are not significantly different after being analyzed using DMRT at α: 0.05 (C: 1st instar - pre-pupa on fresh castor leaves; $P_1$: 1st - 2nd instar on fresh castor leaves, and 3rd – pre pupa on fresh-leaf-based artificial diet; $P_2$: 1st - 2nd instar on fresh leaf-based artificial diet, and 3rd – pre pupa on fresh castor leaves; $P_3$: 1st - 2nd instar on fresh castor leaves, and 3rd – pre pupa on dried-leaf-based artificial diet; $P_4$: 1st - 2nd instar on dried-leaf-based artificial diet, and 3rd – pre pupa on fresh castor leaves; $P_5$: 1st instar – pre pupa on fresh leaf-based artificial diet; $P_6$: 1st instar – pre pupa on dried leaf-based artificial diet)

Table 3. Effects of artificial diets and different rearing methods on the larval weight of *Samia ricini* (mean±SE)

| Treatments | Instar (g) | | | | |
|---|---|---|---|---|---|
| | I | II | III | IV | V |
| C | 0.0142±0.002a | 0.211±0.01b | 0.827±0.030c | 3.209±0.088c | 4.803±0.062c |
| $P_1$ | 0.0118±0.001a | 0.181±0.01ab | 0.952±0.046d | 2.985±0.069b | 3.706±0.072b |
| $P_2$ | 0.0114±0.001a | 0.147±0.006a | 0.795±0.029bc | 2.937±0.075b | 3.64±0.084b |
| $P_3$ | 0.0136±0.001a | 0.704±0.038d | 0.693±0.022ab | 2.883±0.056b | 3.585±0.061ab |
| $P_4$ | 0.0134±0.001a | 0.575±0.019c | 0.678±0.019a | 2.643±0.078a | 3.585±0.042ab |
| $P_5$ | 0.0118±0.002a | 0.176±0.006ab | 0.667±0.018a | 2.599±0.082a | 3.516±0.066ab |
| $P_6$ | 0.0114±0.001a | 0.175±0.009ab | 0.631±0.023a | 2.54±0.069a | 3.431±0.033a |

Note: Means in the same row followed by the same notation indicate results that are not significantly different after being analyzed using DMRT at α: 0.05 (C: 1st instar - pre-pupa on fresh castor leaves; $P_1$: 1st - 2nd instar on fresh castor leaves, and 3rd – pre pupa on fresh-leaf-based artificial diet; $P_2$: 1st - 2nd instar on fresh leaf-based artificial diet, and 3rd – pre pupa on fresh castor leaves; $P_3$: 1st - 2nd instar on fresh castor leaves, and 3rd – pre pupa on dried-leaf-based artificial diet; $P_4$: 1st - 2nd instar on dried-leaf-based artificial diet, and 3rd – pre pupa on fresh castor leaves; $P_5$: 1st instar – pre pupa on fresh leaf-based artificial diet; $P_6$: 1st instar – pre pupa on dried leaf-based artificial diet)

Despite the paucity of studies specifically addressing the direct impact of ricinine alkaloids on eri silkworm growth, the general principle of plant-derived secondary metabolites affecting insect growth, survival, and fecundity has been well documented by Ahamad et al [26] in *Antheraea mylitta*. The presence of these compounds in fresh leaves is likely to contribute to the suboptimal performance of silkworms.

Notwithstanding the potential for some compounds to be damaged during processing, the conversion of fresh castor leaves into powder offers significant advantages. The study on *Bombyx mori* revealed that processing, such as drying, can assist in reducing the concentration or activity of undesirable secondary metabolites. This, in turn, can enhance palatability and digestibility [14]. Artificial diets, which frequently contain powdered plant materials, address the challenges associated with the inconsistent supply and variable quality of fresh leaves [27]. Furthermore, the pulverization of the leaves into a powder form has been shown to increase the surface area and disrupt the integrity of cell structures of the leaves, thereby facilitating the process of the digestion and absorption of nutrients. In the species *B. mori*, the formulation of artificial diets using powdered leaves has been shown to enhance the nutritional efficiency and overall performance of the larval stages [28].

The highest larval weight across all instar stages was consistently observed in the treatment group fed fresh castor leaves (see Table 3 for details). Larvae fed an artificial diet exhibited significantly different weights compared to those fed fresh castor leaves, suggesting that the artificial diet might not have fully met the larvae's nutritional needs at those specific instar stages. These results align with those reported in previous studies [29], which also documented significant weight loss in larvae fed artificial diets across all instar stages. As demonstrated in [30], L-ascorbic acid deficiency is considered as a contributing factor to such weight decreases. It is evident that ensuring proper nutrition is paramount for successful silkworm rearing [31]

Larval weight was found to be significantly determined by the type of feed across different instars. Initial finding indicated that treatments $P_3$ and $P_4$, which involved natural feeding during the first and second instar, resulted in higher larval weights compared to other treatments. However, a subsequent switch to an artificial diet led to a decrease in larval weight in $P_3$ and $P_4$ relative to other treatments. Conversely, treatments $P_1$ and $P_2$, which transitioned from an artificial diet back to natural leaves, demonstrated an increase in larval weight during the third to fifth instars. This pattern suggests that the artificial diet may not have adequately met the nutritional needs of the larvae during the later stages. These results align with those reported by [32], who observed significant weight loss in larvae fed exclusively with an artificial diet throughout instars I-V, potentially linked to L-ascorbic acid deficiency, as highlighted by [33].

The weight of the larvae can be increased by carefully balancing the ingredients in the artificial diet [29]. However, the results of this study indicate that the artificial diets employed in treatments $P_2$, $P_4$, and $P_6$ resulted in lower larval weights in comparison to $P_1$, $P_3$, and $P_5$. This phenomenon may be attributed to the fact that the artificial diet using fresh castor leaves is more susceptible to mold contamination than diets using castor leaf powder. The higher water content of fresh castor leaves, when not processed into powder, likely accelerates the growth of mold in the artificial diet, thereby exerting a negative impact on larval development.

The success of larvae in pupating and forming cocoon structures is a key economic parameter in silkworm cultivation. The impact of artificial diets on cocoon yields was identified to be significant ($F = 8.88$; $df = 6$; $P < 0.05$). The artificial diet comprising castor leaves powder in the early instar, combined with fresh castor leaves in the late instar ($P_2$), resulted in the highest cocoon yields in comparison to other treatments. Furthermore, significant differences in cocoon weight were observed ($F = 7.3$; $df = 6$; $P < 0.05$), with treatment $P_1$ exhibiting the highest results. P1 also exhibited the highest shell ratio and artificial diet affected the shell ratio ($F = 2.06$; $df = 6$; $P < 0.05$). Adult emergence was found to be significantly higher in treatment P5 compared to other treatments ($F=2.80$; $df=6$; $P<0.05$). Furthermore, artificial diets influenced the wingspan of male ($F=5.43$; $df=6$; $P<0.05$) and female ($F=17.38$; $df=6$; $P<0.05$) adults, with $P_1$ demonstrating the widest female wingspan and $P_4$ showed the widest male wingspan. In Vietnamese eri silkworm strains exhibited an outstanding performance in cocoon parameters [32].

The pupation rate in this study was notably high across all treatments, particularly in $P_2$ (62%). This finding is in a sharp contrast to the results reported in [20], where larvae experienced total mortality at the final instar, resulting in a pupation rate of only 0.65%. The elevated pupation rates observed in this study suggest that the artificial diet formulations successfully met the nutritional requirements of the larvae, thereby facilitating their development to the pupal stage

Despite the high pupation rate, the cocoon weight produced in this study was suboptimal in comparison to other research. Even the cocoon weight of the control group (1.61 g) was significantly lower than the one as reported by [34] at 2.66 g. This disparity might be attributed to environmental factors such as temperature and humidity. Typically, the ideal rearing environment for eri silkworms is characterized by a temperature of 26-28°C and humidity of 75-85%. In our study, the room temperature was measured at 30.85°C with humidity level of 60.73%. It has been recognized that elevated temperatures in silkworm rearing can lead to delayed molting, increased susceptibility to disease, and mortality [35]. Consequently, the suboptimal temperature and humidity conditions in this study likely impeded eri silkworm growth.

The proportion of silk in a cocoon can be expressed as the cocoon shell ratio (shell weight / cocoon weight). In this study, the control group exhibited the highest shell ratio in comparison to the other treatments. Treatment $P_1$ (14.84%) was similar to the control, while both control and $P_1$ exhibited a significant difference from the remaining treatments. Although treatment $P_6$ exhibited the highest adult emergence rate, the wingspan of the resulting moths was found smaller in comparison to other treatments. Some moths in $P_6$ exhibited curly wing characteristics, indicating incomplete wing expansion. In contrast, the moths in the $P_1$ treatment primarily displayed fully stretched wings, which was consistent with the observed larger wingspan in both males and females in comparison to the other treatments.

### *3.2. Eri silkworm development after fed with different artificial diets and different rearing method*

The developmental parameters observed in *Samia ricini* included developmental duration, mortality, fecundity, and hatchability (see Table 4). The developmental duration data were obtained by measuring the total length of the egg, larval, and pupal periods of *S. ricini*. Larvae fed naturally exhibited a prolonged mean developmental time in comparison to those fed artificial diets. In treatments $P_1$ and $P_2$, the duration of the first and second instar stages was notably longer than in the other treatments, which was also the case for the pupation period (see in Table 5).

Table 4. Effects of artificial diets and different rearing methods on the development of *Samia ricini* (mean±SE)

| Treatment | Parameter | | |
|---|---|---|---|
| | Development time (day) | Larval Mortality (%) | Egg Fecundity |
| C | 44.95 | 5.49 ± 0.91ab | 147.8 ± 11.06c |
| $P_1$ | 47.79 | 7.41 ± 2.71ab | 79.2 ± 5.83d |
| $P_2$ | 49.62 | 4.23 ± 0.58a | 70.8 ± 7.13c |
| $P_3$ | 36.53 | 5.46 ± 1.26ab | 45.4 ± 6.26ab |
| $P_4$ | 36.8 | 9.14 ± 1.54b | 55.6 ± 10.55bc |
| $P_5$ | 40.23 | 13.28 ± 0.84c | 32.3 ± 3.86a |
| $P_6$ | 41.11 | 13.99 ± 2.53c | 28.6 ± 3.66a |

Note: Means in the same row followed by the same notation indicate the results that are not significantly different after being analyzed using DMRT at α: 0.05 (C: 1st instar up to pre-pupa on fresh castor leaves; $P_1$: 1st - 2nd instar on fresh castor leaves, and 3rd – pre pupa on fresh-leaf-based artificial diet; $P_2$: 1st - 2nd instar on fresh leaf-based artificial diet, and 3rd – pre pupa on fresh castor leaves; $P_3$: 1st - 2nd instar on fresh castor leaves, and 3rd – pre pupa on dried-leaf-based artificial diet; $P_4$: 1st - 2nd instar on dried-leaf-based artificial diet, and 3rd – pre pupa on fresh castor leaves; $P_5$: 1st instar – pre pupa on fresh leaf-based artificial diet; $P_6$: 1st instar – pre pupa on dried leaf-based artificial diet)

Table 5. Effects of artificial diet on the developmental time of *Samia ricini* (days) (mean±SE)

| Treatment | Eggs (day) | Instar Duration (day) | | | | | Pupal (day) | Total (day) |
|---|---|---|---|---|---|---|---|---|
| | | 1st | 2nd | 3rd | 4th | 5th | | |
| C | 8 | 4 ± 0.00a | 3± 0.00a | 3± 0.00a | 3± 0.00a | 3± 0.00a | 20.95±0.31c | 44.95±0.17d |
| $P_1$ | 8 | 5.64 ±0.05b | 3.57±0.05b | 3.34±0.06b | 3.55±0.06b | 4.25±0.07b | 19.44±0.47b | 47.79±0.16e |
| $P_2$ | 8 | 6.06±0.24c | 5.31±0.06c | 4.32±0.09c | 4.42±0.08c | 4.64±0.07c | 16.87±0.32a | 49.62±0.11f |
| $P_3$ | 8 | 5.20±0.06d | 6.01±0.07d | 5.80±0.05d | 5.70±0.05d | 5.82±0.05d | 17.38±0.33a | 36.53±0.11a |
| $P_4$ | 8 | 5.54±0.05e | 6.18±0.06d | 5.53±0.06e | 5.61±0.05d | 5.94±0.07d | 17.78±0.28a | 36.8±0.12a |
| $P_5$ | 8 | 6.84±0.05f | 6.30±0.04e | 6.14±0.07f | 6.21±0.07e | 6.74±0.09e | 18.41±0.77ab | 40.23±0.14b |
| $P_6$ | 8 | 7.05±0.053g | 7.08±0.14f | 6.10±0.08g | 6.12±0.05e | 6.76±0.12e | 17.33±1.25a | 41.11±0.10c |

Note: Means in the same row followed by the same notation indicate results that are not significantly different after being analyzed using DMRT at α: 0.05 (C: 1st instar up to pre-pupa on fresh castor leaves; $P_1$: 1st - 2nd instar on fresh castor leaves, and 3rd – pre pupa on fresh-leaf-based artificial diet; $P_2$: 1st - 2nd instar on fresh leaf-based artificial diet, and 3rd – pre pupa on fresh castor leaves; $P_3$: 1st - 2nd instar on fresh castor leaves, then 3rd – pre pupa on dried-leaf-based artificial diet; $P_4$: 1st - 2nd instar on dried-leaf-based artificial diet, and 3rd – pre pupa on fresh castor leaves; $P_5$: 1st instar – pre pupa on fresh leaf-based artificial diet; $P_6$: 1st instar – pre pupa on dried leaf-based artificial diet)

Table 6. Effects of artificial diets and different rearing methods on the larval mortalities (mean±SE) of *Samia ricini*

| Treatments | Instar (g) | | | | |
|---|---|---|---|---|---|
| | I | II | III | IV | V |
| C | 12.00±1.22b | 9.08±2.84abc | 0.00±0.00a | 2.67±1.65a | 3.67±1.53a |
| $P_1$ | 9.00±4.00ab | 18.87±5.07c | 2.42 ±1.49abc | 4.10±2.82a | 1.67±1.67a |
| $P_2$ | 9.00±3.32ab | 8.53±2.87ab | 1.18±1.18ab | 2.42±1.49a | 0.00±0.00a |
| $P_3$ | 2.00±1.22a | 2.00±1.22a | 10.21±5.48bc | 11.90±3.53a | 1.18±1.18a |
| $P_4$ | 4.00±1.87ab | 5.05±3.87ab | 10.69±2.64c | 13.80±5.51a | 12.14±4.73ab |
| $P_5$ | 12.00±3.16b | 10.16±1.95abc | 9.11±3.45abc | 11.76±4.42a | 23.38±4.09bc |
| $P_6$ | 10.00±1.12ab | 14.67±3.21bc | 7.52±1.23abc | 6.61±3.01a | 31.17±10.24c |

Note: Means in the same row followed by the same notation indicate results that are not significantly different after being analyzed using DMRT at α: 0.05 (C: 1st instar up to pre-pupa on fresh castor leaves; $P_1$: 1st - 2nd instar on fresh castor leaves, and 3rd – pre pupa on fresh-leaf-based artificial diet; $P_2$: 1st - 2nd instar on fresh leaf-based artificial diet, and 3rd – pre pupa on fresh castor leaves; $P_3$: 1st - 2nd instar on fresh castor leaves, and 3rd – pre pupa on dried-leaf-based artificial diet; $P_4$: 1st - 2nd instar on dried-leaf-based artificial diet, and 3rd – pre pupa on fresh castor leaves; $P_5$: 1st instar – pre pupa on fresh leaf-based artificial diet; $P_6$: 1st instar – pre pupa on dried leaf-based artificial diet).

The duration of instar I to V larvae in treatment $P_1$ in this study was similar to the 20.09 days as reported in a study of eri silkworms fed an artificial diet of silk mate L4M [36]. In contrast, treatments $P_5$ and $P_6$, where larvae were fed artificial diets throughout all larval phases, exhibited the longest larval periods. These results align with those reported by [37], who found that eri silkworms fed an artificial diet at all stages had a larval duration of 30.30 days. The shortest larval duration was observed in the control group (16 days), which is consistent with the findings of [33], who reported a larval period of 20.78 days when eri silkworms were reared on castor leaves at all instar stages. The difference in larval period between larvae that were fed on artificial diets and those that were fed on fresh castor leaves may be attributed to variations in food intake and nutrient composition, thereby leading to differences in larval development.

The artificial diet treatment $P_2$ resulted in the lowest mortality rate (21.13%) in comparison to the other treatments. This figure represents the accumulated average mortality across all instar stages of *S. ricini* larvae. The highest mortality rate was observed in treatment $P_6$, which utilized an artificial feed based on fresh castor leaves for all instars, with a mortality rate of 69.96% (54 out of 100 larvae). Table 6 illustrates the mortality rate for each instar in the different artificial diet treatments.

The high mortality observed at the final instar stage with the artificial diets suggests that the nutrient content of the feed was inadequate to meet the larvae's nutritional requirements. The symptoms exhibited by fifth-instar larvae in treatments $P_5$ and $P_6$, including vomiting, diarrhea, decreased appetite, and eventual death, may be indicative of a pathogenic bacterial infection or an adverse reaction to the feed. It has been demonstrated that larvae that do not receive adequate nutrition become more susceptible to disease. Contrastively, treatment $P_2$, which combined fresh castor leaves during instars I-II with the artificial diet, provided sufficient nutrients, resulting in lower rates of mortality. Nutrition plays a vital role in the silkworm's development and growth and ultimately impacts silk production [38].

### 3.3. Evaluation of artificial diet quality

A significant difference in larval haemolymph protein levels was observed among the different artificial diets ($F=3.93$; $df=6$; $P<0.05$). Treatment $P_2$ exhibited the highest protein levels in comparison to the other treatments (see Table 7). Feeding larvae with artificial diet based on fresh castor leaves resulted in significantly higher protein levels in comparison to larvae fed with artificial diet based on castor leaf powder. While $P_2$ exhibited the highest protein levels, these levels were not significantly different from those observed in the control and $P_1$ treatments. This finding is consistent with the results of *Attacus atlas* artificial diet study [19]. These differences in protein levels in the larval haemolymph in each treatment are indicative of variations in the nutritional content of the provided feed, which we investigated further through proximate and calorimeter bomb tests to assess the quality of diet.

Table 7. Protein content ((mean±SE)) of larval hemolymph of eri silkworm after fed using artificial diets with different treatments

| Treatments | n | Protein content (μg/ml) |
|---|---|---|
| C | 5 | 27.26 ± 10.30b |
| $P_1$ | 5 | 17.96 ± 10.99ab |
| $P_2$ | 5 | 27.51 ± 6.14b |
| $P_3$ | 5 | 5.72 ± 0.78a |
| $P_4$ | 5 | 4.01 ± 1.17a |
| $P_5$ | 5 | 2.92 ± 0.34a |
| $P_6$ | 5 | 1.50 ± 0.85a |

Note: Means in the same row followed by the same notation indicate results that are not significantly different after being analyzed using DMRT at α: 0.05 (C: 1st instar up to pre-pupa on fresh castor leaves; $P_1$: 1st - 2nd instar on fresh castor leaves, and 3rd – pre pupa on fresh-leaf-based artificial diet; $P_2$: 1st - 2nd instar on fresh leaf-based artificial diet, and 3rd – pre pupa on fresh castor leaves; $P_3$: 1st - 2nd instar on fresh castor leaves, and 3rd – pre pupa on dried-leaf-based artificial diet; $P_4$: 1st - 2nd instar on dried-leaf-based artificial diet, and 3rd – pre pupa on fresh castor leaves; $P_5$: 1st instar – pre pupa on fresh leaf-based artificial diet; $P_6$: 1st instar – pre pupa on dried leaf-based artificial diet)

### 3.4. Proximate and bomb calorimeter test results

The calorie value of fresh castor leaves was found to be higher than that of artificial diet (see Table 8). This is in accordance with several parameters in the treatment of fresh castor leaves, which has demonstrated higher results in comparison to other treatments. The caloric value is primarily derived from macronutrient contents such as proteins, carbohydrates, and lipids. Fresh castor leaves are recognized as a rich and balanced source of these essential nutrients for *Samia ricini*. Studies conducted on the biochemical attributes of castor leaves have emphasized the presence of significant crude protein, lipid, and carbohydrate content, which collectively contribute to their high energy value [27].

### *3.5. Correlation of hemolymph protein to effective rate of rearing, cocoon weight, and developmental time Samia ricini*

A positive correlation was identified between hemolymph protein content in larvae and the effective rate of rearing, cocoon weight, and developmental time (see Table 9). The relationship between protein content and developmental time demonstrated a linear correlation, while the other parameters exhibited a nonlinear relationship with an optimum point.

Table 8. Proximate and bomb calorimeter test results of artificial diets for eri silkworm

| Contents | Treatment | | |
|---|---|---|---|
| | P1 (Artificial Diet with castor leaves powder) | P2 (Artificial Diet with Fresh Castor Leaves) | Castor Leaves |
| Water Contents (% w/w) | 75.68 | 83.13 | 9.32 |
| Ash Contents (%w/w) | 0.47 | 1.46 | 8.23 |
| Carbohydrates (%w/w) | 17.14 | 10.66 | 44.58 |
| Total Fat (%w/w) | 0.20 | 0.10 | 0.91 |
| Protein (%) | 6.33 | 4.65 | 36.96 |
| Calories (Kal/g) | 1.81 | 1.16 | 6.30 |

Note: The data obtained from the facility was in averages and no statistical analysis

Table 9. Regression and correlation of hemolymph protein to effective rate of rearing, cocoon weight, and developmental time *Samia ricini*

| Interaction | r | Pattern | Equation |
|---|---|---|---|
| ERR | 0.93 | Logarithm fit | $y = 2.51 + 1,21 lnx$ |
| Cocoon Weight | 0.87 | Quadratic fit | $y = 1.23 + 3.84x – 1.02x^2$ |
| Developmental Time | 0.82 | Linnear | $y = 3.792 + 3.66x$ |

Protein plays a crucial role in the metabolic processes of eri silkworms in which it can determine larval duration, cocoon quality and weight, adult emergence, and fecundity. Research on *Cricula trifenestrata* artificial diet, note that glycine, an amino acid, plays a pivotal role in the citric acid cycle, which produces energy [39]. Therefore, inadequate protein intake has the potential to adversely impact the metabolism of *Samia ricini*. Proteins have been demonstrated to be essential for the optimization of silk quality [40]. To enhance the success rate of the larvae, the addition of fermented molasses may be prospective to attract and to increase palatability, as well as to provide an energy source for growth and development [41].

Feed nutrition exerts a significant impact on larval growth and development, with protein intake being essential for larval metabolism [4]. Insufficient hemolymph protein levels have been demonstrated to compromise larval metabolism, which consequently hinders optimal growth and development. Furthermore, the moisture content of the feed is crucial. Previous studies in *B. mori* suggest that the ideal moisture content of artificial feed should be comparable to that of natural feed [42]. In addition to nutrients, water contributes to larval growth and development and provides the necessary texture to artificial feed. Consequently, the ratio of ingredients and water in the formulation is of importance. The $P_2$ treatment exhibited the highest moisture content (83.13%), which is likely to have supported larval metabolism and development. However, it is noted that elevated moisture content can also increase the risk of mold contamination.

The direct correlation between the higher caloric value of fresh castor leaves and improved silkworm parameters underscores the importance of energy input for optimal development. An increase in the caloric intake from fresh leaves has been demonstrated to result in greater allocation of energy towards biomass accumulation, then resulting in increased larval weights [43,44]. This enhanced growth during the larval stages is critical, as it directly influences subsequent economic traits. Adequate food consumption, including sufficient caloric intake, has a direct impact on the performance, mating success, and reproduction of silkworms [45]. Ultimately, larvae consuming a diet with higher caloric value tends to produce heavier cocoons, exhibits better shell ratios, and achieves higher adult emergence rates. This, in turn, contributes to increased raw silk production and overall sericultural productivity [46,47]. It can thus be concluded that the higher caloric density of fresh castor leaves plays a fundamental role in driving the superior growth and yield observed in eri silkworms.

## 4. Conclusion

This study conclusively concludes that an artificial diet combining fresh castor leaves in early instar stages with fresh castor leaves in late instar stages yielded optimal results, effectively mirroring the performance achieved by exclusively rearing silkworms on fresh castor leaves. However, our analysis of hemolymph protein content unequivocally indicates that further refinement of the artificial diet formula is imperative to significantly enhance critical economic parameters such as cocoon weight, fecundity, and egg hatchability. Concurrently, rigorous management of optimal temperature and humidity, achieved through precise control of rearing room conditions, is crucial for ensuring successful silkworm development and maximizing productivity.

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