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Black Soldier Fly Prepupae, Pupae, and Imago (*Hermetia Illucens*) Grew in Mixed Containing Fermented Coconut Pulp (FCP) and Fermented Rumen Contents (FRC)

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Abstract. *The increasing generation of organic waste has highlighted the need for sustainable bioconversion technologies, including the use of Black Soldier Fly (Hermetia illucens) larvae as efficient decomposers. This study evaluated the effects of fermented coconut pulp (FCP) and fermented rumen contents (FRC) as rearing substrates on the morphometric characteristics of BSF prepupae, pupae, and imago. A Completely Randomized Design (CRD) with five substrate formulations and 40 replications per treatment was applied. Morphometric parameters, including body length, body width, body weight, and adult abdominal dimensions, were analyzed using one-way ANOVA. The results indicated that different substrate formulations did not significantly affect any morphometric parameter ($P > 0.05$). Nevertheless, numerical variations were observed, with the substrate containing 85% FCPand 15% FRC (T3) tending to produce greater body dimensions, while the substrate containing 80% FCPand 20% FRC (T4) showed the greatest abdominal width. Overall, fermented coconut pulp, either alone or combined with fermented rumen contents, provided comparable nutritional conditions that supported normal BSF growth and development. These findings suggest that both organic waste materials can serve as promising alternative substrates for sustainable BSF cultivation and organic waste bioconversion.*

Keyword: *Hermetia Illucens, Fermented coconut pulp, Fermented rumen contents, Morphometrics, Prepupae, Pupae*

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INTRODUCTION

The increasing generation of organic waste has become a major environmental challenge, particularly in developing countries where rapid population growth and agro-industrial activities continuously increase the amount of biodegradable waste produced. Improper management of these residues contributes to environmental pollution, greenhouse gas emissions, unpleasant odors, and the proliferation of pathogenic microorganisms that threaten public health. Consequently, sustainable waste management strategies that simultaneously reduce waste accumulation and recover valuable resources have become a global priority. Among the available approaches, bioconversion has emerged as an environmentally friendly technology that transforms organic waste into valuable biomass through the activity of microorganisms and insects (Herlinda & Sari, 2021; Mabruroh et al., 2022; Putri et al., 2023; Surendra et al., 2020; Siddiqui et al., 2024).

Black Soldier Fly (*Hermetia illucens*) is recognized as one of the most efficient insects for organic waste bioconversion because its larvae can rapidly convert diverse organic substrates into protein- and lipid-rich biomass while substantially reducing waste volume. In addition to producing high-quality biomass for animal feed, BSF cultivation generates frass that can be utilized as an organic fertilizer, thereby supporting circular bioeconomy systems. Previous studies have reported that BSF larvae are capable of reducing organic waste by more than 70–80%, although the efficiency of waste conversion and biomass production largely depends on the nutritional composition and physicochemical properties of the rearing substrate (Barragán-Fonseca et al., 2017; Lalander et al., 2019; Gold et al., 2020; Siddiqui et al., 2024).

Among the numerous organic substrates available for BSF cultivation, fermented coconut pulp (FCP) and fermented rumen contents (FRC) were selected because their nutritional characteristics are expected to complement each other. Coconut pulp is an abundant agro-industrial by-product containing residual carbohydrates, lipids, and dietary fiber; however, its relatively high lignocellulosic content limits nutrient digestibility when used directly as a rearing substrate. Conversely, rumen contents contain partially digested plant materials, microbial protein, minerals, and cellulolytic microorganisms that can improve nutrient availability after fermentation. Fermentation using Effective Microorganisms-4 (EM4) further enhances substrate quality by degrading structural

carbohydrates, reducing crude fiber, and increasing nutrient accessibility for insect larvae (Hidayati, 2017; Palma et al., 2019). Therefore, combining FCP and FRC is expected to provide a more balanced carbon-to-nitrogen ratio and a more suitable nutritional environment than either substrate used individually, thereby supporting efficient larval development and biomass formation.

Morphometric development of BSF, including the prepupal, pupal, and imago stages, reflects the cumulative efficiency of nutrient assimilation during larval growth. Body length, body width, and body weight are commonly used as biological indicators to evaluate substrate suitability because these parameters represent nutrient allocation during metamorphosis. Although numerous studies have investigated the effects of food waste, fruit waste, vegetable residues, and livestock manure on BSF performance, relatively few have evaluated fermented coconut pulp and fermented rumen contents as combined substrates. Moreover, previous studies have predominantly focused on larval biomass production, waste reduction efficiency, or nutritional composition, while morphometric responses during the prepupal, pupal, and imago stages remain insufficiently explored (Gold et al., 2020; Surendra et al., 2020; Siddiqui et al., 2024).

Therefore, this study aimed to evaluate the effects of different combinations of fermented coconut pulp (FCP) and fermented rumen contents (FRC) on the morphometric characteristics of Black Soldier Fly (*Hermetia illucens*) prepupae, pupae, and adults. The findings are expected to provide additional scientific evidence regarding the utilization of fermented agro-industrial and livestock wastes as alternative substrates for sustainable BSF cultivation and to support the development of environmentally friendly organic waste bioconversion systems.

LITERATURE REVIEW

Black Soldier Fly (*Hermetia Illucens*) has become one of the most promising insects for organic waste bioconversion because of its ability to efficiently convert a wide range of organic materials into high-protein biomass while simultaneously reducing waste volume. This dual function has positioned BSF as an important component of sustainable waste management and circular bioeconomy systems. According to Surendra et al. (2020), the efficiency of BSF bioconversion is largely influenced by the nutritional quality of the rearing substrate, making substrate selection a critical factor in insect production.

Substrate quality determines nutrient availability, feed digestibility, and biomass accumulation throughout the larval stage. Palma et al. (2019) reported that substrates containing high levels of structural fiber generally reduce larval growth because complex carbohydrates are difficult to digest. Consequently, pretreatment technologies such as fermentation are increasingly applied to improve substrate digestibility by reducing lignocellulosic components and increasing the availability of soluble nutrients for BSF larvae.

Among agro-industrial residues, coconut pulp is widely available and still contains considerable amounts of carbohydrates, residual oil, and moderate protein. However, its high crude fiber content limits its direct utilization as a rearing substrate. Fermentation has been reported to improve the nutritional quality of coconut pulp by increasing nutrient availability and decreasing crude fiber, thereby enhancing its potential as a substrate for BSF cultivation (Palma et al., 2019).

In addition to coconut pulp, rumen contents represent an abundant livestock by-product containing partially digested plant materials, microbial protein, minerals, and cellulolytic microorganisms. After fermentation, these nutrients become more accessible and may complement the nutritional deficiencies of coconut pulp. Trihendarsa et al. (2025) demonstrated that combining fermented coconut dregs with fermented rumen contents supported satisfactory larval growth, indicating that both substrates can be utilized synergistically as alternative rearing media.

Morphometric characteristics, including body length, body width, and body weight during the prepupal, pupal, and adult stages, are important indicators of nutrient utilization and developmental performance. Since prepupae and pupae no longer actively feed, their morphometric characteristics primarily reflect nutrient reserves accumulated during the larval stage. Therefore, morphometric evaluation provides valuable information regarding the suitability of different substrates for supporting BSF development (Dieng et al., 2019).

Although previous studies have demonstrated the potential of fermented organic substrates for BSF production, most investigations have focused on larval biomass, waste reduction efficiency, and nutritional composition. Only a limited number of studies have evaluated morphometric responses throughout the prepupal, pupal, and adult stages. Trihendarsa et al. (2025) reported that different combinations of fermented coconut dregs

and fermented rumen contents produced relatively similar prepupal and pupal growth, suggesting that substrate nutrient balance may be more influential than individual substrate proportions.

Based on these findings, information regarding the influence of different proportions of fermented coconut pulp and fermented rumen contents on the complete morphometric development of *Hermetia Illucens*, including prepupal, pupal, adult, and abdominal characteristics, remains limited. Therefore, the present study was conducted to evaluate the effects of different substrate formulations on body length, body width, and body weight throughout the developmental stages of BSF. The results are expected to provide additional scientific evidence supporting the utilization of fermented agro-industrial and livestock wastes as sustainable substrates for BSF cultivation.

RESEARCH METHODS

Study Site and Experimental Design

This study was conducted from April to June 2024. Morphometric measurements were performed at the Reproduction Laboratory, Faculty of Animal Science, Universitas Brawijaya, Malang, Indonesia, whereas Black Soldier Fly (Hermetia illucens) rearing was carried out under controlled conditions in Kedungkandang District, Malang. Throughout the experiment, ambient temperature ranged from 27–30°C with relative humidity maintained at 65–75%, which falls within the optimal range for BSF development.

A Completely Randomized Design (CRD) consisting of five dietary treatments with 40 replications per treatment was employed, resulting in 200 experimental units. Each unit consisted of a plastic rearing container containing 2 kg of substrate and 1,000 newly hatched larvae. Larvae were reared for 15 days until reaching the prepupal stage. One prepupa was randomly selected from each experimental unit and subsequently monitored through the pupal and adult stages for repeated morphometric measurements. Consequently, 40 individuals per treatment (200 individuals in total) were evaluated.

Preparation of Fermented Substrates

The experimental substrates consisted of fermented coconut pulp (FCP) and fermented rumen contents (FRC). Coconut pulp was obtained from local coconut-processing industries, whereas fresh rumen contents were collected immediately after slaughter from a commercial slaughterhouse. Coconut pulp was selected because it remains rich in residual carbohydrates, lipids, and structural fiber, whereas rumen

contents contain partially digested plant materials, microbial protein, minerals, and cellulolytic microorganisms capable of improving nutrient availability after fermentation. These complementary characteristics were expected to provide a more balanced nutritional substrate for BSF larvae than either material alone. Both substrates were fermented separately using Effective Microorganisms-4 (EM4) and molasses as microbial inoculant and carbon source, respectively. The mixtures were fermented anaerobically for seven days at room temperature before being homogenized and mixed according to the treatment formulations.

Based on previous studies indicating that BSF larvae can tolerate moderate inclusion levels of livestock-derived organic residues without adversely affecting growth, five substrate formulations were established to evaluate the gradual substitution of fermented coconut pulp with fermented rumen contents while maintaining a constant substrate weight of 2 kg per experimental unit. The treatments consisted is

a) T0 (100% FCP)
b) T1 (95% FCP+ 5% FRC)
c) T2 (90% FCP+ 10% FRC)
d) T3 (85% FCP+ 15% FRC)
e) T4 (80% FCP+ 20% FRC)

Corresponding to FRC inclusion levels of 0, 5, 10, 15, and 20%, respectively. This treatment gradient was designed to determine whether incremental substitution of FCP with FRC could improve larval morphometric performance while maintaining substrate stability. The fermented substrates were characterized based on their proximate nutritional composition obtained from previous analyses. Fermented coconut pulp contained approximately 13.1% crude protein with reduced crude fiber following EM4 fermentation (Hidayati, 2017), whereas fermented rumen contents are recognized as a source of microbial protein, minerals, and partially degraded fiber that may enhance nutrient utilization by BSF larvae. Therefore, combining these substrates was expected to provide a more balanced carbon-to-nitrogen ratio and improve nutrient availability during larval growth.

Rearing Procedures and Morphometric Measurements

Approximately 2.5 g of BSF eggs obtained from a healthy laboratory colony were incubated under identical environmental conditions. After hatching (3–4 days), first-

instar larvae were randomly allocated to the experimental units, with each container receiving 1,000 larvae. Larvae were maintained on their respective substrates for 15 days. Environmental temperature and relative humidity were monitored daily using a digital thermo-hygrometer to ensure uniform rearing conditions throughout the study.

Morphometric observations were conducted during the prepupal, pupal, and adult stages. Body length (mm) was measured using a digital caliper from the anterior margin of the head to the posterior end of the abdomen. Body width (mm) was measured at the widest body region using the same instrument. Body weight (g) was determined using a digital analytical balance with an accuracy of 0.001 g after removing residual substrate attached to the body surface. Measurements were repeatedly performed on the same randomly selected individual from each experimental unit.

Statistical Analysis

Morphometric data obtained from the prepupal, pupal, and adult stages were analyzed separately using one-way Analysis of Variance (ANOVA) based on a Completely Randomized Design (CRD) at the 95% confidence level. The statistical model applied was:

$$Yij = \mu + \tau i + \varepsilon ij$$

where (Yij) is the observation of the j-th replication receiving the i-th treatment, (μ) is the overall mean, (τi) is the treatment effect, and (εij) is the experimental error. When significant treatment effects were detected ($P < 0.05$), Duncan's Multiple Range Test (DMRT) was performed to compare treatment means. Statistical analyses were conducted using IBM SPSS Statistics.

RESULTS AND DISCUSSION

1. Prepupa

Based on the research conducted, data on prepupae as shown in Table 1.

Table 1. The size of the prepupa from the fermented coconut with fermented Rumen Contents

Treatment	Length (mm)	Width (mm)	Weight (g)
T0	16.75±0.03	4.53±0.02	0,13±0,005
T1	16.85±0.02	4.59±0.008	0,13±0,001
T2	16.55±0.03	4.43±0,02	0,12±0,001
T3	16.94±0.02	4.66±0.02	0,13±0,0009
T4	16.64±0.02	4.48±0.004	0,13±0,0006

The morphometric characteristics of Black Soldier Fly (*Hermetia illucens*) prepupae reared on different combinations of fermented coconut pulp (FCP) and fermented rumen contents (FRC) are presented in Table 1. Mean prepupal body length ranged from 16.55 to 16.94 mm, body width from 4.43 to 4.66 mm, and body weight from 0.12 to 0.13 g. Although slight numerical variations were observed among treatments, one-way analysis of variance (ANOVA) indicated that substrate composition had no significant effect on prepupal body length, body width, or body weight ($P > 0.05$). These results indicate that replacing fermented coconut pulp with fermented rumen contents at inclusion levels up to 20% did not alter prepupal morphometric development under the conditions of the present study.

The absence of significant differences among treatments suggests that all substrate formulations provided comparable nutritional conditions to support normal prepupal development. During the prepupal stage, larvae cease feeding and rely entirely on nutrient reserves accumulated during the larval period to support metamorphosis. Therefore, prepupal morphometric characteristics largely reflect the efficiency of nutrient assimilation before the onset of metamorphosis rather than the immediate composition of the substrate. Since all experimental substrates were fermented prior to use, the fermentation process may have improved nutrient availability by reducing structural fiber and increasing the accessibility of digestible nutrients, allowing larvae in all treatments to accumulate sufficient reserves for normal growth despite differences in substrate composition.

The relatively similar morphometric responses observed in this study are consistent with previous reports demonstrating that BSF larvae are highly adaptable to a wide range of organic substrates when their nutritional requirements are adequately fulfilled. Lalander et al. (2019) reported that larval growth is influenced more by the overall balance and availability of nutrients than by the specific origin of the substrate. Similarly, Lagnaro et al. (2021) found that substrates with comparable nutritional quality frequently produce similar larval growth and morphometric characteristics despite differences in raw material composition. Bellezza Oddon et al. (2024) further emphasized that balanced nutrient availability following substrate processing, particularly fermentation, contributes to stable larval development and minimizes variation in body size. Therefore, the non-significant differences observed in the present study likely indicate that all combinations

of fermented coconut pulp and fermented rumen contents were capable of providing sufficient nutrients to sustain normal prepupal growth, rather than suggesting a superior effect of any individual treatment.

2. Pupae

Based on the research conducted, data on pupae as shown in Table 2.

Table 2. The size of the pupa from the fermented coconut with fermented Rumen Contents

Treatment	Parameter		
	Length (mm)	Width (mm)	Weight (g)
T0	17.84±0.02	4.63±0,02	0,14±0,0005
T1	17.94±0.02	4.69±0.007	0,14±0,0015
T2	17.64±0.02	4.52±0,02	0,13±0,0011
T3	18.05±0.02	4.75±0,02	0,15±0,0025
T4	17.75±0.02	4.58±0,004	0,14±0,0006

The morphometric characteristics of Black Soldier Fly (Hermetia illucens) pupae reared on different combinations of fermented coconut pulp (FCP) and fermented rumen contents (FRC) are presented in Table 2. Mean pupal body length ranged from 17.64 to 18.05 mm, body width from 4.52 to 4.75 mm, and body weight from 0.13 to 0.15 g. Although slight numerical variation was observed among treatments, one-way analysis of variance (ANOVA) showed that substrate formulation had no significant effect on pupal body length, width, or weight ($P > 0.05$). These findings indicate that substituting fermented coconut pulp with fermented rumen contents up to 20% did not significantly influence pupal morphometric development.

The absence of significant differences among treatments is biologically reasonable because the pupal stage represents a non-feeding phase in which growth is no longer supported by nutrient intake from the substrate. Instead, pupal development depends entirely on nutrient reserves accumulated during the larval stage, which are mobilized to support tissue remodeling and metamorphosis into the adult insect. Since all substrate formulations were able to sustain normal larval development, the amount of stored nutrients available for metamorphosis was likely sufficient across treatments, resulting in comparable pupal body length, width, and weight despite differences in substrate composition.

These findings suggest that fermentation improved the nutritional quality of both coconut pulp and rumen contents to a level that adequately fulfilled the physiological requirements of BSF larvae before pupation. Consequently, replacing a portion of fermented coconut pulp with fermented rumen contents did not substantially alter nutrient reserves available during the pupal stage. Similar observations have been reported by Lalander et al. (2019), who concluded that BSF development depends primarily on the overall availability of digestible nutrients rather than the specific origin of the substrate. Likewise, Ha et al. (2025) reported that morphometric responses tend to remain similar when substrates possess comparable nutritional quality and digestibility. Bellezza Oddon et al. (2024) further emphasized that balanced nutrient availability supports stable biomass accumulation during larval growth, allowing successful metamorphosis even when different organic substrates are utilized.

Overall, the present results indicate that all combinations of fermented coconut pulp and fermented rumen contents provided sufficient nutritional reserves to support normal pupal development. Therefore, the non-significant differences observed among treatments most likely reflect the physiological ability of BSF to efficiently utilize stored nutrients during metamorphosis rather than differences in the nutritional value of the experimental substrates.

3. BSF

Based on the research conducted, data on BSF as shown in Table 3.

Table 3. The size of the BSF body from the fermented coconut with fermented Rumen Contents

Treatment	Parameter		
	Length (mm)	Width (mm)	Weight (g)
T0	17.54±0.03	3.45±0,03	0,05±0,0003
T1	17.35±0.02	3.32±0,02	0,04±0,0003
T2	17.24±0.02	3.15±0,02	0,04±0,0003
T3	17.64±0.02	3.54±0,02	0,05±0,0003
T4	17.44±0.03	3.34±0,03	0,05±0,0003

The morphometric characteristics of adult Black Soldier Fly (*Hermetia Illucens*) reared on different combinations of fermented coconut pulp (FCP) and fermented rumen contents (FRC) are presented in Table 3. The mean adult body length ranged from 17.24 to 17.64 mm, body width ranged from 3.15 to 3.54 mm, and body weight ranged from

0.04 to 0.05 g. Numerically, treatment P3 (1,700 g FCP+ 300 g FRC) produced the greatest body length (17.64 ± 0.02 mm) and body width (3.54 ± 0.02 mm), whereas treatments P0, P3, and P4 exhibited similar body weights (0.05 ± 0.0003 g). However, one-way analysis of variance (ANOVA) showed that the different substrate formulations did not significantly affect adult body length, width, or weight ($P > 0.05$).

The consistently greater body length and width observed in P3 suggest that the combination of 85% fermented coconut pulp and 15% fermented rumen contents may have provided a more balanced nutrient composition for structural development. Fermented coconut pulp supplies carbohydrates and residual lipids as energy sources, whereas fermented rumen contents contribute nitrogen, minerals, and partially degraded fibrous materials that become more accessible after microbial fermentation. This nutritional complementarity may have supported the formation of structural tissues during metamorphosis, resulting in slightly larger adults. Nevertheless, because these differences were not statistically significant, they should be interpreted as numerical tendencies rather than evidence of treatment superiority.

The present results are consistent with previous studies demonstrating that BSF exhibits considerable developmental plasticity when reared on different organic substrates. Lalander et al. reported that BSF can maintain relatively stable growth performance across a wide range of organic substrates provided that essential nutritional requirements are fulfilled. Likewise, Bellezza Oddon et al. (2024) emphasized that balanced nutrient availability is more important than increasing the proportion of a single substrate component in determining insect growth. Similarly, Ha et al. observed that differences in substrate composition frequently produce only minor numerical variation in adult body size when the nutritional characteristics of the substrates are relatively comparable.

4. The Size of BSF Abdoment

Based on the research conducted, data on BSF Abdoment as shown in Table 4.

Table 4. The size of the BSF Abdoment from the fermented coconut with fermented Rumen Contents

Treatment	Parameter	
	Length (mm)	Width (mm)
T0	6,44±0,03	2,77±0,37
T1	6.24±0.03	2,77±0,43
T2	6,15±0,03	2,80±0,31
T3	6.54±0,02	2,66±0,80
T4	6.34±0.02	2,9±0,42

The abdominal morphometric characteristics of adult Black Soldier Fly (*Hermetia illucens*) reared on different combinations of fermented coconut pulp (FCP) and fermented rumen contents (FRC) are presented in Table 4. Mean abdominal length ranged from 6.15 to 6.54 mm, whereas abdominal width ranged from 2.66 to 2.90 mm. Although slight numerical variation was observed among treatments, one-way analysis of variance (ANOVA) showed that substrate formulation had no significant effect on abdominal length or width ($P > 0.05$). These results indicate that the different proportions of fermented coconut pulp and fermented rumen contents produced comparable abdominal morphometric characteristics in adult BSF.

The similarity in abdominal dimensions among treatments is consistent with the developmental biology of BSF. Adult flies no longer consume substantial nutrients and depend almost entirely on nutrient reserves accumulated during the larval stage to complete metamorphosis and maintain adult body structures. Consequently, abdominal morphology reflects the efficiency of nutrient assimilation during larval development rather than the direct effect of the substrate during adulthood. Because all experimental substrates were fermented and provided sufficient nutrients to support normal larval growth, the resulting abdominal dimensions remained relatively uniform across treatments.

Abdominal size is commonly considered an indicator of overall body development because the abdomen contains major internal organs, including the digestive and reproductive systems. Nevertheless, abdominal morphometric measurements alone cannot be used to infer reproductive performance without additional observations such as fecundity, egg production, fertility, or egg hatchability. Therefore, the present findings

should be interpreted as evidence of normal morphological development rather than differences in reproductive capacity among treatments.

The present results agree with previous studies reporting that balanced nutrient availability during the larval stage supports normal adult morphogenesis even when different organic substrates are used. Lalander et al. (2019) demonstrated that BSF larvae reared on nutritionally adequate substrates developed into adults with comparable body morphology despite differences in substrate composition, while Bellezza Oddon et al. (2024) emphasized that substrate quality influences insect growth through nutrient balance and digestibility rather than through the dominance of individual substrate components. Collectively, these findings support the conclusion that fermented coconut pulp and fermented rumen contents provided adequate nutritional conditions to sustain normal abdominal development in adult *H. illucens*.

CONCLUSION

The present study demonstrated that different combinations of fermented coconut pulp (FCP) and fermented rumen contents (FRC) did not effect the morphometric characteristics of Black Soldier Fly (*Hermetia illucens*) during the prepupal, pupal, and imago stages, including body length, body width, body weight, and imago abdominal dimensions. These findings indicate that all substrate formulations provided comparable nutritional conditions capable of supporting normal BSF growth and morphometric development. From a practical perspective, the results demonstrate that fermented coconut pulp can be partially substituted with up to 20% fermented rumen contents without adversely affecting BSF development, providing a sustainable alternative for utilizing agro-industrial and livestock wastes as rearing substrates.

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