Performance of Black Soldier Fly, *Hermetia illucens*, Larvae during valorization of organic wastes with changing quality

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Abstract. Organic wastes are one of the major problems in many cities. A common treatment for the wastes usually by sanitary landfill, composting, or burning. However, many cities of developing countries lack the effort to collect, separate, and transform organic waste lead to an increasing pile of organic wastes and the cost of treatment. Bioconversion of organic wastes by larvae of black soldier fly larvae (BSFL) (*Hermetia illucens*) into versatile prepupae could be considered as a solution for this problem as this process give economic value to organic wastes. Studies in Indonesia showed the high potency of this insect to apply for managing organic wastes. However, most of the studies only focused on one type of wastes which is only available from standardizing industrial systems, not municipal systems, and small industries which are the main organic producers in Indonesia. In this study, BSFL was fed only one type of organic wastes showed a shorter development period. Changes in feedstock quality from low protein to high protein material generally produced lower survival rates and weight with a longer development period. The result indicated the importance of the nutrition content early period feedstock to produce high-quality larvae and more sustainable organic waste management.

1. Introduction

One of the main challenges caused by the increasing human population is the increasing amount of waste generated through economic activities. It has been estimated that by 2025, the total number of the world population lives in the cities will be double from 49% of the total population recorded in 2000 [1]. Among wastes produced by human Study by FAO showed an estimation of 1.6 tonnes of food wastes produced worldwide in 2007 from production to consumption [2]. These piles of wastes are taking up space in landfills, a common way to manage the wastes, while may hasten the spread of pathogens, produce noxious odors, and significantly contribute to global CO₂ production [2]. Various methods are already conducted by researchers to reduce the number of organic wastes send to landfills. One of the methods by applying a biological agent to process organic wastes, such as the black soldier fly (*Hermetia illucens*). This wasp-like fly, which originated from the new world, almost found in every part of the world [3][4] which has great ability to utilize various types of organic wastes ranging from cellulose-
rich rice husk [5] and cassava peel [6] too soft material like bananas [7] and tofu dreg [8] also from food waste [9][10][11][12] to livestock [13][14][15] and human feces [16][17].

Recently, due to its nature as decomposer and ability to be mass production in small space and low cost [18][19], this species has been applied as a possible agent to recycling nutrients available in organic wastes [20][21][22]. Studies showed the biomass produced through the digestion of organic waste rich in protein and fat [19] which can be applied as an alternative material for the production of feed for the aquaculture [23][24][25], livestock [26], poultry [27][28], industrial material like biodiesel and sugar [29] even future human food [30].

However, one of the challenges applying this species as industrial material is the nutritionally heterogeneous nature of organic wastes in space and time [31]. Studies on insects, especially in herbivorous insects, showed the impact of variation in the nutrient content of their food on life-history and fitness and could become the selective force for insect population [32][33][34].

In this study, we simulated the change of nutritional condition of feeding material of black soldier fly larvae during their development period and observed the impact on some life history and fitness traits.

2. Methods

Insect

The black soldier larvae used in this study were originated from a colony established in May 2017 from the Laboratory of Animal Physiology and Entomology, Universitas Islam Negeri Sunan Gunung Djati Bandung. Larvae were kept inside the laboratory with 12 L:12 D photoperiod, average temperature 18-26°C, and relative humidity 67-80%.

Feeding regimes

Larvae were divided into 5 groups, namely (1) group A as a control group in which larvae were fed with commercial chicken feed, (2) group B in which larvae were fed with tofu dreg, (3) group C in which larvae were fed with fruit wastes, (4) group D in which larvae were fed with tofu dreg at the beginning then replaced with fruit wastes, (5) group E in which larvae were fed with fruit wastes at the beginning then replaced with tofu dreg. Fruit wastes used in this study were a combination of papaya, mango, banana, and avocado.

Research procedure

In this study, one hundred, 6-days-old, black soldier fly larvae (BSFL) were used for each feeding group and replicated 5 times. Larvae were kept inside a plastic box which already filled with 100-gram feeding material. Feeding materials were replaced with fresh material every 3 days. As for group D and E, the replacement of types of feeding material was conducted 8 days after first fed.

During the study, survival rate and weight were measured every 3 days (at the same period of feeding replacement). At the end of the study sex ratio of the adult, flies were observed. The observation was conducted until more than 50% of larvae metamorphosed into prepupae.

Data analysis

The difference in the larvae weight among groups was analyzed by One Way ANOVA with a confidence level of 95%. The analysis was conducted by PRISM 8.

3. Results

Growth period

Black soldier fly undergoes a complete metamorphosis from larvae to imago. This study showed faster development time in the control group (group A) and group D as the larvae spend less time to reach imago. On the other hand, group C spend the longest time to reach imago (Fig. 1).
Figure 1 Development pattern of black soldier fly larvae fed with various feeding material. The red arrow showed the time when the feeding regime was replaced.

**Body Weight**

Larvae fed with chicken feed and tofu dreg followed with fruit waste had significantly higher final weight than other groups (Table 1). On the other hand, larvae fed with fruit waste followed by tofu dreg had the lowest final weight.

| Age (days) | Chicken feed | Tofu dreg | Fruit waste | Tofu dreg - fruit waste | Fruit waste - tofu dreg |
|-----------|--------------|-----------|-------------|------------------------|------------------------|
| 6         | 0.015 ± 0.001 a | 0.014 ± 0.001 a | 0.016 ± 0.001 a | 0.015 ± 0.001 a | 0.015 ± 0.001 a |
| 10        | 0.143 ± 0.003 a | 0.163 ± 0.009 b | 0.087 ± 0.004 c | 0.053 ± 0.001 d | 0.044 ± 0.002 d |
| 13        | 0.167 ± 0.003 a | 0.114 ± 0.003 b | 0.086 ± 0.003 c | 0.087 ± 0.002 cd | 0.059 ± 0.002 e |
| 17        | 0.163 ± 0.005 a | 0.129 ± 0.003 b | 0.124 ± 0.003 b | 0.178 ± 0.064 a | 0.104 ± 0.003 c |

The different letters showed a significant value based on One Way Anova and Tukey test at a confidence level of 95%.
**Sex Ratio**

In general, significantly more females produced in groups A and B while more males produced in groups C and E. On the other hand, group D produced a more balanced proportion of adult males and females (Fig. 2).

![Figure 2 Sex ratio of black soldier fly imago](attachment:image.png)

**4. Discussion**

**Effect of feeding regime with development time**

Studies showed the effect of diet on development time on black soldier fly larvae [11][13][14][35][36]. The larvae group fed on high protein material at an early period (group A, B, and D) showed shorter development time. This result agreed with some previous studies [37][38]. Longer development time of larvae fed on fruit waste may be related to the lower energy and imbalance on protein, and fat content which are necessary for many developmental processes in insect larvae [14][39][40][41][42][43][44].

This study also showed the importance of early consumption of high protein by larvae which may be related to fast growth strategy commonly used by insect larvae when balanced nutrients are available to be consumed [45].

**Effect of feeding regime on weight**

Larvae fed with high protein material at an early stage of their development showed the highest body weight. It seems that the combination of tofu dreg and fruit waste provided much balance nutrients than other feeding regimes. Studies in insect herbivores showed the importance of micronutrient, especially amino acid to growth [40]. Fruit waste at the last stage of development might provide more energy through nonstructural carbohydrates (starch and sugar) which are supplied by fruit wastes. Larvae converted this carbohydrate converted into lipids and stored them in the fat body [46]. Some studies also showed the ability of *H. illucens* to use fruit wastes as sources for lipid production [42][47][48].

**Effect of feeding regime to sex ratio**

The result of this study agreed with previous studies that showed a more female-biased sex ratio while larvae fed on high protein content diets [41][49][50]. On the other hand, more balanced nutrients more likely to produce a 1:1 or slightly male-biased sex ratio [51][52]. Although the possibility of nutritional variability may produce a difference in the mortality between sexes [53], further studies are needed to conduct to gain more understanding of the mechanism of sex ratio in this species.

**5. Conclusion**

It was concluded that *H. illucens* can convert diets with changing nutrient content into biomass that can be used as animal feed. Early protein consumption seems to be the key factor in the development and growth of larvae. This strategy can be used for the production of black soldier fly larvae in regions with a high variation of available diets for larvae.
Acknowledgment

This study was funded in part by grant of Bantuan Peningkatan Mutu Penelitian Terapan DIKTIS Kemenag granted to corresponding author and grant of P3MI to last author in 2018.

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