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Bioconversion of Rice straw waste by black soldier fly larvae (*Hermetia illucens* L.) : Optimal feed rate for biomass production

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Abstract
This study focuses on the application of black soldier fly larvae (*Hermetia illucens* L.) as a bioconversion agent of the rice straw to reduce amount of waste while in the same time produced larvae biomass. In this study, larvae were fed with rice straw at six different feed rates (12.5, 25, 50, 100, and 200 mg/larvae/day) until larvae reached prepupal stage. During study, relative growth, relative consumption rate, and waste reduction level were measured. Daily feeding of 200 mg of grinded rice straw per larvae resulted in the highest prepupal dry weight (15.59 ± 0.01 mg), lowest developmental time (39 ± 0.1 days), but lowest waste reduction efficiency (10.85 ± 0.0005%). Highest waste reduction efficiency was recorded by larvae feed rate of 12.5 mg/larvae/day (31.53 ± 0.01%) and decreased with higher feeding rate. This study showed the possibility of production of insect larvae biomass as through bioconversion process of agricultural waste rich with lignocellulose.

Keywords: Bioconversion, feed rate, *Hermetia illucens*, rice straw

1. Introduction

The increase in productivity of rice in Indonesia created new problem due to increasing postharvest wastes. It is estimated that 4 to 5 ton dry rice stalk are produced per hectare after each harvest with a total annual production of 100 million ton [49]. Common practices for handling rice straw waste is by burning, or used as feed for livestock or mulched in the rice fields which provides low economic return and potentially caused another problems such as release of carbon fraction to the atmosphere that contributes to global warming [1, 2, 25], reduce livestock quality due to application of rice straw as low quality feed material [24, 44, 46], provide medium for pest species and source of various agricultural diseases [20].

Several studies have shown about the potency of this material to be valorized as a raw material for organic based products due to its high content of cellulose, hemicellulose, lignin [18, 47], a small amount of protein [11], and a significant amount of carbon [32]. Various attempts also had been carried out to utilize the waste as a raw material for animal feed through chemical treatment [40], compost [20], soil protection [31] bioethanol production [4], biogas production [7], or microbial protein [14]. Although these approaches showed high promises to increase the economic value of rice straw, their application at farmers level are still challenging due to the requirement of the specific material, equipment, and skill while the products relatively have limited markets.

Another potential approach is the application of organisms to convert organic wastes into biomass and simple organic materials, a concept known as bioconversion [22, 37]. One of the bioconversion agents is Black Soldier Fly Larvae (BSFL) (*Hermetia illucens* L.). Various studies have shown the ability of BSFL to convert organic wastes into biomass which could be harvested for its protein and fatty acid content [3, 6, 9, 35, 43, 50]. Harvested protein and fatty acids could be applies as a substitute for fish meal and animal fed [5, 12, 15, 36], source of biofuel [26, 52], and sugar for bioethanol [27] which may provided additional income for smallholder farmers.

The application of BSFL as a bioconverter of agricultural waste in Indonesia is still limited to oil palm waste and have shown to be promising [39]. Unlike oil palm waste, the content of rice straw is primarily lignocellulosic which has a different level of stress for the digestive system of BSFL. A previous study by Kim *et al.* [21] found several digestive enzymes that allow BSFL to digest various organic materials.

Zheng *et al.* [52] showed BSFL able to digest rice straw with the help of microorganisms. However, to the best of our knowledge, our study is the first study that investigated the direct application of BSFL as a bioconverter of rice straw.

This study experimentally test the hypothesis in which BSFL is able to digest and convert lignocellulosic materials into biomass. There is a threshold in the amount of feed where the development time of larva is constant with the increase in feed rate and there is a maximum amount of waste that larvae are able to digest within their developmental period before larva pupation.

2. Materials and Methods

2.1 Animal Specimen

Larvae of black soldier fly were obtained from eggs produced by adult population reared in a container (1 m x 1 m) at constant temperature (28 ºC, 70% RH) in the Laboratory of Environmental Toxicology, School of Life Sciences and Technology, Bandung, Indonesia. Adult population was originated from larvae fed with grilled rice straw. The study was conducted from March to August 2015.

2.2 Waste Material

Fresh rice straw waste originated from conventional rice farm at Souther Bandung. Rice straw were packed inside air-tight plastic bags and transfer to laboratory within collection day. Before apply as feed source for BSFL, all rice straw samples were kept inside frezeer to prevent decomposition by microorganism. Prior application, rice straw removed from freezer, dried at room temperature, and grinded by food mill. Material obtained then thoroughly mixed with water (60% moisture) to created feeding material for BSFL.

2.3 Treatments

Six days old larvae were used in this study. Each treatment (with three replicates for each treatment) contained 200 larvae fed with five different daily feed rates: 12.5, 25, 50, 100, and 200 mg/day/larva (wet weight, 60% moisture content). The larvae were initially placed onto the prepared feed in a plastic cup (height 11 cm, diameter 6 cm) and covered by a black sheet. The lid of the cup contained holes to allow air circulation. In order to prevent oviposition of other flies and parasitoid, a mosquito mesh was clamped between box and lid. The feed for larva were prepared, weighed, and kept frozen 24 hours before treatment to prevent decomposition process.

Sampling and feeding was conducted every three days. During sampling and feeding, the remaining larvae was transferred into another glass already filled with next feed. Residual material of previous glass was dried at 105 ºC to dry mass determine.

Feeding of larvae was continued until more than 50% of the larvae of each treatment had developed into prepupae. Five prepupae were randomly sampled from each treatment and determined for their dry mass and protein content in the same way as larvae.

All experiments were conducted at room temperature and relative humidity (28-30 ºC, RH = 65-75%, 12 hours photoperiod).

2.4 Growth and waste reduction

Relative growth rate of larvae was calculated by Waldbauer's formula [45]

$$RGR = \left[\frac{\text{Dry weight}_{(t2)} - \text{Dry weight}_{(t1)}}{\text{Dry weigth}_{(t1)}}\right] \Big/ (t_2 - t_1)$$

Relative consumption rate was calculated by Waldbauer's formula [45].

$$RCR = \left[\frac{\text{Dry weight of remained food}_{(t2)} - \text{Dry weight}_{(t1)}}{\text{Dry weigth}_{(t1)}}\right] \Big/ (t_2 - t_1)$$

In order to measure overall material reduction, the time of larvae required to reduce amount of food include in calculation along with overall degradation (D) of waste. All of those variables was defined as Waste Reduction Index (WRI) which measured by formulae developed by Dienar *et al.* [9]:

$$WRI = \frac{D}{t} \times 100 \qquad (1)$$

$$D = \frac{W - R}{W} \qquad (2)$$

where, *D* represents overall material degradation; W = total amount of food provided during course of experiment (t); and R = total amount of residue during course of experiment.

The ability of larva to digest rice straw was measured by Efficiency of Digested Feed (ECD) based on the relationship developed by Scriber and Slansky [42] and modified by Dienar *et al.* [9],

$$B = (I - F) - M \qquad (3)$$

$$ECD = B/(I - F) \qquad (4)$$

Where, *B* represents total amount of food use for growth; I = total amount of food offered during experiment; F = total amount of residue during experiment (undigested food + excretory food); and M = amount of food metabolized by larvae (calculated by mass balance). Dry weight was used for all calculation.

2.5 Data analysis

One-way ANOVA, with subsequent Duncan tests were applied to determine the differences in development time, substrate consumption, WRI and ECD among various treatments ($p < 0.05$).

3. Results And Discussion

Larva fed with 200 mg grinded rice straw per day had the highest prepupal dry weight with shortest development time (average 13.64 mg and 38 days, respectively). Decreasing food ratio produce lower prepupal weight and longer development time. Larva fed on food ratio less than 100 mg/larva/day had similar development time although larva fed on 50 mg/larva/day Prepupal dry weight was similar between larva fed with 12.5 and 25 mg/day/larvae (Figure 1).

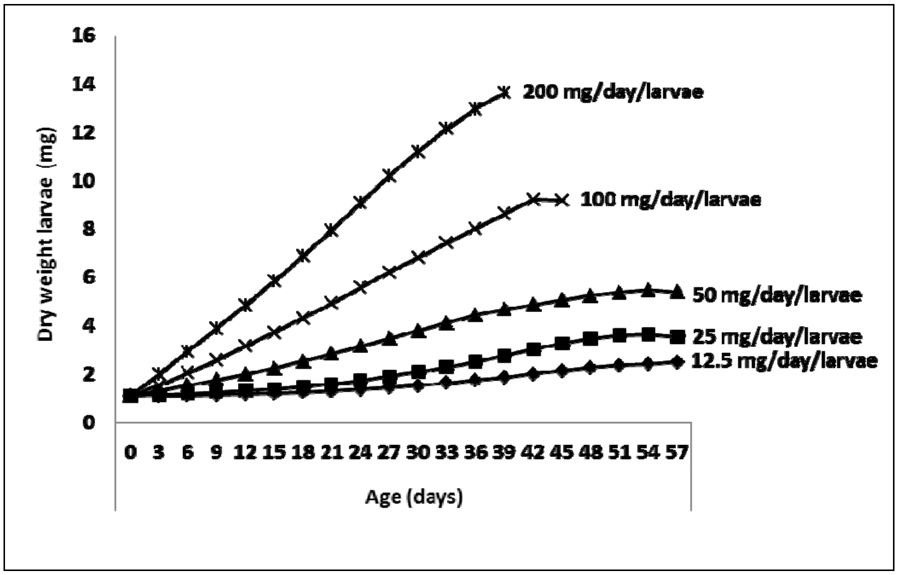

Fig 1: Change in dry weight of *H. illucens* larvae with development time and final weight of the prepupae.

Application of different feed rate in general, except for group fed with 100 mg and more rice straw/day/larvae, produced similar growth pattern as larva slowly gained weight then followed by sharp decreased of weight gain when larvae metamorph into prepupae (Figure 2).

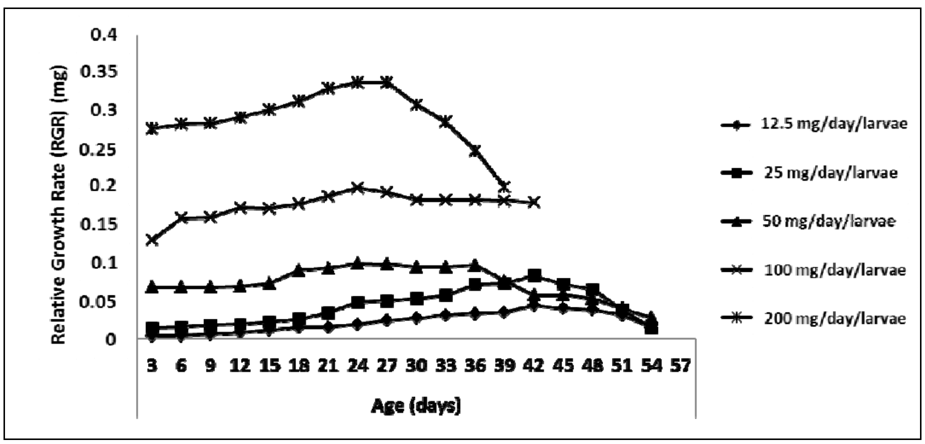

Fig 2: Relative Growth Rate (RGR) of Black Soldier Larvae (in dry mass) feed on different ration of rice stalk.

Even though there were differences on the RGR, the Relative Consumption Rate (RCR) of group 100 and 200 were similar. The results indicated that 100 mg/day/larvae probably the highest consumption ability of larvae per day as additional feed ration did not increase consumption (Figure 3).

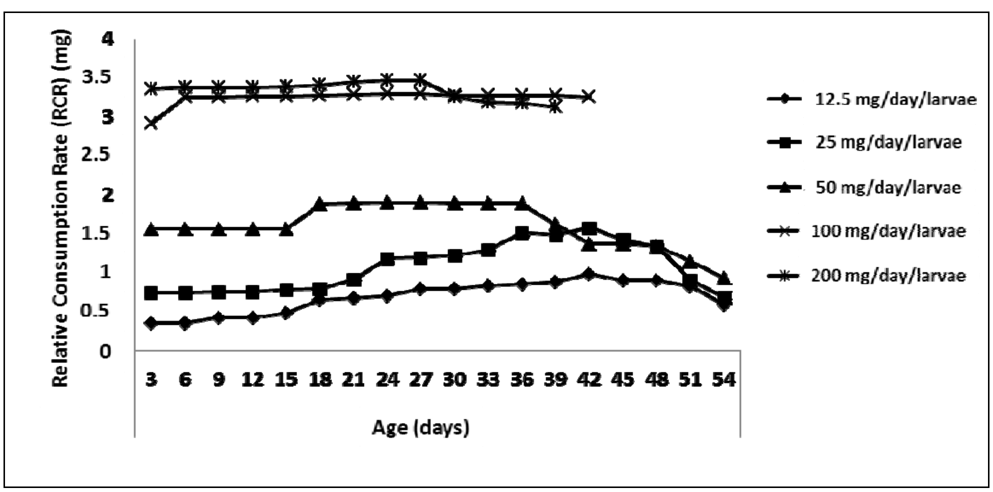

Fig 3: Relative Consumption Rate (RCR) of Black Soldier Larvae (in dry mass) feed on different ratio of rice stalk.

Development time of BSFL fed on rice stalk ranged from 38 to 54 days with higher feeding rate significantly reduced the development time. However, fast growing BSFL consumed less substrate, ranged from 9.5 to 31.53% that related to less substrate reduction. On the other hand, higher ECD recorded by the group fed with highest and lowest amount of feeding material. However, high ECD on larva fed on 12.5 g rice straw followed by high mortality rate which could be caused by strategy to scarcity of food and low food quality. High ECD generally also followed by high WRI except for feed rate 200 mg/larvae/day (Table 1).

Table 1: Larval development time (egg to prepupa, in days) estimation of growth rate, substrate consumption, relative waste reduction index (WRI, overall reduction/days), efficiency of conversion of digested food (ECD, %), and survival rate. Daily feeding rates 12.5, 25, 50, 100, and 200 mg rice straw powder (60% moisture) per larva per day.

	12.5	**25**	**50**	**100**	**200**
	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
Development time (days)	54.10 ± 0.57c	54.20 ± 0.57c	53.20 ± 0.57c	41.10 ± 1b	38.20 ± 1.15a
Substrate consumption (%)	31.53 ± 0.74d	24.13 ± 0.060c	18.36 ± 0.02b	18.65 ± 0.01b	9.58 ± 0.01a
ECD (%)	9.92 ± 0.01c	5.69 ± 0.01a	5.67 ± 0.01a	6.11 ± 0.01b	10.85 ± 0.01d
Survival rate (%)	51.21 ± 2.29a	74.24 ± 2.62b	80.90 ± 1.19c	91.79 ± 1.17d	98.27 ± 1.13e
WRI	0.58 ± 0.01d	0.45 ± 0.01c	0.34 ± 0.01b	0.44 ± 0.01c	0.24 ± 0.01a

Average values followed by the same letter at same row do not vary significantly (p<0.05)

This study showed that *H. illucens* able to consume and digest rice straw as their main food sources. Previous studies on similar subjects was applied rice straw mixed with restaurant wastes and glucose as feed material of BSFL [28, 52]. However, as rice straw contain high lignin-celulose, final biomass of larva produced was less than larvae fed on another organic waste rich with protein and lipid. The result was lower than ~35 mg dry weight obtained by study of Diener *et al*. [9] and ~16 mg dry weight Rachmawati *et al*. [39]. Main caused of this phenomenon is lack of energy sources for growth. Even though gut of BSFL contained microbes produced cellulase for degradation of lignin [21, 23], convertion of lignocellulose into simple sugar molecules required certain amount of initial energy. Addition of sugar, hydrolitic enzymes, and innoculate the material with certain type of bacteria originated from BSFL might overcome this problem however with additional production cost which is unsuitable for low income farmers [28, 48].

Under this condition, larva adjust their energy budget and prioritize energy allocation to growth and metabolism which lower their relative growth rate (RGR) [13, 16, 41]. In this study, RGR at feed rate lower than indicated slow growth rate of larvae under food scarcity (Figure 2). By using this strategy, larvae prioritized their strategy to allocate more energy to keep good health and resume growth after food scarcity is over (known as compensatory growth) [10, 29]. However, as the level of food scarcity became longer and the quality of food was not changed, larva changed their strategy to prioritize growth. This trend was demonstrated at day 18 as larvae of group 12.5 and 50 mg increased their growth and consumption rate (Figure 2 and 3). This strategy come with the cost of self-maintenance [16, 21] like reduced immune function [8], shorter life span [33], poor performance [34], and possible low offspring quality which could hinder sustainability for larva production. This phenotypic plasticity follow strategy of holometabolous insect when dealing with food scarcity period longer than their normal development time [19]. Based on this, 15 to 18 days maybe considered as a threshold for maintaining good quality of BSFL during food restriction.

Our study showed that larva provided with limited amount of feed migrates out as soon as their prepupal weight reached ~ 2 to 4 mg dry weight. This weight maybe considered as a critical weight defined by Nijhout and William [38]. This critical weight also indicated the lowest weight of harvested.

Prior to pupation, larva stops their feeding in order to produce prothoracicotropic hormone (PPTH) that is necessary for metamorphosis process into pupa. This condition create time lag and weight change during this period, which related to the quality and amount of food consume, may explain the differences in prepupal weight between group of this experiment and with result from other studies.

In term of larva life cycle, there is possibility that the shortest development time (38 days) may be further reduced by increasing the amount of feed ratio more than 200 mg rice straw/larva/day as the threshold for development time still needed to be studied further.

4. Conclusion

In brief, black soldier fly larvae has the ability to digest and survive when fed with raw rice straw. Survivorship of larvae, biomass produced, and time of development positively are related to the amount of feeding material provided to the larvae. However, larvae provided with large amount of feeding material consumed less fraction of substrate which is not beneficial in term of application for waste reduction. Daily rate of 200 mg grinded rice straw may be considered as the best ratio as it meets the requirement of short development time, considerable prepupal weight, low mortality rate, good fraction of organic matter degradation, and high of consumption rate efficiency. Using this number as reference value system then as sytem of rice farming waste management could be build by simple mass balance (Figure 4).

Application for small farm could provided additional income for farmers not only from selling larvae biomass as source of high value fish feed but also application of compost produced as growth medium for high value crops (such as exotic crops), and leached water produced through bioconversion process (Putra, unpublished data).

Combination of waste treatment and generation of valuable product makes this technology as a promising tool for waste management in low and middle-income countries. This technology offers possible income generation without high investment cost while reduce environmental impact and production cost of local rice farming.

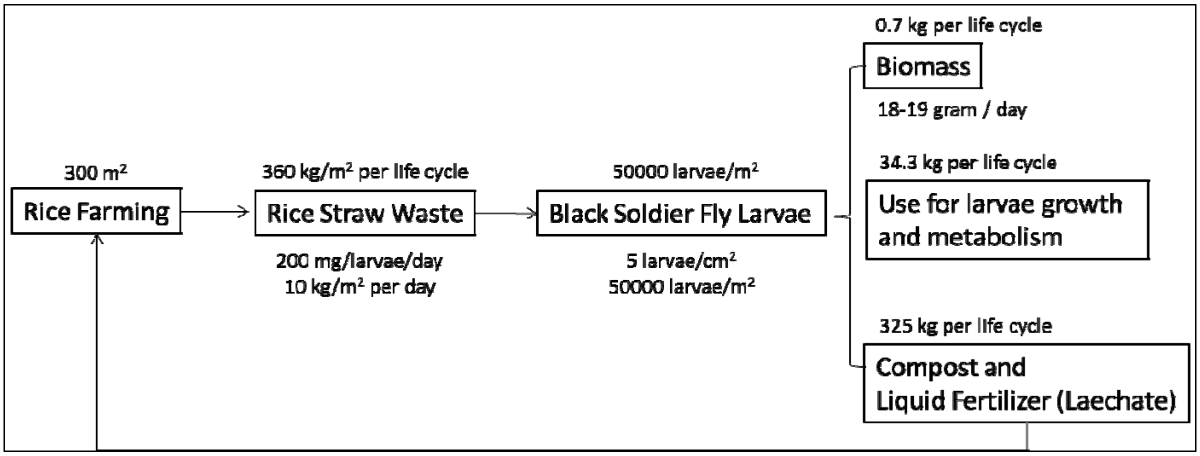

Fig 4: Possible management of rice straw waste with bioconversion by Black Soldier Fly Larvae

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