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Growth of black soldier larvae fed on cassava peel wastes, An agriculture waste

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Abstract
In Indonesia, growing cassava based food production significant amount of cassava peel wastes which has huge potential to cause significant environmental and health problems. Studies had showed the ability of Black Soldier Fly (BSF) larva (*Hermetia illucens*) to live in various type organic wastes made them as one of potential biological agents for bioconversion of household, industrial, and agricultural organic wastes. wastes. In order to apply this insect as bioconverter of cassave peel wastes, some growth variabels of BSF fed on cassave peel wastes were measured. In this study, two hundreds 6-day-old larvae were fed on cassava peel with a variation of 12.5, 25, 50, 100, and 200 mg/larva/day, replicated three times, until pupation. Results showed negative relation betweenfeeding rate and proportion of substrat consumption while negative relation recorded between feeding rate with efficiency of feed conversion. Highest propotion of feed used for metabolism recorded at lowest feeding rate. Best waste reduction index (2.79 ± 0.15), development time to pupation, and feeding duration recorded on feeding rate of 100 mg/larvae/day. Based on this study, it could be concluded best feeding rate for growth of BSF larvae is 100 mg/larvae/day.

Keywords: Bioconversion, cassava peel, Black Soldier fly larvae, growth, waste reduction

1. Introduction

Cassava is one of Indonesia's main agricultural commodities whose potential with total production of 21 million tons in 2011 from 0.95 million hectares of harvest area [14]. Mature root of cassava consisted of three distinct regions: central vascular core, the cortex (flesh) and phelloderm (peels). Both central vascular core and cortex are used as materials for various food products leaving large amount of peels as wastes [1]. Cassava peel account for 8-15% of total dry matter of the root [4]. Cassava peels composition, depend on region, consisted of 20-31% hemicelluloses, 16,42% cellulose [35], and 6-8% lignin [17]. Other than fiber, peels also contains 81.9-93.9% organic matter and 4.1-6.5% crude protein [15]. Analysis of mineral content of cassava peel indicated following mineral content: 48,7% C; 1% N; 1,1% K; 1,6% P; 0,16% NO_3; 0,15% Na; 0,9% Ca; 125 mg/kg Zn; 15 mg/kg Cu; 180 mg/kg Mn; 16,7 mg/kg Pb; 48,7% C/N; and 52,6% ash [1].

Cassave peel have been applied as feedstuff for various livestock with various encouraging results [2, 3, 29, 40]. This material also evaluated as raw material for biogas production [1], reducing sugar [30], or bioethano [15]. However, due to its hydrocyanic acid (HCN) content which is harmful for monogastric and requirement of specific material and knowledge for fermentation, most of peels are discarded as wastes.

Another approach to manage organic wastes by bioconversion. Usually, microorganisms are applied to reduce organic wastes biomass into microorganism biomass, metabolism by products, and waste residue. However, the needs of specific bioreactor, additional energy, specific skill, and limitation of product application made this methods unattractive for smallholder farmers. Recently, another approach of organic wastes reduction have been developed by application of sacrophages, such as insects larvae. Black Soldier Fly (*Hermetia illucens*) larvae (BSFL) is one of cosmopolitan insect species in which their larvae had been known for their ability to consume various types of organic wastes [5, 16, 41]. Biomass of larvae produced from their feeding activities furtherly applied as protein source for animal feed [22], fatty acid for biodiesel [16, 41], bioethanol [32], while residue of bioconversion could be applied asfertilizer [10].

However, most of studies of bioconversion by *H. illucens* larvae focused on metabolism waste, such as animal and human feses [66, 46] or human food wastes [74, 75].

Few attempts were conducted on agricultural wastes [57, 37] eventhough it produces in significant numbers annually. In Indonesia potency of biomass wastes produces estimated aroung 146.7 millions ton per year originated from residue of paddy, rubber wood, sugarcane, palm tree, tree felling, and other agricultural wastes.

In order to apply this larvae as bioconversion agent of cassava peel, it is necessary to understand ability of larvae to consume the feeding material and its effect to growth and development.

2. Materials and Method

2.1 Black Soldier Fly Larvae

Black Soldier Fly Larvae (BSFL) were obtainedfrom population kept in Laboratory of Environmental Toxicology, School of Life Sciences and Technology. All larvae kept in contast temperature of 26-28 °C, relative humidity of 65-70%, and 12:12 hour day:night period. Larvae used in this study were 6 days old larvae feed with feeding material made of combination of cassava peel, manure mixed with water. Study was conducted from March to June 2015.

2.2 Waste Material

Fresh cassava peels waste originated from local food industry at South Bandung. Peels were packed inside air-tight plastic bags and transfer to laboratory within collection day. Upon arrived at laboratory, all peels were kept inside freezer with temperature 4 °C to prevent decomposition by microorganism. Prior application as feeding material, peels removed from freezer, dried at room temperature, and grinded by food mill. Grinded cassava peel then sieve with sieve number 2 and thoroughly mixed with water (60% of total weight to create feeding material for BSFL.

2.3 Treatments

Treatment method was based on modified Diener's method [10]. In this study, BSFL were fed on cassava skin with variation of 12.5; 25; 50; 100; and 200 mg/ day/larva (furtherly state as feeding group in this paper). Larvae kept inside containers 11 cm in height and 8 cm in diameter, wrapped with black plastic sheet at side and lid. Ten holes, with size of 15 mm each, were punctured at the lid to provide oxygen to BSFL.Two hundreds larvae were kept in the container and all treatments replicated three times. The amount of feed was calculated in accordance to the number of remaining larvae at each container. Larvae were transferred to new container filled with required feeding material, prepared in same day, every three days. At each transfer, 5 larvae were sampled for body weight measurement. Study was conducted until at least 50% of total pupae population reached pupation, which also identified at development time to pupation.

2.4 Growth and Feed Conversion

Growth of the larvae was observed as change in body weight until pupation while in the same time required to complete development into pupae also measured for each feeding group. The feed conversion ability was calculated using the formula developed by Scriber & Slansky [36], which is

$B = (I - F) - M$

$ECD = B/(I - F)$, where B is total feed used for growth of larvae, I is the total feed during experiment, F is total feed residue during experiment (undigested food + excretory product), M is total feed metabolized by larvae. All materials were calculated in dry weight (mg).

2.5 Waste Reduction

Waste reduction index (WRI) showed level of waste reduced during specific time. Itwas calculated in its dry weight by using the formula [10]:

$$WRI = \frac{D}{t} \times 100$$

$$D = \frac{W - R}{W}$$

Where D is the total feed degradation, W is the total feed during test time (t), while R is the residue during the test.

2.6 Data Analysis

All data was analyzed using one-way ANOVA at a significant rate of 0.05, and followed by Duncan test for post hoc test.

3. Results and Discussion

Different feeding rates influenced development time as larvae fed with both 100 mg/day and 200 mg/day had the lowest development time among study groups (20 days). While longest development time achieved by larva fed with lowest feeding rate (54 days) (Table 1).

This result indicated ability of *H. illucens* to use low quality diet, like cassava peel, as their main food sources. Compare with other studies, best larvae development time recorded in this study was similar to larvae feed on high protein and high lipid material [31] and slower than larvae fed on high protein commercial chicken feed [10]. Under food scarcity, development time of larvae recorded on this study also slower than studies reported by Ooninincx *et al*. [31] and Diener *et al* [10].

It seems that cassava peels could provide required proteins and carbohydrates which critical for development [6, 25, 37, 39]. However differences on nutrient content of feeding materials may lead to different growth patterns (growth rate plasticity) especially under both malnutrition and food scarcity condition [11, 24].

Table 1: The insect growth dynamics and estimation of substrate utilization

	12.5		25		50		100		200	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Development time (days)	53.67d	0.58	41.67c	0.58	29.33b	0.58	20a	1	20a	1
Feeding duration (days)	17.89	0.19	13.89	0.19	9.78	0.19	6.67	0.33	6.67	0.33
Substrat consumption (%)	36.82e	0.66	31.47d	0.6	22.58c	0.6	18.59b	0.4	9.29a	0.8

Longer development time could be affected by weight larvae, as direct result of food quality, as studies showed timing of maturation in insects is determined by the time when larvae reaching a critical developmental stage [27]. At this stage, there is a shift in hormonal level which limit the development to a defined time period until the onset of pupation [26, 34]. Under this condition, final weight of larvae is relatively constant (critical weight) as larvae terminate their feeding in preparation for pupation (defined as critical weight) termination of feeding, and initiation of metamorphosis [39].

Time required to achived the critical weight is defined the development time of larvae [26, 28]. It is also hypothesized that there was a nutritional imbalance at lower feeding rate which led to an increase in consumption to compensate nutrient deficient (indicated by longer feeding duration and higher substract consumption) [6, 7].

In this study, under optimal nutritional condition (feeding group 100 and 200) critical weight of larvae was higher than larvae fed on rice straw [37] and oil palm kernel [57] and much lower than larvae fed on high protein diet like chicken feed [19]. It seems, protein content in cassava peel provide better food resources than rice straw and palm kernel.

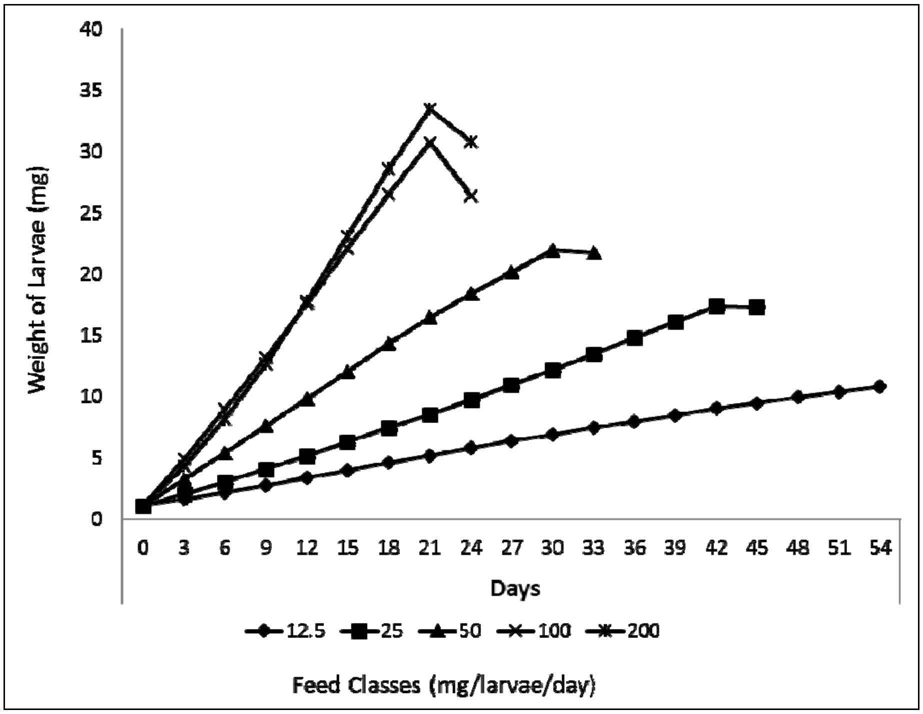

Fig 1: Change in weight of Black Soldier Flies Larvae Fed with different feeding rate
(Note: observation was finished when 50% of larvae attained pupation, except class 12.5 in which observation was finished due to all individual was sampled)

In our study, the larvae also carried out pupation under malnutrition (feeding group 25 and 50) even though their final weight was much lower than final weight of other groups (Fig. 1). This mechanism is known as "intercalary" molts [26]. This mechanism is independent of size and might also independent of brain that produces prothoracicotropic hormone (PTTH) which drives the ecdysteroid surges needed for molting [8, 38].

Unfortunately, this study did not carry out any observation on the mechanism even though protein may hold important factor for metamorphosis [39].

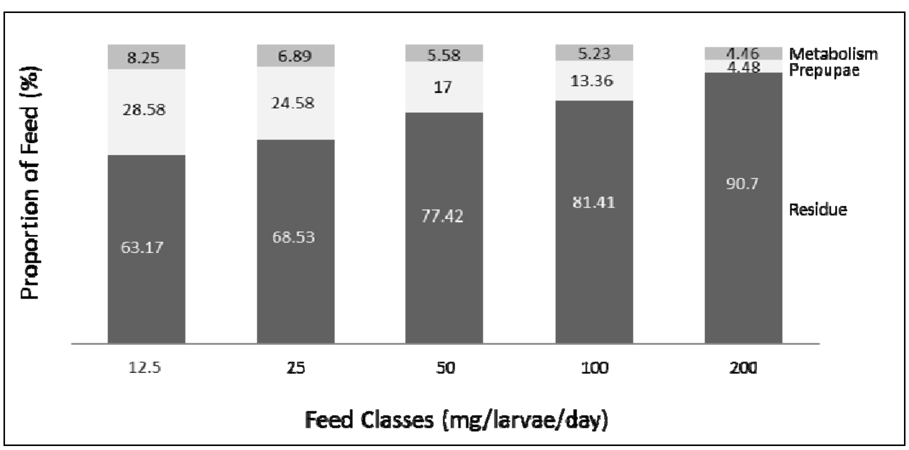

Fig 2: Relative proportion of feed (five feed treatment: in dry weight) used for metabolism and growth

This study showed larvae with food scarcity (12.5 mg/day) had to adjust their energy budget and prioritize energy allocation to growth and metabolism [12, 13]. This explained significant proportion of feed used for metabolism and growth in this group (Fig. 2) and lower conversion of food into final biomass (Fig. 3). On the other hand, larva with abundance resource only used part of their resource to growth and metabolism while most of resource left unconsumed.

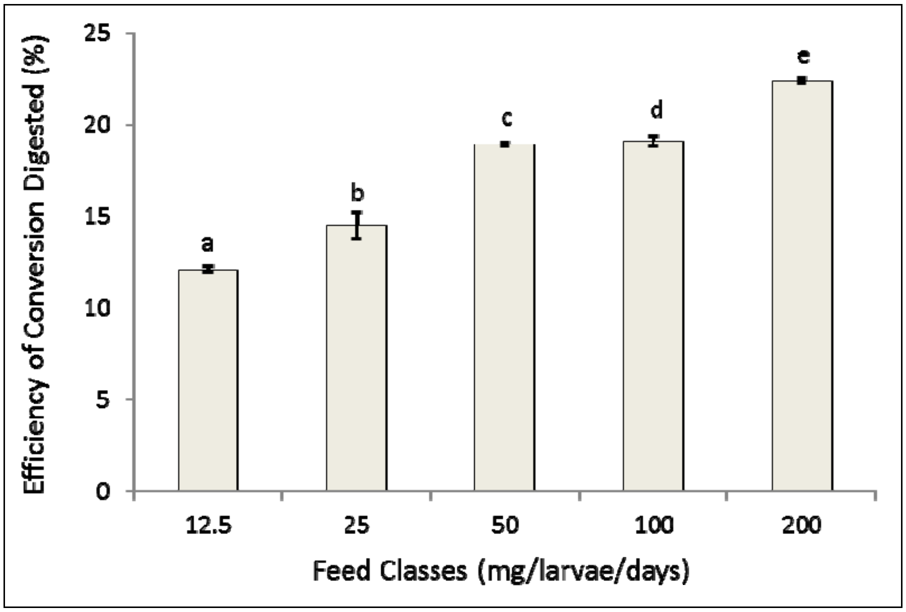

Fig 3: Efficiency of Conversion of Cassava Peel into Biomass by Black Soldier Fly Larvae

Larvae allocate more energy in order to reduce mortality rate and to resume growth after food scarcity is over (known as compensative growth) [11, 18]. However, as the level of food scarcity become longer and the quality of food was not changed, larva changed their strategy to prioritize growth in order to metamorph into pupae with cost of self-maintenance [13, 19], like reducedimmunity [9], shorter life span [21], poor performance [40], and possible low adult quality which could hinder sustainability of population and production of pupae biomass.

4. Conclusion

It can be concluded that 100 mg cassava peel/day/larvae is the optimum feeding rate for BSFL which produce pupae in shortest time with significant weight. This study also indicated phenomenons of "critical weight", "intercalary molt", and "compensative growth" which may explain plasticity feeding resources of BSFL. Further study required test this hypothesizes in order to development efficient application of BSFL as bio converter of cassava peel, a low quality agriculture wastes.

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