

## Growth Performance of Black Soldier Fly Larvae (*Hermetia illucens*) Fed on Some Plant Based Organic Wastes

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### ABSTRACT

Insect larvae, such as black soldier fly larvae (BSFL: *Hermetia illucens*) are currently being considered as an alternative source of protein for poultry and aquaculture industry while in the same time apply as agent to manage organic wastes. In order to achieve both roles it is necessary to develop an appropriate farming methodology and understand the impact of different feeding source for the insect production. The study explored the effect of three local plant based organic wastes as feedstocks to growth of the black soldier fly larvae. Separate feeding trials on horse manure (HM), vegetable wastes (VW), and tofu dreg (TD) was done and their effect on BSF larvae growth rate, larvae weight and total prepupal yield in grams content analysed. The efficiency of the BSF larvae to consume and ability to reduce the waste load of the different substrates was also evaluated. The result showed all materials suitable as feeding material for black soldier fly. Difference on physical properties and chemical content affected the development time, harvested biomass, efficiency of digestivity, and efficiency of biomass production which is unique for each type of waste. This study provide base line information for future development of plant based organic waste management.

## 1. Introduction

For decades, researchers worldwide have developed an alternative method to manage organic wastes through decomposition by macrofauna such as black soldier fly larva (BSFL), earthworm, house fly, and mealworm (Beard and Sands 1973; El Boushy 1991; Ndegwa and Thompson 2001; Ramos-Elorduy *et al.* 2002; Elissen *et al.* 2006; Diener *et al.* 2009), in which black soldier fly considered as the best candidate. The black soldier fly is native species of neotropic and now found worldwide through human-mediated dispersal and natural dispersal (Callan 1974; Marshall *et al.* 2015). Studies showed that larva of this species has the ability to consume wide range of organic wastes such as agricultural wastes (Manurung *et al.* 2016; Supriyatna *et al.* 2016), animal and human remains (Tomberlin *et al.* 2005; Pujol-Liz *et al.* 2008), fish offal (St-Hilaire *et al.* 2007a), food waste (Diener *et al.* 2011; Nguyen *et al.* 2013; Oonincx *et al.* 2015a), as well human and livestock feces (Fatchurochim *et al.* 1989; Myers *et al.* 2008; Banks *et al.* 2014; Oonincx *et al.* 2015b). Due to this ability and the

fact that the species also able to be mass-produced (Sheppard *et al.* 2002), this species has been studied as decomposer that recycling nutrients in organic wastes into their biomass which is high in protein and fat, a process called bioconversion (Sheppard *et al.* 1994; Diener *et al.* 2009; Li *et al.* 2011; Surendra *et al.* 2016). The biomass of the larvae is applicable as part of ingredient of feed for aquaculture, livestock, and poultry industries (Newton *et al.* 1977; St-Hilaire *et al.* 2007b; Hariadi *et al.* 2014; Li *et al.* 2016; Magalhaes *et al.* 2017; Renna *et al.* 2017; Schiavone *et al.* 2017) even as potential source for biodiesel (Zheng 2012).

In Indonesia, large numbers of various types of organic wastes produced by local small to large scale agroindustry, tourism, and household. Each of them has unique nature and properties. Restaurant waste, for example, containing parts of animal and plant which rich in carbohydrate and relatively similar amount of protein and fat while on the other hand fruits and vegetables rich in carbohydrate with significantly low-fat content. Studies showed that each type of diet would affect the development, productivity, some life history traits, and chemical composition of the biomass (Tomberlin *et al.* 2002; Oonincx *et al.* 2015a; Tschirner and Simon 2015; Cammack and Tomberlin 2017). Among organic wastes produces in Indonesia,

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plant-based wastes are considered the most dominant and produced by various human activities.

This study was designed to imitate the real condition in Indonesia in term of variation of plant-based organic wastes produced. The objectives of this experiment were 1) to determine the effects of diet composition on growth, development time, and prepupal weight and 2) to compare the consumption efficiency when larva fed with different types of waste materials. The results of this study could be applied as guideline to design optimum and sustain integration system between plant-based organic wastes bionconversion system to activities that produced the wastes.

## 2. Materials and Methods

### 2.1. Animal Specimen

Larvae of the black soldier fly (in this manuscript will be state as BSFL) were obtained from eggs purchased from commercial BSF farms in Sumedang and Depok (both areas are in West Java) in order to augmented egg produced by black soldier fly colonies kept in Laboratory of Environmental Toxicology, School of Life Sciences and Technology, Bandung, Indonesia. All eggs were kept on substance made of commercial chicken feed (60% moisture) and kept at constant temperature (28°C, 70%RH) in a container (50 cm x 25 cm x 10 cm) at Laboratory of Environmental Toxicology, School of Life Sciences and Technology, Bandung, Indonesia prior used.

### 2.2. Waste Materials

In this study, three types of organic wastes were tested. Those wastes were (1) vegetable wastes (VW) originated from a local traditional market in Bandung, (2) house manure (HM) from horse stall at Ecopasantren Daarut Tauhid, Paronpong, West Bandung, and (3) tofu dreg (TD) from local tofu maker in Summersari, Bandung.

Prior application, all wastes kept in closed plastic bags and stored in the refrigerator with temperature -5°C to prevent decaying by microorganism activities.

### 2.3. Feed Rates

Each treatment (three replicates for each treatment) contained 200 of six days old larva (hand counted). Larvae fed with five different daily food rates: 12.5, 25, 50, 100, and 200 mg/larvae/day (wet weight) for each wastes material.

The larvae were initially placed onto the prepared food within plastic cup (height 12 cm, upper diameter 7 cm, lower diameter 5 cm) and covered with a black plastic sheet to protect them from light disturbance. Dark cloth (diameter 0.01 mm) was clamped between box and lid to prevent infestation of feeding materials by other flies and parasitoid attacks. Twenty four hours prior used, food rations were prepared, weighed, and kept frozen.

Data collection and feeding were performed every three days. At the same time, the larvae were transferred into another glass already filled with next feed. The residual material of previous glass was dried at 60°C for dry mass determine.

Feeding of larvae was continued until more than 40% of all larvae metamorphosed into prepupal (Tomberlin *et al.* 2002) while weighing of larvae continued until each larvae metamorphosed into prepupae. All prepupal were removed daily from each container and weighed, then placed in a plastic container for further rearing process into adults. Prepupal and pupal were held in the same incubator in which the larvae were reared and were monitored for adult emergence daily (Cammack and Tomberlin 2017).

### 2.4. Digestibility

Ability of larvae to digest wastes used in this study was determined by calculating Efficiency of Digested Feed (ECD) based on terminology of Scriber and Slansky (1981).

$$B = (I-F) - M \quad (1)$$

$$ECD = B/I - F \quad (2)$$

where B = assimilated food used for growth (measured as prepupal biomass), I = total food offered during the experiment, F = residue in the experimental cup (undigested food + excretory products), and M = assimilated food metabolized (calculated by mass balance). All figures are given in mg (dry weight). Higher ECD indicates better food conversion efficiency. All collected data compared with the result of Diener *et al.* (2009) which considered as the most common method to produce larvae for feedstock material.

### 2.5. Mass Balance

Mass balance is an approach to design biomass production system and to predict the digestibility of diet. Based on this approach, the total number of feed consumed by larva was divided into three output, the mass of diet material that use to maintain homeostasis of larva, the mass of undigested diet material, and the harvested biomass (Figure 1).

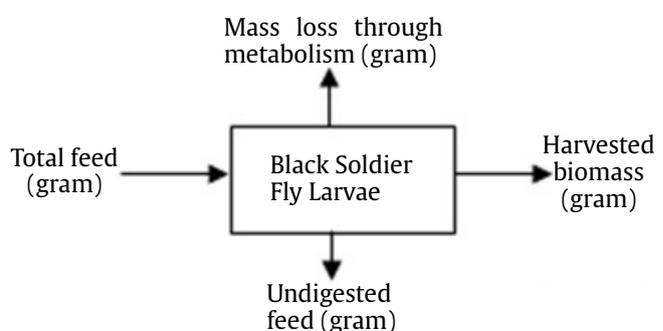


Figure 1. Mass balance model of bioconversion of organic waste into body biomass of black soldier fly larva

## 2.6. Data Analysis

One way ANOVA ( $P \leq 0.05$ ) with subsequent Tukey HSD tests was applied to detect difference on data collected among all treatments.

## 3. Results

### 3.1. Development Time

Larvae spent less time with increasing amount of feeding material provided to them. Larvae fed with organic wastes spent more time in development compared with the ones fed on chicken feed (except for BSFL group fed with TD at rate 12.5 mg/larva/day).

Among organic wastes applied in this study, BSFL group fed with TD had shorter development time. However, all treatment groups have similar development time when fed with rate of 100 mg/larvae/day and more (Figure 2).

### 3.2. Prepupal Weight

There were two groups of larvae based on the prepupal weight. Prepupal weight of HM and VW groups was similar on the trend and value for all feed rate. On the other hand, both TD and CF groups had significantly heavier prepupal than groups HM and VW (ANOVA,  $p < 0.05$ ) (Figure 3).

Feeding BSFL with TD on the feed rate more than 25 mg/larvae/day produced slightly heavier prepupal compared to other groups (ANOVA,  $p > 0.05$ ) (Figure 3).

### 3.3. Digestibility

Vegetables waste and CF more easier to be digested and converted into energy at lower feed rate. On the other hand, efficiency of conversion increased with higher feed rate until reached a threshold when larvae fed with TD while there was stable digestibility of horse manure (Figure 4).

Larvae fed with TD on the feed rate of 50 mg/larvae/day and vegetable waste on the rate of 12.5 mg/larvae/day showed highest conversion efficiency (48.1% and 49.54%, respectively) (ANOVA,  $p < 0.05$ ).

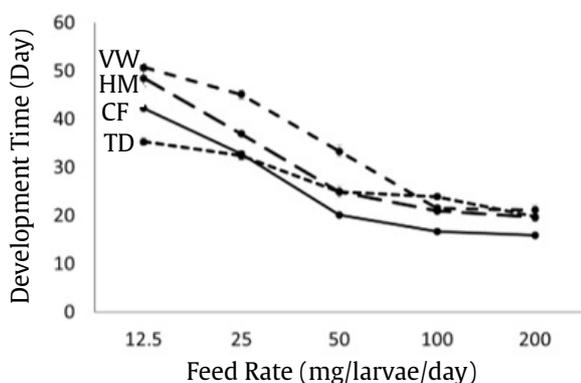


Figure 2. Developmental time of black soldier fly larvae fed on different organic wastes. TD: Tofu Dreg, VW: Vegetables Waste, HM: Horse Manure, CF: Chicken Feed (Diener 2009)

### 3.4. Mass Balance

All treatment showed different pattern of proportion of feed converted into prepupal biomass, used for metabolism, and undigested. On average, most of feed converted into residue except for VW group (Figure 5).

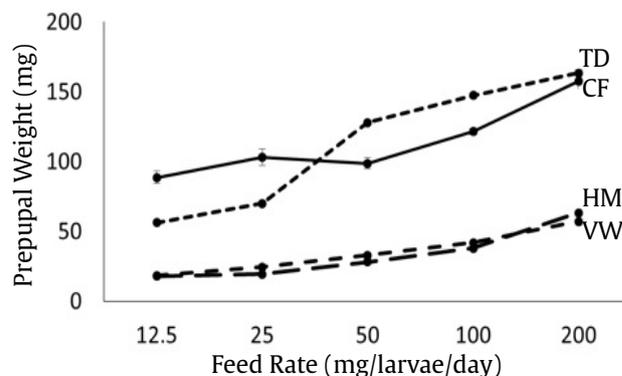


Figure 3. Prepupal weight of black soldier fly larvae fed on organic wastes. TD: Tofu Dreg, VW: Vegetables Waste, HM: Horse Manure, CF: Chicken Feed (Diener 2009)

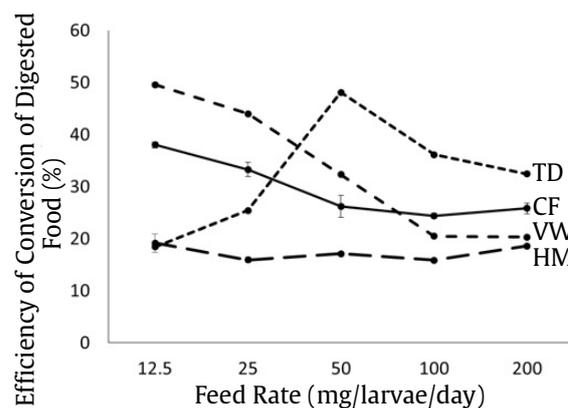


Figure 4. Efficiency of Conversion of Digested Food (ECD) of black soldier fly larvae fed on organic wastes. TD: Tofu Dreg, VW: Vegetables Waste, HM: Horse Manure, CF: Chicken Feed (Diener 2009)

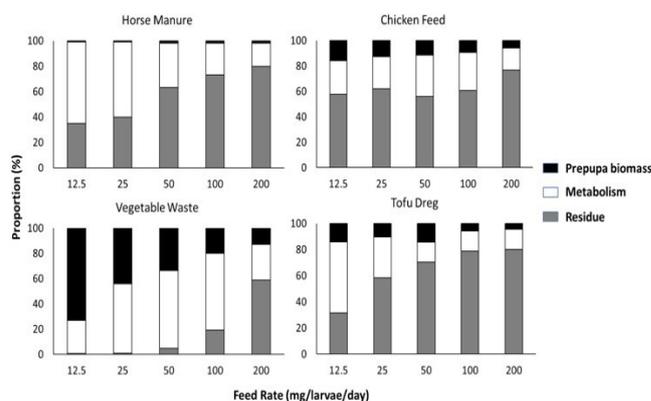


Figure 5. Proportion of feed converted into prepupal biomass, used for metabolism, and residue by black soldier fly larvae fed on organic wastes. TD: Tofu Dreg, VW: Vegetables Waste, HM: Horse Manure, CF: Chicken Feed (Diener 2009).

## 4. Discussion

### 4.1. Developmental Time

The developmental time of BSFL in this study generally shorter than some of previous studies (Table 1). It seems BSFL able to consume and digest plant based organic wastes tested in this study as their only nutrient source.

Slower development time of the larvae fed on organic wastes could be related to the quality of the food especially balance in nutritional contents. Unlike chicken feedstock which designed with all nutritional content for growth, all organic wastes characterized with the abundance of particular nutrition while lacking in others. Nutritional imbalance in diet could lead to an increase in consumption period of insect larvae to compensate for deficient in the nutrients especially proteins and carbohydrates (Nijhout 2003; Wright *et al.* 2003; Lee *et al.* 2004; Simpson *et al.* 2006; Banks *et al.* 2014). Lack of particular nutrient influenced the time when larvae reaching a critical developmental stage (Nijhout 1981). In insect, larval weight is attained to the critical stage of development (Nijhout and Williams 1974; Blakley and Goodner 1978; Keena 2005). At this stage, a shift in hormonal level occurs that induce further development (Berreuer *et al.* 1979; Raubenheimer and Simpson 1997).

### 4.2. Prepupa Weight

During larvae stage, insect consumes a large quantity of food as a reserve for adult stage. The weight of larvae highly depends on the composition of food. The study by Tschirner and Simon (2015) showed the importance of protein to produce heavier larvae which explained the lighter prepupal weight of HM and WM (both are rich in complex carbohydrate) compared to CF and TD (both are rich in protein and simple carbohydrate) (Li *et al.* 2012).

Prepupal weight highly affects the growth, survival, and biological traits related to reproduction of adult flies (Roper *et al.* 1996; Blackmore and Lord 2000; Liu *et al.* 2008). As for black soldier fly, lower prepupal weight could hamper the sustainability of bioconversion process as they will produce adults with lower reproduction ability (Gobbi *et al.* 2013). The result also indicated that the development time could be reduced by applying more food to the larvae as the larvae compensate low food quality by consuming more food to obtain the required amount of specific nutrition.

### 4.3. Efficiency of Conversion

The ECD level recorded in this study was relatively higher than most of the previous studies (Table 2). The result of this study agreed with the conclusion of previous studies indicated lower ECD when BSFL consumed the low quality and/or quantity diet.

Table 1. Comparative data on development time for black soldier fly larvae on various substrates

Reference	Substrate	Development time (days)
Myers <i>et al.</i> (2008)	Dairy manure	28-30
Li <i>et al.</i> (2011)	Dairy manure	<31
Gobbi <i>et al.</i> (2013)	Meat meal	33
Gobbi <i>et al.</i> (2013)	Hen feed	15
Manurung <i>et al.</i> (2016)	Rice straw	38-52
Supriyatna <i>et al.</i> (2016)	Cassava peel	20-54

Table 2. Comparative data on efficiency of conversion of digested-feed (ECD) for black soldier fly larvae on various substrates

Reference	Substrate	ECD (%)
Manurung <i>et al.</i> (2016)	Rice straw	5.69 – 10.85
Supriyatna <i>et al.</i> (2016)	Cassava peel	12 – 21
Abduh <i>et al.</i> (2017)	Rubber seed	12.5 – 25.9
Abduh <i>et al.</i> (2017)	<i>Pandanus tectorius</i> fruits	6.3 – 27.4

### 4.4. Mass Balance

This study showed variation in proportion of feed converted into prepupal biomass, used for metabolism, and residue. In general, based on the mass balance, increasing feed rate produced more residue during bioconversion process of all material tested while the proportion for metabolism was decreased with more food.

We proposed two hypotheses to explain this pattern: (1) Under abundance food source, high amount of food also increase the amount of major organic substances (i.e protein, amino acid, lipid) for growth which is in total higher than the required amount. Under this condition there was possibility high amount of left over diet which became residue, (2) During food scarcity, larva adjusts their energy budget and prioritize energy allocation to maintain their homeostasis by increasing metabolism of consumed food and reduce excretion (Roff 2001; Glazier 2002; Hou 2014). Larvae also increase their consumption rate thus reducing the amount of residue (Couture *et al.* 2016). By applying this strategy larvae keep their good health which allowing them to resume growth after food scarcity is over (known as compensatory growth) (Mangel and Munch 2005; Dmitriew 2011).

On the other hand, when larvae feed with vegetable waste at low feed rate most of the food convert into biomass. This result may relate to moisture and water content of vegetables allowing larvae to use smaller energy for metabolism and concentrated to biomass. At high feed rate, mass of undigested vegetables easily loss through transpiration. Further studies are required to test this hypothesis.

## 5. Conclusion

This study showed composition of nutrient of the organic waste affects the development of BSFL. However, providing BSFL with large amount of feed may overcome the negative effect of low quality feed to developmental time despite small pupa size and possible negative effect of reproduction.

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