

## GROWTH OF BLACK SOLDIER FLY LARVAE (*Hermetia illucens*) FED WITH PAK CHOI (*Brassica chinensis*) AND CARP (*Cyprinus carpio*) RESIDUES

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### ABSTRACT

One main drawback of the local animal industry is the inavailability of affordable and sustainable protein supply for the livestock. Insect larvae, such as the Black Soldier Fly (*Hermetia illucens*) larvae (BSFL), have been considered as a protein source which can be produced at a large scale using low cost organic wastes as feeding material. This study was designed to determine the response of BSFL to various waste combinations of vegetable and animal remains, Pak Choi (*Brassica chinensis*) residues (S) and carp (*Cyprinus carpio*) fish offal (I). A total of 540 BSFL were fed with 100 mg/larvae/day combination of vegetable wastes: animal waste 70%: 30% (S > I), 50%: 50% (S = I), and 30%: 70% (S < I). Among the feed combinations, the S < I group showed the best results as it produced the significantly highest weight of BSFL at 122.8 mg/larvae and approximate digestibility of 62.01%, with the least pupae mortality rate at 4.29%.

**Keywords:** bioconversion, biomass, *Brassica chinensis*, *Cyprinus carpio*, *Hermetia illucens*

### INTRODUCTION

As the communities develop and increase in size, the amount of waste generated by the human population also increases. In the year 2000, about 49% of the total world population lived in cities and generated more than three million metric tons of daily waste (e.g., household items, food waste, packaging, ash) and this number is predicted to double in 2025 (Hoornweg *et al.* 2013). In 2007, the amount of food waste generated worldwide as the result of economic activities, from production to consumption, was estimated at 1.6 Gtonnes (FAO 2013). These wastes are taking up space in landfills, as the most common waste management practice, thereby contributing to the spread of pathogens, production of noxious

odors, and a significant amount of CO<sub>2</sub> (Zhang *et al.* 2019).

For several decades, researchers worldwide have developed a method to process organic matter away from landfills using biotic decomposer such as black soldier fly larvae (BSFL), earthworm, house fly, and mealworm (Beard & Sands 1973; El Boushy 1991; Ndegwa & Thompson 2001; Ramos-Elorduy *et al.* 2002; Elissen *et al.* 2006; Diener *et al.* 2009), in which BSFL is considered as the best candidate.

The black soldier fly is of Neotropic origin and now spread in all warmer regions through natural and human-mediated dispersal (Callan 1974; Marshall *et al.* 2015). This species can colonize a wide range of organic wastes, including agricultural wastes (Manurung *et al.* 2016; Supriyatna *et al.* 2016), animal and human remains (Tomberin *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;

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Oonincx *et al.* 2015a), as well as human and livestock feces (Myers *et al.* 2008; Banks *et al.* 2014; Oonincx *et al.* 2015b). Due to its biological characteristic and being easily mass-produced (Sheppard *et al.* 2002), this species has been studied to recycle the nutrients found in organic wastes to be converted into protein-rich and fat-rich biomass (Sheppard *et al.* 1994; Diener *et al.* 2009; Li *et al.* 2011; Surendra *et al.* 2016). Through the bioconversion process, the species is applied as a feed ingredient for aquaculture, livestock, and poultry industries (Newton *et al.* 1977; St-Hilaire *et al.* 2007b; Li *et al.* 2016; Magalhaes *et al.* 2017; Renna *et al.* 2017; Schiavone *et al.* 2017).

However, the heterogeneity of available organic material created a challenge to the optimization and implementation of this system, especially in the municipal areas. Restaurant waste, for example, containing animal and plant matters which are rich in carbohydrate and a similar amount of protein and fat, while mixed fruits and vegetables are rich in carbohydrate with significantly low-fat content. In Indonesia, most organic wastes are produced through economic activities in the traditional and modern markets which are dominated by vegetables and animal remains. Applying these heterogeneous resources as diet for black soldier fly would affect the development, productivity, some life-history traits, and chemical composition of the biomass (Tomberlin *et al.* 2002; Oonincx *et al.* 2015a; Tschirner & Simon 2015; Cammack & Tomberlin 2017).

This study was designed to imitate the real condition in Indonesia as a model for other similarly developed tropical countries in which different organic wastes are produced. The objectives of this experiment were 1) to compare the consumption efficiency of BSFL to diet combination of vegetable waste and animal residues, and 2) to determine the effects of diet composition on its growth, development time, pupae survival, and on the adult sex ratio. The results of this study could be used as the basis for diet manipulation in optimizing waste reduction, converting organic materials to insect biomass, and sustainability of the bioconversion system using municipal organic wastes.

## MATERIALS AND METHODS

### Animal Specimen

This study used the seven-day old larvae of the black soldier fly that were obtained from eggs purchased from a BSF farm in Sumedang, West Java. All the eggs were kept on the substance made of commercial chicken feed (60% moisture) and kept at constant temperature (28 °C, 70% RH) in a container (50 x 25 x 10 cm) at the Laboratory of Environmental Toxicology, School of Life Sciences and Technology, Bandung, Indonesia.

### Treatment

Each treatment (with nine replicates) contained 60 larvae fed with 100 mg/day/larvae (wet weight, 60% moisture content) of a diet combination of vegetable wastes (*Brassica chinensis*) and carp (*Cyprinus carpio*) fish offal. The treatments were composed of diet combination ratios of fish offal: vegetable wastes, namely; 30 : 70 ( $S > I$ ), 50 : 50 ( $S = I$ ), and 70 : 30 ( $S < I$ ), and replicated 3 times. The seven-day old larvae were initially placed into a plastic cup (with a height of 12 cm, upper diameter 7 cm, lower diameter 5 cm) filled with feeding material, and covered with a black sheet. The lid of the cup contained holes to allow air circulation. To prevent oviposition of other flies and parasitoids, a round dark cloth with diameter 0.01 mm was clamped between box and lid. The diet for larvae was prepared, weighed, and kept frozen 24 hours before the treatment to prevent the decomposition process. All cups were kept in a shady area. Sampling and feeding were conducted every three days (Diener *et al.* 2009; Lalander *et al.* 2019) during which period the remaining larvae were transferred into another glass already filled with the next feed. The residual material of the previous glass was dried at 60 °C for dry mass determination.

Feeding was conducted until more than 40% of all larvae metamorphosed into prepupae (Tomberlin *et al.* 2002; Lalander *et al.* 2019) while weighing was conducted until all larva metamorphosed into prepupae. All prepupae were removed daily from each container and weighed, then placed in a plastic container for

further rearing process into an adult. Prepupae and pupae were held in the same incubator in which the larvae were reared and monitored for adult emergence daily (Cammack & Tomberlin 2017).

## Data Analysis

### *Larvae Growth Rate and Productivity*

Future production of insect larvae through the bioconversion method highly depends on the larvae growth rate. In this study, the growth rate of each larva was determined by daily biomass change (Waldbauer 1968), with the following formula:

$$\text{Growth rate} = B/t \quad (1)$$

where:

B = weight gain (mg)

t = development time (days)

On the other hand, the productivity of determined by formula:

$$\text{Productivity} = [\text{Dry weight of larvae}/(t \times V)] \quad (2)$$

where:

t = larvae rearing period (day)

V = volume of rearing container (dm<sup>3</sup>)

In this study, the volume of reactor applied was 0.414 dm<sup>3</sup>.

### *Consumption ability*

The ability of larvae to consume diet was determined by AD (Approximate Digestibility), ECD (Efficiency of Conversion of Digested-feed), WRI (Waste Reduction Index), and proportion of diet used for metabolism, converted into biomass, and undigested.

AD parameter was used to determine the effectiveness and larvae ability to digest the diet which could be measured by the formula:

$$\text{AD} = (I-F)/I \times 100\% \quad (3)$$

where:

AD = approximate digestibility

I = initial weight of diet (mg)

F = weight of residue (undigested food + excretions) (mg)

The ability of larvae to digest each diet composition was measured by ECD based on the formulae of Scriber and Slansky (1982) and modified by Dienar *et al.* (2009):

$$B = (I - F) - M0 \quad (4)$$

$$\text{ECD} = B/(I - F) \quad (5)$$

where:

B = the total amount of food use for growth

I = total amount of food offered during the experiment

F = total amount of residue (undigested food + excretory food)

M = amount of food metabolized by larvae (calculated by mass balance)

Dry weight was used in all calculations.

To measure overall material reduction, the time of larvae development was required to reduce the amount of food included in calculation along with overall degradation (D) of waste. All of those variables were defined as Waste Reduction Index (WRI) which was determined by the formula:

$$\text{WRI} = [(I-F)/I \times 100]/t \quad (6)$$

where:

I = initial weight of diet (mg)

F = total amount of residue (undigested food + excretory), and larvae rearing period (day)

### *Mass Balance*

Mass balance is one approach to design the biomass production system and to predict the digestibility of the diet. In this approach, the total amount of feed consumed by larvae was divided into three outputs: the mass of diet material that is used to maintain homeostasis of larvae, the mass of undigested diet material, and the harvested biomass (Fig. 1).

### *Statistical Analysis*

One way ANOVA ( $P \leq 0.05$ ) with subsequent Tukey HSD tests were applied to detect the difference of the means among all treatments.

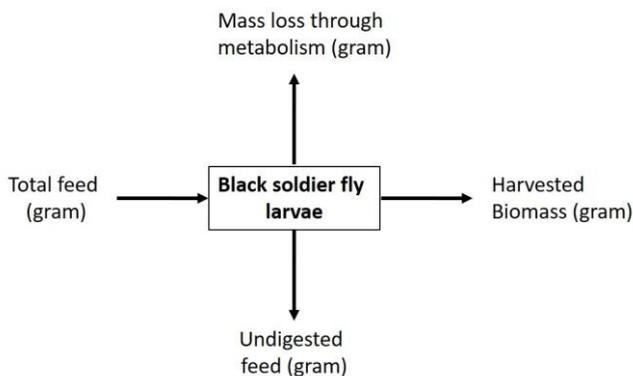


Figure 1 Mass balance model of organic waste bioconversion into body biomass of the BSFL

## RESULTS AND DISCUSSION

### Black Soldier Fly Larvae Growth

The pattern of larval growth among treatments was relatively similar as all prepupae reached pupal stage on day 21 for all treatments. Moreover, the larval weight of group  $S < I$  was the highest, followed by  $S = I$  and  $S > I$ . On average, the final weight of harvested prepupae was 122.80 mg for group  $S < I$  which was significantly higher than group  $S = I$  (113.88 mg) and  $S > I$  (106.89 mg) (Fig. 2).

Diet quality affects the growth and development time of BSFL (Furmant *et al.* 1959; Myers *et al.* 2008; Diener *et al.* 2009; Oonincx *et al.* 2015a,b). The development time to reach the prepupae stage could range from two weeks, under optimal condition, to more than 3 months if the food is limited (Table 1). In this study, the

development time of BSFL to reach the prepupae stage was shorter than most of the other studies (Tabel 1). Higher protein and fatty acid content in the fish offal might have provided the necessary nutrients for the larval growth and metabolism (Cammack & Tomberlin 2017). On the other hand, those nutrients might have also encouraged the diversity of bacterial species, some of which might be associated with BSFL and promote larval growth and development (Dong *et al.* 2009; Yu *et al.* 2010, 2011; Jeon *et al.* 2011; Zheng *et al.* 2013). Furthermore, other bacteria unassociated with BSFL, such as *Escherichia coli*, *Salmonella enterica*, and *Pseudomonas marginalis*, could have been killed by the antimicrobial substance produced by BSFL and then used as a source of nutrition (Erickson *et al.* 2004; Liu *et al.* 2008; Park *et al.* 2015).

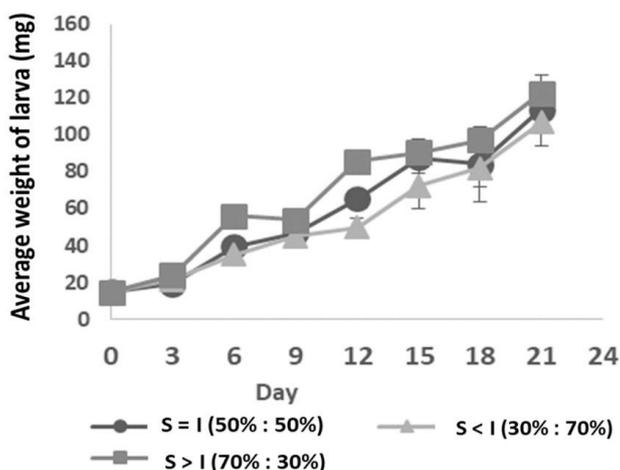


Figure 2 Growth pattern of BSFL

Table 1 Comparative data on development time and efficiency of conversion of digested-feed (ECD) for black soldier fly larvae on various substrates

Reference	Substrate	Development time (days)	ECD (%)
May (1961)	Housefly medium	18	-
Myers <i>et al.</i> (2008)	Dairy manure	28-30	-
Diener <i>et al.</i> (2009)	Chicken feed	16-42	24.4 - 38.0
Sealey <i>et al.</i> (2009)	Dairy manure	120	
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	5.69 - 10.85
Supriyatna <i>et al.</i> (2016)	Cassava peel	20-54	12 - 21
Abduh <i>et al.</i> (2017a)	Rubber seed	-	12.5 - 25.9
Abduh <i>et al.</i> (2017b)	<i>Pandanus tectorius</i>	-	6.3 - 27.4
This study	Combination of vegetables and fish offal	21	17.33 - 22.53

### Productivity

Among all treatments, larvae of group S < I had the significantly highest productivity (11.15 mg/larvae/day/dm<sup>3</sup>) compared to group S > I (9.69 mg/larvae/day/dm<sup>3</sup>) and group S = I (9.42 mg/larvae/day/dm<sup>3</sup>) (Fig. 3).

The larva of group S < I which contained more protein has produced significantly larger prepupae (ANOVA, P < 0.05). This conformed with earlier studies of Diener *et al.* (2009), Cammack & Tomberlin (2017). Furthermore, the higher moisture content might have also contributed to the higher prepupae biomass of group S < I.

### Consumption Ability

The consumption-ability of the larva was determined by waste reduction index (WRI),

efficiency of conversion of digested-feed (ECD), and approximate digestibility (AD). Among treatments, the group S < I produced the significantly highest WRI (3.13) which indicated a higher preference of larvae to richer protein- and-lipid- containing diet (Fig. 4). The level of waste reduction was similar to chicken feed (Diener *et al.* 2009) however, it was higher than rice straw feed (Manurung *et al.* 2016) and rubber seed feed (Abduh *et al.* 2017a) which indicated the effect of diet composition to the level of consumption by BSFL.

The effectiveness of larvae to convert digested diet into biomass was measured by ECD. In this study, the ECD ranged between 17.33 to 22.53% with group S < I showing the lowest ECD (17.33%) while also recording the significantly highest AD at 62.01% (Table 2).

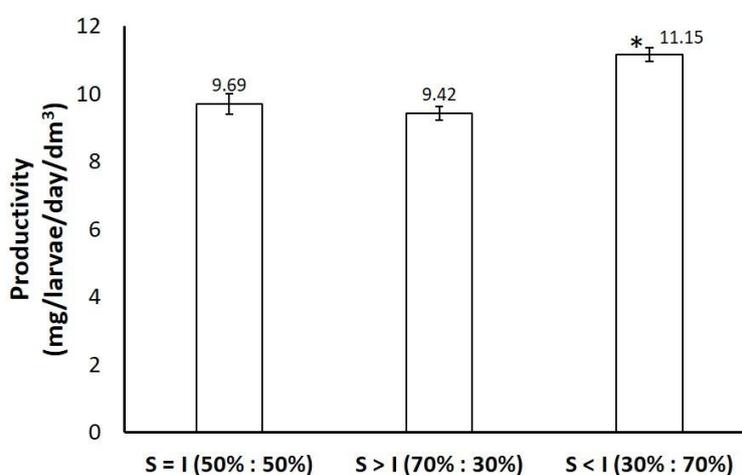


Figure 3 Productivity of BSFL

Note: \* = significant at P &lt; 0.05).

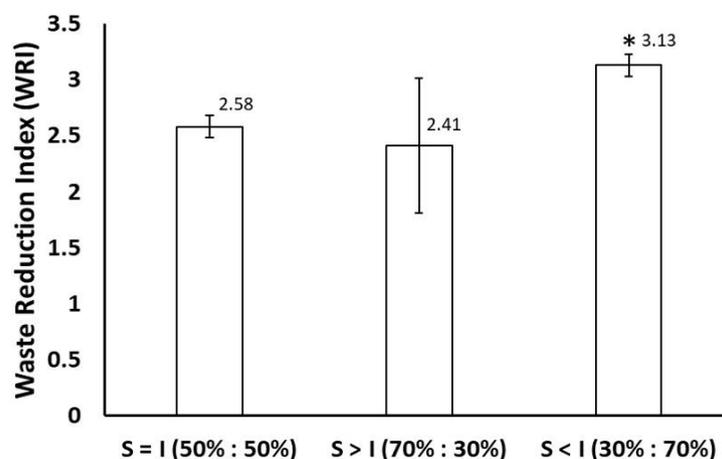


Figure 4 Waste Reduction Index of diet  
Note: \* = significant at P < 0.05).

Table 2 ECD and AD among different diet regimes

Diet (100 mg/larva/day)	ECD (Efficiency of Conversion of Digested-Feed)	AD (Approximate Digestibility)
S = I (50 : 50)	22.53%	49.46%
S > I (70 : 30)	21.56%	45.45%
S < I (30 : 70)	17.33%	62.01%

The ECD level recorded in this study was relatively higher than most of the previous studies (Table 1). ECD decreased when the quality of diet decreased (higher proportion of undigested material, such as cellulose and hemicellulose). However, in this study, the larval group that consumed the protein has showed lower ECD than the group that consumed a cellulose-rich diet. Higher ECD manifested by BSFL receiving an inferior diet could be a strategy to obtain more nutrients by increasing the consumption rate (Couture *et al.* 2016).

On the other hand, as shown by AD, a diet with higher protein content was more readily digested by the larvae which compensated for its lower ECD. This compensation allowed the S < I group to produce heavier harvested prepupae.

### Mass Balance of Bioconversion Process

Around 4.45 to 6.66% of the substrate was transformed into biomass, 40.99 to 55.35% was used for metabolism, and 37.99 to 54.55% was undigested. The highest transformation rate of the substrate into biomass was recorded on the group S < I (Fig. 5).

High transformation rate of the substrate to biomass in group S < I indicated the importance of food digestibility in the production of more biomass.

### Pupae Mortality and Adult Sex Ratio

The level of pupae mortality was low at all groups, around 4.29 to 10.71%. The highest mortality was recorded at group S > I (10.71%) while the lowest in group S < I (4.29%) (Fig. 6). The level of pupae mortality in this study was lower than those of Tomberlin *et al.* (2002) and Gobbi *et al.* (2013) and of Cammack and Tomberlin (2017). Generally, the larvae of black soldier fly are fed on a wide variety of substrates on the larval stage and accumulate a large store of fat to reduce and/or eliminate the need for the adult to feed (Sheppard *et al.* 2002). For this reason, the quality of diet plays a key role in the development of adults during the pupae stage. To produce high quality food, the larvae of insects tend to consume a balanced diet that is optimum for its growth and development (Gobbi *et al.* 2013). This study showed that larvae reared with the high protein diet combined with complex carbohydrates (from vegetable wastes) could significantly reduce the level of pupae mortality. Furthermore, the juice produced by the degradation of fish offal maintained the diet moisture which highly influenced pupae mortality, particularly for fly species (Cickova *et al.* 2012; Cammack & Tomberlin 2017).

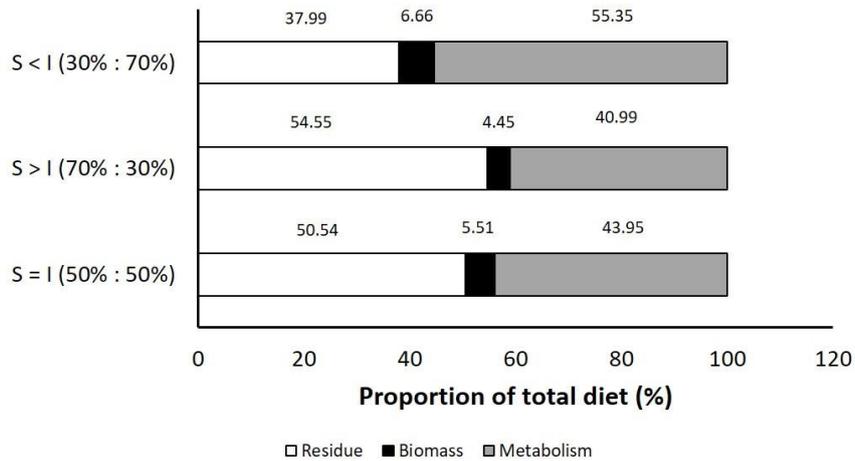


Figure 5 Proportion of digested feed used for metabolism, converted into biomass, and left undigested (residue)  
 Note: \* = significant at P < 0.05).

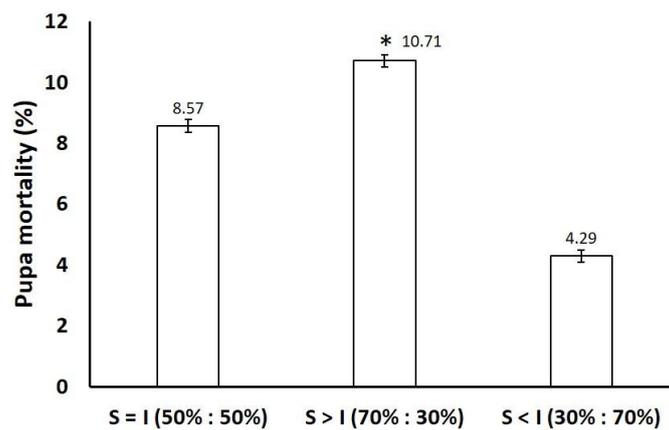


Figure 6 Percentage of pupae mortality  
 Note: \* = significant at P < 0.05).

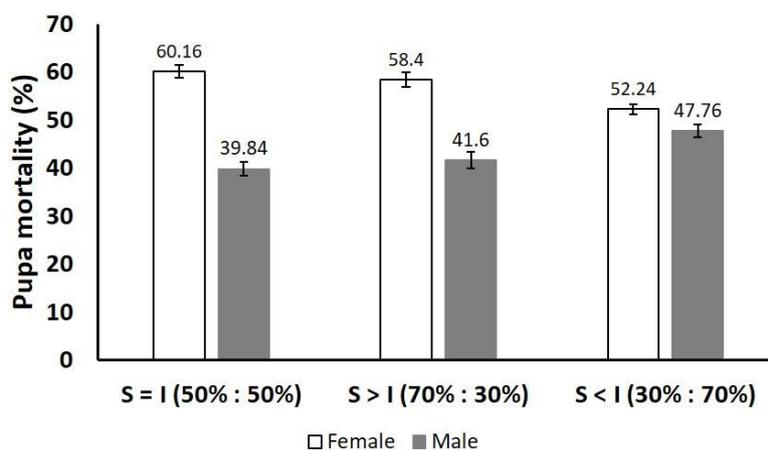


Figure 7 Proportion of adult female and male

More female adults were produced than the males for all groups although the proportion was more balanced in S < I group with strong differences manifested by the group receiving the balanced feed (Fig. 7). The female-biased sex ratio showed in this study confirmed some earlier studies (Tomberlin *et al.* 2002; Zarkani &

Miswati 2012; Gobbi *et al.* 2013). However, other studies reported a more male-biased adult proportion when larva were fed with artificial feeds (Ma *et al.* 2018; Meneguz *et al.* 2018).

Variables like pH of the substrate, nutritional variability, and feeding regime were probably the factors that govern the sex ratio of adult flies

(Quezada-Garcia *et al.* 2014; Ma *et al.* 2018; Meneguz *et al.* 2018). Strong differences on the sex ratio related with substrate differences in this study are against some previous studies reporting the insignificant effect of diet quality and content to the sex ratio of black soldier fly (Tomberlin *et al.* 2002; Gobbi *et al.* 2013; Diener *et al.* 2015). However, this result agrees with the study of Zarkani & Miswati (2012) in the tropical region which provides some evidences of the effect of environmental factor to the final stage of the developmental period of BSFL.

## CONCLUSIONS

Diet combination consisting of a higher proportion of protein and lipid has resulted in a higher weight of harvested prepupae and the lowest mortality of the black soldier fly which was probably due to a higher consumption rate and approximately higher digestibility. To improve the sustainable production of the feed, further studies are suggested on the rearing environment of larvae particularly, on reducing the mortality rate of pupae brought about by the bioconverted waste that is high in protein and lipid; on the effect of various waste materials to biomass production and sustainability; and on the composition of biomass.

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