EFFECT OF FEEDING BLACK SOLDIER FLY LARVAE (*Hermetia illucens*) WITH COMBINATION OF VEGETABLE WASTE OF PAK CHOI (*Brassica chinensis*) AND FISH OFFAL OF CARP (*Cyprinus carpio*) ON GROWTH AND MORTALITY

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ABSTRACT

One of the main obstacles of the local animal production system is the availability of affordable and sustainable protein supply. Insect larvae, such as Black Soldier Fly larvae (BSFL) (*Hermetia illucens*), have been considered as a protein source, which could be produced on large scale with low cost since organic wastes could be used as feeding material for them. This study was designed to determine the response of BSFL to various waste combinations of vegetable and animal remains Pak Choi (*Brassica chinensis*) (S) and fish offal of carp (*Cyprinus caprio*) (I)). In this study, a total number 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). The result showed group S < I showed the best combination to be applied as it produced significantly the highest weight of BSFL (122.8 mg/larvae) and Approximate Digestibility (62.01%) with least pupae mortality rate (4.29%) compared with other groups.

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

INTRODUCTION

The amount of waste generated by the human population increases as their communities develop and increases their size. In the year 2000, about 49% of the total world population lived in cities and generated more than three million metric tons of waste (e.g., household items, food waste, packaging, ash) daily (Hoornweg *et al*. 2013), and this number is predicted to double in 2025. The FAO estimates 1.6 Gtonnes of food waste were generated worldwide in 2007 as the result of economic activities, from production to consumption (FAO 2013). These wastes are taking up space in landfills, as the most common waste management practice, contributing to the spread of pathogens, producing of noxious odors, and producing a significant amount of CO_2 (contributor number three of global production) (FAO 2013).

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 such as 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, Oonincx *et al*. 2015a), as well human and livestock feces (Myers *et al.* 2008, Banks *et al.* 2014, Oonincx *et al*. 2015b). Due to its biological characteristic and the fact that it can be able to be mass-produced (Sheppard *et al*. 2002), this species has been studied to recycle nutrients presents in organic waste to convert into biomass rich in protein and fat (Sheppard *et al*. 1994, Diener *et al*. 2009, Li *et al*. 2011, Surendra *et al*. 2016), which is applicable as a part of the ingredient of feed 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) on the process known as bioconversion.

However, the heterogeneous nature of organic material provides the challenge to the optimization and implementation of this system, especially in the municipal area. 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 were produced through economic activities in the traditional and modern markets which are dominated by vegetables and animal remains. Applying these heterogeneous resources as the 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 similar developed tropical countries in which different organic wastes were produced. The objectives of this experiment were 1) to compare the consumption efficiency of BSFL to diet combination of vegetable waste and animal remain, and 2) determine the effects of diet composition on growth, development time, survival ship of pupae, and adult sex ratio. The results of this study could be used as the base of diet manipulation to optimize waste reduction, conversion of these materials to insect biomass, and sustainability of bioconversion system for municipal organic wastes.

MATERIALS AND METHODS

Animal Specimen

Larvae of the black soldier fly were obtained from eggs purchased from a BSF farm in Sumedang, West Java. All eggs were kept on the substance made of commercial chicken feed (60% moisture) and kept at constant temperature (28^oC, 70%RH) in a container (50 cm x 25 m x 10 cm) in Laboratory of Environmental Toxicology, School of Life Sciences and Technology, Bandung, Indonesia.

Treatment

Seven-day old larvae were used in this study. Each treatment (with nine replicates for each treatment) contained 60 larvae fed with 100 mg/day/larvae (wet weight, 60% moisture content) of a diet combination of vegetable wastes (*Brassica chinensis*) and fish offal of carp (*Cyprinus caprio*). Diet combinations ratio applied in this study were 30:70, 50:50, and 70:30 (fish offal : vegetable wastes). All treatment noted as S>I, S=I, and S<I, respectively, and replicated 3 times. The larvae were initially placed into a plastic cup (height 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, dark cloth (diameter 0.01 mm) was clamped between box and lid. The diet for larvae was prepared, weighed, and kept frozen 24 hours before treatment to prevent the decomposition process. All cups kept in a shady area. Sampling and feeding were conducted every three days. (Diener *et al*. 2009, Lalander *et al.*, 2019)During sampling and feeding, 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^oC for dry mass determine.

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 each 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 pupas 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:

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

where B = weight gain (mg), t = development time (days). On the other hand, the productivity of determined by formula

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

Where t = larvae rearing period (day) and V = volume of rearing container (dm^3). In this study, the volume of reactor applied was 0.414 dm^3.

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 formula:

$$AD = (I-F)/I \times 100\% \qquad (3)$$

where AD = approximate digestibility, I = initial weight of diet (mg), and F = weight of residue (undigested food + excretory) (mg).

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

$$B = (I - F) - M \qquad (4)$$

$$ECD = B/(I - F) \qquad (5)$$

Where B represents 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); and M = amount of food metabolized by larvae (calculated by mass balance). Dry weight was used for all calculations. To measure overall material reduction, the time of larvae 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:

$$WRI = [(I-F)/I \times 100]/t \qquad (6)$$

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 number 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 (Figure 1).

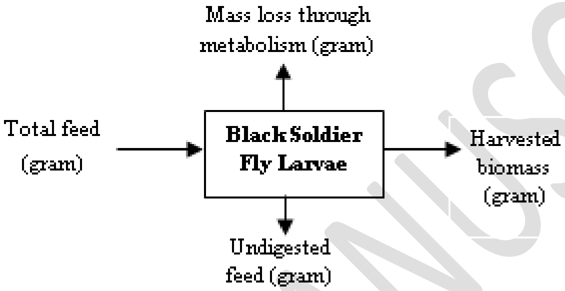

Figure 1 Mass balance model of bioconversion of organic waste into body biomass of BSFL

Statistical Analysis

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

RESULTS AND DISCUSSION

Black Soldier Fly Larvae Growth

The pattern of larvae growth among treatments was relatively similar as all prepupae reached on day 21 for all treatments. In general, the weight of larvae of group S<I was the highest and 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 (*ANOVA*, $p<0.05$) than group S=I (113.88 mg) and S>I (106.89 mg) (Figure 2).

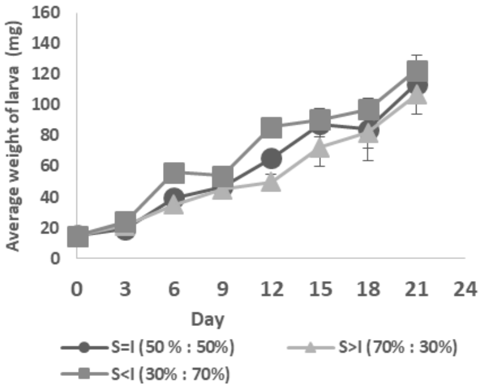

Figure 2 Growth pattern of BSFL

It is well known that the quality of diet will affect 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). The development time of BSFL to reach the prepupae stage recorded in this study was shorter than most of the other studies (Tabel 1). Higher protein and fatty acid content in fish offal could be provided necessary nutrients for growth and metabolism for the larvae (Cammack & Tomberlin 2017). On the other hand, those nutrients could encourage the diversity of bacterial species. Some of these bacteria may 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 be killed by the antimicrobial substance produced by BSFL then used as a source of nutrition (Erickson *et al.* 2004, Liu *et al*. 2008, Park *et al*. 2015).

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 *et al.* (2008)	Dairy manure	28-30	-
Diener *et al.* (2009)	Chicken feed	16-42	24.4 – 38.0
Sealey *et al.* (2009)	Dairy manure	120	
Li *et al*. (2011)	Dairy manure	<31	
Gobbi *et al*. (2013)	Meat meal	33	
Gobbi *et al*. (2013)	Hen feed	15	
Manurung *et al*. (2016)	Rice straw	38-52	5.69 – 10.85
Supriyatna *et al*. (2016)	Cassava peel	20-54	12 – 21
Abduh *et al.* (2017a)	Rubber seed	-	12.5 – 25.9
Abduh *et al*. (2017b)	*Pandanus tectorius*	-	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 significantly highest productivity compared to group S>I (9.69 mg/larvae/day/dm^3) and group S=I (9.42 mg/larvae/day/dm^3) (Figure 3).

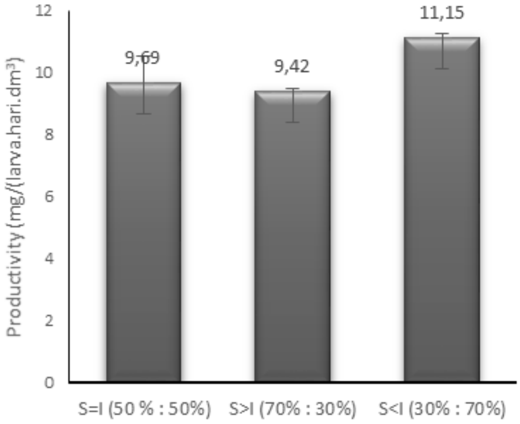

Figure 3 Productivity of BSFL

The larva of group S<I (contained more protein) produced significantly larger prepupae (*ANOVA*, $p<0.05$) which is agreed with the earlier studies (Diener *et al.* 2009, Cammack & Tomberlin, 2017). Furthermore, higher moisture may also contribute to higher prepupae biomass of group S<I.

Consumption Ability

In this study, the consumption-ability of the larva was determined by WRI, ECD, and AD. Among treatments, significant highest WRI was recorded in the group S<I (*ANOVA*, $p<0.05$), which indicated a higher preference of larvae to richer protein and lipid content diet (Figure 4). The level of waste reduction was similar to chicken feed (Diener *et al.* 2009) while it was higher than rice straw (Manurung *et al*. 2016) and rubber seed (Abduh *et al*. 2017a) which indicated the effect of diet composition to a level of consumption by BSFL.

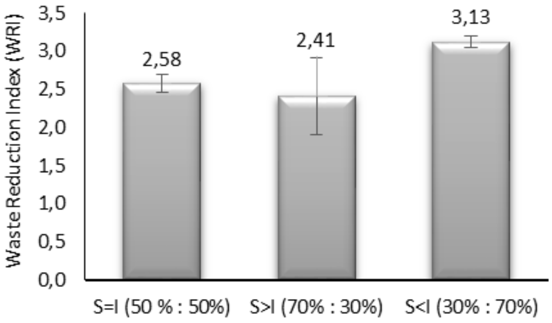

Figure 4 Waste Reduction Index of diet

The effectiveness of larvae to convert digested diet into biomass was measured by ECD. In this study, the ECD was ranged between 17.33 to 22.53% with group S<I that showed the lowest ECD (Table 2). On the other hand, significantly highest AD was recorded in group S<I (*ANOVA*, $p<0.05$)(Table 2).

Table 2 ECD and AD among different diet regimes

Diet (100mg/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). Based on the data present in the bibliography, ECD decreased when the quality of diet decreased (higher proportion of undigested material, such as cellulose and hemicellulose). However, in this study, the larva group consumed diet which higher in protein showed lower ECD than group consumed a cellulose-rich diet. Higher ECD showed 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, a diet with higher protein content much easier to be digested by larvae (showed by AD) which compensated the 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 (Figure 5).

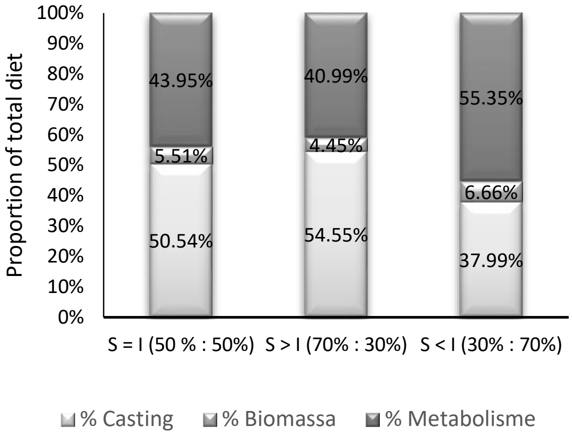

Figure 5 Proportion of digested feed used for metabolism, converted into biomass, and left undigested

High transformation rate from the substrate to biomass on group S<I showed the importance of food digestibility to produce 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 while the lowest in group S<I (Figure 6). The level of pupae mortality in this study was lower than Tomberlin *et al.* (2002) and Gobbi *et al*. (2013) but lower than the results of Cammack and Tomberlin (2017). In general, the larvae of black soldier fly were fed on a wide variety of substrates on the larval stage and accumulated 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, the larvae of insects tend to consume a balanced diet that was optimum for its growth and development (Gobbi *et al*. 2013). This study showed the larvae rearing diet with high protein 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 moisture of diet which highly influenced pupae mortality, especially for fly species (Cickova *et al*. 2012, Cammack & Tomberlin 2017).

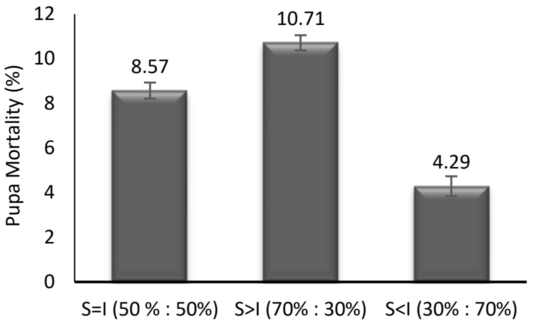

Figure 6 Percentage of pupae mortality

Adult females produced more than the males for all group although the proportion more balanced in S<I group with strong differences showed at group received balance feed (Figure 7). The female-biased sex ratio showed in this study in agreement to some earlier studies (Tomberlin *et al.* 2002, Zarkani & Miswati 2012, Gobbi *et al*. 2013) although other studies reported a more male-biased adult proportion when larva fed with artificial feed (Ma *et al.*2018, Meneguz *et al.* 2018).

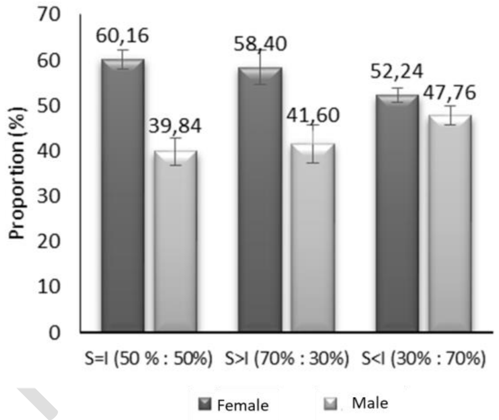

Figure 7 Proportion of adult female and male

Variables like pH of the substrate, nutritional variability, and feeding regime had been reported as the possible factors that govern the sex ration of adults (Quezada-Garcia *et al.* 2014, Ma *et al.* 2018, Meneguz *et al.* 2018). Strong differences on the sex ratio with differences on the substrate found in this study were 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 agreed with the study of Zarkani & Miswati (2012) that was conducted in the tropical region which provides some evidence of the effect of environmental factor to the final stage of the developmental period in BSFL.

CONCLUSION

Diet combination consisted of a higher proportion of protein and lipid producing a higher weight of harvested prepupae and lowest mortality of black soldier fly due to higher consumption rate and approximate digestibility. Further studies to improve the larvae rearing environment may improve the sustainability of larvae, by reducing the mortality rate of pupae biomass produced by bioconverting waste high in protein and lipid. The studies on the effect of various waste materials with a similar composition to biomass production, sustainability, and chemical composition of biomass should also be conducted.

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