Growth of Black Soldier Fly (*Hermetia illucens*) Larvae Fed on Spent Coffee Ground

Agus Dana Permana*, Jessica Esther N. Ramadhani Eka Putra

1School of Life Sciences and Technology, Institut Teknologi Bandung, Jalan Ganesha No. 10 Bandung 40132 Indonesia

*email: agus@sith.itb.ac.id

**Abstract.** *Hermetia illucens* or Black Soldier Fly (BSF) is one of the bioconversion agents that can convert any kind of organic wastes. In this research, BSF larvae was used as a bioconversion agent on spent coffee ground, the main waste in coffee industry. This study aimed to determine the effect of spent coffee ground as feed on larval growth and the ability of BSF larvae in converting this spent based on ECD (Efficiency of Conversion Digestive) and WRI (Waste Reduction Index). The 7-day old larvae was nourished on spent coffee grounds with different feeding rate (200, 100, 50, 25, dan 12.5 mg/larvae/day). The results showed that larvae with feeding rate at 200 mg/larvae/day had shortest development time (25.6 ± 2.19 days), highest ECD value (5 ± 0.49%), highest pupae biomass (14.8 ± 2.15 mg), highest grow rate (1.41 ± 0.17 mg/day), but low WRI (0.997 ± 0.11). Additional proximate analysis exhibited that pupae contain total protein up to 33.45%. We concluded that BSF larvae has slow growth rate but can convert spent coffee ground.

1. **Introduction**

Coffee is the most widely consumed beverage in the world. Indonesia is the top 4 country in producing coffee and the 10th in consuming coffee. In Indonesia, the number of coffee consumption has reached 13.5 kg/capita/year [1]. The brewing process of coffee left a residue which isn’t solved in coffee beverage called as spent coffee ground [2]. Spent coffee ground production itself has reached 6 million tons a year and this number keep increasing due to a recently popular brewing method which has no spent coffee ground waste in the coffee drink [2].

It has been reported that spent coffee ground has high N & P contents which is useful for compost or plant growth medium [3]. However, the used of spent coffee ground as a compost is less effective due to its crude fiber, oil, and caffeine which allegedly inhibit plant growth [3]. Another research explained that spent coffee ground could also be used for a material of charcoal briquette, biodiesel, and a mix to livestock feed. Yet, this is also less effective due to its process that requires specific materials, equipment, and skill. Therefore, there is still much of spent coffee ground wasted and it may cause another problem to the environment [4].

To overcome this problem, spent coffee grounds could be converted into useful product using a bioconversion agent, such as *Hermetia illucens* (BSF/black soldier fly). On its larval phase, BSF are among the most efficient insect at converting organic material feeds into biomass. This biomass contains high and economically nutrient value, such as proteins and lipids [5]. Though, biomass...
production by BSF larvae is affected by the quality of organic material consumed by the larvae [6]. Therefore, this study was conducted to determine the impact of spent coffee ground in BSF larvae development and its ability to convert spent coffee ground.

2. Materials and Methods
2.1. Materials
Spent coffee ground was obtained from a coffee shop in Dago area, Bandung. The spent coffee ground were packed inside airtight plastic bag, transferred to laboratory, and kept inside the refrigerator to prevent decomposition by microorganism. Spent coffee ground was removed from refrigerator and left until it reached the room temperature.

2.2. Methods
Adults BSF were reared in a 1 m x 1 m x 1 m screen cage under laboratory conditions with 12 L : 12 D photoperiod. A medium comprised mixture of water and hen feed was placed inside the cage. Strips of cardboard were placed on top of the medium to provide a laying eggs sites for the flies. Once the eggs hatched, the larvae were kept inside the medium until 7 days before proceed for further experiment. Each treatment (5 replicates/treatment) contained 75 larvae that fed by spent coffee ground at different feeding rate (12, 5, 25, 50, 100, and 200 mg/larvae/day). Larvae were placed inside plastic cup and covered by a black fabric.

Sampling and feeding were done every four days. Five larvae were randomly taken from each cup as a subsample. The selected larvae were weighed then dried in oven at 60 °C for 24 h. The leftover feed was counted as residue and weighed and dried in oven. New feed was given based on the feeding rate and the number of larvae on each treatment.

Sampling and feeding were done until larvae develop into prepupal phase. Larvae that have turned into prepupae were weighed for wet mass and dried in oven (60°C, 24 hours) for its dry mass. Prepupae and feed (spent coffee ground) were collected for proximate analysis conducted at The Ruminant Animal Nutrition Laboratory and Animal Feed Chemical, University of Padjadjaran, Jatinangor.

2.3. Data Analysis
Larval growth was measured by biomass gain and expressed in Growth Rate (GR) following the equation below:

\[ GR (mg/day) = \frac{(final \ weight - initial \ weight)}{time} \]

Time refers to days needed for larvae to reach prepupal phase during the experiment. Higher GR indicates a faster growth of larvae.

Not only, Survival Rate (SR), likewise was measured to determine the effect of the feed following the equation below:

\[ SR (%) = \frac{numbers \ of \ larvae \ survive}{numbers \ of \ initial \ larvae} \times 100\% \]

This formula based on the assumption that larvae taken for subsample were considered survive.

Larvae ability to convert feed into its biomass was calculated by ECD (Efficiency Conversion of Digestive Feed) with the equation below [7]:

\[ ECD (%) = \frac{ECI}{AD} \times 100\% \]

\[ ECI (%) = \frac{B}{(T - R)} \times 100\% \]
AD (%) = \frac{(T - R)}{R} \times 100\%

Remarks:
ECI = efficiency conversion of ingestive feed
AD = approximate digestibility
B = larvae final biomass (mg)
T = total feed (mg)
R = residue (mg)

Larvae ability to reduce feed (organic matter) was calculated by WRI (Waste Reduction Index) with the equation below [5]:

\[
\text{WRI} = \frac{(T-R)}{T} \times \frac{1}{t} \times 100
\]

Remarks:
T = total feed (mg)
R = residue (mg)
t = feeding time (days)

2.4. Statistical Analysis
Data among various treatments were statistically analyzed with one-way ANOVA with Post-Hoc Tuckey. Statistical analysis was done to determine the difference in development time, GR, SR, ECD, and WRI (p<0.05). All analysis was done with IBM SPSS software.

3. Results and Discussion

3.1. Spent Coffee Ground Nutritional Content
Nutritional content of spent coffee ground that used in this study is shown on Table 1. Nutritional content analysis in spent coffee ground is needed because it affects the growth and survival of BSF larvae [6]. From proximate analysis, it shows that spent coffee ground has low protein to carbohydrate ratio, high crude fiber content, and low crude lipid content.

| Table 1 | Spent Coffee Ground Nutritional Content |
|----------|-----------------------------------------|
| Nutritional Content | Spent Coffee Ground (％) |
| Ash       | 0.13 |
| Protein   | 19.93 |
| Crude fiber | 18.8 |
| Crude lipid | 1.98 |
| Carbohydrate | 59.16 |

*Analysis was done based on dry mass

3.2. Larval Growth
Larvae fed with 200 mg/larvae/day showed the fastest development time (32.6 ± 2.19 days) with highest growth rate (1.41 ± 0.17 mg/days) among other treatments. This study revealed that larvae stage has longer development period compare with those fed on artificial hen fed or mix between hen fed and meat meal [5, 6]. Apparently, the amount of the feed had an impact to larval growth (Table 2). There was a tendency that bigger feed quantity supports a higher and faster larval growth. The quantity of the feed or feeding rate has affected BSF growth and development since larval to adult phase. The higher feed portion increased the opportunity of the larvae to get all the nutrition needed to growth. Hence, higher feed portion affects the growth of BSF larvae positively [8].

At 12.5 mg/larvae/day feeding rate, larvae didn’t complete the development time and didn’t reach its prepupal phase. Larvae fed at that rate had 100% mortality on the 16th day of treatment. For that
reason, survival rate on 12.5 mg/larvae/day feeding rate has the lowest value compared to others. This phenomenon happened due to less quantity of the feed. Evaporation of the feed in the plastic cup where the larvae placed happened faster in small quantity feed. High speed of feed evaporation has a negative impact on larval growth and could lead into high mortality rate [6].

### Table 2 Larval Growth based on development time, GR (Growth Rate), and SR (Survival Rate)

| Feeding Rate (mg/larva/day) | Development time (days) | Growth Rate (mg/day) | Survival Rate (%) |
|-----------------------------|-------------------------|----------------------|-------------------|
| 12.5                        | 54.2 ± 1.78a            | 0.114 ± 0.02a        | 19.2 ± 2.6a       |
| 25                          | 53.4 ± 2.19a            | 0.271 ± 0.02b        | 82.4 ± 3.45b      |
| 50                          | 43 ± 2.82b              | 0.244 ± 0.03b        | 97.87 ± 0.73c     |
| 100                         | 32.6 ± 2.19c            | 0.331 ± 0.03b        | 94.13 ± 2.02c     |
| 200                         | 32.6 ± 2.19c            | 1.41 ± 0.17c         | 97.6 ± 1.46c      |

Mean values followed by different letter indicated significant different within treatment (p<0.05)

The nutritional balance of feed and primary nutrition content such as protein and crude lipid affects growth rate of larvae. Protein is needed as a precursor on larval growth. Lipid is needed for development and food reserve for adult life of BSF. Balanced nutrition on feeding gives larvae less time to complete its nutritional requirements for entering the next phase (prepupal phase) [8]. Previous study on BSF larvae growth fed by mixed waste (vegetable, fruits, and human leftover food), the growth rate reached 1.53-1.8 mg/day [9]. Compared to that study, larval growth fed by spent coffee ground is considered low. Mixed waste used in the previous study has more balance nutrition components compared to spent coffee ground so the larval growth rate was higher. In addition, spent coffee ground has a low protein and lipid, which are important on larval growth.

3.3. Efficiency of Conversion Digestive Feed (ECD)

ECD defines larvae efficiency in converting the digestive feed into its biomass. ECD value depends on AD (Approximate Digestibility) value. AD defines amount of feed assimilated by BSF larvae [10]. The results of this study showed that AD values were decreasing while feed quantity was increasing (Table 3). In the limited nutrition condition, bigger quantity of feed will increase consumption rate of larvae. Higher consumption rate resulted in increasing rate of passage food in larvae’s gut and the amount of food assimilated is small [10, 11].

### Table 3 AD and ECD value of BSF larvae feed with spent coffee ground

| Feeding Rate (mg/larva/day) | Approximate Digestibility (%) | Efficiency Conversion of Digestive Feed (%) |
|-----------------------------|-------------------------------|---------------------------------------------|
| 12.5                        | 64 ± 1.92a                    | 3.47 ± 0.21a                                |
| 25                          | 60.52 ± 1.67b                 | 3.27 ± 0.25a                                |
| 50                          | 48.17 ± 1.27c                 | 2.71 ± 0.21a                                |
| 100                         | 29.9 ± 2.35d                  | 4.72 ± 0.77b                                |
| 200                         | 25.36 ± 1.28e                 | 5 ± 0.49b                                   |

Mean values followed by different letter indicated significant different within treatment (p<0.05)

An inverse relationship between AD and ECD was observed in this experiment. Low value of AD describes the small amount value of feed assimilated in its body. In that case, larvae will increase their digestive system efficiency to get all the nutrition needed and so ECD increase. A low ECD value was also obtained when larvae increase their metabolic cost. Feed digested by larvae was not converted into biomass yet it was used for metabolism [10]. This may affirm the prior explanation about the mass balance, when the small quantity of feed is given, high proportion of feed is used for metabolism. Previous study reported that chicken feed for larvae feed have ECD value ranged 24.4-38% [5]. In contrast, spent coffee ground has a low ECD value. Low ECD value from spent coffee ground was
caused by low protein to carbohydrate ratio compared to the chicken fed. Protein and carbohydrate are needed by larvae for their growth (biomass production) but an excessive amount of carbohydrate will lower the conversion efficiency (ECD) of BSF larvae. An excessive amount of carbohydrate is not converted into biomass (used for growth). It is converted to lipid instead of food reserve, which is used by BSF on its next phase (prepupae until adult) [12].

3.4. Waste Reduction Index (WRI)
WRI describes the ability of larvae in reducing organic material. Higher WRI value indicates a high ability of larvae in reducing organic material. The result of experiment of WRI can be seen on Table 4.

Table 4 WRI on spent coffee ground

| Feeding Rate (mg/larva/day) | WRI      |
|----------------------------|----------|
| 12.5                       | 4.00 ± 0.12a |
| 25                         | 1.28 ± 0.08b |
| 50                         | 1.04 ± 0.06c |
| 100                        | 0.83 ± 0.09d |
| 200                        | 1.00 ± 0.11d |

Mean values followed by different letter indicated significant different within treatment (p<0.05)

Bigger quantity of feed given to larvae will lower ability of larvae in reducing spent coffee ground. Small quantity of feed made larvae in underfeeding condition. This condition increased the consumption rate of larvae and uses more proportion of the feed to get all the nutrition so the residue amount is so small (related to the mass balance depicted in Figure 1) [10].

According to prior study of chicken feed, the WRI value has a range value of 1.1 – 3.8 [5]. Similar WRI value was also obtained in this experiment. This means that BSF larvae have the same ability to reduce chicken feed and spent coffee ground. It is concluded that BSF larvae could reduce spent coffee ground.

3.5. Bioconversion and Optimum Feeding Rate
BSF is known of its ability to convert protein until 40-50% when it reaches pupa phase [13]. From proximate analysis (Table 5), it is proved, that BSF converted protein almost 2 times from the feed yet, and it hasn’t reached 40%. This indicates that BSF could be another potency on spent coffee ground application. But it still needs improvement to enhance the protein value of the pupa. The protein content of the pupae obtained from this study is lower compare with those fed on hen feed [5] and fed on dairy manure [8].

Table 5 Nutritional Content of BSF Pupae

| Nutritional Content | BSF Pupae (%) |
|---------------------|---------------|
| Ash                 | 3.44          |
| Protein             | 33.45         |
| Crude fiber         | 2.62          |
| Crude lipid         | 7.64          |
| Carbohydrate        | 52.85         |

Based on the parameters of development time, WRI value and prepupae biomass, the feeding rate of 200 mg/larvae/day was the most optimum feed rate even though the WRI value obtained was not the highest value (Fig. 1). The optimum feeding rate is different from that of the fed chicken feeding for BSF larvae, 100 mg/larvae/day giving the optimum feeding rate, and when the dose is added, it does not accelerate the growth of the larvae [5].
Figure 1 Optimum-feeding rate for BSF growth using spent coffee ground

Acknowledgements
We thank to Mr. Suyitno who helped for BSF rearing and Dr. A. Faizal for his valuable comments on this manuscript as well as the reviewers for their valuable input to improve this paper. This study was funded by P3MI LPPM ITB 2017 and Pengabdian kepada Masyarakat LPPM ITB 2017.

Reference
[1] Pusat Data dan Sistem Informasi Pertanian 2015 Outlook Kopi Sekretariat Jenderal Kementerian Pertanian. Jakarta
[2] Mussatto SI, Machado EMS, Martins S and Teixeira JA 2011 Production, Composition, and Application of Coffee and Its Industrial Residues Food Bioproces Technol 4 661-672
[3] Gomes T, Pereira JA, Ramalhosa E, Casal S. and Baptista P 2013 Effect of fresh and composted spent coffee grounds on lettuce growth, photosynthetic pigments and mineral composition. In VII Congreso Ibérico de Agroingeniería y Ciencias Hortícolas Madrid 26-29 August 2013 (pp. 1-5) SECH e SEAgIng
[4] Vega Rocio C, Loarca-Piña G, Vergara-Castañeda H and Oomah BD 2015 Spent Coffee Grounds : A Review on Current Research and Future prospects Trends in Food Science & Technology 45(1) 24-36
[5] Diener S, Zurbrügg C and Tocknera K 2009 Conversion of organic material by black soldier fly larvae—Establishing optimal feeding rates Waste Management & Research 27 603-610
[6] Gobbi P, Martinez-Sanchez A and Rojo S 2013 The Effect of Larval Diet on Adult Life-History Traits of the Black Soldier Fly, Hermetia illucens (Diptera :Stratiomyidae). Eur. J. Entomol. 110(3) 461-468
[7] Waldbauer GP 1968 The Consumption and Utilization of Food by Insects Advances in
Insects Physiology 5 229-288

[8] Myers HM, Tomberlin JK, Lambert BD and Kattes D 2008 Development of Black Soldier Fly (Diptera : Stratiomyidae) Larvae Fed Dairy Manure Environ. Entomol 37(1) 11-15

[9] Hahn DA 2005 Larval Nutrition Affects Lipid Storage and Growth, but Not Protein or Carbohydrate Storage in Newly Eclosed Adults of the Grasshopper Schistocerca americana Journal of Insect Physiology 51 1210-1219

[10] Kusumah MS 2016 Pengembangbiakan Black Soldier Fly, Hermetia illucens L. (Diptera: Stratiomyidae) Sebagai Sumber Pakan Ikan Skripsi Sarjana Rekayasa Pertanian ITB

[11] Kerkut GA and Gilbert LI 2013 Regulation : Digestion, Nutrition, Excretion. USA : Pergamon Press

[12] Cohen AC 2015 Insect Diets Science and Technology, 2nd ed US CRC Press

[13] Le Gall M and Behmer ST 2014 Effects of Protein and Carbohydrate on an Insect Herbivore: The Vista from a Fitness Landscape Integrative and Comparative Biology 54(5) 942-954

[14] Wardhana AH 2016 Black Soldier Fly (Hermetia illucens) sebagai Sumber Protein Alternatif untuk Pakan Ternak Wartazoa 26(2) 69-78