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# Growth performance and nutritional composition of black soldier fly, *Hermetia illucens* (L), (Diptera : Stratiomyidae) reared on horse and sheep manure

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# Growth performance and nutritional composition of black soldier fly, *Hermetia illucens* (L), (Diptera : Stratiomyidae) reared on horse and sheep manure

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**Abstract**. Black soldier fly, *Hermetia illucens* is one organism that widely used to reduce organic wastes through bioconversion process by employing the ability of larvae to consume various types of organic waste. This process changes organic waste into larvae and prepupae biomass which was developed in order to provide an alternative protein source for animal feedstock. In this study, the effect of four experimental diets was analyzed, namely horse manure, a mixture of horse manure and vegetable waste, sheep manure and a mixture of sheep manure and vegetable waste. Newly hatched larvae were inoculated on experimental diets. The sample of 20 larvae from 5 replicates were measured and weighed until pupae stage. Nutrient content of prepupae reared on experimental diet were analyzed proximately. The effects of diet on development time, growth of the larval stages, mortality, fecundity and nutritional composition of black soldier fly were studied. Results indicate that a mixture of horse manure and vegetable waste diet was the best of the four diets in terms of the shorter of development time, the lowest of percentage mortality, the highest of fecundity and the better of nutritional content. In conclusion, both of morphological development and the nutritional ingredient of black soldier fly were highly determined by the used growing substrates and food ingested. Addition of vegetable waste can improve growth performance and nutritional content of black soldier fly when reared on horse and sheep manure.

## 1. Introduction

Black soldier flies (*Hermetia illucens*) play a significant role in recycling many forms of organic waste and other accumulated nutrients in the environment. The larvae of black soldier flies able to broken down various organic matter including food waste in ecosystem [1][2]. This Species also recorded as an agricultural-waste consumer such as coffee pulp [1], palm kernel meal [3] and rice straw waste [4] or organic waste materials like market waste, municipal organic waste or dewatered faecal sludge [5]. Larvae of black soldier flies also consume livestock manure such as chicken manure [6], pig manure [7], and dairy manure [2]. In digestion process, this larvae assimilate nutrients of the organic matter. Through this process, black soldier fly larva cuts down the amount of organic waste, so indirectly decline the pollution potential [8].

More than half of the nutrients contained in feed are excreted as manure [9]. The black soldier fly consume and convert residual manure proteins and other nutrients into their valuable biomass, which is a high quality animal protein feedstuff [10]. The larvae and prepupae of black soldier fly have a high protein and fat content [11] that can be used to support growth a lot of livestock such as blue tilapia fish [12] and pigs [13]. The environmental impact of animal husbandry could decline significantly if black soldier fly larvae are used in order to eliminate livestock manure and reused as livestock feed. Organic matter as growing substrate greatly affects the development of black soldier fly and various biological traits [14].

The present study focused on analyzing the influence of horse and sheep manure substrates toward nutrient composition and growth performance of black soldier fly larvae and to quantify the suitability of horse and sheep manure as feed for black soldier fly larvae. In this study we determined the biological parameters of black soldier fly by focusing on the following specific objectives: the effect of diet treatment (a) development time of larval, pupal and adult stages, (b) mortality (c) size and weight gain, and (d) nutrient composition of the substrates and prepupae from all treatment.

## 2. Material

The larvae of black soldier fly were derived from eggs produced by adult colony raised in a 1 $m^3$ cages in the laboratory of animal physiology and entomology, Biology Departement, Faculty of Science and Technology, Universitas Islam Negeri Sunan Gunung Djati Bandung, Indonesia. Fresh horse and sheep manure were collected at the conventional farms at East Bandung. Fresh vegetable wastes were collected from traditional market Ujung Berung East Bandung. This study was carried out from July to September 2017.

## 3. Methods

The 3 days-old larvae were transferred to the trial plastic cup (height 12 cm, diameter 8 cm) filled by experimental diet. The cups were covered by a black sheet allowing aerobic circulation. The four experimental diets were: (A) pure horse manure, (B) a mixture of horse manure and vegetable waste (50%:50% in ratio), (C) pure sheep manure and (D) a mixture of horse manure and vegetable waste (50%:50% in ratio). The trial was conducted in five replications. Each treatment contain 100 larvae fed with 100 mg/larvae/day daily feed rates.

The trials were carried out under room conditions (temperature: 27 ± 2ºC, relative humidity: 65 ± 5%, and photoperiod Light : Dark = 12 : 12 hours). Every three days, the old growing substrates were replaced with new fresh experimental diets. The surviving larvae, prepupal, pupal and adult was counted as a measure of mortality. Development time of larval, prepupal, pupal and adult (male and female) was recorded continuously until all individuals died. The sample of 20 larvae from each 5 replicates were used to measure the length, width and weight. Proximate and mineral analysis of the diets and prepupae of black soldier fly were performed at nutrition laboratory of Padjajaran University. Nutrition content was determined according to common methods for crude nutrient analysis as describe by AOAC (1995).

The statistical analysis was conducted using SPSS software, version 22.0. The statistical differences in were determined by a one-way analysis of variance (ANOVA) followed by Tukey tests with a threshold value (α) of 0.05.

## 4. Result and Discussion

There were a difference among development time of black soldier flies reared on each experimental diet at all stages (larval, prepupal, pupal, and adult). Development time of black soldier fly reared on experimental diets presented in Table 1. Black soldier flies reared on horse manure had the highest total development time (larval to adult) among treatments (112.8 days). The lowest total development time was black soldier fly reared on a mixture of horse manure and vegetable waste (91.4 days). The time taken for larval stage was the longest than other treatment. This study demonstrates that horse and sheep manure are suitable as growing substrate for black soldier fly larval, although it takes longer development time than previous study. Black soldier flies have a relatively long life cycle about 40-45 days, with the larval stage lasting 22-24 days at 27-30ºC [7] [14] [15]. Larvae reared on pig manure generally took the longest time in reaching the wandering stage compared to other diets (fruit and vegetable, kitchen waste, liver, fish) [16]. The development of black soldier fly depends on many factors, such as the nutritional value of a resource that impact the development rate. Development of the black soldier fly will differ across organic wastes provided. There are a lot of factors that responsible for the differential development observed between diets. The most influential factor was likely the nutritional content difference of animal and plant based diets [17].

**Table 1.** Population development time parameters (Mean ± SE) for black soldier fly reared at each experimental diets.

| | The Development time (Mean ± SE) | | | |
|---|---|---|---|---|
| Stage | A | B | C | D |
| larval | 42.00±1.34[b] | 28.60±3.53[a] | 42.80±0.58[b] | 30.40±3.43[a] |
| prepupal | 22.60±3.60[a] | 21.40±2.66[a] | 26.00±3.75[a] | 24.80±2.54[a] |
| pupal | 24.60±2.69[a] | 22.60±0.24[a] | 18.80±1.62[a] | 23.00±1.38[a] |
| adult | 23.60±2.25[b] | 18.80±0.87[ab] | 16.50±1.17[a] | 18.70±0.77[ab] |
| male | 24.80±2.97[a] | 19.40±0.68[a] | 18.40±1.78[a] | 18.60±1.12[a] |
| female | 22.40±1.78[b] | 18.20±1.16[ab] | 14.60±0.68[b] | 18.80±0.66[ab] |

Different superscript letters within the same parameter indicate significant differences ($P \leq 0.05$)

The difference of nutritional content would support why larvae reared on horse manure and sheep manure took longer development time than other treatments. Proximate composition of larval growing substrates and prepupal of black soldier fly presented in Table 2. The feeding activity of black soldier fly is done in the larval stage, with larvae acquiring a large fat body to use as energy for pupation, adult survival, and reproduction [14]. In general, black soldier flies larvae are capable of converting substrate with low nutritional content into biomass prepupa that is rich in higher nutrients such as crude protein (35.38 – 46.59%), crude lipid (10.97-14.09%), and energy (≈ 4603 Kcal/kg), as well as low ash and fiber content so it has potential to be used as animal feed.

The larvae of the black soldier fly were not only able to survive and even develop in pure manure but were also capable of significantly reducing the manure biomass. The development of larvae much faster and the nutrient content being higher when vegetable waste was added to the manure. This study were along with previous study that concerning the treatability of faecal sludge, the larvae developed much faster when market waste was added to the faecal sludge to enhance the nutritive value of the larvae's feed source [5].

The mortality of black soldier flies at each stage (larval to adult) in our study were relatively low suggesting its high survival rate high (Table 3). This result indicates that the tested manure types were suitable as growing substrate or feed for the larval of black soldier flies. Life history characteristics of black soldier fly such as survival rate and development time, are determined by a variety of factors such as food source [18], feed nutrition content [19], temperature [17], relative humidity [20] and genetic strain [21].

**Table 2.** Proximate composition of larval growing substrates and prepupal of black soldier fly

| | A | | B | | C | | D | |
|---|---|---|---|---|---|---|---|---|
| | Substrate | Prepupal | Substrate | Prepupal | Substrate | Prepupal | Substrate | Prepupal |
| Parameters | | | | | | | | |
| Dry Matter (%) | 91.03 | 88.02 | 88.05 | 94.96 | 90.84 | 95.49 | 89.59 | 94.11 |
| Ash (%) | 14.15 | 19.63 | 21.80 | 14.22 | 21.45 | 15.35 | 20.45 | 15.48 |
| Crude protein (%) | 10.13 | 35.38 | 13.89 | 46.59 | 13.34 | 40.00 | 16.43 | 44,13 |
| Crude fiber (%) | 19.90 | 2.19 | 23.40 | 3.41 | 20.77 | 2.09 | 17.64 | 2.56 |
| Crude lipid (%) | 3.87 | 14.09 | 3.51 | 12.98 | 4.27 | 10.97 | 3.87 | 9.73 |
| Carbohydrates (%) | 51.97 | 28.71 | 37.40 | 22.85 | 40.17 | 31.59 | 41.61 | 28.10 |
| Gross Energy (Kcal/kg) | 3553 | 4603 | 3269 | 4270 | 34.58 | 4557 | 3280 | 4270 |
| Minerals | | | | | | | | |
| Calcium (%) | 2.42 | 3.03 | 3.43 | 2.28 | 3.83 | 2.48 | 2.18 | 2.38 |
| Phospor (%) | 0.59 | 0.82 | 0.74 | 0.78 | 0.42 | 0.54 | 0.64 | 0.35 |

**Table 3.** Percentage mortality (± SE) of black soldier fly reared at each experimental diets

| | Mortality (%) | | | |
|---|---|---|---|---|
| Stage | A | B | C | D |
| larval | 22.60±4.32 $^{a}$ | 14.40±4.77 $^{a}$ | 29.60±6.40 $^{a}$ | 22.40±2.89 $^{a}$ |
| prepupal | 26.40±3.72 $^{ab}$ | 14.60±4.82 $^{a}$ | 32.40±5.55 $^{b}$ | 22.60±3.04 $^{ab}$ |
| pupal | 26.60±3.70 $^{ab}$ | 14.60±4.82 $^{a}$ | 33.00±5.43 $^{b}$ | 22.60±3.04 $^{ab}$ |
| adult | 26.60±3.70 $^{ab}$ | 15.20±5.14 $^{a}$ | 34.00±5.35 $^{b}$ | 22.60±3.04 $^{ab}$ |
| male | 26.60±3.70 $^{ab}$ | 15.20±5.14 $^{a}$ | 34.00±5.35 $^{b}$ | 22.60±3.04 $^{ab}$ |
| female | 26.60±3.70 $^{ab}$ | 15.20±5.14 $^{a}$ | 34.00±5.35 $^{b}$ | 22.60±3.04 $^{ab}$ |

Different superscript letters within the same parameter indicate significant differences ($P \leq 0.05$)

This study demonstrates that the resulting larvae were much fatter, longer and wider on a mixture of manure and vegetable waste than when feeding on manure only (Table 4, 5, 6). Certain bacteria, such as *Bacillus natto*, are present in poultry manure and promote larval growth and development [22] [23]. Larval survival of black soldier flies is not influenced by bacterial presence, but addition of *Bacillus subtilis*, a species present in the gut, does increase prepupal weight [23] [24].

**Table 4.** Larval length (mm) of black soldier fly developing at four experimental diets

| Larval age (day) | Length (mm) | | | |
|---|---|---|---|---|
| | Horse manure | Horse manure and vegetable waste | Sheep manure | Sheep manure and vegetable waste |
| 4 | 8.98±0.17$^{b}$ | 5.57±0.09$^{a}$ | 10.60±0.15$^{c}$ | 10.72±0.15$^{c}$ |
| 6 | 12.50±0.24$^{c}$ | 8.08±0.36$^{a}$ | 11.31±0.19$^{b}$ | 10.50±0.27$^{b}$ |
| 8 | 11.62±0.19$^{b}$ | 10.04±0.25$^{a}$ | 11.90±0.22$^{b}$ | 12.38±0.34$^{b}$ |
| 10 | 12.10±0.21$^{bc}$ | 11.29±0.29$^{ab}$ | 10.44±0.25$^{a}$ | 12.61±0.17$^{c}$ |
| 12 | 10.95±0.23$^{b}$ | 10.97±0.21$^{a}$ | 10.93±0.17$^{a}$ | 12.60±0.28$^{b}$ |
| 14 | 11.77±0.20$^{b}$ | 11.14±0.22$^{ab}$ | 10.71±0.22$^{a}$ | 12.83±0.23$^{c}$ |
| 16 | 11.72±0.21$^{b}$ | 11.75±0.14$^{b}$ | 10.85±0.32$^{a}$ | 13.22±0.12$^{c}$ |
| 18 | 11.95±0.35$^{a}$ | 11.79±0.21$^{a}$ | 10.96±0.16$^{a}$ | 12.36±0.63$^{a}$ |
| 20 | 12.41±0.20$^{b}$ | 11.43±0.28$^{a}$ | 11.68±0.36$^{ab}$ | 13.98±0.14$^{c}$ |
| 22 | 12.50±0.17$^{ab}$ | 12.93±1.53$^{b}$ | 11.76±0.15$^{a}$ | 12.79±0.28$^{b}$ |
| 24 | 12.41±0.23$^{ab}$ | 12.69±0.29$^{ab}$ | 11.89±0.31$^{a}$ | 12.86±0.26$^{b}$ |

Different superscripts in the same row indicate significant differences between means ($P \leq 0.05$)

**Table 5.** Larval width (mm) of black soldier fly developing at four experimental diets

| Larval age (day) | Width (mm) | | | |
|---|---|---|---|---|
| | Horse manure | Horse manure and vegetable waste | Sheep manure | Sheep manure and vegetable waste |
| 4 | 2.40±0.05$^{b}$ | 1.37±0.06$^{a}$ | 2.25±0.05$^{b}$ | 2.60±0.0$^{cb}$ |
| 6 | 3.52±0.09$^{c}$ | 2.01±0.17$^{a}$ | 2.95±0.05$^{b}$ | 2.68±0.08$^{b}$ |
| 8 | 3.10±0.07$^{b}$ | 2.79±0.04$^{a}$ | 3.26±0.08$^{b}$ | 2.59±0.08$^{b}$ |
| 10 | 2.94±0.06$^{b}$ | 2.79±0.08$^{ab}$ | 2.60±0.08$^{a}$ | 3.15±0.08$^{c}$ |
| 12 | 2.59±0.08$^{a}$ | 2.78±0.11$^{a}$ | 2.54±0.07$^{a}$ | 3.29±0.07$^{b}$ |
| 14 | 2.82±0.10$^{a}$ | 3.24±0.08$^{b}$ | 2.84±0.09$^{a}$ | 3.02±0.09$^{b}$ |
| 16 | 2.96±0.09$^{a}$ | 3.44±0.06$^{b}$ | 2.96±0.09$^{a}$ | 3.52±0.06$^{b}$ |
| 18 | 2.98±0.08$^{q}$ | 3.30±0.08$^{b}$ | 2.82±0.08$^{a}$ | 3.04±0.08$^{b}$ |
| 20 | 3.51±0.10$^{c}$ | 3.25±0.07$^{b}$ | 2.49±0.07$^{a}$ | 3.34±0.06$^{bc}$ |
| 22 | 3.36±0.07$^{c}$ | 3.04±0.08$^{b}$ | 2.60±0.06$^{a}$ | 3.24±0.06$^{c}$ |
| 24 | 3.48±0.08$^{b}$ | 2.79±0.08$^{a}$ | 2.56±0.07$^{a}$ | 3.38±0.11$^{b}$ |

Different superscripts in the same row indicate significant differences between means ($P \leq 0.05$)

**Table 6.** Larval weight (mm) of black soldier fly developing at four experimental diets

| Larval age (day) | Weight (g) | | | |
|---|---|---|---|---|
| | Horse manure | Horse manure and vegetable waste | Sheep manure | Sheep manure and vegetable waste |
| 4 | 0.017±0.000$^{b}$ | 0.014±0.000$^{a}$ | 0.023±0.000$^{c}$ | 0.048±0.000$^{d}$ |
| 6 | 0.031±0.000$^{c}$ | 0.023±0.000$^{a}$ | 0.025±0.000$^{b}$ | 0.074±0.000$^{d}$ |
| 8 | 0.032±0.000$^{c}$ | 0.026±0.000$^{a}$ | 0.027±0.000$^{a}$ | 0.071±0.000$^{c}$ |
| 10 | 0.040±0.000$^{c}$ | 0.028±0.000$^{b}$ | 0.027±0.000$^{a}$ | 0.075±0.000$^{d}$ |
| 12 | 0.040±0.000$^{c}$ | 0.030±0.000$^{b}$ | 0.028±0.000$^{a}$ | 0.080±0.000$^{d}$ |
| 14 | 0.040±0.000$^{c}$ | 0.032±0.000$^{b}$ | 0.030±0.000$^{a}$ | 0.075±0.000$^{d}$ |
| 16 | 0.040±0.000$^{c}$ | 0.037±0.000$^{b}$ | 0.031±0.000$^{a}$ | 0.069±0.000$^{d}$ |
| 18 | 0.045±0.000$^{c}$ | 0.040±0.000$^{b}$ | 0.037±0.000$^{a}$ | 0.054±0.000$^{d}$ |
| 20 | 0.047±0.000$^{c}$ | 0.046±0.000$^{b}$ | 0.034±0.000$^{a}$ | 0.052±0.000$^{d}$ |
| 22 | 0.046±0.000$^{b}$ | 0.047±0.000$^{c}$ | 0.033±0.000$^{a}$ | 0.051±0.000$^{d}$ |
| 24 | 0.045±0.000$^{b}$ | 0.047±0.000$^{c}$ | 0.032±0.000$^{a}$ | 0.052±0.000$^{d}$ |

Different superscripts in the same row indicate significant differences between means ($P \leq 0.05$)

Larvae fed manure were shorter, weighed less, and took longer to develop compared with others growing substrate such as fruit and vegetable, kitchen waste, liver, and fish [16]. The development of black soldier flies larvae on vegetable waste was faster than on restaurant waste and less substrate had to be fed because vegetable waste was substantially higher in moisture. In natural populations, black soldier flies at larval stage are adapted and preferred to decompose decaying organic materials. Rearing system of black soldier flies larvae on vegetable waste could provide a high quality insect resource with potential for being alternative in animal feed [25].

Based on this study, it could be concluded that horse and sheep manure were suitable as growing substrate or feed for the larval of black soldier flies. In addition, the mixture of manure with vegetable waste in a 50%:50% proportion is a good combination for prepupal biomass production.

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