Jurnal Biodjati 5(2):191-198, November 2020

e-ISSN : 2541-4208
p-ISSN : 2548-1606

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

STUDY ON SEX DETERMINATION AND IMPACT OF SEX RATIO TO REPRODUCTION SUCCESS IN BLACK SOLDIER FLY

Ramadhani Eka Putra[1]*, Nindia Safa'at[2]

Received : September 05, 2020
Accepted : October 31, 2020

DOI: 10.15575/biodjati.v5i2.9472

[1,2]School of Life Science and Technology, Institut Teknologi Bandung (ITB) Jl. Ganesha No. 10, Bandung 40132 Indonesia

e-mail:
*[1]ramadhani@sith.itb.ac.id
[2]nindiasafaat2@gmail.com

**Corresponding author*

Abstract. *Black soldier fly (BSF) (Hermetia illucens) is an insect species utilized as the converter of organic wastes into biomass applicable as a potential replacement of protein and lipid sources of animal feed and other industrial use. The limiting factor for the sustainability of this activity is the number of eggs produced as all processes started from the egg. Variable of quality of the substrate eaten during the larval stage and the proportion of males and females are among the decisive factors for egg production. The purposes of this study were to understand the impact of the substrate to the production of a particular sex, to developed a simple method by comparing the weight and size of the pupae to distinguished between male and female, and to find the best sex ratio to produce the highest number of eggs. In this study, two types of substrates (balance proportion of macronutrient and high in protein and lipid were applied as feeding material for larvae. The weight and length of each pupa produced were measured and the pupae kept inside separated containers to find the morphological differences between male and female pupae. Adult insects produced from pupae then used for study on the effect of sex ratio on reproduction success. The adults kept inside 1 x 1 x 1 m screen cages with 3 groups of sex ratio (male : female) which were 50%:50% (P1), 60%:40% (P2), and 40%:60% (P3). The probability of sex was analyzed by logistic regression while the number of eggs estimated by the weight of the egg batch. The result showed substrate used in this study is not affect the sex ratio of adult flies, weight of pupae as the best predictor for sex of the adult flies, and the best ratio of male and female for egg production was 40:60.*

Keywords: *black soldier fly, egg production, pupa, sex ratio, sex separation*

Citation
Putra, R. E. & Safa'at, N. (2020). Study on Sex Dtermination and Impact of Sex Ratio to Reproduction Success in Black Soldier Fly. *Jurnal Biodjati*, 5(2), 191-198.

INTRODUCTION

Black soldier fly (*Hermentia illucens*) is known worldwide as one of the biological agent applied to convert the biodegradable wastes into biomass high in protein and lipid (Kinasih et al., 2018) which applicable as part of animal feed and other industrial need (Li et al., 2011; Gobbi et al., 2013). Unlike other insect species, this species able to be kept in a container and reared in a closed ecosystem which made it one of the conversion processes of biodegradable wastes by BSF to be developed at an industrial level.

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One of the most important critical factors in the continuity of bioconversion activities is the availability of the eggs (Pastor et al., 2015). Several studies have been conducted related to the egg production of BSF in a natural condition (Sheppard et al., 2002), semi-artificial (Booth & Sheppard, 1984), and complete artificial (Zhang et al., 2010). Studies also have shown that some factors related to the egg production in artificial light duration exposure (Hoc et al., 2019), the density of the adult in the cage (Hoc et al., 2019), temperature and humidity (Tomberlin & Sheppard, 2002; Park et al., 2010), and light intensity and sources (Zhang et al., 2010; Nakamura et al., 2016; Oonicx et al., 2016; Heussler et al., 2018). These studies showed the importance of more in-depth research on biological parameters that optimize the reproduction of BSF. Furtherly, most of the studies were conducted in the temperate regions where temperature became the limiting factor for year-round production and the more natural condition setting for egg production is least likely to be achieved while the study in tropics on the reproduction aspects of BSF hardly found.

This study was designed to assess the influence of the substrate on the sex ratio of adults and the impact of sex ratio proportion to egg production. For sex ratio, data on the length and weight of the pupae were collected to develop a simple mathematic model for distinguished the male and female at the pupa stage.

MATERIALS AND METHODS

Study Area

This study was conducted at Environmental Toxicology, School of Life Sciences and Technology, Institut Teknologi Bandung in the room with the temperature of 25-27°C and relative humidity of 60-80%. The experiment was conducted from November 2018 to Januari 2019.

Black Soldier Fly

Black soldier fly used in this study was originated from the colony kept in the Laboratory of Entomology, School of Life Sciences and Technology, Institut Teknologi Bandung.Two thousand larvae (7-days old) divided into 2 groups fed with different types of the substrate which were substrate A and B (Table 1) ad libitum until they reach pupa.

Table 1. Treatment scheme of feed composition on the black soldier fly

Group	Fish offal (%)	Banana skin (%)	Tofu dreg (%)
A	30	30	40
B	50	-	50

Sex Determination

For determine the sex of adult, 655 and 640 pupa developed from all surviving larvae which fed with substrat A and substrat B., respectively, were used. The weight of each pupa was measured by analytical balance and the length was measured by a digital caliper (Mitatuyo, precision level 0.01 mm). After weight and length measurement, each pupa photographed (Nikon EOS) then placed inside individual vial with the hole on the cover to provided airflow. All vials then kept inside the dark chamber to prevent the negative effect of the sunlight.

Effect of Sex Ratio to Egg Production

200 adult BSF of each substrate group were kept inside screen cage with size 1 x

1 x 1 m with the ratio of equal numbers of male and female (P1), male-biased (P2), and female-biased (P3) (Table 2). Ovitrap was made from wood planks which placed above substance made of a mixture of tofu dreg and aged yogurt as oviposition attractant (Wardhana, 2016). Eggs collected from ovitraps started after 3 days and weighed. Egg collection conducted until all fly died.

Table 2. Sex ratio scheme of egg production study

Group	Male ratio (%)	Female ratio (%)
P1	50	50
P2	60	40
P3	40	60

Data Analysis

The effect of the substrate to sex ratio of adult was tested by one-way ANOVA. Prediction of sex of adult by pupae geometric (length and weight) was tested by logistic regression. In this test, sex was designed as a dependent variable, data on weight and length of the pupae as an independent variable using data compiled from pupae of group A and B. All statistic were conducted by JASP software (an open-source statistical analysis). with P value < 0.05 as a significant factor (Walpole et al., 2012).

RESULTS AND DISCUSSION

Effect of the Substrate Type to the Sex Ratio of Adult

There was no significant effect of the substrate on the sex ratio of adult flies produced as both substrates produced about 50% adult males (P-value = 0.136, P > 0.05) (Figure 1).

In this study, both feed types consumed by larvae showed no effect on the sex ratio of adults. Studies showed that sex ratio of adults could be affected by the physical condition of the substrate (Gobbi et al., 2013; Holmes et al., 2013), the acidity of the substrate (Ma et al., 2018), feeding system (Meneguz et al., 2018), and proportion of dietary protein and carbohydrate (Cammack & Tomberlin, 2017; Barragan-Fonseca et al., 2019). It seems that the addition of banana skin did not significantly alter the substrate condition and nutritive value while it has relatively low nutritive value for larva due to low digestibility (Nyakeri et al., 2017; Isibika et al., 2019).

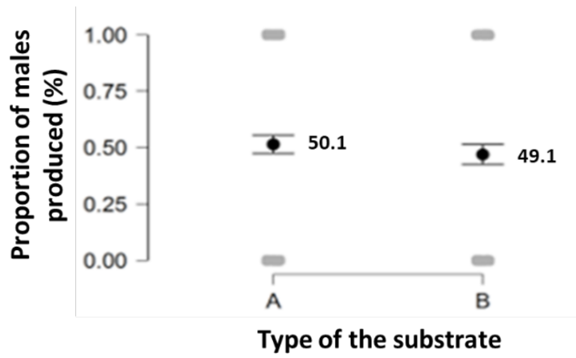

Figure 1. The proportion of males produced from larvae fed on different types of substrate study

Prediction of Adult Sex Type Based on the Weight of Pupae

The quantitative analysis showed a significant correlation between sex type and weight of the pupa (P-value = 0.00001, P-value < 0.001) and the model able to predict the correct sex type at 62.2% (Figure 2). Based on the model, female adults more likely to be originated from heavier pupae at 0.12 grams as the middle point of the probability of male and female.

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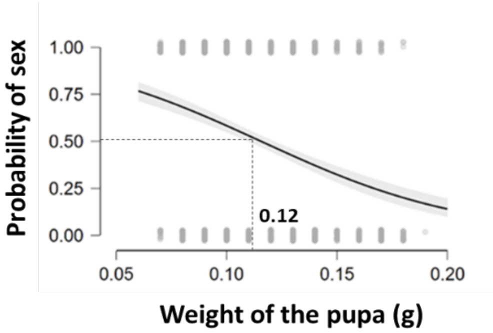

Figure 2. Probability of sexbased on the weight of pupa (male probability at 1 and female probability at 0). Data was compiled from treatment groups A and B.

Prediction of Adult Sex Type Based on the Length of the Pupa

The quantitative analysis also showed a significant correlation between sex type and length of the pupa (P-value = 0.00001, P-value < 0.001) and the model able to predict the correct sex type at 61,9% (Figure 3). Based on the model, females tend to be produced from the pupa with a higher length.

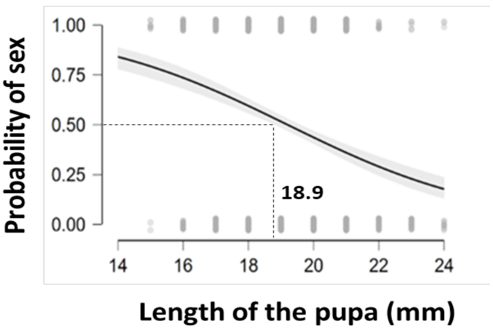

Figure 3. Probability of sex-based on length of pupa (male probability at 1 and female probability at 0). Data was compiled from treatment groups A and B.

Further analysis showed a strong correlation between weight and length of the pupa (Pearson correlation test = 0,807) and the correlation between these variables was a positive correlation (Figure 4).

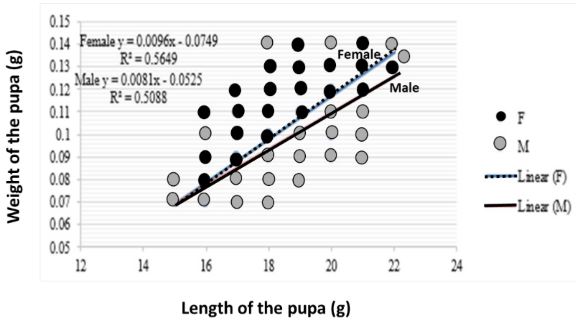

Figure 4. Correlation between weight and length of the pupa.

Studies showed the differences in the size and weight of the male and female pupae in various fly families. The size of female pupae could be bigger (Testa et al., 2013) or smaller (Braet et al., 2015) which depends on the life history of the species, growth rate, and time spent in the larva period. Beside provides a possible simple method to distinguish between male and female, the result also indicates some phenomenon which may use of mass rearing of this species, such as (1) the existence of resource competition between sex (Partridge & Farquhar, 1983) and/or (2) differential in the utilization of the resource (Temeles & Kress, 2003).

Egg Production of *Hermetia illucens* at Different Males : Females Ratio

More eggs was produced in the female dominated group while less eggs produced in the group with balance sex ratio and male biased group, if the larvae fed with balance feed composition (treatment A). On the other hand, egg production among different ratio groups was more distinct in treatment group B as a balanced proportion of males and females (P1) produced the least number of the egg (Figure 5).

Although the addition of banana skin in the substrate did not alter the sex ratio, it produced a positive effect on the egg production of the male-dominated population. This result may relate to the proportion of protein and carbohydrate, of the substrate, as both macromolecules are the most important nutrients for insect physiology related to survival, growth, and reproduction (Aguila et al., 2013; Nash & Chapman, 2014) especially for species with a nonfeeding adult stage (Arrese & Soulages, 2010) and for those that depend on the protein acquire during larvae for protein needs at adult stage (Waldbauer et al. 1984). Lower egg production in substrate A may indicate that the quality of the substrate was lower than substrate B as suboptimal substrate usually produces a low-quality female (Gobbi et al., 2013; Barragan-Fonseca et al., 2019).

Higher egg production at the female-dominated population showed in this study was different from the result of Hoc et al. (2019). This may relate to the mating behavior of this species in which males only attracted to active female and female-only mates once (Giunti et al., 2018). In the population when females only mating once, there is a mechanism of female choice in which females only choose the physiological superior male to prevent the production of low fitness progeny (Jones & Ratterman, 2009). There is a possibility that the quality of the males, in this study, relatively similar thus each male had a similar chance to mate with a female. Another possibility may relate to the time of adult emergence. A study by Tomberlin & Sheppard (2002), showed that females oviposit the eggs from third to sixth day (life expectancy for female BSF is 7 days) and supported by Giunti et al. (2018) that reported 1-day old females always rejected a mating attempt from males. In this study, we observed that adults did not emergence at the same time in which may increase the possibility of lower quality males to mate. However, further study should be conducted to test these hypotheses.

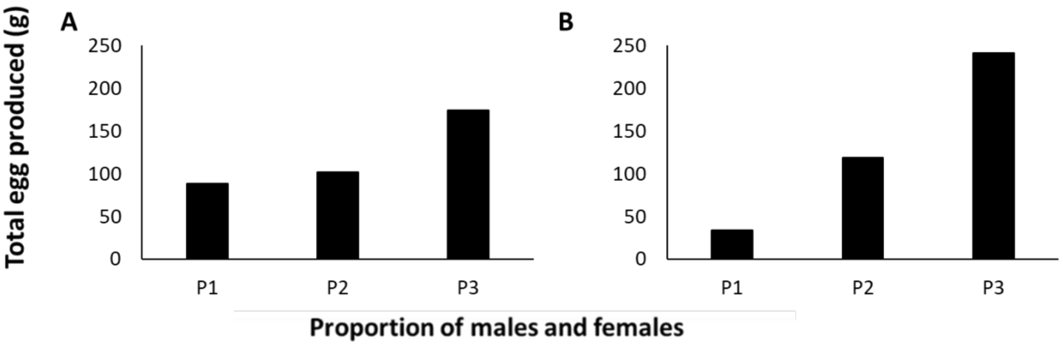

Figure 5. Number of eggs produced by different ratio of males and females (P1-P3) from larvae feed with different substrate (Group A and B)

The types of adult sex types can be predicted by morphometric of the pupa as the longer (more than 18 mm) and heavier pupa (more than 0.12 grams) tend to be a female. This information can be applied to improve the total egg production by creating a stack of pupa consisted of more females than males (a female-biased ratio).

ACKNOWLEDGEMENTS

This study partly funded by Hibah Kompetensi 2019 (2/E1/KP.PTNBH/2019) granted to corresponding author.

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