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Research Article

Reproduction and Production Traits of Eggiest of Black Soldier Fly Reared in Different Enriched Media and its Potential as Poultry Feed

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Abstract | The aim of this study is to investigate the reproduction traits of eggiest of black soldier flies (*Hermetia Illucens*) in different enriched media and its potential as a poultry feed. A total of 1000 one-day-old eggiest were weighed (pool of 200 eggiest per single weight), randomly divided into groups of five and allocated in 5 bio ponds (12 cm x 15 cm x 10 cm) (5 replicated per dietary treatments). The treatments enriched media was formulated as follows: T0: 100% dried poultry waste; T1: mixed up-fermented dried poultry waste 75% and commercially laying hens feed (starter); T2: mixed up-fermented dried poultry waste 50% and 50% commercially laying hens feed (starter); T3: mixed up-fermented dried poultry waste 25% and 75% commercially laying hens feed (starter); T4: 100% and commercially laying hens feed (starter). A statistical analysis was conducted using analysis of variance using Proc Mixed with general linear model (GLM) using SAS studio for academics Online Edition (https://odamid-apse1.oda.sas.com/SASStudio/). Comparing two results, it can be seen that the different enriched media gave gradually significant differences ($p < 0.05$) in the egg weight, number of eggs of BSF success to hatch, hatchability, length, width, and number of change becoming flies at period shown. Conclusively it has been shown that the different enriched media gave positive effect until 75% replacement. Those treatment controls are not recommended.

Keywords | Black soldier fly, Feed, Fermentation, Health, Reproductive

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INTRODUCTION

The world is facing an extensive growth in population, which is predicted approximately to reach 9 billion people in 2045, the global food demand will rise by 200% from 2010 to 2050, where animal feed and production is expected to increase by 60% (He *et al*., 2023). In contrast, future shortages for yellow maize, wheat, and soybean were estimated to be approximately reduced by only 65%, 35%, and 50%, respectively. Nowadays, a lot of researchers has been undertaken to determined alternative as source of materials. One of the potential that can be replaced is black soldier flies. A total 200-300 billion globally of black soldier flies, *Hermetia illucens* are estimated to be farmed annually and expected to grow (Rowe, 2020).

Black soldier fly (BSF) larvae, well-known as biomass converting waste that helps to reduce ecological pollution from biomass into protein and fat rich insect biomass (Tavares *et al*., 2022). Maggot is generally known as a decaying organism because of its habit of consuming organic materials. Maggot chews his food with his hook-shaped mouth. Maggots can grow on decaying organic matter in temperate and tropical regions (Tavares *et al*., 2022). The livestock sector is also still faced with the problem of livestock manure which has the potential to cause

environmental pollution. Livestock manure waste actually has relatively potential to be used as organic fertilizer (Dewi *et al*., 2017). However, it requires a composting process which takes up to two months to get the fertilizer organic (Barragan-Fonseca *et al*., 2017). The length of the processing process makes breeders less interested in utilizing livestock manure as organic fertilizer. Livestock manure waste which continues to accumulate will certainly cause environmental pollution and potentially raises public health problems (Permana *et al*., 2020).

Nowadays, BSF extensively uses increasingly expensive protein sources used in poultry and livestock compound diet formulation such as fish meal and soybean meal which holds the potential to alleviate the production of animal and feed security. In response to the continuous demand for meat and eggs, a novel alternative protein resource as animal feed, edible insects are globally appealing (Adli *et al*., 2023a). Utilizing feed that can improve egg, meat, and health production is remarkable. Life cycle of the BSF was divided into four stages as follows: Egg, larva, pupae, and adult stage.

Reproductive selection is a selection and powerful that can lead to adaptations of reproductive tracts (Munsch-Masset *et al*., 2023). To improve production, the management of reproduction should be establishing to improve the knowledge, especially of factors that can influence fertility and reproductive output, as this may directly improve mass productions (Munsch-Masset *et al*., 2023). The female BSF oviposit only around the edges of the larval food source (EPPO, 2019). The current data revealed that there is no consistent data of nutritional quality and reproductive profile of BSF (EPPO, 2021). Therefore, exploration of the inconsistency such as protein, fat, and fiber in different media is essential. Furthermore, there is a lack of information related to the reproductive profile of BSF. The aim of this research project has therefore been to try and investigate the reproduction traits of eggiest of black soldier flies (*Hermetia illucens*) in different enriched media and its potential as a poultry feed.

MATERIALS AND METHODS

Experimental design

The experiment was performed using the BSF established at the PT. Dioola Karya Indonesia and Faculty of Animal Science, Universitas Brawijaya. Collected BSF eggs were incubated and the one-day-old larvae were harvested and fed using enriched media until the end of the cycle. A total of 1000 one-day-old eggiest were weighed (pool of 200 eggiest per single weight), randomly divided into groups of five and allocated in 5 bio ponds (12 cm x 15 cm x 10 cm) (5 replicated per dietary treatments). Each bio pond was filled with 100 g of media (0.1 g/larva) that underwent an acclimatization chamber in order to avoid a thermal shock. The bio pond was placed in the climatic room with controlled conditions (Temperature: approximately 26°C). Each day, the boxes were checked and substrate was individually mixed with an artificial mixer. A total, 250 g of feed was added every three days in all the replicates until the pre-pupae appearance (18 days of age). The treatments enriched media was formulated as follows: T0: 100% dried poultry waste; T1: mixed up-fermented dried poultry waste 75% and commercially laying hens feed (starter); T2: mixed up-fermented dried poultry waste 50% and 50% commercially laying hens feed (starter); T3: mixed up-fermented dried poultry waste 25% and 75% commercially laying hens feed (starter); T4: 100% and commercially laying hens feed (starter). The commercial feed taken from PT. Malindo feed mill Tbk. consisted yellow maize, dried distiller grain soluble (DDGS), meat-bone-meal (MBM), corn gluten meal (CGM), Di-calcium phosphate (DCP), salt, and crude palm oil (CPO) can be seen in the Table 1.

Table 1: Nutrient content of laying hens (starter).

Parameters (%)	
Dry Matter	87.00
Moisture	13.00
Ash	8.00
Crude Protein	20.00
Crude Fibre	6.00
Fat	3.00
Calcium	0.80
Phosphorus	1.20
Lysine	1.00
Methionine	0.50
Methionine + cysteine	0.80
Threonine	0.75
Tryptophan	0.20

Based on the label of PT. Malindo feedmill

Reproductional traits of eggiest

At the end of the trial, a total of two hundred representative samples were collected (200 eggiest/ replicated) at 8-day intervals. The parameters observed are egg weight (gr), the number of egg of BSF success to hatch (pcs), the number of egg BSF fail to hatch (pcs), hatchability (%), length (µm), width (µm), number of change becoming flies at period shown (male and female) (%), and male and female ratio of flies. Then, a representative sample was collected and identified using a biological microscope (Olympus CX33).

Statistical analysis

A statistical analysis was conducted using analysis of variance using Proc Mixed with general linear model (GLM) using SAS studio for academics online edition (https://odamid-apse1.oda.sas.com/SASStudio/). An

error was expressed as standard error mean (SEM). At the end, probabilities values were subjected in the Duncan Multiple Range Test. The following model was used (Sholikin *et al*., 2023).

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where Yij was parameters observed, μ was the overall mean, Ti the effect of different levels of media used, and eij the amount of error number. The treatments enriched media was formulated as follows: T0: 100% dried poultry waste; T1: mixed up-fermented dried poultry waste 75% and commercially laying hens feed (starter); T2: mixed up-fermented dried poultry waste 50% and 50% commercially laying hens feed (starter); T3: mixed up-fermented dried poultry waste 25% and 75% commercially laying hens feed (starter); T4: 100% and commercially laying hens feed (starter). and the significance threshold was established at $p < 0.05$ using the was used to compare the means. All analysis was carried out in six replications and the significance of difference was defined as the 5% level (p <0.05). At the end, probabilities values were subjected to the Duncan Multiple Range Test (DMRT) (Sholikin *et al*., 2023).

RESULTS AND DISCUSSION

Effects of the reproduction traits of eggiest of different media its potential as poultry feed

As an overall perspective, from this data we can see that Table 2 depicted hit the peak value at the 100% and commercially laying hens feed (starter). As Table 2 shows, there is a moderately significant difference across the whole reproductive profile of maggot eggs ($p < 0.05$) (Table 2) and Figure 1). Comparing these results, it can be seen that the different enriched media reached the peak value for the number of eggs of BSF success to hatch, hatchability, egg become male flies, and ratio both male and female (Table 2 and Figure 2). To begin with, there is an oscillation of the egg weight results. Subsequently, the different media had been successfully giving significant results on the egg weight by (1.13; 1.45; 0.21; 0.62, respectively) (Table 2). Those the treatment control presented plateaued results across whole the periods shown. ($p > 0.05$) (Table 2). The present study confirms previous findings from Van Huis (2013) and contributes additional evidence that suggest Maggot can grow on media containing nutrients in accordance with their life needs. Another research has several practical applications. Firstly, it makes it easy to breed. Despite its exploratory nature, this study offers some insight into the breed of these BSF flies, the success of breeding determined from the growth medium (Julita *et al*., 2022). Subsequently, when the fly's reproduction process occurs, the fly will like a typical growing medium and when he likes the scent, then flies want to live and reproduce in the media (Sindermann *et al*., 2021). *Hermetia illucens* reproduce sexually. According to Liu *et al*. (2022) that sexual reproduction is also called generative reproduction. Sexual reproduction involves the fusion of 2 gametes (sperm and ovum) to form a zygote. In general, the gamete cells come from 2 different parents (male and female). Egg-laying of the maggot was more likely to be conducted on the animal protein-based substrate (Putra and Safa'at, 2020).

In detail, the differences between number of eggs of BSF success to hatch and number of eggs of BSF fail to hatch are highlighted in Table 2. Comparing the two results, it can be seen that there were minimal changes between number of eggs of BSF success to hatch and number of eggs of BSF fail to hatch ($p < 0.05$) (Table 2). The result reaches the highest point at T4 (197.80%) and T1 (8.60%) for number of eggs of BSF success to hatch and number of eggs of BSF fail to hatch, respectively. In contrast, these two results hit the lowest point at T0 (0.00%) (Table 2 and Figure 4). BSF larvae grow very fast organic wastes such as poultry manure. When the maggot is ripe, it takes 3-4 days to be used as feed by drying and milling process. However, the strong smell of the animal protein-based substrate acted as trigger liking behavior, which is an important part of mating behavior. For instance, egg trapping in nature may also improve the efficiency of egg production in mass-rearing facilities (Freitas, 2019).

Table 2: The reproduction profile of black soldier fly after reared different media.

	T0	T1	T2	T3	T4	SEM	R^2
Egg weight (gr)	0.00^{a}	1.13^{ab}	1.45^{ab}	0.21^{b}	0.62^{ab}	0.73	0.33
Those number of egg of BSF success to hatch (pcs)	0.00^{a}	191.40^{a}	194.40^{ab}	195.40^{b}	197.80^{bc}	12.02	0.99
Those number of egg of BSF fail to hatch (pcs)	0.00^{a}	8.60^{d}	5.20^{cd}	4.60^{bc}	2.20^{ab}	12.1	0.46
Hatchability (%)	0.00^{a}	95.70^{a}	97.20^{ab}	97.80^{ab}	98.90^{b}	3.00	0.99
Length (μm)	0.00^{a}	210.37^{b}	208.18^{b}	211.57^{b}	207.95^{b}	41.92	0.99
Width (μm)	0.00^{a}	47.99^{b}	48.21^{b}	47.75^{b}	47.69^{b}	5.82	0.98
Egg become male flies (%)	14.00^{a}	29.00^{ab}	31.00^{ab}	36.00^{b}	37.00^{b}	0.12	0.76
Egg become female flies (%)	10.00^{a}	43.00^{b}	50.00^{b}	42.00^{b}	40.00^{b}	2.33	0.98
Male and female ratio	1.4	0.67	0.62	0.85	0.92	0.45	0.98

Gr, gram; pcs, pieces.

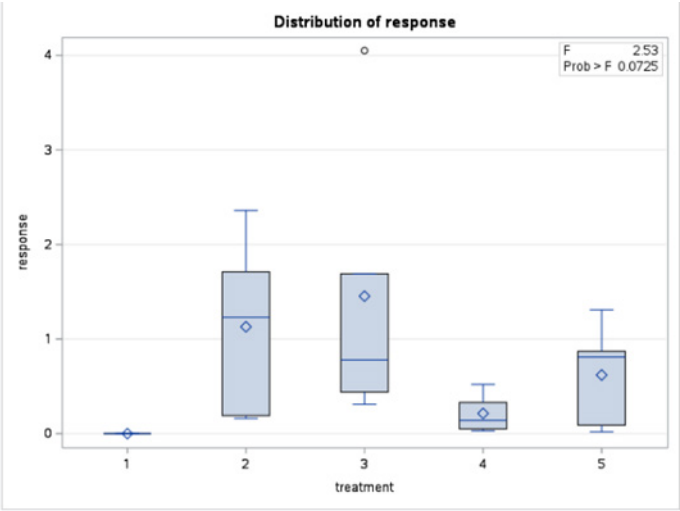

Figure 1: Distribution of response in egg weight (gr).

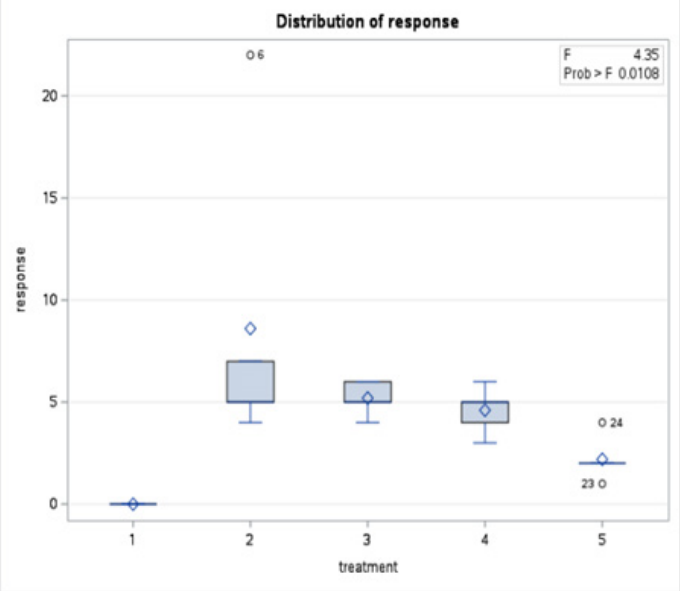

Figure 2: Distribution of response in Those number of egg of BSF fail to hatch (pcs).

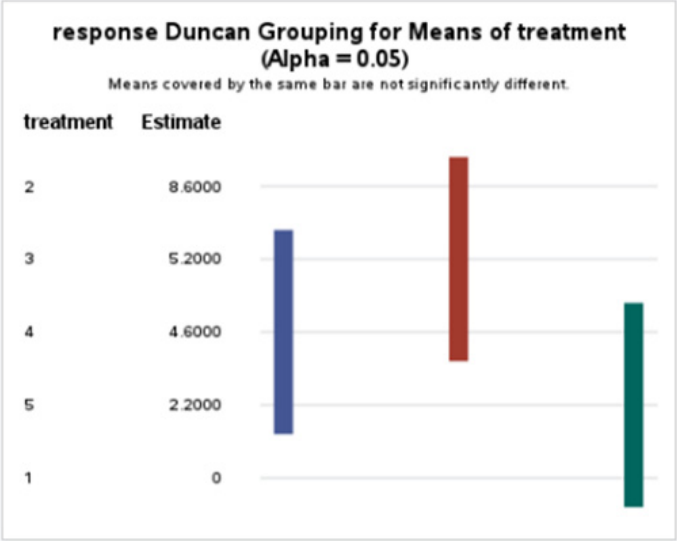

Figure 3: Response Duncan grouping for means of those number of egg of BSF fail to hatch (pcs).

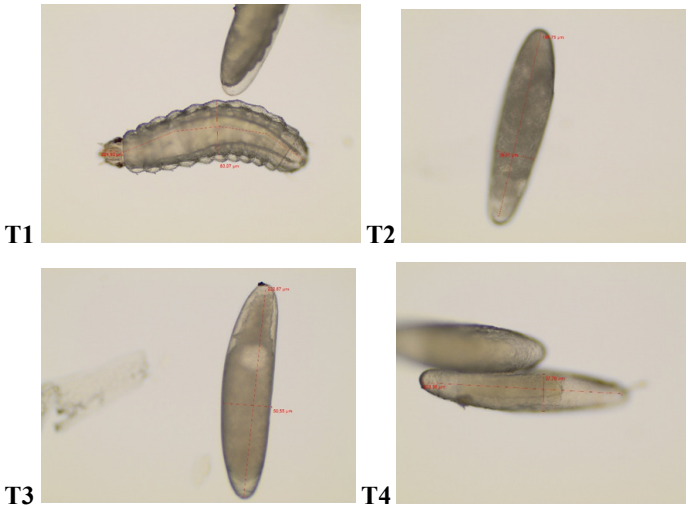

Figure 4: the larvae bsf after growth in the enriched different media.

Larvae BSF can consume and degrade a number of organic materials contained in waste up to 70%. Many factors influence the success of maggot cultivation. Things that affect maggot production in the media provided are the environmental conditions of maggot cultivation and the nutrient content of the ingredients. Judging from the environmental conditions, maggot likes humid environmental conditions (Hoc *et al.*, 2018; Barragan-Fonseca *et al.*, 2017). Likewise, with the nutrient content in the maggot growing media. Optimum nutrient content is very important for maggot biomass growth, according to, suitable material for maggot growth is material that contains a lot of organic matter. *Hermetia illucens* cycle is relatively short, around 40 days. The metamorphosis phase consists of the egg phase 2 to 3 days, the larval (maggot) phase 18 days, the pre-pupa and pupa phase 3 days, the young black soldier flies phase 3 days, the adult black soldier flies phase occurs 8 mating, two to three days after mating the female will lay eggs, females die after laying eggs, males die after mating.

From the Figures 3 until 10 have shown there is no significant difference among different stages of the maggot. According to Makkar *et al.* (2014), larger larvae are ideal when used for mixed feed ingredients or pellet raw materials because they are able to meet the quantity production (Hoc *et al.*, 2019).

Maggot has been used as a poultry feed supplement several times conducted including research by Hong *et al.* (2020), showing that the use of maggot as f feed has a significant effect on growth with a value of 6.51±0.32. The impact of using maggot can also be seen in improving the health status of animals. This can be seen from the increasing number of cells of red blood cells, white blood cells, and the number of cells that carry out phagocytic activity (Ottoboni *et al.*, 2020). Adult maggots do not eat,

but only need water because nutrients are only needed for reproduction during the larval phase (Laksanawimol *et al*., 2023). *Hermetia illucens* in its life cycle does not perch on food that is directly consumed by humans. In adulthood, the main food is flower extract, while at a young age the food comes from the food reserves in the body (Binsin *et al*., 2023). Breeding is done sexually, where the female contains the eggs, then the eggs are placed on a clean surface, but close to a suitable food source for the larvae (Chemello *et al*., 2022). At the beginning of the stage the larvae need more nutrients and media to grow that larvae to pupa stages (Chemello *et al*., 2022).

The most preferred feed source seems to be fermented commercial layer feed. Male BSF flies only live a maximum of 5 days, after mating they die. Meanwhile, the females die at most 8 days after laying their eggs (Julita *et al*., 2020). From egg to spawn again. Maximum total of 45 days (depending on various conditions) (Gougbedji *et al*., 2021). The lifespan of the black soldier flies relatively rapidly while the reproductive system is high, at present ration one female can reproduce one egg cluster which consists of 500-900 larvae (Klüber *et al*., 2023; Barragán-Fonsec (2018). The life span since the eggs hatch into eggiest is approximately at 9 days, and on the 17th day (7 days) Baby larvae will reach a weight of 5000-6000 times their original weight or become adult larvae with an average weight of 0.16 gram/heads (Oonincx *et al*., 2016).

CONCLUSIONS AND RECOMMENDATIONS

Conclusively it has been shown that the different enriched media gave positive effect until 75% replacement. Those treatment controls are not recommended.

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NOVELTY STATEMENT

The novelty of this research is there is a lack of information related to the reproductive profile of BSF as an animal feed. The sexually-related activities of BSF adults were a lack of information observed. A majority of the females preferred to perch on the ovipositional apparatus and fly around the illuminated area compared to the very low activities of the mated males. The BSF adults displayed different behavioral responses to the specific substrate.

AUTHOR'S CONTRIBUTION

NI, APAY, and FA contributed to collecting data, validation, data analysis and preparing the manuscript. HES, DA contributed to the research design, revised the manuscript and supervision. All authors read and approved the final version of the manuscript in the present journal.

Ethical approval

Ethical approval for the study was given by the health research ethics committee, Faculty of Medicine, Universitas Brawijaya number 247/EC/KEPK/08/2023 with date approval 23 August 2023. The Faculty of Medicine, Universitas Brawijaya following ethical review on the declaration of Helsinki through the following research protocol.

Conflict of interest

The authors have declared no conflict of interest.

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