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Full Length Research

Bioconversion of fermented tofu waste into high protein alternative feed using black soldier fly (*Hermetia illucens*)

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ABSTRACT: This study determines the life cycle and nutritional content of Black Soldier Fly (*Hermetia illucens)* larvae cultured on fermented tofu waste. The materials used in the study were tofu pulp, fermented tofu pulp, tempeh yeast, and the eggs of *H. illucens* fly. This study used a Completely Randomized Design with four treatments with five replications. The treatments were T_0: tofu pulp (0% yeast tempeh); T_1: fermented tofu pulp (0.25 % yeast tempeh); T_2: fermented tofu pulp (0.5% tempeh yeast), and T_3: fermented tofu pulp (0.75 % tempeh yeast). The variables measured included: the life cycle, dry matter conversion, dry matter extraction, and proximate composition of *H. illucens* larvae. The collected data were processed using variance analysis. The results showed that the *H. illucens* fly's life cycle follows: from laying eggs to hatching is four days, and growing into adult larvae takes 26-28 days. The dry matter conversion was 11.9 to 22.1%, and dry matter extraction was 55.8 to 67.5%. There was no effect ($p>0.05$) of adding tempeh yeast on crude protein, ether extract, crude fibre, and ash content. The result showed that the conversion and extraction of dry matter media were higher in the more media. The fermentation of tofu pulp did not increase the nutritional value of *H. illucens* larvae.

Keywords: Conversion, extraction, fermentation, *Hermetia,* tofu pulp.

INTRODUCTION

Feed is the most expensive of the total production costs, around 50 to 70% (Thirumalaisamy *et al*., 2016). One of the classic problems in animal husbandry is the high cost of protein feed ingredients, especially fish meals. Fish meal is one of the main components of animal feed, which has not been fully substituted either for nutritional reasons or because of the guarantee of its availability (Husain and Serdiati, 2014; Manangkot *et al*., 2014; Okah and Onwujiariri, 2012). Efforts to reduce food costs are to find ingredients that do not compete with humans are cheap, have high nutritional value, are available continuously, are liked by livestock, and are not harmful to livestock that eats them (Blair, 2011; Ravindran, 2013; Mallick *et al*., 2020). Various attempts have been made to replace fish meals by using conventional and unconventional feed ingredients to replace fish meals. Non-conventional feed generally refers to any feed that has not been used traditionally to feed livestock and is not used commercially in animal feed production (Amata, 2014). One of the potential alternative feed ingredients is *H. illucens* larvae as a protein feed source because the larvae have excellent nutritional content, namely 42% crude protein, and 35% ether extract (Newton *et al.*, 2005). The value is comparable to fish meals and soybeans. In addition, black soldier fly larvae have a high protein content and are used as an alternative feed source for livestock (Dabbou *et al*., 2018; Liu *et al*., 2008). In their life cycle, *H. illucens* flies have five stages: egg, larval, prepupa, pupa, and adult. Of the five stages, the larval and prepupa stage was often used as fish feed (Newton *et al*., 2005; Diener *et al*., 2009). The time to reproduce from eggs to the post-feeding stage may vary depending on the food source and the appropriate environmental conditions (Tschirner and Simon, 2015; Liland *et al.,* 2017). Bioconversion is converting or changing an organic material into other valuable products. It has added value by taking advantage of events and the

biosynthesis or the formation of biolysis or solutions (Abduh *et al*., 2018; ur Rehman *et al*., 2017). In the tofu production process, tofu and waste are produced (solid and liquid waste). Tofu pulp is a solid waste with a crude protein content of about 21.29% (Yusnawati *et al*., 2019). Furthermore, efforts to improve the quality of solid tofu waste are carried out through fermentation to make tempeh gembus (Simanjuntak *et al*., 2021). The fermentation uses tempeh yeast to increase the protein content of the tofu pulp (Afifah *et al.,* 2019). According to Denardi-Souza *et al.* (2018), mould protein biomass formation in fermentation will increase the substrate's protein and amino acid levels. Increasing the nutrient of media containing tofu pulp fermentation is also expected to obtain larvae *H. illucens* that contain high nutrients. Therefore, the study purposed to determine the life cycle of *H. illucens*, dry matter conversion, dry matter extraction, and nutritional content (dry matter, crude protein, ether extract, ash, and crude fibre) of black soldier fly larvae.

MATERIALS AND METHODS

Research on the bioconversion of fermented tofu waste into high-protein alternative feed using the *H. illucens* fly was conducted at the Laboratory of Animal Nutrition and Feed Science at the Faculty of Animal and Agriculture Science, Diponegoro University, Semarang, Central Java, Indonesia. Tofu pulp, tempeh yeast (*Rhizopus oligosporus)*, and wild black soldier fly eggs were used as experimental materials. Tofu wastes were obtained from Sumedang tofu artisans located at Dewi Sartika Timur street, Sukorejo Village, Gunungpati District, Semarang City, Indonesia. The eggs of *H. illucens* fly were obtained by catching wild female flies that lay their eggs in quail droppings on a quail farm located in Kalisidi Village, West Ungaran District, Semarang Regency, Central Java. Larvae were reared on two different media, 500 and 300 g, for *H*. *illucens* life cycle parameters, dry matter extraction, and dry matter conversion. Furthermore, the larvae production in both media was mixed for nutritional quality testing. The study used a completely randomized design with four treatments and five replications, namely:

T_0: tofu pulp with tempeh yeast 0% (control)
T_1: fermented pulp waste with tempeh yeast 0.25%
T_2: fermented pulp waste with tempeh yeast 0.5%
T_3: fermented pulp waste with tempeh yeast 0.75%

The observations made include:

1. The time length takes for a BSF egg to hatch.
2. The time length takes for the prepupa to form (the prepupa will come out of the media itself for the pupation period).
3. Dry matter conversion (DMC) is the number of dry matter diets converted to prepupae dry matter expressed in percentage. It was calculated using the formula Barry (2004):

$$\text{DMC}\ (\%) = \frac{\text{larvae dry matter}}{\text{tofu pulp dry matter}} \times 100$$

4. Dry matter extraction (DME) is a percentage of the diet consumed by dry matter, it was calculated using the following formula Barry (2004):

$$\text{DME}\ (\%) = (1 - \left(\frac{\text{dry matter residual}}{\text{dry matter diet}}\right)) \times 100$$

5. The nutritional content of *H. illucens* (dry matter, crude protein, ether extract, crude fibre, and ash) of black fly larvae was measured by the AOAC method (AOAC, 1990).

Data were analyzed using analysis of variance (ANOVA) applying SPSS 16.0 statistical software. Duncan's multiple range tests examined the differences between the treatments′ means (Steel *et al*., 1997). The significance level was $p<0.05$.

RESULTS AND DISCUSSION

Life cycle of the *Hermetia illuscens* fly

This study's life cycle of the *H*. *illucens* fly is as follows; the egg phase to hatch takes 3-4 days. The flies were caught on Monday at 10.00 am, and then on Thursday around 4.00 pm, the eggs were hatched. The larval to prepupa phase takes 24-28 days, depending on the level of larvae density in the growing medium. The average final weight for black soldier fly larvae generally increased with the addition of greater amounts of manure (Liu et al 2008). Moreover, larvae fed with adequate media faster to reach the pupa period. Meanwhile, the pupal period to become a fly takes approximately 12-14 days. According to Myers *et al.* (2008), who examined larval development on three types of diet (food), the larval stage lasts 22 to 24 days at 27°C, and that adult flies appear after the next 18 to 21 days.

Dry matter conversion and extraction

Measurement of the conversion and extraction of dry matter in this study was carried out twice, and the results are shown in Table 1. The treatments did not affect the dry matter conversion (DMC) or extraction (DME) ($p>0.05$) significantly. The first conversion (DMC1) measurement used an enormous amount of media, namely 500 grams. The other (DMC2) with fewer media was 300 grams. Therefore, the value of DMC1 tended to be higher than DMC2, namely for the treatment of T_0 (17.2 vs 14.4%), T_1 (15.9 vs 11.9%), T_2 (18.7 vs 16.0%), and T_3 (22.1 vs 15.6%), respectively. Likewise, the dry matter extraction, DME1 tend to be higher than DME2, namely for treatment of T_0 (61.5 vs 55.8%), T_1 (59.6 vs 58.5%), T_2 (59.2 vs 61.3%), and T_3 (67.5 vs 62.5%), respectively.

Table 1. The Dry Matter Conversion and Dry Matter Extraction of Fermented Tofu Waste by *Hermetia illucens* Larvae.

Variable	Treatments				SEM	p
	T0	T1	T2	T3		
DM Conversion 1 (%)	17.2	15.9	18.7	22.1	0.90	0.07
DM Conversion 2 (%)	14.2	11.9	16.0	15.6	1.31	0.73
DM Extraction 1 (%)	61.5	59.6	59.2	67.5	1.53	0.20
DM Extraction 2 (%)	55.8	58.5	61.3	62.5	1.79	0.59

Note: DM = Dry Matter; SEM = Standart Error Mean.

Table 2. The Nutrients Content of *Hermetia illucens* Larvae (%)

Variables	Treatments				SEM	p
	T0	T1	T2	T3		
Ash	14.19	13.51	13.99	13.87	0.303	0.898
Ether extract	26.27	27.00	25.05	25.43	0.404	0.336
Crude fibre	23.96	22.00	21.52	21.36	0.403	0.070
Crude protein	34.57	37.17	39.07	38.72	0.713	0.090

Note: SEM: Standart error mean.

The dry matter conversion and extraction values tend to be higher on more media; this indicates that larvae can grow better in lower-density media to increase the availability of *H. illucens* nutrients'. This follows Liu *et al*. (2008) that the mean final weight for black soldier fly larvae generally increased with a more significant number of media. There was no effect on fermented tofu pulp conversion and dry matter extraction ($p>0.05$). Its shows that the larvae can effectively convert the organic matter of the medium into a different medium, that is, the organic matter of the larvae. The dry matter conversion was 17.2%, indicating that the media of dry matter converted into larvae was 17.2%. After the conversion of organic waste by *H. illucens* larvae, organic waste accumulation is reduced, and pollution and environmental harm are decreased (Liu *et al*., 2019). In comparison, the dry matter extraction obtained was 61.9%, meaning that the dry matter consumed by the larvae was 61.9%. This result is higher than the study by Newton *et al*. (2005), and Myers *et al.* (2008) that larvae can reduce waste accumulation by up to 50%.

Larvae of *H. illucens* convert organic waste into edible biomass; proteins, lipids, peptides, amino acids, chitin, vitamins, and polypides. Proteins and amino acids have been used to produce animal feed, fish meal substitutes, and highly digestible feed ingredients. Compared with typical energy-source plants, BSF has the advantages of high fertility and a short life cycle. In addition, they can convert manure and domestic waste into clean energy, utilising organic waste resources (Liu *et al.,* 2019).

The nutrition of *H. illucens* fly larvae

This study's nutritional contents were ash, ether extract, crude fibre, and crude protein, as shown in Table 2. The results showed that tempeh yeast's addition did not affect the ash content, ether extract, crude fibre, and crude protein of larvae ($p>0.05$). However, the treatment tends to increase the crude protein and decrease the crude fibre. The increase in crude protein content of larvae is thought to be due to increasing the protein value of fermented tofu pulp as larvae feed. In contrast, the highest protein and fat content of *H. illucens* larvae were 39.07 and 27.00%, respectively, these results are lower than the study of Sheppard *et al.* (1994), i.e., crude protein by 42%; and 35% ether extract. The low value due to the materials or media used are different. Food availability can affect the traits of larval and adult life history (Myers *et al*., 2008). Food quality affects insect growth rates and positively correlates with larval length and percentage survival (De Haas *et al*., 2006).

Conclusion

The findings in this study showed that the conversion and extraction of dry matter media were higher in the more media. However, tofu pulps fermentation did not increase the nutritional value of *H*. *illucens* larvae.

CONFLICT OF INTEREST

The author declares no competing interests.

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