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BIOCONVERSION OF FERMENTED BARLEY WASTE BY BLACK SOLDIER FLY *Hermetia illucens* L. (DIPTERA; STRATIOMYIDAE)

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Abstract. *Black Soldier Fly Larvae (BSFL), Hermetia illucens (Diptera: Stratiomyidae) are widely used as bioconverter agents for various organics waste, and BSF pupae are often used as fodder for poultry and fish, because the BSF pupae have a high protein content. This study focused on applying BSFL as a bioconversion agent of the fermented barley waste to convert it to larvae biomass. Prior to application, barley waste was fermented either using effective microorganisms-4 (EM4), leachate, and water for seven days. The fermented barley waste was applied as feeding material for BSFL at the rate of 100 mg/larvae/days. As control commercial chicken fed (CF) was applied as feeding material at a similar feeding rate. During this study, waste reduction index (WRI), and efficiency of digested feed (ECD) were calculated, and the protein content in the BSF prepupae was analyzed. The results of this study showed that BSFL fed with CF produces the shortest development time (27 days), and high consumption rate. BSFL fed with barley waste fermented with EM4 (BE) and Leachate (BL) produces a larval period of 31 and 30 days respectively, and statistically those were not significantly different from control. This study showed that treatments of BE and BL, produced a very high larval survival rates, 98.67% and 97.00% respectively, and those two treatments were not statistically different from the control (96.67%). Although the control treatment resulted in a higher WRI compared to the other treatments, but the ECD of BE and BL treatments were higher than the ECD of the control. From this study, it can be concluded that BSFL has a good ability to convert fermented barley waste as well as controls, and the prepupae has a high protein content (42%), so BSFL fed with fermented barley waste has the opportunity to be used as a fed for poultry and fish.*

Keywords: *barley, bioconversion, biomass, efficiency, husks*

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INTRODUCTION

Various agro-industrial waste is often used for animal feed, including palm kernel mills, cassava from tapioca factories, wine factory waste, tofu dregs, and barley waste. The waste can be directly given to ruminant animals, but some types of agro-industrial waste require a fermentation process before being used as animal feed. Barley waste is the most considerable waste generated from several agroindustries, about 85% of the total waste produced (Lima, 2010; Mathias et al., 2014). Barley waste consists of 15-26.2%

protein and 70% fiber, which is divided into three fractions: cellulose (15.5-25%), hemicellulose (especially arabinoxylans, 28-35%), lignin (approx. 28%), lipids (3.9-10%), ash (2.5-4.5%), vitamins, amino acids, and phenolic compounds (Mussato et al., 2006; Lima, 2010; Robertson et al., 2010; Aliyu et al., 2011; Mathias et al., 2014). Nowadays, barley waste is managed, on a very limited scale, for animal feed due to its high protein content of the material which produced a positive effect on cattle weight (Chrisdiyanto et al., 2018). Since a significant amount of barley waste is produced daily which is higher than the cattle requirement it is necessary to develop an alternative management strategy for this material. In the last decade, there have been many studies on the biowaste conversion process using *Hermetia illucens* L. (Diptera: Stratiomyidae), commonly known as black soldier fly (BSF). *H. illucens* larvae can convert several organic wastes into body biomass rich in protein (more than 30% crude protein) and lipid (30% crude fat). In addition, *H. illucens* are auspicious because a source of protein for animal and fish feed and become an alternative to conventional feed. Several studies (Makkar et al., 2014; Lock et al., 2015; Veldkamp et al., 2015; Gasco et al., 2016; Ji et al., 2016; Renna et al., 2017; Schiavone et al., 2017) showed that BSF larvae (BSFL) are applicable as an alternative substitute for fish meal and soybean meal in poultry, pork, and fish feed in almost all parts of the world and also provide business opportunities (Van Huis et al., 2013; Kelemu et al., 2015; Dobermann et al., 2017).

BSF is also applicable to decomposed agricultural waste containing high lignocellulose although relatively inefficient (Manurung et al., 2016; Supriyatna et al., 2017; Liu et al., 2018; Chia et al., 2018). This condition is due to (1) the lack of a digestive enzyme to necessary digest lignocellulose (Ohkuma, 2003; Amin et al., 2017) and (2) the low nitrogen content of the substrate (Meneguz et al., 2019). There were several strategies to improve the digestibility of the lignocellulose rich substrate by BSFL, such as (1) pre-treatment of the substrate through the application of microbes or chemicals which hydrolyzed the lignocellulose, (2) addition of nitrogen-rich material to the substrate, and (3) combination with lignin-consumer, such as *Tenebrio molitor* (Wang et al., 2017).

In this study, a pre-treatment process, substrate fermentation was applied to improve the digestibility of the substrate. Unlike other studies which applied specific types of microbes, that may impractical for small-scale and low-budget system application, a much common microbial consortium (named Effective Microorganism 4), leachate, and water (utilized microbes naturally occur on barley waste). Thus, the purpose of this study was to assess the effectiveness of pre-treatment of barley waste for the bioconversion process by BSFL.

MATERIALS AND METHODS

Study Site

This research was conducted in the Laboratory of Environmental Toxicology, School of Life Sciences and Technology, Institut Teknologi Bandung (SITH-ITB), for seven months, in September 2020-March 2021.

Black Soldier Fly Larvae

BSFL obtained from a population were kept in the Laboratory of Environmental Toxicology, School of Life Science and Technology, Institut Teknologi Bandung. The larvae used in this study were 6 days old larvae. All larvae were kept at room temperature (18-

30°C), relative humidity 60-90%, and 12:12 hour day: night period.

Waste Material

Barley waste was originated from the soya milk industry in Cimahi, Indonesia. Barley waste consists of barley husks (BH) and barley seeds. Before application as feeding material, barley waste and BH were fermented by 5% EM4 (PT. Songgolangit Persada), 0.5% leachate as a byproduct of tomato waste bioconversion by BSF larvae, and distilled water for 5 days. EM4 and leachate were dissolved in sterile distilled water, then barley waste and BH were added to fermentation solution, then stored in the laboratory (room temperature). The solution was filtered and stored as an ingredient for increasing the water content of the substrate. The experimental diets for this study were: husks fermented by EM4 (HE), barley spend ground fermented by EM4 (BE), husks fermented by leachate (HL), barley spend ground fermented by leachate (BL), husks fermented by water (HW), barley seeds fermented by water (BW), and chicken fed (CF) was used as a control.

Bioconversion Study

BSFL aged six days were fed by all treatment with a daily feeding rate of 100 mg/day/larvae. A hundred larvae were used for each treatment in three replications (Diener et al., 2009). Larvae were placed inside a plastic cup (5 cm x height 8 cm) and covered by gauze. Every three days, larvae were transferred to another cup with the required feeding material. The residue from the previous cup was weighed and dried in an oven at 60°C for 72 hours to determine the dry mass. The study was conducted until at least 60% of total larvae reached prepupal (Tomberlin et al., 2002; Diener et al., 2009). All prepupal were removed daily from each cup and weighed, then dried in an oven at 60°C for 72 hours to determine the dry mass.

Substrate Consumption

Substrate consumption was feeding consumption during an experiment and it was calculated in its dry base (db) weight (Diener et al., 2009) :

$$\text{Survival rate} = \frac{numbers\ of\ larvae\ survive}{numbers\ of\ initial\ larvae} \times 100\%$$

Survival Rate

Survival rate was measured to determine total larvae survival during the experiment following the equation below (Myers et al., 2008):

$$\text{Survival rate} = \frac{numbers\ of\ larvae\ survive}{numbers\ of\ initial\ larvae} \times 100\%$$

Waste Reduction

Waste reduction index (WRI) is the ability of larvae to reduce feeding substrates during a specific time. Higher values show a greater ability of larvae to reduce substrate or organic matter. The formula follows the equation below (Diener et al., 2009):

$$\text{WRI} = \frac{total\ feeding\ (db) - residue\ (db)}{total\ feeding\ (db)} \div time \times 100\%$$

The efficiency of digested feed

The efficiency of digested feed (ECD) is based on terminology Scriber & Slanssky (1981).

$$\text{ECD} = \frac{biomass\ (db)}{total\ feeding\ (db) - total\ residue\ (db)} \times 100\%$$

Biomass and Chemical Analysis of Larvae

The biomass of larvae was measured from the final weight of larvae, and it was calculated in a dry base (Diener et al., 2009). Chemical analysis of larvae with proximate analysis was performed at Pusat Pelayanan Basic Science (PPBS) of Padjajaran University.

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Data analysis

Data collection among treatments was statistically analyzed with one-way ANOVA at a significant rate of 0,05 and followed by the Tukey test for post hoc test. All analysis was done with IBM SPSS 25 software.

RESULTS AND DISCUSSION

Larval Growth

The development time of larvae fed with chicken feed was lower than other groups (27 ± 0.00 days), followed by barley waste fermented by leachate (30 ± 1.00) days and EM4 (31 ± 0.00 days). The larval period of those 3 treatments was not a statistically significant difference. On other hand, larvae fed on fermented barley required less development time than the group fed on fermented husk (47-52 days) (Table 1).

The highest survival rate (98.67±0.67%) was recorded from the group fed with barley waste fermented by EM4 (BE). Statistically, all treatments resulted in a survival rate that was not significantly different, except for the barley husk fermented with water (HW) (Table 1). Substrate consumption by BSF larvae was varied from 45.07% in BE treatment to 52.78 in the BW treatment. The value of substrate consumption between treatments was not significantly different from the results of statistical analysis (ANOVA, p=0.001).

The longer development time for BSFL fed on barley waste may be related to the high lignin and fiber of barley endosperm (the main component of barley waste) (Lynch et al., 2016). BSFL do not have lignin-degrading enzymes in their intestines so that the larvae will find it challenging to digest substrates containing lignocellulose (Kim et al., 2011; Zheng et al., 2012). Under malnourished conditions, BSFL can prolong the life cycle (Dortmans et al., 2017). This development time of BSFL fed on barley waste substrate was longer than Liu et al. (2018) (average development time = 14.97 days) and Chia et al. (2018) (development time = 16-21 days). Differences between studies may be due to variations in the quantity and quality of feed given to larvae and also this study used the old and dried barley waste while previous studies applied the fresh barley waste. Studies showed that hard material may significantly reduce the ability of BSFL to digest it (Gobbi et al., 2013; Nguyen et al., 2013).

Table 1. Development time, survival rate, and substrate consumption of Hermetia illucens (HE = husk/EM4; BE = barley/EM4; HL = husk/leachate; BL = barley/leachate; HW = husk/water; BW = barley/water; CF = chicken feed).

Parameter	HE	BE	HL	BL	HW	BW	CF
Development time (day)	52.00 ± 1.00a	31.00 ± 0.00cd	48.00 ± 2.00ab	30.00 ± 1.00cd	47.00± 0.00b	32.00 ± 0.88c	27.00 ± 0.00d
Survival rate (%)	91.33 ± 0.67ab	98.67 ± 0.67a	81.67 ± 0.88ab	97.00 ± 1.73a	68.33± 13.20b	79.33 ± 3.28ab	96.67 ± 0.33a
Substrate consumption (%)	45.67 ± 0.91bc	45.07 ± 0.58c	46.93 ± 0.65bc	50.93 ± 0.81abc	49.54± 1.30bc	52.78 ± 0.46ab	57.52 ± 0.16a

Larval Biomass and Proximate

The highest larval biomass was the barley fermented by EM4 (BE) (55.46 mg), and the lowest was husks fermented by EM4 (HE) (28.62 mg). The chicken feed substrate analysis results were not significantly different from the fermented barley substrate but were significantly different from the fermented husk substrate. The difference between husk and barley substrate showed significantly different

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results, but differences in pre-treatment (fermentation) did not show significantly different (Figure 1). The pattern proportion of feed converted into prepupal biomass, used for metabolism, and undigested or residue were different in all treatjment. The highest prepupal biomass generated by group CF and most of feed converted into residu (Figure 2). Proximate analysis was carried out on EM4 fermented barley treatment because it was the best result compared to other treatments. The proximate results obtained were crude protein (42.86%), crude fat (32.43%), and ash (3.87%) (Table 2).

H. illucens larvae's ability to convert barley waste substrates is due to the digestive enzymes in their intestines, such as amylase, protease, and lipase, which work most effectively in hydrolyzing the substrate. In addition to these enzymes, there are leucine arylamidase, α-galactosidase, β-galactosidase, and α-mannosidase, which are not present in *Musca domestica* flies but work very effectively on *H. illucens* larvae (Kim et al., 2011). In addition to *H. illucens* larvae's ability to degrade the substrate, this study used fermented substrates to make it easier for larvae to digest the substrate as they had high lignin and fiber content.

The proximate analysis of *H. illucens* fed with barley waste showed a high content of crude protein (42.86) (Table 2). This indicates that BSF larvae are very suitable for use as poultry and fish feed (Schiavone et al., 2017; Renna et al., 2017; Henry et al., 2015). Zheng et al. (2013) stated that *H. illucens* could increase the protein and fat content in organic waste that could be used for animal feed. Makkar et al. (2014) and Henry et al. (2015) revealed that BSF larvae produce body biomass with high protein and fat content, so it is very suitable to be used as animal feed. Elwert et al. (2010) reported that the weight of chicken cultivated with feed containing 4.7-6.6% BSF larvae was comparable to the weight of chicken fed with a fish meal (3%) as a source of animal protein. Moula et al. (2018) reported that the weekly weight of Ardennaise chickens fed on commercial feed substituted by 8% of BSF larvae was slightly higher than control. Cullere et al. (2016), stated that *H. illucens* larvae meal can replace conventional soya bean meal and soya bean oil in the feed for growing broiler quails, thus confirming it as a promising source of insect protein for the feed industry.

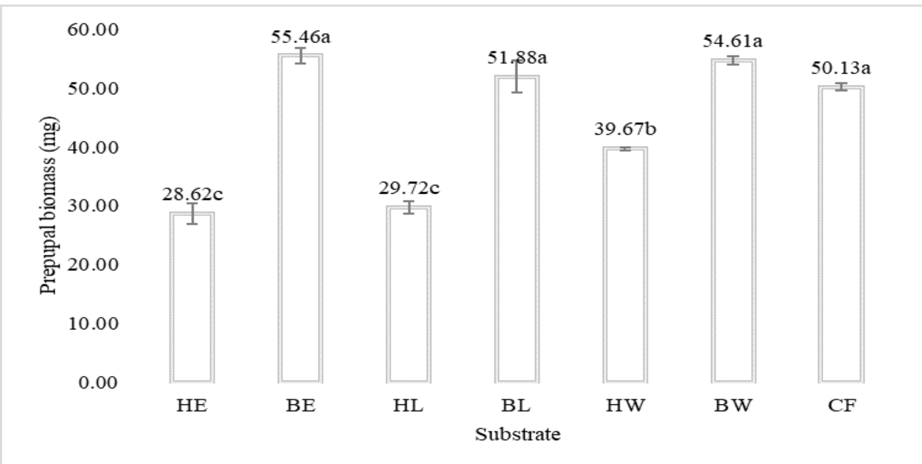

Figure 1. Prepupal weight of black soldier fly larvae fed on barley waste. (husk/EM4, HE; barley/EM4, BE; husk/leachate, HL; barley/leachate, BL; husk/water,

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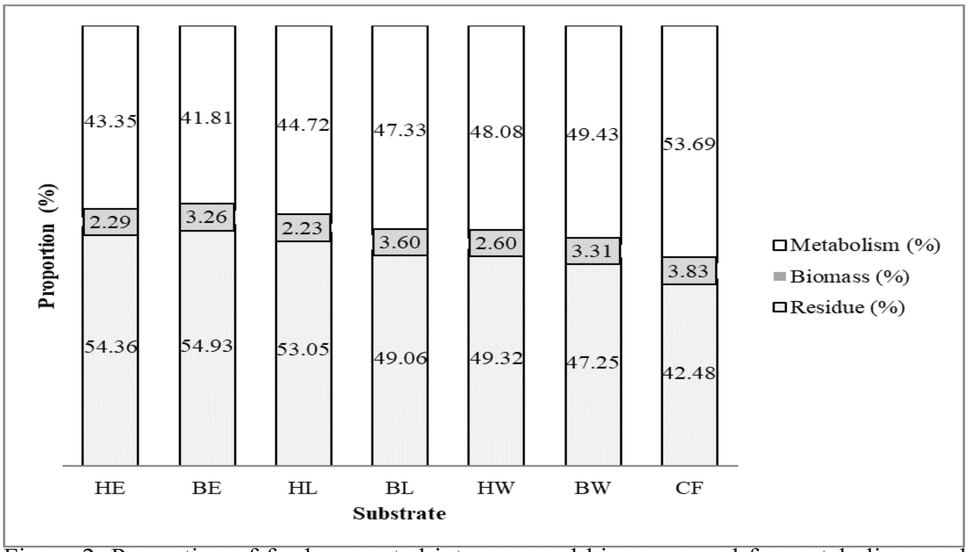

Figure 2. Proportion of feed converted into prepupal biomass, used for metabolism, and residue by black soldier fly larvae. (husk/EM4, HE; barley/EM4, BE; husk/leachate, HL; barley/leachate, BL; husk/water, HW; barley/water, BW; chicken

Table 2. Chemical composition of black soldier fly prepupae on barley fermented by EM4 (BE)

Chemical composition	Prepupae (%)
Water content	15.34
Crude protein content	42.86
Crude fat content	32.43
ash content	3.87

Waste Reduction Index (WRI) and Efficiency of Digested Feed (ECD)

The WRI value was applied to express the amount of substrate ingested by larvae in a certain period. In contrast, the ECD value represents the conversion potency of a substrate or ingested food into larval biomass (Diener et al., 2009). The highest WRI value of this study was 2.74 ± 0.01 which was recorded from the group fed with the chicken feed and the lowest WRI value was the group fed with husk fermented by EM4 (0.99 ± 0.03). The highest ECD value was recorded from the group fed with barley fermented by EM4 (7.24 ± 0.05%) and the group fed with husk fermented by leachate has the lowest (4.76 ± 0.15%) (Table 3).

Table 3. Waste reduction index (WRI) and Efficiency of Conversion of Digested Food (ECD) of black soldier fly larvae fed barley waste. (HE = husk/EM4; BE = barley/EM4; HL = husk/leachate; BL = barley/leachate; HW = husk/water; BW = barley/water; CF = chicken feed).

Parameter	HE	BE	HL	BL	HW	BW	CF
WRI	0.09 ± 0.03c	1.80 ± 0.02b	1.12 ± 0.06c	2.13 ± 0.13b	1.21 ± 0.03c	2.04 ± 0.08b	2.74 ± 0.01a
ECD (%)	5.02 ± 0.31b	7.24 ± 0.05a	4.76 ± 0.15b	7.08 ± 0.33a	5.61 ± 0.35ab	6.30 ± 0.27ab	6.66 ± 0.09ab

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The high fiber content (70%) in barley waste needs to be fermented to break down cellulose and lignin. EM4 as a product of effective microorganisms contains cellulose-degrading fungi, Lactobacillus, phosphorous solubilizing bacteria, Streptomyces, and lactic acid bacteria seem to be effective in breaking down the fiber in barley waste. Leachate from the bioconversion of tomato waste by BSF larvae also contains various microorganisms that may be able to break down the fiber in barley waste, although in more detail the types of microorganisms in leachate still need to be investigated. Barley husk is part of the barley seed called lemma and palea and is the hardened seed coat (Brenan et al., 2019). Barley husk-like rice husk which is processed into flour is often used as additional feed for chickens and ducks. The nutritional content of barley husk is much lower than barley waste and has a high starch content (Gujral et al., 2018; Grant et al., 2020). This may have an effect that causes BSF larvae to have difficulty digesting barley husk even though it has been fermented with EM4 or leachate.

This study observed the benefit of a pre-treatment process for bioconversion of barley waste into the biomass of *H. illucens*. The pre-treatment process applied in this study produced the substrate consumption rate ranging from 45.07-57.52%, indicating an incomplete fermentation process of the substrate so that *H. illucens* larvae still have difficulty digesting the substrate. However, the biomass obtained was high compared to several other studies with a similar feeding rate and substrate properties (high lignin and cellulose) (Table 4). Some organic waste such as coconut dregs which contain high lignocellulose has also been successfully used as feed for *H. illucens* larvae and as oviposition site of adult females of black soldier fly (Hasan & Dina, 2019). It seems that the high protein content of the substrate compensates for the lack of digestibility on the substrate which is indicated by the high crude protein content of the prepupae. However, interestingly the protein content in larvae is not directly related to the feed consumed (Simpson & Simpson, 1990).

Table 4. Comparative data on larval biomass of black soldier fly on various substrates

Reference	Substrate	Biomass (mg)
Manurung et al. (2016)	Rice straw	13.64
Supriyatna et al.(2017)	Fermented rice straw	13.68
Liu et al. (2018)	Brewer's waste	34.54
Liu et al. (2018)	Pig manure	13.98
This study	Barley waste	28.62 – 55.46

The protein content and amino acid profile of prepupae are not dependent on protein content and quality of the substrates they are feed (Spranghers et al., 2017; Barragan-Fonseca et al., 2018). For example, Liu et al., (2018) reported that prepupae fed with standard diet and barley waste had similar protein content, whereas the protein content of the standard diet was only 70% than barley waste. A possible explanation for this is larvae fed with a standard diet could further increase protein assimilation to maintain protein homeostasis than larvae fed with barley waste, and Simpson & Simpson (1990) stated this phenomenon as a compensatory response to nutritional deficiencies or imbalances often found in phytophage insect species. However, Nguyen et al., (2015) reported that *H. illucens* fed with high protein content such as liver or meat produced a higher protein content in the pupae (60%), and this protein content was higher than that of *H. illucens* which fed the vegetable waste (38%).

From the results of this study, it can be concluded that the larvae of *Hermetia illucens* L. have the potential to convert fermented barley waste into their body biomass, and the resulting prepupae contains high protein, which can be used as poultry feed. However, there is a need for further study to compare the fermentation treatment of barley waste using bacteria or fungi that have a specific ability to degrade lignin to ascertain that the substrate is entirely degraded and more comfortable to digest by *H. illucens* larvae.

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