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Original article

The use of probiotics in fermenting food wastes for production of black soldier fly larvae (*Hermetia illucens* L.; Diptera: Stratiomyidae)

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Abstract

Larvae of the black soldier fly (BSF) can process a wide variety of organic wastes and convert them into larval body primarily consisting of protein. Production of larvae with high-quality protein content is affected the rearing system, one of them is quality of feeding substrates. This study was aimed to evaluate the effect of food wastes fermented by probiotic culture as the feeding substrates for producing the BSF larvae. The probiotic cultures used were *Lactiplantibacillus plantarum* E2 and *Limosilactobacillus fermentum* F5, while the food wastes were derived from a seafood restaurant. Four rearing substrates were compared: food wastes fermented by *L. plantarum* E2 (P1), food wastes fermented by *L. fermentum* F5 (P2), food wastes fermented by consortium (*L. plantarum* E2 *and L. fermentum* F5) (P3), and food wastes without probiotics (P0). Before the feeding substrates were applied, the food wastes were fermented by the probiotics for 10 days. Six-day-old BSF larvae were reared for 14 days on each fermented substrate. The larval growth performance observed were fresh weight, length and width, while the nutrient content of the larvae was protein and fat content. The waste reduction index was also measured. The data showed that the best larval growth performance was shown in P3 substrate, with fresh weight of 287.27 g, larva length and width of 19.93 mm and 3.53 mm, respectively. In P3 substrate also could produce the larvae with the best nutrient content, with protein and fat content by 12.53% and 16.09%, respectively. The waste reduction index value of P3 substrate was 6.12%. The duration of BSF larvae rearing required to be extended to obtain an increased average protein content. The findings of this study could be helpful for improving the quality of BSF larvae by applying a consortium of probiotic cultures in fermenting the feeding substrates.

Keywords: *Lactiplantibacillus plantarum*, *Limosilactobacillus fermentum,* protein content, nutrient content.

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Introduction

The larvae of black soldier fly (BSF), *Hermetia illucens* (Linnaeus, 1758) (Diptera: Stratiomyidae), currently, has an important role in developing the waste management system, especially organic wastes. Besides they can convert a range of organic wastes from vegetable waste, food waste, animal carcasses and manure into compost (Cintaningtya, et al. 2020), the larvae of BSF also contain a high nutritional value particularly the protein content about 30-45% which then can be used as part of animal feed (Indarmawan, 2014). The use of insects as a source of protein has been widely studied. Protein sourced from insects is more economical, and environmentally friendly (van Huis, 2013).

The growth of BSF larvae can be optimized by modifying the nutrient composition and condition of the substrate. The combination of biofloc (aquaculture sludge) and fermented wheat bran at ratio 6:4 was the ideal feeding substrate for BSF larvae by resulting crude protein and crude lipid content were 53.58% and 3.96%, respectively (Zhang et al., 2023). Another study showed that the best performance of BSF larvae was cultivated in a mixed of leachate and wheat bran and brewers' spent grain. This substrate could increase the fat dan protein content of the larvae significantly (Grossule et al., 2020). Furthermore, restaurant leftover had an increased crude protein weight and dry matter yield of the BSF larvae as well as substrate reduction index compared to those in the other substrates (wheat bran, millet waste, and fruit waste) (Opoku et al., 2023). This implies that the larvae reared in a better nutrient composition was able to convert a greater proportion of energy in the substrate into insect biomass.

Microorganisms in the gastrointestinal tracts (GIT) of animals contributes to the growth performance and health significantly, which also applies to BSF (de Smet et al., 2018). Substrate composition including the native microbes with probiotic properties influences on the BSF larval microbiome. Probiotics contributed to the BSF larvae growth especially in improving the efficiency of waste conversion and nutritional value (Jordan & Tomberlin, 2021). The use of probiotics during rearing of the BSF larvae is one of main tool that can be used for altering the gut microbiome (microbiome engineering). Some studies that used probiotics in supporting the BSF larvae growth have completely reviewed by Gorrens et al. (2023). *Bacillus valezensis* EEAM 10B incorporated in the BSF larval feeding substrate successfully improved the larval biomass weight, bioconversion rate, and protein content by 0.95 g, 27.22% and 39.3%, respectively. Also, this probiotic could modulate the microbiome community in the larval gut (Pei et al., 2022). In this current study, probiotics were applied as

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starter cultures in fermenting the food wastes for the BSF larvae substrates. *Lactiplantibacillus plantarum* E1 and *Limosilactobacillus fermentum* F5 were probiotics derived from wine coffee fermentation and yogurt, respectively (Wikantyasti, 2021; Mustamin, 2022). These probiotics are expected can help to accelerate the fermentation process so that it can form a good substrate for the BSF larvae growth, as well as these probiotics can colonize in the GIT of the larvae. The purpose of this study was to evaluate the effect of food wastes fermented by probiotic culture as the feeding substrates for producing the BSF larvae.

Methods

Probiotic culture preparation

Lactiplantibacillus plantarum E2 and *Limosilactobacillus fermentum* F5 which have been characterized as probiotic candidates were a culture collection of Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya. Each probiotic isolate was grown in 10 mL de Man, Rogosa and Sharpe (MRS) broth and incubated at 37°C for 24 hours. After that, the probiotic inoculum was centrifuged at 5,000 rpm for 10 minutes at room temperature. The pellet was then resuspended in MRS broth and incubated at 37°C for 24 hours until the cell number of 10^6 cell/mL was achieved.

Food waste fermentation

The food wastes were obtained from seafood restaurant which was mainly consisted of fish wastes (restaurant leftover). The wastes were cut into several small pieces and mashed. The fermentation of food waste was carried out by adding the probiotic cultures (100 mL) into 500 g of food waste (Daten & Ardyati, 2018; Awasti et al., 2020), which was divided into four treatments: food wastes fermented by *L. plantarum* E2 (P1), food wastes fermented by *L. fermentum* F5 (P2), food wastes fermented by consortium (*L. plantarum* E2 *and L. fermentum* F5) (P3), and food wastes without probiotics (P0). The volume ratio of probiotic consortium used was 1:1 (v/v) with cell density of 10^6 cell/mL (Awasthi et al., 2020). The food waste or later called feeding substrate was stirred gently using spatula and then allowed to be fermented for 10 days in closed containers to allow anaerobic condition established at room temperature (Rostini et al*.,* 2022). The non-sterile food wastes were used in this study to maintain the nutrient content of the substrates which is sensitive to high temperature, as well as the indigenous microbes were expected to be involved in the fermentation process. The initial pH and temperature as well as the nutrient content of the substrate were not set. The room temperature observed was in range of 20-30°C with the humidity of 50-55%. This treatment was conducted in triplicates. The number of lactic acid bacteria (LAB) was counted by using Total Plate Count in MRS agar containing $CaCO_3$ 1% at the end of fermentation. The data were expressed as the mean of the level of increasing cell density (%) ± standard deviation and visualized in a graph with y-axis as the increase of cell density (%), and x-axis as feeding substrate types. The value of pH and temperature was also monitored every day (Lindgren & Pleje, 1983; Somroo et al., 2019).

BSF larvae cultivation

The BSF eggs were weighed in a ratio of 1:0.05 in which 1 kg of feeding substrate was laid 5 g of BSF eggs. The eggs were laid on a paper with a mesh screen, or a hollow box to make the hatched eggs easy to creep into the substrates. Six-day-old larvae (2.4 g) or around 1000 larvae were transferred to the fermented feeding substrate. The BSF larvae were cultivated for 14 days in each treatment of fermented feeding substrates. After the prepupa appears (around 1% in total), the larvae were ready for harvesting (Somroo et al*.,* 2019). The larval growth performance observed included fresh weight, and size (length and width), while the nutritional value was protein and fat content. To evaluate the waste conversion efficiency, the waste reduction index (WRI) was also be measured. WRI was defined as the index of waste reduction by larvae per day. The WRI was calculated based on the remaining substrates in each treatment (Aswati et al*.,* 2020) and calculated based on the equation 1 (Diener et al*.*, 2009).

$$\text{Waste reduction index (WRI)} = \frac{D}{t} \times 100$$

$$D = \frac{W-R}{W} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1)$$

D : Decreasing of total feed
t : Time required for the larvae feed on the substrate (day)
W : Initial weight of feed (g)
R : Final weight of feed (g)

Protein content analysis

The determination of total protein content using the Kjeldahl method. The sample (2 g) was mashed and put into a 30 mL Kjedahl flask and added 7.5 g K_2SO_4, 0.3 g HgO and 15 mL concentrated H_2SO_4. Then the sample destruction was carried out until a clear green color was obtained. Subsequently, the sample was cooled and put into the distilled flask. After that, the sample was added with 60 mL of distilled water and 20 mL of 50% NaOH solution and distillate was collected in Erlenmeyer which had previously been filled with 20 mL H_2SO_4 0.1 N and 3 drops of red metal indicator which was then distilled until 75 mL of distillate was collected. Then the contents of the Erlenmeyer flask were titrated with 0.1 N NaOH until a yellow soluble color was obtained and the protein content was calculated based on the N content in the sample multiplied by the conversion factor (Formula 2) (Association of Official Agricultural Chemist, 2005).

Crude protein (%) = % Kjeldahl N x F.....(2)
F : The N Protein correction factor is 5.70 for soybeans and 6.25 for fishery products

Fat content analysis

A sample of 5 g was wrapped using filter paper and placed on a Soxhlet extraction device mounted on top of

the condenser and flasks below. Hexane solvent was used, and reflux was done until the solvent drops into the fat flask. The solvent in the fat flask was distilled and collected. The fat flask containing extracted fat was then dried using an oven at 105°C for ± 5 hours. Then the fat flask was cooled in a desiccator for 20 - 30 mins and then weighed. The percentage of fat content can be calculated through calculations based on Association of Official Agricultural Chemist (2005) in the formula 3.

$$\text{Fat content (\%)} = \frac{\text{Final weight (g)} - \text{initial weightl (g)}}{\text{sample weight (g)}} \; x\; 100\% \quad \text{.....3)}$$

Data analysis

All experiments were conducted in triplicates, and the data expressed in mean ± standard deviation (larval fresh weight, WRI, protein and fat content) were analyzed using one-way ANOVA with SPSS 16.0 software with significance level of $p \leq 0.05$. Further test was conducted if there was a significant different using Tukey HSD.

Results

Fermentation of food wastes

Fermented food waste was used as a feeding substrate for BSF larvae. During the fermentation process, the density of LAB was increased. The feeding substrate fermented by consortium probiotics showed the highest increase of LAB density by 231%, while substrate without probiotics (P0) had the lowest LAB density (Figure 1). The probiotic cultures added in the food wastes was able to use the carbon sources to support their growth. Each probiotic culture in a consortium form was not inhibited each other (data not shown).

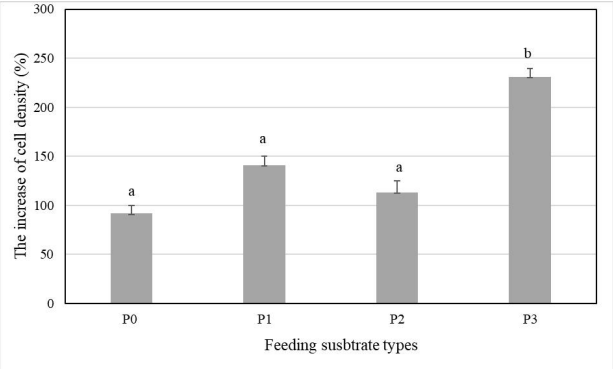

Figure 1. The increase of cell density of lactic acid bacteria duri fermentation of feeding substrate. P0: Substrate fermented without probioti P1: Substrate fermented by *L. plantarum* E2, P2: Substrate fermented by *fermentum* F5, P3: Substrate fermented by consortium (*L. plantarum* E2 an *fermentum* F5)

A constant increase of temperature in each feeding substrate also occurred during fermentation (Figure 2). The increasing of LAB density correlated with the increase the substrate temperature. In agreement with LAB density, feeding substrate fermented by probiotic consortium (P3) demonstrated the highest temperature, while the substrate without probiotics (P0) was the lowest one. The pH value of the feeding substrate during 10 days of fermentation decreased constantly in all treatment (Figure 3). The highest pH reduction was observed in P3 treatment followed by treatment of P2, P1 and P0. This pH reduction was correlated with an increase in LAB cell density.

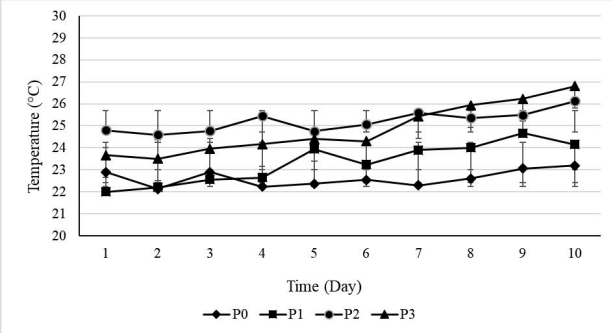

Figure 2. The temperature of feeding substrate during fermentation for 10 days. P0: Substrate fermented without probiotics, P1: Substrate fermented by L. plantarum E2, P2: Substrate fermented by L. fermentum F5, P3: Substrate fermented by consortium (L. plantarum E2 and L. fermentum F5)

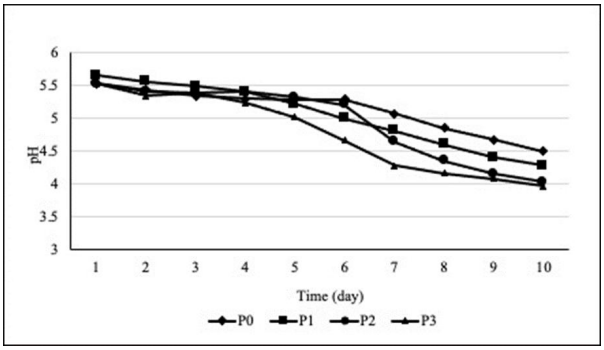

Figure 3. The pH of feeding substrate during fermentation. P0: Substrate fermented without probiotics, P1: Substrate fermented by L. plantarum E2, P2: Substrate fermented by L. fermentum F5, P3: Substrate fermented by consortium (L. plantarum E2 + L. fermentum F5)

The BSF larvae growth performance

The effect of probiotic cultures in the BSF larvae growing media on growth performance can be determined by observing larval weight, length and width at the end of cultivation. The addition of probiotic cultures as fermentative agent in the food wastes (P1, P2, and P3) affected the weight of BSF larvae compared to control treatment (P0) (Figure 4). The weight of BSF larvae in P3 treatment significantly increased ($p<0.05$) by 287.27 g compared to the control treatment and it was the highest weight value among all the treatments. However, the BSF larvae weight in the P1 and P2 treatment did not show significant improvement compared to the control treatment ($p>0.05$).

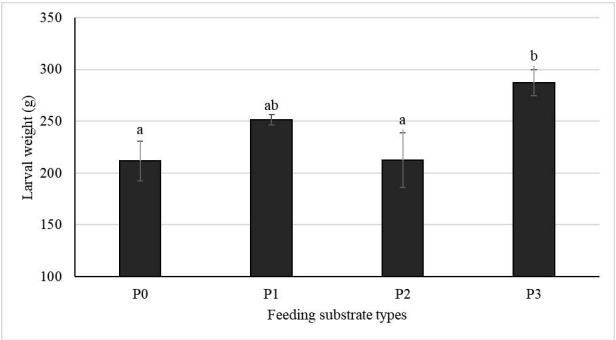

Figure 4. The weight of BSF larvae cultivated in different feeding substrate types. P0: Substrate fermented without probiotics, P1: Substrate fermented by *L. plantarum* E2, P2: Substrate fermented by *L. fermentum* F5, P3: Substrate fermented by consortium (*L. plantarum* E2 and *L. fermentum* F5). Different letters indicate statistically significant differences (P≤0.05).

The use of probiotic cultures to ferment the food wastes did not affect the larval size (length and width) (Figure 5). However, the longest larvae were observed in P3 treatment by 19.93 mm. Likewise, the width of larvae was also relatively similar among treatments (p>0.05).

The waste reduction index (WRI)

The food wastes were successfully converted into biomass of BSF larvae shown by the reduction of waste weight. The highest WRI was observed in the P3 treatment by 6.12%, followed by P2 treatment by 2.23%, P1 treatment by 2.17%, and P0 treatment by 2.15% (Table 1).

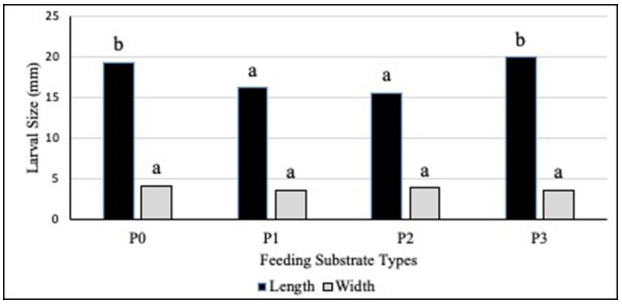

Figure 5. The size (length and width) of BSF larvae cultivated in different feeding substrate types. P0: Substrate fermented without probiotics, P1: Substrate fermented by L. plantarum E2, P2: Substrate fermented by L. fermentum F5, P3: Substrate fermented by consortium (L. plantarum E2 and L. fermentum F5). Different letters indicate statistically significant differences (P≤0.05)

Table 1. Waste reduction index during BSF larvae cultivation for 14 days

Treatment	Initial Weight (g)	Final Weight (g)	Reduction (g)	Decreasing of total feed (g)	WRI (%)
P0	500	349.45	151	0.30	1,50a
P1	500	348.09	152	0.30	2.17a
P2	500	343.63	156	0.31	2.23a
P3	500	71.26	429	0.86	6.12b

Nutritional content of BSF larvae

The use of probiotic cultures in the feeding substrates of BSF larvae did not significantly (p>0.05) affect the protein content of the larvae (Figure 6). However, the highest protein content was found in P3 treatment by 12.53 %, followed by treatment of P2, P0, and P1.

Likewise in protein content, the fat content was also showed a similar trend which was no significant difference among treatments (p>0.05). The highest fat content was found in P3 treatment, which was 16.09%, while the lowest fat content was found in P2 treatment by 13.15% (Figure 6).

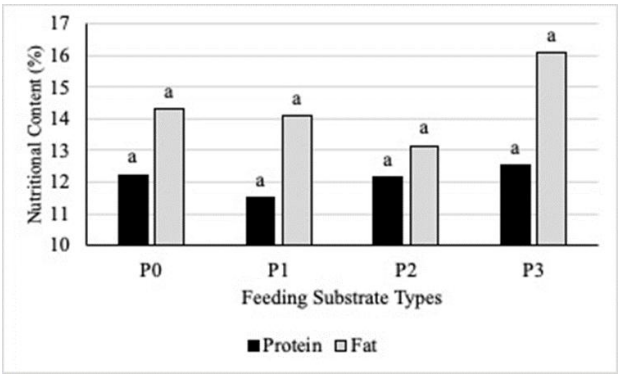

Figure 6. The protein and fat content of BSF larvae cultivated in different feeding substrate types. P0: Substrate fermented without probiotics, P1: Substrate fermented by L. plantarum E2, P2: Substrate fermented by L. fermentum F5, P3: Substrate fermented by consortium (L. plantarum E2 + L. fermentum F5). Similar letters indicate statistically no significant differences (P>0.05)

Discussion

The growth of BSF larvae was positively affected by the presence of probiotic cultures in the feeding substrates. Proteolytic activity and acidification ability was considered as important criteria for starter culture criteria. Proteolytic activity of lactic acid bacteria plays a role in breaking down complex proteins in the substrate into essential amino acids required by the BSF larvae (Luparelli et al., 2022). Probiotics also produced organic acids and other antimicrobial substances that could control the growth of specific pathogens (Suardana et al., 2007). The increase in the number of probiotic cells after 10 days of fermentation indicated that the probiotic was beneficial in triggering the larval growth, especially if the probiotics was used in consortium form. A synergistic effect was expected to be occurred in the probiotic consortium. One isolate can protect the other isolate that sensitive to a certain substance through by reducing the concentration of inhibiting substance through producing specific and non-specific protective factors (Yanti & Dali, 2013). Since LAB produces lactic acid as the primary metabolite product, the higher number of LAB in the consortium treatment, the more lactic acid will be produced which in turn could increase the digestibility of BSF larvae towards the substrate. The lactic acid will help the BSF larvae to digest and absorb the nutrients containing in the growing substrates. Furthermore, the probiotics contained in fermented substrates also plays a role in maintaining intestinal health of BSF larvae (Setyawan et al., 2014).

The temperature in the feeding substrate can affect the BSF larvae growth (Tomberlin *et l*., 2012). If the temperature on the substrate is more than 27°C, the growth of larvae will decrease. Conversely, if the temperature reaches 36°C or above then larvae cannot survive (Tomberlin et al., 2002). The increase in temperature in the BSF growth substrate might be caused by the high content of crude fiber in the food wastes that will be hydrolyzed by bacteria as the energy sources. This process causes heat generation that will increase the temperature of the substrate. The temperature was also increased in the control treatment (P0). This shows that

the bacteria in food waste play a role in the fermentation process because the substrate is not sterile. The higher the number of cells, the accumulation of organic acid content produced will also increase, thereby reducing the pH value of the substrate (Isroi, 2008). This high acid content can be beneficial because the growth of pathogens can be inhibited (Suardana et al., 2007).

The use of LAB probiotics to ferment the BSF larvae growth substrate can trigger the fermentation rate, which can lead to an increase in nutrient content that can support the increase in BSF larvae weight. The availability of nutrients rich in protein and carbohydrates will result in good growth for maggots (Sabdo et al., 2018; Mokolensang et al., 2018). Some factors that can affect the weight of BSF larvae were the condition of the substrate/growth medium, the condition of cultivation environment, and the nutrient content of the feeding substrate (Agustinus & Minggawati, 2019). Substrates with high moisture content can inhibit larval growth (Sabdo et al., 2018). The ideal water content for BSF larvae growth is 60% (Sabdo *et el*., 2018). In general, the weight of BSF larvae is in a range of 220 mg (Somroo et al., 2019). The morphology of BSF larvae is elliptical in shape with a yellowish to blackish color on the head where basically length of BSF larvae can reach up to 2 cm at the age of 10 days after hatching with a maximum length of 2.5 cm (Fahmi, 2015). In this study, the length of the larvae ranges from 15.53 – 20.03 mm (Figure 4), which in a range of previous study (14 - 24 mm) (Rumondang et al., 2019).

The use of LAB probiotics to ferment the growth substrate for BSF larvae can trigger the rate of fermentation so that it can cause an increase in nutritional content which can support an increase in the weight of BSF larvae. The availability of nutrients rich in protein and carbohydrates will provide good growth for maggots (Sabdo et al., 2018; Mokolensang et al., 2018). The addition of probiotics to the fermentation substrate/growth medium for BSF larvae was able to help the digestion of BSF larvae so that they absorb nutrients more quickly in the substrate/growth medium for BSF as indicated by the weight ranking of the maggots, this is in accordance with the research results of Wang & Shelomi (2017). The probiotics found in fermented products also play a role in maintaining intestinal health in BSF larvae. Apart from that, the role of the amylase enzyme in hydrolyzing starch into glucose and an energy source for bacterial growth also greatly supports LAB growth (Suciati et al., 2016). The number of LAB which increases greatly in the consortium treatment will increase the production of lactic acid which helps the digestibility of the substrate/growth medium by BSF larvae.

The WRI value indicated the rate of larval consumption (Nugraha, 2019). The higher the substrate consumption value, the higher the WRI value. Instead, the more remaining substrate, the WRI value will also decrease. This study showed that the WRI was reduced when fermented with single bacteria, and the consumption rate increased twofold when the substrate was fermented using consortium bacteria. This can be caused by fermented wastes with two different bacteria increased the efficiency of the larvae in eating substrates. In other words, the results of fermentation with the application of probiotic consortium also affected the rate of larval consumption.

Based on the National Research Council (NRC) in Giri et al. (2007), the protein content in the feed needed in fish farming ranges from 25-55%. Therefore, protein value obtained in this study was not met the requirement for fish meal. The low of protein content in this study can be caused by the lack of duration of BSF larvae cultivation. Harvesting of BSF larvae can be conducted until day 18 (Amran et al., 2021). Some efforts that can be made to increase the protein content of BSF larvae include increasing the cultivation time and enrich the food waste with additional ingredients that contain high protein. The use of rice bran, vegetable waste, and coconut pulp as additional ingredients can increase the protein content of the substrate by 20-26% (Sunarto et al., 2001). Vegetable waste can also be used to increase the protein content of the substrate by 12.64% (Muktiani et al., 2007). According to the NRC (National Research Council, 1993 in (Giri et al., 2007) the recommended fat content in fish meal is 4-18%. The fat content in the BSF larvae was affected by water content in larval body (Utami, 2013). The higher the water content contained, the lower the fat content (Kantun et al., 2012). Therefore, the fat content in the BSF larvae was still within the standard value for fish feeds.

Conclusion

The BSF larvae cultivated in the feeding substrate fermented by probiotic consortium (*L. plantarum* E2 and *L. fermentum* F5) produced the larvae biomass with the best performance especially weight (287.27 g), length of 19.93 mm, width of 3.53 mm, and waste reduction index value of 6.12. Likewise, the average value of protein and fat content of the substrate fermented by probiotic consortium showed the highest value by 12.53% and 16.09%, respectively. Some efforts are required to be elucidated to increase the protein content of the BSF larvae.

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References

Agustinus F., Minggawati I., 2019. Pengaruh Pemanfaatan Batang Pisang (*Musa paradisiaca)* dengan Komposisi yang Berbeda untuk Menumbuhkan Maggot *(Hermetia illucens). Jurnal Ilmu Hewani Tropika (Journal of Tropical Animal Science*), 8(1):9–12.

Amran M., Nuraini N., Mirzah M, 2021. Pengaruh Media Biakan Fermentasi dengan Mikroba yang Berbeda Terhadap Produksi Maggot Black Soldier Fly (*Hermetia illucens). Jurnal Peternakan*, 18:41. doi: 10.24014/jupet.v18i1.11253.

Awasthi M.K., Liu T., 2020. Manure Pretreatments with Black Soldier Fly *Hermetia illucens* L. (Diptera: Stratiomyidae) A Study to Reduce Pathogen content. *Science of The Total*

Environment, 737:139842. doi: 10.1016/j.scitotenv.2020.139842.

Association of Official Agricultural Chemist, 2005. Methods of Analysis. Washington DC.

Nugraha F.A, 2019. Analisis Laju Penguraian dan Hasil Kompos pada Pengolahan Sampah Sayur dengan Larva Black Soldier Fly (*Hermetia illucens)*. Universitas Islam Indonesia. Yogyakarta. Tesis.

Cintaningtya E., Utami B., Nurmilawati M., 2020. Efisiensi Degradasi Sampah Organik oleh Larva Black Soldier Fly. *Jurnal Biologi dan Pembelajarannya (JB&P)*, 7(2): 47–50. doi: 10.29407/jbp.v7i2.15070.

Daten H., Tri Ardyati, 2018. Potensi Penambahan Probiotik (*Lactobacillus pentosus* K50) untuk Meningkatkan Kualitas Pakan Ikan Air Tawar. *Biotropika - Journal of Tropical Biology*, 6(2):63–67. doi: 10.21776/ub.biotropika.2018.006.02.04.

Darwisito S., Junior M.Z., Sjafei D.S., 2008. Feeding Broodstock on A Diet Containing Vitamin E and Fish Oil Improve Eggs and Larval Quality of Nile Tilapia (*Oreochromis niloticus*). *J. Akuakultur Indonesia*, 7(1):1. doi: 10.19027/jai.7.1-10.

De Smet J., Wynants E., Cos P., Van Campenhout L., 2018. Microbial Community Dynamics during Rearing of Black Soldier Fly Larvae (*Hermetia illucens*) and Impact on Exploitation Potential. *Applied and Environmental Microbiology,* 84(9):e02722-17.

Diener S., Zurbrügg C, Tockner K., 2009. Conversion of Organic Material by Black Soldier Fly Larvae: Establishing Optimal Feeding Rates. *Waste Management & Research*. 27(6): 603–610. doi: 10.1177/0734242X09103838.

Fahmi M. R., 2015. Optimalisasi Proses Biokonversi dengan Menggunakan Mini-Larva *Hermetia illucens* untuk Memenuhi Kebutuhan Pakan Ikan. doi: 10.13057/psnmbi/m010124

Giri N.A., Suwirya K., Pithasari AI, Marzuqi M., 2007. Pengaruh Kandungan Protein Pakan terhadap Pertumbuhan dan Efisiensi Pakan Benih Ikan Kakap Merah (*Lutjanus argentimaculatus*). *Jurnal Perikanan Universitas Gadjah Mada,* 9(1)*:55–61. doi: 10.22146/jfs.64.*

Gorrens, E., Lecocq, A., De Smet, J., 2023. The Use of Probiotics during Rearing of *Hermetia illucens*: Potential, Caveats, and Knowledge Gaps. *Microorganisms*, 11(2), p.245. https://doi.org/10.3390/ microorganisms11020245.

Grossule V, Vanin S, Lavagnolo M.C. Potential Treatment of Leachate by *Hermetia Illucens* (Diptera, Stratyomyidae) Larvae: Performance Under Different Feeding Conditions. 2020. *Waste Management & Research*. 38(5):537-545. doi:10.1177/0734242X19894625.

Grossule V, Vanin S, Lavagnolo M.C. Potential Treatment of Leachate by Hermetia illucens (Diptera, Stratyomyidae) Larvae: Performance under Different Feeding Conditions. *Waste Management & Research*, 2020;38(5):537-545. doi:10.1177/0734242X19894625

Indarmawan, 2014. Hewan Avertebrata sebagai Pakan Ikan Lele. Jurusan Biologi Fakultas Matematika Dan Ilmu Pengetahuan Alam Universitas Jenderal Soedirman. Purwokerto. Skripsi.

Isroi, 2008. Kompos. Balai Penelitian Bioteknologi Perkebunan Indonesia. Bogor

Jordan H.R., Tomberlin J.K., 2021. Microbial Influence on Reproduction, Conversion, and Growth of Mass Produced Insects. *Current Opinion on Insect Science*, 48: 57-63.

Kantun W, Adam Malik A., Harianti, 2012. Kelayakan Limbah Padat Tuna Loin Madidihang (*Thunnus Albacares*) Untuk Bahan Baku Produk Diversifikasi. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 18(3):303-314. doi: DOI: 10.17844/jphpi.2015.18.3.303

Lalander C., Diener S., Magri M.E., 2013. Faecal Sludge Management with the Larvae of the Black Soldier Fly (*Hermetia Illucens*) from A Hygiene Aspect. T*he Science of the Total Environment,* 458–460, 312–318. doi: 10.1016/j.scitotenv.2013.04.033.

Lalander, C., J. Senecal, M. Gros Calvo, L. Ahrens, S. Josefsson, K. Wiberg & B. Vinnerås. 2016. Fate of pharmaceuticals and pesticides in fly larvae composting. *Science of The Total Environment*. 565:279–286.

Lindgren S., Pleje M., 1983. Silage Fermentation of Fish or Fish Waste Products with Lactic Acid Bacteria. *Journal of the Science of Food and Agriculture*, 34(10):1057–1067. doi: 10.1002/jsfa.2740341005.

Li Q., Zheng L., Qiu N., 2011. Bioconversion of Dairy Manure by Black Soldier Fly (Diptera: Stratiomyidae) for Biodiesel and Sugar Production. *Waste Management (New York, NY)*, 31(6):1316–1320. doi: 10.1016/j.wasman.2011.01.005.

Luparelli A.V., Saadoun J.H., Lolli V., Lazzi C., Sforza S., Caligiani A., 2022. Dynamic Changes in Molecular Composition of Black Soldier Fly Prepupae and Derived Biomasses with Microbial Fermentation. *Food Chemistry: X*, *14*, p.100327.

Mustamin A., 2022. Isolasi dan Karakterisasi Bakteri Asam Laktat Asal Fermentasi Kopi (*Wine Coffee*) yang Berpotensi sebagai Kandidat Probiotik. Departemen Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Brawijaya. Malang. Skripsi.

Opoku O., Hamidu J.A., Adjei-Mensah B., Quaye B., Sasu P., Benante V., Amankrah M.A., Donkoh A. Atuahene C.C., 2023. Alternative organic wastes as substrates and its impact on growth, proximate chemical and mineral composition of black soldier fly (*Hermetia illucens*) larvae for subsistent animal feed. *Journal of Applied Animal Research*: 51:(1): 564-572. doi: 10.1080/09712119.2023.2249545.

Pei Y., Zhao S., Chen X., Zhang J., Ni H., Sun M., Lin H., Liu X., Chen H., Yang S., 2022. *Bacillus velezensis* EEAM 10B Strengthens Nutrient Metabolic Process in Black Soldier Fly Larvae (*Hermetia illucens*) via Changing Gut Microbiome and Metabolic Pathways. *Frontiers in Nutrition*. 9:880488.

Rachmawati D., Samidjan I., 2013. Efektivitas Substitusi Tepung Ikan dengan Tepung Maggot Dalam Pakan Buatan terhadap Pertumbuhan dan Kelulushidupan Ikan Patin (The Effect Of Substitution of Fish Meal With Maggot Meal in Artificial Feed for Growth And Survival Rate of Catfish). *Saintek Perikanan : Indonesian Journal of Fishries Science and Technology*, 9(1):62–67. doi: 10.14710/ijfst.9.1.62-67.

Rety, M. 2016. Pengaruh variasi konsentrasi pelet sayur kubis dan sawi sebagai sumber pakan terhadap pertumbuhan ikan nila (*Oreochromis niloticus*) pada kolam semen di desa Beran, Bantul. Sanata Dharma Univesity. Tesis

Rostini T., Jaelani A., Ali M., 2022. Pengaruh Lama Fermentasi terhadap Karakteristik, Kandungan Protein dan Serat Kasar Tongkol Jagung. *Ziraa'ah Majalah Ilmiah Pertanian*, 47(2):257. doi: 10.31602/zmip.v47i2.7302.

Rumondang R., Batubara J.P., Sriwahyuni E., 2019. Pengaruh Media yang Berbeda Pengaruh Media Yang Berbeda Terhadap Pertumbuhan Lalat Black Soldier Fly (*Hermetia illucens*). *Prosiding SEMDI-UNAYA (Seminar Nasional Multi Disiplin Ilmu UNAYA)*, 3 (1):163–171.

Sabdo A. Y., Priscilia D. M., 2018. Penggunaan Larva Black Soldier Fly (Bsf) Dalam Pengolahan Limbah Organik. Seameo Biotrop, Bogor.

Setyawan A.A., Sukanto S., Widyastuti E., 2014. Populasi Bakteri Asam Laktat pada Budidaya Ikan Nila yang Diberi Pakan Fermentasi Limbah Pertanian dengan Suplemen Enceng Gondok dan Probiotik. *Scripta Biologica*, 1(1):87–91. doi: 10.20884/1.sb.2014.1.1.32.

Somroo A. A., Ur Rehman K., Zheng L., 2019. Influence of *Lactobacillus Buchneri* on Soybean Curd Residue Co-Conversion by Black Soldier Fly Larvae (*Hermetia illucens*) for Food and Feedstock Production. *Waste* Management, 86:114–122. doi: 10.1016/j.wasman.2019.01.022.

Suardana I.W., Suarsana I. N., Sujaya, K. Wiryawan K. G., 2007. Isolasi dan Identifikasi Bakteri Asam Laktat Dari Cairan Rumen Sapi Bali Sebagai Kandidat Biopreservatif Isolation and Identification of Acid Lactic Bacteria From Bali Cattle's Gastric Fluid As A Potential Candidate of Biopreservative. *J. Vet*, 8.

Suciati, P., W. Thahjaningsih, E. D. Masithah & H. Pranomo. 2016. Aktivitas Enzimatis Isolat Bakteri Asam Laktat dari Saluran Pencernaan Kepiting Bakau (*Scylla* spp.) sebagai Kandidat Probiotik. *Jurnal Ilmiah Perikanan dan Kelautan,* (8):2.

Suciati R., 2017. Efektifitas Media Pertumbuhan Maggots *Hermetia illucens* (Lalat Tentara Hitam) sebagai Solusi Pemanfaatan Sampah Organik. *Biosfer*: *Jurnal Biologi dan Pendidikan Biologi*, 2(1):8–13. doi: 10.23969/biosfer.v2i1.356.

Tomberlin J. K., Sheppard D. C., Joyce J. A., 2002. Selected Life-History Traits of Black Soldier Flies (Diptera: Stratiomyidae) Reared on Three Artificial Diets. *Annals of the Entomological Society of America*, 95(3):379–386. doi: 10.1603/0013-8746(2002)095[0379:SLHTOB]2.0.CO;2.

Utami, D.A.T. 2013. Variasi kombinasi tepung labu kuning (*Cucurbita moschata d.*) dan tepung azolla (*Azolla pinnata r.br.*) pada kecerahan warna ikan koi (*Cyprinus carpio l.*). *J. Ilm. Biol.* 1.

van Huis A., 2013. Potential of Insects As Food and Feed in Assuring Food Security. *Annual Review of Entomology*, 58(1):563–583. doi: 10.1146/annurev-ento-120811-153704.

Wang, Y. S. & M. Shelomi. 2017. Review of Black Soldier Fly (*Hermetia illucens*) as animal feed and human food. *Foods*. 6:91.

Wikantyasti N. U. D., 2021. Isolasi dan Identifikasi Bakteri Asam Laktat Dari Produk Yogurt Lokal yang Berpotensi sebagai Probiotik. Departemen Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Brawijaya. Malang. Skripsi.

Yanti I. W. D., Abdurrahim F. D., 2013. Characteristics of Lactic Acid Bacteria Isolated during Bakasang Fermentation Process. *Jurnal Pengelolaan Hasil Perikanan Indonesia*, 16(2): 133-141.

Zhang H., Sun T., Tan H., Lv X., Liu W., Luo G., 2023. Using Black Soldier Fly Larvae (*Hermetia Illucens*) Converted the Bioflocs Produced with Shrimp Solid Waste. *Aquaculture*. 568(2023): 739329.