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Growth and Development Performance of *Hermetia illucens* L. (Diptera: Stratiomyidae) Larvae on Fermented Palm Kernel Meal (PKM) Substrate

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ABSTRACT

Larvae of *Hermetia illucens* (Black Soldier Fly/BSF) are considered agents of bioconversion of organic waste, including by-products of agroindustrial waste. Palm kernel meal (PKM), a palm oil mill waste contains high lignocellulose, making it difficult for BSF larvae to digest in which pre treatment process is required. This study aims to analyze the growth and development performance of BSF larvae in fermented PKM waste using EM4 and molasses as the pretreatment process. Five (5) days old BSF larvae were reared in PKM waste, which was fermented with EM4 and molasses for 2, 3, and 4 days (F2, F3, and F4) and with water for four days (FA) while chicken feed (PA) applied as control. During this study, growth rate, average weight, developmental period, survival rate, and larval development period. Feed efficiency and feed reduction analyzed by efficiency of conversion of digested food (ECD), feed conversion ratio (FCR), waste reduction index (WRI) and substrate consumption rate (SCR). The growth and development performance of F2 group was the best among other treatment groups which is similar to larvae fed on chicken feed as a control. All treatments showed high larval survival rates (99.72-100.00%). On the other hand, the best best reduction efficiency recorded in F3. Based on these result it can be concluded that pretreatment of PKM by EM4 and molasses is applicable to be improve the quality of PKM as feeding material for BSF larvae.

1. Introduction

Organic waste management by macrophage has been emerging as a promising method to solve waste problem in municipal and industry. One of the most promising macrophage is black soldier fly (BSF) (*Hermetia illucens* L.). This insect originated from South American savanna (Liu *et al.* 2019) which undergoes a complete metamorphosis process (holometabola) which consists of the life stages of egg–larvae–prepupae–pupae–adult (Dzepe *et al.* 2020). This insect spend most of their adult phase to mating which limit their distribution to organic wastes pile while lacking interaction to fresh food. This behavior made them not considered as vector disease to human and animals (Banks *et al.* 2014; Oliveira *et al.* 2016; Liu *et al.* 2017). On the other hand, the larval phase of BSF insects is known as saprophagous (Diener *et al.* 2011; Liu *et al.* 2019; Scala *et al.* 2020). Black soldier fly larvae has capability to consume various types of organic wastes such as household organic waste, kitchen waste, and even agriculture and industry to animal waste and humans (Bondari and Sheppard 1987; Sheppard *et al.* 1994; Newton *et al.* 2005; Diener *et al.* 2011; Li *et al.* 2011; Zheng *et al.* 2012a, 2012b, Lalander *et al.* 2015; Nguyen *et al.* 2015; Manurung *et al.* 2016; Abduh *et al.* 2017a, 2017b; Jucker *et al.* 2017; Spranghers *et al.* 2017; Kinasih *et al.* 2018; Bava *et al.* 2019; Julita *et al.* 2019; Liu *et al.* 2019; Elsayed *et al.* 2020).

The ability of BSF larvae to consume various types of organic waste is due to the diverse and unique microbial communities inhibits the gut of larvae (Lee *et al.* 2014, 2016; De Smet *et al.* 2018). The final products of consumption are biomass that rich in protein and fat (Liu *et al.* 2019) which applicable as aniumal fed (St-Hilaire *et al.* 2007) and the residue which can also be used as good-quality compost (Liu *et al.* 2019).

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Indonesia is known as one of the largest palm oil-producing countries in the world, according to BPS (2020), the production of palm oil and palm kernel in Indonesia can reach 44,759,147 and 8,951,829 tons per year. Based on palm kernel production, the by-product of 45% palm kernel meal (PKM) can become waste (Pasaribu 2018). Oil and palm kernel production waste in PKM is still used as an alternative feed for livestock because the nutritional content is still good. PKM contains protein (14-17%), lipid (9.1-10.5%) and fiber (12-18%), but there is also a lignin content of 13.6% (Sekoni *et al.* 2008; BPTP 2015). The protein and lipid content of PKM are important for the growth and development of H. illucens larvae (Julita *et al.* 2018; Gao *et al.* 2019; Liu *et al.* 2019). However, BSFL larvae does not have specific enzymes for digesting lignin material (Kim *et al.* 2011) and may highly depend on the microbial symbiont to convert lignocellulose into simple sugars or monosaccharides (Kim *et al.* 2011; Lee *et al.* 2014). Studies showed that by only depending on the internal symbiont, the larval developmental time is longer and size of final biomass is smaller than BSF larvae fed on feeding material low in lignin (Manurung *et al.* 2016; Supriyatna *et al.* 2016; Abduh *et al.* 2017a; Palma *et al.* 2019).

Another appoach has been developing to overcome this challenge by fermentation of feeding material. Microbe may alter the structure of feeding material made it more nutritious and easier to digest (Bianco *et al.* 2020). Studies showed the benefit of the fermentation of lignocellulose material to growth of BSF larvae (Gao *et al.* 2019; Liu *et al.* 2021; Permana *et al.* 2021, 2022). However, there is lack information on the effect of fermentation duration of lignocelulose material to growth dan development performance of BSF larvae. Furtherly, the study on the effect of fermentation of high protein feeding material to growth performance of BSF larvae also lacking. PKM is considered a material that rich in protein while also has significant lignocellulose which made it a suitable material to answer research questions. Furtherly, the result of this study may provides additional knowledge on the application of BSF larvae for agricultural wastes that rich in lignocellulose.

2. Materials and Methods

2.1. Study Site

This research was conducted in the School of Life Sciences and Technology, Institut Teknologi Bandung (SITH-ITB), Toxicity Test Laboratory from January-August 2022.

2.2. Materials

Hermetia illucens' eggs used in this study were mass-reared in the School of Life Sciences and Technology, Institut Teknologi Bandung (SITH-ITB), Toxicity Test Laboratory. Eggs were hatching on chicken feed, and then the 5-day-old *H. illucens* larvae were taken and grown on the treatment of palm kernel meal substrates. Palm kernel meal (PKM) is obtained from a palm oil industrial factory in North Sumatra while chicken feed (Hi Pro – Vite 511®) purchased from local poultry shop. EM4 and molases were obtained from local agricultural shop.

2.3. Pretreatments

Pretreatment was carried out by fermentation using livestock EM4 and molasses. The fermentation solution is made with a ratio of EM4: molasses: distilled water 1: 1: 1000 ml (according to the packaging for animal feed). The fermentation solution is mixed with the PKM substrate with a ratio of PKM substrate: fermentation solution, namely 1:2.2. Fermentation is carried out using tightly closed plastic and stored at room temperature in Toxicity Test Laboratory with fermentation duration of 2, 3 and 4 days, whereas, without fermentation EM4 and molasses are only made by mixing PKM substrate and water with the same ratio of 1:2.2 and stored in tightly closed plastic for four days. The ratio between PKM and water is essential because substrate moisture affects the growth and development of *H. illucens* larvae. The comparison of PKM and water used in this study refers to Caruso *et al.* (2014).

2.4. Treatment

BSF larvae were divided into 5 feeding treatment gropus, which was PKM fermented with EM4 and molasses for two days (F2), three days (F3), four days (F4), and PKM soaked in water for four days (FA). Chicken feed (PA) was used as a control to compare PKM substrates. Each groups had five replications and 15 larvae for each replication. The larvae provided with feeding material with rate of 100 mg/larvae/day.

2.5. Growth and Development

The growth rate was analyzed by calculating the difference between the larvae's final average weight and the larvae's initial average weight divided by the length of time the larvae reached the prepupae (dry weight) (Bava *et al.* 2019). The fresh larvae were dried in the oven at 60°C for 48 hours to obtain dry weight. Both fresh and dry larvae were weighed for six samples of larvae from each replication using the OHAUS PX224/E digital analytical balance with an accuracy of 0.0001 gram. In contrast, the fresh and dry weight of the substrate and residue after drying in an oven at 105°C for 24 hours was weighed using an AMSTECH digital balance with an accuracy of 0.01 gram. Each fresh and dry weight of larvae and residue was measured once every three days when changing feed. Larval development time was analyzed by calculating the length of time the larvae became prepupae by determining when 40% of the larvae had become prepupae (Meneguz *et al.* 2018), however, feeding continued up to 60%. The survival rate of larvae is obtained from the percentage of larvae that survive from the beginning and end of trials by calculation based on the following equation:

$$\text{SR}\ (\%) = \frac{\text{number of larvae survive}}{\text{number of initial larve}} \times 100\%$$

2.6. Digestion and A Reduction Efficiency

Digestibility of larvae on feed given was analyzed by calculating ECD (efficiency of conversion of digested food) and FCR (food conversion ratio) based on the following equation:

$$\text{ECD} = \frac{\text{prepupal biomass (B)}}{\text{total feed (I)-total residu (F)}} \times 100\%$$

Reduction efficiency was analyzed by calculating WRI (waste reduction index)and SCR (substrate consumption rate) based on the following equation:

$$\text{WRI} = \frac{\text{feed degradation (D)}}{\text{feed duration (t)}} \times 100\%$$

$$\text{D} = \frac{\text{total feed (W)}}{\text{total feed (W)-residue (R)}} \times 100\%$$

$$\text{SCR} = \frac{\text{initial feed (W1)-residue (W2)}}{\text{initial feed (W1)}} \times 100\%$$

2.7. Mass Balance

Determination of the estimated level of substrate digestibility for mass rearing using the mass balance approach, namely by knowing the proportion of residue, biomass, and metabolism with dry weight data. The following approach to organic substrate bioconversion by BSF larvae is modelled in the following illustration (Figure 1) (Kinasih *et al.* 2018).

2.8. Data Analysis

The statistical analysis used was the Kruskal-Wallis test to analyze significant differences in growth rate, biomass growth, length, development period, and survival rate of ECD, FCR, WRI and SCR. The Kruskal-Wallis test was accomplished because the data did not meet the normality and homogeneity assumptions after the normality test using the Shapiro-Wilk test and the homogeneity test using the Levene test. Then, further analysis was done using Pairwise-Wilcoxon to find significant differences between treatments (Dzepe *et al.* 2020; Purba *et al.* 2021). Correlation analysis of ECD-FCR and WRI-SCR was also performed using the Spearman correlation test (Kawasaki *et al.* 2022; Ma *et al.* 2022). Each with a 95% confidence level and using the R Studio application version 4.2.0.

3. Results

3.1. Growth

Larvae had the highest growth rate in chicken feed. However, the growth rate between the four treatments of fermented PKM and pretreated with water was not significantly different and lower than chicken feed as a control. Nevertheless, among PKM treatments applied in this study, 2-day fermented PKM (F2) had a higher growth rate (Figure 2).

3.2. Development

The development performance of BSF larvae was analyzed by the survival rates and development time to reach the prepupal phase. Larvae performed the shortest time to develop into prepupae in chicken feed (PA) and were not significantly different from 2-day (F2) and 3-day (F3) fermented PKM (Table 1). On the other hand, larvae fed in PKM pretreated with water (FA) had a longer larval developmental time than all treatments and control. However, all treatments showed high larval survival rates and were not significantly different from chicken feed (PA).

3.3. Efficiency of Conversion

BSF larvae had high conversion efficiency (ECD) in chicken feed (PA). They were significantly different with 4-day fermented PKM and PKM pretreated with water. There is no significant difference among PKM treatments. However, the results showed that conversion efficiency tends to be low in 4-day fermented PKM and PKM pretreated with water compared to chicken feed (Figure 3).

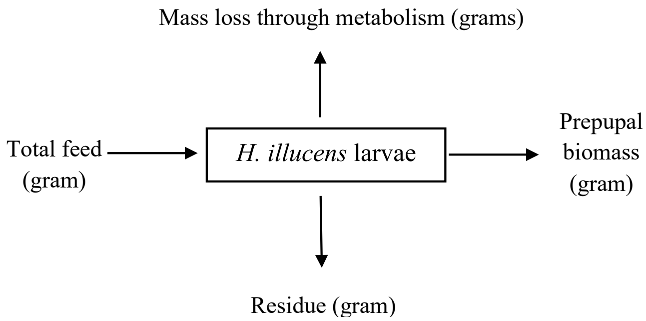

Figure 1. Main concept of mass balance in *Hermetia illucens* larvae

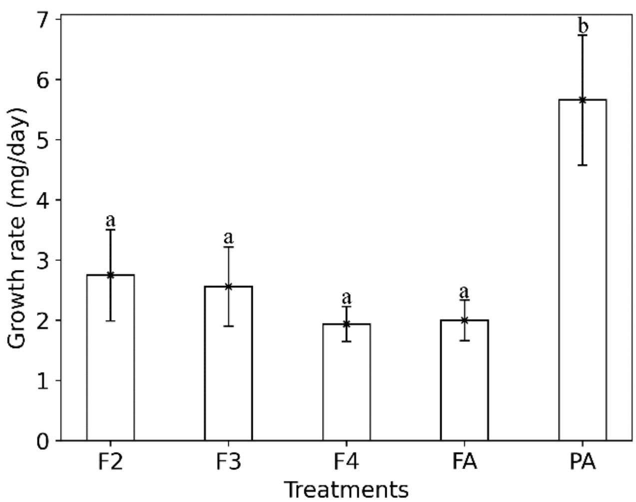

Figure 2. The growth rate of BSF larvae fed on fermented palm kernel meal (PKM). Graph bars followed by different letter had the value significantly different among others (P<0.05)

Table 1. Development time of *H. illucens* larvae on fermented PKM and chicken feed

Feed	Larval development time (days)	Larval survival rate (%)
2-days fermented PKM	17.00±0.00[a]	99.88±0.44[a]
3-days fermented PKM	17.60±1.34[a]	99.87±0.48[a]
4-days fermented PKM	20.60±1.34[b]	99.72±1.09[a]
4-days water soaked PKM	23.60±1.34[c]	99.80±0.76[a]
Chicken feed	15.80±1.64[a]	100.00±0.00[a]

Larval development time and survival rate values followed by different letter on the same column vary significantly (P<0.05)

On the other hand, the food conversion ratio (FCR) was used to measure the ratio of feed converted to biomass. The more efficient larvae convert feed to biomass, usually followed by less feed needed to consume. The result showed that larvae fed on chicken feed (PA) also had the lowest FCR. On the other hand, larvae fed on PKM pretreated with water showed a higher ratio among all PKM treatments and were significantly different (Figure 3).

Spearman's correlation test a strong negative correlation (p-value < 0.05; R > 0.70), which means there were feed influences in the larval digestibility efficiency (Figure 4). This result indicated that higher FCR will followed by low ECD.

3.4. Efficiency of Reduction

The efficiency of reduction performance was analyzed by waste reduction index (WRI) and substrate consumption rates (SCR). The highest waste reduction index was achieved by chicken feed (PA) and significantly different with fermented PKM and PKM pretreated with water (p-value > 0.05), which also showed the highest substrate consumption rates.

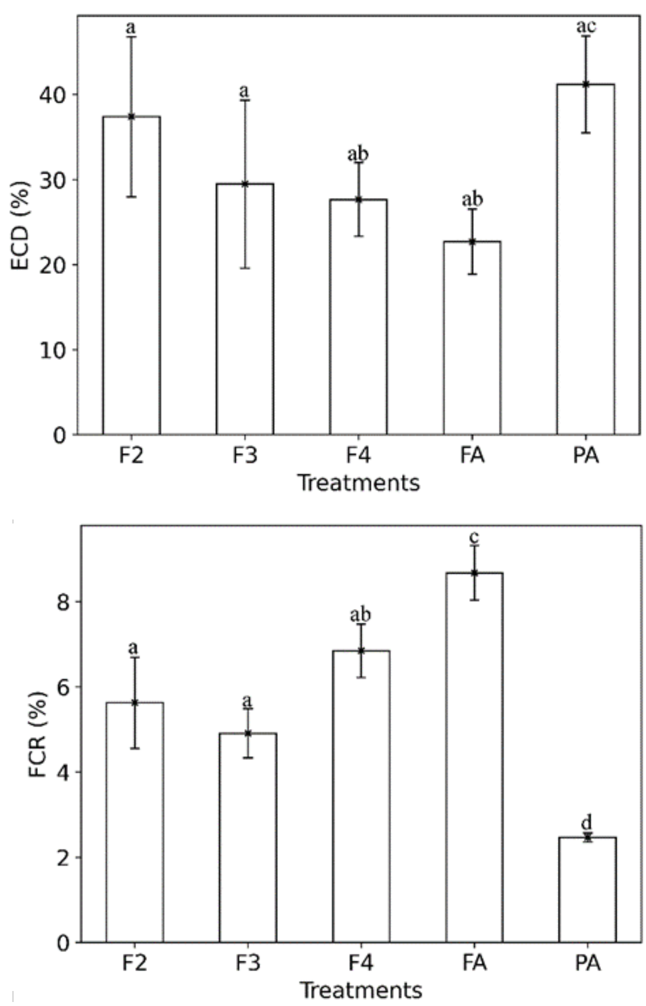

Figure 3. The efficiency of conversion of digested food (ECD) (left) and food conversion ratio (FCR) of BSF larvae fed on fermented palm kernel meal (PKM) and commercial chicken feed. Graph bars followed by different letter had the value significantly different among others (P<0.05)

Among the PKM treatments, 3-day fermented PKM showed the highest substrate consumption rate, though the substrate consumption rate was not significantly different with PKM soaked in water (Figure 5). Compared to control, the substrate consumption rate of treatment significantly lower.

Spearman 's correlation was also analyzed to confirm the relationship between WRI and SCR (Figure 6). It showed a medium positive correlation between WRI and SCR (R = 0.79), which means the index reduction has a correlation with substrate consumption rates that shows the higher the ability of larvae to eat the feed given will affect a higher reduction index.

3.5. Mass Balance

In summary, all the performances of growth and development of BSF larvae fed with fermented PKM, soaked in water and chicken feed for mass production can be shown in mass balance. Mass balance as a proportion of feed used for biomass, metabolism and the residue were analyzed (Figure 7). The results showed that the range of the proportion of feed for biomass in PKM treatments (fermented and soaked in water) was 7.93–12.08%. Moreover, the proportion of chicken feed for biomass was 17.95%. The proportion of metabolism in PKM treatments was 20.09–27.16%, and in chicken feed was 25.73%. Using this mass balance, the proportion of residue in PKM treatments was

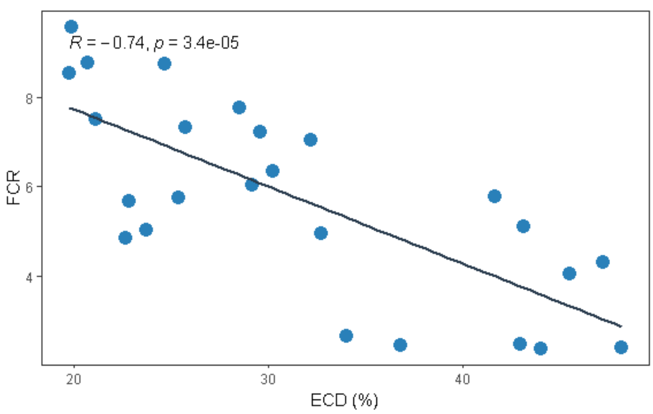

Figure 4. Spearman's Correlation of ECD and FCR of BSF larvae fed on palm kernel meal (PKM) and chicken feed (PA). Each dot represented one data from all treatment groups and control

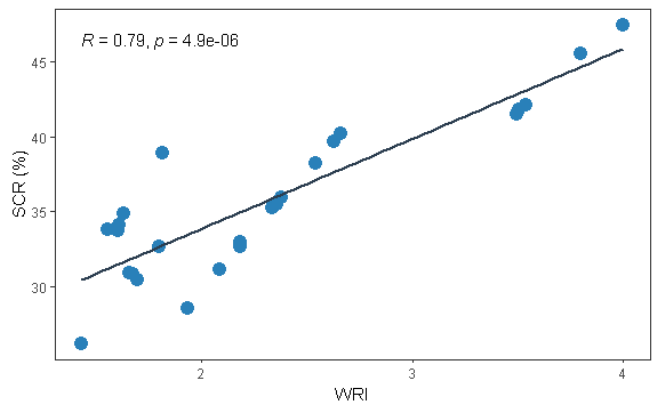

Figure 6. Spearman's Correlation of WRI and SCR of *H. illucens* larvae fed on palm kernel meal (PKM) and chicken feed (PA). Each dot represented one data from all treatment groups and control

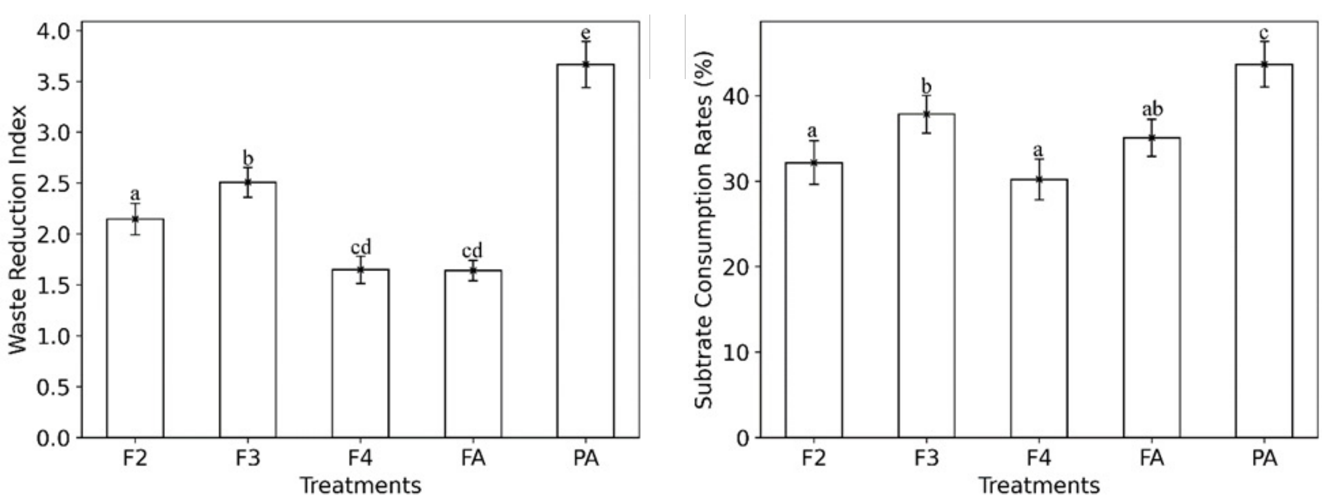

Figure 5. The waste reduction index (WRI) (left) and substrate consumption rate (SCR) (right) of BSF larvae fed on fermented palm kernel meal (PKM) and commercial chicken feed. Graph bars followed by different letter had the value significantly different among others (P<0.05)

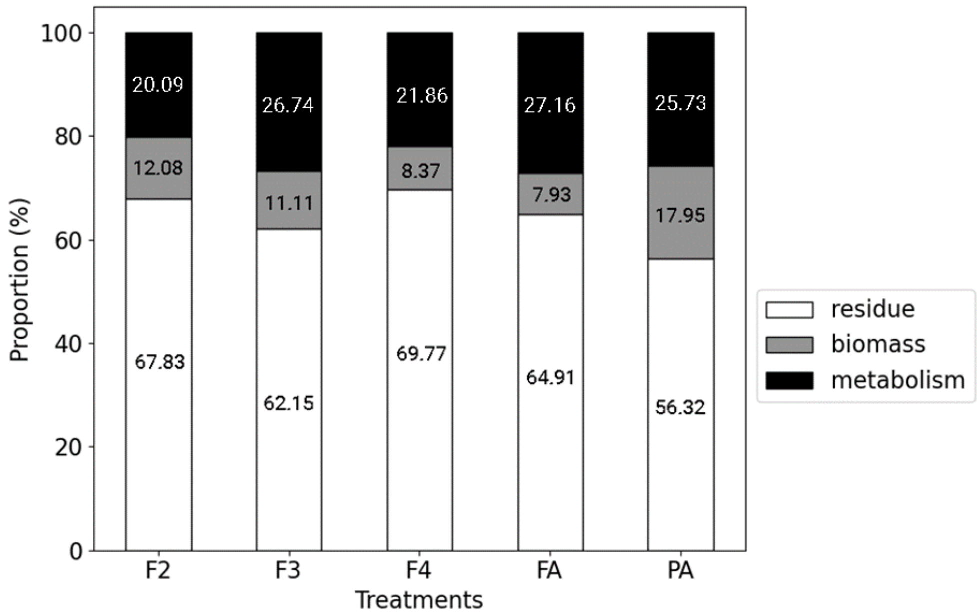

Figure 7. The proportion of feed used for biomass, metabolism and left as residue of BSF larvae fed on fermented palm kernel meal (PKM)

62.15–69.77%. Instead, in chicken feed, it was 56.32%. The proportions were various, and it can be influenced by the type of feed given by different pretreatment.

4. Discussion

4.1. Growth

The results showed that BSF larvae fed with chicken feed (PA) had a higher growth rate than palm kernel meal (PKM). It could be related to nutrient content of feeding material, especially protein and carbohydrate. The chicken feed contains 21–23% protein and a maximum crude fiber of 5% (Simanjuntak 2018) while fresh PKM contains 21–23% crude fiber and 14–19.24% crude protein (Ezieshi and Olomu 2007; Sekoni *et al.* 2008; BPTP 2015). Studies showed that low ratio of protein and carbohydrate improve the growth rate (Barragan-Fonseca *et al.* 2019; Putra *et al.* 2020; Eggink *et al.* 2023). Chicken feed has high carbohydrate to provide energy needed either for biomass production and egg production (Al-Kendi and Al-Gubary 2022).

On the other hand, PKM generally consists of three forms of crude fiber: hemicellulose, cellulose, and lignin and the lignin content is about 12.0–13.6% (Sekoni *et al.* 2008; Caruso *et al.* 2014), whereas chicken feed does not contain lignin. High fiber tends to affect low digestibility in larvae. This condition could trigger the compensatory growth (Supriyatna *et al.* 2016), which makes the larvae allocate the energy obtained to maintain its viability to continue its development, by reducing the proportion of energy to biomass.

Even though it has been given pretreatment with fermentation using commercially available EM4 and molasses, the growth rate is similar with the pretreated without EM4 and molasses. There are three possible hypotheses: (1) the concentration of EM4 in the livestock used in this study was too low. Study by Firdausy *et al.* (2022) reported the improvement of growth rate of BSF when organic waste pretreated by EM4 fermentation. Hence, for further research could be design to find the best amount of EM4 application; (2) the duration of fermentation. The longer the fermentation is likely to be ineffective because it may reduce other nutrient content, too, such as protein (Mulyono *et al.* 2019). However, it is good for converting crude fiber consists of hemicellulose, cellulose, and lignin into simple sugars (Caruso *et al.* 2014); (3) types of microbes. The result on the growth rate of PKM fermented by commercial EM4 and naturally occurring microbes was not signficantly different. This could indicated that microbes of commercial EM4 relatively unsuitable for fermentation of PKM. Exploring the naturally occurring microbes in the future research could solve this problem.

Compare to other study on other lignocellulose feeding materials, BSF larvae growth rate recorded in this study was higher than some materials (e.g. coconut testa, banana peel, spent coffee ground) (Table

2). This result may showed the benefit fermentation to provides necessary nutrition for BSF larvae.

Another possible explanation of this finding is the availability of the protein on the feeding material. For example, coconut Testa had 7% protein and 17% crude fiber (Appaiah *et al.* 2014; Putra *et al.* 2020), banana peel had 1% protein and 30% crude fiber (Anhwange *et al.* 2009; Putra *et al.* 2020) and spent coffee ground had 19.93% protein and 18.8% crude fiber (Permana *et al.* 2018). All of these materials has lower protein content than PKM (14.0-19.24%) and brewery by-products (20.05%) which produced better growth rate than previous materials. This protein could be used by BSF larvae as material for improve their growth rate as omnivore insect, like BSF larvae, more likely to regulate protein than carbohydrate (Wang *et al.* 2018). By this, this insect more likely to suffer growth defficiency when received insufficinent amount of protein (Simpson and Raubenheimer 2005).

4.2. Development

The result increasing developmental time with increasing fermentation time. It could related to decreasing nutritional content of substrate. According to Gustika (2022, unpublished data), the percentage of carbohydrates in fresh PKM decreased from 62.85% to 24.32% while protein content decreased from 16.05% to 7.22% after fermentation for seven days. It is aligned with Myers *et al.* (2008) and Miranda *et al.* (2021) that the quality and quantity of feed influence the development time of larvae such as feed rate, moisture, and nutrients (carbohydrate, protein, fat and fiber). Significant increasing of development time related to physiological requirement of protein for development as larvae required specific amount of accumulated protein to continue their development into next phase (Oonincx *et al.* 2015; Barragan-Fonseca *et al.* 2018; Gold *et al.* 2018; Lalander *et al.* 2019). On the other hand, fermentation duration did not have significant negative impact to mortality of larva which indicated the level of nutrition of PKM fullfilled minimum requirement for larva.

Table 2. Comparative data on the growth rate of H. illucens larvae on fermented PKM with various lignocellulose substrates

Feed	Growth rate (mg/day)	Reference
Coconut Testa	0.07	(Putra *et al.* 2020)
Banana peel	0.08	(Putra *et al.* 2020)
Spent coffee ground	1.41	(Permana *et al.* 2018)
4-days fermented PKM	1.94	This study
2-days fermented PKM	2.75	This study
Winery by-product	6.00	(Meneguz *et al.* 2018)
Brewery by-product	14.00	(Meneguz *et al.* 2018)

In comparison with the previous study used lignocellulose substrate, 2-day fermented PKM larval development time was shorter than most of result from various studies (Table 3). However, it is slightly longer than fermented soybean husk (Permana *et al.* 2022) and brewery's spent grain (Liu *et al.* 2018). Longer developmental time could be related to higher protein content and availability of carbohydrate (from digestible carbohydrates) from substrate as both soybean husk and brewery's spent grain relatively lack of lignin compare to PKM. During feeding process some of the hemicellulose could digested by microbes and increased the carbohydrate content providing energy source for larva. This condition could altered the protein : carbohydrate ratio to suitable level for larva development which tend to by low protein and high carbohydrate (Barragan-Fonseca *et al.* 2019, 2020). On the other hand, the result on development period confirmed the growth rate plasticity oft BSF larvae which can consume varied nutrient compositions but will take longer to develop when fed in poor-quality substrates (Dmitriew 2011; Banks *et al.* 2014).

4.3. Efficiency of Conversion

The efficiency of conversion of digested feed (ECD) is essential as information for the utilization

Table 3. Comparative data on the development time of BSF larvae on fermented PKM with various lignocellulose substrates

Feed	Development time (days)	Survival rate (%)	Reference
Brewery's spent grain	14.97	98.00	(Liu *et al.* 2018)
Fermented soybean husk	15.00	98.80	(Permana *et al.* 2022)
2-days fermented PKM	17.00	99.88	This study
Fermented banana peel	19.20	98.30	(Permana *et al.* 2022)
4-days fermented PKM	23.60	99.80	This study
Fermented maize waste	24.20	93.00	(Gao *et al.* 2019)
7-days fermented PKM	28.00	96.00	(Gustika 2022; unpublished data)
Fermented barley waste	31.00	98.67	(Permana *et al.* 2021)
Rice straw	38.20-54.20	51.21-98.27	(Manurung *et al.* 2016)

of H. illucens larvae on a production scale because it is related to the biomass (yield) of larvae and the nutritional content produced so that it can be reused as alternative feed. The ECD value determines feed conversion efficiency used to consider the quality and quantity of organic substrates used for the growth of BSF larvae (Supriyatna *et al.* 2016; Pliantiangtam *et al.* 2021; Permana *et al.* 2022).

The result showed short fermentation duration improved the digestibility of PKM close to digestibility of commercial chicken feed. This value significantly better than application of naturally occuring microbes (FA treatment). It seem that commercial E4 include microbes with ability to digest fiber This is presumably due to pretreatment by EM4 and molasses may decrease the crude fiber of PKM and increase the larvae's digestibility (Pamintuan *et al.* 2020; Permana *et al.* 2021). Thus, the 2-day fermented PKM can be considered for BSF larvae growth substrate.

Furthermore, feed conversion efficiency can also be seen from the FCR (food conversion ratio) to determine the feed ratio the larvae can convert into biomass. The more efficiently the larvae use feed for processing bioconversion, the smaller the FCR (Broeckx *et al.* 2021). This study also confirmed the negative correlation between ECD dan FCR. Low FCR indicated that the substrate was unsuitable for feeding material (Nyakeri 2017) and this furtherly explained low growth rate of larvae for substrate with low FCR. However, in this study the correlation between both value was not strong which indicated that consumed feed not all converted into biomass especially on the low quality feeding material.

4.4. Efficiency of Reduction

This study showed correlation between substrate digestibility, growth rate, dan the rate of waste reduction. Just as nutritional composition affects the digestibility of larvae, so does the waste reduction ability of larvae. In this study, we found similar WRI among treatment groups However, the substrate consumption was not significantly different from that of PKM soaked in water. The similarity indicated that fermentation may not significantly change the palatability of PKM. In addition, the lowest consumption rate obtained at 4-day fermented PKM could related to lower nutrition content and increased acidity of the substrate due to additional acid produced by fermentation (Lim *et al.* 2019). Based on this result, if the purpose of application of BSF larvae is to reduce the waste then the 3-day fermented PKM can be considered.

There was negative correlation between WRI and SCR yet the correlation was not strong. It could be caused that not all reduction caused by consumption by larvae. Thus, there is possibility that eventhough the FCR is high, however the substrate physical condition may not change much or converted into other material that not significantly lighter than previous material.

4.5. Mass Balance

The relatively high proportion of biomass indicates that the substrate utilized for the formation of biomass and high conversion efficiency. BSF larvae consumed the chicken feed more likely to convert it into biomass than treatment groups. High consumption rate also correlated to the amount of residues which were the lowest.

Among treatment groups, BSF larvae feed on the PKM soaked in the water for 4 days (FA) utilized most of the substrate for metabolism. There are several hypothesis to explain this result

- Higher metabolism could related to the effort of larvae to maintaining homeostasis through energy generating. Study showed that unsuitable substrate could lead to increasing allocation of food for energy (Barragan-Fonseca *et al.* 2018; Laganaro *et al.* 2021).
- Change in substrate conditions such as acidity, water content, and bulk density. Keeping PKM inside the water could alter the physical properties of substrate by increasing the water content and bulk density and effecting the BSFL larvae (Bekker *et al.* 2021; Yakti *et al.* 2023). High water content and bulk density reduce the aeration for larvae which lead to increasing effort for homeostasis (Palma *et al.* 2018; Lalander *et al.* 2020). In more extreme case, some larvae could be drown (Lalander *et al.* 2020).

In conclusion, short fermentation duration of PKM by commercial EM4 could be considered as pretreatment from BSF larvae substrate to improve the digestibility of substrate. Fermentation may alters the substrate nutrient content and physical properties which influence the growth performance of BSF larvae. Further study should carried out to find the most optimum concentration of EM4 for pretreatment of PKM for BSF larvae substrate.

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