THE EFFECT OF POLYSTYRENE PAPER STYROFOAM FEED ON SURVIVORSHIP AND GROWTH OF *Tenebrio molitor* L.

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ABSTRACT

Polystyrene Paper Styrofoam (PSP) styrofoam waste is one type of plastic waste that is difficult to degrade. However, recent research reported that *Tenebrio molitor* L. can degrade PSP Styrofoam. The degradation depends on the composition of PSP styrofoam as a part of feeding. This research aimed to get the composition of good feed to increase the degradation rate of the PSP styrofoam waste, but it still had a good effect on the survivorship and growth of *T. molitor* L. larvae. This research was an experimental laboratory and was designed using a control and seven treatments with 3 replications. The parameters observed were survivorship, growth in body length, biomass, and waste degradation rate. The data obtained was analyzed using ANOVA and Duncan test. The results showed that differences in PSP styrofoam waste feed had a significant effect on survivorship ($p=0.00$), on the growth of body length ($p=0.002$) and biomass ($p=0.00$), and waste degradation rate ($p=0.02$). The research revealed that the lower the composition of PSP styrofoam and the higher the composition of the concentrate combination, the higher the survivorship, growth, and waste degradation rate. The result shows that 1 kg *T molitor* L. larvae can degrade 0.25 kg of PSP styrofoam waste for 60 days. The findings can be used to solve the problem of styrofoam pollution.

Keywords : degradation, larvae, styrofoam, *Tenebrio molitor* L.

INTRODUCTION

Styrofoam is a product made from raw polystyrene and is one of the plastic types. The number of styrofoam waste products increases as the human population increases. Polystyrene is divided into three types: Expanded Polystyrene (EPS), Extruded Polystyrene (XPS), and Polystyrene Paper (PSP). Generally, PSP styrofoam is used as a food tray because of the advantages of its flexible material, can maintain the freshness and temperature of the ingredients in it, and is practical, and cheap (Swamilaksita et al., 2018). PSP styrofoam also has a weakness, its nature is difficult to degrade and takes about 500 years to fully decompose. A once-designed PSP styrofoam design increases the waste that can pollute the soil, water, and air (Bilal et al., 2021). These things cause PSP styrofoam waste which must be solved. However, based on recent research, there are some biological agents that have the potential to degrade PSP Styrofoam, such as bacterial (Ho et al., 2018) and fungus (Ho et al., 2018; Onodera et al., 2001).

Tenebrio molitor L. larvae is an insect (Coleoptera) that reported has high potential to degradate the PSP styrofoam waste (Peng et al., 2020; Tsochatzis et al., 2021) and others type of plastic (Peng et al., 2020; Zhang et al., 2022) . In society, the potential for these larvae is still not fully understood and properly harnessed. *T molitor* larvae are known as mealworm which has characteristic golden-yellow and holometaboles (Hong et al., 2020). *T molitor* larvae reported has capability to degraded PSP styrofoam due to the larvae's intestine containing bacterial symbions which produce extraculicular enzyme to degrade the PSP styrofoam fragment to small molecule (Fabreag & Familara, 2019). The aspect of nutritions is main factor to optimize microbial activity of the larvae (Palmer et al., 2022). However, there is no data regarding feeding composition using PSP styrofoam waste on survivorship and growth of the larvae, and capability the larvae to degraded the waste. Degradation of PSP styrofoam will be faster due to availability of proper nutrition in feed which stimulate to optimize microbial activity in the produce degradation enzymes (Fabreag & Familara, 2019). Feeding composition using concentrate design as a source for metabolic energy (Ferrari et al., 2019) and fresh chayote as a source of minerals and water (Sakung et al., 2020; Subchan et al., 2022).

This research is to investigate the effect of different composition of polystyrene paper (PSP) styrofoam waste feed on survivorship, growth, and waste degradation rate of the *T molitor* larvae. By investigating the impact of styrofoam feed on the survivorship and growth of *Tenebrio molitor* larvae, the study sheds light on the feasibility of using these larvae as a means of degrading styrofoam waste. This research is significant as it offers insights into the efficacy of utilizing *Tenebrio molitor* larvae as a bio-degradation for styrofoam waste, potentially leading to the development of eco-friendly waste management strategies. Furthermore, by addressing the environmental concerns associated with styrofoam pollution, the study contributes to ongoing efforts to reduce plastic waste and promote sustainability in waste management practices.

MATERIALS AND METHODS

This research was conducted from February to April, 2021 at the Center Development Advance Sciences and technology (CDAST) University of Jember. This research uses Completely Randomized Design (CRD) consisting of control and seven treatments with 3 replications. The feed composition used included Control (K): (90% concentrate + 10% chayote), P1 (100% PSP styrofoam), P2 (90% PSP styrofoam + 10% chayote), P3 (80% PSP styrofoam + 10% concentrate + 10% chayote), P4 (70% PSP styrofoam + 20% concentrate + 10% chayote), P5 (60% PSP styrofoam + 30% concentrate + 10% chayote), P6 (50% PSP styrofoam + 40% concentrate + 10% chayote), P7 (45% PSP styrofoam + 45% concentrate + 10% chayote). Feeding quantity (grams) adjusted by 0.08 x latest total wet biomass x 7 days x feed percentage (concentrate/ PSP styrofoam/ chayote).

Preparation phase

Tools and materials that were used including container (23×9.5×6 cm^3) for *T molitor* larvae used during the research. The analytic balance are required to count biomass and PSP styrofoam waste mass. An electronic digital caliper to measure the larvae body length. The dry styrofoam PSP waste taken from the final processing place must be soaked in a mixture of water and charcoal chaff (1:1) for the previous three days (served as adsorben) and then cut into pieces.

Tenebrio molitor L. larvae were purchased from a farmer in Jember. *Tenebrio molitor* L. larvae were reared in a plastic tray (37×30×11 cm^3) fed with concentrate so that at the time of sorting it has reached the desired length and wet biomass of 2-2.6 cm and 0.07-0.14 grams. The final test research pan used were 24 pieces, with 50 larvae in each pan. Aliquot pans as many as 8 pieces, with 125 larvae in each pan. The total larvae used amounted to 2400 individuals.

THE RESEARCH STAGE

Parameters observed were survivorship (%), length growth (cm/week) as well as biomass (gram/week), and degradation rate (gram/week). Measurement once a week for 6 weeks. The survivorship is shown from the quantity of the larvae and pupas in the final weeks of research. The growth is indicated by the increase in the body length or biomass between the last week and first. The degree of degradation is measured based on the mass of the beginning and the end of the styrofoam PSP styrofoam in each pan per week.

Preparation of the aliquot methods

The process of obtaining dried biomass by baking 25 larvae/week from 8 aliquot pans in oven at 65 (±0.5)˚C. Once it is an oven, it is measured totally dry biomass and divided by 25. The wet biomass in 8 pans is compared to aliquot, compared with an estimated biomass of biomass that leads to a regression equation. Each treatment has a different allometric regression equation. This regression equation, used as a formula for finding dry biomass in a final test (24 pans). Allometric equations for measuring dry biomass are (Daba & Soromessa, 2019):

$$y = a + bX$$

Note:

- y : dry biomass
- a : constant
- b : coefficient
- X : wet biomass

Data analysis

The data obtained from the research was then analyzed by Analysis of Variance (Anova) and Duncan test using the SPSS 25 program.

RESULTS AND DISCUSSION

After carrying out 1 control and 7 treatments, the results of the comparison of differences in mass, survivorship and level of degradation are obtained as shown in Figure 1.

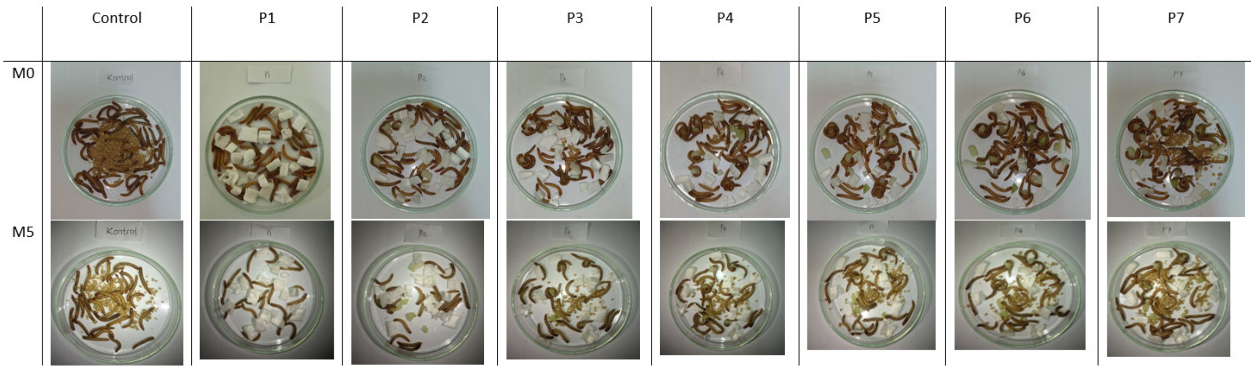

Figure 1 differences in mass, survivorship and level of degradation.

The results of the comparison of differences in length of *Tenebrio molitor* as shown in Figure 2.

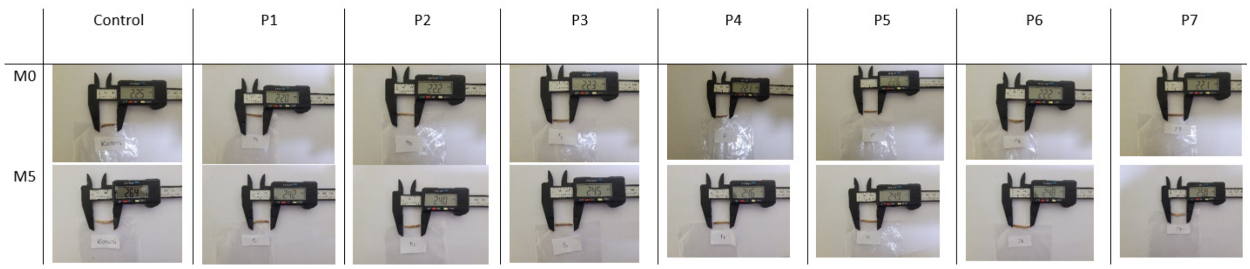

Figure 2 differences in length of *Tenebrio molitor* at week 0 (M0) and week 5th (M5) for all treatments.

T molitor larvae's survivorship

Based on data analyse using Anova, it shows that the difference composition of PSP styrofoam waste feed was significant effect (F = 1787; df = 7; p = 0.000) on the survivorship (%) of the *T molitor* larvae. Table 1 shows that Control and P7 had the greatest survivorship (61.33% and 57.33% respectively). P7 shows no difference significantly with P5 (54.67%) and P6 (55.33%). P1 (42%) shows the smallest survivorship and no difference significantly with P2 (44.67%). P2 shows no difference significantly with P3 (48%) and P4 (48.67%).

Table 1. Differences the *T. molitor* survivorship (%) among treatments

Treatments	Mean ± SD (*)
K	61.33 ± 3.06^{d}
P1	42.00 ± 2.00^{a}
P2	44.67 ± 2.31^{ab}
P3	48.00 ± 2.00^{b}
P4	48.67 ± 2.31^{b}
P5	54.67 ± 4.16^{c}
P6	55.33 ± 3.06^{c}
P7	57.33 ± 6.78^{cd}

*) Note: Note: mean and standard deviation (SD) followed by the same alphabet indicate a different result based on Duncan test 5%.

The research revealed that higher the composition of PSP styrofoam waste feed, the lower composition of concentrate which effected on decreasing the survivorship. The low percentage of survivorship due to the composition of the feed given imprecise and lacking in several essential nutrient substances. The concentrate contains carbohydrate that will be converted into glucose molecules. The glucose being a source of energy will be absorbed by intestine larvae and used for metabolism process and mobility. If the amount of glucose exceeds the body's needs, it will be stored in the form of glycogen or fat as energy reserves. The research findings indicate that an increase in the proportion of PSP styrofoam waste feed leads to a decrease in the concentration of concentrate. To optimize degradation while maintaining the well-being of *Tenebrio molitor*, it may be beneficial to carefully balance the feed composition, ensuring that essential nutrients are provided in sufficient quantities. By adjusting the feed composition to provide adequate nutrition, we can enhance degradation rates without compromising the health and survival of the larvae. The best feed composition identified was P7 (45% PSP styrofoam + 45% concentrate + 10% chayote).

Growth of body length

Based on the results of the Anova test, it shows that the difference in the composition of PSP styrofoam waste feed had a significant effect (F = 5.5; df =7; p = 0.002) on the growth of body length (cm/week) *T molitor* larvae.

Table 2. The differences growth of body length (cm/week) of *T molitor* larvae among treatments

Treatments	Mean ± SD (*)
K	0.381 ± 0.108[b]
P1	0.026 ± 0.042[a]
P2	0.097 ± 0.133[a]
P3	0.003 ± 0.002[a]
P4	0.050 ± 0.167[a]
P5	0.034 ± 0.072[a]
P6	0.004 ± 0.088[a]
P7	0.180 ± 0.015[a]

*) Note: Note: mean and standard deviation (SD) followed by the same alphabet indicate a different result based on Duncan test 5%.

Based on Table 2, shows that the various composition treatments of the PSP styrofoam waste feed have different results. Control had the greatest growth of body length (0.381 cm/week), while the P3 (0.003 cm/week) showed the smallest growth of body length and no different significantly with P1 (0.026 cm/week), P2 (0.097 cm/week), P4 (0.05 cm/week), P5 (0.034 cm/week), P6 (0.004 cm/week), P6 (0.004 cm/week), P6 (0.004 cm/week), P6 (0.004 cm/week), P6 (0.004 cm/week), and P7 (0.18 cm/week).

Based on the research, it was found that the different treatments of PSP styrofoam waste feed had a significant effect on growth of body length (cm/week) and biomass (grams/week) of *T molitor* larvae. A good feed composition was contained a high percentage of concentrate. This is because the concentrate contains essential amino acids such as lysine which plays a major role in protein synthesis and methionine which plays a role in tissue growth (Q. Yang et al., 2020) and nitrogen balance (Ferrari et al., 2019). Thus, a deficiency in the percentage of concentrate will cause a deficiency of essential amino acids which can lead to a decline in the protein synthesis process and small growth.

Growth of biomass

Based on the results of the Anova test, it shows that the difference in the composition of PSP styrofoam waste feed had a significant effect ($F = 27.895$; $df = 7$; $p = 0.00$) on the growth of biomass *T molitor* larvae.

Table 3. The differences growth of biomass (grams/week) of *T molitor* larvae among treatments.

Treatments	Mean ± SD (*)
K	0.0155 ± 0.0026[c]
P1	0.0013 ± 0.0004[a]
P2	0.0026 ± 0.0007[a]
P3	0.0043 ± 0.0006[a]
P4	0.0016 ± 0.0008[a]
P5	0.0013 ± 0.0004[a]
P6	0.0042 ± 0.0014[a]
P7	0.0088 ± 0.0032[b]

*) Note: mean and standard deviation (SD) followed by the same alphabet indicate a different result based on Duncan test 5%.

Based on Table 3, shows that the various composition treatments of the PSP styrofoam waste feed have different results. Control had the greatest growth of biomass (0.0155 grams/week), while P1 (0.0013 grams/week) and P5 (0.0013 grams/week) showed the smallest growth of biomass and no different significantly with P2 (0.0026 grams/week), P3 (0.0043 grams/week), P4 (0.0016 grams/week), and P6 (0.0042 grams/week), but different significantly with P7 (0.0088 grams/week).

Giving a high PSP styrofoam feed composition and a low concentrate feed composition gives a consequence a fewer available and digestible amino acids. In addition, the results of the depolymerization of PSP styrofoam carbon will be converted into 47.7% CO_2, 49.2% feces, and only 0.5% assimilated as biomass (Matyja et al., 2020). Matter this shows that PSP styrofoam as feed only provides a little energy but can still make *T molitor* larvae grow in length (cm/week) and biomass (grams/week). *T molitor* larvae fed with high composition PSP styrofoam did not form many folds in its body structure (S. S. Yang et al., 2018). These are the reasons why Control (90% concentrate + 10% chayote) and P7 (45% PSP styrofoam + 45% concentrate + 10% chayote) continued to show good growth. P1 to P6 show different growth with ideal conditions. This can occur due to internal factors such as the digestibility of each individual.

Degradation rate of the PSP styrofoam waste

Based on the results of Anova test, it shows that the difference in the composition of PSP styrofoam waste feed had a significant effect ($F = 2.33$; $df = 7$; $p = 0.02$) on degradation rate (grams/week).

Table 4. The differences degradation rate of PSP styrofoam waste by the *T molitor* larvae among treatments

Treatments	Mean ± SD (*)
K	0.00 ± 0.00 [a]
P1	0.09 ± 0.12 [ab]
P2	0.18 ± 0.23 [ab]
P3	0.25 ± 0.36 [b]
P4	0.25 ± 0.36 [b]
P5	0.26 ± 0.37 [b]
P6	0.29 ± 0.38 [b]
P7	0.33 ± 0.38 [ab]

*) Note: mean and standard deviation (SD) followed by the same alphabet indicate a different result based on Duncan test 5%.

Based on Table 4, shows that the various composition treatments of the PSP styrofoam waste feed had different results in the degradation rate (grams/week) of PSP styrofoam. P7 (0.33 grams/week) shows no different significantly with P1 (0.09 grams/week), P2 (0.18 grams/week), P3 (0.25 grams/week), P4 (0.25 grams/week), P5 (0.26 grams/week), P6 (0.29 grams/week), and at the Control treatment there is no degradation. Based on the research, it was found that the different feed treatments had a significant effect on the degradation rate (grams/week) of PSP styrofoam waste. The *T molitor* larvae can digest polystyrene which is the raw material for PSP styrofoam because their intestines contain Exiguobacterrium sp. YT2 strain that secretes extracurricular enzymes. This enzyme can catalyze the depolymerization process of styrofoam fragments and also reducing the size of the molecules (Matyja et al., 2020). The degradation process of PSP styrofoam that occurs in the intestines of *T molitor* larvae causes changes in the chemical structure of polystyrene from hydrophobic to hydrophilic so that it can be digested. Enzymatic activity is also carried out by gut microbes. Urbanek, et al. (2020) found that there were seven classes, Gammaproteobacteria, Bacilli, Clostridia, Acidobacteria, Actinobacteria, Alphaproteobacteria and Flavobacteria, which were the most abundant microbiome groups in the intestine of *T. molitor*, while the dominant genera were Enterobacter, Lactococcus and Enterococcus. While Brandon et al. (2021) discovered eight unique gut microorganisms associated with PS biodegradation including Citrobacter freundii, Serratia marcescens, and Klebsiella aerogenes.

The PS degradation process occurs because *T. molitor* as the host and the microbiome in its gut collaborate to create an environment conducive to the plastic biodegradation process. This was proven by Brandon, et al. (2021) that *T. molitor* secretes emulsifying factors (30–100 kDa) that mediate the bioavailability of plastics. The gut microbiome of *T. molitor* secretes factors (<30 kDa) that enhance respiration on polystyrene (PS).

Based on the results that have been obtained, it shows that the lower the composition of PSP styrofoam feeding, the higher the concentrate causes the waste degradation rate (grams/week) to be higher. In addition, the higher nutrient can optimize microbial activity in producing extracurricular enzymes (Sari et al., 2019) so that the degradation rate of PSP styrofoam waste is faster. Based on the difference in feed composition of the eight treatments, P1 (100% PSP styrofoam) showed the lowest degradation rate is 0.09 grams/week, while K (0% PSP styrofoam) no degradation process occurs. P7 (45% PSP styrofoam + 45% concentrate + 10% chayote) showed the highest degradation rate of styrofoam PSP waste is 0.33 grams/week or 0.047 grams/day. P7 used 50 larvae so each individual can degraded 0.00094 grams/day/individual. It means, 4.432 individuals or roughly 1 kg *T molitor* larvae can degrade 0.25 kg of PSP styrofoam waste for 60 days. This composition study is important because if *T. molitor* larvae only uses styrofoam (100% PSP), it shows changes in larval development caused by a decrease in insufficient food supply (Matyja, et al., 2020).

Tenebrio molitor still has significant potential to be applied in outdoor waste management, albeit requiring further research and careful approaches. Although typically found in its natural habitats, some studies support the adaptability of these beetles to new environments, including outdoor settings. Recent studies also suggest that *Tenebrio molitor* may have the potential to degrade non-organic waste in outdoor environments, such as styrofoam. Based on research conducted by Ribeiro et al., (2018), although *Tenebrio molitor* depends on abiotic conditions throughout its life, it turns out that *Tenebrio molitor* has a high adaptability. *Tenebrio molitor* is able to live in extreme dry conditions and can survive by eating substances with low water content. With the appropriate technological developments and interdisciplinary cooperation, the implementation of *Tenebrio molitor* in outdoor waste management can be an effective and sustainable alternative. Additionally, it necessitates environmental regulation and the development of monitoring and control techniques.

Optimizing the feed composition to enhance the degradation rate of *Tenebrio molitor* involves several key steps. Firstly, initial research on larval nutritional requirements is conducted to understand the food requirements necessary for optimal growth and activity. Subsequently, various feed ingredients that potentially meet these needs are systematically evaluated, including various types of protein sources, carbohydrates, fats, and additional nutrients. Following this, a series of feeding trials is conducted to test different combinations and proportions of feed ingredients in effectively promoting larval growth and activity. Throughout the trials, parameters such as feed consumption rate, larval growth, and waste degradation efficiency are continuously monitored and evaluated. By employing this approach, the feed composition can be adjusted and optimized to achieve the best outcomes in terms of waste degradation rate by *T. molitor*. This method enables the development of more effective and efficient feed formulations, thereby enhancing the potential application of larvae in organic waste management overall.

The different treatment of PSP styrofoam waste feed had a significant effect on the survivorship (%) of *T molitor* larvae. The addition of high feed supplements could improve the larval body condition. The nutrient needs of the larvae will also be met so as to increase the probability of survivorship (%) of *T molitor* larvae. On the other hand, feeding the PSP styrofoam waste with a high composition to the larvae, will not be able to finished their life cycle (Yang et al., 2018). This condition can happen because the lack of nutrients in the feed will cause *T molitor* larvae to not grow to the standard threshold and cause death (Wu et al., 2018). This research lasted for 42 days or 6 weeks, while *T molitor* larvae could only survive well for one month trial with styrofoam feed (Yang et al., 2015). These are some of the situations that caused *T molitor*'s survivorship to become low.

CONCLUSION

The composition of PSP styrofoam, consentrate, and chayote affected highly significant on survivorship ($p<0.001$) and growth ($p<0.01$) of *T molitor* and PSP styrofoam degradation rate ($p=0.02$). The treatment which contains lower the composition of the PSP styrofoam, the higher the composition concentrate which contains high nutrients, resulting higher in survivorship, the body length growth, biomass growth, and degradation rate. The good composition of feed after control (P0) is P7 (45% PSP styrofoam + 45% concentrate + 10% chayote). *T molitor* in the treatment of P7 showed a survival rate of 57.33 (6.78) % and dry biomass growth of 0.0088 (0.0032) grams/week. The result shows that 1 kg *T molitor* L. larvae can degrade 0.25 kg of PSP styrofoam waste for 60 days. Therefore, these findings indicate substantial potential for promoting sustainable waste management practices. reducing the negative impacts of styrofoam pollution on the environment. The feed composition P7 is considered the optimal blend for mitigating the negative environmental impact of styrofoam pollution, as it exhibits a high degradation rate while also maintaining a balanced nutritional profile for the larvae and preserving their viability. The efficient degradation process by *T. molitor* not only reduces the amount of styrofoam waste polluting the environment but also produces residues that are more easily biodegradable. This can alleviate pressure on landfills and mitigate the risk of environmental contamination. Furthermore, the use of *T. molitor* as a waste degradation agent also holds promise for larger-scale applications. Implementing *T. molitor*-based waste management systems in places such as waste treatment facilities or areas with high levels of styrofoam pollution could yield significant impacts in reducing the amount of waste ending up in the environment.

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