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Effect of Expanded Polystyreneon Growth and Development Time of *Tenebrio molitor*

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Abstract. Many studies have reported that mealworms can ingest plastic during their larval stage. The purpose of this study was to investigate the effects of expanded polystyrene consumption on the growth and development of mealworms during their larval stage. We found that the growth rate is significantly different between mealworms consumed wheat bran and mealworms consumed expanded polystyrene ($p < 0.001$). The transformation into pupa occurred faster among the mealworms consumed expanded polystyrene than those consumed wheat bran ($p < 0.001$). However, the survival rate was not significantly different between the two groups of mealworms (p = 0.786). Based on the data, we conclude that mealworms consuming expanded polystyrene have a slower weight gain and a shorter development period than mealworms consuming wheat bran, but the expanded polystyrene does not affect the survival rate before the transformation into pupa. Practically, we anticipate that mealworms can be a resource for the sustainable and eco-friendly removal of expanded polystyrene waste.

Additional key words: average weight, cox proportional hazard model, mealworms, plastic waste, pupation

Introduction

Plastic products have various usages in human life. However, plastics have very low biodegradability in general, resulting in plastic waste which has adverse impacts on the global ecosystem. The Ministry of Environment in South Korea (2017) reported that the plastics produced from 1950 to 2015 weighed about 8.3 billion tons total, and it was estimated that about 5.8 billion tons of them have been discarded. Among the 5.8 billion tons discarded, about 4.6 billion tons (79%) were buried or abandoned, about 700 million tons (12%) were incinerated, and only about 500 million tons (9%) were recycled. However, 80% of the recycled products are estimated to be incinerated or discarded after one recycle. To this end, the true recycling rate might be much less than 9% (Geyer et al., 2017). Moreover, waste import bans in China resulted in the suspension of plastic waste collections for recycling in the metropolitan areas in 2018, thus the problem of plastic wastes was aggravated.

Styrofoam is expanded polystyrene which is a type of plastic. It is made by injecting hydrocarbon gas into polystyrene (PS) granules and is made of 95% air. Expanded polystyrene is very light and easy to mold, and it has good thermal insulation. Due to these characteristics, expanded polystyrene is used in various ways in real life, such as insulation for walls or refrigerators.

But expanded polystyrene breaks down easily and turns into microplastics, causing ocean pollution (Lebreton et al., 2019). Expanded polystyrene made up about 75% of large debris and about 98% of small debris in marine ecosystems (Hong et al., 2014). For this reason, there are increasing concerns about the environmental pollution caused by expanded polystyrene.

Many studies have reported that mealworms can ingest plastic during their larval stage. If mealworms consume expanded polystyrene and grow normally, it can contribute

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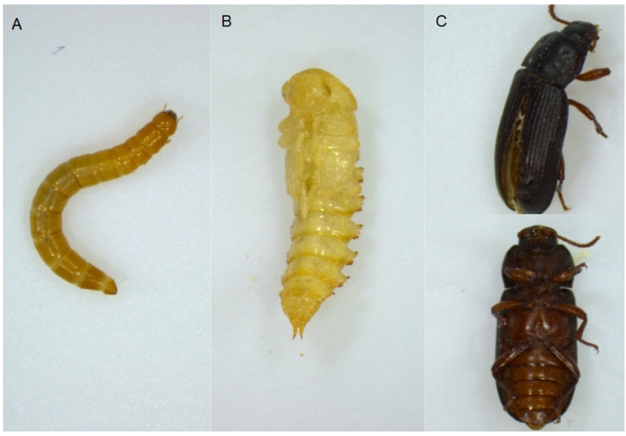

Fig. 1. A mealworm development stage. (A) Larva, (B) Pupa, and (C) Adult.

to reducing expanded polystyrene wastes. Mealworms are the larva of *Tenebrio molitor* L which belongs to the family Coleoptera, and they are completely metamorphic insects that undergo metamorphosis in the following order: egg, larva, pupa, and adult Fig. 1). They consume food during the larval period, the longest period of its life cycle, and they store energy for the next stage of metamorphosis (Yang et al., 2022; Zhong et al., 2023). Yang et al. (2015) reported that mealworms can survive for about a month when consuming only expanded polystyrene and presented evidence that polystyrene is biodegradable based on changes in the chemical and physical properties of the plastic. Moreover, mealworms can metabolize about 50% of the ingested PS waste into easily decomposable low-molecular-weight compounds and carbon dioxide with an intestinal residence time of about 12 to 15 hours, after ingesting PS wastes (Yang et al., 2018).

The plastic waste problem is a global issue, as most of the waste is sent to a landfill or incinerated, causing secondary problems. Since the discovery of mealworms' ability to consume plastic, many studies have been conducted, but not much is known about the effect of consuming expanded polystyrene on the growth and development of mealworms. There were no studies using a statistical model to compare the growth rate and using survival analysis for estimating and comparing the development and survival rates of mealworms. This study focuses on the effect of feeding expanded polystyrene on the growth and development of mealworms, relative to feeding wheat bran. If mealworms grow and develop normally, feeding expanded polystyrene can contribute to reducing expanded polystyrene wastes and providing nutrients to humans.

Materials and Methods

1. Mealworm and environment condition

Mealworms, larvae of *Tenebrio molitor* L, were observed in this study. The larvae were purchased from a website (Mealworm Nara, South Korea), and they had an average weight of 181.2 mg per mealworm estimated base on 200 mealworms. Two hundred mealworms in good condition were selected and distributed into 10 Petri dishes (125 × 125 × 20 mm; i.e., 20 mealworms per dish). Five Petri dishes were randomly selected, and larvae in these five Petri dishes were fed 5 g of wheat bran per dish. Larvae in the other five Petri dishes were fed 2 g of expanded polystyrene per dish. The smaller amount of expanded polystyrene was fed because its low density occupied a large space in the Petri dish.

The larvae were placed in a chamber (VS-1203PFC, Visionbionex, South Korea) with an air temperature of 25 ± 0.2°C and relative humidity of 30 ± 5% (TE-201, CAS, South Korea), without light. To maintain moisture in the Petri dishes, 0.5 g of lettuce was provided every 4 days. The pupae were transferred to a plastic container to prevent kin predation. The weight changes of the larvae and the changes to pupae were observed and recorded every day at 5 PM from March 18 to April 11, 2024.

It was confirmed that the mealworms that were used ate the expanded polystyrene (Fig. 2). Only larvae ate the expanded polystyrene. during the experiment period, and the mealworms consumed an average of 0.182 g of the provided 2 g of expanded polystyrene per Petri dish. The amount of bran consumed could not be determined because it was mixed with mealworm excrement, making it difficult to obtain accurate data.

2. Statistical analysis

It was hypothesized that mealworms consuming expanded polystyrene grow or transform into pupae slowly than

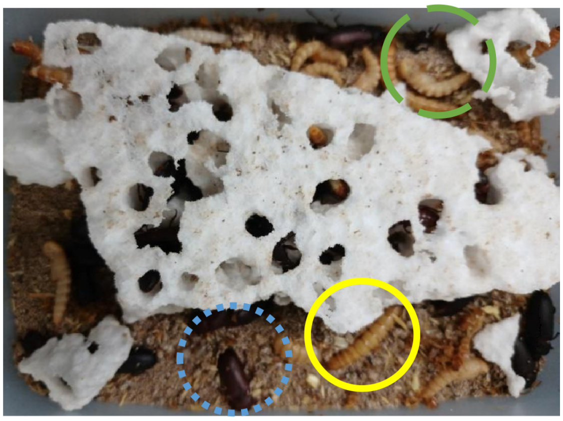

Fig. 2. Mealworms eating expanded polystyrene. Yellow circle: Larva; Green circle: Pupa; Blue circle: Adult.

mealworms consuming wheat bran.

There were two kinds of response variable for comparing the two food groups, wheat bran and expanded polystyrene. The first kind was the weight (mg) averaged within each experimental unit (Petri dish) before transformation into pupa. The linear mixed-effects model was used to estimate the expected change in the averaged weight per day. The model accounted for repeated measures of each experimental unit and different numbers of mealworms measured in each experimental unit for each day. Under the mixed-effects model, the null hypothesis was that the two food groups have the same growth rate, and the alternative hypothesis was that they have different growth rates. More specifically, the expected weight was modeled by $\mu = \beta_0 + \beta_1$ group $+ \beta_2$ day $+ \beta_3$ group $\times$ day, where group is the dummy variable (0 for wheat bran and 1 for expanded polystyrene). The statistical hypothesis test was formulated as H_0: $\beta_3 = 0$ versus H_1: $\beta_3 \neq 0$. We performed the hypothesis test under the model at a significance level of 0.05 (i.e., H_0 is rejected when $p < 0.05$).

The second kind of response variable was the time elapsed before an event occurred, where the first event of interest was the transformation into pupa and the second event of interest was death before transformation into pupa. The survival analysis with the Cox proportional hazard model was used to compare the time elapsed before each event occurred, between the two food groups. The model accounted for censored data. When analyzing the amount of time before the transformation into pupa, death was treated as censoring in the survival analysis. When analyzing the amount of time before death, the transformation was treated as censoring. The rate of transformation or death was modeled as $h(t) = \gamma_0 + \gamma_1 \times$ group, where group is the dummy variable (0 for wheat bran and 1 for expanded polystyrene). The statistical hypothesis test was formulated as H_0: $\gamma_1 = 0$ versus H_1: $\gamma_1 \neq 0$ at a significance level of 0.05.

All statistical analyses were performed in R Version 4.3.0 (R Core Team, 2023), the lme4 and lmerTest packages were used for the mixed-effects model (Bates et al., 2015; Kuznetsova et al., 2017), and the survival package was used for the survival analysis (Therneau, 2023; Therneau and Grambsch, 2000).

Results

The average weight on the first day was 183.8 mg (95% CI: 170.4-197.2) for the bran group and 178.5 mg (95% CI: 165.1-191.9) for the expanded polystyrene group. The estimated difference -5.3 mg (95% CI: -27.5-16.9) was not statistically significant (p = 0.597). Using the daily weight measurement, the mixed-effects model estimated that the expected weight increases by 2.09 mg per day (95% CI: 1.68-2.49) for the bran group and 0.68 mg per day (95% CI: 0.025-1.34) for the expanded polystyrene group. The difference in growth rate (the slope of the average weight per day) was significantly different ($p < 0.001$), and it is presented in Fig. 3.

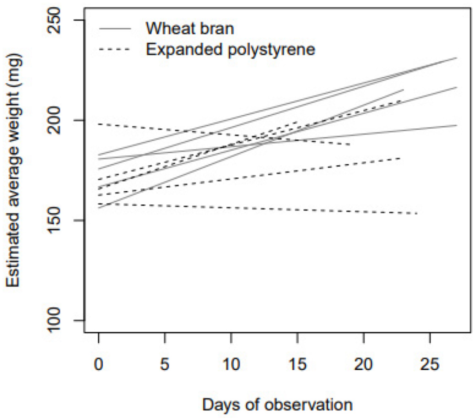

Fig. 3. Estimated average fresh weight of 10 experimental units (20 mealworms per container on the first day) with respect to day. The estimated regression lines (overall average weight) are 169.5 + 2.086 × days for the wheat bran group and 170.2 + 0.685 × days for the expanded polystyrene group.

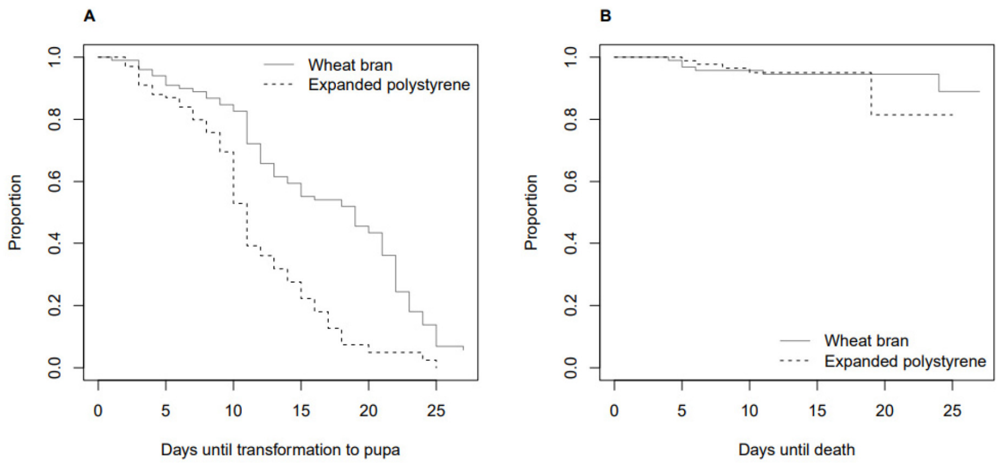

Fig. 4. Days to transformation into pupa (A) and days to death, if died before transformation into pupa (B).

The Cox proportional hazard model estimated that, until the mealworms transform into pupae, the expanded polystyrene group is about 2.56 times more likely to transform into pupae at any point in time when compared to the Bran group (95% CI: 1.88-3.49). The proportional hazard assumption was satisfied for the event of transformation (p = 0.57) and for the event of death (p = 0.20). Fig. 4A shows that the transformation into pupa occurred faster in the expanded polystyrene group when compared to the bran group ($p < 0.001$). Fig. 4B shows that death before transformation into pupa was rare, and the survival rate was similar between the two groups ($p = 0.786$).

Discussion

The weight gain of the mealworms that consumed expanded polystyrene was lower when compared to the mealworms that consumed wheat bran (Fig. 3), and the survival rate was similar between the two groups (Fig. 4B). It was reported that the survival rate of mealworms that consumed wheat bran was over 90% (Peng et al., 2020; Wang and Tang, 2022; Zhong et al., 2023), and the data observed in this study were consistent with the data reported in literature. Furthermore, the studies of Zielińska et al. (2021) and Nukmal et al. (2018) reported that expanded polystyrene consumption did not affect the health status of mealworms. This study showed that, although expanded polystyrene consumption slowed the weight gain in mealworms, it did not fatally affect their growth and survival, and the data showed that it can accelerate the time to transformation into pupa. To this end, expanded polystyrene can be an alternative food source for mealworms, and mealworms can be used to reduce expanded polystyrene waste in a sustainable and eco-friendly way.

Zhong et al. (2023) reported that the mealworms that consumed only plastic food experienced negative effects on their development, such as delaying or preventing pupation. In this study, however, the mealworms that consumed expanded polystyrene transformed into pupa faster than those that consumed wheat bran (Fig. 4A). This different result may be due to factors such as the timing of providing expanded polystyrene, air temperature, relative humidity, container size, and population density. Wang and Tang (2022) kept mealworms (developmental stage: instar 5－7) in the breeding room at a temperature of 26 ± 1°C and a relative humidity of 65% ± 5%. In their study, three hundred larvae were randomly divided into two groups (polystyrene foam group and wheat bran control group), the first group was fed with a 1.0 g polystyrene foam blocks (1.5－2.0 cm thickness), and the second group was fed with wheat bran as a sole diet. If the goal is to use mealworms as a food source, a combination of expanded polystyrene and wheat bran may be a compromise for balancing the growth rate and the time until transformation into pupa, and the combination improved the plastic consumption rate of mealworms (Wang and Tang, 2022). As such, an extension of this study can be finding an optimal combination of expanded polystyrene, wheat bran, and other foods.

Expanded polystyrene with low density and low hardness was used in our study. It is a limitation of this study which could not determine the mealworms' normal growth, development, and ability to biodegrade when consuming other types of plastic. Yang et al. (2022) investigated mealworms that consumed polyethylene (PE) of various molecular weights and crystallinity, and their results showed that PE with low molecular weight and low crystallinity was consumed and biodegraded easily. According to Gu (2023), historical damage was observed in epithelial cells in the hindgut of the mealworms after consuming PE, resulting in the epithelial layer being about 33 μm wide, but the damage by PE consumption was less severe when compared to another type of plastics consumption. In contrast, the most histological damage was observed in epithelial cells of the hindgut after the mealworms consumed polyurethane (PU), where the thickness of the epithelial layer was 38 μm. Further studies are needed to investigate the effects of plastic types with various molecular weight and crystallinity on the growth and development of mealworms.

Our data showed that expanded polystyrene can sustain the life of mealworms when compared to the wheat bran. Farmers can reduce the cost of raising mealworms by recycling expanded polystyrene instead of feeding wheat bran which is more expensive. Additionally, the excrement converted into organic matter by decomposing expanded polystyrene can be used as plant fertilizer or feed, making agriculture more sustainable.

Mealworms are insects that have recently been in the spotlight, not only as an insect which consumes plastic wastes, but also as an edible insect. Zielińska et al. (2021) reported that the mealworms that consumed expanded polystyrene had lower fat (24.05 ± 0.55%) and carbohydrate contents (2.95 ± 0.15%) but higher protein (48.66 ± 0.92%) and ash contents (4.81 ± 0.22%) than the mealworms that consumed conventional food, and no significant differences in nutrient composition were observed in the super mealworms (*Zophobas morio*). According to this data, mealworms fed with expanded polystyrene can be an alternative source of high-protein and low-calorie products. However, in order to use mealworms as food, more studies are needed on the edibility of mealworms that have consumed expanded polystyrene from the perspectives of safety and health. The larval period of mealworms is typically about 90 to 120 days, and one limitation of this study is the short experiment period (25 days). Though this study was long enough to detect the difference in growth rate and development rate between the two groups, any potential long-term effect of feeding expanded polystyrene could not be studied.

In summary, the mealworms that consumed expanded polystyrene showed slower growth (measured by weight), faster development (measured by time to transformation into pupa), and similar survival rate when compared to the mealworms that consumed wheat bran. To this end, after confirming the long-term effect, mealworms can be a potential solution for reducing expanded polystyrene waste while providing nutrients to humans. However, there are various types of plastics, and future studies are needed to determine whether or not mealworms can consume all types of plastic and show similar results as shown in this study.

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발포폴리스티렌이 *Tenebrio molitor*의 성장 및 발달 기간에 미치는 영향

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적 요. 많은 연구에 따르면 *Tenebrio molitor*은 유충 단계에서 플라스틱을 섭취할 수 있다고 보고되었다. 이 연구의 목적은 *T. molitor* 유충의 성장과 발달에 발포폴리스티렌 섭취가 미치는 영향을 조사하는 것이다. 밀기울을 섭취한 유충의 성장률은 발포폴리스티렌을 섭취한 유충의 성장률보다 더 좋았고($p < 0.001$) 발포폴리스티렌을 섭취한 유충의 번데기로 전환되는 기간은 밀기울을 섭취한 유충의 번데기로 전환되는 기간보다 더 빨랐다($p < 0.001$). 하지만 두 처리구간 생존율은 유의미한 차이가 없었다($p = 0.786$). 이 결과에 따르면 발포폴리스티렌을 섭취한 유충은 체중 감소와 짧은 발육기간이 특징이지만 생존하는 것에는 문제가 없었다. 따라서 우리는 *T. molitor*가 플라스틱 폐기물의 지속 가능하고 친환경적인 제거를 위한 주요 자원이라는 결론을 내렸다.

추가 주제어 : 평균 체중, Cox proportional hazard model, 밀웜, 플라스틱 쓰레기, 번데기