



Biodegradation of polyethylene (PE) microplastics by mealworm larvae: Physiological responses, oxidative stress, and residual plastic particles

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ABSTRACT

Biodegradation of petroleum-derived plastics in mealworms has been identified with different plastic products. To date, however, the responses of mealworm larvae to plastic biodegradation have received comparatively little attention. Herein, we tested the physiological responses of mealworm larvae after biodegradation of polyethylene (PE) microplastics and evaluated the impact of supplementing co-diet bran on the PE biodegradation and mealworm homeostasis. Significant weight loss, biomass reduction, and decreased survival rates were found for the PE-fed larvae after the six-week test. The efficient removal of PE, molecular weight reduction of residual polymers, and thermal decomposition signals demonstrated the depolymerization and biodegradation. Supplementing bran significantly enhanced the biomass levels and the survival of larvae but disturbed the PE removal in the intestinal tracts. The frass (excrement) from the larvae fed with PE and PE + bran feedstocks both contained undigested residual microplastics of different sizes with no accumulation of nanoplastics. Additionally, higher levels of reactive oxygen species, antioxidant enzyme activities, and lipid peroxidation were detected for larvae fed with pure PE, whereas the oxidative stress levels could be mitigated by supplementing co-diet bran. These results provide methodological insights into assessing the physiological responses of invertebrates after plastic ingestion or under micro(nano)plastic exposure.

1. Introduction

Persistent plastics are the major environmental pollutants of our time (Allen et al., 2022; Amaral-Zettler et al., 2020; Tournier et al., 2020). The plastic production is still growing rapidly with the escalating market demand for low-cost commodity thermoplastic polymers (Allen et al., 2022; Nelson et al., 2022), among which polyethylene (PE) polymer, chemically expressed as “[CH₂ – CH₂]_n”, makes up the largest market share (i.e., 36%) (PlasticsEurope, 2022). Commercial PE products, including high, low, and linear-low density PE (HDPE, LDPE, and LLDPE), are produced by given molding processes (e.g., injection, extrusion, rotation, thermoforming, etc.). The desired physical properties make PE products ideal for diverse applications, ranging from food packaging and sterile medical devices to construction uses (Chamas et al., 2020). Nevertheless, the chemical inertness, macromolecular

structure, and hydrophobicity of PE polymers also influence their biodegradability, which makes them particularly difficult to attack by microorganisms and enzymes after escaping into ecosystems. At current growth rates, plastic waste accumulation in the natural environment and landfills is projected to reach 12,000 million metric tons by 2050 with continuous inputs (Geyer et al., 2017). Plastic waste not only alters the bio-function and biodiversity of ecosystems, but it also endangers human health and food security (Amaral-Zettler et al., 2020; Li et al., 2021; Nelson et al., 2022).

Upon release into the environment, the plastic residue undergoes mechanical, thermal, and chemical fragmentation (e.g., weathering, photolysis, and microbial decomposition), leading to the production of smaller plastic debris such as micro(nano)plastics (MNPs). These MNPs are bioavailable to a wide range of soil and aquatic organisms (e.g., land snails, earthworms, groupers, nematodes, etc.) and are distributed in

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numerous compartments throughout the natural environment. Further, MNPs can cause physical damage to biological tissues (such as intestinal and fat tissues), induce oxidative damage, and trigger inflammatory responses in living organisms even further (Lin et al., 2022; Liu et al., 2022; Wang, L. et al., 2022; Wang, Q. et al., 2021; Wang, X. et al., 2020; Zhang et al., 2022). They can also act as vectors for various pollutants (sulfamethoxazole, phenanthrene, heavy metals, etc.), releasing toxic compounds, bioaccumulating in organisms, and disrupting food webs (Sun et al., 2021, 2022; Wang, H. et al., 2022). As a result, the hazards of exposing creatures to environments containing MNPs have emerged as a major environmental concern.

A promising and environmentally friendly strategy to overcome the accumulation of plastic waste is to depolymerize and biodegrade plastics *in vivo* via biological and enzymatic approaches (Tournier et al., 2020; Wei and Zimmermann, 2017; Yang, S.-S. et al., 2022). In recent years, researchers have discovered that several insect species within the darkling beetle (Coleoptera: Tenebrionidae) and pest moth (Lepidoptera: Pyralidae) families can voluntarily masticate natural or human-made polymers, fragment macro-scale plastic waste into micron-scale particles, and obtain carbon sources and energy from plastic degradation products and intermediates. Of these, the most studied species is yellow mealworms (larvae of *Tenebrio molitor*, Coleoptera: Tenebrionidae), which are an ideal model organism to investigate plastic biodegradation because of their rapid growth rates, wide commercial availability, and remarkable plastic biodegradation capacity (Brandon et al., 2018; Lou et al., 2021; Peng et al., 2023; Yang, L. et al., 2021; Yang, S.-S. et al., 2021; Yang, S.-S. et al., 2022). Up to 70% of the tested plastics can be digested and removed in about 12 h after passage through the larval intestines when the mealworm larvae are fed LDPE foam products as their exclusive diet, suggesting a highly-efficient synergistic system to biodegrade PE and an appealing approach to convert plastic waste into valuable insect biomass (Peng et al., 2021, 2023; Sanchez-Hernandez, 2021). Mealworm larvae are also commercially reared to produce sustainable food proteins and animal fats for the livestock industry and human consumption in Europe, Asia, and America (Bovera et al., 2015; Hong et al., 2020; Thévenot et al., 2018).

Recently, digestive biofragmentation of ingested plastics into MNPs has been discovered in the gastrointestinal systems of a wide range of organisms, including Antarctic krill, snails, and earthworms (Dawson et al., 2018; Kwak and An, 2021; Song et al., 2020; Wang, L. et al., 2021). Similarly, the rapid biodegradation of plastics (e.g., PE products) in mealworm larvae includes plastic biofragmentation processes by mealworm mouthparts and intestines (Pivato et al., 2022; Sanchez-Hernandez, 2021). Smaller-sized MNPs might be generated and accumulated during these processes, but the understanding of the physiological responses of mealworm larvae to plastic biodegradation and the influence of potential MNP exposure on plastic-degrading invertebrates is currently very limited. We hypothesize that (a) the ingestion and degradation of microplastics may lead to a certain degree of negative physiological responses in plastic-degrading mealworm larvae (*Tenebrio molitor*), which are similar to those of invertebrates without plastic-degrading abilities (e.g., Antarctic krill, snails, and earthworms); (b) oxidative stress and damage will still be induced in the mealworm larvae after the ingestion of microplastics, despite their extraordinary capacity for plastic biodegradation; and (c) residual undigested plastic particles will be retained and accumulate in the larval excrement as a result of biofragmentation and incomplete digestion.

In this work, we present extensive data on the physiological responses and antioxidant performance of mealworm larvae after ingestion of PE microplastics. We identified the consumption, removal, and rapid biodegradation of ingested PE microplastics by mealworm larvae within 6 weeks. We also tested the physiological responses of the mealworm larvae after PE biodegradation, including the survival rate, average weight, and biomass levels (i.e., water, total carbohydrate, fat, and protein content). The indicators associated with oxidative stress, including reactive oxygen species (ROS), antioxidant enzyme activities,

and lipid peroxidation, were systematically investigated. The effects of the co-diet bran on enhancing mealworm survival and mitigating oxidative stress were elucidated to provide critical information. Further, particle-analyzing methods were applied to determine the size distribution of residual plastic particles derived from biofragmentation and incomplete digestion. Our study highlights the need for more experimental studies on the physiological responses of plastic-degrading invertebrates exposed to micro(nano)plastics and the environmental impact of plastic biodegradation residue.

2. Material and methods

2.1. Plastic feedstocks and chemicals

Commercially available PE microplastics (color: white; high purity) with the weight-average molecular weight (M_w) of 152.5 ± 6.6 kDa were purchased from Zhonglian Petrochemical Co. (Guangdong, China) (Fig. S1). These PE microplastics belong to the medium molecular-weight PE polymers, which have been demonstrated to be capable of being depolymerized by mealworm larvae (Yang, S.-S. et al., 2022). Before the test, the PE microplastics with powder sizes lower than ~ 300 μm were obtained by sieving using stainless steel mesh in order to fit the mouthpart of the mealworm larvae and facilitate particle ingestion. Pre-test characterizations demonstrated that the PE materials were additive- and plasticizer-free with external standard methods (Table S1). Food-grade jelly, an adhesive to make the PE + bran mixture feedstock, was obtained from Gurado Food Co. (Shanghai, China). The additional reagents, including 1,2,4-trichlorobenzene (1,2,4-TCB, GC grade purity >99.9%), were purchased from Sigma-Aldrich Co. (Shanghai, China).

2.2. Preparation of plastic degradation

Mealworm larvae (larval instar: 3–4; initial average weight: 73.8 ± 1.3 mg/lava) and their regular food (co-diet bran; C: H: O: N ratio of 40.1: 7.7: 47.3: 3.4) were purchased from Binzhou Mealworm Breeding Co. (Shandong, China). The mealworm larvae were in rapid growth periods and thus sensitive to external environmental stress. This mealworm source has also been demonstrated to be capable of degrading various plastics, including polystyrene (PS), polyvinyl chloride (PVC), polypropylene (PP), and polylactic acid (PLA) (Peng et al., 2020a; Peng et al., 2021; Peng et al., 2022; Yang, S.-S. et al., 2020; Yang et al., 2018b).

In this study, four experimental conditions were prepared ($n = 3$; 100 larvae for each replicate): a) PE, b) PE + bran, c) bran, and d) unfed (starved). The jelly was used to make the PE + bran feedstock (Text S1), with the mass ratio of PE microplastics versus bran at 1:5 (w/w). To study the growth, physiological responses, and biodegradation performance of mealworm larvae, three replicates are well accepted among scientists, including entomologists, biologists, and environmental scientists, when a large number of larvae is used (e.g., 50 or more) (Brandon et al., 2020; Brandon et al., 2018; Ding et al., 2023; Lou et al., 2020; Lou et al., 2021; Yang, L. et al., 2021; Yang et al., 2018a; Yang et al., 2019; Yang, S.-S. et al., 2022; Yang, Y. et al., 2020). The proportion of PE microplastics to bran (1:5) was set based on previously published studies (Peng et al., 2021; Wu and Criddle, 2021; Yang et al., 2018a, 2018b), which could help achieve relatively high plastic consumption and sustainable mealworm growth during experiments. The main component of the jelly adhesive is digestive carrageenan, and the proportion of the jelly adhesive was low. According to our observations, the impact of the added jelly adhesive on the survival and development of mealworms was supposed to be negligible. The larvae chosen at random were incubated in glass containers with smooth insides (15 cm \times 15 cm \times 6 cm), which were kept in a constant temperature incubator (25 °C and 70% humidity). Each glass container was initially prepared with 100 depurated larvae and 1.0 g of corresponding feedstock. During the 6-week test period, when the feedstock in the container was almost

depleted, another 1.0 g was provided to make the feedstock continuously available. The dead larvae and exoskeletons were removed from the glass container every day to prevent cannibalism and any possible intraspecific diseases, as observed in the breeding plant (Brandon et al., 2018; Peng et al., 2019). The experimental setup and methods referred to previously established protocols (Wu and Criddle, 2021).

2.3. Plastic consumption, removal, and biodegradation

Total frass yield and plastic consumption were determined when the recently added feedstock was completely consumed near day 42. The calculation procedures were documented in Text S2. To collect enough frass for characterizations, more glass containers were prepared under the same incubation conditions. Extraction experiments were conducted to measure the water-extracted fraction (C_w), ethanol-extracted fraction (C_e), and 1,2,4-TCB-extracted fraction (C_t) of the frass residue following the procedures established in previous studies (Peng et al., 2021; Peng et al., 2022; Yang, S.-S. et al., 2021). The PE removal was further estimated based on the frass yield, the weight of total plastic consumption, and the C_t value (Text S2).

High-temperature gel permeation chromatography (HT-GPC, 1260 Infinity II HT GPC, Agilent Technologies Inc., U.S.A.) analysis was performed to identify the biodegradation of the ingested PE microplastics. In this study, we only conducted HT-GPC analysis to verify the depolymerization and biodegradation because biodegradation of commercial PE products by mealworms has been demonstrated via other analyses in previous studies (Brandon et al., 2018; Yang, L. et al., 2021; Yang, S.-S. et al., 2022). The operational procedures were documented in Text S3.

2.4. Physiological performance of mealworm larvae after PE biodegradation

The survival index of larvae, including the survival rate (SR) and average weight (AW) of mealworms, was measured by week during the 6-week test period (Text S4). The changes in biomass levels of mealworm larvae, including the water, total carbohydrate, fat, and protein content, were determined after the 6-week period. The tests of physiological indexes were all performed in triplicate ($n = 3$). The mealworm larvae were randomly selected, euthanized, crushed under liquid nitrogen conditions, and prepared for testing. The methods were referred to food safety testing standards and documented in the supporting information (Text S5) (Peng et al., 2022; Turck et al., 2021; Zhang et al., 2019). The indexes of physiological responses, including the SR, AW, water, total carbohydrate, fat, and protein content, were further normalized to evaluate the overall performance of the mealworm larvae versus control (Text S6), with the results presented using the star map.

2.5. Reactive oxygen species, antioxidant enzymes, and lipid peroxidation levels in mealworm larvae

At the end of the 6-week test, the levels of reactive oxygen species (ROS) in the larval bodies were determined using an assay kit from Sigma-Aldrich Co. (Shanghai, China). In brief, the mealworm larvae were randomly selected, carefully cleaned, and ground by a glass grinder with homogenate buffer, followed by cell disruption using a cell disruptor. The homogenate was then centrifuged, and the supernatant was incubated at 37 °C to prepare for bicinchoninic acid protein quantification and ROS fluorescence detection using a fluorescence microplate reader (VICTOR Nivo Multimode microplate reader, PerkinElmer, U.S.A.) (Liu et al., 2022; Yang, L. et al., 2022). The lipid peroxidation level indicated by malondialdehyde (MDA) and antioxidant enzyme activities, including superoxide dismutase (SOD), glutathione-S-transferase (GST), and catalase (CAT), were determined using enzyme assay kits from Abbkine Scientific Co., Ltd. (Shanghai, China). The experimental procedures followed the instructions of the manufacturers (Text S7). The overall antioxidant performance of the

mealworm larvae in response to PE biodegradation was further calculated and evaluated, with the results presented using the star map.

2.6. The size distribution of residual particles

To identify the size distribution of the residual plastic particles after digestion and biodegradation, the PE MNPs in the frass samples were recovered. In brief, the frass was first digested using a 30% w/w H₂O₂ solution with gentle heating (70 °C) for at least 12 h to thoroughly digest the organic matters (Peng et al., 2022; Song et al., 2020). After the digestive solution was clear, the mixture was filtered through a polyamide filter (pore size: 100 nm) to collect the residual plastic particles. The sizes of the residue plastic particles (nominally >100 nm) were measured using laser particle analyzer (Mastersizer 3000, Malvern Panalytical, UK) based on the polymer volume with a measurement range of 0.01–3500 μm.

2.7. Statistics analysis

In this study, the differences among groups were assessed by statistical analysis of variance (ANOVA) and student's t-test with Tukey's correction. The significance level was set at 0.05. The statistical analysis was performed on Origin Pro 2021 software (OriginLab Corp., MA, U.S.A.). All error values were reported as the mean ± standard deviation of experimental replicates.

3. Results and discussion

3.1. Plastic consumption, removal, and biodegradation

The PE + bran-fed mealworm larvae were observed to be more aggressive in ingesting the feedstock than the PE-fed larvae (Fig. S2). It is likely that the co-diet bran increased the affinity of mealworm larvae for the PE feedstock. The average specific plastic consumption rates (SPCRs; i.e., the mass of plastics consumed per 100 live larvae per day) of the PE-fed larvae and PE + bran-fed larvae were 43.9 ± 2.0 and 67.7 ± 2.3 mg PE 100 larvae⁻¹ d⁻¹, respectively, during the test period (Fig. 1a and Table S2), indicating that the addition of wheat bran as a co-diet significantly ($p < 0.05$) increased the PE consumption of the mealworm larvae. Similar observations were reported by Brandon et al. (2018) when plastic-fed mealworms received bran as a cofeed (Brandon et al., 2018). This might be due to the extra carbon sources and energy provide by bran digestion, thereby stimulating mealworm larvae to consume the PE feedstock. Nevertheless, the SPCR of the PE + bran-fed larvae in our study (67.7 ± 2.3 mg PE 100 larvae⁻¹ d⁻¹) was lower than the SPCR of mealworm larvae fed on a PLA (bio-based degradable plastic) plus bran diet (i.e., >125 mg PLA per 100 larvae d⁻¹) reported in previous research (Table S2) (Peng et al., 2021). The difference revealed that bio-based degradable plastics (e.g., PLA) were more favorable to the plastic-degrading mealworm larvae than non-degradable plastics (e.g., PE, PS, PVC, etc.). The frass yield, an indicator of feedstock digestibility (Wu and Criddle, 2021), was significantly different ($p < 0.05$) between mealworm larvae fed with pure PE microplastics and the PE + bran mixed feedstock (Fig. 1a), whereas the frass yield of the PE + bran larvae was not statistically different ($p > 0.05$) from the bran-fed larvae (0.61 ± 0.01 versus 0.58 ± 0.02). The results of the frass yield suggested that pure PE powders were relatively difficult for the mealworm host and gut microbiome to digest. Future research is required to identify the reason and determine whether the linear structure or crystallinity of PE polymers affects the digestibility in the recently reported plastic-degrading insect larvae, including *Zophobas atratus* (Peng et al., 2022), *Tenebrio obscurus* (Peng et al., 2019; Yang, S.-S. et al., 2021), and *Galleria mellonella* larvae (Bombelli et al., 2017; Lou et al., 2020; Sanluis-Verdes et al., 2022; Wang, S. et al., 2022).

Extraction experiments were performed to determine the water-, ethanol-, and 1,2,4-TCB-extracted fractions of the frass of mealworm

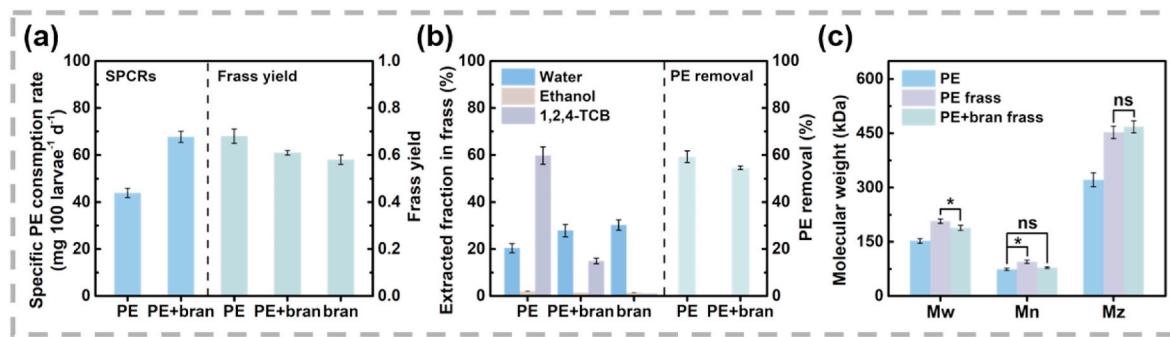


Fig. 1. Consumption, removal, and biodegradation of ingested PE microplastics by mealworm larvae within the 6-week period. (a) SPCRs and frass yields of mealworm larvae fed with unlimited feedstocks under different diet conditions. (b) Extracted fractions in the frass, and PE removal (%) by the mealworm larvae with and without bran supplementation. (c) Changes in M_w , M_n , and M_z of the PE microplastics before and after biodegradation by the mealworm larvae. The results are represented with the mean \pm standard deviation ($n = 3$). The symbols * and ns denote a statistically significant difference ($p < 0.05$) and no significant difference, respectively.

larvae and the PE removal (Fig. 1b). As shown in Fig. 1b, the frass derived from the PE-fed and PE + bran-fed larvae both contained a relatively high level of water-extracted fraction (i.e., $20.4 \pm 1.9\%$ and $27.9 \pm 2.6\%$, respectively) as observed for the frass from larvae fed with normal feed bran, implying that hydrophilic products and water-soluble compounds were generated during the PE digestion. Interestingly, the PE + bran-fed larvae had a slightly lower PE removal efficiency than the PE-feed larvae ($54.6 \pm 0.7\%$ versus $59.3 \pm 2.5\%$) (Fig. 1b), which could be attributed to competitive digestion between the components in the mixed feedstock. The results could also suggest that over 54% of the ingested PE microplastics were efficiently digested and removed after passage through the larval intestine with a retention time of less than 24 h. However, this value was significantly lower than the PLA removal efficiency by yellow mealworms (i.e., $>81.5\%$) in 4–5 larval instars, as reported in previous studies (Peng et al., 2021). These findings further validated that mealworm larvae derived carbon sources and energy from non-degradable plastics (e.g., PE) less efficiently than from bio-based degradable plastics (e.g., PLA).

HT-GPC analysis was used to characterize the depolymerization of the PE polymers and determine the effect of co-diet bran on polymer biodegradation. For the mealworm larvae fed with pure PE microplastics, the residual PE polymer extracted from frass exhibited a significant increase ($p < 0.05$) in the number-average molecular weight (M_n), weight-average molecular weight (M_w), and size-average molecular weight (M_z) compared to those of the original PE polymer, i.e., by $28.3 \pm 5.8\%$, $35.6 \pm 3.7\%$, and $40.6 \pm 5.3\%$, respectively (Fig. 1c and Table 1), demonstrating the depolymerization and biodegradation. The M_n , M_w , and M_z provide information about the lower, average, and higher molecular weight fractions of the polymer, respectively (Wu and Criddle, 2021; Yang, L. et al., 2022; Yang, S.-S. et al., 2022). Therefore, the increase in the M_n , M_w , and M_z values indicated that the PE polymers with lower molecular weights were depolymerized more efficiently and rapidly than those with higher molecular weights. The relatively slower biodegradation of PE with high molecular weights in the larval gut microenvironment triggered random internal scissions and yielded heterogeneous mid-chain and/or oligomeric polymers, thereby resulting in the accumulation of residual polymer macromolecules with increased molecular weights (Inderthal et al., 2021; Peng et al., 2020b). It is worth noting that the depolymerization pattern of PE by the mealworm larvae

in this study was different from those observed in PS, PVC, and polypropylene (PP) biodegradation in previous research, which might be associated with the complex physiochemical properties of PE polymer (Brandon et al., 2018; Peng et al., 2020a; Yang, L. et al., 2021; Yang, S.-S. et al., 2020). Additional research is needed to identify the impact of polymer properties (e.g., crystallinity degree, branching structures, etc.) on the depolymerization and biodegradation processes of PE polymers.

By contrast, the depolymerization of the ingested PE polymers was significantly perturbed in the PE + bran-fed mealworm larvae (Fig. 1c). Notably, the M_n increase was minor (i.e., $6.1 \pm 2.8\%$) compared to the increases in M_w and M_z values ($23.7 \pm 4.7\%$ and $45.4 \pm 5.2\%$) (Table 1). The result might be due to that the gut microbiota responsible for degradation of polymers of low and medium molecular weights were influenced by the digestion of co-diet bran. The niches of specific plastic-degrading functional microbes were perturbed, thereby causing different molecular weight changes and biodegradation efficiency within the gut microenvironment of mealworm larvae. Overall, except as a global pollution problem, waste polymer products are also a low-cost, carbon-rich, and easily obtained feedstock. Deciphering the specific plastic-degrading functional microbes and related enzymatic pathways is critical from an environmental standpoint for developing sustainable strategies to realize recycling and upcycling of plastic waste and address the entire plastics dilemma, which requires additional in-depth research in the future.

3.2. Physiological responses of mealworm larvae to PE biodegradation

The SR curves were significantly different for the mealworm larvae under different diet conditions based on Kaplan-Meier (K-M) survival analysis (Fig. 2a). When the feedstocks were provided at libitum, the overall survival of mealworm larvae fed on the PE + bran diet was relatively normal in comparison to the mealworm larvae fed on the pure PE diet but still inferior to that of bran-fed larvae (Fig. 2a). The final SR of the larvae on the PE + bran diet was $87.3 \pm 1.5\%$, while the bran-fed larvae had a SR of $95.3 \pm 1.2\%$ (Fig. 2c). These results indicated that biodegradation and mineralization of PE polymers caused a negative impact on larval survival even though the normal feed bran was supplemented. Previous studies by Lou et al. (2021) and Yang S.-S. et al. (2022) indicated that biodegradation of commercial PE products in

Table 1

Characterization of the depolymerization of PE microplastics after biodegradation. The M_w , M_n , and M_z values are presented as the mean \pm standard deviation.

	M_w (kDa)	M_w increase (%)	M_n (kDa)	M_n increase (%)	M_z (kDa)	M_z increase (%)
PE microplastics	152.5 ± 6.6	—	73.9 ± 3.1	—	321.9 ± 19.2	—
PE frass	206.8 ± 5.7	35.6 ± 3.7	94.8 ± 4.3	28.3 ± 5.8	452.6 ± 16.9	40.6 ± 5.3
PE + bran frass	188.7 ± 7.1	23.7 ± 4.7	78.4 ± 2.1	6.1 ± 2.8	468.1 ± 16.7	45.4 ± 5.2

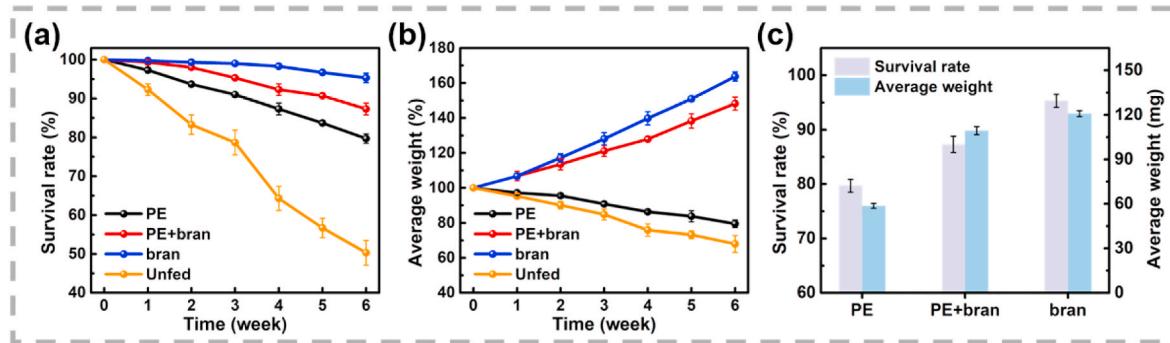


Fig. 2. The physiological response of the mealworm larvae under different diet conditions (PE, PE + bran, bran, unfed). The changes in (a) the SR (%) and (b) AW (%) of the mealworm larvae over the 6-week period; (c) the final SR (%) and AW (mg) of the mealworm larvae with unlimited feedstocks after 6 weeks of continuous feeding.

mealworm larvae could generate different degradation products and intermediates belonging to long-chain acids, esters, ketones, and phenolic compounds (Lou et al., 2021; Yang, S.-S. et al., 2022). Therefore, the influence of PE biodegradation on larval survival and physiology could be associated with the toxicity derived from the degradation intermediates and products, the physical damage caused by the plastic particles within the larval gut microenvironment, or other unknown reasons.

The AW is a direct indicator of larval growth and could be used to show the physiological responses of mealworm larvae in rapid growth periods to PE biodegradation. As expected, the AWs of the bran-fed larvae and PE + bran-fed larvae both increased progressively over time and were not statistically different in the first three weeks, but the growth rate diverged in the later test period (Fig. 2b). After 6 weeks of continuous feeding, the accumulated AW increase of the bran-fed larvae was $63.7 \pm 2.6\%$, while that of the larvae on the PE + bran diet was $48.2 \pm 3.7\%$ (Fig. 2c). By contrast, the PE-fed larvae underwent weight loss and growth inhibition during the test period, demonstrating that mealworm larvae could not subsist on the pure plastic diet (e.g., PE polymer) as an exclusive food and carbon source for the long term. However, it is worth mentioning that the final AW of the PE-fed larvae was still higher than that of the unfed larvae (Fig. 2b), suggesting that digestion of the non-degradable PE polymer supplied limited carbon sources for maintaining the larval metabolism and physiological function. These observations further confirmed that the co-feeding bran was indispensable for the mealworm larvae to achieve growth and complete the whole life cycle (Yang, L. et al., 2021; Yang, S.-S. et al., 2021).

Mealworm larvae are not only a highly efficient plastic-degrading

candidate, but they are also a sustainable source of food protein and animal fat for the livestock industry and human consumption (Bovera et al., 2015; Hong et al., 2020; Thévenot et al., 2018). However, previous studies on mealworm-mediated plastic biodegradation did not investigate the changes in the typical nutrient content of larval biomass after the plastic degradation and mineralization (Brandon et al., 2018; Lou et al., 2021; Yang, S.-S. et al., 2020; Yang, S.-S. et al., 2022; Yang et al., 2018b). In this study, the water, total carbohydrate, fat, and protein content of mealworm larvae were also tested as direct indicators to determine the physiological responses to PE biodegradation (Fig. 3). After 6 weeks of incubation, the larvae groups fed on PE + bran and sole bran diets showed no significant difference ($p > 0.05$) in the water content (i.e., $61.3 \pm 0.9\%$ and $62.2 \pm 0.7\%$, respectively), while that of the larvae fed with the pure PE feedstock (i.e., $59.1 \pm 0.7\%$) was slightly lower (Fig. 3a). This observation could suggest that the mealworm larvae fed with pure PE polymers were mildly dehydrated after a long period of continuous PE feeding. Further, the levels of total carbohydrate and fat content of the PE-fed larvae were also reduced to $4.5 \pm 0.2\%$ and $8.9 \pm 0.3\%$, respectively, after 6 weeks of continuous feeding (Fig. 3a and b). This could be due to the mealworm larvae not receiving sufficient energy and nutrients via biodegradation and mineralization of the ingested PE polymers, which was similar to the performance of great wax moth larvae (*Galleria mellonella* L.) during PE feeding (Lou et al., 2020). Since the PE polymer is devoid of key nutrients such as nitrogen source, vitamins, and trace elements (Yang et al., 2018a), the PE-fed mealworm larvae needed to continuously drain their energy reserves and stored nutrients to maintain metabolism and finish the PE biodegradation processes, resulting in final malnutrition, decreased AW, and

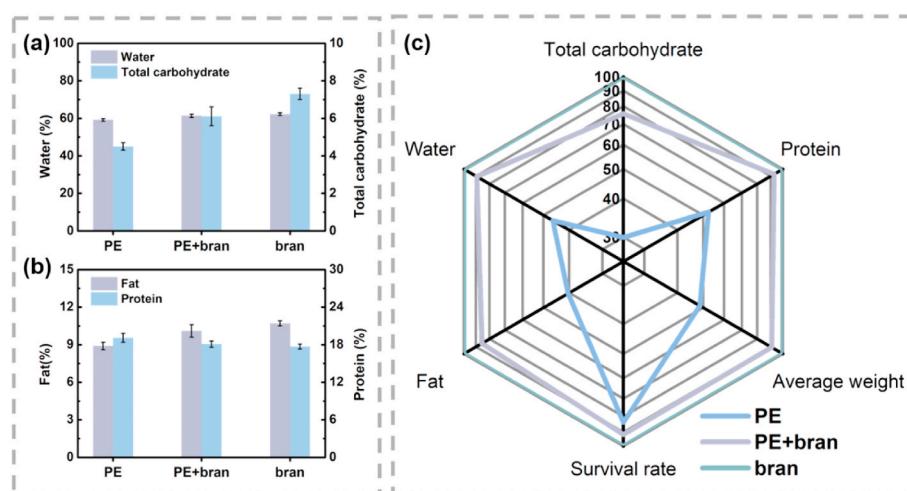


Fig. 3. Changes in biomass levels and comprehensive evaluation of the physiological performance of mealworm larvae to PE biodegradation. (a) The water and total carbohydrate content (%) of the mealworm larvae after 6 weeks; (b) the fat and protein content (%) of the mealworm larvae after 6 weeks; (c) the overall performance of mealworm larvae after 6 weeks of continuous feeding on different diets, including the SR, AW, water, total carbohydrate, fat, and protein content. The results are represented with the mean \pm standard deviation ($n = 3$).

low SR at the end of the period. In comparison, the fat content of the PE + bran-fed larvae ($10.1 \pm 0.5\%$) and the bran-fed larvae ($10.7 \pm 0.2\%$) was not significantly different (Fig. 3b), but the total carbohydrate content of the PE + bran-fed larvae was lower than that of the bran-fed larvae ($6.1 \pm 0.5\%$ versus $7.3 \pm 0.3\%$) (Fig. 3a). The ingestion of microplastics could cause intestinal blockage and/or physical damage to organisms, which would result in a decreased uptake of nutrients (Ouyang et al., 2021). Therefore, the slightly lower carbohydrate content of mealworm larvae fed with the PE plus bran mixed food could be attributed to intestinal blockage caused by undigested or incompletely digested plastic particles. Nevertheless, the biomass nutrient status of the PE + bran-fed larvae was relatively normal compared to that of the mealworm larvae fed with pure PE powders (Fig. 3). These findings confirmed that the plastic plus bran diet was more sustainable in the long run for mealworm larvae growth and plastic biodegradation.

On the other hand, the protein content of the PE-fed larvae ($19.1 \pm 0.7\%$) was comparatively higher than that of the PE + bran-fed ($18.1 \pm 0.5\%$) and bran-fed larvae ($17.7 \pm 0.4\%$) (Fig. 3b). The result could be due to the decomposition of total carbohydrate and stored fat content being of higher priority than that of the protein content, thus leading to the relative increased level of protein content in the mealworm larvae. Additionally, more enzymes that might be functional for PE biodegradation (e.g., hydrolase, oxidase, etc.) needed to be biosynthesized within the mealworm body to engage in digestion and biodegradation of the ingested plastics, thereby causing the protein content to increase within the mealworm larvae (Peng et al., 2022; Tschatzis et al., 2022).

The overall physiological responses of mealworm larvae to PE biodegradation were assessed, with bran-fed larvae serving as a control. The star map shows that the larvae responded differently with and without the co-diet bran supplementation during the test period (Fig. 3c). For the PE-fed mealworm larvae, the survival, growth, and biomass levels all shrank notably compared to the bran-fed larvae. In contrast, the mealworm larvae fed with PE + bran mixed feedstocks showed better physiological performance after 6 weeks of continuous PE feeding, although the level of total carbohydrate was still slightly lower than that of the bran-fed larvae (Fig. 3a). These results verified that the PE plus bran diet could be more sustainable for long-term PE biodegradation and carbon resource recovery for the mealworm larvae, but the digestion of PE would still cause mild negative impacts on the metabolic turnover, survival, and growth of mealworms.

3.3. The oxidative stress in mealworm larvae after plastic biodegradation

After 6 weeks of continuous feeding, the mealworm larvae showed distinct ROS levels under different dietary conditions (Fig. 4a). In

general, a high ROS level was observed in the PE-fed larvae (5.07×10^6 fluorescence intensity/mg protein), while the ROS level of the PE + bran-fed and bran-fed larvae was lower, i.e., 4.66×10^6 and 4.50×10^6 fluorescence intensity/mg protein, respectively. Previous research indicated that exposure to polystyrene microplastics (PS-MP) could induce adverse effects (physical damage, increased ROS levels, and toxicity) on soil invertebrates (*Eisenia fetida*) with no or poor plastic biodegradation capacity (Liu et al., 2022). This finding revealed that biodegradation of PE microplastics could also induce serious oxidative stress within mealworm larvae with the high capacity of plastic biodegradation. Excessive production of intracellular ROS could attack the cellular structure and deteriorate biomacromolecule function (e.g., membrane lipid), resulting in serious oxidative damage and metabolic dysbiosis (Shao et al., 2018).

We analyzed the changes in MDA content in the mealworm body to better understand the level of oxidative damage in mealworm larvae. As with the ROS level, the MDA content was significantly elevated ($p < 0.05$) for both mealworm larvae fed with PE and PE + bran feedstocks versus the bran-fed control (Fig. 4a), indicating that lipid peroxidation and oxidative damage occurred in the larval body. Similarly, ROS and MDA accumulation were also discovered in earthworms (*Eisenia fetida*) exposed to mulch film-derived PE microplastics, which influenced gene expression and caused DNA damage in tissues (Cheng et al., 2020; Wang, Q. et al., 2021). Oxidative damage could promote apoptosis and lead to the abnormal development of organisms (Zhang et al., 2021; Zou et al., 2020). Therefore, the physiological responses of the PE-fed larvae (i.e., SR, AW, and biomass levels) and the respective metabolic dysbiosis presented in this study (Fig. 3c) might be associated with the increased MDA level within mealworm larvae. Interestingly, the MDA content in the larvae fed on the PE + bran diet was lower than that of the PE-fed larvae (Fig. 4a). The result implied that the oxidative stress and lipid peroxidation derived from plastic ingestion and biodegradation could be effectively mitigated by bran supplementation.

To further examine the activity of antioxidant enzymes in mealworms against oxidative stress, the concentrations of typical antioxidant enzymes, including SOD, GST, and CAT, were determined after 6 weeks of feeding. Notably, the SOD concentration in the PE-fed larvae was about 82% higher than the bran-fed larvae, i.e., 105.65 ± 3.69 versus 57.94 ± 2.87 U/g fresh weight (Fig. 4b). As with the SOD level, the enzyme activities of GST and CAT were also enhanced in the larvae fed with PE as an exclusive food source (Fig. 4b–c). SOD is essential for the elimination of the superoxide anion (O_2^-), and GST scavenges metabolites of lipid peroxidation in organisms (Jia et al., 2017; Wu et al., 2011). These results demonstrated that the ingestion and biodegradation of PE polymers triggered defenses of antioxidant systems to scavenge free

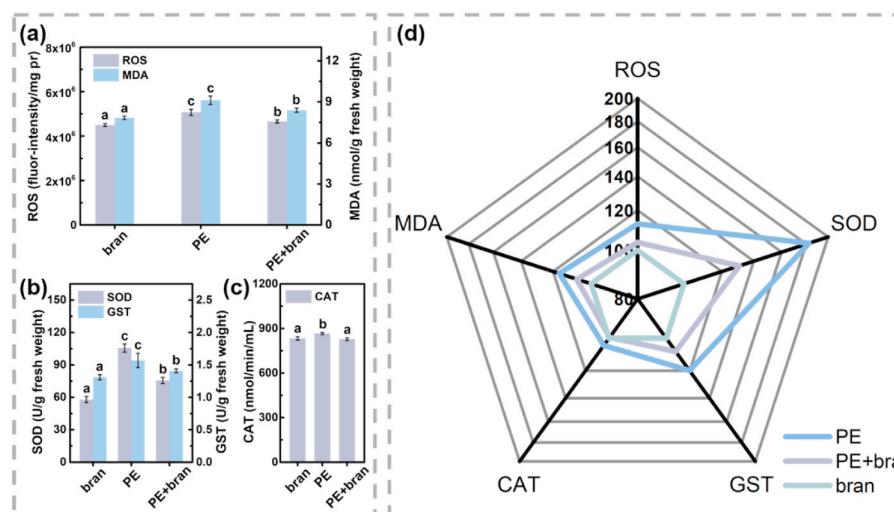


Fig. 4. Characterization of the antioxidant performance of mealworm larvae under different feeding conditions. (a) The levels of reactive oxygen species (ROS, fluorescence intensity/mg protein) and malondialdehyde (MDA, nmol/g fresh weight) in the larvae after 6 weeks; (b) the levels of superoxide dismutase (SOD, U/g fresh weight) and glutathione-S-transferase (GST, U/g fresh weight) in the larvae after 6 weeks; and (c) the level of catalase (CAT, nmol/min/mL) in the larvae after 6 weeks. (d) The overall antioxidant performance of mealworm larvae in response to PE biodegradation. The results are presented as the mean \pm standard deviation ($n = 3$). Different letters above the columns denote significant differences.

radicals and protect larval tissues from oxidative damage in mealworm larvae. However, the antioxidant enzymes could not counteract the ROS generation, leading to increased levels of oxidative stress in the mealworm larvae (Fig. 6). On the other hand, the enzyme activities of SOD, GST, and CAT in the PE + bran-fed larvae were all reduced as expected, compared to the mealworm larvae fed with pure PE microplastics, which was consistent with the result of the MDA level (Fig. 4).

Taken together, the overall antioxidant performance of mealworm larvae in response to PE biodegradation was shown in the star map (Fig. 4d). Global oxidative stress and damage were found for the PE-fed larvae, while the supplementation of co-diet bran could effectively alleviate the oxidative damage to close to the baseline, implying that plastics plus bran feedstocks caused less negative impact and toxicity to mealworm larvae during PE ingestion and biodegradation. Future studies should examine the effects of bran supplementation on mealworms, superworms, and waxworms from different geological sources using additional petro-based and bio-based plastics (e.g., PS, PP, PVC, PLA, etc.). It should be noted that the antioxidant performance tested in this study was performed using batches of entire mealworm larvae and therefore has little resolution at the organ level. The oxidative stress and responses in specific organs of mealworm larvae need further in-depth evaluation in future studies.

3.4. The size distribution of residual plastic particles

Residual plastic debris could be generated since the retention time of plastics in the gut of mealworms was limited (i.e., ~12 h) (Brandon et al., 2020; Yang, S.-S. et al., 2022). To elucidate the influence of bran supplementation on the size of plastic particles in the egested frass residue, we measured the size frequency distribution of residual PE particles using particle analyzing apparatus with a measurement range of 0.01–3500 μm.

After PE biodegradation, different sizes of residual PE particles (i.e., 5–300 μm) were generated for both PE-fed larvae and PE + bran-fed larvae with a wide particle size distribution (Fig. 5), which fell in the range of microplastics (100 nm–5 mm) (Wang, L. et al., 2021; Wang, L. et al., 2020). The absence of PE nanoplastics in the egested frass removed our concerns about the generation of nanoplastics during biodegradation by mealworm larvae. Due to the large specific surface area, nanoplastics could react with functional microorganisms and enzymes in highly efficient ways, resulting in the rapid removal of these

nano-sized particles in the gut microcosms with no accumulation. The finding is of scientific significance since nanoplastics pose more biological and environmental risks to organisms directly or indirectly in the environment, e.g., by interacting with biomolecules to inactivate their biofunction, increasing membrane permeability, and inducing inflammatory responses and cellular toxicity (Dawson et al., 2018; Li et al., 2021; Min et al., 2020; Sun et al., 2021, 2022; Taghavi et al., 2021; Wang, J. et al., 2020).

The volume percentage of residual PE particles reached a peak at a similar size of 143.897 μm and 126.652 μm, respectively, for the PE-fed and PE + bran-fed larvae groups (Fig. 5). However, a wider size-frequency distribution of residual particles was found in the frass of PE + bran-fed mealworm larvae, and submicron-sized plastic particles were discovered (i.e., 5–12 μm). This finding could suggest that the biofragmentation and biodegradation of PE were significantly perturbed by the digestion of co-diet bran in the gut microcosms, as indicated in the results of plastic removal and depolymerization (Fig. 1b–c). As a result, the residual plastic particles of submicron size finally accumulated in the frass of the mealworm larvae. Future studies should investigate the influence of co-diet supplementation on the biofragmentation and biodegradation of other major petrochemical plastics.

4. Conclusion

The results of this study first demonstrate that the removal and biodegradation of PE microplastics negatively influence the physiological performance, growth, and homeostasis of mealworm larvae (*Tenebrio molitor*). After 6 weeks of continuous PE feeding, significant weight loss, biomass reduction (water, total carbohydrate, fat, and protein content), and decreased survival rates were found for the mealworm larvae fed with pure PE microplastics. The efficient removal of PE (>54%), molecular weight reduction of residual polymers, and thermal decomposition signals demonstrated the depolymerization and biodegradation. However, oxidative stress and lipid peroxidation were induced in the mealworm larvae despite their remarkable capacity for PE removal and biodegradation (Fig. 6). Supplementing bran significantly enhanced the biomass levels and the survival of larvae but slightly disturbed the PE removal efficiency in the gut microenvironment. The frass (excrement) from mealworm larvae fed with PE and PE + bran feedstocks contained undigested residual microplastics of different sizes (>5 μm) with no accumulation of nanoplastics after PE biodegradation. Additionally, higher levels of reactive oxygen species, antioxidant enzyme activities (including SOD, GST, and CAD), and lipid peroxidation were detected for larvae fed with pure PE, whereas the oxidative stress levels could be effectively alleviated by supplementing co-diet bran. Our findings provided insights into assessing the physiological performance and function of invertebrates under environmental exposure to microplastics and nanoplastics.

CRediT authorship contribution statement

Bo-Yu Peng: Conceptualization, Methodology, Formal analysis, Investigation, Methodology, Writing – original draft. **Yazhou Xu:** Formal analysis, Methodology, Validation. **Ying Sun:** Formal analysis, Validation. **Shaoze Xiao:** Conceptualization, Methodology, Formal analysis, Validation. **Jingjing Sun:** Methodology, Validation. **Zheng Shen:** Validation, Funding acquisition. **Jiabin Chen:** Validation, Funding acquisition. **Xuefei Zhou:** Methodology, Funding acquisition, Validation, Writing – review & editing, Funding acquisition. **Yalei Zhang:** Conceptualization, Methodology, Validation, Project administration, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

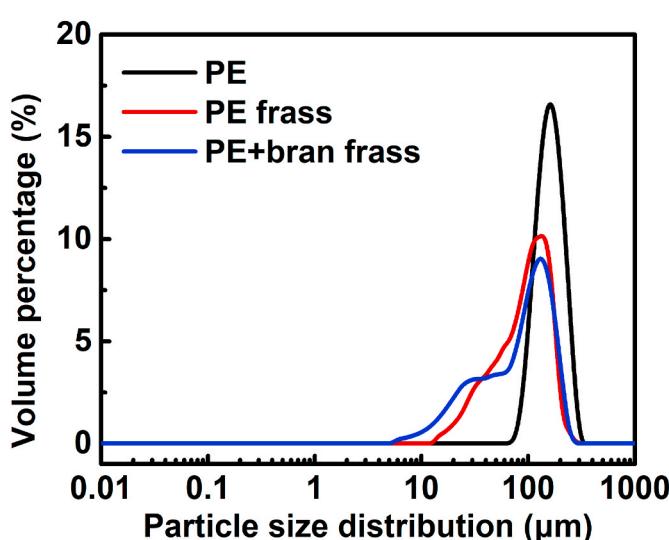


Fig. 5. Characterization of the size-frequency distribution of the residual PE polymers extracted from the frass of the PE-fed and PE + bran-fed larvae, respectively. The distribution was expressed based on the polymer volume. The result was presented with logarithmic spacing (range: 0.01–3500 μm).

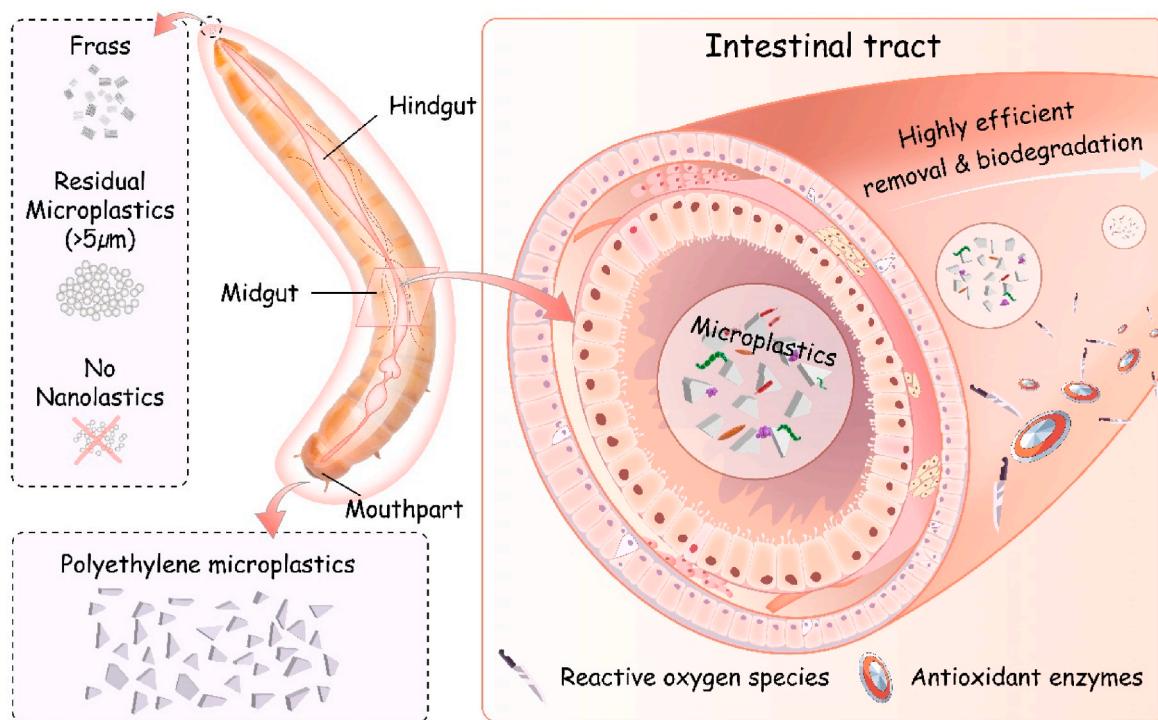


Fig. 6. A schematic diagram of the physiological responses of mealworm larvae after digestion and biodegradation of PE microplastics.

the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jclepro.2023.136831>.

Abbreviation list

AW	Average weight
CAT	Catalase
C _e	Ethanol-extracted fraction
C _t	1,2,4- trichlorobenzene-extracted fraction
C _w	Water-extracted fraction
GCMS	Gas chromatography mass spectrometry
GST	Glutathione-S-transferase
HT-GPC	High-temperature gel permeation chromatography
HDPE	High-density polyethylene
LDPE	Low-density polyethylene

LLDPE	Linear-low density polyethylene
MDA	Malondialdehyde
MPs	Microplastics
M _n	Number-average molecular weight
MNPs	Micro(nano)plastics
M _w	Weight-average molecular weight
M _z	Size-average molecular weight
NPs	Nanoplastics
1,2,4-TCB	1,2,4-trichlorobenzene
PE	Polyethylene
PLA	Polylactic acid
PP	Polypropylene
PS	Polystyrene
PVC	Polyvinyl chloride
ROS	Reactive oxygen species
SPCR	Specific plastic consumption rate
SR	Survival rate

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