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RESEARCH ARTICLE

Effect of biodegrading polyethylene, polystyrene, and polyvinyl chloride on the growth and development of yellow mealworm (*Tenebrio molitor*) larvae

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Abstract

Yellow mealworm (*Tenebrio molitor* L.) larvae can depolymerize and degrade polyethylene (PE), polystyrene (PS), and polyvinyl chloride (PVC). In this study, mealworms were utilized to biodegrade PE, PS, and PVC. Additionally, the effects of plastic degradation on the growth and development of yellow mealworm larvae were investigated by investigating the physiological indices and nutritional components of the larvae after plastic degradation. The results showed that degradation of plastics (PS, PE, and PVC) was promoted at a feeding amount of 0.50 g. However, the degradation of PVC at this concentration increased the mortality of yellow mealworms. In contrast, the degradation of a small amount of PS (0.10 g) promoted the nutritional value of crude protein ($45.7 \pm 2.08\%$) and phosphorus ($1.23 \pm 0.04\%$), had a lower larval mortality rate ($7.90 \pm 1.10\%$), and thus did not have a significant effect on the growth and development of yellow mealworms.

Keywords Economic insects · Plastic degradation · Quality · Nutrition

Introduction

Plastic waste is increasing exponentially, which is causing serious damage to the environment. Moreover, such high levels are toxic to humans and organisms (Horton et al. 2017) and represent a huge challenge for environmental management (Elsawy et al. 2017; Li et al. 2016). The major categories of plastic waste in the environment include polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET), and polyurethane (PUR) (Plastics-Europe 2019; Wu et al. 2016). Among these polymers, PE, PP, PS, and PVC are non-hydrolyzable plastics that are more resistant to enzymatic depolymerization or degradation (Inderthal et al. 2021). PE is stretch resistant, easily processed, and considered the most ecologically problematic owing to its high molecular weight, strong hydrophobicity, and high chemical and biological inertness (Brandon et al. 2018, Vimala and Mathew 2016). It is widely used in agricultural films and packaging materials, and in recent years, it has also been widely used as a flame retardant (Rochman et al. 2014). PS has the characteristics of good thermal insulation, radiation resistance, and high oil yields from pyrolysis (Rutkowski and Kubacki 2006). It is often used to make thermal insulation materials, including household appliances, takeout boxes, and industrial commodities (Artetxe et al. 2015). PVC is one of the most common general-purpose plastics worldwide. It is widely used in building materials, industrial products, daily necessities, artificial leather, pipes, wires and cables, and packaging films (Xu and Jiang 2019). Currently, the main treatment methods for plastics include landfilling, incineration, and chemical treatment; however, these methods cannot fundamentally resolve the pollution problem (Nobbs and Gregson 2000). In addition, plastic structural monomers and additives, such as plasticizers, can affect human health by stimulating the senses, impairing immune function, and causing endocrine disorders, asthma, genetic diseases, cancer, and many other diseases (Wright and Kelly 2017). Therefore, developing a cost-effective treatment method that can avoid secondary pollution is of vital importance for solving the

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"white pollution" problem caused by plastic products in the environment (Shen et al. 2020).

Yellow mealworm (*Tenebrio molitor* L.) larva is a nutrient-rich and plastic-degradable insect (Peng et al. 2020, 2019). The larval lifespan is about 2 months (Luo et al. 2012). The protein content of yellow mealworm larva ranks first among all types of live animal protein feeds; thus, this larva is known as the "protein feed treasure house" (Selaledi et al. 2020). It is widely used in feed, food, medicine, health products, and antibacterial protein products (Allen et al. 2012) and has a high comprehensive utilization value. Yellow mealworm is omnivorous and can be used to treat organic waste based on its ability to transform it into proteins, fats, and other nutrients. Many researchers have conducted a series of studies to verify the mealworm's plastic degradation mechanism (Liu et al. 2022; Lou et al. 2021; Zhong et al. 2022). Brandon et al. (2018) used high-temperature gel permeation chromatography (HT-GPC), proton nuclear magnetic resonance (^{1}H-NMR), and Fourier transform infrared spectroscopy (FTIR) to analyze the degradation of PE by yellow mealworms. They removed the PE debris and collected the egested frass after the degradation and found that yellow mealworm could convert 49.0 ± 1.4% of the plastic into CO_2. The molecular weight of residual polymer (Mn) decreased by 40.1 ± 8.5% in mealworms fed PE relative to that in PE samples. NMR and FTIR analyses revealed chemical modifications consistent with the degradation and partial oxidation of the polymer (Brandon et al. 2018). Yang et al. (2015a, b) and Peng et al. (2020) obtained similar results for the degradation of PS and PVC plastics by the same methods (Peng et al. 2020; Yang et al. 2015a). In addition, bacterial strains that can effectively degrade plastics were successfully isolated from the intestinal flora of yellow mealworms (Yang et al. 2015b), indicating that the intestinal flora actually consumed the ingested plastics. These important findings provide a strong basis for using yellow mealworms to degrade plastics.

In summary, previous studies have primarily investigated the degradation of plastics by yellow mealworms while few have investigated changes in the nutritional components of yellow mealworms after consuming plastics. Since the protein-rich yellow mealworm larvae hold many economic benefits for practical production, its effective degradation of plastics and the effects of plastics on its growth and nutrient composition should be further investigated. For example, whether the ability of yellow mealworm to efficiently degrade plastic has an effect on its growth should be further resolved. Therefore, the purpose of this study was to examine the optimal transformation efficiency and most suitable type of plastic among three types by determining the optimal contents in the feed at one time and exploring the effect of degradable plastics on the physiological and nutritional indicators of the yellow mealworm. Under the premise of ensuring the health of the yellow mealworm, the best method of reducing degradable plastics is researched and the sustainability of plastic degradation is analyzed. This work provides a data reference and scientific basis for further research on how to improve the efficiency of plastic biodegradation on a large scale to resolve the "white pollution" problem in a green and sustainable way.

Materials and methods

Preparation of yellow mealworm, plastics, and other materials

Yellow mealworms used in this experiment were obtained from the Institute of Ecology, Sichuan Agricultural University. These larvae were self-raised for more than three generations with a standard culture substrate (70% wheat bran, 25% corn, and 5% soybean) to adapt to the laboratory environment, the temperature and relative humidity were maintained at 25 ~ 28 °C and 60% ~ 70%. Under these conditions, 6–7 instar larvae with body length of 1.20 ± 0.50 cm and body weight of 0.05 ± 0.00 g were selected for the experiment, and they were fasted for 48 h before being fed the experimental diet. The PE plastic film used in this study was purchased from Huahua Environmental Protection Technology Co., Ltd. The plastic film is low-density PE (LDPE) with a molecular weight less than 10,000, and the main components were 30% starch and 70% PE. Before serving to the mealworms, the film was cut into 2.5 cm × 2.5 cm pieces, each weighing 0.05 g. The PS used in the study was expanded PS (EPS) purchased from Hebei Depeng Building Materials Co., Ltd., and the main ingredients were 96% polystyrene, 1% talc, 1% calcium stearate, 1% butane, and 1% others. The EPS was cut into cubes of approximately 4 cm × 5 cm × 1 cm, each weighing 0.1 g. The PVC used in this study was in film form and purchased from Chengdu Shuangyi Packaging Co. The main components of PVC included polyvinyl chloride and plasticizers, flame retardants, antiaging agents, adhesives, and chlorides. It was cut into 2 cm × 3 cm pieces, each weighing 0.05 g per serving. The above materials were washed with distilled water and freeze-dried (SCIENTZ-12 N, Ningbo Xinzhi Biotechnology Co., Ltd.) for later use before being provided to mealworms. Wheat bran was purchased from the Chengdu Market in Sichuan Province and dried before use in the experiment, and the particle size was less than 5 mm. The box for feeding the yellow mealworm was composed of tinfoil produced by "Dolly Industries." The tinfoil was folded into carton with dimensions of 25 cm × 25 cm × 5 cm and a thickness of 0.20 cm for use.

Experimental design

Ten experimental diets were compared: PE-A, PE-B, PE-C, PS-A, PS-B, PS-C, PVC-A, PVC-B, PVC-C, and CK (fed wheat bran only). In each "A," "B," and "C" treatment, 0.10 g, 0.50 g, and 1 g of the corresponding plastic substance were added, respectively. A total of 120 yellow mealworms were fed 30 g wheat bran, and plastic was added to each treatment. PE and PS were added according to a previous study, and the maximum degradable PE and PS mass for 120 yellow mealworms was approximately 1 g when wheat bran was added for 32 days (Brandon et al. 2018), and PVC was also added according to this quantity. Three replicates were used for each treatment, and a total of thirty feeding boxes were implemented. The feeding methods and conditions followed those described by Luo et al. (2012). The temperature and relative humidity were maintained at 25 ~ 28 °C and 60% ~ 70%, respectively, and the feed moisture content was maintained at 18%. In order to control the relative humidity, all wheat bran was exposed to the sun to prevent soft rot and mites from killing the yellow mealworms. Yellow mealworms of the same instar stage were selected for plastic degradation under different treatments for 35 d. During this period, the larval weight and mortality were recorded. The pupae, dead larvae, and shed skin were removed from all treatments every 5 days. After 35 days, the remaining plastic was removed using tweezers and weighed (because mealworms are borehole food plastic consumers, the remaining plastic was easily selected), and other samples were collected, freeze-dried, and ground into powder, which was placed in tinfoil bags for storage. Anhydrous silica gel was placed around the sample bags as the drying agent. The samples were stored in sealed boxes at room temperature prior to analysis (Luo et al. 2012).

Analysis method

Moisture was measured using the vacuum cryodesiccation method, and approximately 0.10 g of yellow mealworm samples was taken and wrapped in tinfoil bags of known weight, placed in a freeze dryer to dry, removed after 5 days, and weighed. The moisture content was calculated as follows: yellow mealworm (%) = (water weight/sample weight) × 100%. The protein content was determined using the Kjeldahl method (Kjeldahl Apparatus (KDN-1)), with a protein-to-nitrogen conversion factor of 6.25. Total phosphorus was determined using the colorimetric method (GB/T 6437) (UV Spectrophotometer (UV-1800)), and total potassium was measured using flame photometry (GB 9836–88) (Flame Photometer (6400A)). To reduce reagent errors and operational errors, a 10% reagent blank was used in the experiment, and three parallel samples were prepared for each sample. The plastic degradation rate was obtained according to the following formula:

$$\text{Plastic degradation rate\%} = (M_a - M_b)/M_a \times 100\% \tag{1}$$

where M_a is the mass of the added plastic and M_b is the mass of the plastic after being degraded by the yellow mealworm.

Statistical analysis

The means and standard deviations were computed with Microsoft Excel 2016. Analysis of variance (ANOVA) was performed based on Duncan's new multiple range method in SPSS 22.5 software, with a value of $p < 0.05$ considered significant. In addition, "Origin 2021" was used to illustrate the obtained data.

Results and discussion

Plastic degradation rate

As the yellow mealworm degrades plastic, approximately 40% of the reduced plastic mass is converted to carbon dioxide and approximately 50% of the residue is digested into feces (Brandon et al. 2018; Yang et al. 2015a). This demonstrates that yellow mealworms can degrade plastic effectively rather than simply changing the plastic volume. To some extent, the degradation rate of plastics indicates the conversion effect of yellow mealworms on plastics (Brandon et al. 2018). Because the degradation rate of plastic by mealworm may affect its growth and development, this study analyzed the plastic degradation rate of yellow mealworm. For PE, the degradation rate reached 20.7 ± 2.62% in the PE-B treatment, and significant differences were observed among the three treatments ($p < 0.05$) (Fig. 1, Table S1). In the PE-C treatment, the conversion rate was significantly reduced to 9.00 ± 1.41%. For PS, the highest degradation rate was obtained in the PS-B at 15.3 ± 0.41% and the lowest was obtained in the PS-A treatment at 13.7 ± 1.25% (Table S2). For PVC, the conversion rate of yellow mealworms was lower than that of the PE and PS treatments. In the PVC-A treatment, the degradation rate was only 7.33 ± 0.47%, while in the PVC-B treatment, the highest degradation rate of 11.8 ± 0.98% was obtained (Table S3). Among the three plastic polymers selected for the experiment, the PS conversion effect was the most stable and relatively higher than that of the other two polymers. The ability to degrade PS is widespread in yellow mealworms (Yang et al. 2018b). Researchers have used yellow mealworms to degrade different types of PS and found that the same PS (EPS) used in this study could be degraded to the maximum, although an increase in yellow mealworm mass was not observed (Urbanek et al.

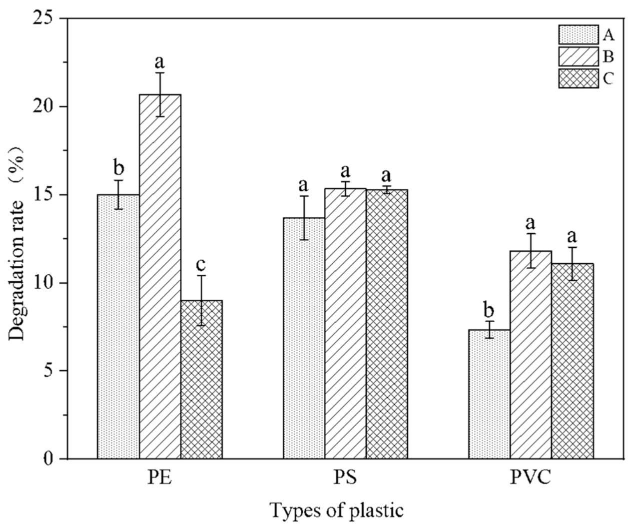

Fig. 1 The conversion rate of *Tenebrio molitor* to 3 different types of plastic. Note: PE, mealworms fed polyethylene; PS, mealworms fed polystyrene; PVC, mealworms fed polyvinyl chloride; A treatment: feeding corresponding plastic 0.1 g; B treatment: feeding corresponding plastic 0.5 g; C treatment: feeding corresponding plastic 1 g. Vertical T bars indicate standard deviations between three replicates. Means followed by different letters among A, B, and C treatments within each type of plastic indicate significant differences (Duncan's, $p<0.05$)

2020). This may be because wheat bran was not added to the plastic control of the previous study, which resulted in a shortage of nutrients for the mealworm, thus affecting its normal growth. This may be related to the low density of PS, which was convenient for the yellow mealworms to drill in the material (Przemieniecki et al. 2019), which increased the ingestion rate of PS. The texture of PE and PVC is hard, which was not conducive for feeding and degradation by yellow mealworm larvae. When the PE dosage was 1 g, the conversion rate was the lowest. This may be because PE is toxic to yellow mealworm (Jiang 2018). When yellow mealworms ingested too many PE particles, toxicity accumulated in the body, which affected their health and growth. This would significantly affect subsequent ingestion. Among the different plastic treatments, the conversion efficiency was the best when the plastic mass was 0.50 g. In all controls, yellow mealworms were treated with less than 1 g of plastic, which differs from the findings of Brandon et al. (Brandon et al. 2018). This may be because a sufficient amount of wheat bran was added at one time in this experiment. In the case of sufficient feed, yellow mealworms may prefer to consume wheat bran and reduce plastic intake.

When the weight of plastic was suitable, the yellow mealworm was more easily able to convert plastic, and some experiments also showed that when PS and wheat bran were fed to yellow mealworm in a certain ratio, the amount of PS consumed increased significantly (Abdel-Shafy and Mansour 2016; Yang et al. 2018a). The conversion rate of yellow mealworms to the three types of plastics was less than 100%, which indicated that the plastic conversion ability of yellow mealworm was limited.

Weight change trends and mealworm's mortality

The weight change of yellow mealworms during plastic degradation can directly reflect their growth. In the PE-degraded treatments, the body weight gain of the larvae reached a maximum in PE-B at 35 days; however, adding too much PE inhibited the growth of the larvae (Fig. 2a). During the degradation of PS, the growth of yellow mealworm was significantly inhibited by the PS-A treatment, which may be due to the lower mortality and greater competition in the PS-A treatment (Fig. 2d), which affected the monomer insect weight. There was no significant difference between the PS-B and CK groups (Fig. 2b). The results of the PVC treatment were quite different from those of the PS and PE treatments. In PVC-A and PVC-C, the weight of the yellow mealworms reached a maximum. After 14 days, significantly lower weight gain was observed in the CK group while higher weights were observed in the yellow mealworms in PVC-C. At 35 days, the weight gain in PVC-C was $31.6 \pm 0.59\%$ while that for the PE treatment was $25.6 \pm 0.43\%$, which was greater than that of the PS treatment. The most apparent reason for the weight gain of yellow mealworms fed PVC may be due to the following reasons. Wheat bran is a common feed for yellow mealworms and has more nutrition than PVC and thus is more conducive to the growth and development of yellow mealworms. The worst conversion effect was observed for PVC. At this time, yellow mealworms prefer to consume more nutritious wheat bran, and the proportion of wheat bran ingested was more significant than that of plastic; therefore, the weight gain is more pronounced. Yellow mealworms had a greater effect on PS plastic conversion. Compared with the other treatments, wheat bran consumption is reduced. The intake of less wheat bran and more plastic led to a poor nutritional status and, therefore, less weight gain. Studies have shown that microplastics entering the body can cause unavoidable damage to the intestinal tract (von Moos et al. 2012; Watts et al. 2014). When yellow mealworms take in more plastic, intestinal damage will be more significant, which is highly unfavorable to the absorption of nutrients in the intestine, resulting in less weight gain.

Studies have shown that the mortality of yellow mealworms treated with plastics (PE, PS, and PVC) was significantly higher than that of the control experiments (Fig. 2d), indicating that eating plastic reduces the survival rate of yellow mealworms. The larval mortality rate increased with an increase in the plastic mass concentration, regardless of the type of plastic. The lowest mortality of yellow mealworms

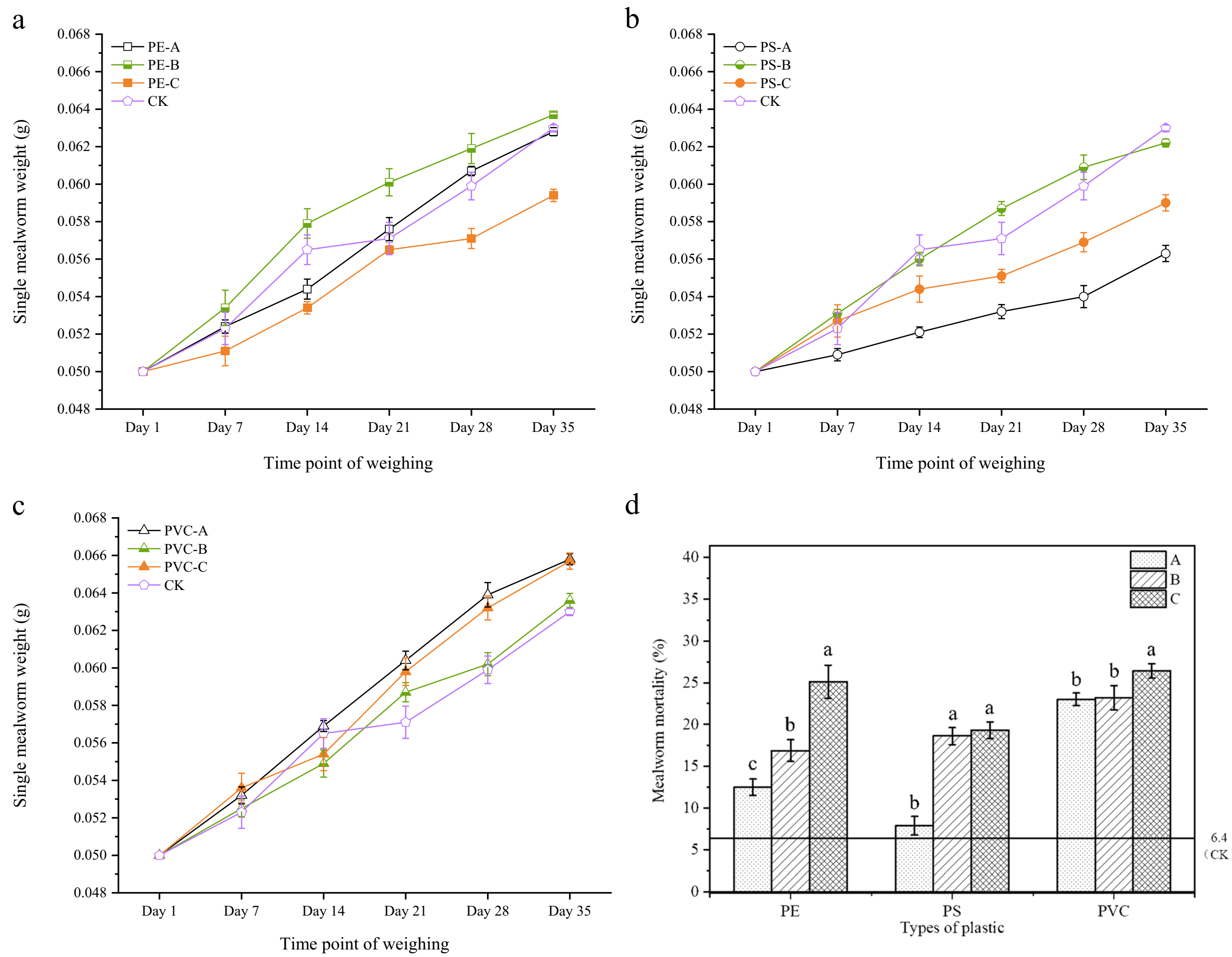

Fig. 2 Single mealworm weight in PE treatment (**a**), PS treatment (**b**), and PVC treatment (**c**) and mealworm's mortality (**d**) in the experiment. Note: CK, mealworms fed wheat bran; PE, mealworms fed polyethylene; PE-A, mealworms fed PE 0.1 g; PE-B, mealworms fed PE 0.5 g; PE-C, mealworms fed PE 1 g; PS, mealworms fed polystyrene; PS-A, mealworms fed PS 0.1 g; PS-B, mealworms fed PS 0.5 g; PS-C, mealworms fed PS 1 g; PVC, mealworms fed polyvinyl chloride; PVC-A, mealworms fed PVC 0.1 g; PVC-B, mealworms fed PVC 0.5 g; PVC-C, mealworms fed PVC 1 g. Vertical T bars indicate standard deviations between three replicates. Means followed by different letters among A, B, and C treatments within each type of plastic indicate significant differences (Duncan's, $p < 0.05$)

eating PS was $7.90 \pm 1.10\%$, whereas the mortality of yellow mealworms eating PVC was as high as $26.4 \pm 0.87\%$ (Table S2). This may be because the PS used in this experiment was in direct contact with food and did not release a large number of harmful substances at room temperature. The inhibition to the growth of yellow mealworms was less than that caused by PVC. Therefore, yellow mealworms that eat PS grow well and have a low cannibalism rate. PVC and PE contain more carcinogens, such as plasticizers, and plastic toxicity accumulates in the body after eating, causing harm to the body and reducing the lifespan of yellow mealworms. If it is necessary to reduce the mortality of yellow mealworms, artificial cross-mating can be conducted to create resistant to plastic consumption yellow mealworm strains.

Although the yellow mealworms fed PVC had a significant weight gain, based on the higher mortality rate of PVC, it can be concluded that the consumption of PVC will seriously affect the production of yellow mealworms. Studies have also confirmed that compared with other plastics, PVC can significantly affect both the intake and feeding rate of *Daphnia magna Straus*, where it has a stronger toxic effect and causes higher mortality and oxidative stress (Browne et al. 2013, Lithner et al. 2009).

Yellow mealworm nutritional indexes

Yellow mealworms have a high nutritional value because of their high protein content; thus, they can be utilized as feed ingredients. Therefore, measuring the protein content during the growth of yellow mealworms is an important indicator of their nutritional value. The crude protein content of yellow mealworms in PE was significantly higher than that in the other two plastic treatments (Fig. 3a) and reached 55.2 ± 1.35% in the PE-A treatment (Table S1). The crude protein content of yellow mealworms in the PS treatments was the second highest and reached 45.7 ± 2.08% in the PS-A treatment (Table S2). The crude protein content of yellow mealworms in the PVC treatment was the lowest and reached 30.8 ± 1.57% in the PVC-C treatment (Table S3). Except for the PE-A treatment, the protein content of the other treatments was lower than that of the control, indicating that plastic has an adverse effect on the accumulation of protein by yellow mealworm. Although the purpose of transforming plastics has been achieved, it also reduces the nutritional value of yellow mealworms. A lack of crude protein in the feed will inhibit growth, slow tissue repair, and reduce immunity (Sun et al. 2019). According to the requirements of "Grass Carp compound fed standard SC/T1024-2002" (SC/T1024 2002), the crude protein content of fish feed must be higher than 30% and the crude protein content of edible fish feed must be higher than 28%. The crude protein content of all treatments reached the standards for edible fish and fingerling feeds.

The different mass concentrations of these three plastics led to significant differences in the crude protein content of yellow mealworms ($p < 0.05$), indicating that the lower the plastic quality, the higher the crude protein content. The intestinal flora is closely related to eating habits. After ingesting different types of plastics, there were significant differences in the main intestinal flora (Przemieniecki et al. 2019). The crude protein content of yellow mealworms that ingested 0.10 g PE was higher

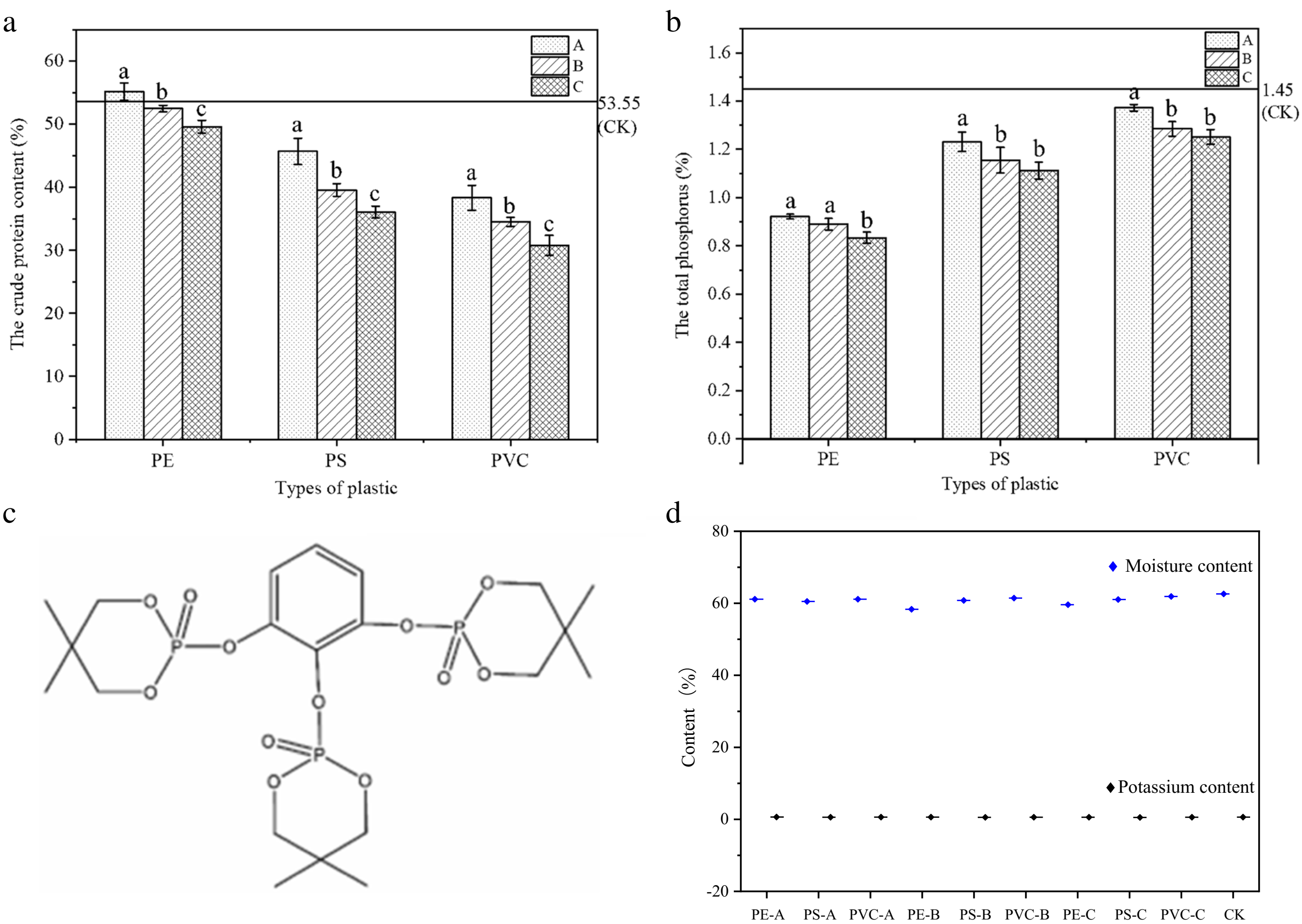

Fig. 3 The crude protein content (**a**), the total phosphorus content (**b**), the structure of phosphorus flame retardant (**c**), and moisture and potassium content (**d**) in yellow mealworm. Note: CK, mealworms fed wheat bran; PE, mealworms fed polyethylene; PS, mealworms fed polystyrene; PVC, mealworms fed polyvinyl chloride. A treatment: feeding corresponding plastic 0.1 g; B treatment: feeding corresponding plastic 0.5 g; C treatment: feeding corresponding plastic 1 g. Vertical T bars indicate standard deviations between three replicates. Means followed by different letters among A, B, and C treatments within each type of plastic indicate significant differences (Duncan's, $p < 0.05$)

than that of the CK. A small amount of PE does not affect the protein content of yellow mealworms but promotes the accumulation of protein, which indicates that consuming a little amount of plastic has a positive effect on the accumulation of nutrients in the body. Specific changes in the intestinal flora can be further analyzed to find the most beneficial method to the growth of yellow mealworms and the degradation of plastics.

The phosphorus content in yellow mealworms is higher than that in many other foods and can be used in feed or fertilizer production. Determining the phosphorus content of yellow mealworms can reflect its nutritional value. The phosphorus content of yellow mealworms ingesting plastic was significantly lower than that of the whole wheat bran (Fig. 3b). Phosphorus in yellow mealworm is mainly derived from wheat bran (Juan et al. 2013). The decrease in phosphorus content may be due to the decrease in the intake of wheat bran by the yellow mealworm ingesting plastic. The phosphorus content of yellow mealworm that ingested PE was the lowest at less than 1%, while that of mealworms that ingested PVC was the highest at 1.37 ± 0.13% (Table S3). The phosphorus content of yellow mealworms ingesting PS was higher than 1%, and there was no significant difference in phosphorus content between the treatments with different mass concentrations of each plastic ($p > 0.05$). The higher content of phosphorus in the PS and PVC treatments may be because the flame retardant contained in these two materials is phosphorus (Salasinska et al. 2020) (Fig. 3c). The plastic that enters the intestinal tract of the yellow mealworm is broken down by intestinal flora, which breaks the chemical bonds, and the resulting phosphorus supplements the phosphorus that is not taken in from wheat bran (Peng et al. 2020).

There was no significant difference in the water content of the different treatments of yellow mealworm ingesting different plastics, which were all within the range of 60.0 ± 2.00% (Fig. 3d).

The potassium content in yellow mealworm can provide high-quality potassium as food and is a nutritional indicator. The experimental results showed that there was no significant difference in the total potassium content of each treatment (Fig. 3d), which were all within the range of 0.60 ± 0.04%. The type and quality of ingested plastics did not have a significant impact on the total potassium content of the yellow mealworms.

Conclusions

The degradation efficiency of yellow mealworm is different for different types of plastics. At a moderate dosage (0.50 g), the treatment efficiency of the three different types of plastics reached the highest degradation rate. However, the increase in plastic dosage did not have a significant impact on yellow mealworm biomass, and the mortality of yellow mealworm increased. Among them, feeding with PVC had the greatest impact on the yellow mealworm, and the mortality rate was significantly increased. Among the different types of plastic, PS had little effect on the nutritional value of the yellow mealworm; therefore, a small amount of PS plastic is the most suitable material for the continuous transformation of plastic by yellow mealworm.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11356-022-24957-8.

Author contribution Ling Jin: writing—original draft and conceptualization. Peng Feng: data curation. Zhang Cheng: methodology, review and editing, funding acquisition. De Wang: supervision, review, and editing.

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Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate The manuscript did not contain any reporting studies involving human data.

Consent for publication The manuscript does not contain any individual person data.

Conflict of interest The authors declare no competing interests.

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