The Effect of Polyethylene Terephthalate Microplastics on the Growth of Mice

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Abstract. Over the course of the previous decade, there has been a growing apprehension regarding the presence of MPs. This concern has arisen because of the detrimental effects of MPs pollution on the well-being of both animals and people. This study explores the potential implications of MPs contamination on mice. This study examined the impact of microplastics on mice by feeding four food experiment; P0 (pellet BR1), P1 (potato from Pujon Farm), P2 (potato mixed 300 µg PET) and P3 (potato mixed 600 µg PET). Body weight, feces weight, and left feed were observed in this study. Food contaminated with microplastics (MPs) has a negative effect on appetite, feces weight and body weight of mice. This process has an impact on reducing the weight of feces, appetite, body weight of mice and weight of mice. It is suspected that MPs in rat digestion increase concentrations of mice corticosterone metabolites and carcass fat, suggesting an adaptation of metabolism to this situation. Another impact is expected the decrease in microbial richness in the intestines of mice, causing a decrease in mice weight and body weight of mice. The high concentration of MPs entering the digestive tract can also reduce the rats' appetite.
Keywords: Contaminated *Solanum tuberosum* L *in vivo* study, poison, *Rattus norvegicus* (Berkenhout, 1769), toxicity.

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1 Introduction

The issue of plastic pollution has emerged as a significant and urgent concern within the frame of environmental matters. Based on statistical data, it has been determined that the global production of plastic reached approximately 370 × 10^6 t by the year 2022 [1]. Furthermore, an estimated quantity of (4.8 to 12.7) 10^6 t of plastic pieces is observed to enter the ocean on an annual basis [2]. The emergence of microplastics (MPs), which are plastic particles with a diameter of less than 5 mm, can be attributed to the widespread manufacture and extensive utilization of plastic materials on a global scale [3]. Presently, there is proof that these tiny pieces of plastic release harmful plastic additives and can absorb different chemicals. It acts as sinks for poisonous compounds, making them more bioavailable, toxic, and easy to transport in terrestrial environment [4]. According to that statement, the presence of MPs or plastic particles in terrestrial environments and their impact on species has raised significant environmental concerns, prompting expedited research efforts in this area.

Over the course of the previous decade, there has been a growing apprehension regarding the presence of MPs. This concern has arisen because of the detrimental effects of MP pollution on the well-being of both animals and people. MPs have been observed to be consumed by a wide range of animal species, including rabbit [5], shrimp [6], fish [7] and even wild bird [8], resulting in their transfer along the food chain. Moreover, after being consumed, poisons and microorganisms that are absorbed by MPs or accumulated on their surface have the potential to impact the overall health of the host organism [9]. Numerous experiments have been conducted on living organisms to investigate the dispersion patterns of MPs. Furthermore, the extent of distribution and research conducted on parliamentarians in relation to mice remains limited.

MPs have been found to have an impact on mice. Exposure to polystyrene (PS) MPs, one of the most common types of MPs, led to insulin resistance (IR) in mice fed with normal chow diet (NCD) or high-fat diet (HFD) [10]. PS MPs also caused damage to the intestinal barrier function and dysbiosis of the gut microbiota in mice [11]. Additionally, exposure to compositional MPs PS, polypropylene (PP), polyethylene (PE), polyethylene terephthalate (PET or PETE), polystyrene (PS), and polyvinyl chloride (PVC) resulted in colon tissue damage and inflammatory response in mice, indicating health effects [12]. While the effects of MPs on human health are still being studied, there is evidence to suggest that MPs consumption could provoke immune and stress responses, induce reproductive and developmental toxicity, and cause renal and hepatic lesions [13, 14]. Overall, these findings highlight the potential toxicity of MPs and the need for further research to understand their impact on both animals and humans.

MPs research on animals has been extensively studied in recent years. Studies have shown that MPs are now ubiquitous in the environment and are increasingly reported in biota across the globe [15]. There is a wide range of model organisms used in studying MPs, including both aquatic and terrestrial species [16]. The effects of MPs on animal health have been investigated, and it has been found that MPs ingestion can provoke immune and stress responses, as well as induce reproductive and developmental toxicity [17]. However, there is still limited research impact MPs on mice, with most studies analyzing the effects on model organisms and inferring the likely impacts on humans. Further research is needed to explore the potential implications of MPs contamination on mice in more rigorous clinical studies. Mice were fed MPs in this study. The result showed that MPs could build up in the gut and cause problems with the gut barrier, the bacteria, and metabolism in mice. The study's findings give some new information about the possible dangers of MPs exposure to land-based environments and basic information about how MPs might affect human health.

2 Materials and methods

2.1 Experimental animal and housing condition

Eight-week-old male *Rattus norvegicus domestica* (Berkenhout, 1769). Each mouse was placed in a glass cage (300 mm × 300 mm × 300 mm) with a wire floor during the study period. Under each cage, a plastic tray filled with sawdust was placed, which was covered with a sheet of paper. All animals were placed in experimental animal cages, Pharmacology Laboratory, Faculty of Medicine, University of Muhammadiyah Malang, campus 2 (coordinates: S 7°57'27.1332" and E 112°36'50.7312”) at constant temperature (28 °C ± 2 °C), humidity (55 % ± 10 %), and an 8 hour light/16 hour dark cycle (lights on at 09:00 and off at 17:00). Mice were given feed according to treatment and given tap water (mixed with filtered water from a blender potato) ad libitum. The experiment was carried out in accordance with the description of ethical approval No. E.5a/211/KEPKUMM/VII/2023, issued by the Health Research Ethics Commission, Faculty of Medicine, University of Muhammadiyah Malang.

2.2 Feed preparation

Potato (*Solanum tuberosum* L.) as mouse feed were purchased from potato farm at Maron hamlet, Pujon Kidul village, Pujon subdistrict, Malang Regency (coordinate: S 7°51'20.0664” and E 112°28'18.4152"). Male mice were purchased from Rattus Breeding Centre (coordinate: S 8°0'22.1508" and E 112°37'40.1592"). PET – MPs (used plastic bottles) were crushed using a blender until smooth and filtered using a 5 mm filter. Feed formula was prepared as follows: P0 (pellet BR1), P1 (Potatoes Pujon Kidul farm), P2 (potatoes and 300 µg PET per day) and P3 (potatoes and 600 µg PET per day). The dose of 600 µg PET per day refers to Li *et al*. [18]. This study used eight mice per treatment with a total of 32 mice.

Feed consumption per mouse was 5 g in 1st wk, and 20 g in 2nd wk until finish the research. Potato feed on the 1st wk is given in the form of thick porridge, namely potatoes blended and filtered. Filtered water is mixed as drinking water. In the 2nd wk, the feed is given in the form of thick porridge plus potatoes cut into 2 mm pieces, and in the 3rd wk it is given in the form of potatoes cut into 2 mm pieces. Mice will be adapted to feeding BR1 pellets for 7 d, before being given special treatment (P0 to P3).

The BR1 is a feed produced by PT Japfa Comfeed – Indonesia. The authors in related research reported that the gross energy (GE) in BR1 feed was 4 131.24 %, lower than potatoes from Pujon Farm, which was 4 359.75 %. The authors have also reported that potatoes at Pujon Farm were declared contaminated with MPs particle and Stanum (Sn) amounting to 0.05 g^{-1}, and 0.490 8 mg L^{-1}. This MPs and Sn contamination is higher than samples from two other potato cultivation areas in Malang Raya.

2.3 Score observation

Status of mice were monitored in this study. Body weight, feed left, and feces were measured using an electronic scale (PE-1600, Mettler Ltd., Tokyo, Japan). Body weight and feed left were measured every week for 3 wk, while feces were measured at 1st wk, 2nd wk, and 3rd wk. Feed consumption per animal was determined by determining the known weight and weighing the amount of food remaining after 7 d. Water consumption is measured in the same way. Survival rates were assessed throughout the animal's lifetime. Deaths due to natural causes were recorded. Autopsies were carried out on all mice, except for rotten or partially eaten carcasses and all organs were subjected to macroscopic examination.

2.4 Statistical analysis

Data are expressed as means ± SD. Differences were tested by analysis of variance (ANOVA) and TUKEY post hoc test. The data were tested statistically for normality using Shapiro–Wilk (S–W) tests and to test the homogeneity of variance using the Levene test. Then the comparison test for body weight, feces weight and fed left were analyzed using SPSS 18 [19, 20].

3 Result and discussion

3.1 Body weight

The effects of different feed experiment on weight of mice are shown in Figure 1. In contrast, there was a decrease in weight at P0 compared to P1, P2 and P3 ($P < 0.05$). It is suspected that the decrease in weight was due to the emergence of energy balance disorders in experimental mice as a form of impact caused by MPs disturbances. Moreover, comparison of rat weight (g) in treatment P1 was significantly different from treatment P0 ($P < 0.05$), but not significantly different from treatments P2 and P3 ($P > 0.05$). However, the comparison of rat weights (g) in group P2 was significantly different from treatment P0 and P3 ($P < 0.05$), but not significantly different from treatment P1 ($P > 0.05$). Meanwhile, the comparison of mouse weight (g) in treatment P3 was significantly different from treatments P0 and P2 ($P < 0.05$), but not significantly different from treatment P1 ($P > 0.05$). P2 and P3 had a high concentration of MPs. PET MPs were found to have had effects on body weight in mice. In one study, mice were exposed to various levels of MPs for 90 d, and it was observed that the body weight gain was significantly reduced in male mice compared to the control group [14]. Another study showed that exposure to MPs resulted in concentration-dependent reductions in hematological markers in mice, indicating potential impacts on overall health and physiology [21]. Moreover, impact caused by MPs is thought to affect probiotics and metabolism. The association between resveratrol and *Lactococcus lactis* [(Lister 1873) Schleifer *et al.* 1986], a probiotic, was found to decrease body weight in mice and modulate the gastrointestinal microbiota [22].

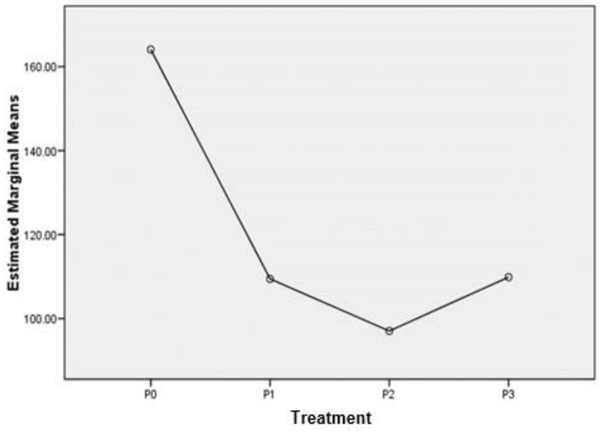

Fig. 1. Mouse weight at different treatment.

The presence of MPs on potatoes affected the weight loss of mice in the treatment P0 compared P1. In this research, GE in P0 (BR1) feed was 4 131.24 %, lower than P1 (potatoes from Pujon Farm), which was 4 359.75 %. However, P1 clearly showed the highest decrease in mouse weight compared to P0 ($P < 0.05$). P1 was potatoes using conventional farming from Pujon, Indonesia. Agricultural land was fairly high contributor of MPs to agricultural products [23, 24] and consuming product contaminated MPs induces a variety of toxic effects [17]. This shows that the presence of MPs in P1 inhibits metabolism and oxidation in mice in this study. MPs have been found to disturb metabolic pathways such as arachidonic acid metabolism and can lead to damage in organs such as the hepatopancreas in shrimp [25] and the liver, kidney, and spleen in chickens [26]. Meanwhile, MPs have been studied for their potential impact on the weight of mice. Tissue accumulation of MPs in mice could lead to widespread health risks [27]. However, it is important to note that this study was met with concerns regarding the entry of MPs into mouse tissues and the appropriateness of biomarkers used to assess biological responses.

Another study focused on the weight of gaming mice and its impact on muscle activity, performance, and user preference [28]. That study showed the weight of the mouse influenced muscle activity and performance, with the 60 g mouse receiving the highest overall preference score. Additionally, selection for increased or decreased body weight in mice resulted in changes in muscle fiber number and diameter, indicating the complexity of how weight changes can affect cellular structure [29]. The number of MPs consumed does affect the weight of mice. High concentrations of MPs led to significant weight loss in mice, while lower concentrations resulted in little change or even weight gain [30].

Mouse weight at the time of observation on 16/10/2023, 03/10/2023, 26/09/2023, and the highest on 19/09/2023 (Figure 2). The weight of the mouse on 09/19/2023 showed a significant difference with observations on 09/26/2023, 10/03/2023, and 10/16/2023 ($P < 0.05$). Mouse weight on 09/26/2023 was significantly different from 09/19/2023 ($P < 0.05$), but not significantly different from observations on 10/03/2023 and 10/16/2023 ($P > 0.05$). Likewise, a comparison of mouse weight on 10/03/2023 showed a significant difference from 09/19/2023 ($P < 0.05$), but not significantly different from observations on 09/26/2023 and 10/16/2023 ($P > 0.05$). Whereas on 10/16/2023, the mouse weight was significantly different from 09/19/2023 ($P < 0.05$), but not significantly different from observations on 09/26/2023 and 10/03/2023 ($P > 0.05$). This is confirmed by the results of research which shows that P2 treatment reduces the weight of mice the lowest, although in P3 treatment the weight of mice shows an increase (Figure 1). Additionally, mice treated with relatively low concentrations of MPs became overweight, with excessive lipid accumulation and a better appetite [31]. These findings suggested that PET MPs could have negative effects on body weight in mice, highlighting the need for further research on the potential health risks associated with MPs exposure.

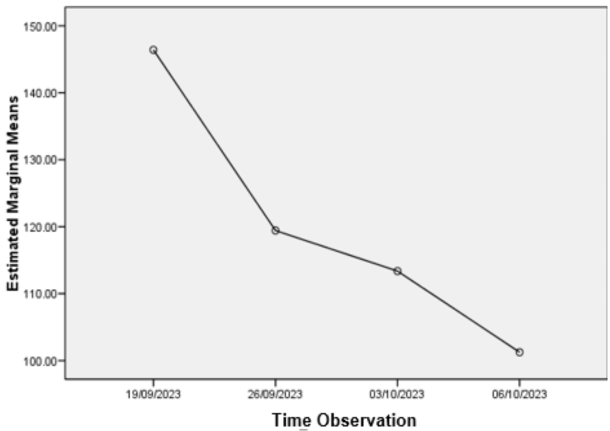

Fig. 2. Mouse weight at different time observation.

3.2 Feces weight

There was a significant difference in Mouse Feces (g) between groups P0, P1, P2 and P3 (Figure 3). The results indicated that the type of feed and the presence of MPs contamination in the feed swallowed by mice can affect the weight of the feces. There was a significant difference in fecal weight in mice treated with P0 compared to those treated P1, P2, and P3. Moreover, there was a significant decrease when treated based on Figure 3, observation (26/09/2023, and 03/10/2023) shows the order of Mouse Feces (g) from lowest to highest as follows: Mouse Feces at the time of observation on 26/09/2023, and the highest on 03/10/2023. Weight gain and feces weight in mice were found to be influenced by various factors. P0 is significantly different from P1, P2 and P3 ($P < 0.05$). Comparison of Mouse Feces (g) in P1, significantly different from the P0 group ($P < 0.05$), but not significantly different from P2 and P3 ($P > 0.05$). Comparison of Mouse Feces (g) at P2, significantly different from P0 ($P < 0.05$), but not significantly different from P1 and P3 ($P > 0.05$). Comparison of Mouse Feces (g) at P3, significantly different from P0 ($P < 0.05$), but not significantly different from P1 and P2 ($P > 0.05$). One study found that weight cycling, which refers to the phenomenon of weight gain after dieting, specifically altered the composition and function of the mouse fecal microbiota [32]. Another study examined the effects of low and high food availability on mice and found that low food availability caused higher concentrations of fecal corticosterone metabolites, suggesting an adaptation of metabolism to this situation [33]. Additionally, a study on the transplantation of fecal samples from lean mice to obese mice found that prolonged supplementation with feces from lean mice altered bacteria species diversity and richness and caused increased feces weight gain in mice on a high-fat diet [34].

Meanwhile, giving MPs PET as rat feed (P2) and (P3) showed the lowest fecal weight. MPs exposure in mice has been shown to have an impact on feces weight. Treatment with high concentrations of MPs increased the numbers of gut microbial species, bacterial abundance, and flora diversity, which may contribute to changes in feces weight [11]. Additionally, oral ingestion of MPs led to an increase in liver weight and general liver index, indicating potential disruption in liver function [35]. These findings suggest that MPs can affect the gastrointestinal system and may alter feces weight in mice.

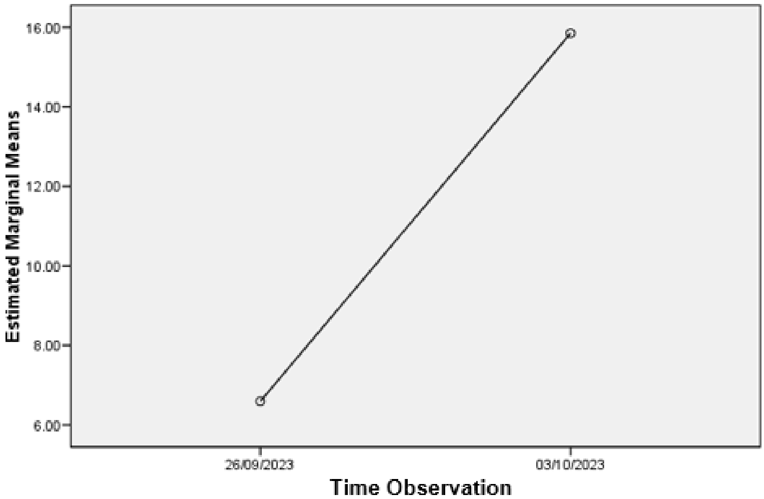

Fig. 3. Feces weight at different treatment.

3.3 Feed left

The effects of different feed experiment on left feed are shown in Figure 4. Feed left (g) pada P0 significantly different P1, P2 and P3 ($P < 0.05$). Feed left everyday (g) at P1, significantly different from the P0 group ($P < 0.05$), but not significantly different from P2 and P3 ($P > 0.05$). Feed left at P2, significantly different from P0 ($P < 0.05$), but not significantly different from P1 and P3 ($P > 0.05$). Moreover, Feed left at P3, significantly different from P0 ($P < 0.05$), but not significantly different from P1 and P2 ($P > 0.05$). The feed mixed 300 µg of MPs PET was consumed most frequently by mice compared to other treatments. The surprising thing is that the BR1 pellet type feed that many mice leave behind. Followed by Pujon Farm potatoes and feed mixed PET 600 µg. MPs has been found to have an impact on feed left by mice. MPs have been found to have an impact on appetite in mice. Exposure to PET MPs resulted in insulin resistance (IR) in mice feed with normal chow diet (NCD) or high-fat diet (HFD), accompanied by increased plasma lipopolysaccharide and pro-inflammatory cytokines [36]. Additionally, exposure to MPs caused a decrease in the richness and diversity of gut microbiota, particularly an increase in the relative abundance of Gram-negative bacteria [10]. The alteration of gut microbiota composition was associated with metabolic disorders and changes in metabolic pathways [37]. Furthermore, the exposure to MPs induced inflammatory response and colon tissue damage in mice, leading to changes in the dynamics of intestinal flora [12]. These findings suggest that MPs can disrupt appetite regulation and metabolic processes in mice, highlighting the potential health risks associated with MPs exposure. MPs in feed are thought to affect lean mass which impacts the appetite of mice. Appetite in mice is influenced by various factors.

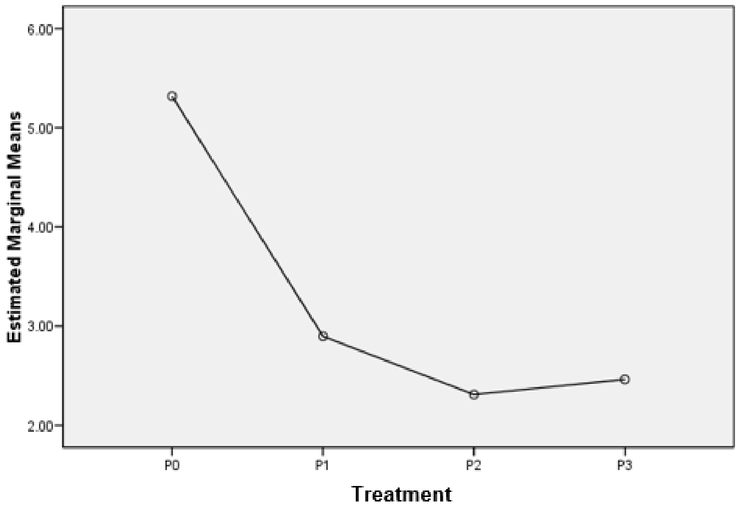

Fig. 4. Feed remained at different treatment.

4 Conclusion and recommendation

The data obtained has confirmed that feed contaminated with MPs has a negative effect on appetite, feces weight and body weight of mice. This process has an impact on reducing the weight of feces, appetite, and weight of feces. It is suspected that MPs in rat digestion increase concentrations of fecal corticosterone metabolites and carcass fat, suggesting an adaptation of metabolism to this situation. Another impact is expected the decrease in microbial richness in the intestines of mice, causing a decrease in fecal weight and body weight of mice. The high concentration of MPs entering the digestive tract can also reduce the rats' appetite. However, the authors are still preliminary and need to be studied further. Future research should be conducted to observe serum glutamic oxaloacetic transaminase (SGOT) or aspartate aminotransferase (AST), and serum glutamic pyruvic transaminase (SGPT) or alanine aminotransferase (ALT). Likewise, liver histopathology analysis and calculation of the number of antibody expressions analyzed using immunohistochemistry (IHC) are planned in *Rattus norvegicus.* The aim of this action should be considered by the Food and Drug Supervisory Agency (BPOM) and the National Standardization Agency - Indonesia (BSN) to improve food standards in Indonesia, particularly MPs contamination.

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