Assessment of ANKOM Daisy^{II} Incubator for measuring microplastic degradability in rumen environment

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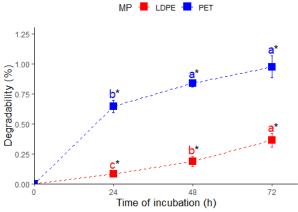


Figure 1 – Ruminal degradability (%) of polyethylene terephthalate (PET), and low-density polyethylene (LDPE) microplastics (MPs) at 24, 48 and 72 hours. An asterisk donates a statistical difference from 0 (p-value <0.05, Wilcoxon). Different superscripts are statistically different within MP polymer (p-value < 0.05, Kruskal-Wallis). Error bars indicate the Standard Error of the Mean. Microplastics (MPs) were detected in ruminant feeds, faeces, follicular fluid, blood, milk, and meat [1-3]. Ruminants themselves may provide an approach for tackling these pollutants, given the presence of microorganisms in rumens possessed hydrolytic enzymes capable of degrading natural polyesters such as cutin [4]. The similarities between plant-cell molecules (e.g., cutin) and plastics (e.g., polyethylene terephthalate, PET) propel researchers to investigating the capacity of rumen microbiota to degrade plastics. However, a reliable methdology to evaluate this ability has not been established [4, 5].

This study aimed to assess the use of ANKOM Daisy^{II} Incubator as a potential instrument for measuring the ability of rumen microbiota, in rumen environment, to degrade two common polymers in farms, PET and low-density polyethylene (LDPE).

Rumen fluid from Piemontese bulls was collected from a slaughterhouse. Three jars were allocated to different incubation periods; 24 (jar 1), 48 (jar 2), and 72 (jar 3) hours, each containing 0.5 g of each type of MPs

weighed in filter bags in six replicates, two empty bags as a blank, 20 g of ground Total Mixed Ration, and 2000 mL of rumen fluid- buffer mixiture. All the jars were placed in a modified ANKOM Daisy^{II} Incubator [6] at 39 °C to determine degradability of MPs calculated as weight change before and after incubation with adjustments made based on the weight change in the blanks. The experiment was triplicated.

The results demonstrated that the ANKOM Daisy^{II} Incubator identified various levels of MP degradability, ranging from 0.08 to 0.97 % (p-value < 0.05, Wilcoxon), among different MPs and incubation periods. The lowest degradability of 0.08 %, shown in LDPE with 24 hours incubation, was also succesfully distinguished from 0 (p-value = 0.0038, Wilcoxon). Significant differences were noted within both MP groups (p-value < 0.05, Kruskal-Wallis), indicating that this application provided insight into the alteration of MP degradability during the incubation. Moreover, this technique revealed that the levels of degradability statistically differed by the type of MPs, proving the polymer-specific activity of rumen microbiota.

This study using the ANKOM Daisy^{II} Incubator underscore its reliability as a method for evaluating the degradability of MPs by rumen microbiota in rumen environment. We anticipate our study to be a starting point for more sophisticated methodology for understanding this promissing ability of ruminants. Future efforts to refine this technique and explore new methods will be crucial to develop sustainable strategies to combat MPs pollution in agriculture.

[1] I. van der Veen, L. M. van Mourik, M. J. M. van Velzen, Q. R. Groenewoud and H. A. Leslie, "Plastic Particles in Livestock Feed, Milk, Meat and Blood," Environ. Health, 2022.

[3] N. Grechi, R. Franko, R. Rajaraman, J.B. Stöckl, T. Trapphoff, S. Dieterle, T. Fröhlich, M.J. Noonan, and M. de A. M. M. Ferraz, "Microplastics are present in women's and cows' follicular fluid and polystyrene microplastics compromise bovine oocyte function in vitro (pre-print)," 2023.

[6] R. Fortina, S. G Patrucco, S. Barbera and S. Tassone. "Rumen Fluid from Slaughtered Animals: A Standardized Procedure for Sampling, Storage and Use in Digestibility Trials," Methods and Protocols, 5(4), 59, 2022.

^[2] N. Beriot, J. Peek, R. Zornoza, V. Geissen, and L.E. Huerta, "Low density-microplastics detected in sheep faeces and soil: a case study from the intensive vegetable farming in southeast Spain," Sci. Total Environ., vol. 755, pp.142653, 2021.

^[4] F. Quartinello, K. Kremser, H. Schoen, D. Tesei, L. Ploszczanski, M. Nagler, S. M. Podmirseg, H. Insam, G. Piñar, K. Sterflingler, D. Ribitsch, and G. M. Guebitz, "Together is better: the rumen microbial community as biological toolbox for degradation of synthetic polyesters," Front. Bio-eng. Biotechnol., vol. 9, 2021.

^[5] H. Galyon, S. Vibostok, J. Duncan, G. Ferreira, A. Whittington, K. Havens, J. McDevitt, and R. Cockrum, "Digestibility Kinetics of Polyhydroxyalkanoate and Poly(butylene succinate-co-adipate) after In Vitro Fermentation in Rumen Fluid," Polymers, vol. 14, 2022.

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