In vitro gas production technique applied to measure the impact of low-density polyethylene microplastics on lamb ruminal activity

In vitro gas production technique applied to measure the impact of low-density polyethylene microplastics on lamb ruminal activity

K. Abid[1], S. Barbera[1], S. Glorio Patrucco[1], H. Kaihara[1], and S. Tassone*[1]
[1] Department of Agriculture, Forestry and Food Sciences, University of Turin, Largo Braccini 2, 10095 Grugliasco, Italy.

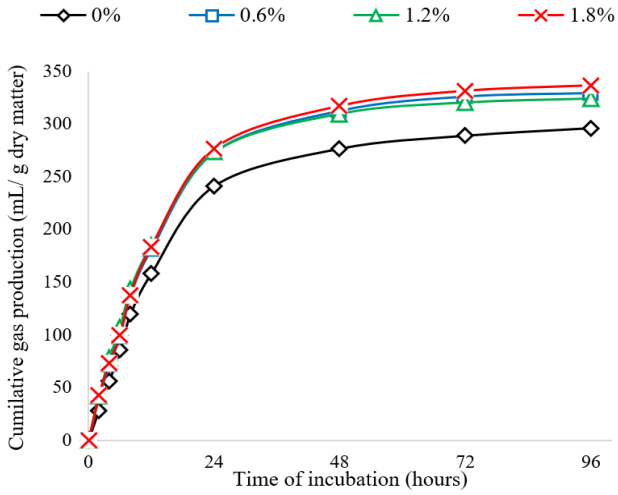

Figure 1 - Impact of different concentrations of low-density polyethylene on *in vitro* gas production during incubation of concentrate with lamb rumen fluid.

The proliferation of microplastics (MPs) in ruminant digestive system [1] and feeds has raised significant concerns [2], necessitating urgent attention and precise assessment techniques to understand their impact on rumen function. This study was conducted to explore the capacity of the *in vitro* gas production (GP) technique, commonly used for rumen fermentation assessment, to investigate the influence of low-density polyethylene (LDPE), the predominant plastic in agriculture [3], on rumen activity and degradability.

Rumen fluid was collected from five lambs before morning meal using a rubber stomach tube inserted into the rumen through the oesophagus. Rumen samples were prepared according to the official method [4]. Samples of 200 mg of concentrate fortified with virgin LDPE sourced from industrial company and measuring under 2 mm in dimensions, at varying concentrations (0, 0.6, 1.2 and 1.8%) were inoculated with 30 mL of buffered rumen fluid in triplicate into serum bottles. Blanks containing only buffered rumen fluid were also prepared in triplicate to correct gas production from the buffered rumen. All bottles were immediately sealed and incubated for 96 hours in a shaking-water bath at 39 °C and 120 rpm. Gas production was measured after 2, 4, 6, 8, 12, 24, 48, 72, and 96 hours, and data were fitted with an exponential model [5]. Additionally, post fermentation assessments were conducted to evaluate concentrate dry matter degradability, rumen ammonia-nitrogen levels, rumen protozoa population, microbial crude protein and microbial efficiency.

The *in vitro* GP technique successfully detected and quantified the significant impact of LDPE contamination on gas production (Fig. 1), rumen ammonia-nitrogen levels, fermentation rates and microbial efficiency across all doses. Particularly at the highest LDPE dose, gas emissions, rumen ammonia-nitrogen levels and fermentation rates increased by 15, 12 and 18%, respectively, while microbial efficiency decreased by 16%. The effect of LDPE contamination on microbial crude protein, rumen protozoa population, and concentrate dry matter degradability was noted only at the highest LDPE dose, resulting in decreases of 8.1, 16.4 and 4.6%, respectively.

These findings underscore the potential of the *in vitro* GP technique in studying the impact of LDPE on rumen functionality and concentrate utilization in controlled laboratory conditions by mimicking rumen fermentation processes. Further research on the utilization of *in vitro* GP technique can be conducted to understand the impact of different MP polymers present in feeds on the digestive physiology of ruminants.

[1] 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 southeastt Spain," *Sci. Total Environ.,* vol. 755, pp.142653, 2021.
[2] S.S. Maganti, and R.C Akkina, "Detection and characterisation of microplastics in animal feed," *Online J. Anim. Feed Res.*, vol 13(5), pp. 348-356, 2023.
[3] I. Sa'adu, and A. Farsang, "Plastic contamination in agricultural soils: a review," *Environ. Sci. Eur.*, vol 35(1), pp. 13, 2023.
[4] KH.Menke, and H. Steingass, "Estimation of the energetic feedvalue obtained from chemical analysis and *in vitro* gas production using rumen fluid," *Anim. Res. Dev.,* vol 28, pp 7–55, 1988.
[5] J. France, J. Dijkstra, M.S. Dhanoa, S. Lopez, and A. Bannink, "Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed *in vitro*: derivation of models and other mathematical considerations". *Br. J. Nutr*, vol 83(2), pp. 143-150, 2000.

* e-mail: Sonia.Tassone@unito.it