Degradation of Microplastics in an In Vitro Ruminal Environment

fermentation

MDPI

Article

Degradation of Microplastics in an In Vitro Ruminal Environment

Sonia Tassone [1,*], Rabeb Issaoui [2], Valentina Balestra [3], Salvatore Barbera [1], Marta Fadda [4], Hatsumi Kaihara [1], Sara Glorio Patrucco [1], Stefania Pragliola [5], Vincenzo Venditto [5] and Khalil Abid [1,*]

[1] Department of Agricultural, Forest and Food Sciences, University of Turin, Largo P. Braccini 2, 10095 Grugliasco, Italy; salvatore.barbera@unito.it (S.B.); hatsumi.kaihara@unito.it (H.K.); sara.gloriopatrucco@unito.it (S.G.P.)
[2] Independent Researcher, Tunis 1068, Tunisia; rabeb.issaoui@etudiant-issbat.utm.tn
[3] Department of Environment, Land and Infrastructure Engineering, Politecnico di Torino, Corso Duca degli Abruzzi, 24, 10129 Torino, Italy; valentina.balestra@polito.it
[4] Quantum Metrology and Nano Technologies Division, Istituto Nazionale di Ricerca Metrologica (INRiM), Strada delle Cacce 9, 10135 Torino, Italy; fadda.marta93@gmail.com
[5] Department of Chemistry and Biology "A. Zambelli", University of Salerno, Via Giovanni Paolo II, 132, 84084 Fisciano, Italy; spragliola@unisa.it (S.P.); vvenditto@unisa.it (V.V.)
* Correspondence: sonia.tassone@unito.it (S.T.); khalil.abid@unito.it (K.A.)

Abstract

Microplastic (MP) pollution is an emerging concern in ruminant production, as animals are exposed to MPs through air, water, and feeds. Ruminants play a key role in MP transmission to humans via animal products and contribute to MP return to agricultural soil through excreta. Identifying effective strategies to mitigate MP pollution in the ruminant sector is crucial. A promising yet understudied approach involves the potential ability of rumen microbiota to degrade MPs. This study investigated the in vitro ruminal degradation of three widely distributed MPs—low-density polyethylene (LDPE), polyethylene terephthalate (PET), and polyamide (PA)—over 24, 48, and 72 h. PET MP exhibited the highest degradation rates (24 h: 0.50 ± 0.070%; 48 h: 0.73 ± 0.057%; and 72 h: 0.96 ± 0.082%), followed by LDPE MP (24 h: 0.03 ± 0.020%; 48 h: 0.25 ± 0.053%; and 72 h: 0.56 ± 0.066%) and PA MP (24 h: 0.10 ± 0.045%; 48 h: 0.02 ± 0.015%; and 72 h: 0.14 ± 0.067%). These findings suggest that the ruminal environment could serve as a promising tool for LDPE, PET, and PA MPs degradation. Further research is needed to elucidate the mechanisms involved, potentially enhancing ruminants' natural capacity to degrade MPs.

Keywords: low-density polyethylene; microplastics; polyamide; polyethylene terephthalate; rumen microbiota; ruminal degradability

Academic Editor: Cláudia Braga Pereira Bento

Received: 24 June 2025
Revised: 21 July 2025
Accepted: 23 July 2025
Published: 31 July 2025

Citation: Tassone, S.; Issaoui, R.; Balestra, V.; Barbera, S.; Fadda, M.; Kaihara, H.; Glorio Patrucco, S.; Pragliola, S.; Venditto, V.; Abid, K. Degradation of Microplastics in an In Vitro Ruminal Environment. *Fermentation* **2025**, *11*, 445. https://doi.org/10.3390/fermentation11080445

1. Introduction

Plastic pollution has emerged as one of the most critical environmental challenges of the 21st century. Global plastic production has surged from 2 million Mg in 1950 to over 400 million Mg annually, with cumulative waste generation expected to reach 11 billion Mg by 2025 and 26 billion Mg by 2050 [1]. Despite global efforts to manage this crisis, current recycling systems remain inefficient—only 9% of plastic waste is recycled, while 22% is mismanaged, 49% landfilled, and 20% incinerated, leaving the majority (91%) to persist in the environment [1]. Without intervention, plastic waste could exacerbate ecological and health risks.

Once released into the environment, plastics undergo progressive fragmentation through physical and chemical degradation processes, including ultraviolet photodegradation (sunlight breaks polymer bonds), mechanical abrasion (wind, waves, and sediment wear down plastic), oxidative degradation (oxygen reactions weaken plastic structure), and abiotic weathering (temperature fluctuations and hydrolysis contribute to breakdown), generating microplastics (MPs, plastic with dimensions between 1 μm and 5 mm) [2,3]. Nowadays, MPs are ubiquitous, representing a serious concern for both aquatic and terrestrial ecosystems; in addition, their small size and hydrophobic surfaces allow them to adsorb toxic chemicals, including persistent organic pollutants and heavy metals, amplifying their harm [4]. This contamination poses a dangerous threat to both animal and human health [5].

Ruminants, mainly including cattle, sheep, and goats, are significant participants to the global MPs cycle due to their widespread farming practices and digestive physiology. These animals are primarily exposed to MPs through three major pathways: MPs suspended in the air, particularly in intensive farming environments, can be inhaled by livestock, leading to respiratory and systemic exposure; ingestion via drinking water from water sources contaminated with MPs, including surface runoff from plastic-laden agricultural fields; and consumption of contaminated feed often containing MPs from degraded agricultural plastics, soil contamination, or processing/packaging materials [6,7]. MPs on farms may also derive from the fragmentation of commonly used plastics, primarily composed of low-density polyethylene (LDPE) or polyamide (PA) [3,8]. Potential sources include agricultural plastics such as hay bale wraps, silage covers, mulching films, irrigation pipes, and plastic-coated fertilizers used in feed production and crop cultivation [8,9]. Moreover, ruminants can ingest large plastic debris that could be broken down into MPs during the digestion process [2].

MPs were found inside ruminant bodies (blood, follicular fluid, and sperm) indicating systemic circulation and potential reproductive risks [10–12]. MPs can be passed to humans through the consumption of contaminated animal products such as milk (due to mammary gland uptake) and edible tissue (via bioaccumulation in muscle and organs) [12–15]. MPs can also return to the environment from ruminants through their feces [15–17]. Human exposure to MPs through animal-derived food products is expected to increase due to the rising global demand for animal-based foods, driven by population growth, which is projected to reach 9.7 billion by 2050 [18,19]. It is important to note that, given current scientific and technological limitations, there are no effective methods for the large-scale collection or removal of MPs from the environment. Despite their widespread presence, strategies for reducing or degrading MPs in situ remain largely undeveloped, making intervention and remediation extremely challenging at this time. Therefore, it is crucial to identify effective strategies to mitigate MP pollution in ruminant sectors. One promising but understudied solution could come from the ruminants themselves.

In vitro studies have demonstrated that rumen microbiota possess the capacity to hydrolyze various synthetic polyester MPs, including both powder and film forms of polyethylene terephthalate (PET), the biodegradable polyester polybutylene adipate-co-terephthalate, and the bio-based polyester poly(ethylene furanoate) [20–22]. Notably, the hydrolytic activity observed in whole rumen fluid was substantially higher than that achieved using purified enzymes or supernatants from individual microbial isolates, indicating the synergistic effect of the complex microbial community present in the rumen. The initial stage of polyester degradation was detected within 24 h of incubation, underscoring the rapid onset of microbial activity [20]. Furthermore, both in situ and in vitro investigations by Galyon and colleagues [21] have substantiated the ability of rumen microbiota to degrade additional biodegradable polyesters, such as polyhydroxyalkanoates

and poly(butylene succinate-co-adipate), whether these polymers were tested individually or as blends. In these studies, the onset of degradation was similarly observed within the first 24 h, further confirming the efficiency and versatility of rumen microbial consortia in initiating the breakdown of diverse polyester-based plastics [22].

Given the urgent need to address microplastic pollution in livestock systems, and the promising yet insufficiently explored capacity of rumen microbiota to degrade MPs, this study aimed to evaluate the in vitro degradation potential of rumen microbiota against three widely prevalent MPs: low-density polyethylene, polyamide, and polyethylene terephthalate. This study assumes that the rich rumen microbiota can contribute the degradation of MPs, as it contains enzymes such as cutinases, which are typically involved in cutin hydrolysis. Since certain polymers, like polyethylene terephthalate, share structural similarities with natural fibers, it is hypothesized that the microbiota may also be capable of breaking them down. This represents the first study aimed at exploring the potential of the rumen as a MPs biodigester.

2. Materials and Methods

2.1. Microplastic Samples

LDPE, PA, and PET MPs were provided by a private company specialized in the production and sale of thermoplastic polymers. MPs were particles with a length-to-diameter ratio of less than 3, in accordance with the classification outlined by the European Chemicals Agency [23]. All three polymers were white and declared the absence of any additives. The company provided the analysis certificates for the three MPs (ISO 13320 [24]). LDPE MP had an average real value of granulometry of 643 ± 80 µm and a density of 0.918 g/cm^3 (ISO 1183 [25]). PA MP had an average real value of granulometry of 993 ± 100 µm and a bulk density of 0.45 g/cm^3 (ASTM D1895 method A [26]). PET MP had an average real value of granulometry of 522 ± 221 µm and a bulk density of 0.57 g/cm^3 (ASTM D1895 method A).

The MP size distributions are presented in Figure 1 along with a stereomicroscope (EZ4, Leica, Leica Microsystems GmbHm Wetzlar, Germany) picture for each polymer.

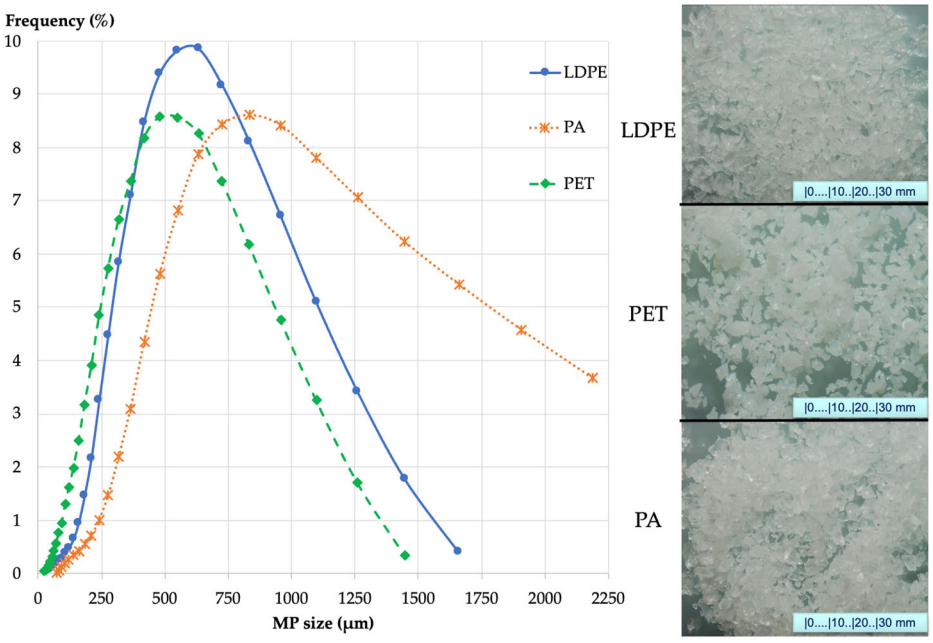

Figure 1. Microplastic (MP) size distribution (in µm) of LDPE (low-density polyethylene), PET (polyethylene terephthalate), and PA (polyamide) provided by a private company on the left. Stereomicroscope pictures of LDPE, PET, and PA MPs used in the experiment on the right.

2.2. Rumen Fluid Collection and Buffer Rumen Fluid Preparation

An experimental run was conducted weekly for three weeks, with 2 L of rumen fluid collected from three healthy Piedmontese bulls immediately after slaughter at a slaughterhouse, following the standardized protocol of Fortina et al. [27]. All donor bulls, approximately 16 months old, were fed the same daily diet consisting of 2 kg of barley straw, 4 kg of ryegrass hay, and 10 kg of concentrate. The concentrate contained 68.9% corn meal, 11.7% soybean meal, 5.9% beet pulp, 5% bran, 1% soybean oil, 1% extruded flaxseeds, 0.5% hydrogenated fat, 3.5% calcium carbonate, 0.5% buffer, 0.9% multi-acid, and 1% urea. The rumen fluid was transported to the laboratory in a pre-warmed thermic bottle at 39 °C within 20 min to preserve its integrity. Upon arrival, the rumen fluid was filtered through three layers of cheesecloth and mixed with the buffer solution described in the "In Vitro True Digestibility using the ANKOM DAISY^{II} Incubator (Method 3)" [28] under continuous CO_2 flushing at 39 °C to simulate the ruminal environment.

2.3. Microplastics Degradability in In Vitro Ruminal Environment

In vitro ruminal fermentation to assess LDPE, PET, and PA MP degradability by rumen microbiota was performed with the Ankom Daisy^{II} Incubator (AD^{II}, Ankom Technology Corporation Fairport, Macedon, NY, USA), a thermostatically controlled chamber (at 39 °C) containing 4 rotating digestion jars that simulate the ruminal environment. The protocol followed was an adaptation of the method for feed digestibility provided by ANKOM Technology, "In Vitro True Digestibility using the ANKOM DAISY^{II} Incubator (Method 3)" [28].

In brief, each jar (1, 2, 3, and 4) was loaded with LDPE, PET, and PA MP samples, each previously weighed (500 mg) and sealed in F57 ANKOM bags (25 µm pore size; Ankom Technology Corporation Fairport, Macedon, NY, USA) using a thermos-sealer. Each jar contained 6 replicate bags for each polymer and 2 empty bags totaling 20 bags per jar. Jars 1, 2, and 3 were filled with buffered rumen fluid (1600 mL of buffer solution and 400 mL of rumen fluid) and 20 g of dried and 2 mm ground diet provided to the rumen fluid donor bulls (TMR). Jar 4, intended to assess potential MP loss, contained only the buffer solution (1600 mL). Before the incubation, each jar was saturated with CO_2 to maintain anaerobic conditions. The bags were removed after 24 h of incubation from jar 1, after 48 h from jar 2, and after 72 h of incubation from jar 3. From jar 4, which contained 6 bags of each polymer type and only the buffer solution, two bags per polymer were removed at 24, 48, and 72 h of incubation to assess potential MP weight loss. At the end of each incubation period, both bags with MPs and empty bags were removed and washed in cold tap water (15 min) to clean them from buffered ruminal fluid residues. After washing, samples were dried at 60 °C in a forced-air oven for at least 6 h. The experimental design is illustrated in Figure 2.

MP degradability, corrected for any variation in empty bag weight, was measured by weight loss [29,30] using a five-decimal place precision balance (CP225D, Sartorius AG, Göttingen, Germany) and calculated as follows:

$$\text{MP degradability } (\%): \left(\frac{(MP\ weight_{PreInc} - MP\ weight_{PostIncCorrected})}{MP\ weight_{PreInc}}\right) \times 100$$

where

$$\boldsymbol{MP\ weight_{PreInc}} = (\boldsymbol{MP + Bag})\ \boldsymbol{weight_{PreInc}} - \boldsymbol{Bag\ weight}$$

$$\boldsymbol{MP\ weight_{PostInc}} = (\boldsymbol{MP + Bag})\ \boldsymbol{weight_{PostInc}} - \boldsymbol{Bag\ weight}$$

$$\boldsymbol{MP\ weight_{PostIncCorrected}} = \boldsymbol{MP\ weight_{PostInc}} - (\boldsymbol{Rumen\ fluid\ residue\ on\ the\ bag + MP\ loss})\ \boldsymbol{weight}$$

Rumen fluid residue on the bags and MP loss were quantified using a control group —bags containing MPs incubated with buffer solution, without ruminal fluid. Additionally, MPs were visually inspected under a stereomicroscope before and after incubation to assess physical changes.

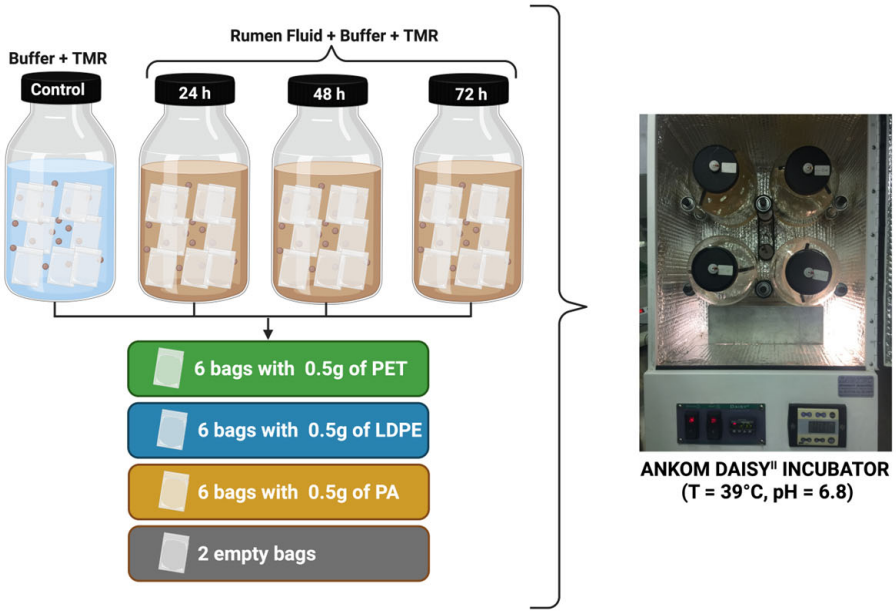

Figure 2. Summary of microplastics biodegradability in vitro ruminal fermentation. LDPE = low-density polyethylene; PET = polyethylene terephthalate; and PA = polyamide.

2.4. Chemical Analysis of LDPE Microplastic Before and After Ruminal Degradation

The chemical structure of LDPE MP, aimed at detecting ruminal fluid activity, was investigated using multiple analytical techniques.

Fourier Transform Infrared (FT-IR) Analysis: Infrared spectra of LDPE MP samples before and after degradation were recorded using a Tensor 27 Bruker spectrometer (Ettlingen/Rheinstetten, Germany, 2.0 cm^{-1} resolution) equipped with a deuterated triglycine sulfate detector and a Ge/KBr beam splitter. The frequency scale was internally calibrated to 0.01 cm^{-1} using a He–Ne laser (Helium-Neon laser, Thorlabs Inc., Newton, NJ, USA). Thirty-two signal scans were averaged to minimize noise. Prior to analysis, samples were prepared by evaporating a toluene polymer solution onto KBr plates at room temperature. The deposits were vacuum-dried at room temperature, and solvent evaporation was monitored via FT-IR analysis.

Carbon-13 Nuclear Magnetic Resonance (^{13}C NMR) Analysis: NMR spectra of LDPE MP samples were obtained using a Advance 300 spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany, ^{13}C, 75 MHz) at 353 K. Samples were prepared by dissolving 10 mg of LDPE MP in 0.5 mL of tetrachloro-dideuteroethane (TCDE), with hexamethyldisiloxane (HMDS) as the internal chemical shift reference.

Size Exclusion Chromatography (GPC/SEC) Analysis: Molecular weight distribution and dispersity were determined by gel permeation chromatography (GPC) at 140 °C using 1,2,4-trichlorobenzene as the solvent. Polystyrene standards with narrow molecular weight distributions were used for calibration. Measurements were conducted on a PL GPC 210 system with four 4 PL-Gel Mixed A Columns, a refractive index detector, and a DM 400 data manager (Viscotek, Church Stretton, Shropshire, UK).

2.5. Statistical Analysis

Data from three experimental runs (n = 240) were analyzed using RStudio (version 2022.07.2) to assess the ruminal degradation of LDPE, PET, and PA MPs over different incubation periods (24, 48, and 72 h). As the data did not follow a normal distribution, non-parametric tests were employed. For each type of MP and incubation time, a Wilcoxon test was applied using the "wilcox.test" function from the "stats" package (version 4.2.2) to determine whether degradability differed significantly from zero. To compare degradability among LDPE, PET, and PA MPs across incubation times, the Kruskal–Wallis test was applied using the "kruskal" function from the "agricolae" package (version 1.3-5). Pairwise comparisons were adjusted using the Bonferroni correction to account for multiple testing. Results are reported as means ± SE. Data visualization was carried out using the "ggplot2 package" (version 3.5.1).

3. Results

As illustrated in Figure 3, PET MP exhibited the highest level of ruminal degradation, followed by LDPE and PA MPs. At 24 h, PA and LDPE MPs demonstrated similar degradation rates. For PET MP, degradability was significantly different from zero at all three incubation times ($p < 0.0001$). Moreover, the degradability of PET MP at 72 h was significantly greater than at 24 h, with values of 0.50 ± 0.070%, 0.73 ± 0.057%, and 0.96 ± 0.082% at 24, 48, and 72 h, respectively. Regarding LDPE MP, degradability was significantly different from zero at 48 h ($p = 0.001$) and 72 h ($p < 0.001$), showing a significant increase over time (24 h = 0.03 ± 0.020%; 48 h = 0.25 ± 0.053%; and 72 h = 0.56 ± 0.066%). However, the degradability of LDPE MP at 72 h was only comparable to that of PET MP at 24 h. In contrast, PA MP exhibited a degradability significantly different from zero only at 24 h ($p = 0.020$), but values remained close to zero throughout the incubation period (24 h = 0.10 ± 0.045%; 48 h = 0.02 ± 0.015%; and 72 h = 0.14 ± 0.067%). Although the weight loss at 72 h was greater than at 24 h, the high variability precluded the degradability from being statistically significant at this time point. The apparent decrease in PA degradation at 48 h represents normal experimental variation, as confirmed by the lack of statistical significance. This temporary fluctuation does not affect the overall findings, which show that PA exhibits strong resistance to degradation throughout the 72 h timeframe.

Figure 4 presents stereomicroscope pictures of LDPE, PET, and PA MPs before and after an incubation of 0, 24, 48, and 72 h. The stereomicroscope images show a progressive darkening or browning effect, specifically in the PA MP samples, that becomes increasingly apparent at the 48 and 72 h time points compared to the initial 0 and 24 h samples. This color change is very light in both the LDPE and PET MPs samples, which maintain a relatively consistent appearance across all time points.

From a chemical perspective, although ruminal environments are known to initiate the degradation of polymers such as PET and PA MPs [20,31], their impact on LDPE MP remains poorly understood [22,32]. To address this knowledge gap, comprehensive analyses were conducted on LDPE MP residues recovered after incubation with ruminal fluid. These analyses included FT-IR, ^{13}C NMR spectroscopy, and GPC/SEC performed at multiple incubation time points for comparative evaluation. FT-IR spectroscopy highlights the presence of functional groups that can be generated on the surface of LDPE MP samples due to the ruminal degradation processes [32–35].

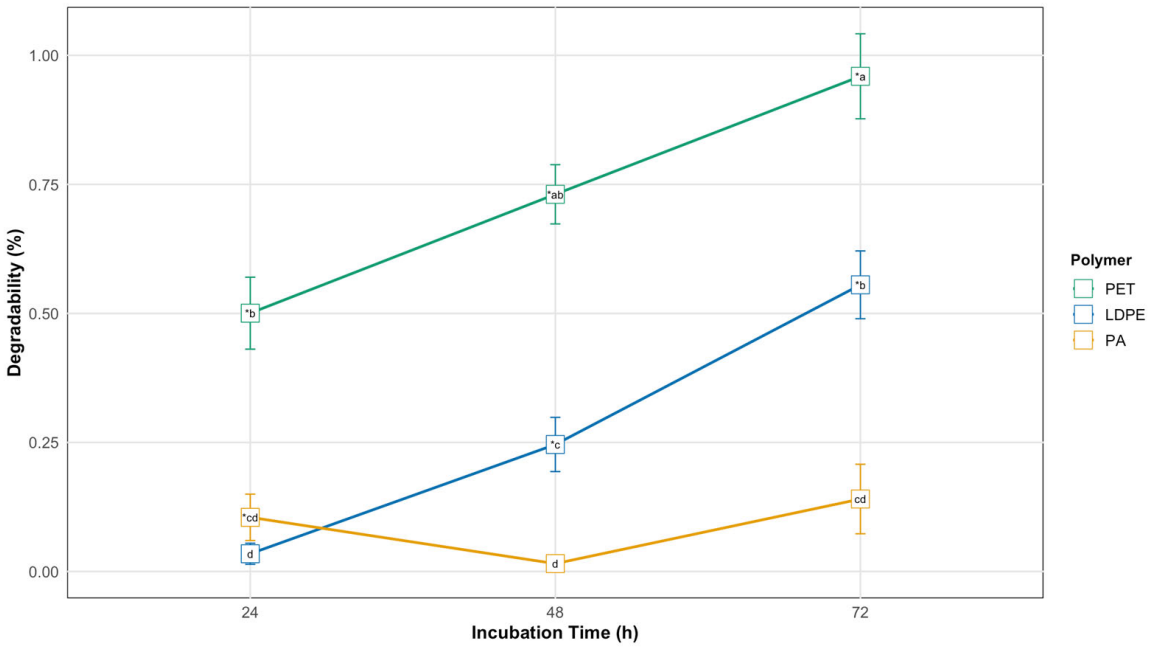

Figure 3. Ruminal degradability (%) of PET (polyethylene terephthalate), LDPE (low-density polyethylene), and PA (polyamide) polymers during in vitro incubation with buffered rumen fluid (incubation times: 24, 48, and 72 h). To test whether degradability was significantly greater than 0%, a Wilcoxon test was applied (* = p-value < 0.05). To compare degradability differences among the three polymers across incubation times, a Kruskal–Wallis test was applied (a–d = p-value < 0.05).

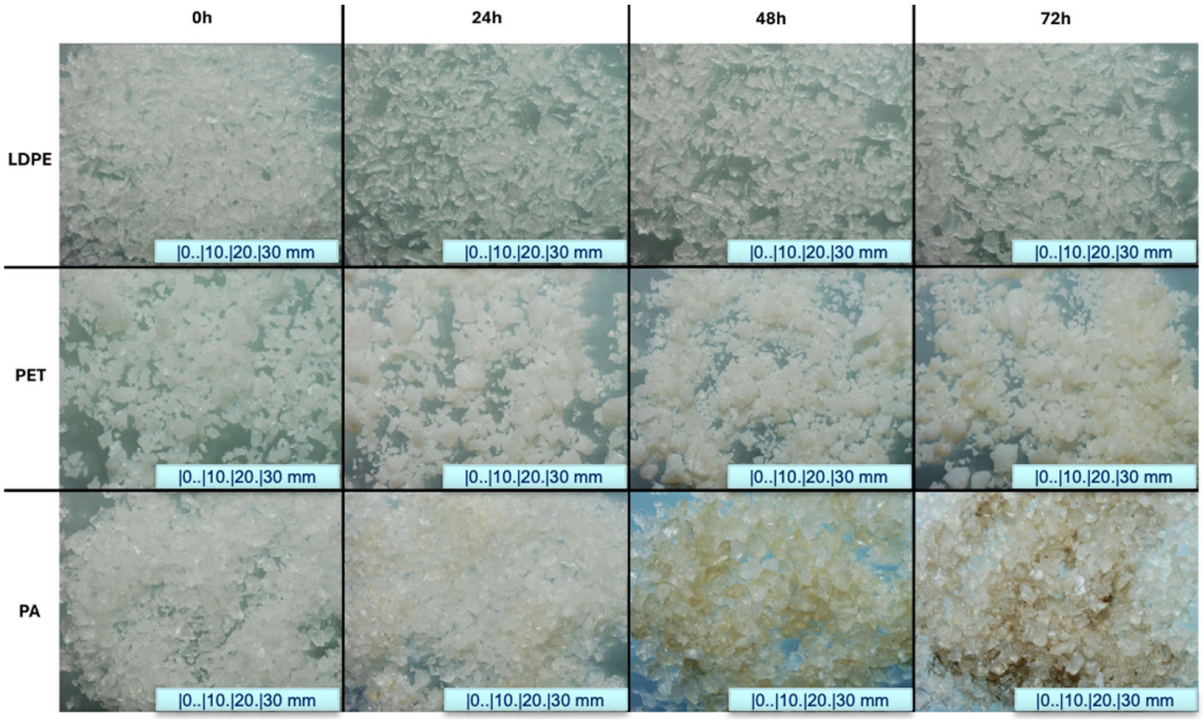

Figure 4. Stereomicroscope pictures of LDPE (low-density polyethylene), PET (polyethylene terephthalate), and PA (polyamide) MPs in the in vitro incubation with rumen fluid (incubation times: 0, 24, 48, and 72 h).

Figure 5 presents the FT-IR spectra of LDPE MP residues recovered after 24, 48, and 72 h of incubation in ruminal fluid, along with spectra of LDPE MP incubated in the buffer for 72 h and untreated LDPE MP. The upper portion of Figure 5 illustrates the spectral

region between 4000 and 2000 cm^{-1}, while the lower portion covers the 2000–400 cm^{-1} range. Additionally, the 24 h sample was included to assess early indications of degradation trends, if there were any.

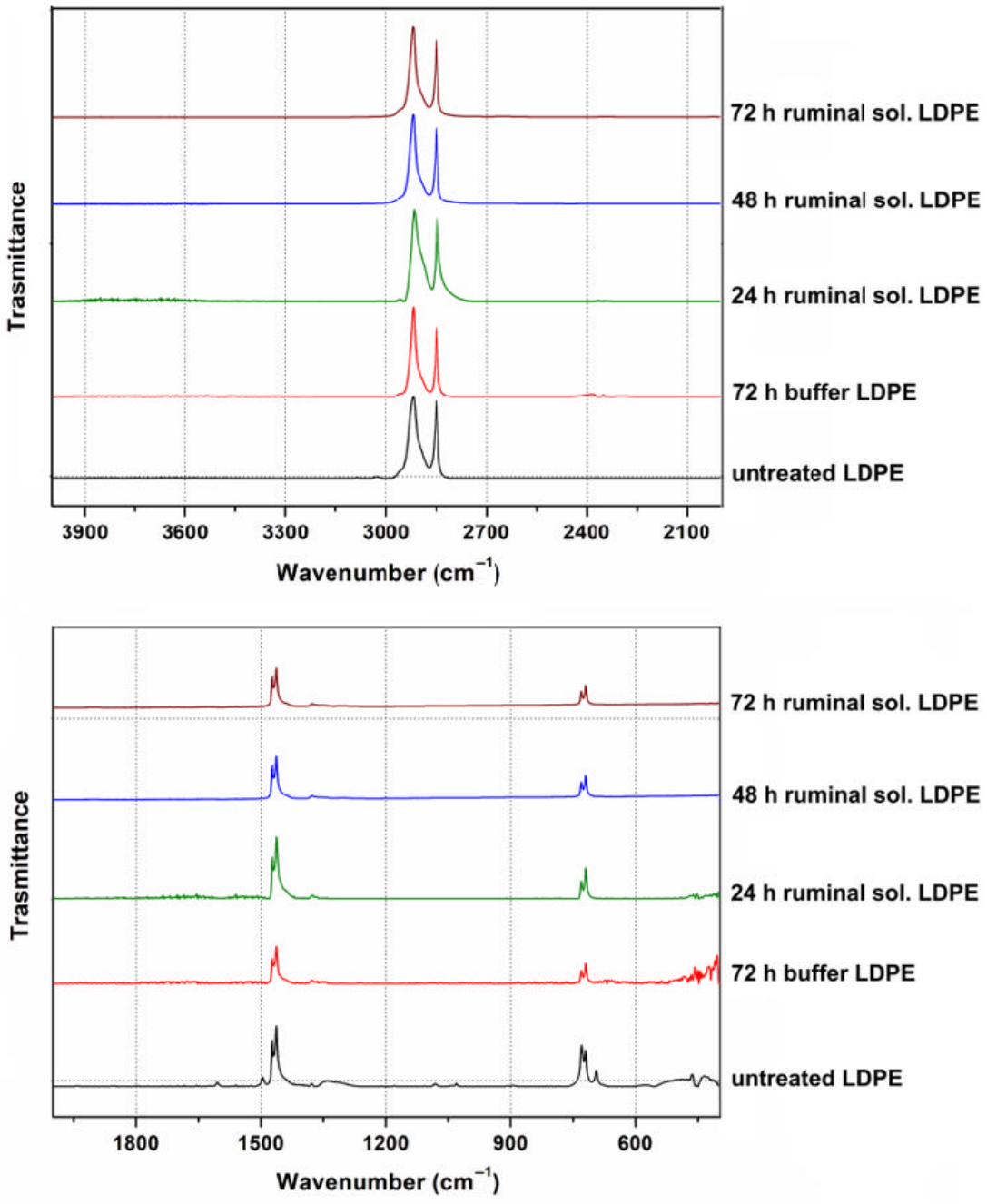

Figure 5. Fourier transform infrared spectra of LDPE (low-density polyethylene) samples at 0, 24, 48, and 72 h of incubation in buffered rumen fluid exhibit peaks exclusively at the characteristic absorption bands of the polymer. The upper figure illustrates the spectral region between 4000 and 2000 cm^{-1}, while the lower figure covers the 2000–400 cm^{-1} range.

The analysis confirmed the presence of the LDPE polymer in all samples. Notably, the spectra of residues from rumen fluid incubation (24–72 h) and buffer incubation (72 h) were identical, showing only characteristic LDPE MP absorption bands without detectable structural modifications. Specifically, these spectra exhibited peaks exclusively at the characteristic absorption bands of LDPE polymer, including peaks at 2918 and 2850 cm^{-1} (asymmetric and symmetric C–H stretching vibrations); at 1472 and 1463 cm^{-1}, associated

with CH_2 scissoring vibrations; and at 729 and 720 cm^{-1}, attributed to CH_2 rocking vibrations [36,37]. No oxidative signatures (e.g., carbonyl groups) were observed. In contrast, the untreated LDPE MP sample displayed additional non-polymeric peaks (~1050 cm^{-1}) likely from additives, a finding inconsistent with the supplier's certified specifications.

The results obtained from the FT-IR analysis were further confirmed by ^{13}C NMR spectroscopy, which has been used to investigate the microstructure of the polymer chains. The ^{13}C NMR data showed that no significant alterations occurred in the polymer microstructure following the incubation in rumen fluid. As an illustrative example, in Figure 6, the spectra of untreated and degraded LDPE MP residues after 72 h of incubation are compared.

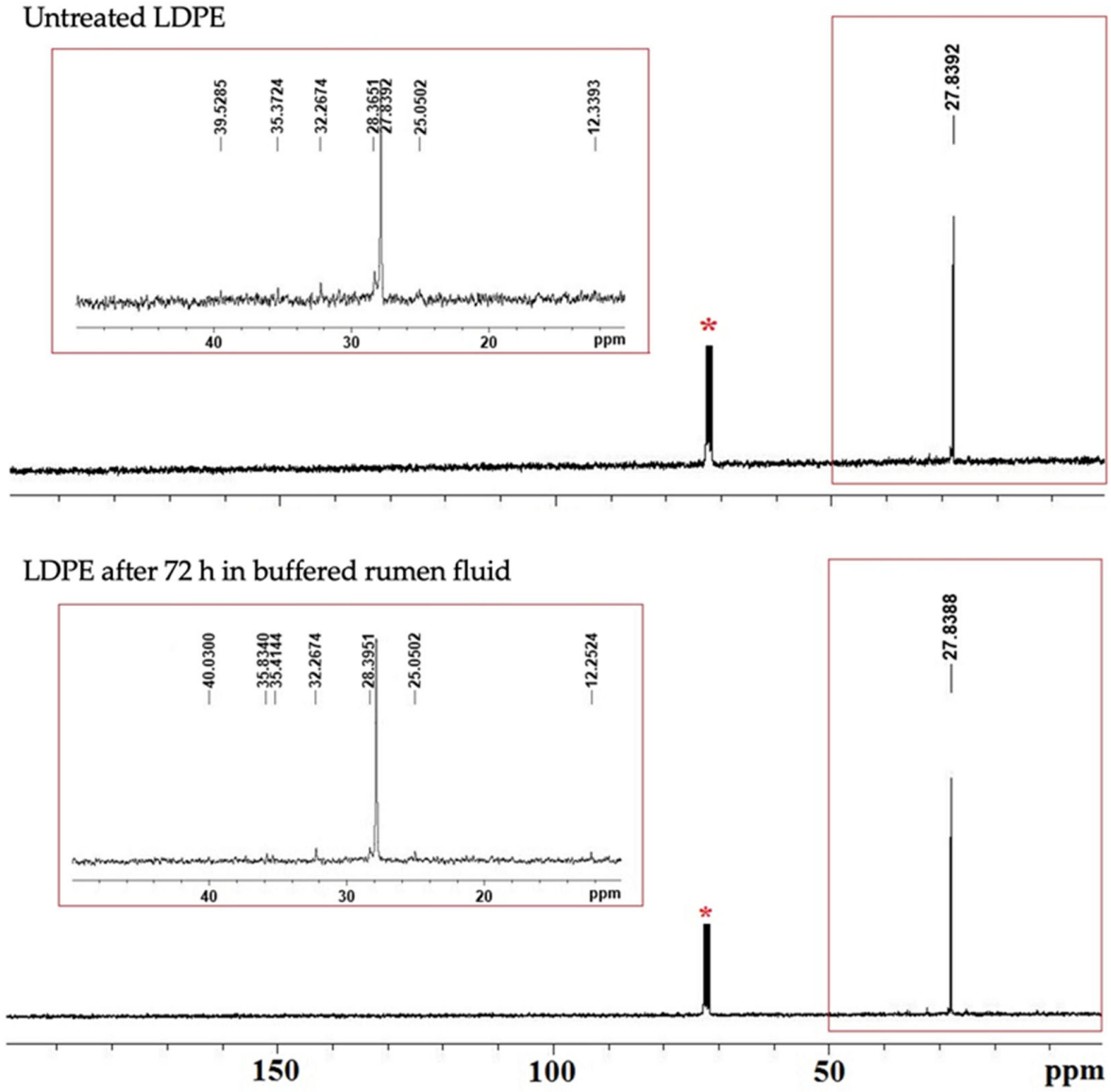

Figure 6. ^{13}C NMR spectra of LDPE (low-density polyethylene) samples—either untreated or incubated for 72 h in buffered rumen fluid—show no significant alterations in the polymer microstructure after incubation. * Solvent: tetrachlorodideuteroethane; scale: hexamethyldisiloxane.

Size Exclusion Chromatography analysis was carried out to evaluate whether exposure to the ruminal environment could influence the molecular weight of the PE MP samples. It is known that degradation processes can sometimes lead to an increase in the average molecular weight, potentially due to the preferential degradation or loss of lower-molecular-weight chains [38–40].

However, the spectra of the untreated LDPE MP sample and the LDPE MP sample incubated in buffered rumen fluid for 72 h (Figure 7a,b) show no significant differences in either the average molecular weight or dispersity. This indicates that incubation in rumen fluid does not induce a reduction in polymer molecular weight, nor does it lead to the extraction of low-molecular-weight chains. It should be noted that SEC does not provide information on low-molecular-weight additives, i.e., compounds with molecular weights significantly lower than those of the polymer chains.

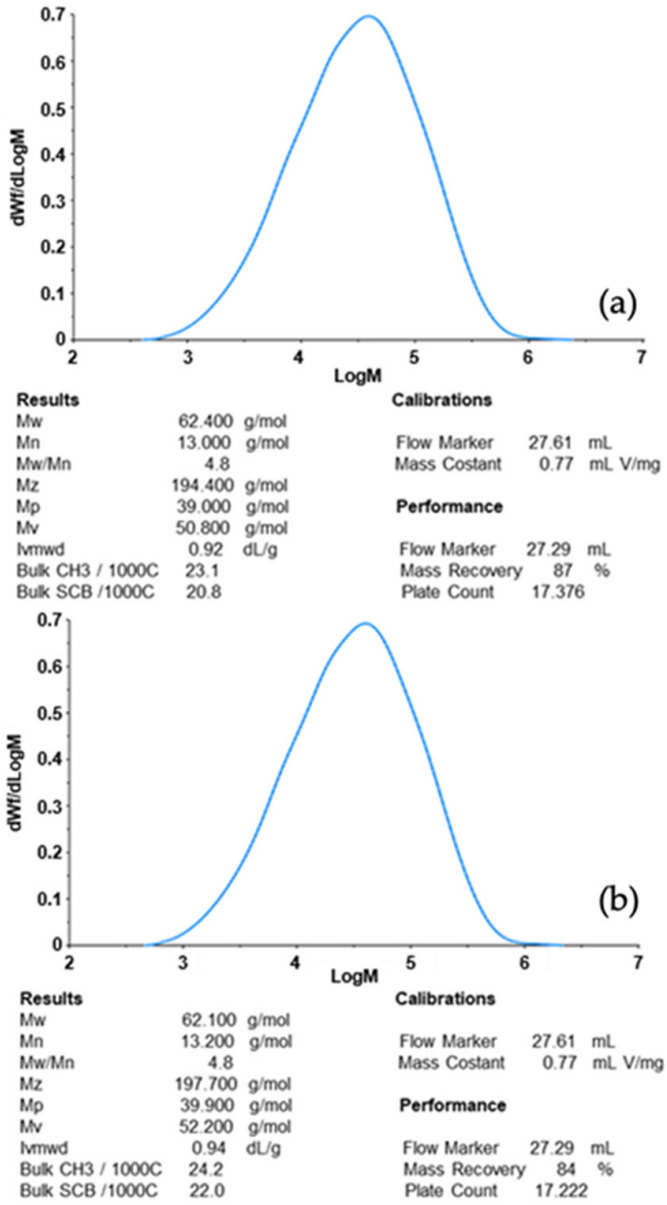

Figure 7. GPC/SEC trace of LDPE (low-density polyethylene) sample: (**a**) untreated sample; (**b**) incubated in buffered rumen fluid for 72 h.

4. Discussion

This study offers one of the first insights into the potential of rumen microbiota to degrade synthetic polymers—specifically LDPE, PA, and PET MPs—under conditions closely resembling the ruminal environment. The results demonstrated that PET MP exhibited the highest degradability (0.96 ± 0.082% after 72 h), followed by LDPE MP (0.56 ± 0.066% at 72 h) and PA MP (0.10 ± 0.045% at 24 h).

The higher degradability of PET MP compared to LDPE and PA MP can be attributed to the similarities in structure between PET MP and cutin [20,31]. Rumen microbiota naturally produces cutinases (EC 3.1.1.74), enzymes that hydrolyze cutin—a primary component of plant cuticles situated between the primary cell wall and the surface wax [20,41]. Both cutin and PET MP are polyesters containing ester bonds, making them susceptible to hydrolysis by cutinases [31]. The observed degradation of PET MP is consistent with the findings of Quartinello et al. [20], who demonstrated that rumen microbiota could partially degrade PET MP, along with two other bio-based polyesters, PBAT and PEF. In their study, the degradability of PET MP increased with prolonged incubation times (24, 48, and 72 h), consistent with our results. The rumen's microbial consortium appears more effective than isolated enzymes, explaining why PET MP degradation exceeds rates reported for purified cutinases [20]. The specificity for PET MP over LDPE and PA MP aligns with the absence of ester bonds in polyethylene and polyamide structures, making them resistant to cutinase-mediated hydrolysis [20].

LDPE MPs are generally considered chemically inert. However, efforts have been made to isolate microorganisms capable of degrading LDPE MPs, with selected microbial strains emerging as a potential solution for waste biodegradation. LDPE polymer is highly recalcitrant to degradation due to its hydrophobic, saturated C-C backbone and high molecular weight [42]. Rumen microbiota may introduce carbonyl (C=O) or hydroxyl (-OH) groups via partial oxidation, detectable by FT-IR [43], and cause a limited reduction in molecular weight, suggesting a slow depolymerization [38]. Wanapat et al. [32] reported a 2.66% mass loss in LDPE MP after prolonged in vitro ruminal incubation (30 days, rumen-derived Pseudomonas aeruginosa) and a 0.80% loss after 10 days, which aligns with our findings after 3 days of exposure. Our results revealed a low but statistically significant weight loss (0.56% at 72 h); in contrast, the in situ trial by Galyon et al. [21] reported no detectable degradation of LDPE macroplastics. Huerta Lwanga et al. [44], by SEM imaging, revealed surface pitting but no bulk erosion. Additives (e.g., antioxidants, UV stabilizers) in commercial LDPE polymers further inhibit microbial action [45]; moreover, degradation is highly dependent on rumen retention time (typically 24–48 h for cattle) and particle size (microplastics > macroplastics) [32,39]. The chemical analysis of the effects of the ruminal environment on LDPE MP by three different approaches (FT-IR, ^{13}C NMR, and GPC/SEC) indicates that ruminal fluid incubation did not reduce polymer molecular weight or release low-molecular-weight chains. Notably, GPC/SEC analysis cannot detect low-molecular-weight additives (i.e., compounds with molecular weights far below the polymer range). These results suggest that LDPE MP retains its molecular integrity under tested conditions, demonstrating a high resistance to rumen-mediated degradation. Thus, the observed mass loss in LDPE MP samples is likely attributable to the extraction of additives from the polymer matrix rather than substantive polymer degradation.

PA MP degradability in the ruminal environment appears to be low as PA polymers (nylon-6, nylon-6,6) are generally resistant to abiotic hydrolysis due to their high crystallinity and strong hydrogen-bonded networks [46] but can occur via the enzymatic action of microbial proteases/amidases [20]. Rumen microbiota (e.g., Clostridium, Prevotella) secrete enzymes that cleave the PA's amide linkages, though rates are low [31]. Brunner et al. [47], by NMR, detects oligomer formation (e.g., ε-caprolactam from nylon-6), confirming partial depolymerization. FT-IR studies show the carbonyl index (C=O) increases over time, suggesting oxidative surface degradation [43]. Also, additives (e.g., plasticizers) in commercial PA plastic may inhibit microbial activity [20], so degradation is highly dependent on the PA type (aliphatic vs. aromatic) and rumen conditions (pH, retention time). Our results demonstrated minimal degradation, likely

attributable to the short incubation period (24–48 h), which was selected to reflect typical rumen retention times.

The progressive darkening or browning effect in the PA samples that becomes increasingly apparent at the 48 and 72 h time points compared to the initial 0 and 24 h samples suggests several possibilities: Chemical degradation, as PA MP may be more susceptible to oxidative processes or other chemical reactions under the experimental conditions [48]; surface modifications and browning could indicate changes in surface chemistry or the formation of degradation products [49]; a material specific response, as only PA shows this effect while LDPE and PET remain unchanged, suggesting this is a polymer-specific phenomenon rather than a general experimental artifact [39]. This visual change in the PA MP is particularly important because it provides a clear, observable indicator that some form of transformation is occurring during the incubation period. The time-dependent nature of the browning (appearing after 24 h and intensifying by 72 h) suggests this is a progressive process that could have implications for understanding microplastic behavior and fate in environmental or biological systems. The unknown cause highlights an important area for further investigation, as understanding this browning mechanism could provide insights into PA microplastic stability and potential environmental or biological interactions.

Microplastics are now ubiquitous in the environment, and currently, no effective methods exist to collect and destroy them before their progressive breakdown into even more hazardous nanoplastics. Nanoplastics pose greater risks because they can readily penetrate cellular membranes in animals, humans, and plants. Our previous research examined the effects of microplastic ingestion on ruminant fermentation dynamics and related indicators [50–52], but we had not yet explored a potentially crucial ecological service that ruminants may provide for humanity. While all organisms, including ruminants, inevitably consume microplastics, the unique rumen environment may offer degradation capabilities that could help address this global challenge. This study confirms that ruminants can indeed degrade certain microplastics, albeit to a limited extent. These findings open an important research avenue focused on harnessing these natural, widespread biological systems as potential bioreactors for reducing environmental microplastic contamination.

5. Conclusions

This preliminary study investigated the ability of rumen microbiota to degrade three different microplastics—low-density polyethylene, polyethylene terephthalate, and polyamide —under in vitro conditions. The results revealed significant differences in degradation efficacy, with PET MP exhibiting the highest susceptibility to microbial breakdown, while LDPE and PA MP showed marked resistance. These findings highlight the potential of rumen microbial consortia as a biological tool for mitigating MP pollution. Future research should focus on elucidating the underlying mechanisms, including the identification of hydrolysis by-products generated during biodegradation, as well as the specific microorganisms and enzymes involved. Expanding investigations to include other commonly occurring environmental MP polymers will be essential, along with determining whether complete mineralization is achieved and evaluating the potential health risks associated with MP-derived by-products in ruminants. A deeper understanding of these processes could ultimately enhance the natural degradative capacity of ruminants, which are chronically exposed to MPs through their diet, water, and environment.

Author Contributions: Conceptualization: S.B., S.G.P. and S.T.; methodology: S.B., S.G.P., S.P., S.T. and V.V.; investigation: S.G.P., S.P. and V.V.; formal analysis: S.G.P., K.A., H.K., S.P., V.V. and S.T.; chemical evaluation: S.P. and V.V.; data curation: K.A., S.G.P. and H.K.; writing—original draft preparation: S.G.P. and S.T.; writing—review and editing: K.A., S.B., V.B., M.F., S.G.P., H.K., R.I., S.P.,

V.V. and S.T.; visualization: S.G.P.; supervision: S.B. and S.T.; project administration: S.B. and S.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Geyer, R.; Jambeck, J.R.; Law, K.L. Production, Use, and Fate of All Plastics Ever Made. *Sci. Adv.* **2017**, *3*, e1700782. [CrossRef]
2. Dong, X.; Liu, X.; Hou, Q.; Wang, Z. From Natural Environment to Animal Tissues: A Review of Microplastics (Nanoplastics) Translocation and Hazards Studies. *Sci. Total Environ.* **2023**, *855*, 158686. [CrossRef]
3. Klimasz, M.; Grobelak, A. Migration of Microplastics in Agriculture and Marine Ecosystem: Biotechnology Approaches. In *Biotechnology of Emerging Microbes*; Elsevier: Amsterdam, The Netherlands, 2024; pp. 127–142. ISBN 978-0-443-15397-6.
4. Kumari, A.; Rajput, V.D.; Mandzhieva, S.S.; Rajput, S.; Minkina, T.; Kaur, R.; Sushkova, S.; Kumari, P.; Ranjan, A.; Kalinitchenko, V.P.; et al. Microplastic Pollution: An Emerging Threat to Terrestrial Plants and Insights into Its Remediation Strategies. *Plants* **2022**, *11*, 340. [CrossRef]
5. Corte Pause, F.; Urli, S.; Crociati, M.; Stradaioli, G.; Baufeld, A. Connecting the Dots: Livestock Animals as Missing Links in the Chain of Microplastic Contamination and Human Health. *Animals* **2024**, *14*, 350. [CrossRef]
6. Glorio Patrucco, S.; Rivoira, L.; Bruzzoniti, M.C.; Barbera, S.; Tassone, S. Development and Application of a Novel Extraction Protocol for the Monitoring of Microplastic Contamination in Widely Consumed Ruminant Feeds. *Sci. Total Environ.* **2024**, *947*, 174493. [CrossRef]
7. Khan, A.; Qadeer, A.; Wajid, A.; Ullah, Q.; Rahman, S.U.; Ullah, K.; Safi, S.Z.; Ticha, L.; Skalickova, S.; Chilala, P.; et al. Microplastics in Animal Nutrition: Occurrence, Spread, and Hazard in Animals. *J. Agric. Food Res.* **2024**, *17*, 101258. [CrossRef]
8. Borreani, G.; Tabacco, E. Plastics in Animal Production. In *A Guide to the Manufacture, Performance, and Potential of Plastics in Agriculture*; Elsevier: Amsterdam, The Netherlands, 2017; pp. 145–185. ISBN 978-0-08-102170-5.
9. Urli, S.; Corte Pause, F.; Crociati, M.; Baufeld, A.; Monaci, M.; Stradaioli, G. Impact of Microplastics and Nanoplastics on Livestock Health: An Emerging Risk for Reproductive Efficiency. *Animals* **2023**, *13*, 1132. [CrossRef]
10. Grechi, N.; Franko, R.; Rajaraman, R.; Stöckl, J.B.; Trapphoff, T.; Dieterle, S.; Fröhlich, T.; Noonan, M.J.; De A. M. M. Ferraz, M. Microplastics Are Present in Women's and Cows' Follicular Fluid and Polystyrene Microplastics Compromise Bovine Oocyte Function in Vitro. *eLife* **2023**, *12*, RP86791. [CrossRef]
11. Grechi, N.; Ferronato, G.A.; Devkota, S.; Ferraz, M.A.M.M. Microplastics Are Detected in Bull Epididymal Sperm and Polystyrene Microparticles Impair Sperm Fertilization. *Biol. Reprod.* **2024**, *111*, 1341–1351. [CrossRef]
12. van der Veen, I.; van Mourik, L.M.; van Velzen, M.J.M.; Groenewoud, Q.R.; Leslie, H.A. Plastic Particles in Livestock Feed, Milk, Meat and Blood. 2022. Available online: https://www.greenme.it/wp-content/uploads/2022/07/sutdio_microplastiche.pdf (accessed on 2 April 2025).
13. Bahrani, F.; Mohammadi, A.; Dobaradaran, S.; De-la-Torre, G.E.; Arfaeinia, H.; Ramavandi, B.; Saeedi, R.; Tekle-Röttering, A. Occurrence of Microplastics in Edible Tissues of Livestock (Cow and Sheep). *Environ. Sci. Pollut. Res.* **2024**, *31*, 22145–22157. [CrossRef]
14. Rbaibi Zipak, S.; Muratoglu, K.; Buyukunal, S.K. Microplastics in Raw Milk Samples from the Marmara Region in Turkey. *J. Consum. Prot. Food Saf.* **2024**, *19*, 175–186. [CrossRef]
15. Shelver, W.L.; McGarvey, A.M.; Billey, L.O. Disposition of [^{14}C]-Polystyrene Microplastics after Oral Administration to Lactating Sheep. *Food Addit. Contam. Part A* **2024**, *41*, 1132–1143. [CrossRef]
16. Beriot, N.; Peek, J.; Zornoza, R.; Geissen, V.; Huerta Lwanga, E. Low Density-Microplastics Detected in Sheep Faeces and Soil: A Case Study from the Intensive Vegetable Farming in Southeast Spain. *Sci. Total Environ.* **2021**, *755*, 142653. [CrossRef]
17. Wu, T.; Shu, X.; Wang, C.; Li, W.; Zhu, D.; Wang, J.; Zhang, Y.; Yang, X.; Wang, X. Microplastic Pollution of Threatened Terrestrial Wildlife in Nature Reserves of Qinling Mts., China. *Global Ecol. Conserv.* **2024**, *51*, e02865. [CrossRef]
18. Henchion, M.; Moloney, A.P.; Hyland, J.; Zimmermann, J.; McCarthy, S. Review: Trends for Meat, Milk and Egg Consumption for the next Decades and the Role Played by Livestock Systems in the Global Production of Proteins. *Animal* **2021**, *15*, 100287. [CrossRef]
19. Lackner, M.; Branka, M. Microplastics in Farmed Animals—A Review. *Microplastics* **2024**, *3*, 559–588. [CrossRef]

20. Quartinello, F.; Kremser, K.; Schoen, H.; Tesei, D.; Ploszczanski, L.; Nagler, M.; Podmirseg, S.M.; Insam, H.; Piñar, G.; Sterflingler, K.; et al. Together Is Better: The Rumen Microbial Community as Biological Toolbox for Degradation of Synthetic Polyesters. *Front. Bioeng. Biotechnol.* **2021**, *9*, 684459. [CrossRef]
21. Galyon, H.; Vibostok, S.; Duncan, J.; Ferreira, G.; Whittington, A.; Cockrum, R. Long-Term in Situ Ruminal Degradation of Biodegradable Polymers in Holstein Dairy Cattle. *JDS Commun.* **2022**, *4*, 70–74. [CrossRef]
22. Galyon, H.; Vibostok, S.; Duncan, J.; Ferreira, G.; Whittington, A.; Havens, K.; McDevitt, J.; Cockrum, R. Digestibility Kinetics of Polyhydroxyalkanoate and Poly(Butylene Succinate-Co-Adipate) after In Vitro Fermentation in Rumen Fluid. *Polymers* **2022**, *14*, 2103. [CrossRef]
23. European Chemicals Agency (ECHA). Introductory Guidance on the CLP Regulation, Version 3.0, January 2019. Available online: https://www.moew.government.bg/static/media/ups/categories/attachments/clp_introductory_en51c9cac19256d5fb7615c619842ec3f6.pdf (accessed on 24 March 2025).
24. *ISO13320*; Particle Size Analysis—Laser Diffraction Methods. International Organization for Standardization: Geneve, Switzerland, 2020.
25. *ISO1183*; Plastics, Mehods for Determining the Density of Non-Cellular Plastics. International Organization for Standardization: Geneve, Switzerland, 2019.
26. *ASTM D1895*; Standard Test Method for Apparent Density, Bulk Factor, and Pourability of Plastic Materials. ASTM International: West Conshohocken, PA, USA, 2024.
27. Fortina, R.; Glorio Patrucco, S.; Barbera, S.; Tassone, S. Rumen Fluid from Slaughtered Animals: A Standardized Procedure for Sampling, Storage and Use in Digestibility Trials. *Methods Protoc.* **2022**, *5*, 59. [CrossRef]
28. ANKOM Technology In Vitro True Digestibility Using the ANKOM DAISYII Incubator 2023. Available online: https://www.ankom.com/sites/default/files/2024-08/Method_3_InVitro_D200_D200I.pdf?srsltid=AfmBOoomvCEN7ibYOc6Re2bhEPhokN9Le3E7A-4D0CQvMdQIcCQ6_AIR (accessed on 13 February 2025).
29. Mohanan, N.; Montazer, Z.; Sharma, P.K.; Levin, D.B. Microbial and Enzymatic Degradation of Synthetic Plastics. *Front. Microbiol.* **2020**, *11*, 580709. [CrossRef]
30. Venkatesh, S.; Mahboob, S.; Govindarajan, M.; Al-Ghanim, K.A.; Ahmed, Z.; Al-Mulhm, N.; Gayathri, R.; Vijayalakshmi, S. Microbial Degradation of Plastics: Sustainable Approach to Tackling Environmental Threats Facing Big Cities of the Future. *J. King Saud Univ. Sci.* **2021**, *33*, 101362. [CrossRef]
31. Din, S.U.; Kalsoom; Satti, S.M.; Uddin, S.; Mankar, S.V.; Ceylan, E.; Hasan, F.; Khan, S.; Badshah, M.; Beldüz, A.O.; et al. The Purification and Characterization of a Cutinase-like Enzyme with Activity on Polyethylene Terephthalate (PET) from a Newly Isolated Bacterium Stenotrophomonas Maltophilia PRS8 at a Mesophilic Temperature. *Appl. Sci.* **2023**, *13*, 3686. [CrossRef]
32. Wanapat, M.; Muslykhah, U.; Matra, M.; Dagaew, G.; Suriyapha, C.; Sommai, S.; Pongsub, S.; Phupaboon, S. Biodegradation of Low-Density Polyethylene Plastics by Cellulolytic Pseudomonas Aeruginosa Isolated from the Rumen of Swamp Buffalo and the in Vitro End-Product Characteristics. *Environ. Technol. Innov.* **2025**, *38*, 104175. [CrossRef]
33. Zhang, Z.; Cui, Q.; Chen, L.; Zhu, X.; Zhao, S.; Duan, C.; Zhang, X.; Song, D.; Fang, L. A Critical Review of Microplastics in the Soil-Plant System: Distribution, Uptake, Phytotoxicity and Prevention. *J. Hazard. Mater.* **2022**, *424*, 127750. [CrossRef]
34. Harshvardhan, K.; Jha, B. Biodegradation of Low-Density Polyethylene by Marine Bacteria from Pelagic Waters, Arabian Sea, India. *Mar. Pollut. Bull.* **2013**, *77*, 100–106. [CrossRef]
35. Hasan, F.; Shah, A.A.; Hameed, A.; Ahmed, S. Synergistic Effect of Photo and Chemical Treatment on the Rate of Biodegradation of Low Density Polyethylene by *Fusarium* sp. AF4. *J. Appl. Polym. Sci.* **2007**, *105*, 1466–1470. [CrossRef]
36. Smith, B.C. The Infrared Spectra of Polymers II: Polyethylene. *Spectroscopy* **2021**, *36*, 24–29. [CrossRef]
37. Krimm, S.; Liang, C.Y.; Sutherland, G.B.B.M. Infrared Spectra of High Polymers. II. Polyethylene. *J. Chem. Phys.* **1956**, *25*, 549–562. [CrossRef]
38. Kumar Sen, S.; Raut, S. Microbial Degradation of Low Density Polyethylene (LDPE): A Review. *J. Environ. Chem. Eng.* **2015**, *3*, 462–473. [CrossRef]
39. Albertsson, A.-C.; Bánhidi, Z.G. Microbial and Oxidative Effects in Degradation of Polyethene. *J. Appl. Polym. Sci.* **1980**, *25*, 1655–1671. [CrossRef]
40. Albertsson, A.-C.; Erlandsson, B.; Hakkarainen, M.; Karlsson, S. Molecular Weight Changes and Polymeric Matrix Changes Correlated with the Formation of Degradation Products in Biodegraded Polyethylene. *J. Polym. Environ.* **1998**, *6*, 187–195. [CrossRef]
41. Xin, A.; Fei, Y.; Molnar, A.; Fry, S.C. Cutin:Cutin-Acid Endo-Transacylase (CCT), a Cuticle-Remodelling Enzyme Activity in the Plant Epidermis. *Biochem. J.* **2021**, *478*, 777–798. [CrossRef]
42. Restrepo-Flórez, J.-M.; Bassi, A.; Thompson, M.R. Microbial Degradation and Deterioration of Polyethylene—A Review. *Int. Biodeterior. Biodegrad.* **2014**, *88*, 83–90. [CrossRef]
43. Gajendiran, A.; Krishnamoorthy, S.; Abraham, J. Microbial Degradation of Low-Density Polyethylene (LDPE) by Aspergillus Clavatus Strain JASK1 Isolated from Landfill Soil. *3 Biotech* **2016**, *6*, 52. [CrossRef]

44. Huerta Lwanga, E.; Mendoza Vega, J.; Ku Quej, V.; Chi, J.D.L.A.; Sanchez Del Cid, L.; Chi, C.; Escalona Segura, G.; Gertsen, H.; Salánki, T.; Van Der Ploeg, M.; et al. Field Evidence for Transfer of Plastic Debris along a Terrestrial Food Chain. *Sci. Rep.* **2017**, *7*, 14071. [CrossRef]
45. Kumar, M.; Chen, H.; Sarsaiya, S.; Qin, S.; Liu, H.; Awasthi, M.K.; Kumar, S.; Singh, L.; Zhang, Z.; Bolan, N.S.; et al. Current Research Trends on Micro- and Nano-Plastics as an Emerging Threat to Global Environment: A Review. *J. Hazard. Mat.* **2021**, *409*, 124967. [CrossRef]
46. Wei, R.; Tiso, T.; Bertling, J.; O'Connor, K.; Blank, L.M.; Bornscheuer, U.T. Possibilities and Limitations of Biotechnological Plastic Degradation and Recycling. *Nat. Catal.* **2020**, *3*, 867–871. [CrossRef]
47. Brunner, I.; Fischer, M.; Rüthi, J.; Stierli, B.; Frey, B. Ability of Fungi Isolated from Plastic Debris Floating in the Shoreline of a Lake to Degrade Plastics. *PLoS ONE* **2018**, *13*, e0202047. [CrossRef]
48. Hu, K.; Zhou, P.; Yang, Y.; Hall, T.; Nie, G.; Yao, Y.; Duan, X.; Wang, S. Degradation of Microplastics by a Thermal Fenton Reaction. *ACS EST Eng.* **2022**, *2*, 110–120. [CrossRef]
49. Zhao, X.; Wang, J.; Leung, K.M.Y.; Wu, F. Color: An Important but Overlooked Factor for Plastic Photoaging and Microplastic Formation. *Environ. Sci. Technol.* **2022**, *56*, 9161–9163. [CrossRef] [PubMed]
50. Tassone, S.; Barbera, S.; Kaihara, H.; Glorio Patrucco, S.; Abid, K. First evidence of the effects of polyethylene terephthalate microplastics on ruminal degradability and gastro-intestinal digestibility of mixed hay. *Animals* **2024**, *14*, 2139. [CrossRef] [PubMed]
51. Tassone, S.; Kaihara, H.; Barbera, S.; Glorio Patrucco, S.; Issaoui, R.; Abid, K. Low-density polyethylene microplastics in the rumen: Implications for rumen fermentation dynamics and utilization of concentrate feed. *Animals* **2025**, *15*, 297. [CrossRef]
52. Abid, K.; Aroua, M.; Barbera, S.; Glorio Patrucco, S.; Kaihara, H.; Mahouachi, M.; Ben Saïd, S.; Tassone, S. Effect of microplastic contamination on in vitro ruminal fermentation and feed degradability. *Anim. Sci. J.* **2025**, *96*, e70063. [CrossRef] [PubMed]