Influence of particle size and density on mean retention time in the rumen of dairy cows

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ABSTRACT

Increasing rumen-undegraded protein is one challenge of ruminant nutrition to both meet protein requirements of animals and reduce nitrogen excretion in the environment by increasing nitrogen efficiency. Industrial processes using heat or tanning to reduce rumen protein degradation have certain limitations, such as difficulty in balancing low ruminal degradation and high intestinal digestibility. Reducing the mean retention time (MRT) in the rumen by varying the size and density of particles may be another promising way to increase the rumen-undegraded protein proportion of concentrate feeds and improve the effectiveness of industrial processes. Spherical plastic particles of 3 mean diameter sizes (1, 2, and 3 mm) and 4 densities (0.9, 1.1, 1.3, and 1.5) were used to study the combined effect of size and density on the MRT of particles without interactions with microbial fermentations. Dynamics of fecal excretion of particles were monitored over 106 h (17 sampling times) in a Latin square experiment with 4 lactating cows. Cumulative particle excretion curves were fitted to a double exponential model to calculate total MRT in the digestive tract (TMRT), MRT in 2 compartments (MRT1 and MRT2), and retention time in the intestines' tubular section (TT). Differences in density had a quadratic effect, with densities of 1.1 and 1.3 yielding lower TMRT (29.5 and 31.2 h, respectively) than the densities of 0.9 and 1.5 (TMRT = 64.0 and 51.2 h, respectively). Similar responses were observed for MRT1, which was assumed to be the ruminal MRT for densities 1.1 and 1.3 (8.9 and 10.5 h, respectively) compared with densities 0.9 and 1.5 (39.6 and 22.6 h, respectively). Differences in diameter had a linear effect on TMRT (12.9 h longer for 3 mm than for 1 mm) and on TT. A combined effect of size and density was

observed and particle size had no effect on TMRT when density was 1.1 to 1.3; however, outside this range, an increase in particle diameter increased TMRT. Consequently, a density of 1.2 to 1.3 is optimal for the escape of particles. As smaller particles of concentrates lose functional specific gravity more rapidly than larger particles due to their higher fermentation rate, our results, obtained with plastic particles, suggest that a diameter slightly greater than 3 mm seems a compromise to delay the start of fermentation and allow for rapid passage through the reticulo-omasal orifice.

Key words: mean retention time, rumen, plastic particle, dairy cow

INTRODUCTION

Increasing the proportion of RUP in feed, especially in protein-rich supplements such as oil seed meals or legume grain, is one challenge for the nutrition of high-producing dairy cows to increase protein-use efficiency and reduce nitrogen excretion in the environment by increasing nitrogen efficiency. Digestion of concentrate feeds in the rumen depends on their degradation rate and retention time.

To increase the proportion of RUP, feed manufacturers developed processes to reduce the degradation rate of concentrates. The main processes are heat treatments (Whitelaw et al., 1961; Sherrod and Tillman, 1964; Krizsan et al., 2017) and tanning with formaldehyde (Vérité et al., 1977); however, heat treatment can reduce intestinal digestibility or become ineffective at protecting feeds when industrial processes are not well monitored (Broderick et al., 1991). Formaldehyde tanning is highly effective (Barry, 1976) in reducing the ruminal degradability of protein and preserving high intestinal digestibility; however, most feed manufacturers have stopped using it due to lack of social acceptance and carcinogenic risks for feed factory employees. New processes based on plant-based tanning or essential oils were tested, but they have variable efficacy in vivo (Min et al., 2003; Waghorn, 2008; Bayourthe and Ali-Haimoud-Lekhal, 2014).

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Manipulating retention time in the rumen has not received much attention until recently. However, decreasing ruminal retention time can improve the efficiency of protection processes by increasing the probability that particles escape the rumen before extensive microbial degradation. The combination of short retention time in the rumen and short-term protection could result in high escape protein of concentrates. Physical characteristics, such as size and functional specific gravity, are known to influence the retention time of concentrates in the rumen (Lechner-Doll et al., 1991). Functional specific gravity corresponds to particle density associated with volumes occupied by liquids and gases, which evolve during hydration and digestion (Sutherland, 1986; Wattiaux et al., 1993).

Studying the influence of particles' physical characteristics on their retention time requires using inert particles because their size and density can be strictly controlled and they do not interact with microbial fermentation. Several studies using this approach have investigated the influence of physical characteristics, such as size and density, on the mean retention time (MRT) of plastic particles (King and Moore, 1957; Campling and Freer, 1962; Welch and Smith, 1978; DesBordes and Welch, 1984; Ehle and Stern, 1986; Murphy et al., 1989; Kaske and Engelhardt, 1990; Prigge et al., 1990; Clauss et al., 2011) in the entire digestive tract and in the rumen. The general observations of these studies are that the MRT of particles negatively correlates with particle size and that a density of 1.1 to 1.4 minimizes MRT in the rumen. These results, however, come from studies that used low-producing animals (dry cows, steers, or sheep), whereas it is well established that both ruminal and total MRT of particles decrease as intake increases (Verite et al., 1987); therefore, high-producing dairy cows may have different responses to particle size and density. Most of these studies aimed to estimate the MRT of forage particles, so the size tested (length mean 6.5 mm) was larger than the size of concentrate particles. Only Murphy et al. (1989) examined the influence of size and density of plastic particles in the range close to concentrates; however, the experimental diet of their study (95% of rice straw) could not be applied to cover the high requirements of lactating cows, as rice straw is a low-energy, low-protein, and low-digestible feed.

We explored the combined effect of the size and density of plastic particles on the retention time in different parts of the gastrointestinal tract of lactating cows and tested the hypothesis that a certain size and density combination would yield the lowest MRT for particles in the rumen. The range of variation in size and density was chosen to encompass the values generally observed for concentrates.

MATERIALS AND METHODS

Principle of the Measurements

Four diameter sizes (0.5, 1, 2, and 3 mm) and 4 densities (0.9, 1.1, 1.3, and 1.5) of plastic particles, resulting in 16 size $\times$ density combinations, were tested with 4 lactating cows in a Latin square design during four 1-wk periods. Particles were pulse-dosed in the rumen of each cow at each period. After dosing, excretion of particles was monitored by collecting all fecal material. The number of plastic particles was determined using a method to separate plastic particles from feces (adapted from DesBordes and Welch, 1984) and an innovative image analysis, based on a color-separation of particles followed by an ImageJ counting (ImageJ 1.47v software; US National Institutes of Health, Bethesda, MD). Excretion dynamics were adjusted to a double exponential model (Grofum and Williams, 1973) to calculate total mean retention time (TMRT) in the gastrointestinal tract and the 3 compartments identified by the model: 2 mixing compartments, the first associated with the rumen, and the intestines' tubular section. Mean retention time for the 3 sizes (1, 2, and 3 mm) and 4 densities (0.9, 1.1, 1.3, and 1.5) of plastic particles in the entire digestive tract and its 3 compartments is presented in Appendix Table A1.

Animals, Diets, and Experimental Design

Four multiparous cows [mean of 727 kg of BW (SD = 30.5); 29.9 kg of milk/d (SD = 6.8); 21.9 kg of DMI/d (SD = 2.5)] were used in a Latin square experiment on the experimental farm of Méjusseume (Le Rheu, France). All cows were fitted with large (i.e., 123-mm diameter) rumen cannulas at least 1 yr before the beginning of the experiment and were confirmed pregnant before the experiment to avoid differences in DMI due to estrus. The cows were housed in individual stalls equipped with automatic troughs and were milked twice a day at 0700 and 1700 h. The experiment lasted 54 d, including an adaptation period of 28 d. The cows were fed ad libitum during the first 14 d to determine individual ad libitum DMI. For the following 14 d, cows were adapted to restricted feeding, which was set to 95% of each cow's individual DMI to ensure constant DMI during the trial. Access to the troughs was restricted to four 1-h periods (starting at 0200, 0800, 1400, and 2000 h) per day to spread DMI throughout the day. Cows had free access to water and a feed block. Meals contained maize silage, concentrates with minerals, tanned soybean meal, and chopped dehydrated alfalfa (Table 1). Procedures related to surgery, care, and use of animals for the experiment were approved by an

Table 1. Chemical composition and nutritional values of feeds (g/kg, unless otherwise indicated)

Characteristic	Maize silage	Concentrate with mineral ¹	Tanned soybean meal	Chopped dehydrated alfalfa
Amount in the diet (%)	60	15	15	10
DM	376	919	905	924
OM	965	877	928	883
CP	66	102	506	189
NDF	422	196	102	420
ADF	239	79	61	299
ADL	24	8	4	110
Starch	332	420	65	39
Fatty acids	29	39	26	29
Net energy (UFL ² /kg of DM)	0.91	0.96	1.2	0.63
MP				
g of PDIE/kg of DM ³	63	91	248	95
g of PDIN/kg of DM ⁴	41	69	361	119

¹Contained (in %) 18.3 wheat, 18.3 maize, 18.3 barley, 13.2 wheat feed flour, 18.3 beet pulp, 0.9 rapeseed oil, 3 sugar cane molasses, 0.9 salt, 2 AdiSodium (Bluestar Adisseo Co., Ltd., Beijing, China), and 6.8 minerals (5.5% P, 27% Ca and 4% Mg).

²UFL = unité fourragère lait; amount of net energy for milk production contained in 1 kg of a reference barley (87% DM, 2,700 kcal of ME), based on the INRA feeding system (INRA, 2007).

³PDIE = truly digestible (dietary + microbial) protein when available energy decreases microbial growth.

⁴PDIN = truly digestible (dietary + microbial) protein when available degradable nitrogen decreases microbial growth.

animal committee of the French Ministry of Agriculture (no. 201606132002401), in accordance with European Directive 2010/63/UE.

Plastic Particles

Particles used in the experiment were a mix of polypropylene and barium sulfate (manufactured by CRITT Polymères Picardie, Verneuil-en-Halatte, France). The barium sulfate was added to the polypropylene during manufacturing at 0, 21.87, 37.77, and 49.99%, to obtain final densities of 0.9, 1.1, 1.3, and 1.5, respectively. Four colorants, orange, red, purple, and white, were also added to identify the 4 density treatments samples when counting particles in fecal matter for the densities 0.9, 1.1, 1.3, and 1.5, respectively. These 3 components (polypropylene, barium sulfate, and colorant) were mixed together and heated (from 120 to 190°C) by a co-rotating, twin-screw extruder (Clextal, Firminy, France) before being extruded as 3-mm diameter wire. The wire was cooled in 15°C water then cut to 3 mm in length. At this stage, 2 kg of each density were retained for the experiment. The remaining particles were ground (Micronis, Colayrac-Saint-Cirq, France) and sieved (Agrial, La-Selle-en-Luitré, France) to obtain 3 ranges of diameters: 0.3 to 0.6, 0.9 to 1.2, and 1.8 to 2.5 mm, hereafter referred to as 0.5, 1, and 2 mm, respectively. The global shape of 0.5-, 1-, and 2-mm particles were coarse spheres.

Fecal Sampling

The TMRT within the gastrointestinal tract was determined by monitoring dynamics of particle excretion

in the feces over four 1-wk periods. In each period, cows received all 4 densities of only 1 size of particle. At the beginning of each period (0800 h on Monday, d 29, 36, 43, and 50), approximately 8,000 particles of 3 or 2 mm (2,000 per density), 66,000 particles of 1 mm (16,500 per density), or 528,000 particles of 0.5 mm (132,000 per density) were introduced into the rumen through the cannula according to the experimental design. Total fecal output was collected 17 times during the first 106 h postdosing (6, 9, 12, 16, 20, 24, 28, 32, 36, 40, 48, 54, 60, 72, 82, 96, and 106 h). For the sampling times from 6 to 40 h, total fecal outputs were collected and treated. After the 40-h sampling time, a representative subsample equal to the amount of feces collected over 4 h was first taken. Thus, feces from the samplings at 48, 54, 60, 72, 82, 96, and 106 h were weighed, thoroughly mixed, and then 50, 66, 66, 33, 40, 28, and 40% of their total weights, respectively, were collected and treated.

Treatment of Fecal Samples and Photography

After feces were collected and weighed, most fecal matter was eliminated by wet sieving under high water pressure through 7 successive sieves (8, 4, 2.5, 1.5, 0.8, 0.5, and 0.2 mm). Only the material on the sieve corresponding to the particle size introduced in the rumen was collected (i.e., the 2.5-mm sieve for 3-mm particles, the 1.5-mm sieve for the 2-mm particles, the 0.8-mm sieve for the 1-mm particles, and the 0.5- and 0.2-mm sieves for the 0.5-mm particles). Collecting particles in different sieves based on their size avoided confounding particles from a given period, as a small fraction of particles (less than 10 particles of all densities per sam-

pling) were still being excreted during the subsequent period (6, 9, and 12 h after the dosing of the subsequent period). Fecal residues and plastic particles retained on the sieves were dried at 80°C for 48 h and stored at ambient temperature in aluminum containers before counting (Figure 1).

After storage, the amount of fecal residue remaining among the 3-mm particles was small enough that it could be photographed directly. The samples were spread on a black fabric under a digital camera (Panasonic Lumix DMC-FZ30, Osaka, Japan) and photographed. The black background prevented color interference during image analysis. For the 2-, 1-, and 0.5-mm particles, the amount of fecal residue recovered in the corresponding sieves was too large to photograph all of it directly. Thus, each sample (fecal residue + particles; mean mass of 400 g) was floated in a bucket with 8 L of water at ambient temperature during 5 min. Two grams of Span 20 (Croda International Plc, Goole, UK) was added to decrease surface tension. The plastic particles heavier than water (densities 1.1, 1.3, and 1.5) sank to the bottom of the bucket and were recovered after removing the fraction lighter than water. This fraction, containing particles of density 0.9, and the fecal residues were skimmed off the water surface and then floated in another bucket with 8 L of boiling water to heat the fecal residues overnight. The hot fecal residues sank, and the plastic particles were skimmed off the water surface. These particles were spread on the black fabric under the camera and photographed. Each image was identified by period, date, cow, and sampling time.

The method used to recover particles of different densities and remove fecal residues before image analysis was developed for this study. We considered degrading fecal residue with acid or enzymes, but the quantities to be treated were too large and the time required was too long to use this technique. Using ethanol instead of water recovered only 80% of a known quantity of particles of density 0.9 added to feces and removed only 50% of fecal residues. The water-based recovery method described (adapted from DesBordes and Welch, 1984) recovered more than 99% of particles with a density of 1.1, 1.3, and 1.5 and all sizes of particles during a pilot study in which particles were added to fecal material. The only challenge was recovering particles of density 0.9 from fecal residues. Using water only at ambient temperature also caused fecal residues to sink to the bottom of the bucket, but the process of recovering all particles of 0.9 density took more than 2 d. Using boiling water instead shortened the process to 1 night and also recovered all of the plastic particles.

Rumination of plastic particles was also considered, as chewing can reduce the size of the particles or de-

form them greatly. During the first period, ruminated particles were identified by chewing marks. Clear chewing marks were detected only on particles of 0.9 density (less than 1% of them) and only for the last 3 sampling times (82, 96, and 106 h). No chewing marks were detected on particles of other densities, even those 3 mm in size. Consequently, ruminated and nonruminated particles were not distinguished in subsequent periods.

Image Analysis

The images were first processed with Adobe Photoshop CS5 software (Adobe Inc., San Jose, CA), which separated each image by particle color into 4 images, each showing the particles of 1 color (i.e., 1 density). Next, the number of particles in each image was counted using ImageJ 1.47v software (US National Institutes of Health). For the sampling times between 48 and 106 h, the same percentages used to subsample feces were used to extrapolate the number of particles excreted in the complete fecal sample. Preliminary analyses of 3-mm particles of all densities indicated that the image analysis was sufficiently accurate (100.5%, SD = 2.4%). However, the analysis did not accurately distinguish 0.5-mm particles from fecal residues remaining in the samples. Consequently, only results for 1-, 2-, and 3-mm particles are presented. Figure 1 shows a diagram of plastic particle manufacturing, the experimental design, and the recovery procedure.

Model of Particle Excretion

We used the number of particles counted at each sampling time to adjust our data to a cumulative percentage excretion curve to avoid irregularity due to defecations. The cumulative percentage is an expression of the number of particles counted compared with the number of particles introduced into the rumen at the beginning of each period. As the initial double exponential model of Grovum and Williams (1973) considers the kinetics of concentration, we integrated this initial model for considering the cumulative excretion curve according to the following equation:

$$Y = (C_0/k_2) - \left(\frac{[(C_0 \times k_1)/(k_1 - k_2)] \times \{\exp[-k_2 \times (t - TT)]/k_2 - \exp[-k_1 \times (t - TT)]/k_1\}}{k_1 - k_2} \right),$$

where C_0 is the adjusted plastic particle number, k_1 and k_2 are rate constants of the 2 models' compartments, **TT** is the time calculated for the first appearance of plastic particles in the feces, and t is the sampling time in h after particles were introduced in the rumen.

1. Particle manufacturing

Mix of different proportions of polypropylene and barium sulfate + 1 color per density

Particles 3 mm in size and 4 different densities (0.9 – 1.1 – 1.3 – 1.5)

Grinding and sieving of particles of each density

Particles of 3 different sizes (2, 1, and 0.5 mm) and 4 different densities

2. Fecal sampling

- Four cows in a Latin square: design of four periods

One period and one cow: particles of only one size at 4 different densities

17 fecal samplings over 106 h

3. Sieving

Sieve size (mm)

8
4
2.5
1.5
0.8
0.5
0.2

Fecal samples sieved under high-pressure water

Particle recovery with fecal residues:

3 mm – 4 densities
2 mm – 4 densities
1 mm – 4 densities

0.5 mm – 4 densities

Drying

4. Density separation in water for particles size 2, 1, and 0.5mm

Dry fecal residues with particles of one size and 4 densities

Ambient-temperature water

Fecal residues with particles of density 0.9

Particles of densities 1.1, 1.3, and 1.5

Boiling water

Fecal residues Discarded

Particles of density 0.9

Photography

5. Image analysis (example for 3-mm size and 1.1, 1.3, and 1.5 densities particles with dry feces)

Isolating particles of each density by their color

Counting with ImageJ software

Figure 1. Diagram of the manufacturing of plastic particles, fecal sampling, the separation procedure, and image analysis for particles of different sizes and densities. ImageJ from US National Institutes of Health (Bethesda, MD; <https://imagej.nih.gov/ij/>).

The MRT in the 2 compartments was calculated by inverting their rate-constant coefficients: **MRT1** = 1/k₁ and **MRT2** = 1/k₂. The TMRT in the digestive tract was calculated as the sum of all 3 times: TMRT = MRT1 + MRT2 + TT.

The use of Grovum and Williams' model allowed us to obtain physiological correspondence of the different compartments. Based on the study of Mambrini and Peyraud (1997), we considered MRT1 as the time required for particles to flow out of the rumen toward distal digestive compartments, TT as the transit time of concentrates in the intestines' tubular section, and MRT2 as the time spent in the cecum, as well as in other compartments or processes that were not clearly identified. Mambrini and Peyraud (1997) demonstrated that some of MRT2 is due to the time required to comminute large forage particles in the rumen. MRT2 is also due in part to ruminal processes such as particle mixing.

The Grovum and Williams double exponential model was also chosen based on comparison between different modes and previous work on the subject (Mambrini, 1990; Mambrini et al., 1994). Those authors compared different models with the same data set and found that models from Grovum and Williams (1973) and Ellis (1978), with a time-dependent passage rate following a gamma 2 or 3 distribution (E2 or E3), adjust best to data with the most precise estimation of parameters of the equation. We adjusted our data on these 3 models and the Grovum and Williams' one fitted best (SEM of 22, 28, and 34 respectively for Grovum and Williams, E2, and E3 respectively).

Statistical Analyses

Split-plot ANOVA was performed to estimate nested effects of the densities on the particle recovery rate, MRT1, MRT2, TT, and TMRT using the MIXED model procedure of SAS Enterprise Guide 6.1 (SAS Institute Inc., Cary, NC):

$$Y_{ijkl} = \mu + P_i + A_j + S_k + D_l + (SD)_{kl} + (AD)_{il} + e_{ijkl}$$

where Y_{ijkl} is the adjusted response variable of the model, μ is the overall mean, P_i is the effect of period i ($n = 4$), A_j is the random effect of animal j ($n = 4$), S_k is the effect of particle size k ($n = 3$), D_l is the effect of particle density l ($n = 4$), $(SD)_{kl}$ is the effect of the interaction between size k and density l , $(AD)_{il}$ is the random effect of the interaction between animal i and density l , and e_{ijkl} is the residual error. The MRT1 and TMRT data were analyzed after being log transformed to ensure normal distribution of their residuals.

To highlight interactions between size and density on MRT, orthogonal contrasts [linear (L) and quadratic (Q)], and the interactions [quadratic $\times$ linear (Q $\times$ L), quadratic $\times$ quadratic (Q $\times$ Q) and linear $\times$ linear (L $\times$ L)] were tested to study the overall shape of response of size and density on different MRT in the gastrointestinal tract. Significance was declared at $P < 0.05$.

RESULTS

Particle Recovery Rate

The recovery rate depended on the size and density of the particle. The lowest recovery rate (23.1%, SD = 11.7) was observed for particles of density 0.9. For 2- and 3-mm particles, the recovery rate was highest for those of density 1.1 (90.1%, SD = 8.3), followed by those of density 1.3 (73.6%, SD = 3.8) and 1.5 (68.6%, SD = 2.9). For 1-mm particles, the mean recovery rate of those with densities 1.1 to 1.5 (41.4%, SD = 13.4) was 36 percentage points lower than that of the other 2 sizes combined (Table 2).

Response of TMRT and its Compartments to Particle Size and Density

The MRT1 averaged 39% of TMRT, but TMRT was correlated with MRT1 (TMRT = $1.02 \times \text{MRT1} + 23.1$; $R^2 = 0.92$), with similar standard deviations for each variable (22.58 and 21.11 h for TMRT and MRT1, respectively), which implied that variations in MRT1 were mirrored in TMRT. Both TMRT and MRT1 had a quadratic response to density (Table 3); TMRT and MRT1 were shortest for particles of densities 1.1 and 1.3 and longest for those of density 0.9. Similar quadratic trends were observed for MRT2, which was shortest for particles of densities 1.1 and 1.3 and longest for those of density 1.5. Particle density had no influence on TT.

Increasing particle size linearly increased TMRT and TT. As particle size increased from 1 to 3 mm, TMRT and TT increased linearly by 13.9 and 1.5 h, respectively. Unlike density, particle size had no significant effect on MRT1 and MRT2.

Table 2. Percentage of plastic particles recovered¹ for each combination of size and density of particles

Density	Size (mm)		
	1	2	3
0.9	9.3	37.5	22.5
1.1	26.0	98.5	81.8
1.3	48.0	69.8	77.3
1.5	50.2	65.9	71.3

¹Particles recovered over a period of 106 h.

Table 3. Mean retention times (MRT, h) in the entire digestive tract and its 3 compartments (MRT1, MRT2, and TT) for the 3 sizes (1, 2, and 3 mm) and 4 densities (0.9, 1.1, 1.3, and 1.5) of plastic particles, with *P*-values of the contrasts

Item ¹	MRT (h)										Contrasts (<i>P</i> -value) ²					
	Size (mm)					Density					Main					
											Size			Density		
	1	2	3	SEM	0.9	1.1	1.3	1.5	SEM	L	Q	L	Q	L	Q	Q × Q
TMRT	38.0	43.1	50.9	5.15	64.0	29.5	31.2	51.2	5.77	<0.001	0.77	0.10	<0.001	0.04	0.56	0.89
MRT1	16.0	20.6	24.6	5.00	39.6	8.9	10.5	22.6	5.79	0.14	0.95	0.20	<0.001	0.09	0.98	0.87
MRT2	9.8	9.1	10.7	1.45	10.6	7.8	7.5	13.5	1.67	0.68	0.52	0.29	0.03	0.36	0.62	0.81
TT	12.3	13.4	15.6	1.01	13.8	12.8	13.3	15.1	1.07	<0.001	0.50	0.21	0.08	0.54	0.02	0.21

¹TMRT = total MRT; MRT1 = MRT in the first compartment; MRT2 = MRT in the second compartment; TT = time until plastic particles first appear in the feces.
²L = linear; Q = quadratic; L × Q = linear size × quadratic density; L × L = linear size × linear density; Q × Q = quadratic size × quadratic density.

Effect of Interaction between Size and Density on TT and TMRT

The increase in TMRT as particle size increased was greater for densities 0.9 and 1.5 than for densities 1.1 and 1.3. The effect of particle density on TMRT was greatest for 3-mm particles and much smaller for 1-mm particles (quadratic × linear interaction, *P* < 0.04; Figure 2). At densities of 0.9 and 1.5, 3-mm particles had a mean MRT 31 and 17.5 h longer, respectively, than those of 1-mm particles. No difference was observed for densities of 1.1 and 1.3, indicating that particle size had no effect on TMRT for this range of density.

The incremental increase in TT with particle size was even greater as particle density increased (linear density × linear size interaction, *P* < 0.02; Figure 3). The slope of the relation between TT and size increased by 0.4, 0.9, 1.9, and 3.5 h/mm of diameter for densities 0.9, 1.1, 1.3, and 1.5, respectively. The denser and larger the particles were, the longer the TT.

DISCUSSION

Fecal Recovery of Plastic Particles and Reliability of the Method

Recovery rates of particles in our study were consistent with those of other studies (King and Moore, 1957; DesBordes and Welch, 1984; Ehle and Stern, 1986; Murphy et al., 1989). Those studies' highest recovery rates for densities 1.0 to 1.5 (70.6%, mean of the 4 studies; Figure 4) were similar to our recovery rates for 2- and

Figure 2. Influence of relative density (0.9–1.5) and diameter (1–3 mm) on total mean retention time in the entire digestive tract.

3-mm particles in the same range of density (81.9%, Table 2). This indicates that our innovative method for image analysis is effective for counting particles.

Recovery rates in our study for 2- and 3-mm particles were lower for densities 0.9 and 1.5 (30.0 and 68.6%, respectively) than for densities 1.1 and 1.3 (90.2 and 73.6%, respectively; Table 2). This agrees with data from the literature, which also observed lower recovery rates for particles with a density lower than 1.0 (average of the studies: 17.7%; Figure 4) or higher than 1.5 (average of the studies: 46.7%). This low recovery rates for 2- and 3-mm particles may be related to the increase in TMRT (Figure 5) rather than a problem with the method. The low recovery rate for particles of density 0.9 may indicate that this density is too low for sedimentation to occur in the rumen and for particles to pass out of the rumen through the reticulo-omasal orifice, as observed by Campling and Freer (1962), DesBordes and Welch (1984), Ehle and Stern (1986), and Welch (1990). Those authors observed slow and uniform excretion of particles with densities lower than 1.0 when feces were sampled 10 d after the particles were inserted into the rumen. The period over which feces were collected may explain these low rates of particle recovery. Similar to other studies using plastic particles (King and Moore, 1957; Kaske et al., 1992) or concentrates with lactating cows (Mambrini and Peyraud, 1997; Zebeli et al., 2007; Krämer et al., 2013), our collection period lasted 106 h; however, a longer collection period might have increased the particle recovery rate. The longer periods reported in the literature (mean

8.3 d; Welch and Smith, 1978; Welch, 1982; DesBordes and Welch, 1984; Ehle and Stern, 1986; Murphy et al., 1989; Kaske and Engelhardt, 1990; Prigge et al., 1990; Clauss et al., 2011) did not facilitate complete recovery of particles of low or high density. In our study, the experimental design and collection of particles in different sieves based on their size avoided confounding particles from a given period if they were still being excreted during the subsequent period. Nevertheless, these low recovery rates might have induced an underestimation of the MRT, as all particles have not been recovered (i.e., some particles were still in the digestive tract with, therefore, a longer MRT) but relative MRT between size-density combinations still indicate those that escape the rumen the fastest.

The recovery rate of 1-mm particles with densities of 1.1 to 1.5 was 30 percentage points lower in our study than in other studies (King and Moore, 1957; DesBordes and Welch, 1984; Ehle and Stern, 1986; Murphy et al., 1989), and we observed no linear relationship between the particle recovery rate and TMRT as we did for the 2 other sizes ($R^2 = 0.08$; Figure 5). This indicates that the low recovery rate of 1-mm particles might have resulted from a problem with the method, as these particles displayed static electricity when sieved. Consequently, they could have clung to fecal residues during water separation despite the surfactant used to avoid this problem. In contrast, the 2- and 3-mm particles were likely large enough to overcome static electricity during water separation. Thus, results for 1-mm particles must be taken with more caution than the others, despite the consistent results.

Finally, based on observations during the first period of our trial, we decided not to distinguish ruminated and nonruminated particles, as the proportion of ruminated particles was very low during this period. For the same reason, the number of particles reduced by rumination (i.e., particles passing through their recovering sieve) have not been recorded. However, other studies (DesBordes and Welch, 1984; Murphy et al., 1989; Clauss et al., 2011; Lauper et al., 2013) have reported high proportion of ruminated particles. We assumed that our low proportion of ruminated particles might be linked to higher DMI of our animals compared with literature. Nevertheless, it cannot be precluded that the nonseparation of ruminated and nonruminated particles might have influenced our MRT.

Figure 3. Influence of relative density (0.9–1.5) and diameter (1–3 mm) of plastic particles on the time until they first appear in the feces, according to the model of Grovum and Williams (1973), equivalent to mean retention time in the intestines' tubular section (Mambrini and Peyraud, 1997).

Influence of DMI on TMRT of Plastic Particles

In our study, the TMRT of plastic particles of densities 0.9 to 1.3 was much shorter (44 h) than reported in previous studies (76.3 h; Figure 6). Voluntary DMI was much higher in our study, which used high-producing

Figure 4. Influence of the relative density of plastic particles on the percentage of particles recovered from the digestive tract of cattle. Lines connect particles with the same size in a given experiment (hatched lines = particles size ≤ 3 mm; solid lines = particles size between 3 and 5 mm; dotted lines = particles size > 5 mm). ■ = DesBordes and Welch (1984); ● = Ehle and Stern (1986); Δ = King and Moore (1957); $\blacktriangle$ = Murphy et al. (1989); ○ = present study.

dairy cows (157 g of DM/kg of $BW^{0.75}$) than in the previous studies conducted with dry cows, steers, or sheep on maintenance diets (mean 84 g of DMI/kg of $BW^{0.75}$; Welch and Smith, 1978; Ehle and Stern, 1986;

Kaske and Engelhardt, 1990; Prigge et al., 1990; Clauss et al., 2011). It is well established that the TMRT of forage particles decreases as DMI increases (Colucci et al., 1982; Shaver et al., 1986). We observed the same relation for the MRT1 of plastic particles, with shorter MRT than those of other studies due to higher DMI (Figure 7). Allen (1996) also reported that an increase in voluntary DMI decreased MRT in the reticulo-rumen, probably through specific mechanisms that control flow through the reticulo-omasal orifice. Consequently, the restriction on feed intake (95% of DMI) may have resulted in a slight increase of the TMRT but because the restriction applied was not severe, the effect on TMRT must be minimal.

Figure 5. Percentage of particles recovered as a function of their mean retention time (h) in the digestive tract for the size 1 (●), 2 (○), and 3 mm (◇). Each dot represents the mean for all 4 cows for a particle size-density combination.

Influence of Density on the MRT of Plastic Particles

The TMRT was shortest for densities 1.1 to 1.3 and sharply increased for densities 0.9 and 1.5. This response curve agrees with data from the literature, for which the shortest TMRT were generally observed for particles of densities 1.1 to 1.4 (Figure 6). King and Moore (1957) observed the shortest MRT for particles of density 1.24, whereas Campling and Freer (1962) observed that TMRT increased for particle densities of 1.4 or more. DesBordes and Welch (1984) observed the

Figure 6. Influence of the relative density of plastic particles on the total mean retention time in cattle. Lines connect particles with the same size in a given experiment (hatched lines = particles size ≤ 3 mm; solid lines = particles size between 3 and 5 mm; dotted lines = particles size > 5 mm). Note: We calculated total mean retention time for the studies of DesBordes and Welch (1984) and Murphy et al. (1989) by adjusting their cumulative percentage of particles recovered with the model of Grovum and Williams (1973). $\square$ = Campling and Feer (1962); $+$ = Clauss et al. (2011); $\blacksquare$ = DesBordes and Welch (1984); $\bullet$ = Ehle and Stern (1986); $\triangle$ = King and Moore (1957); $\blacktriangle$ = Murphy et al. (1989); $\circ$ = present study.

shortest MRT for particles of density 1.42 and TMRT increasing moderately from densities 1.4 to 1.7 and more rapidly from 1.7 to 2.15.

Figure 7. Rumen mean retention time of plastic particles as a function of DMI of animals on maintenance diets ($\blacklozenge$, $\diamond$, $\bullet$, and $\times$) and lactating cows ($\circ$). Each dot represents the mean of rumen mean retention time for particles of all sizes and densities tested in each study. $\bullet$ = Ehle and Stern (1986); $+$ = Clauss et al. (2011); $\blacklozenge$ = Kaske and Enghardt (1990); $\times$ = Prigge et al. (1990); $\diamond$ = Welch and Smith (1978); $\circ$ = present study.

As for TMTR, MRT1 was shortest for densities 1.1 and 1.3 and increased for densities 0.9 and 1.5. This is consistent with Ehle and Stern (1986), Kaske and Engelhardt (1990), and Murphy et al. (1989), who reported minimum MRT in the entire digestive tract and the rumen for the same range of densities (1.34, 1.44, and 1.34, respectively). Therefore, it appears that particles with densities ranging from 1.1 to 1.4 have the shortest MRT in the rumen. Particles with a density higher than 1.4 or 1.5 probably separate from the rumen fiber mat better than particles with a lower density, but they are not readily transported with liquids once they are in the ventral section of the rumen, which could explain the longer MRT, as Campling and Freer (1962) suggested. A density of 1.0 or lower is too low to allow sedimentation from the rumen fiber mat and, thus, passage of particles out of the rumen.

Influence of Particle Size on the MRT of Plastic Particles

The influence of particle size on TMRT is consistent with results of other studies, where increasing the size of particles increases MRT (Campling and Freer, 1962;

Welch and Smith, 1978; Welch, 1982; Ehle and Stern, 1986; Kaske and Engelhardt, 1990; Clauss et al., 2011). From a meta-analysis of literature data, Lechner-Doll et al. (1991) demonstrated an exponential response curve for TMRT as particle size increases from 1 to 20 mm. In our study, TMRT had a linear response to particle size; however, we tested a smaller range of sizes that is consistent with that of concentrate particles. Therefore, the shape of the response does not necessarily contradict those of other studies.

Prigge et al. (1990) found that MRT in the rumen increased for nylon particles with diameters larger than 3 mm (19.3, 20.3, and 60.1 h for 1, 3, and 5 mm, respectively), which is consistent with our results. The critical size threshold theory, first suggested by Poppi et al. (1980), established a threshold of 3 to 4 mm for cattle, although it may vary with the level of intake and the type of diet (Van Soest et al., 1986). In our study, particle size was smaller than the critical size, and the intake levels were high; thus, the lack of effect of particle size on MRT1 was not surprising.

We observed a linear increase in TT as particle size increased. Ehle and Stern (1986) and Siciliano-Jones and Murphy (1986) reported that particle size had no influence on TT [average 16.3 h (SD = 1.3) for density 1.34 and 31.5 h (SD = 4.2) for density 2.3; Ehle and Stern, 1986] for diameters of 3 to 12 mm, which indicates that particles smaller than 3 mm might transit the tubular section of the digestive tract faster. More studies are required to confirm this hypothesis; however, the influence of TT on TMRT remained smaller than that of MRT1.

Overall Response of MRT to the Size and Density of Plastic Particles

Our experiment was designed to study the interaction between particle size and density on MRT. Two interactions were detected. Particle size appears to have little effect on TMRT and MRT1 when particle density is 1.1 to 1.3. For particle densities outside of this range, the smaller the size, the shorter are the TMRT and MRT1. Murphy et al. (1989) performed an experiment with similar range of size (2–5 mm) and density (1.1, 1.34, and 1.77), which was in part consistent with ours, with size appearing to have less effect on rumen MRT for density 1.34 than for the 2 other densities (1.1 and 1.77). For densities 1.1 and 1.77, the influence of size is not as clear as in our study, as particles of size 2 mm had shorter MRT than 5 mm for density 1.1 but reversed order for density 1.77. However, Murphy et al. (1989) used an experimental diet based on 95% of rice straw, which cannot be used to feed lactating cows with high requirements, as rice

straw is low digestibility feed; thus, comparison between the study of Murphy et al. (1989) and ours must be taken with caution, as they refer to very different experimental conditions.

The TT increased as particle size and density increase, and the larger and denser the particle, the longer the TT. As mentioned, few studies have focused on the influence of the size of plastic particles on TT (Ehle and Stern, 1986; Siciliano-Jones and Murphy, 1986). These studies observed no size effect but did observe a density effect consistent with our results: particles of density 1.0 were retained an average of 18 to 19 h, and denser particles were retained 3 to 8 h longer (Kaske and Engelhardt, 1990).

Application to Feed Particles

Movement of a feed particle in the rumen and its passage through the reticulo-omasal orifice depends on processes such as mastication, hydration, comminution, colonization, and degradation. As time passes, the particle can pass more easily as some of its characteristics change, such as size, moisture content, and functional specific gravity (**FSG**). Retention time of feed particles in the rumen also depends strongly on fermentation and degradation, which are the factors that influence dynamics of the FSG of feed particles the most among the factors that influence their buoyancy (Lechner-Doll et al., 1991). First, fermentation produces gases on particle surfaces, which in turn decreases particle density (Hooper and Welch, 1985; Sutherland, 1986; Lechner-Doll et al., 1991; Wattiaux et al., 1992). Then, once degradation ends, a particle's FSG increases again as gas content decreases and the proportion of its higher-density components increases. Wattiaux et al. (1992) observed in vitro a decrease in FSG from 1.12 at 3 h after incubation to 1.05 at 9 h for alfalfa hay due to fermentation gases, and then an increase up to 1.31 at 30 h when the gases were released.

Plastic particles are biologically inert. Their original size and density tend to remain constant regardless of their MRT; thus, their dynamics do not accurately represent how feed particles pass through the rumen. However, accurate description of MRT response curves, by testing the ranges of size and density generally observed for particles in the rumen, enabled us to identify characteristics required for particles to leave the rumen as quickly as possible to limit their degradation. Because the FSG of concentrates begins to decrease once they arrive in the rumen, the closer that particle density is to 1.3, the more time it will take for its density to decrease to less than 1.1. Therefore, a density ranging from 1.2 to 1.3 seems to be an optimum to minimize the retention time in the rumen.

Concerning particle size, many authors (Bjorndal et al., 1990; Michalet-Doreau and Cerneau, 1991; Wattiaux et al., 1992; Bhatti and Firkins, 1995; Wadhwa et al., 1998) have shown that degradation rate decreases as the surface-to-mass ratio of particles increases. Consequently, large particles ferment more slowly than small particles and require more time to degrade (Bjorndal et al., 1990). They can escape the rumen less degraded than smaller particles, provided their MRT is not too long. A particle with a size near the critical threshold (i.e., 3 mm and no more than 4 mm) may have a higher probability of escaping the rumen less degraded. Based on our data, we assume that a spherical concentrate particle with a FSG of 1.2 to 1.3 and a diameter of 3 to 4 mm will more likely leave the rumen without being greatly degraded, thus increasing the proportion of particles escaping the rumen within a given time period.

CONCLUSIONS

This study was designed to describe the combined effect of the size and density of plastic particles on MRT in different parts of the gastrointestinal tract of lactating cows and to determine the size and density combination that passed most rapidly out of the rumen. Description of response curves of MRT enabled us to determine characteristics required for concentrate particles to leave the rumen quickly, to limit their ruminal degradation, and to increase escape protein. Particles with densities of 1.2 to 1.3 had the lowest MRT in the entire digestive tract and in the rumen. Particles of 1 to 3 mm in diameter with this range of density had little influence on MRT. However, given the effect of particle size on the rate of ruminal degradation, a 3- to 4-mm diameter feed particle would have a higher probability of leaving the rumen less degraded. These assumptions require testing feeds subjected to microbial fermentation to assess the increase in RUP in the feed when size and density are fixed. In addition, combining these physical properties with chemical protection, which delays the rate of ruminal fermentation of protein, could be a promising strategy to further increase RUP.

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APPENDIX

Table A1. Mean retention times (MRT, h) in the entire digestive tract and its 3 compartments (MRT1, MRT2, and TT) for the 3 sizes (1, 2, and 3 mm) and 4 densities (0.9, 1.1, 1.3, and 1.5) of plastic particles

MRT ¹ (h)	1 mm				2 mm				3 mm				SEM
	0.9	1.1	1.3	1.5	0.9	1.1	1.3	1.5	0.9	1.1	1.3	1.5	
TMRT	46.4	29.6	30.3	45.8	67.9	29.5	30.3	44.6	77.6	29.5	33.1	63.3	8.3
MRT1	24.5	9.5	10.6	19.2	43.6	8.9	9.9	20.2	50.7	8.3	11.0	28.4	9.0
MRT2	8.2	8.7	8.2	14.2	11.1	6.8	7.5	10.8	12.4	8.0	6.8	15.5	2.9
TT	13.7	11.4	11.5	12.4	13.2	13.8	12.9	13.6	14.5	13.2	15.3	19.4	1.5

¹TMRT = total MRT; MRT1 = MRT in the first compartment; MRT2 = MRT in the second compartment; TT = time until plastic particles first appear in the feces.