The effect of size and density on mean retention time of particles in the gastrointestinal tract of sheep

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The selective retention of particles in the reticulo-rumen and in the gastrointestinal tract distal to the reticulo-rumen was studied in fistulated sheep maintained on a roughage diet. Polyethylene glycol and plastic particles of different lengths (1 and 10 mm) and densities (0.92, 1.03, 1.22 and 1.44 g/ml) were either fed or were introduced into the omasum. The mean retention time in the reticulo-rumen (MRT$_{rr}$) of 1 mm long particles with a density of approximately 1.0 g/ml was about 67 h, that is eight times longer than the MRT$_{rr}$ of fluid; the heavier particles were retained only three times longer than fluid. Particles with a length of 10 mm were retained in the reticulo-rumen 19–28 h longer than 1 mm long particles of the same density. Particles with a length of 10 mm were reduced to smaller particles (0.5–4 mm) due to rumination. Multiple regression analysis indicated that particle density and particle size accounted for 59 and 28% of the total variation of MRT$_{rr}$, respectively. The mean retention time distal to the reticulo-rumen (MRT$_{gat}$) of 1 and 10 mm long particles with a density near 1.0 g/ml was 18–19 h, similar to that of fluid (16 h). The heavier particles were retained about 3–8 h longer.

Particle size and density: Mean retention time: Reticulo-rumen: Sheep

The microbial degradation of plant cell walls is a rather slow process. To achieve a high digestibility of cellulose, ruminants retain feed particles substantially longer than fluid in the reticulo-rumen. The mechanism by which the selective retention of particles is attained is not well understood.

From faecal analysis it has been calculated that feed particles longer than 1–2 mm have a very low probability of leaving the reticulo-rumen compared with small particles (Troelsen & Campbell, 1968; Reid et al. 1977; Welch, 1982; Ulyatt, 1983). This size-dependent retention of particles in the rumen led to the ‘critical size theory’ (Poppi et al. 1980). This concept divides the rumen particles into two pools: a large-particle pool, which cannot pass out of the rumen, and a small-particle pool, which can leave the rumen (Martz & Belyea, 1986). Less attention has been paid to the influence of particle density on mean retention time, although an effect was demonstrated in earlier studies (King & Moore, 1957; Campling & Freer, 1962). It was shown that particles with a density between 1.17 and 1.42 g/ml leave the rumen of cows faster than particles with a higher or a lower density (Durkwa, 1983; desBordes & Welch, 1984; Ehle, 1984; Ehle & Stern, 1986).

The objective of the present study was to estimate the quantitative contribution of particle density and size to mean retention time in the reticulo-rumen (MRT$_{rr}$) and in the gastrointestinal tract distal to the reticulo-rumen (MRT$_{gat}$) of sheep.

MATERIALS AND METHODS

Plastic particles

Plastic particles of different densities were manufactured by mixing polyethylene (PE; DSK 1812, HPPE, BASF, Ludwigshafen) with increasing proportions of barium sulphate (DAB...
6, Merck, Darmstadt) and a dye in a computerized kneader (Haake Buchler System 40, Haake, Karlsruhe, FRG) at a temperature of 150°. The mixtures were then extruded through a 0.7 mm nozzle, and the emerging filaments were rolled on metal frames (100 x 300 mm). These frames were placed under a press, and filaments were cut with parallel steel knives to lengths of 1 and 10 mm respectively. Density of the particles was controlled using a precision balance with density set (Mettler AE 160, Mettler, Gießen); this allowed determination of density differences up to 0.001 g/ml. These plastic particles were similar in size and density to normal feed particles in the reticulo-rumen. The impact strength of the mixtures was measured during the production process by the determination of the torque. The values varied between mixtures of different densities by not more than 15%. Therefore, particles of different densities were comminuted due to rumination to the same extent. Particles (1000) of each density and size were counted and weighed. Particles of length 10 mm of all densities were flexible. The physical properties of the particles are listed in Table 1.

Experimental design

Blackhead sheep (three wethers and one female) weighing between 60 and 75 kg were each fitted with a rumen fistula. Sheep had been adapted over several months to a diet of medium-quality hay (g/kg dry matter: crude fibre 360 (Weende analysis; Nehring, 1960), crude protein (nitrogen x 6.25) 147, ash 90), given ad lib. Water and mineralized salt licks were accessible at all times.

In the experimental periods the animals were kept in metabolism crates. They were fed three times each day (08.00, 13.00 and 17.00 hours) on 800 g hay/meal. The feed refusal was weighed and removed before the next feed. The adaptation period before each experiment was at least 5 d.

The mean retention time (MRT) of fluid and of plastic particles in the total gastrointestinal tract (MRT\textsubscript{GRT})

At 08.00 hours of the 1st day 10000 plastic particles with a length of 1 mm of each density and 1000 particles with a length of 10 mm of each density, mixed with 150 g ground commercial dairy concentrate, were fed to each sheep. The sheep ate this mixture within 5 min.

Rumen fluid volume and the MRT of fluid in the reticulo-rumen were estimated with polyethylene glycol (PEG; molecular weight 4000; Merck, Darmstadt). A single dose of 6 g PEG dissolved in 12 ml water was administered through the fistula. Rumen samples were taken 2, 4, 6, 8, 10, 12 and 14 h after the injection. Samples were stored at 4° and analysed nephelometrically at 90° to the incident light according to Hydén (1955).

Total faecal output was collected on the 1st day at intervals of 3 h, on the 2nd day at intervals of 6 h and twice daily for the following 8 d. The faeces were dried at 105° for 24 h for determination of dry matter. The faeces of each collection period were divided into three equal subsamples: one subsample (33%) of each period was ground in a coffee grinder (K 6; Bosch, Stuttgart) for 45 s. Preliminary studies had indicated that this method did not change the size of the plastic particles. Ground subsamples were sieved through a 500 \(\mu\)m wire-mesh sieve (Retsch, Haan). The plastic particles on the sieve were manually separated from the remaining faecal particles, and sorted according to their density by colour. Subsamples containing larger amounts of plastic particles were separated by density gradients. Salt solutions of increasing density were used to separate the plastic particles by sedimentation and flotation (Hooper et al. 1984). Plastic particles of each density were dried and weighed. The concentration of the marker was determined by calculation as g particles per g faecal dry matter for each density and each initial particle size.
Table 1. Composition and properties of plastic particles

<table>
<thead>
<tr>
<th>Density (g/ml)</th>
<th>Length (mm)</th>
<th>Colour</th>
<th>Diameter (mm)</th>
<th>Weight (g/1000)</th>
<th>PE (g/kg)</th>
<th>Barium sulphate (g/kg)</th>
<th>Dye (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.919</td>
<td>1.05</td>
<td>Red</td>
<td>0.798</td>
<td>0.4785</td>
<td>970</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>0.917</td>
<td>1.00</td>
<td>Grey</td>
<td>0.841</td>
<td>5.2210</td>
<td>970</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>1.025</td>
<td>1.05</td>
<td>Yellow</td>
<td>0.802</td>
<td>0.5577</td>
<td>845</td>
<td>125</td>
<td>30</td>
</tr>
<tr>
<td>1.028</td>
<td>1.00</td>
<td>Magenta</td>
<td>0.813</td>
<td>5.5880</td>
<td>845</td>
<td>125</td>
<td>30</td>
</tr>
<tr>
<td>1.216</td>
<td>1.05</td>
<td>Orange</td>
<td>0.760</td>
<td>0.6152</td>
<td>660</td>
<td>310</td>
<td>30</td>
</tr>
<tr>
<td>1.219</td>
<td>1.00</td>
<td>Dark Red</td>
<td>0.750</td>
<td>5.6767</td>
<td>660</td>
<td>310</td>
<td>30</td>
</tr>
<tr>
<td>1.436</td>
<td>1.05</td>
<td>Turquoise</td>
<td>0.723</td>
<td>0.6276</td>
<td>520</td>
<td>450</td>
<td>30</td>
</tr>
<tr>
<td>1.435</td>
<td>1.00</td>
<td>White</td>
<td>0.720</td>
<td>5.9646</td>
<td>520</td>
<td>450</td>
<td>30</td>
</tr>
</tbody>
</table>

PE, polyethylene.

**MRT of fluid and of plastic particles distal to the reticulo-rumen (MRT_{fur})**

Plastic particles (1000) with a length of 1 mm of each density and 200 plastic particles with a length of 10 mm of each density were mixed and placed in four gelatine capsules (4 ml; WDT, Hannover). The experiment was carried out about 4 weeks after the determination of MRT_{fur}. At 08.00 hours of day 1 capsules were administered manually through the reticulo-omasal orifice between the leaves of the omasum. A single dose of 3 g PEG dissolved in 15 ml water was given into the omasum via a plastic tube. Total faecal output was collected at 3 h intervals on the 1st day, 6 h intervals on the 2nd day and twice daily for the following 3 d. After collection the samples were divided into a one-third (a) and a two-third (b) subsample. Subsample (a) was dried and analysed as described previously. Both 1 and 10 mm particles of each density were weighed. A 1 g subsample of the ground faeces was taken for PEG estimation. To obtain a suitable number of 10 mm particles in the whole faecal sample, subsample (b) was soaked in water for 4 h. Then the slurry was poured into a sieve with a pore diameter of 1 mm (Retsch, Haan) and with a high-pressure water jet the 10 mm particles were separated and weighed for each density.

**Calculations**

Rumen fluid volume was calculated by dividing the amount of PEG added by its concentration at time zero using regression analysis. The MRT of fluid in the reticulo-rumen (MRT_{rr}) was calculated as $MRT_{rr} = k^{-1}$. The proportion of marker leaving the compartment per hour ($k$) can be calculated from the equation

$$ k = (\ln C_i - \ln C_j) \times t^{-1}, $$

where $C_i$ is the marker concentration at zero time calculated by regression analysis and $C_j$ is the marker concentration at sampling time $t$. The MRT_{fur} of fluid was calculated as $MRT_{fur} = MRT_{rr} + MRT_{fur}$.

The MRT_{fur} of plastic particles and the MRT_{fur} of fluid and plastic particles were calculated according to Thielemans et al. (1978):

$$ MRT = (\Sigma C_i \times t \times dt) / (\Sigma C_i \times dt)^{-1}, $$

where $C_i$ is the marker concentration in the sample, $t$ is the time-interval after particle administration at which the sample was taken, and $dt$ is the faecal collection interval.

The MRT_{rr} of plastic particles was calculated as $MRT_{rr} = MRT_{fur} - MRT_{fur}$.

The effects of particle length and particle density on MRT were evaluated by using a block analysis of variance with a blocking factor of sheep and the treatment factors of
Particle density and particle length (Winer, 1971). A multiple regression analysis with two dependent variables was applied to determine the quantitative contribution of particle density and particle size on \( \text{MRT}_{\text{RR}} \).

Results are given as means and standard errors. Significance of differences between the MRT and the faecal recoveries of particles were tested using the Tukey-test for multiple mean comparison (Winer, 1971).

RESULTS

**Feed intake and rumen volume**

For both sets of the experiment, feed intake averaged 1869 (SE 45) g hay/d, i.e. 1699 (SE 41) g dry matter/d. Rumen fluid volume was 13.2 (SE 0.7) litres or 206 (SE 13) g/kg bodyweight for both sets of the experiment respectively.

\( \text{MRT}_{\text{RR}} \)

Applying the analysis of variance a significant effect of particle density on \( \text{MRT}_{\text{RR}} \) was found ( \( P < 0.001 \); Fig. 1). However, the multiple-mean-comparison test showed no significant difference in \( \text{MRT}_{\text{RR}} \) between particles of similar size with densities of 0.92 and 1.03 g/ml.

Particles (1 mm) with a low density (0.92 and 1.03 g/ml) were retained two to three times...
Table 2. Mean retention time in the total gastrointestinal tract (MRT<sub>GIT</sub>) of fluid and of plastic particles with different sizes and densities

(Mean values and standard errors for four sheep)

<table>
<thead>
<tr>
<th>Length (mm)</th>
<th>Density (g/ml)</th>
<th>MRT&lt;sub&gt;GIT&lt;/sub&gt; (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>1</td>
<td>0.92</td>
<td>86.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1.22</td>
<td>56.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>0.92</td>
<td>107.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1.22</td>
<td>85.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means were compared between the different densities within each size; values with different superscript letters were significantly different (P < 0.01); differences between 1 and 10 mm particles of each density were significantly different (P < 0.01).

longer than the heavier particles of the same length (0.92 g/ml: 67.3 (SE 1.4) h; 1.03 g/ml: 65.7 (SE 2.8) h; 1.22 g/ml: 35.5 (SE 5.2) h; 1.44 g/ml: 25.4 (SE 2.9) h). The MRT<sub>RR</sub> of light particles was eight times longer and for small heavy particles three times longer than the MRT<sub>RR</sub> of fluid (8.3 (SE 0.3) h).

Particles with a length of 10 mm were retained significantly longer (P < 0.001) in the reticulo-rumen than 1 mm particles. Most of the 10 mm particles were found comminuted in the faeces to a size of 0.5-4 mm; the amount of unchewed 10 mm particles in the faeces was highest for particles with a density of 1.44 g/ml (0.037 of the administered particles). The MRT<sub>RR</sub> of particles with a length of 10 mm was on average 22.7 h longer than that of the 1 mm particles of the corresponding density; this difference between MRT<sub>RR</sub> of 1 and 10 mm particles was not influenced by particle density.

\[ MRT_{GIT} \]

MRT<sub>GIT</sub> was slightly (P < 0.05) influenced by particle density (Fig. 1); particle length did not have a marked effect on MRT<sub>GIT</sub>. Particles with a density of 0.92 and 1.03 g/ml were retained for 19.9 (SE 2.0) h and 17.7 (SE 1.6) h respectively; these values were in the same range as MRT<sub>GIT</sub> of fluid (16.3 (SE 1.6) h). Heavy particles were retained longer than the light particles (1.22 g/ml: 21.3 (SE 2.2) h; 1.44 g/ml: 26.6 (SE 3.7) h).

\[ MRT_{GIT} \]

The negative correlation between particle density and MRT was somewhat less pronounced for MRT<sub>GIT</sub> than for MRT<sub>RR</sub> due to the slight increase of MRT<sub>GIT</sub> with increasing density (Table 2).

Faecal recovery

When MRT<sub>GIT</sub> was determined, faecal recoveries were significantly higher (P < 0.001) for 1 mm particles (81.8 (SE 2.0) % of the particles fed) than for the 10 mm particles (67.6 (SE 1.5) % of the particles fed; Table 3). This may be explained by the greater comminution of large particles by chewing, leading to a higher loss during sieving of the subsamples. Particles with a higher density had significantly higher faecal recoveries (P < 0.001) than lighter particles due to their shorter MRT.
Table 3. Faecal recoveries of plastic particles of different sizes and densities (% of total weight applied). For the estimation of the mean retention time (MRT) in the gastrointestinal tract (MRT\textsubscript{GIT}), faeces were collected for 10 d after particles were fed; for the estimation of the MRT in the gastrointestinal tract distal to the reticulo-rumen (MRT\textsubscript{Gut}), faeces were collected for 5 d after the administration of the particles into the omasum

(Mean values and standard errors for four sheep)

<table>
<thead>
<tr>
<th>Length (mm)</th>
<th>Density (g/ml)</th>
<th>Faecal recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MRT\textsubscript{GIT}</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>1</td>
<td>0·92</td>
<td>74·9\textsuperscript{a}</td>
</tr>
<tr>
<td>1·03</td>
<td>72·4\textsuperscript{b}</td>
<td>1·3</td>
</tr>
<tr>
<td>1·22</td>
<td>86·3\textsuperscript{c}</td>
<td>1·9</td>
</tr>
<tr>
<td>1·44</td>
<td>89·8\textsuperscript{d}</td>
<td>1·2</td>
</tr>
<tr>
<td>10</td>
<td>0·92</td>
<td>62·2\textsuperscript{a}</td>
</tr>
<tr>
<td>1·03</td>
<td>67·6\textsuperscript{a}</td>
<td>2·1</td>
</tr>
<tr>
<td>1·22</td>
<td>68·9\textsuperscript{a}</td>
<td>3·0</td>
</tr>
<tr>
<td>1·44</td>
<td>71·7\textsuperscript{a}</td>
<td>3·2</td>
</tr>
</tbody>
</table>

Means were compared between the different densities within each size; values with different superscript letters were significantly different ($P < 0·01$); differences between 1 and 10 mm particles of each density were significantly different ($P < 0·01$).

When MRT\textsubscript{Gut} was determined, faecal recoveries were independent of particle length and density and averaged 98·8 (SE 0·7) %.

DISCUSSION

Density of particles

Large feed particles entering the rumen mostly have a density below 1·0 g/ml based on an air-filled interior (Evans \textit{et al}. 1973; Van Soest, 1975; Sutherland, 1988). Particle size decreases during the digestion process due to rumination and microbial breakdown; particle density increases due to hydration, ion-exchange and destruction of cellular space, and values up to 1·4 g/ml have been measured (Hooper & Welch, 1985). To study the influence of density on MRT it was essential in our experiment to use inert plastic particles whose density did not change in the forestomach.

Our findings clearly underline the marked influence of the density of particles on MRT\textsubscript{RR}. Particles of length 1 mm with a density of 1·44 g/ml left the reticulo-rumen 2·6 times faster than those of densities of 0·92 and 1·03 g/ml (Fig. 1). This is in agreement with studies in cows (desBordes & Welch, 1984; Ehle, 1984) and sheep (Lindberg, 1985; Katoh \textit{et al}. 1988) where the highest passage rates were found for particles with a density of 1·38–1·5 g/ml. Particles with very high densities of 1·77 and 2·15 g/ml, on the other hand, were retained longer. However, feed particles normally do not reach a density higher than 1·5 g/ml (Evans \textit{et al}. 1973).

Particle size

Particles 10 mm in length of all densities were retained in the reticulo-rumen of our sheep 19·1–27·9 h longer than the 1 mm long particles. Most of these large particles were found comminuted to a size of 0·5–4 mm in faeces. Although breakdown of large particles is obviously essential for a high outflow rate, rumination alone does not guarantee a high
passage rate. Ruminated particles with a low density may be small enough for passage into the omasum, but they are retained considerably longer in the reticulo-rumen than the heavier particles. Likewise, $MRT_{RR}$ of heavy particles with a length of 10 mm was markedly shorter than that of the 1 mm long particles with low densities, although the larger particles had to be ruminated before outflow from the reticulo-rumen.

**Quantitative contribution of particle density and length to $MRT_{RR}$**

From our findings it was possible to estimate by multiple-regression analysis the quantitative contribution of particle length and density to $MRT$. The first condition for the calculation is a linear decline of $MRT_{RR}$ with increasing particle density between 0.92 and 1.44 g/ml. Such a linear relation may be assumed from our findings (Fig. 1); this was also shown by Ehle (1984) and Lindberg (1985). The second assumption is an exponentially decreasing probability of passage of particles from the reticulo-rumen with increasing particle length. Such a relation was shown for feed particles by Welch & Smith (1978) and Poppi et al. (1980) and, in an additional study, for plastic particles. In these experiments, the reticulo-rumen was emptied and filled with buffer solution. Particles (1, 5, 10, 20 mm with a density of 1.03 g/ml) were given into the ventral sac of the rumen and sedimentation was inhibited largely by bubbling the buffer solution. Particles could leave the reticulo-rumen only at their original size because the sheep did not ruminate during the 4 h experiment. In fact, a negative exponential relation between outflow from the reticulo-rumen and particle size had been found (Kaske, 1987).

Regression analysis using the values of $MRT_{RR}$ (Fig. 1) showed that 87% of the total variation of $MRT_{RR}$ could be explained by the factors particle density and particle size. Other factors obviously had no major effect in our experiment. Particle density accounted for 59% of the total variation of $MRT_{RR}$; particle size determined 28% of the variation of $MRT_{RR}$.

The application of our results for feed particles is justifiable only if the breakdown rate of our plastic particles is comparable to that of feed particles. Using the findings of Ulyatt et al. (1986) we estimated a breakdown rate for feed particles in the range of 0.07/h. The breakdown rate of our plastic particles seems to be comparable with this value. Approximately 24 h after feeding, the probability for passage of particles with a length of 10 mm was the same as that for particles with a size of 1 mm. In further experiments the plastic particles were ruminated at a rate of 0.073/h (M. Kaske, unpublished results).

**$MRT_{RR}$ of feed particles with changing density**

In contrast to our experiment the density of feed particles changes during the digestion process. However, little is known about the rate at which the density of feed particles increases during their stay in the reticulo-rumen. Hooper & Welch (1985) and Nocek & Kohn (1987) placed forage particles in nylon bags in the rumen. The density of feed particles increased rapidly within 1 h after introduction of particles into the rumen from 0.9 g/ml up to approximately 1.1 g/ml. During the following 28 h a further relatively slow increase in particle density was observed. Highest values of about 1.4 g/ml were reached after an incubation period of 52–76 h. Small particles increased in density faster than large particles, and the rate of density change seemed to be different for different feeds; e.g. legume particles changed their density faster than grass particles.

**$MRT_{out}$ of particles of different size and density**

While particle size had no clear influence on $MRT_{out}$ in our experiment, particles with a density of 1.44 g/ml were retained somewhat longer than particles with a density of 1.03 g/ml. These results contrast with the generally accepted concept of similar passage rates
for particles and fluid in the gut of ruminants. Nevertheless, the absolute difference between MRT\textsubscript{Rut} of heavy and light particles (26.6 h and 17.7 h respectively) was small compared with the marked differences in MRT\textsubscript{RR}. Furthermore, the tendency for a longer retention of heavy particles differed considerably between the four animals. However, Campling & Freer (1962) found a stronger influence of particle density on MRT\textsubscript{Rut} in cows; MRT\textsubscript{Rut} of particles with a density of 1.21 g/ml (52 h) was 78.4\% longer than the retention time of particles with a density of 1.02 g/ml (29 h).

**Conclusions**

The passage of particles into the omasum seems to be primarily limited by the velocity of density increase of the particles in the reticulo-rumen. Rumination diminishes particle size rapidly. A high particle density considerably increases the probability of outflow. The preferential passage of the small, heavy particles into the omasum seems to be physiologically sensible, because these particles are mostly sufficiently digested. The longer retention of the light particles, on the other hand, is a prerequisite for efficient digestion of the cellulose in the feed.

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**References**


