

No distinct difference in the excretion of large particles of varying size in a wild ruminant, the banteng (*Bos javanicus*)

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Abstract The forestomach of ruminants and camelids does not only allow a differential excretion of fluids and small particles but also a differential excretion of small and large particles. The question whether larger particles of different size classes are also retained for different time periods, or whether simply a particle-size threshold exists above which all particles of a size higher than this threshold are retained in an undifferentiated manner, has not been addressed so far. We determined the mean retention time (MRT) of different-sized large particles (10 and 20 mm) in three banteng (*Bos javanicus*) on two forage only diets, grass and grass hay. We used cerium-mordanted fibre (10 mm) and lanthanum-mordanted fibre (20 mm) as particle markers,

mixed in the food. Average total tract MRT for large and very large particles at the grass diet was 58 and 56 h and at the grass hay diet 66 and 64 h, respectively. Very large particles moved slightly faster than large particles through the gut of the banteng. Three interpretations are possible: Very large particles are resubmitted to rumination sooner than large particles; ingestive mastication of the particle markers could have reduced the difference in the size of the particle markers; alternatively, particle retention may be governed by a threshold, above which all particles of a size higher than this threshold are retained in an undifferentiated manner. In order to test these possibilities, experiments with fistulated animals would have to be performed.

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Introduction

The forestomach of ruminants and camelids does not only allow a differential excretion of fluids and small particles (Lechner-Doll et al. 1990; Clauss et al. 2006) but also a differential excretion of small versus large particles (Heller et al. 1986; Lirette and Milligan 1989; Cherney et al. 1990; Lechner-Doll et al. 1990; Schwarm et al. 2008); in this process, the selective retention of different-sized particles is not so much a function of particle size per se but a function of particle density (Lechner-Doll et al. 1991). This sequence of excretion, with the shortest retention for fluids, followed by small and then by large particles, gives rise to the question if density-mediated excretion is a function of particle size in general, or if a particle-size threshold exists above which all longer particles are retained in an undifferentiated manner, irrespective of their size. To our

knowledge, this question has not been addressed experimentally with forage-based markers. Here, we report the results of a study we performed with a wild cattle-type ruminant, the banteng (*Bos javanicus*) on the differential excretion of large (10 mm) and very large (20 mm) particles.

Materials and methods

Two feeding trials were performed with three banteng at the Zoological Garden of Berlin. The animals were fed mostly grass during the first trial and mostly grass hay (soaked in water) during the second trial (ratio of grass to grass hay feeding days was 6:1 and 2:5 during the first and second trial, respectively). For a detailed description of the animals, the diets used and amounts ingested, the duration of the adaptation and trial period, the faecal sampling regime, the marker preparation and the analytical procedures, see Schwarm et al. (2008). In addition to the 10-mm particles (mordanted with cerium (Ce)) reported in that study, hay particles of 20 mm length were prepared by grinding dried grass hay through 20-mm square perforated screen (Retsch catalogue no. 03.647.0062) with a retsch mill (SM 2000, Retsch, Haan, Germany). After removing dust and smaller particles by dry sieving (by hand) and incubation with neutral detergent solution, particles were incubated with 27 g lanthanum (La) per 50 g particles in 800 ml distilled water (lanthanum [III] chloride hydrate, $\text{LaCl}_3 \cdot \text{H}_2\text{O}$, Sigma-Aldrich 61490). After mordanting at 37°C and washing with tap water, the fibre was dried at 65°C. The marker dose applied was 0.2 g/kg BM of each mordanted fibre marker; the marker was fed to the animals in a small amount of soaked sugar beet pulp and was consumed completely within 10 to 60 min. After the marker feeding, faeces were collected continuously from the individual animals, noting the time of each defecation. After wet microwave digestion, faeces were analysed for passage markers by inductive-coupled plasma mass spectroscopy (ELAN 6000, PerkinElmer Life and Analytical Sciences, Milano, Italy). The mean retention time (MRT) in the total gastrointestinal tract was calculated according to Thielemans et al. (1978) for the two large particle markers. Results are displayed as mean \pm standard deviation. We used only descriptive statistics because the normality presumption for parametric tests cannot reliably be tested with very small sample sizes.

Results

Without exception, the resulting marker excretion curves for Ce (10-mm particles) and La (20-mm particles) indicated a simultaneous processing of the two particle-size classes (Fig. 1). The mean retention times for Ce (10 mm) did not

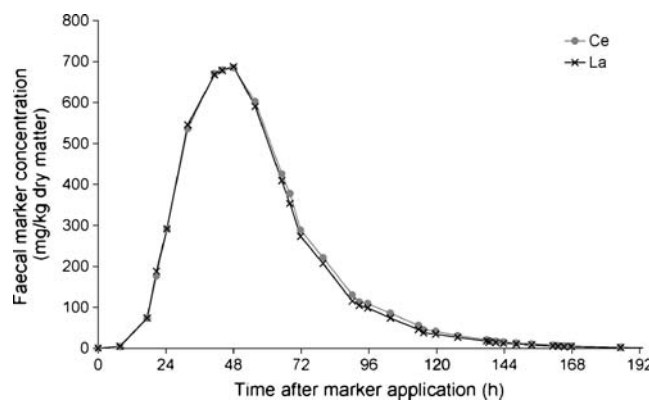


Fig. 1 Excretion pattern of a banteng (*B. javanicus*, animal 3, G) for 10-mm particles (mordanted with cerium (Ce)) and 20-mm particles (mordanted with lanthanum (La))

differ distinctively from those for La (20 mm; first trial, 58 ± 3 vs. 56 ± 3 ; second trial, 66 ± 7 vs. 64 ± 8 ; Table 1). However, the very large (20 mm) particles were always excreted slightly faster (1–3 h) than the large (10 mm) particles, and it can be speculated that with a larger sample size, this tendency would turn out to be a differential excretion of large particles.

Discussion

Both metals, cerium and lanthanum, have a similar atomic mass (139 and 140, respectively) and thus, an effect of marker density on marker excretion appears unlikely (but could occur if different proportions of the rare earth elements were actually bound to the fibres). This assumption was confirmed in a pilot study where both markers mordanted to hay particles of the same length were excreted simultaneously by three roe deer (Albrecht et al., unpublished). Therefore, only two different interpretations remain to explain the result of a similar excretion of large particles. In this study, the animals were fed the marker and ingested it themselves; therefore, ingestive mastication could have reduced differences in particle size to such an extent

Table 1 MRT of 10- and 20-mm particles in the gastrointestinal tract of banteng (*B. javanicus*) on a grass or grass plus grass hay diet

Animal	Diet	MRT (h)	
		10mm	20mm
1	G	56	54
2	G	61	59
3	G	56	55
1	G+H	60	57
2	G+H	74	72
3	G+H	65	63

G grass, G+H grass plus grass hay

that a potential actual difference in the retention of different-sized large particles in the forestomach could not be measured; however, in cattle, ingestive mastication contributes to a lesser extent to the overall particle-size reduction than rumination (McLeod and Minson 1988). The influence of ingestive mastication could only be ruled out by the use of experimental animals that are fitted with a forestomach cannula, by which the markers can be inserted directly into the forestomach without ingestive mastication (e.g. Lechner-Doll et al. 1990). Whilst differences between small (2 mm) and large particles (the 10-mm particles of this study) could be demonstrated in the same animals (Schwarm et al. 2008), the probability of ingestive particle-size reduction could be a function of particle size, and hence, the larger particles of this study might have been more likely to be affected by mastication—which would explain why their retention time was always somewhat shorter than that of the 10-mm particles.

On the other hand, the very similar excretion pattern of different-sized large particles could indicate that in ruminants, density-dependent particle retention is not a continuous function of particle size, but a process governed by a particle-size threshold: Small particles are retained in the fibre raft by the “filter-bed effect” but can become disentangled from the mat by sedimentation and filtration during repeated contraction cycles of the forestomach; in contrast, larger particles above a certain threshold will themselves form the fibre raft by entanglement. Only after having been resubmitted to mastication (rumination) from the fibre raft, these larger (10- and 20-mm-long) particles are reduced in size so that they can filter through the remainder of the raft (Beaumont and Deswysen 1991; Lechner-Doll et al. 1991; Poppi et al. 2001; Hristov et al. 2003; Faichney 2006). The findings of Kaske et al. (1992), who found no significant difference in the rumination and excretion of 10- and 20-mm plastic particles (of varying density) inserted into the rumen of fistulated sheep, support this possibility.

The results of this study would only have been conclusive if, similar to the difference between 2- and 10-mm particles (Schwarm et al. 2008), a difference between the 10- and 20-mm particles would have been found. Given the lack of such a distinct difference, the results might indicate—similar to the findings of Kaske et al. (1992) in sheep—that above a certain size, differences in particle size are of little relevance, because all particles above that size are subject to entanglement in the raft and rumination in a similar fashion. Actually, the slightly faster excretion of 20-mm particles than 10-mm particles could also indicate that very large particles are resubmitted to rumination sooner than large particles. These new hypotheses would have to be tested by experiments with fistulated animals.

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