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The effect of size and density on the mean retention time of particles in the reticulorumen of cattle (*Bos primigenius* f. *taurus*), muskoxen (*Ovibos moschatus*) and moose (*Alces alces*)

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Abstract

Particle passage from the reticulorumen (RR) depends on particle density and size. Forage particle density and size are related and change over time in the RR. Particle density mainly influences sorting in the reticulum, whereas particle size influences particle retention in the fibre mat of stratified rumen contents ('filter-bed' effect). We investigated these effects independently, by inserting plastic particles of different sizes (1, 10 and 20 mm) and densities (1·03, 1·20 and 1·44 mg/ml) in the RR of cattle (*Bos primigenius* f. *taurus*) as a pilot study, and of muskoxen (*Ovibos moschatus*; *n* 4) and moose (*Alces alces*; *n* 2) both fed two diets (browse and grass). Faeces were analysed for plastic residues for 13 d after dosing to calculate mean retention times (MRT). The results confirmed previous findings of differences in absolute MRT between species. Comparing muskoxen with moose, there was no difference in the effect of particle density on the MRT between species but particle size had a more pronounced effect on the MRT in muskoxen than in moose. This indicated a stronger 'filter-bed effect' in muskoxen, in accord with the reports of stratified RR contents in this species *v.* the absence of RR content stratification in moose. Low-density particles were retained longer in both species fed on grass diets, indicating a contribution of forage type to the 'filter-bed effect'. The results indicate that retention based on particle size may differ between ruminant species, depending on the presence of a fibre mat in the RR, whereas the density-dependent mechanism of sedimentation in the RR is rather constant across species.

Key words: Stratification: Rumen physiology: Particle retention: Particle size: Viscosity: Fluid throughput

Ruminants are peculiar among mammalian herbivores because they combine a foregut fermentation system with a specific sorting mechanism[1,2]. This not only facilitates a very efficient reduction in size of ingesta particles[3] but also allows ruminants to consume more feed than other foregut fermenters[4,5]. In contrast to the historical view that this sorting mechanism operates mainly on the size of ingesta particles in the forestomach, like a simple sieve mechanism, it has more recently been understood that the sorting mechanism in the ruminant forestomach operates in particular on the density of ingesta particles[6–9]. Because the size of actual ingesta particles is related to their density[10–13], this density-dependent mechanism automatically ensures that particles are sorted according to their size, even if the separation mechanism does not discriminate particles by size itself. In addition to this density-dependent effect, a 'filter-bed effect' is assumed to operate in domestic ruminants. The rumen contents of domestic ruminants are usually stratified in different layers, with a 'fibre mat' or 'fibre raft' above a more fluid phase[13]. This fibre mat can additionally enhance particle retention, independent of the density-dependent sorting mechanism, by entanglement of particles in the fibre mat that acts as a 'filter-bed' that does not release larger particles[14–16]. Such a mechanism may represent an additional size- or shape-based sorting mechanism in those ruminants whose rumen contents stratify.

Abbreviations: GIT, gastrointestinal tract; MRT, mean retention time; RR, reticulorumen.

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The influence of density on the fate of particles in the ruminant forestomach has repeatedly been investigated with plastic particles of varying size and density in domestic goats and sheep[17–20], and buffalo and cattle[21–30]. The general result of these studies is that larger particles are usually retained in the reticulorumen (RR) for a longer time than shorter particles, possibly due to a 'filter-bed effect', although this assumption has rarely been stated (but see Prigge *et al.*[30]). Note that in plastic particles, variations in length are specifically not linked to variations in density. Moreover, as the specific gravity of particles increases from about 0·92 to about 1·44 g/ml, their retention time decreases, or in other words, denser particles are excreted faster. This indicates that low-density particles are retained in the RR by their buoyancy and that denser particles have a higher probability of leaving the RR. However, once a certain density of approximately 1·50 g/ml is surpassed, the retention time again increases, indicating that very high densities make an expulsion from the RR less likely. This fact is recognised in the application of intraruminal devices, which reliably stay in the RR irrespective of their size, if they are at least 1·8 g/ml[31,32].

Ruminants differ in terms of their forestomach physiology; the two extremes of this range have been termed 'cattle-type' (with stratified RR contents) and 'moose-type' (without stratification in RR contents)[5]. The adaptive significance of this difference remains hypothetical and might be more related to salivary defences against secondary plant compounds in 'moose-type' ruminants and optimisation of microbial harvest from the RR in 'cattle-type' ruminants than to mechanisms of particle retention[5]. Nevertheless, an absence of stratification should also translate into a less pronounced 'filter-bed effect'.

It has been suggested that stratification and the 'filter-bed effect' result in particle sorting in the rumen before sorting in the reticulum in 'cattle-type' ruminants, whereas sorting may be limited to the reticulum in 'moose-type' ruminants[11,12,33]. Lechner *et al.*[34] investigated the retention of small *v.* large particles in muskoxen and moose and did not find a difference between the species using mordanted fibres as particle markers; additionally, faecal particle size – the ultimate measure of the efficiency of the RR sorting mechanism – did not differ between species when fed their natural forages, regardless of whether they were of the 'moose-type' or the 'cattle-type'.

Mordanted fibres have the advantage of closely resembling ingesta in their physical properties; however, they only represent different size classes with similar density. In order to clearly separate the effects of size and density, we conducted additional studies using plastic particles in which the variation of size and density was not inherently linked, using domestic cattle for a pilot trial regarding the method, and muskoxen and moose to test our hypothesis. Because a series of measurements confirmed the fundamental differences between moose *v.* domestic cattle and muskoxen[34–38], a comparison of moose and muskoxen should be particularly appropriate for investigating the effects of different physiological adaptations on RR particle retention mechanisms. On the basis of previous results, our hypothesis was that muskoxen, with their typically stratified rumen content and thus an expected 'filter-bed effect', should not only display a density-dependent particle retention but also a size-dependent particle retention in the RR. In contrast, moose, lacking a rumen content stratification and thus a 'filter bed', should have a similar density-dependent but not size-dependent particle retention.

Materials and methods

We used four adult, fistulated domestic oxen (mean 1238 (SD 39) kg) of the Institute of Animal Science of the University of Bonn, Germany, four fistulated, adult castrated male muskoxen (276 (SD 23) kg) of the Robert G. White Large Animal Research Station, Institute of Arctic Biology, University of Alaska, Fairbanks, AK, USA and two adult, fistulated female moose (345 (SD 13) kg) of the Alaska Department of Fish and Game at the Palmer Research Center, AK, USA (Table 1). All animals had received the rumen fistulas for other studies more than a year before the present experiment. All animals were kept individually (wild ruminants in outdoor pens, oxen in a stable) with *ad libitum* access to water, shade and their respective feed. Adaptation periods to new diets were at least 14 d. The oxen received a diet of grass silage (*n* 4; trials in autumn 2007). The muskoxen received a diet of either mixed browse (*n* 4; *Salix* spp.) or grass hay (*n* 4; *Bromus* sp.) in a crossover design (two trials in June/July 2008). The moose received a diet of mixed browse (*n* 2; mostly *Salix* spp.) in June 2008 and a diet of grass silage (*n* 2; *Bromus* sp.) in October 2008 for *ad libitum* intake. Browse was harvested on a daily basis for the respective animals. All forages were fed whole (i.e. not chopped). Feed intake and proximate composition of the different diets have already been reported[34] and are given in Table 2.

Table 1. Animals used in the present study, location and measurements performed in each species

Species	Animal type	Feeding type	Location	Diet	*n*	Time
Cattle (*Bos primigenius* f. *taurus*)	Domestic	Grazer	Bonn, Germany	Grass silage	4	October 2007
Muskoxen (*Ovibos moschatus*)	Wild	Grazer/intermediate feeder	Fairbanks, AK, USA	Browse	4	June/July 2008
				Grass hay	4	June/July 2008
Moose (*Alces alces*)	Wild	Browser	Palmer, AK, USA	Browse	2	June 2008
				Grass silage	2	October 2008

Table 2. Diets used and DM intake (DMI) during the feeding trials in domestic cattle (*Bos primigenius* f. *taurus*), muskoxen (*Ovibos moschatus*) and moose (*Alces alces*)

(Mean values and standard deviations)

			DMI (kg/d)		DMI ($g/kg^{0.75}$ per d)		Diet				
Species	Diet	*n*	Mean	SD	Mean	SD	DM (%)	CP	NDF	ADF	ADL
Cattle	Grass silage	4	10·1	1·9	48	9	38·7	13·1	56·2	37·9	7·2
Muskoxen	Browse leaves	4	4·8	0·5	70	7	19·6	13·6	29·1	23·3	16·1
	Grass hay	4	2·9	0·9	43	12	87·9	5·0	59·6	38·0	7·4
Moose	Browse leaves	2	5·3	0·2	66	0	30·4	16·2	44·2	30·5	17·9
	Grass silage	2	5·6	0·3	69	6	33·7	14·4	59·2	31·8	2·3

CP, crude protein (in % DM); NDF, neutral-detergent fibre; ADF, acid-detergent fibre (in % DM with residual ash); ADL, acid-detergent lignin (in % DM).

Retention times of fluid and forage particle markers have been described previously for these animals[34,39]. In addition, we applied a set of plastic particle markers similar to those described by Kaske & von Engelhardt[18] and Kaske *et al.*[19]. These particles were of three different densities (1·03, 1·22 and 1·44 g/cm^3) and three different lengths (1, 10 and 20 mm), with a common diameter of 0·7 mm. Polyethylene (high-pressure polyethylene 1840H; Basell, Frankfurt am Main, Germany) and barium sulphate (Honeywell Specialty Chemicals Seelze GmbH, Seelze, Germany) were mixed in different proportions (1·03 g/cm^3: 86:14, w/w; 1·22 g/cm^3: 69:31, w/w; 1·44 g/cm^3: 55:45, w/w).

For each density, three batches with different pigments (yellow UN1750, orange UN2255, red UN3927, white UN0005, beige UN8016, black UN0055, green UN66003, violet UN5046, blue UN5001; COLOR-Service, Hainburg, Germany; at 0·5–1 % of the total mix) were produced. The material was first mixed in a tumbling mixer (and, in the case of high barium sulphate proportions, additionally by hand) and then melted at <200°C and homogenised in a co-rotating twin-screw extruder (Teach-Line® ZK 25 T; Dr Collin, Ebersberg, Germany). The material was extruded as a long string, cooled in water and cut into small pieces using a string granulator (CSG 171/1; Dr Collin). The resulting material was homogenised in the tumbling mixer and then extruded by a single-screw extruder (Teach-Line® E 20 T; Dr Collin) at <150°C using a nozzle with twenty-four openings of 0·7 mm each. To avoid adhesion of the individual strings, they were allowed to cool in 1·8 m vertical descent in air at ambient temperatures, fixed with adhesive tape at each 0·5 m, and coiled by hand.

Plastic strings were cut to specified lengths using paper cutters. We verified densities of the resultant particles with an Ultrapycnometer 1000 (Quantachrome Instruments, Boynton Beach, FL, USA). Low-density particles ranged from 1·02 to 1·03 g/cm^3, intermediate-density particles were 1·20 g/cm^3 and high-density particles were 1·37–1·44 g/cm^3. The 10 and 20 mm particles were flexible.

We applied markers as a pulse dose. In the domestic oxen, the particles were placed by hand on top of the fibre mat in the middle of the rumen. In the wild ruminants, the smaller cannulae did not allow direct placement of the dose by hand. For these animals, we mixed and packed the particles into plastic tubes of the same diameter as the cannula. The mixtures of markers in the tubes were then saturated with water and frozen. The frozen mixture was then removed from the tubes and dosed through the cannula into the upper to middle layer of the rumen contents in the central (neither cranial nor caudal) region. A thawing test with a frozen marker in a 38°C water-bath resulted in complete thawing after 80 s.

All animals received the markers in the morning between 08.00 and 10.00 hours and received their morning feed directly afterwards. Domestic cattle received a dose of 20 g of the marker of each of the nine coloured markers. Although yellow and white particles were easy to tell apart in the raw state, these two colours could not be differentiated in the faeces of domestic cattle (that is, we could not differentiate yellow particles of 1 mm and 1·03 g/cm^3 from white particles of 1 mm and 1·20 g/cm^3). Therefore, in the trials with wild ruminants, the white marker was not used; 1·20 g/cm^3 particles were thus only represented by 1 mm (black) and 20 mm (beige) particles. Also, after analysing the cattle faeces, we decided to increase the marker dose for the wild ruminants (relative to the body mass) to enhance the marker signal, so that the moose received 25 g and the muskoxen received 16 g of each of the eight coloured markers.

Three faecal samples taken from the animals before marker dosage were used for baseline values. After marker dosing, faeces were sampled at progressively increasing intervals: 4 h (day 1–2), 6 h (day 3), 8 h (day 4–5), 12 h (day 6–9) and 24 h (day 11–13); in doing so, all faeces defaecated during the time period were collected, mixed, and a representative subsample (approximately 10 % of the total sample) was taken. All samples were stored frozen at −20°C until analysis.

For analysis, the samples were dried at 60°C and subsequently ground in a regular coffee grinder. Kaske & von Engelhardt[18] had observed that this procedure did not change the size of plastic particles in the faeces, and we made the same observation. Applying a coffee grinder to dried ruminant faeces apparently only disrupts the cohesion of the dried particles but does not change their size.

The dry mass of the sample was determined by weighing; the sample was washed (5–15 min) over a sieve with a pore size of 0·5 mm and dried again. The plastic particles were then sorted out by hand under bright light conditions and a degree of magnification preferred by the person doing the sorting. Plastic particles of each colour were weighed, and the concentration of marker was expressed as g particles/g faecal DM for each density and initial particle size.

Separation of plastic particles from faecal material was much more labour-intensive than we assumed from reports on this method in the literature. Neither washing with detergent solution nor treatment with 72 % H_2SO_4 made sorting in the remaining material easier, and preliminary attempts at separation by density were not successful due to the varying density of digested plant material. The faeces from grass-fed animals were distinctively easier to sort than the faeces from browse-fed animals. Sorting required approximately 3 h per 30–85 g of sample, in addition to the 10 min required for grinding and up to 25 min of washing before sorting. Although we originally intended that all samples were sorted by one investigator, helpers had to be employed. All 127 cattle samples were sorted by the second author, with some support from the first author. Approximately, 50 % of all muskox (n 212) and moose (n 88) samples were sorted by the second author and the rest by five additional helpers, including the first author. No distinction was made whether particles had been ruminated upon or not, but subjectively, it appeared that the majority of the 10 and 20 mm particles had been ruminated.

For the domestic oxen, the results for yellow, white and black particles were not used, due to the difficulties described earlier and due to putative difficulties in retrieving black particles. The same markers had also been given to the fistulated reindeer used in this set of trials[34,39], but the plastic markers were chewed to such an extreme fineness that manual sorting was considered too laborious.

The mean retention times (MRT) for the whole gastrointestinal tract (GIT) (MRT_{GIT}) was calculated according to Thielemans *et al.*[40] as:

$$MRT_{GIT} = \frac{\Sigma t_i C_i dt_i}{\Sigma C_i dt_i},$$

where C_i is the marker concentration in the faecal samples from the interval represented by time t_i (hours after marker administration) and dt_i is the interval (h) of the respective sample,

$$dt_i = \frac{(t_{i+1} - t_i) + (t_i - t_{i-1})}{2}.$$

Faeces were sampled up to 240 h after marker dosage in oxen, 264–288 h in muskoxen and 264–278 h in moose. Apart from individual cases of small, intermediate and heavy particles in the muskoxen on a grass diet, all cases of small, intermediate and heavy particles in the muskoxen on browse and all intermediate and heavy particles in the moose, particle excretion had not terminated at the end of the sampling period. Therefore, the MRT values reported for these particles are truncated, similar to the results from Kaske & von Engelhardt[18].

Several authors confirmed that fluids and particles move more or less in parallel in the distal GIT of ruminants[41–44]. In contrast, Siciliano-Jones & Murphy[45] found differences in the passage of plastic particles of various density and size through the distal GIT of cattle, which might have been due to the inclusion of very high-density particles (1·77 g/ml) in their study. We followed Kaske & von Engelhardt[18] in calculating the MRT for the RR (MRT_{RR}) by subtracting the fluid MRT for the distal digestive tract[34] from the particle MRT_{GIT}; for this procedure, the fluid MRT for the distal digestive tract was calculated as the difference between the fluid MRT_{GIT}, calculated as described earlier, and the fluid MRT_{RR}, as calculated by the decrease the faecal liquid marker concentration C_i with time according to the equation $C_i = a\,e^{-kt_i}$ or $\ln C_i = -kt_i + b$ (fluid MRT_{RR} is then k^{-1})[46].

Data are presented as means and standard deviations. The effects on the retention time were analysed with the General Linear Models module of Statistica version 8.0 (StatSoft (Europe) GmbH, Hamburg, Germany)[47], using particle size and particle density as continuous predictor variables, and species and diet (browse or grass) as categorical factors.We controlled for intake introducing it as a covariate. The models included two-way interaction terms; when these were NS (α-level 0·05), the models were reanalysed without the interactions. The cattle were analysed separately from the muskoxen and moose, because of the differences in the marker sets described earlier. For the cattle results, interaction terms were not used because of the unbalanced experimental design, with only one particle size at the intermediate particle density.

Results

General remarks

All animals appeared to be in good health during the trials. Two muskoxen were unusually reluctant to accept the grass hay, leading to a high standard deviation in feed intake (Table 2), and consequently in the retention parameters. Interestingly, the summer/autumn feed intake of the moose hardly varied between the browse and the grass silage diet.

Cattle

In the cattle, there was a significant effect of particle size ($P<0·0001$ for both the GIT and the RR; this was best seen in 1·44 g/ml particles, in which all size classes were used) and of density ($P<0·0001$ for both the GIT and the RR) on the MRT, with a longer retention of larger and lower-density particles (Table 3 and Fig. 1).

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Table 3. Retention time (MRT) in the gastrointestinal tract (GIT) of cattle (*Bos primigenius* f. *taurus*), muskoxen (*Ovibos moschatus*) and moose (*Alces alces*) for plastic particles of varying density and size
(Mean values and standard deviations)

		MRT GIT (h)															
		1·03 mg/ml						1·20 mg/ml						1·44 mg/ml			
		1 mm		10 mm		20 mm		1 mm		10 mm		20 mm		1 mm		10 mm		20 mm	
Species	Diet	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cattle	Grass silage	–	–	134	4	145	4	–	–	127	5	–	–	81	11	103	6	125	8
Muskoxen	Grass hay	101	15	135	9	152	11	44	12	–	–	131	21	51	11	115	18	113	24
	Browse	57	11	114	33	130	38	32	6	–	–	117	37	36	7	104	25	105	33
Moose	Grass silage	54	1	79	2	81	0	24	5	–	–	44	6	28	6	49	9	41	8
	Browse	27	1	52	7	55	5	20	1	–	–	38	4	21	1	40	1	39	4

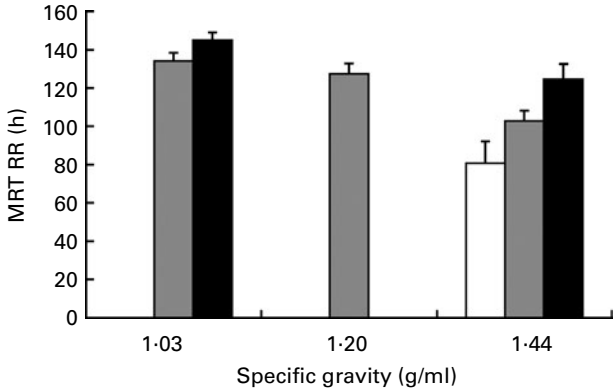

Fig. 1. Retention time (MRT) of plastic particles of varying length and density in the ruminoreticulum (RR) of cattle on grass silage. □, 1 mm; ▩, 10 mm; ■, 20 mm. Values are means, with standard deviations represented by vertical bars.

Muskoxen and moose

In the muskoxen and moose, a similar pattern was evident as seen in the cattle (Table 3 and Fig. 2). However, the moose had generally shorter retention times than the muskoxen. The differences between small (1 mm) and larger particles (10 and 20 mm) were distinct. Retention of the low-density particles (1·03 mg/ml) increased with particle size in the muskoxen on browse, but the differences between the two large particle classes were not distinct for other densities or diets. Retention of the low-density particles (1·03 mg/ml) was notably longer on the grass diet than on the browse diet. In the comparison of muskoxen and moose, species, particle density, particle size, diet and level of intake all had significant effects on the MRT (Table 4).

When comparing the differences in retention in the RR between the small and large particles of any given density, the muskoxen always retained the larger particles longer compared with the smaller ones than the moose (Fig. 3(a), (c) and (e)), which is reflected in the significant interaction term (species × particle size) in Table 4. In contrast, when comparing the differences in retention in the RR between high- and low-density particles of any given size, there was no systematic difference between the muskoxen and the moose. The data for cattle (from the present study) also matched this pattern (Fig. 3(b), (d) and (f)); accordingly, the interaction term (species × particle density) was not significant in the muskoxen–moose comparison.

Discussion

The present study shows that there are not only general differences in the magnitude of the MRT between similar-sized ruminant species[48] and differences in the retention of fluid and the ratio of fluid *v.* small particles[49] but also differences in the mechanics of particle retention. While the influence of particle density appears to be relatively similar across species (Fig. 3(b), (d) and (f)), the difference in retention may occur in relation to particle size (Fig. 3(a), (c) and (e)).

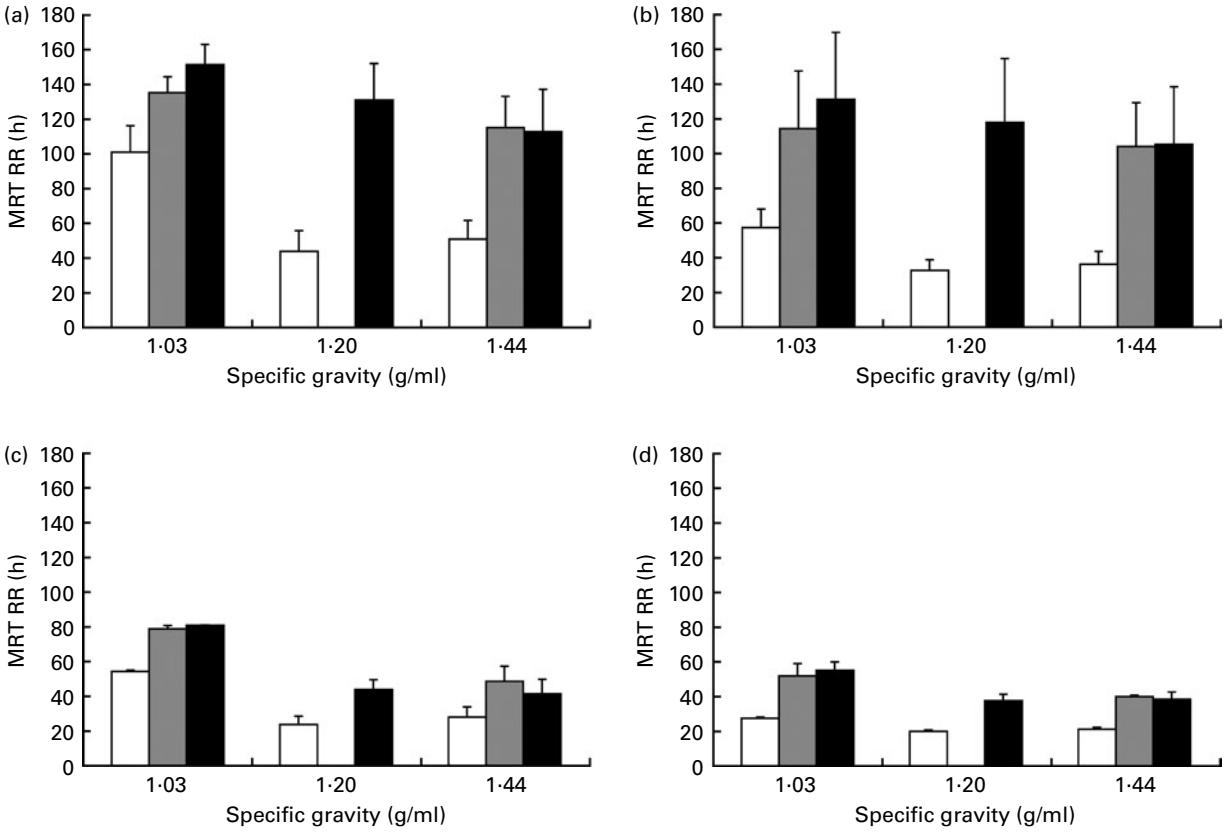

Fig. 2. Retention time (MRT) of plastic particles of varying length and density in the reticulorumen (RR) of muskoxen (*Ovibos moschatus*) on (a) grass hay, (b) browse and of moose (*Alces alces*) on (c) grass silage and (d) on browse. □, 1 mm; ▩, 10 mm; ■, 20 mm. Values are means, with standard deviations represented by vertical bars.

Evidently, caution should be applied when interpreting the results from a comparison of two different individual species[50]. Extrapolation to general rules about 'cattle-type' *v.* 'moose-type' ruminants, or even further interpretation in the sense of generalisations about the digestive physiology of grazing and browsing ruminants, will require evaluation of more species. However, this may prove very difficult in practice, as it will require fistulation of more browsing and grazing wild ruminant species and the relatively laborious sorting of faecal samples as described in the 'Methods' section. The present results only demonstrate two different physiological strategies, which might be linked to other findings in these and other ruminant species. Additionally, the present study was limited by the low sample size (n 2) of available, fistulated moose and the unusual hesitance of some of our muskoxen to readily ingest the grass hay offered; these factors made the intake level a significant contributor to the differences in the MRT and led to a significant interaction term, diet × intake level (Table 4).

Forages ingested by ruminants, regardless of their source, exhibit common characteristics in flotation experiments: the floating fraction being consistently comprised of larger particles and the sedimenting fraction of smaller

Table 4. Significant effects on the mean retention time (MRT) in the gastrointestinal tract (GIT) and the reticulorumen (RR) of muskoxen (*Ovibos moschatus*) and moose (*Alces alces*) for plastic particles of varying density and size

	MRT_{GIT}			MRT_{RR}		
Effect	$F_{1,88}$	P	r^2	$F_{1,88}$	P	r^2
Intercept	64·423	0·0001	0·78	68·122	<0·0001	0·80
Particle density	24·405	0·0001		24·897	<0·0001	
Particle size	77·122	0·0001		78·676	<0·0001	
Species	8·262	0·0051		13·293	0·0005	
Diet (grass/browse)	11·223	0·0012		13·176	0·0005	
Intake level	12·858	0·0006		20·641	<0·0001	
Species × particle size	23·893	0·0001		24·374	<0·0001	
Diet × intake level	13·993	0·0003		14·626	0·0002	

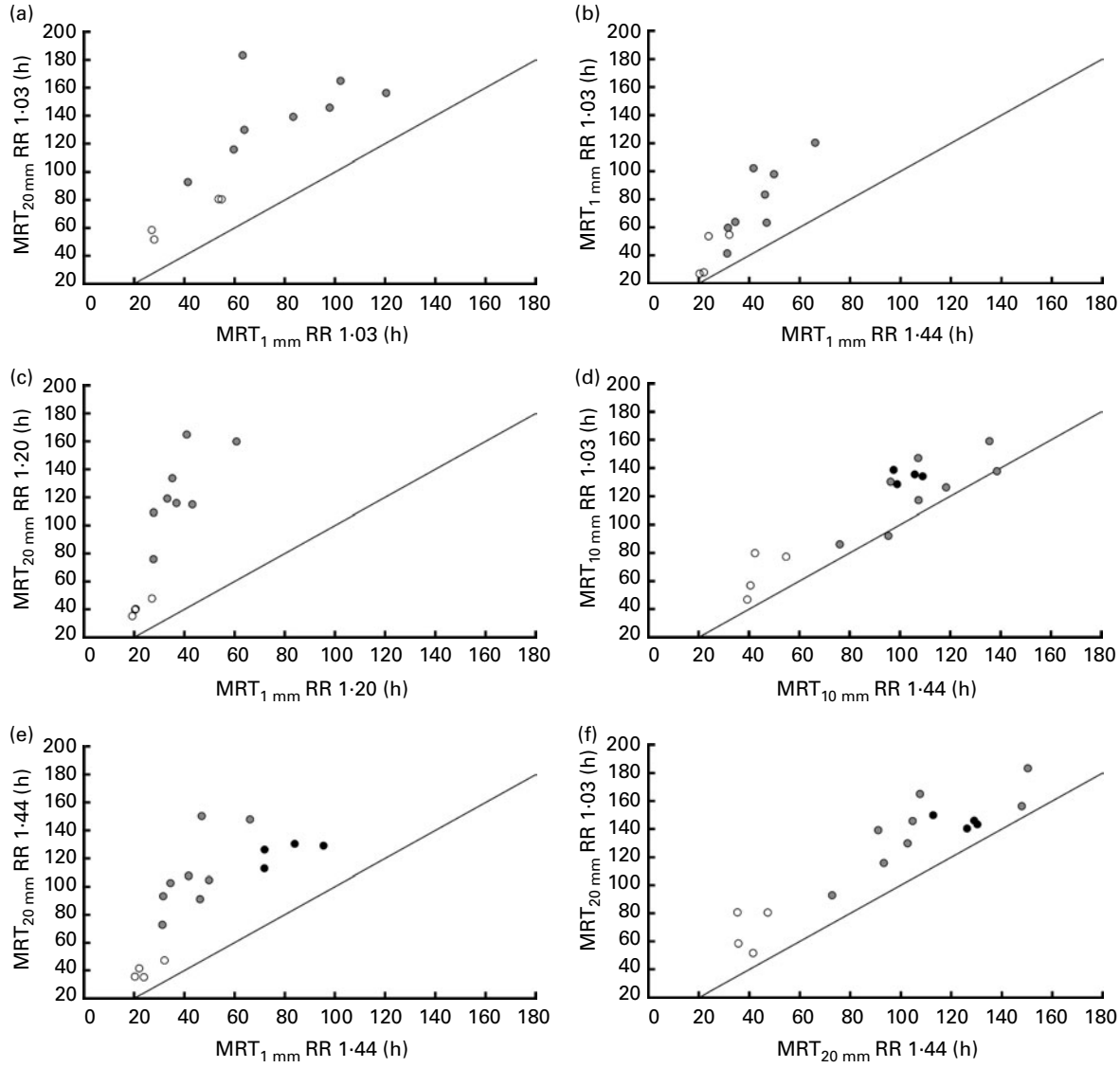

Fig. 3. Relationship of mean retention times (MRT) in the ruminoreticulum (RR) of muskoxen (*Ovibos moschatus*) and moose (*Alces alces*) between small (1 mm) and large (20 mm) particles of ascending density: (a) 1·03 mg/ml (◍, muskoxen; ○, moose); (c) 1·20 mg/ml (◍, muskoxen; ○, moose); (e) 1·44 mg/ml (●, cattle; ◍, muskoxen; ○, moose); between high-density (1·44 mg/ml) and low-density (1·03 mg/ml) particles of ascending size: (b) 1 mm (◍, muskoxen; ○, moose); (d) 10 mm (●, cattle; ◍, muskoxen; ○, moose); (f) 20 mm (●, cattle; ◍, muskoxen; ○, moose). The data for domestic cattle (from the present study) were added where available. The line represents $y = x$.

particles[7,10–13,51,52], most probably due to differences in adhering fermentation gas bubbles[53]. Thus, the process of particle separation by their flotation behaviour, i.e. their density, automatically assures a sorting by particle size and leads to the uniformly small particles that escape the RR of any ruminant species[3,34]. The main location of sorting according to density is the reticulum[6,54], where a relatively high fluid content enables the separation by flotation and sedimentation[11–13]. In domestic cattle, a separation of particles according to density (and hence size) has also been described for the rumen itself, where particles become segregated between the dorsal and the ventral rumen or between the fibre mat and the more liquid phase beneath[13,52,55–59].

Separation due to density and size in the rumen itself has not been demonstrated in rumen contents of domestic sheep[10] or wild ruminants[11,12]. Reasons for this might be that either their rumen contents are inherently homogeneous, or because particles forming the fibre mat are of a heterogeneous nature: Sutherland[10] and Hummel *et al.*[13] suggested that low-density particles in the lower part of the fibre mat can support less-buoyant particles in the upper mat that would not stay in that position by their own buoyancy. This effect, combined with simple physical entrapment especially of elongated particles, creates the 'filter-bed effect', which retains particles in the fibre mat for a longer time than determined by their own disposition alone. Since the proportion of low-density particles in the lower rumen is characteristically higher

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on grass diets[13], one would expect a pronounced filter-bed effect on those diets, especially in particles of lower density that are more susceptible to this effect. The present results are in accord with this expectation, with low-density particles being particularly affected by the difference in the diet (Fig. 2). Similarly, desBordes & Welch[24] concluded that low-density plastic particles are especially subject to rumination, given their propensity to being retained in the fibre mat.

One factor related to the buoyancy of particles is their shape. Lirette *et al.*[60] found that elongated, fibre-like particles are more likely to float than particles of a more cubic nature. Those authors found that these particles also differed in lignin content, which could not only be an indication for advanced digestion but also for an influence of fibre composition on fractionation patterns. Browse, in general, has a higher proportion of lignin in its fibre fraction than grass[48], and a different arrangement of vascular bundles (parallel in grass but branched in browse)[61,62]. Among others, these properties could be responsible for different fracture properties that lead to more elongated, fibre-like particles in comminuted grass and more cubic or polygonal particles in comminuted browse[63–65]. This pattern was also observed in the present study (Fig. 4). Others have suggested that the more cubic shapes of comminuted browse particles are less apt to form fibre mats with intertwined particles[64–66], thereby resulting in less retention of low-density particles in browse *v.* grass diets (Fig. 2).

Durkwa[67], as presented in Murphy *et al.*[21], found little difference in retention or rumination among 1–5 mm particles in cattle. Similarly, differences between 2 and 5 mm plastic particles were not consistent between density classes in cattle[26]. Prigge *et al.*[29] did not find a difference between 1 and 3 mm particles in cattle, but did find a longer RR retention for 5 mm nylon particles, and later reported results from a similar trial, wherein RR retention increased continuously from 1–3 to 5 mm nylon particles[30]. Stetter Neel *et al.*[25] also found a shorter RR retention for 1 mm than for 3 mm nylon particles in cattle, and Kaske *et al.*[19] described an increased RR retention of 1 *v.* 5 mm particles in sheep. In contrast to reported differences between lengths <5 mm, differences between 10 mm and smaller particles were of a larger magnitude[67] (as presented in Kaske *et al.*[19], Murphy *et al.*[21] and in the present study), suggesting that the RR retention of 1–10 mm particles might be a continuous function of particle size, although at times difficult to demonstrate between similar-sized particles.

The differences between larger particles (the 10 and 20 mm particles of the present study) may be less distinct. Similar to the observation of the present study, that the differences in retention between the 10 and 20 mm particles were often small (Fig. 2), Kaske *et al.*[19] observed differences in the retention of 10 and 20 mm plastic particles in the RR of sheep that only tended towards significance, and Schwarm *et al.*[68] and Lechner *et al.*[34] did not find differences in the retention of 10 and 20 mm mordanted fibre particles in wild ruminants. Thus, it appears that at a particle size above 10 mm, little further contribution to retention due to increases in size should be expected. The main potential difference between the 10 and 20 mm particles might be that, at about 20 mm length, passage through the Ostium reticuloomasale is actually physically prevented by particle size[19]; this is in accord with McBride *et al.*[69], who observed the passage of 10 mm particles through the Ostium reticuloomasale (but did not assess 20 mm particles). Therefore, in future studies, when the number of particle sizes that can be investigated is limited, it may be more informative to investigate a combination of 1, 5 and 10 mm particles than 1, 10 and 20 mm particles. For even larger plastic particles, Welch[70] demonstrated that flexible 35 and 70 mm particles could be ruminated and cleared from the rumen in sheep, whereas 300 mm particles could not.

The effect of density on particle retention in the RR is similar in cattle, muskoxen and moose, and we therefore hypothesise that particle separation based on density is a mechanism common to all ruminants. In contrast, there are differences in the effects of particle size. The effect of size may be related to a general difference between species in RR content stratification and formation of a fibre mat. The occurrence of RR content stratification is influenced by the type of forage ingested, grass material tending more towards the formation of a mat and also by the physiology of the animal. Compared with cattle and 'cattle-type' ruminants, moose characteristically have higher RR fluid

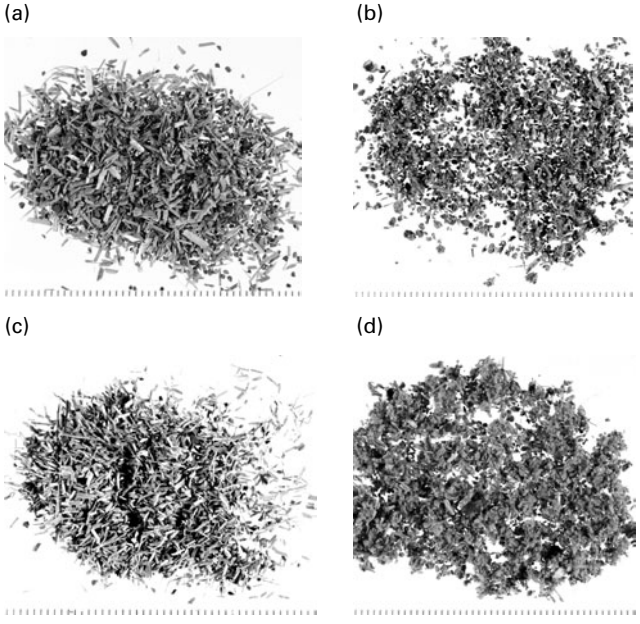

Fig. 4. Faecal particles in muskoxen (*Ovibos moschatus*) fed (a) grass hay and (b) browse, and moose (*Alces alces*) fed (c) grass silage and (d) browse. Note the general difference in shape between grass and browse particles. The scaling is 1 mm.

viscosity, lower RR fluid throughput, less distinct separation of the RR retention of small particles and fluids, more uniform ruminal papillation, smaller differences in the DM content between the dorsal and the ventral rumen, weaker ruminal pillars and absence of an intraruminal gas dome[5,12,34,36,66]. All of these characteristics are associated with limited RR content stratification and a less distinct 'filter-bed effect', and may contribute to the less pronounced effect of particle size on retention in the RR observed in the present study. Moose are additionally characterised by comparatively small omasa[35] and shallow reticular crests[33], both linked to a low RR fluid throughput. Many of these characteristics show some degree of convergence among wild ruminant species having similar natural diets, indicating that species having digestive physiology similar to moose are browsers[71].

To conclude, we propose that a more pronounced 'filter-bed effect' as demonstrated in muskoxen in the present study is one of several[5] advantages the 'cattle-type' ruminants derive from physiological adaptations that enhance RR fluid throughput and rumen content stratification. Due to differences in fermentation characteristics between browse and grass forages, grazers particularly benefit from extended particle retention produced by the 'filter-bed effect'[48].

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