Differential passage of fluids and different-sized particles in fistulated oxen (Bos primigenius f. taurus), muskoxen (Ovibos moschatus), reindeer (Rangifer tarandus) and moose (Alces alces): rumen particle size discrimination is independent from contents stratification. — Source link ↗

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Lechner, I; Barboza, P; Collins, W; Fritz, J; Günther, D; Hattendorf, B; Hummel, J; Südekum, K H; Clauss, M

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Abstract

Ruminant species differ in the degree that their rumen contents are stratified but are similar insofar that only very fine particles are passed from the forestomach to the lower digestive tract. We investigated the passage kinetics of fluid and particle markers (2, 10 and 20 mm) in fistulated cattle (Bos primigenius f. taurus), muskoxen (Ovibos moschatus), reindeer (Rangifer tarandus) and moose (Alces alces) on different diets. The distribution of dry matter in the rumen and the viscosity of rumen fluids suggested that the rumen contents were more stratified in muskoxen than moose. Correspondingly, as in previous studies, the species differed in the ratio of mean retention times of small particles to fluids in the reticulorumen, which was highest in cattle (2.03) and muskoxen (1.97-1.98), intermediate in reindeer (1.70) and lowest in moose (0.98-1.29). However, the ratio of large to small particle retention did not differ between the species, indicating similarity in the efficiency of the particle sorting mechanism. Passage kinetics of the two largest particle classes did not differ, indicating that particle retention is not a continuous function of particle size but rather threshold-dependent. Overall, the results suggest that fluid flow through the forestomach differs between ruminant species. A lower relative fluid passage, such as in moose, might limit species to a browse-based dietary niche, whereas a higher relative fluid passage broadens the dietary niche options and facilitates the inclusion of, or specialization on, grass. The function of fluid flow in the ruminant forestomach should be further investigated.

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3   **Differential passage of fluids and different-sized particles in fistulated oxen (*Bos**

4   ***primigenius* f. *taurus*), muskoxen (*Ovibos moschatus*), reindeer (*Rangifer tarandus*) and**

5   **moose (*Alces alces*): rumen particle size discrimination is independent from contents**

6   **stratification**

7

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22

23   Running head: Particle passage in large ruminants

24

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27   insofar that only very fine particles are passed from the forestomach to the lower digestive

28   tract. We investigated the passage kinetics of fluid and particle markers (2, 10 and 20 mm) in

29   fistulated cattle (*Bos primigenius* f. *taurus*), muskoxen (*Ovibos moschatus*), reindeer

30   (*Rangifer tarandus*) and moose (*Alces alces*) on different diets. The distribution of dry matter

31   in the rumen and the viscosity of rumen fluids suggested that the rumen contents were more

32   stratified in muskoxen than moose. Correspondingly, as in previous studies, the species

33   differed in the ratio of mean retention times of small particles to fluids in the reticulorumen,

34   which was highest in cattle (2.03) and muskoxen (1.97-1.98), intermediate in reindeer (1.70)

35   and lowest in moose (0.98-1.29). However, the ratio of large to small particle retention did

36   not differ between the species, indicating similarity in the efficiency of the particle sorting

37   mechanism. Passage kinetics of the two largest particle classes did not differ, indicating that

38   particle retention is not a continuous function of particle size but rather threshold-dependent.

39   Overall, the results suggest that fluid flow through the forestomach differs between ruminant

40   species. A lower relative fluid passage, such as in moose, might limit species to a browse-

41   based dietary niche, whereas a higher relative fluid passage broadens the dietary niche options

42   and facilitates the inclusion of, or specialization on, grass. The function of fluid flow in the

43   ruminant forestomach should be further investigated.

44

45   **Key words**: stratification, rumen physiology, particle retention, particle size, viscosity, fluid

46   throughput

47

48   **Introduction**

49   Ruminants are peculiar among mammalian herbivores. They achieve exceptionally

50   small ingesta particles due to the repeated mastication (rumination) of ingesta facilitated by a

51   sorting mechanism in their reticulorumen (RR) (Fritz et al. 2009), and can therefore also

52   achieve particularly high digestive coefficients at relatively high food intakes (Clauss et al.

53   2009e; Schwarm et al. 2009b). The RR sorting mechanism has been well investigated in

54   domestic ruminants (reviewed in Beaumont and Deswysen 1991; Lechner-Doll et al. 1991;

55   Allen 1996; Hristov et al. 2003), and is generally described as linked to the stratification of

56   the RR contents. In domestic ruminants, this stratification has been demonstrated repeatedly

57   (reviewed in Hummel et al. 2009).

58   In contrast, such a stratification of RR contents is not a consistent finding in wild

59   ruminants. Hofmann (1973) stated that the RR contents of grazing wild ruminants were

60   stratified, whereas those of browsing wild ruminants were not. Although the general validity

61   of this broad statement remains to be tested, various lines of evidence support this concept.

62   Visual inspections (Hofmann 1973; Nygren and Hofmann 1990; Renecker and Hudson 1990),

63   ultrasonographical visualisation (Tschuor and Clauss 2008) as well as physical analyses

64   (Clauss et al. 2009b; 2009c; Hummel et al. 2009) of the RR contents, the intraruminal

65   papillation pattern (Clauss et al. 2009d), and comparative measurements of fluid and particle

66   retention (Hummel et al. 2005; Clauss et al. 2006b) suggest that in browsing ruminants, the

67   RR contents are stratified to a much lesser degree than in grazing ruminants.

68   On the one hand, the absence or presence of such a stratification has been linked to

69   several other morphological findings in ruminants of different feeding types (summarized in

70 Clauss et al. 2008), providing a general framework for many observations of Hofmann

71 (1989). On the other hand, the question about the functional relevance of the stratification is

72 raised anew by these observations. If browsers lack stratification of RR contents, does this

73 translate into differences in RR function? A study on captive animals suggested that browsers

74 excrete larger particles in their faeces than grazers (Clauss et al. 2002), and this was linked to

75 a less efficient particle size reduction due to the absence of stratification and its retention-

76 facilitating function. However, such a difference in particle size was not evident if free-

77 ranging or naturally fed animals were compared (Renecker and Hudson 1990; Hummel et al.

78 2008a; Clauss et al. 2009c); the evident conclusion was that the reported difference between

79 browsers and grazers was more due to differences in the suitability of their dentitions to

80 comminute diets offered to captive animals, rather than differences in RR physiology.

81 In this study, we aimed to evaluate the retention kinetics of fluids and different-sized

82 particles in ruminants with presumably different degrees of RR contents stratification. The

83 differential retention of fluids and small particles (2 mm) is a well-known difference between

84 ruminants of different feeding types, e.g. between moose (*Alces alces*) and cattle (Renecker

85 and Hudson 1990). The differential retention of large (10 mm) and small (2 mm) particles is a

86 characteristic that sets ruminants apart from nonruminant foregut fermenters (Schwarm et al.

87 2008; 2009c), but has, so far, not been compared between ruminants of different feeding

88 types. Differences in the retention of different large particle classes (e.g., 10 mm vs. 20 mm)

89 have, so far, only rarely been investigated in ruminants. No difference in the retention of these

90 particle classes had been evident in a wild cattle, the banteng (*Bos javanicus*), when both

91 marked particles had been fed to the animals (Schwarm et al. 2009a). However, an effect of

92 ingestive mastication on the different-sized marked particles could not be excluded. In this

93 study, we therefore applied marked fluids and particles of 2, 10 and 20 mm to the animals

94 through a rumen cannula.

95 The marker system was first evaluated in domestic cattle, which are usually considered

96 grazers, and in which the stratification of RR contents has been well described (Hummel et al.

97 2009). Fistulated individuals of muskoxen (*Ovibos moschatus*), reindeer (*Rangifer tarandus*)

98 and moose were available for the comparative evaluation. Various evidence has shown that

99 the RR contents of moose, a strict browser (Schwartz 1992), are not stratified (Nygren and

100 Hofmann 1990; Renecker and Hudson 1990; Tschuor and Clauss 2008; Clauss et al. 2009c;

101 Clauss et al. 2009d). Although the reindeer is usually considered a mixed feeder (Hofmann

102 1985), its rumen shows a very even papillation (Soveri and Nieminen 1995; Josefsen et al.

103 1996; Mathiesen et al. 2000; Soveri and Nieminen 2007) and its RR contents are very

104   homogenous (Westerling 1970; Hobson et al. 1976). Muskoxen feed mainly on grasses and

105   sedges but also include major proportions of browse in their diet (Klein and Bay 1990;

106   Staaland and Olesen 1992; Larter and Nagy 1997). A variety of anatomical and physiological

107   characteristics (reviewed in Clauss et al. 2009a) in this species have been interpreted to

108   indicate a typical "grazer" strategy; however, although their intraruminal papillation pattern

109   indicates a certain degree of RR contents stratification, it is not as distinctive as in other

110   grazers such as cattle (Clauss et al. 2009a).

111   We hypothesised that the differences in the excretion between fluids, small and large

112   particles (measured as the ratio of the retention time of particles vs. that of fluids, and as the

113   ratio of large vs. small particle retention time) would decrease from cattle, muskoxen and

114   reindeer to moose. With respect to the differential excretion of the two large particle classes

115   (10 and 20 mm), we expected that the results would resolve the question whether large

116   particle retention is a continuous function of particle size (i.e., the longer a particle, the longer

117   it is retained) or threshold-dependent (i.e., all particles above a certain size are retained for the

118   same period of time). In order to corroborate assumptions concerning the presence or absence

119   of RR contents stratification, several measurements on physical characteristics of the RR

120   contents were performed. In order to control for effects of diet, the three wild ruminant

121   species were fed a common diet readily accepted by all – mixed willow browse; additionally,

122   cattle, muskoxen and moose received a grass-based diet (hay or silage), which was

123   unfortunately rejected by the reindeer.

124

125   **Materials and Methods**

126   We used four adult, fistulated domestic oxen (mean 1238 kg ± 39 kg standard deviation)

127   of the Institute of Animal Science of the University of Bonn, Germany, four fistulated, adult

128   castrated male muskoxen (276 ± 23 kg) and four fistulated, adult female reindeer (96 ± 12 kg)

129   of the Robert G. White Large Animal Research Station, Institute of Arctic Biology,

130   University of Alaska Fairbanks, and two adult, fistulated female moose (345 ± 13 kg) of the

131   Alaska Department of Fish and Game at the Palmer Research Center (Table 1). With the

132   exception of one reindeer, all animals had received the rumen fistulas for other studies more

133   than one year before this experiment. All animals were kept individually (wild ruminants in

134   outdoor pens, oxen in a stable) with *ad libitum* access to water, shade, and their respective

135   food. Adaptation periods to new diets lasted at least 14 days. Oxen received a diet of grass

136   silage (n=4; trials in autumn 2007). Muskoxen received either a diet of mixed browse (n=4;

137   *Salix* spp.) or grass hay (n=4; *Bromus* sp.); reindeer received either a diet of mixed browse

138   (n=4) or a pelleted compound feed (n=4; D Ration Complete Diet for Reindeer, Alaska

139   Garden and Pet Supply, Anchorage, Alaska); reindeer and muskoxen were fed in a crossover

140   design (two trials in June/July 2008). Moose received a diet of mixed browse (n=2; mostly

141   *Salix* spp.) and a diet of grass silage (n=2; *Bromus* sp.) during June/Octobre 2008 *ad libitum*,

142   and two diets of pre-harvested browse leaves (n=2, *Salix alaxensis* harvested in June; n=2,

143   *Populus balsamifera* harvested in September) during winter 2009 in restricted amounts. It had

144   been our intent to feed the reindeer on grass hay, too, but the reindeer rejected the available

145   material. Browse was harvested on a daily basis for the respective animals, with the exception

146   of winter trials in moose, in which case browse had been harvested in the preceding summer

147   and stored frozen. Apart from the pelleted compound feed used for reindeer, all forages were

148   fed whole (i.e. not chopped). Food intake and crude nutrient composition of the different diets

149   are given in Table 2.

150   Cobalt (Co) was used as fluid marker bound to EDTA. Chromium (Cr), cerium (Ce) and

151   lanthanum (La) served as mordants for marking of different-sized particle fractions – 2-mm

152   particles for Cr, 10-mm particles for Ce, and 20-mm particles for La. Co-EDTA and Cr-

153   mordanted fibres were prepared according to Udén et al. (1980). Ce- and La-mordanted fibres

154   were prepared according to Schwarm et al. (2008; 2009a). The mordanted fibres contained

155   36.9 ± 2.0 g Cr/kg DM, 26.5 ± 2.4 g Ce/kg DM, and 20.9 ± 2.3 g La/kg DM, respectively. We

156   tested the fidelity of markers to their respective particulate fractions by vitro fermentation

157   with standardized sheep rumen inoculum (as in the 'Hohenheim gas test', Menke et al. 1979)

158   followed by treatment with hydrochloric acid and pespin to simulate gastric digestion.

159   Recoveries of marker in the residual material after fermentation alone and after both

160   fermentation and gastric digestion were 104 % and 109 % for Cr, 70 % and 65 % for Ce, and

161   74 % and 71 % for La, respectively. High recoveries indicated that Cr was selectively bound

162   to completely indigestible material whereas lower recoveries of Ce and La indicated lesser but

163   satisfactory binding to digestible and indigestible components of the cell walls.

164   Markers were applied as a pulse dose. In domestic oxen, the dissolved Co-EDTA (10

165   g/animal) and the mordanted fibres (120 g/animal for each mordant) were placed by hand on

166   top of the fibre mat in the middle of the rumen. In the wild ruminants, the smaller cannulae

167   did not allow direct placement of the dose in the rumen. In these animals, Co-EDTA was

168   dissolved in water and frozen in a plastic tube; the mordanted fibres were mixed, packed into

169   plastic tubes with water, and frozen. Markers (2.25 g Co-EDTA and 27 g of each

170   mordant/muskox, 0.8 and 9 g/reindeer, 3.33 and 40 g/moose) were dosed through the cannula

171   into the rumen; in doing so, the frozen marker was pushed into the upper to middle layer of

172   the rumen contents in the central (neither cranial or caudal) region. A thawing test with frozen

173   marker in a 38 °C water bath resulted in complete thawing after 80 seconds. All animals

174   received the markers in the morning between 8 and 10 a.m. and received their morning feed

175   directly afterwards.

176   Three faecal samples taken from the animals prior to marker dosage were used for

177   baseline values. After marker dosing, faeces were sampled at progressively increasing

178   intervals: 4 hours (day 1-2), 6 hours (day 3), 8 hours (day 4-5), 12 hours (day 6-9) and 24

179   hours (day 11-13); in doing so, all faeces defecated during the time period were collected,

180   mixed, and a representative subsample (app. 10% of the total sample) taken. All samples were

181   stored frozen at -20°C until analysis. On days 14 and 15 after marker sampling, rumen

182   contents were sampled in the wild ruminants (in moose only during the summer browse

183   period) using a device based on the design by Tafaj et al. (2001), but with a window that

184   opened along the whole length of the sampling vial. Samples were taken from the dorsal

185   rumen contents first (inserting the probe horizontally) and from the ventral rumen contents

186   afterwards (inserting in a diagonal/vertical way). Following previous protocols (Clauss et al.

187   2009b; 2009c; Hummel et al. 2009), we determined the dry matter (DM) content of the

188   samples, the proportion and mean particle size (MPS) of floating and sedimenting particles,

189   the MPS of the faeces, and the viscosity of the rumen fluid. For moose, these measurements

190   were only available for the summer browse diet.

191   A detailed description of the analysis of faecal samples for the markers is given in

192   Lechner et al. (2009). Analyses were carried out using inductively coupled plasma mass

193   spectrometry of the digested samples. Microwave-assisted, high-pressure digestion with

194   HNO_3 and H_2O_2 was used to dissolve the samples for the analysis. Limits of detection based

195   on the calibration standards were 0.01 µg/L for Co, 0.05 µg/L for Cr, and 0.002 µg/L for Ce

196   and La respectively. This corresponds to 0.06 (Co), 0.3 (Cr) and 0.012 (La, Ce) mg/kg dry

197   mass for a typical sample digest, respectively. Detection limits were at least a factor of 50

198   below the concentrations determined in faeces collected before dosing with markers.

199   The MRT for the whole gastrointestinal tract (MRT_{GIT}) was calculated according to

200   Thielemans et al. (1978) as

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

201

202   With C_i = marker concentration in the faecal samples from the interval represented by time t_i

203   (hours after marker administration) and dt_i = the interval (hours) of the respective sample

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

204

205   Liquid MRTs for the reticulorumen (MRT_{RR}) were calculated as by Grovum and Williams

206   (1973b); this calculation is based on the decrease of the faecal liquid marker concentration C_i

207   with time according to the equation

208    $C_i = a\,e^{-kt_i}$ or $\ln C_i = -k\,t_i + b$

209   Liquid MRT in the RR then is k^{-1}. Because markers can be assumed to move in parallel

210   in the distal gastrointestinal tract of ruminants (empirically confirmed by Grovum and

211   Williams 1973a; Kaske and Groth 1997; Mambrini and Peyraud 1997; Wylie et al. 2000),

212   MRT_{RR} for Cr, Ce and La were calculated by assuming that $MRT_{distal}Co = MRT_{GIT}Co -$

213    $MRT_{RR}Co$ and $MRT_{distal}Cr/Ce/La = MRT_{distal}Co$; hence $MRT_{RR}Cr/Ce/La = MRT_{GIT}Cr/Ce/La$

214    $- MRT_{distal}Cr/Ce/La$.

215   To express differences in the MRT_{GIT} or MRT_{RR} between the individual markers,

216   'selectivity factors' (SF) were calculated according to Lechner-Doll et al. (1990); these

217   represent the ratios of the MRTs of different markers, e.g. the $SF_{GIT}Cr/Co$ would be

218    $MRT_{GIT}Cr/MRT_{GIT}Co$. An SF of 1.00 thus indicates no difference in the retention of two

219   markers; very high SF (e.g. 2.00 and higher) indicate distinct differences in the retention of

220   two markers.

221   Data are presented as means ± standard deviation (SD). Statistical tests were performed

222   with SPSS 17.0 (SPSS Inc., Chicago, IL). Differences in excretion between markers (within a

223   species, on a specific diet) were tested by Repeated Measurements ANOVA followed by

224   paired *t*-tests with Dunn-Sidak adjustment for multiple testing. Differences in marker

225   excretion in the same animals between different diets were tested by paried *t*-tests, as were

226   differences in rumen contents characteristics in the same animals between the dorsal and the

227   ventral rumen contents. Differences in marker excretion and rumen content characteristics

228   between species on similar diets (i.e. muskox, reindeer and moose on browse; cattle, muskox

229   and moose on grass) were tested by one-way ANOVA and subsequent post hoc tests with

230   Sidak adjustment for multiple testing. The significance level was set to 0.05.

231

232   **Results**

233   *General remarks*

234   All animals appeared to be in good health during the trials. Two muskoxen were

235   particularly reluctant to accept the grass hay, leading to high standard deviation in food intake

236 (Table 2), and consequently in the retention parameters. Interestingly, the summer/autumn

237 food intake of the moose hardly varied between the browse and the grass silage diet.

238   *Physical characteristics of RR content*

239   The viscosity of the centrifuged rumen fluid on the browse diet increased from

240 muskoxen (1.29 ± 0.03 mPa s) to reindeer (1.55 ± 0.31 mPa s) and moose (1.76 ± 0.43 mPa

241 s), with the difference between muskoxen and moose being significant (Fig. 1a). In

242 muskoxen, where fluid viscosity was also measured on the grass hay diet, the difference

243 between the two diets was not significant (1.29 ± 0.03 mPa s on browse vs. 1.34 ± 0.12 mPa s

244 on grass hay). In a similar setup, the viscosity of fluid in domestic cattle had been between

245 1.3-1.4 mPa s (Hummel et al. 2009). The average fluid viscosity in reindeer consuming the

246 pelleted feed was much higher than the average viscosity for animals consuming browse

247 (52.14 ± 65.23 mPa s vs. 1.55 ± 0.31 mPa s), but the difference was not significant due to the

248 enormous standard deviation on the pelleted feed.

249   In muskoxen and reindeer on browse diets, dorsal rumen contents had a higher dry

250 matter concentration than ventral rumen contents; in moose, however, this difference was not

251 significant, potentially due to the small sample size (Fig. 1b). For both rumen regions, the

252 three species differed significantly in the dry matter concentration of the rumen content, with

253 moose having the driest, and muskoxen the wettest contents (Fig. 1b). In muskoxen,

254 differences in the DM concentration did not differ significantly between the browse and the

255 grass hay diet (6.3 ± 1.4 % dorsal and 4.5 ± 0.7 ventral on browse vs. 6.3 ± 1.0 % dorsal and

256 4.9 ± 1.0 ventral on grass hay). In reindeer on pellets, differences in DM concentration

257 between the regions were not significant, and the DM concentrations were numerically but

258 not significantly higher in both regions as compared to browse feeding (9.4 ± 1.5 % dorsal

259 and 7.8 ± 0.8 ventral on browse vs. 14.5 ± 5.8 % dorsal and 15.1 ± 5.9 ventral on pellets).

260   On browse, moose had a significantly higher proportion of floating particles in their RR

261 contents than both muskoxen and reindeer (Fig. 1c). Muskoxen on grass had a significantly

262 lower proportion of floating particles in the rumen (4.9 ± 2.5 %) than muskoxen on browse

263 (29.9 ± 5.5 %). The proportion of floating particles did not differ significantly between

264 reindeer on browse (32.3 ± 7.2 %) and reindeer on pellets (19.5 ± 18.4 %).

265   In the three wild ruminant species, ruminal contents that floated in the floatation test

266 (Clauss et al. 2009c; Hummel et al. 2009) had a higher mean particle size than those rumen

267 contents that sedimented in the test (Fig. 1d), which again had a higher mean particle size than

268 the faeces. Differences between the three fractions (dorsal and ventral rumen and faeces) were

269 not significant in moose due to the low sample size. In muskoxen, differences were

270   significant apart from that between floating and sedimenting rumen contents on both diets. In

271   reindeer on browse all differences were significant, but none on pellets. On the browse diets,

272   there was no significant difference in the mean particle size of the floating rumen contents

273   between the species (ANOVA p=0.083); the sedimenting fraction of the rumen contents

274   differed (ANOVA p=0.035) only between muskoxen and reindeer (post hoc p=0.036), with

275   smaller particles in reindeer. The faeces, again, did not differ in particle size between the

276   species (ANOVA p=0.255).

277   *Marker excretion patterns*

278   The faecal marker patterns in the domestic oxen showed three different excretion

279   curves, one for fluids and one for small particles (both well separated but of similar shape,

280   with an immediate increase and a gradual decrease), and with one pattern for 10 and 20-mm

281   particles, with a more gradual increase and a gradual decrease (Fig. 2). MRT_{Cr} was

282   approximately 30 h longer than MRT_{Co}, both in the total GIT (Table 3) or in the RR (Table

283   4), and MRT_{Ce} and MRT_{La} were again approximately 20 h longer than the MRT_{Cr}. All

284   differences in the MRTs of the different markers were significant in cattle, with the exception

285   of the two large particle classes (Ce and La; Table 3 and 4).

286   The faecal marker patterns of muskoxen looked similar to those of domestic cattle (Fig.

287   3). Differences between the individual markers - again except for the two large particles

288   classes -were significant on the browse diet, but not on the grass hay diet; on grass hay, there

289   were very high standard deviations that most likely originated from the variations in food

290   intake (Table 3 and 4). Notably, however, the standard deviations of the SF were not greater

291   on grass hay than on browse, indicating that the relative difference between the individual

292   markers within one animal remained consistent, regardless of absolute differences in MRT.

293   On browse, MRT_{Co} was significantly shorter, but MRT_{Cr} was not different from that on the

294   grass hay diet (Table 3 and 4). SF ratios with MRT_{Co} or MRT_{Cr} in the denominator were often

295   significantly higher on the browse than on the grass hay diet.

296   In reindeer, the different marker excretion peaks appeared to be closer together than in

297   muskoxen (Fig. 4). On browse, differences in MRT between all three particle classes were not

298   significant (Table 3 and 4). MRT_{Co} were higher on browse than on pellets, as was $MRT_{Cr}RR$.

299   Correspondingly, SF ratios with MRT_{Co} or MRT_{Cr} in the denominator were often significantly

300   lower on the browse than on the pelleted diet.

301   In moose, the marker excretion peaks looked similar to the reindeer insofar as fluids and

302   small particles were very similar in their excretion pattern (Fig. 5) and the SF Cr/Co was very

303   low (Table 3 and 4). Comparing the two diets of similar *ad libitum* intake (summer browse

304   and grass silage), longer MRTs were measured on the grass diet, which were, however, only

305   significant for the largest particles, probably due to the small sample size (Table 3 and 4).

306   Similarly, differences between large particle and fluid MRT were evident, but only significant

307   for the browse winter diets. The SF for small particles/fluids was higher on the summer

308   browse than on the grass diet.

309   Comparing the species that received a grass-based diet (cattle, muskoxen, moose),

310   moose tended to have longer $MRT_{Co}GIT$ than cattle (p=0.079); this was not due to differences

311   in the MRT RR but due to significantly longer $MRT_{Co}DIST$ in moose (Table 3). Generally,

312   MRT DIST were longer in moose than in the other species. The $SF_{Cr/Co}$ and $SF_{Ce/Co}$ were

313   similar in cattle and muskoxen, and significantly higher than in moose. The $SF_{La/Co}$ was

314   lowest in moose, intermediate in muskoxen, and highest in cattle (Table 3 and 4). The $SF_{La/Cr}$

315   and $SF_{La/Ce}$ were significantly lower in muskoxen than in the other species.

316   Comparing the species that received a browse diet in summer (muskoxen, reindeer,

317   moose), moose and reindeer had significantly shorter MRTs for the long particles than

318   muskoxen (Table 3 and 4); moose also excreted small particles faster from the RR than the

319   other species (Table 4). Moose tended to have a lower $SF_{Cr/Co}GIT$ than muskoxen (Table 3),

320   and muskoxen had higher $SF_{Ce/Co}$ and $SF_{La/Co}$ than the other species. Reindeer had lower

321   $SF_{Ce/Cr}$ and $SF_{La/Cr}$ than muskoxen and moose, and the $SF_{La/Ce}$ did not differ between the

322   species (Table 3 and 4). There were also no differences in the MRT DIST between the species

323   (Table 3).

324

325   **Discussion**

326   The physical characteristics of the RR contents in the wild ruminants of this study

327   correspond to those reported earlier for these species or other species of similar feeding type

328   (Clauss et al. 2009b; Clauss et al. 2009c; Hummel et al. 2009). They show that although

329   ruminants might differ in the degree their RR contents are stratified, a separation according to

330   buoyancy characteristics can occur in different forages; that regardless of the degree of

331   stratification of RR contents, the RR sorting mechanism is of similar efficiency in different

332   ruminant species, allowing only very fine particles to escape from the RR; that

333   correspondingly the discrimination in the passage of small versus large particles is similar in

334   different ruminant species; and that large particle passage is rather threshold-dependent than a

335   continuous function of particle size. Following Clauss et al. (2001), this leaves us in the

336   dilemma that we observe the rumen sorting mechanism, but without the contents stratification

337   we are used to associate with this mechanism. With respect to the different physiologic

338   strategies of browsers and grazers, the results confirm that it is the ratio of small particle vs.

339   fluid passage that is the major difference between different species and potentially different

340   feeding types.

341   *Potential limitations of this study and methodological aspects*

342   The major limitation of this study is the variation in animal numbers and samples taken

343   between species and treatments. Unfortunately, logistic reasons did not allow consistent

344   sampling of rumen contents via the cannula of all animals on all diets, the use of four rather

345   than two fistulated moose, and the provision of another grass source that would have been

346   accepted by the reindeer. Additionally, the level of food intake was not kept constant in this

347   study; animals were allowed to ingest as much food as they wanted, in order to better mimic

348   natural feeding. In muskoxen, this led to relevant differences in the food intake between the

349   grass hay and the browse diet (Table 2).

350   While measurements on rumen fluid (such as viscosity) are probably not influenced by

351   the sampling method used to collect the fluid - manual or probe sampling in fistulated animals

352   (as performed in this study or in Hummel et al. 2009) versus dissections of the forestomachs

353   of shot animals (Clauss et al. 2009b; Clauss et al. 2009c)-, the probe (but not the manual)

354   sampling via cannula seriously influences results on the whole RR contents. Although, as

355   compared to the rumen sampler of Tafaj et al. (2001), the opening of the rumen probe had

356   been enlarged to a window of approximately 3 x 10 cm (considered the maximum possible

357   without compromising the stability of the sampler), it was evident that during sampling, the

358   very large particles present in the rumen did not enter the probe quantitatively. Therefore,

359   samples taken were most likely biased towards fluids and small particles, making a

360   comparison between the animals of this study feasible, but not with results gathered by other

361   methods.

362   With respect to the mordant markers used in this study, the results of the marker binding

363   assays (see methods) showed that although loss occurred for the Ce- and La-mordants,

364   recoveries after artificial digestion were still reasonably high. As some of the marker will

365   have dissociated from the marked fibres and bound to other (including potentially smaller)

366   particles (Hartnell and Satter 1979; Combs et al. 1990), the retention times of large particles

367   might be somewhat underestimated in this study; however, because marker recovery was very

368   similar for Ce and La, the absence of a difference in the retention of these two markers most

369   likely is a true effect. Although the methods of marker application to feeds vary between

370   studies (Hartnell and Satter 1979; Udén et al. 1980; Crooker et al. 1982; Combs et al. 1990;

371   Bernard and Doreau 2000; Schwarm et al. 2008; 2009a), our findings encourage the further

372 use of rare-earth mordants as passage markers. In particular, the method of preparing Ce-

373 mordants of Schwarm et al. (2008) also used in this study appears to be preferable to the ones

374 described by Udén et al. (1980), Crooker et al. (1982) or Combs et al. (1990). In contrast to

375 the two latter studies, the method of Schwarm et al. (2008) follows the recommendations of

376 Bernard and Doreau (2000) to use neutral detergent residue of forages rather than untreated

377 forages as the material for mordanting. For researchers interested in the method, additional

378 adjustments explained by Mambrini and Peyraud (1997) should be considered.

379   Finally, it should be noted that differences in feeding selectivity, chewing and

380 rumination intensity, forestomach volume and forestomach motility patterns could all have

381 influenced the results reported here. Ideally, such measures should be taken in parallel in

382 future studies.

383   *RR physiology*

384   Differences between fluid and small particle excretion appear to vary between

385 ruminants. The $SF_{Cr/Co}RR$ was in this study highest in cattle (2.03) and muskoxen (1.97-1.98),

386 intermediate in reindeer (1.70) and lowest in moose (0.98-1.29), supporting previous results

387 that this SF is lowest in browsing and highest in grazing ruminants (Clauss and Lechner-Doll

388 2001; Hummel et al. 2005; Clauss et al. 2006b). When comparing published measurements of

389 $MRT_{Cr}RR$ and $MRT_{Co}RR$ (Fig. 6a), it is evident that in species usually associated with a

390 higher proportion of grass in their natural diet, small particles are retained distinctively longer

391 than fluids and/or fluids pass out of the RR distinctively faster than particles.

392   Results of physical analyses of rumen contents in this and previous studies (Clauss et al.

393 2009b; 2009c; Hummel et al. 2009) support the concept that large, grazing species, such as

394 cattle, muskoxen, bison, or addax have a distinct difference in the moisture content between

395 the dorsal and the ventral rumen, and generally a high ruminal moisture content. Browsers,

396 such as moose or roe deer, have more homogenous ruminal digesta in terms of moisture

397 concentration, and their ruminal contents are generally drier than those of grazers (Fig. 1b).

398 Note that the moose of this study were more heterogenous in ruminal moisture concentration

399 than shot and dissected individuals (Clauss et al. 2009c) but still had drier contents than the

400 other species in this study. The higher moisture content in the larger, more grazing ruminants,

401 seems to be associated with a lower rumen fluid viscosity (Fig. 1a), with a more distinct

402 stratification of rumen contents (Tschuor and Clauss 2008; Clauss et al. 2009d), and with

403 larger omasa for fluid re-absorption beyond the RR (Clauss et al. 2006a).

404   The resulting question is what consequences these differences in fluid physiology have

405 for the animals. Due to the common conception that the stratification of rumen contents is

406   important for the selective particle retention and sorting mechanism in the rumen (Poppi et al.

407   2001; Faichney 2006), previous hypotheses linked differences in stratification of rumen

408   contents with differences in the efficacy of particle sorting and the time available for particle

409   digestion. For example, Iason and Van Wieren (1999) and Pérez-Barbería et al. (2004) found

410   a higher fibre digestibility in grazing as compared to browsing ruminants, which corresponds

411   to indications that grazing ruminants have longer particle retention times (Hummel et al.

412   2006; Clauss et al. 2007a) also partially indicated in this study (Table 3 and 4). Another

413   example was the observation by Clauss et al. (2002) that in a dataset of wild ruminants kept in

414   zoos, large browsers had larger particles in their faeces than grazers, which was also linked to

415   the absence of rumen contents stratification and the resulting particle sorting effect in these

416   animals. However, in a subsequent study with aurox (*Bos primigenus taurus*) and giraffe

417   (*Giraffa camelopardalis*), Hummel et al. (2008a) showed that this difference only occurred in

418   zoo animals but not in free-ranging ones, hence indicating that the teeth of browsers are

419   probably less suited for diets offered in captivity than those of grazers. If results of faecal

420   particle sizes of browse-fed animals of this study are compared to those measured in zoo-fed

421   individuals of the same species (Fig. 7), the pattern found by Hummel et al. (2008a) is

422   supported. Differences in faecal particle size in captive browser and grazers might be due to

423   general differences in dental design between the feeding types (Archer and Sanson 2002), and

424   to differences in patterns of tooth wear between captive as compared to free-ranging browsers

425   (Kaiser et al. 2009). Teeth of browsers might be morphologically less suited to the

426   mastication of food traditionally offered in captivity, and their teeth show abrasion-induced

427   wear in captivity that might further compromise their ability to efficiently masticate food.

428   Together with the finding that faecal particle size did not differ significantly between the

429   species of this study, and the dramatic decrease in particle size between the RR and the distal

430   digestive tract in ruminants of any feeding type (Clauss et al. 2009b; 2009c), these results

431   suggest that the RR particle sorting mechanism is equally efficient in different ruminant

432   species, and hence not dependent on the presence of a rumen contents stratification that is

433   reflected in papillation patterns or differential particle-fluid-outflow.

434   This interpretation is corroborated by our findings on the differential particle retention

435   in the RR. The difference between small and large particle excretion from the RR did not

436   seem to be associated with feeding type. The $SF_{Ce/Cr}RR$ was in the range of 1.06-1.47 (Table

437   4), and both extremes were measured in reindeer, with values of the other species in between.

438   The $SF_{La/Cr}RR$ was in the range of 1.03-1.52, with extremes measured in reindeer and moose,

439   and again with values of the other species in between. Comparative data are scarce. Lechner-

440   Doll et al. (1990) measured the MRT RR of 2 mm and 20 mm particles in domestic cattle,

441   sheep and goats. If our own data for 2 and 10 mm particles, or that for 2 and 20 mm particles

442   combined with that study, are plotted in a way similar to Fig. 6a, it seems that there is no

443   systematic difference between the ruminant species with respect to their differential retention

444   of small and large particles (Fig. 6bc). Therefore, ruminants of all feeding types probably

445   share the characteristic of an efficient sorting mechanism in their RR, irrespective of either

446   feeding type or the presence of stratification of their RR contents. This sorting mechanism

447   sets ruminants apart from non-ruminating foregut fermenters such as hippopotamus,

448   peccaries, macropods or colobine monkeys (Schwarm et al. 2008; 2009c). One possible

449   difference between the feeding types could be that the actual particle sorting takes place

450   mainly in the reticulum in strict browsers, but is already prepared to a higher degree within

451   the rumen in grazers (Clauss et al. 2009b; 2009c). This interpretation would conceptually

452   allow for a larger 'filter bed effect' in which small particles are entangled in the ruminal fibre

453   mat (Faichney 2006) in grazers with an according longer delay of the small particles. Because

454   grass particles, in general, can be fermented profitably for a longer time than browse particles

455   (Hummel et al. 2006), such a mechanism could be adaptive and could explain why the small

456   particles – which can leave the reticulum once they reach it – are retained longer in the

457   grazing species, where they are partly entangled in the mat, than in the browsing species,

458   where they move more or less with the fluid (Fig. 6a).

459   Many studies have demonstrated that the functional density or gravity of particles, i.e.

460   their tendency to float or sediment, is the major mechanism that determines their fate in the

461   RR (Sutherland 1988; Beaumont and Deswysen 1991; Lechner-Doll et al. 1991; Dardillat and

462   Baumont 1992; Kaske et al. 1992). Potential differences in the kinetics of density changes

463   between different forages notwithstanding, this study (Fig. 1d) and the preceding ones (Clauss

464   et al. 2009b; 2009c; Hummel et al. 2009) have shown consistently that fragments of different

465   forages can be sorted by flotation/sedimentation according to their size. According to

466   numerous studies, the threshold size of particles for passage out of the rumen is between 1

467   and 4 mm (Poppi et al. 1980; Ulyatt et al. 1986; Shaver et al. 1988; Grenet 1989); however,

468   an additional question is whether particles *above this threshold* are retained in proportion to

469   their respective size, or if they are retained indiscriminately. The results of this study, as well

470   as those of Kaske et al. (1992) and Schwarm et al. (2009a), indicate that above a large particle

471   size threshold (~10 mm), particle size has no further influence on the selective retention or

472   rumination of large particles. However, differences in the retention kinetics of particle size

473   classes below this threshold, e.g. between 4-mm, 2-mm and 0.5-mm particles, have been

474   demonstrated (Spalinger and Robbins 1992).

475   The differences in the shape of the excretion curves of fluids, small and large particles

476   in cattle and muskoxen (Fig. 2-3) indicate that large particles undergo a different treatment in

477   the rumen than fluids and small particles (cf. e.g. Bernard et al. 2000). The immediate, steep

478   increase and gradual, smooth decrease in concentration typical for the fluid and small particle

479   markers is typical for a single continuous-flow stirred-tank reactor (Martínez del Rio et al.

480   1994; Jumars 2000). With respect to the RR, this means that both the fluid and the small

481   particle marker represent a pool which is immediately ready for outflow from the RR, and

482   outflow is only a function of fluid or small particle inflow and, in the case of particles, of

483   mechanical delay due to the 'filter bed effect' of the ruminal fibre mat (Faichney 2006); this

484   corresponds to the 'age-independent' pools described in domestic ruminants (reviewed in

485   Ellis et al. 1999). In contrast, the excretion curves of the two large particle size classes with

486   their more gradual increase and a similar decrease (cf. similar curves for the excretion of

487   marker bound to whole hay in cattle from Mambrini and Peyraud 1997) are typical for a series

488   of continuous-flow stirred-tank reactors (Martínez del Rio et al. 1994; Jumars 2000). In other

489   words, the large particles do not pass through a single pool before leaving the RR but through

490   a series of such pools (representing their different digestion and rumination stages; 'age-

491   dependent pools'); such multiple particle pools have been included in models that represent

492   particle passage through the rumen (Ellis et al. 1999; cf. Spalinger and Robbins 1992). In

493   contrast to markers applied via rumen cannula, large particle markers ingested by another

494   cattle species, the banteng (Schwarm et al. 2008; 2009a) showed an intermediate shape

495   between those for the small and the large particles in this study, with a somewhat steeper

496   increase than decrease. This is most likely the result of ingestive mastication that reduces a

497   part of the labelled large particles in size and hence shifts it to the small particle pool directly

498   ready for rumen outflow. In reindeer and moose, the excretion curve for the larger particles

499   also has such an intermediate shape (Fig. 4-5). In comparison to cattle and muskoxen, this is

500   most likely the consequence of proportionately smaller rumen volumes in these less grass-

501   adapted species (Clauss et al. 2003); less volume available for distribution of large particles

502   probably results in a lower number of (conceptual) 'pools' through which these markers pass

503   before outflow. Note that at low intakes in moose, these curves become more similar to the

504   ones in cattle or muskox (Fig. 5c), indicating that, at lower throughput, more 'serial particle

505   pools' may exist even in moose.

506   The influence of the food intake level on retention parameters, documented in

507 ruminants and nonruminants alike (Clauss et al. 2007a; 2007b; Schwarm et al. 2009c), is also

508 evident in this study (Fig. 8ab). In simple terms, a higher food intake will push ingesta

509 through the gut faster (the effect will be modified by the capacity of the gut to distend).

510 Differences in retention parameters between species must be considered with the respective

511 food intake levels in mind. In this respect, the difference in ingesta retention in moose

512 between the browse and the grass silage diet at comparable levels of intake is particularly

513 striking. Although dry matter intake was nearly identical (Table 2), both fluids and particles

514 were retained longer on the grass diet in moose, and the difference was even significant for

515 the largest particles in spite of the low sample size (Table 3 and 4). While this difference is

516 most likely ultimately adaptive in terms of the time required for fermentation of the respective

517 forages (Hummel et al. 2006), the question arises what proximate mechanism causes this

518 difference. In the lack of experimental data, we can only suggest that differences in the

519 physical characteristics of browse and grass, including the tendency to form a fibre mat, are

520 the underlying cause of this difference (Clauss et al. 2003). It should be noted, however, that

521 the moose had a concomitant increase in fluid retention, and that their SF of small

522 particles/fluids was still significantly lower than that achieved by muskoxen or cattle on grass

523 diets (Table 3 and 4). Actually, the ratio of particle to fluid retention ($SF_{particle/fluid}$) remained

524 comparatively constant across the food intake range in the animals of this study (Fig. 8c).

525 Schwarm et al. (2009c) had noted, in a collection of literature data, that ruminants differ from

526 non-ruminant foregut fermenters in that the $SF_{particle/fluid}$ remains constant with food intake in

527 ruminants. Our results support this observation.

528   *Conclusions*

529   The fact that the $SF_{particle/fluid}$ is relatively constant and appears to be comparatively

530 uninfluenced by the type of forage within a ruminant species might be a reason why ruminant

531 species are limited to certain dietary niches. In this respect, observations on the botanical

532 composition of ruminant diets appear to indicate a bias in niche flexibility when compared to

533 morphophysiological adaptations (Van Wieren 1996; Clauss et al. 2003). While a cattle-type

534 adaptation with a high $SF_{particle/fluid}$ might also allow the use of browse material as long as

535 secondary compounds are not problematic (consider not only the muskoxen, but also e.g. the

536 diets of the wood bison *Bison bison athabascae*, the European bison *Bison bonasus*, the red

537 forest buffalo *Synceros caffer nanus*, or the anoa *Bubalus depressicornis*), a moose-type

538 adaptation with a higher rumen fluid viscosity and a lower $SF_{particle/fluid}$ might prevent a

539 competitive use of grass-based diets. The moose-type adaptation might have been driven by

540 the need to produce salivary defences against secondary plant compounds, with

541 correspondingly large salivary glands that potentially produce a less voluminous and more

542 viscous saliva (Hofmann et al. 2008). Although potentially limited to the reticulum, the

543 particle sorting mechanism is highly efficient also in this ruminant type. The cattle-type

544 adaptation might have been driven by advantages provided by higher fluid throughput

545 through the RR, namely an increased yield of microbial protein (as suggested by Hummel et

546 al. 2008b), and, in addition to the sorting mechanism in the reticulum, a more pronounced

547 stratification of contents with the associated 'filter-bed effect' for a pronounced small particle

548 delay in the rumen.

549

561

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

Species			Feeding type	Location	Diet	n	Time	Measurements
Cattle	*Bos prim. f. taurus*	(domestic)	grazer	Bonn, Germany	Grass silage	4	Oct 2007	MRT
Muskoxen	*Ovibos moschatus*	(wild)	grazer	Fairbanks, Alaska	Browse	4	June/July 2008	MRT, phys
					Grass hay	4		MRT, phys
Reindeer	*Rangifer tarandus*	(wild)	mixed feeder	Fairbanks, Alaska	Browse	4	June/July 2008	MRT, phys
					Grass hay	4		MRT, phys
Moose	*Alces alces*	(wild)	browser	Palmer, Alaska	Browse	2	June 2008	MRT, phys
					Grass silage	2	Oct 2008	MRT
					Browse	2	Feb 2009	MRT
					Browse	2	Mrch 2009	MRT

MRT=mean retention time measured by insertion of markers into rumen cannula

phys=physical characteristics of rumen contents measured by sampling contents via cannula

Table 2. Diets used and absolute and relative dry matter intake (DMI) during the feeding trials in domestic cattle, muskoxen, reindeer and moose. DM = dry matter (in % fresh weight), CP = crude protein, NDF = neutral detergent fibre, ADF = acid detergent fibre, ADL = acid detergent lignin (all in % DM with residual ash)

Species	Diet	n	DMI	rDMI	Diet				
			(kg/d)	(g/kg$^{0.75}$d)	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
Reindeer	Browse leaves	4	2.1 ± 0.6	70 ± 19	19.6	13.6	29.1	23.3	16.1
	Pellets	4	3.3 ± 1.5	124 ± 52	91.3	14.7	33.7	22.7	8.2
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
	Browse (winter)	4	1.6 ± 0.4	20 ± 5	40.0	9.8	31.7	22.4	9.3

753 Table 3. Mean retention times (MRT) in the gastrointestinal tract (GIT) or the distal GIT (DIST) in cattle (*Bos primigenius* f. *taurus*; C), muskoxen
754 (*Ovibos moschatus*; Mx), reindeer (*Rangifer tarandus*; R), and moose (*Alces alces*; Mo) for markers representing fluids (Co), 2-mm particles (Cr),
755 10-mm particles (Ce), and 20-mm particles (La), and the respective selectivity factors (SF; representing ratios of the MRTs of different markers) on
756 diets based on grass (GR), browse (BR) or pellets (Pell); MRT DIST is assumed to be equal for all markers (see text)

Species	Diet	MRT GIT (h)				SF GIT						MRT DIST
		Fluid (Co)	2mm (Cr)	10mm (Ce)	20mm (La)	2mm/fluid (Cr/Co)	10mm/fluid (Ce/Co)	20mm/fluid (La/Co)	10/2mm (Ce/Cr)	20/2mm (La/Cr)	20/10mm (La/Ce)	
Cattle	Grass silage	41 ± 3	73 ± 1	89 ± 2	90 ± 2	1.79 ± 0.10*	2.17 ± 0.12*	2.19 ± 0.12	1.22 ± 0.01*	1.23 ± 0.02*	1.01 ± 0.01	10 ± 1
Muskoxen	Browse leaves	34 ± 3	57 ± 6	77 ± 11	74 ± 8	1.68 ± 0.27*	2.27 ± 0.31*	2.18 ± 0.23*	1.35 ± 0.09*	1.30 ± 0.08*	0.96 ± 0.04	10 ± 2
	Grass hay	49 ± 10	88 ± 28	101 ± 33	93 ± 27	1.77 ± 0.23	2.03 ± 0.25	1.88 ± 0.17	1.15 ± 0.07	1.06 ± 0.06	0.93 ± 0.04	10 ± 2
Reindeer	Browse leaves	38 ± 3	58 ± 8	60 ± 11	59 ± 9	1.52 ± 0.10*	1.58 ± 0.13*	1.56 ± 0.12*	1.05 ± 0.12	1.03 ± 0.11	0.98 ± 0.02	10 ± 2
	Pellets	26 ± 3	38 ± 8	51 ± 11	51 ± 9	1.43 ± 0.26	1.91 ± 0.31	1.93 ± 0.23*	1.35 ± 0.12	1.37 ± 0.19	1.01 ± 0.08	10 ± 2
Moose	Browse leaves	33 ± 5	39 ± 5	52 ± 3	53 ± 2	1.19 ± 0.04	1.59 ± 0.15	1.62 ± 0.21	1.34 ± 0.08	1.37 ± 0.13	1.02 ± 0.03	12 ± 4
	Grass silage	58 ± 0	64 ± 2	76 ± 1	76 ± 0	1.11 ± 0.04	1.32 ± 0.03	1.32 ± 0.01	1.19 ± 0.01	1.19 ± 0.03	1.00 ± 0.01	26 ± 1
	Browse (winter)	74 ± 5	73 ± 3	90 ± 3	89 ± 3	0.99 ± 0.02	1.21 ± 0.03*	1.20 ± 0.03*	1.22 ± 0.02*	1.22 ± 0.02*	1.00 ± 0.00	23 ± 5
C/Mx/Mo[1]	Grass	(Mo > C)	ns	ns	ns	Mo < Mx/C	Mo < Mx/C	Mo < Mx < C	ns	Mx < (Mo)/C	Mx < Mo/C	Mo > C/Mx
Mx/R/Mo[1]	Browse leaves	ns	ns	Mo/(R) < Mx	Mo/R < Mx	(Mo < Mx)	Mo/R < Mx	Mo/R < Mx	R < Mo/Mx	R < Mo/Mx	ns	ns
Muskoxen[2]	BR vs. GR	BR < GR	ns	ns	ns	ns	ns	ns	BR > GR	BR > GR	ns	ns
Reindeer[2]	BR vs. Pell	BR > Pell	ns	ns	ns	ns	(BR < Pell)	BR < Pell	BR < Pell	(BR < Pell)	ns	ns
Moose[2]	BR vs. GR	ns	ns	ns	BR < GR	BR > GR	ns	ns	ns	ns	ns	ns

757 *indicates that the difference in MRT between the respective markers was significant within the species on this diet (repeated measurements-ANOVA and subsequent paired t-
758 tests with Dunn-Sidak adjustment for mulitple testing)
759 [1]tested by one-way ANOVA and subsequent Sidak post hoc tests (brackets indicate differences that only tend towards significance [p between 0.05 and 0.08])
760 [2]tested by paired t-tests (brackets indicate differences that only tend towards significance [p between 0.05 and 0.08]); in moose only summer/autumn diets were compared
761 ns=not significant
762

762 Table 4. Mean retention times (MRT) in the reticulorumen (RR) in cattle (*Bos primigenius* f. *taurus*; C), muskoxen (*Ovibos moschatus*; Mx),

763 reindeer (*Rangifer tarandus*; R), and moose (*Alces alces*; Mo) for markers representing fluids (Co), 2-mm particles (Cr), 10-mm particles (Ce), and

764 20-mm particles (La), and the respective selectivity factors (SF; representing ratios of the MRTs of different markers) on diets based on grass (GR),

765 browse (BR) or pellets (Pell)

Species	Diet	MRT RR (h)				SF RR					
		Fluid (Co)	2mm (Cr)	10mm (Ce)	20mm (La)	2mm/fluid (Cr/Co)	10mm/fluid (Ce/Co)	20mm/fluid (La/Co)	10/2mm (Ce/Cr)	20/2mm (La/Cr)	20/10mm (La/Ce)
Cattle	Grass silage	32 ± 2	64 ± 1	80 ± 2	80 ± 2	2.03 ± 0.12*	2.53 ± 0.14*	2.56 ± 0.14*	1.25 ± 0.02*	1.26 ± 0.03*	1.01 ± 0.01
Muskoxen	Browse leaves	24 ± 4	47 ± 6	68 ± 10	65 ± 7	1.98 ± 0.44*	2.81 ± 0.53*	2.68 ± 0.41*	1.43 ± 0.12*	1.37 ± 0.11*	0.96 ± 0.04
	Grass hay	39 ± 11	78 ± 29	91 ± 33	83 ± 28	1.97 ± 0.22	2.30 ± 0.23	2.11 ± 0.16	1.17 ± 0.08	1.08 ± 0.08	0.92 ± 0.04
Reindeer	Browse leaves	28 ± 2	48 ± 7	50 ± 10	49 ± 8	1.70 ± 0.16*	1.77 ± 0.10*	1.74 ± 0.11*	1.06 ± 0.15	1.03 ± 0.13	0.98 ± 0.03
	Pellets	17 ± 2	28 ± 7	41 ± 10	41 ± 8	1.70 ± 0.48	2.47 ± 0.59	2.48 ± 0.43*	1.47 ± 0.16	1.50 ± 0.25	1.02 ± 0.09
Moose	Browse leaves	21 ± 1	27 ± 1	40 ± 1	41 ± 2	1.29 ± 0.04	1.91 ± 0.14	1.96 ± 0.22	1.48 ± 0.07	1.52 ± 0.13	1.03 ± 0.04
	Grass silage	32 ± 1	38 ± 3	50 ± 3	51 ± 2	1.21 ± 0.06	1.59 ± 0.03	1.59 ± 0.00	1.31 ± 0.04	1.32 ± 0.06	1.00 ± 0.02
	Browse (winter)	52 ± 4	51 ± 3	67 ± 4	67 ± 4	0.98 ± 0.03	1.30 ± 0.04*	1.29 ± 0.03*	1.32 ± 0.01*	1.32 ± 0.01*	0.99 ± 0.00
C/Mx/Mo[1]	Grass	ns	ns	ns	ns	Mo < Mx/C	Mo < Mx/C	Mo < Mx < C	ns	Mx < Mo/C	Mx < Mo/C
Mx/R/Mo[1]	Browse leaves	ns	Mo < R/Mx	Mo/R < Mx	Mo/R < Mx	ns	R/(Mo) < Mx	R/(Mo) < Mx	R < Mo/Mx	R < Mo/Mx	ns
Muskoxen[2]	BR vs. GR	BR < GR	ns	ns	ns	ns	ns	(BR > GR)	BR > GR	BR > GR	ns
Reindeer[2]	BR vs. Pell	BR > Pell	BR > Pell	ns	ns	ns	(BR < Pell)	BR < Pell	BR < Pell	(BR < Pell)	ns
Moose[2]	BR vs. GR	ns	ns	ns	BR < GR	ns	ns	ns	ns	ns	ns

766 *indicates that the difference in MRT of between the respective markers was significant (repeated measurements-ANOVA and subsequent paired t-tests with Dunn-Sidak

767 adjustment for mulitple testing)

768 [1]tested by one-way ANOVA and subsequent Sidak post hoc tests (brackets indicate differences that only tend towards significance [p between 0.05 and 0.08])

769 [2]tested by paired t-tests (brackets indicate differences that only tend towards significance [p between 0.05 and 0.08]); in moose only summer/autumn diets were compared

770 ns=not significant

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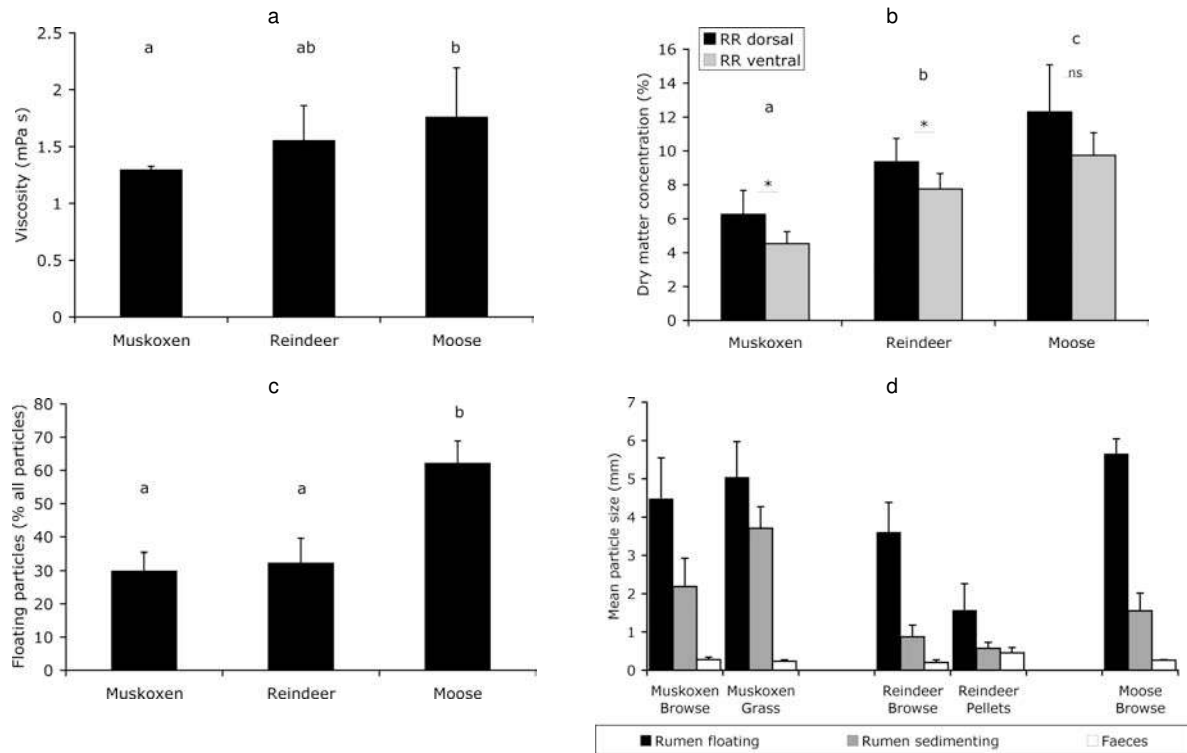
773
774 Figure. 1. Mean ± SD of muskoxen (*Ovibos moschatus*, n=4), reindeer (*Rangifer tarandus*,
775 n=4) and moose (*Alces alces*, n=2) of a) Viscosity of centrifuged rumen fluid on browse diets,
776 with two measurements per individual. Differences between moose and muskoxen are
777 significant. b) Dry matter concentration in the dorsal and ventral rumen contents on browse
778 diets, with two measurements per individual. Differences between rumen regions are
779 significant in muskoxen and reindeer only; differences between species are significant for
780 both dorsal and ventral contents. c) Proportion of floating particles in the rumen (as a
781 proportion of all particles) on browse diets. Differences between moose and
782 muskoxen/reindeer are significant. d) Mean particle size in the floating and sedimenting
783 rumen contents and the faeces on different experimental diets.

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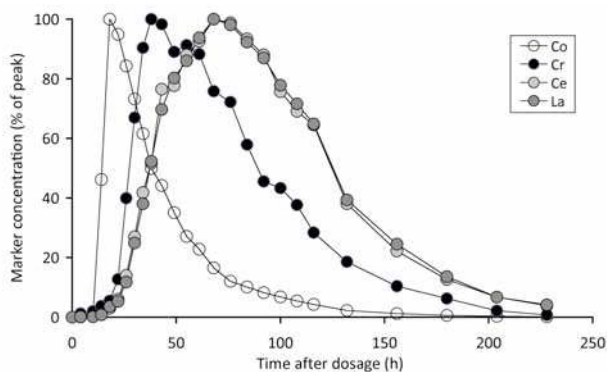
Figure 2. Excretion pattern of markers (fluid: Co-EDTA, peak 351 mg/kg DM; 2-mm particles: Cr, peak 529 mg/kg DM; 10-mm particles: Ce, peak 242 mg/kg DM; 20-mm particles: La, peak 208 mg/kg DM) in an individual domestic ox fed grass silage ad libitum. Note the distinct differences in excretion between fluids, small and large particles, but the absence of difference between the two large particle classes, and the difference in the shape of the ascending part of the excretion curve.
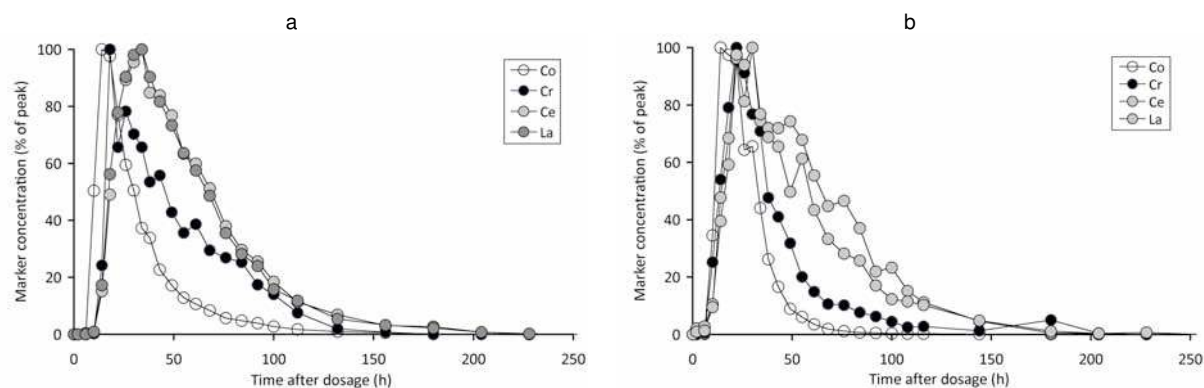
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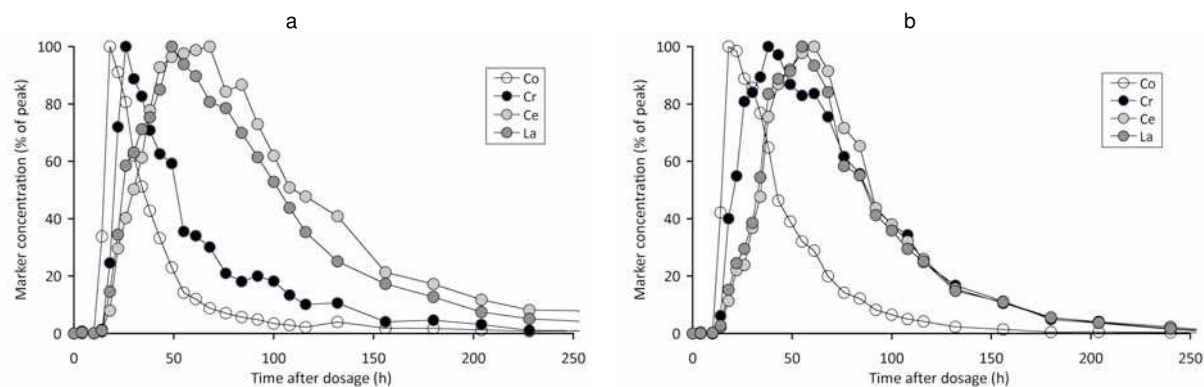
794 Figure 3. Excretion pattern of markers (fluid: Co-EDTA; 2-mm particles: Cr; 10-mm
795 particles: Ce; 20-mm particles: La) in an individual muskoxen (*Ovibos moschatus*) fed a)
796 willow browse (peaks in mg/kg DM: Co 134, Cr 329, Ce 83, La 81) and b) brome grass hay
797 (peaks in mg/kg DM: Co 125, Cr 282, Ce 176, La 151). Note the distinct differences in
798 excretion between fluids, small and large particles, the general absence of difference between
799 the two large particle classes (with a slightly earlier decrease in the excretion curve of the
800 largest particles), and the difference in the shape of the ascending part of the excretion curve.
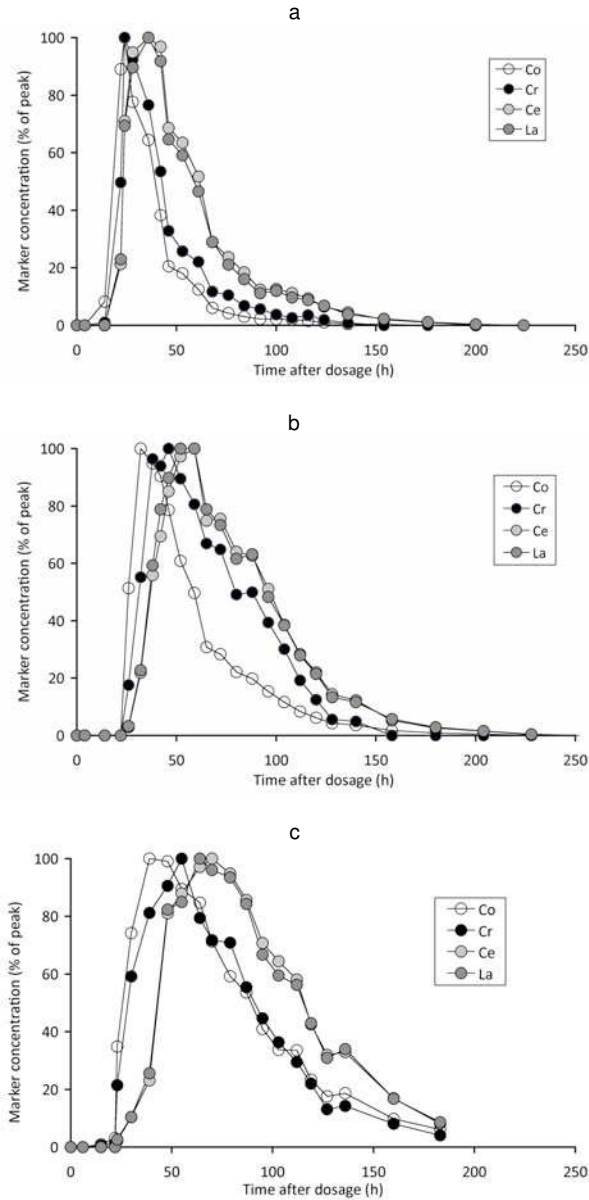801
802

803 Figure 4. Excretion pattern of markers (fluid: Co-EDTA; 2-mm particles: Cr; 10-mm
804 particles: Ce; 20-mm particles: La) in an individual reindeer (*Rangifer tarandus*) fed a)
805 willow browse (peaks in mg/kg DM: Co 130, Cr 263, Ce 112, La 104) and b) a pelleted
806 compound food (peaks in mg/kg DM: Co 160, Cr 315, Ce 80, La 82). Note the similar
807 excretion of fluids and small particles, the absence of difference between the two large
808 particle classes, and the difference in the shape of the ascending part of the excretion curve.
809

809

810
811 Figure 5. Excretion pattern of markers (fluid: Co-EDTA; 2-mm particles: Cr; 10-mm

812 particles: Ce; 20-mm particles: La) in an individual moose (*Alces alces*) fed a) willow browse

813 in summer (peaks in mg/kg DM: Co 264, Cr 743, Ce 255, La 232), b) brome grass silage in

814 autumn (peaks in mg/kg DM: Co 135, Cr 248, Ce 147, La 128) and c) various browse in

815 limited amounts in winter (peaks in mg/kg DM: Co 05, Cr 350, Ce 174, La 153). Note the

816 longer retention on grass silage as compared to willow browse in spite of similar food intake,

817 the similar excretion of fluids and small particles, the absence of difference between the two

818 large particle classes, and the difference in the shape of the ascending part of the excretion

819 curve.

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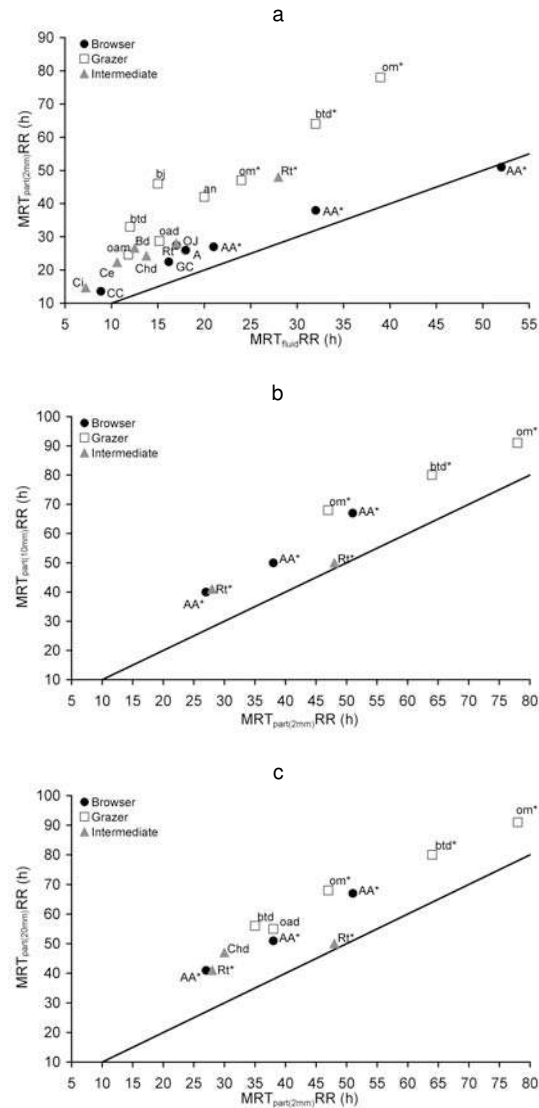
821   Figure 6. Relationship of mean retention times (MRT, in h) in the reticulorumen (RR) of

822   different ingesta fractions in wild ruminant species of different feeding type. a) fluids vs. 2-

823   mm particles (data collection from Flores-Miyamoto et al. 2005; Clauss et al. 2006b with

824   additional data from ; Hummel et al. 2008b; Schwarm et al. 2008; and this study) - note the

825   generally increased difference between small particles and fluid excretion in grazing

826   ruminants; b) 2-mm vs. 10-mm particles (data from this study) – note the uniformity of the

827   relationship across species; c) 2-mm vs. 20-mm particles (data from Lechner-Doll et al. 1990;

828   and this study) – note again the uniformity of the relationship across species. Data from this

829   study marked by *; black line denotes x=y. Species key: AA *Alces alces*, an *Addax*

830   *nasomaculatus*, Bd *Bubalus depressicornis*, bj *Bos javanicus*, btd *Bos taurus f. domesticus*,

831   CC *Capreolus capreolus*, Ce *Cervus elaphus*, Chd *Capra hircus f. domesticus*, Ci *Capra ibex*,

832   GC *Giraffa camelopardalis*, oad *Ovis ammon f. domesticus*, oam *Ovis ammon musimon*, OJ

833   *Okapia johnstoni*, om *Ovisbos moschatus*, Rt *Rangifer tarandus*.
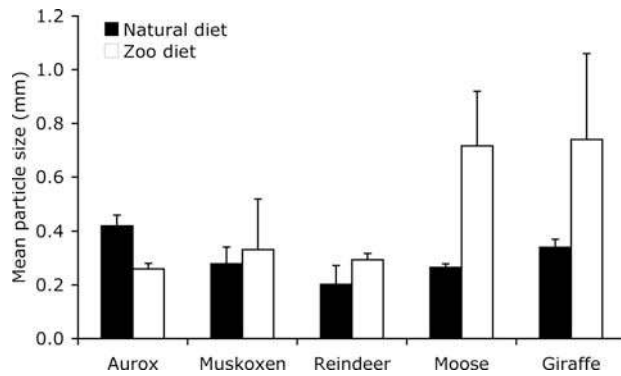
834

835 Figure 7. Comparison of mean particle size of faeces of free-ranging or naturally fed and zoo-
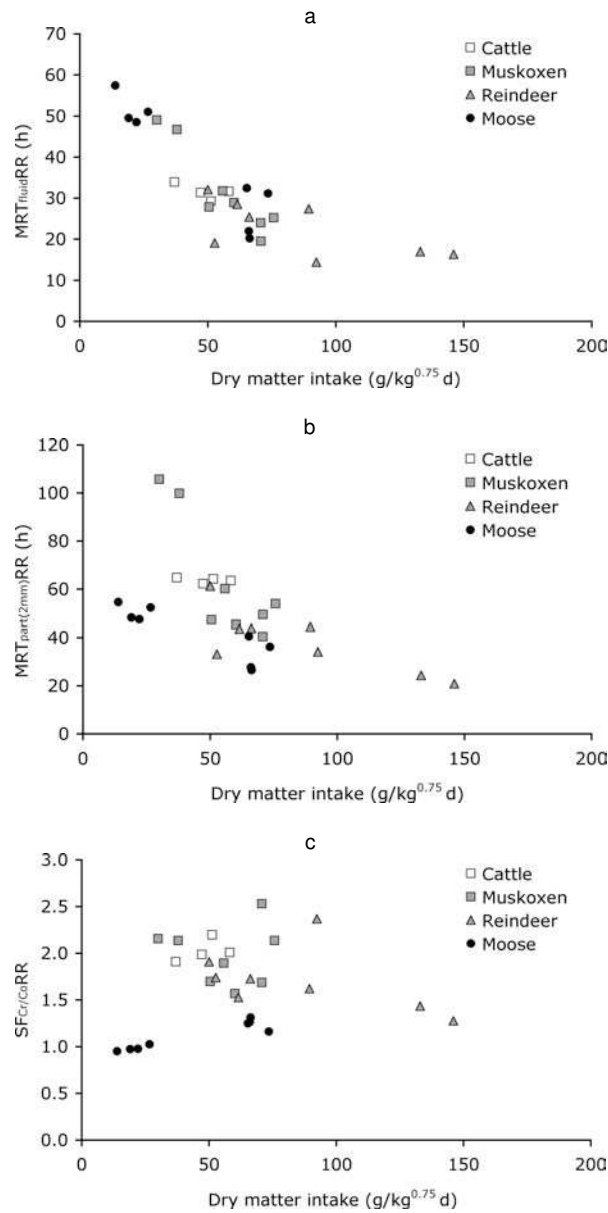
836 fed ruminants. Data from Hummel et al. (2008a; aurox Bos taurus and giraffe Giraffa

837 camelopardalis), browse diets fed in this study and zoo data from Fritz et al. (2009; muskoxen

838 Ovibos moschatus, reindeer Rangifer tarandus, moose Alces alces). Note the large difference

839 in the two strictly browsing species.

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843 Figure 8. Association of dry matter intake (DMI, in $g/kg^{0.75}$ d) and the mean retention time
844 (MRT) of a) fluid, b) 2-mm particles, and c) the 'selectivity factor' (SF) of the two in the
845 reticulorumen (RR) of cattle (*Bos taurus*), muskoxen (*Ovibos moschatus*), reindeer (*Rangifer*
846 *tarandus*), and moose (*Alces alces*). Note that both fluid and particle retention time decrease
847 with increasing food intake, but that their quotient remains relatively constant.