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Article

Accepted Version

Journal of Animal Physiology and Animal Nutrition

Comparative selective retention of particle size classes in the gastrointestinal tract of ponies and goats

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Accepted 09.05.2017

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SUMMARY

There is a discrepancy in the literature on potential digesta separation mechanisms in horses, with both a selective retention of fine and of large particles postulated in different publications. To assess the net effect of such mechanisms, we fed ponies on a hay-only diet a pulse dose of whole (unchopped) marked hay together with a solute marker, collected faeces on a regular basis, measured marker concentrations in whole faeces and in their large (2.0-16 mm), medium (0.5-1.0 mm) and small (0.063-0.25 mm) particle fraction, and calculated the corresponding mean retention times (MRT). For comparison, the same experiment was performed in goats. In goats, as expected, MRT_{solute} (35 h) was significantly shorter than $MRT_{particle}$ (51 h); only a very small fraction of particle marker was excreted as large particles (2%); and the MRT of these large particles was significantly shorter than that of small particles (with a relevant difference of 8.6 h), indicating that those few large particles that escape the rumen do so mostly soon after ingestion. In ponies, MRT_{solute} (24 h) did not differ from $MRT_{particle}$ (24 h); a higher fraction of particle marker was excreted as large particles (5%); and the MRT of these large particles was longer than that of small particles (but with a non-relevant difference of less than 1 h). These results indicate that no relevant net separation of digesta phases occurs in horses, and that selective particle retention mechanisms in the large intestine are unlikely to represent important characteristics of the horse's digestive physiology.

Keywords equid, ruminant, digesta retention, particle size, colon

Introduction

Mechanisms for the selective retention or expulsion of certain particle size fractions are an important feature of the digestive physiology of many herbivores. Sieve analyses of gut contents typically reveal selective retention mechanisms for large particles in the forestomach of functional ruminants (i.e., taxonomic ruminants and camelids) (Lechner-Doll and von Engelhardt, 1989; Clauss et al., 2017), and selective retention mechanisms for small particles in the caecum of small hindgut fermenters (Björnhag, 1972; Lanyon and Sanson, 1986; Vispo and Hume, 1995). The relevance of such mechanisms lies in the fact that, due to surface-volume-relationships, larger particles are digested at a slower rate than smaller particles (Bjorndal et al., 1990). Therefore, the retention of large particles in the ruminant forestomach is coupled with the mechanism of particle size reduction via rumination to enhance the digestibility of these particles,

whereas the selective expulsion of larger particles in hindgut fermenters has been interpreted as a strategy to rid the digestive tract of difficult-to-digest material in order to enhance the animal's potential for higher food intake (Hume and Sakaguchi, 1991).

In horses, a similar observation on selective retention of small particles in the dorsal large colon was published based on sieve analysis of gut contents, which the authors interpreted as an adaptation to maintain large populations of bacteria in the fermentation chamber (Björnhag et al., 1984; Sperber et al., 1992). The equine large intestine has a distinct anatomy with two prominent 'narrow points' that suggest a functional interpretation in the sense of retention mechanisms (Fig. 1A). Other observations, based on passage experiments with polyethylene particle markers of different sizes rather than sieve analysis, led to the conclusion that large particles are selectively retained in the caecum and colon as compared to small particles (Argenzio et al., 1974), as summarized in Fig. 1B, and descriptions of colonic motility in horses appeared to match this pattern (Sellers et al., 1982). To our knowledge, the conceptual problem of explaining the value of a selective retention of both, small and large particles (even though at different sites of the digestive tract), has not been solved, and the adaptive value of such a double mechanism remains obscure. Whether these putative mechanisms lead to a selective net retention of any particle size category has not been investigated.

The differential retention of different-sized particles can be investigated using passage markers of different sizes, either applied via a tube or fistula or offered via food for regular ingestion. Especially in ruminants and camelids, differences in the retention of different-sized markers are evident even when animals ingest these markers via food (Dittmann et al., 2015). An example of a typical excretion pattern for markers ingested via food is displayed in Fig. 2A, where it is evident that a cattle-type ruminant (*Bos javanicus*) retains larger particles of 10 mm for a longer time in its digestive tract than smaller particles of less than 2 mm (Schwarm et al., 2008). An implicit prerequisite for the interpretation is that differences in marker particle size are not extinguished due to ingestive mastication. The presence of large particles in the forestomach of ruminants, with an overall mean particle size of 8-10 mm in the dorsal rumen contents (Clauss et al., 2009a), corroborates this concept. In contrast, when feeding the same marker set to a domestic horse (Fig. 2B), no difference in the excretion pattern between the different-sized markers was evident. However, in horses, the more intensive ingestive mastication (Janis et al., 2010; Dittmann et al., 2017), together with their particularly efficient dental design (Rensberger, 1973), could reduce labelled long particles that are fed to the animals to such a degree that the result no longer represents different-sized particles. This concern is supported by the mean particle size observed in horse faeces of 0.5-1.9 mm (Carmalt et al., 2005; Fritz et al., 2009; Zwirglmaier et al., 2013; Clauss et al., 2014; Gunnarsdottir et al., 2014), which, due to the absence of rumination, can be used as a proxy for their chewing efficiency during ingestion (Carmalt and Allen, 2008). It is also supported by the finding that horses destroy a higher proportion of seeds during ingestive mastication than cattle (Janzen, 1982). Therefore, feeding different-sized particle markers to horses may not be suitable to yield insight into putative selective retention mechanisms in living animals.

Several approaches could be used to overcome this methodological problem. Sets of markers could be applied to the digestive tract either via stomach tube, or via caecal fistula (Argenzio et al., 1974; Udén et al., 1982). Although mean retention times (MRT) of solute and particle markers have been assessed in this way in horses (Argenzio et al., 1974; Udén et al., 1982; Drogoul et al., 2000), different-sized particles were, to our knowledge, only included once in such protocols, in the form of polyethylene markers (Argenzio et al.,

1974). Alternatively, the animals' own natural chewing behaviour could be used for the fractionation of the labelled particle marker, if they are fed whole hay labelled with this marker. The faeces are subsequently analysed for marker excretion patterns in different particle size fractions. For example, if small particles were retained selectively in the horse digestive tract, then the MRT for a particle marker derived from the small particle fraction of horse faeces should be longer than the MRT for the same marker derived from the large particle fraction of the same set of faeces. We tested this hypothesis in six Shetland ponies, evaluating three different chemical markers each bound to unchopped hay. Additionally, we repeated the same experiment in a ruminant species, using six goats. In ruminants, large particles escape the forestomach (and hence comminution via rumination) mostly only directly after ingestion (Lauper et al., 2013); particles that are retained longer are ruminated more often and are hence reduced in size more distinctively (Udén, 1978). Therefore, we hypothesized that in goats, MRT derived from the large particle fraction in the faeces would be shorter than those derived from the small faecal particle fraction.

Materials and methods

Experimental design

Six individuals each of ponies (October 2015) and goats (November 2015) were kept individually and fed unchopped hay from a single batch. After three weeks adaptation to the diet and three days adaptation to individual confinement, each animal was offered a portion of whole hay marked with three different markers as well as a solute marker. Total faecal collection was performed for each animal by collecting faeces regularly in defined intervals and weighing the total amount excreted. Food intake was recorded for 9 days in ponies and 10 days in goats by weighing food offered and leftover. Individual faecal samples were divided into three subsamples. One subsample was used to compose a pooled faecal sample of the whole collection period, and used to determine the mean faecal particle size (FPS) by wet sieving. The second subsample was used to analyse the dry matter content of each defecation and passage marker concentrations in whole faeces. The third subsample was initially separated into three particle fractions (large, intermediate, small) by wet sieving, which were then each submitted to passage marker analysis. The resulting MRT of the different faecal fractions were compared between species, particle size fractions, and markers.

Animals and diet

Six adult nonpregnant female Shetland ponies (Mean ± SD; 164 ±31 kg, 12.8 ±2.9 years; submitted to regular dental controls) and six adult nonpregnant female boer goats (56 ±7 kg, 4.2 ±0.8 years) were housed individually, each animal in a pen of 9.6 m^2 indoor and 9 m^2 outdoor area. The indoor area had a concrete flooring, and each animal was provided a rubber mat as resting area; the outdoor area had a paved stone flooring. No further bedding material was provided. Hay was offered in large feeding bowls placed on the ground. Each for the pony and the goat trial, a composite hay sample was composed from samples taken from every bale of hay used in the respective trial. Hay was analysed using standard methods (VDLUFA, 2012) for dry matter (VDLUFA method 3.1), total ash (method 8.1), crude protein (method 4.1.1) ether extracts (method 5.1.1), neutral detergent fibre and acid detergent fibre (without residual ash, methods 6.5.1 and 6.5.2, analysed with Ankom Fiber Analyzer). The nutritional composition of the grass hay is summarized in Table 1. Gas production in the Hohenheim Gas Test (Menke et al., 1979; VDLUFA method 25.1) was 39 and 47 ml/200 mg DM in the part of the hay batch fed to ponies and goats, respectively. Based on refusal analysis, goats appeared to have fed more selectively than ponies (Table 1); however, due to the fact that the hay ingested by each animal was not sampled and analysed individually, and the variation within the batch (as

evident from the differences in nutrient levels between the two experiments), selective feeding was not analysed further.

Marker preparation and feeding

Cobalt(Co)-EDTA prepared according to Udén et al. (1980) was used as a solute marker. Particle markers were prepared following descriptions of Schwarm et al. (2008); (2009a). Whole, unchopped hay (of the same batch) was placed into nylon bags, washed with a common laundry detergent in a washing machine for 1.5 h, and subsequently dried at 60°C. For marking with chromium (Cr), 190 g of hay was incubated at 100°C for 24 h with 23 g $Na_2Cr_2O_7$ in 4.5 l distilled water; after washing, the hay was incubated with 95 g ascorbic acid in distilled water at room temperature for one hour. Finally, the hay was washed and dried at 60°C. For marking with cerium (Ce) and lanthanum (La), incubation took place at 37°C for 24 h, either 150 g hay with 111 g $CeCl_3 7H_2O$ in 3 l distilled water, or 180 g hay with 133 g $LaCl_3 7H_2O$ in 3.7 l distilled water, each followed by washing and drying at 60°C. The marker concentrations, in three batches of marked hay, were 16.01 g Cr/kg DM, 34.03 g Ce/kg DM and 33.57 g La/kg DM. Ponies received 15 g of Cr-mordanted hay and 25 g of each Ce- and La-marked hay mixed in some handfuls of untreated hay; goats received 5.1 g and 2 × 8.5 g, respectively (all marker hay weight on an as-fed basis). After 30 minutes, any refused feed was removed, and 1.5 g (ponies) or 0.5 g (goats) Co-EDTA dissolved in water and soaked into a handful of pelleted food was offered by hand to each animal and was always eaten completely. Because we did not analyse the leftovers from the mix of marked and unmarked hay removed after the 30 minutes, we could not calculate the exact amount of marker ingested, and hence also could not calculate faecal marker recovery rates. Based on the total amount of marker excreted, the ponies ingested 11.6 ± 5.7, 14.4 ± 7.0 and 16.6 ± 8.6 g as fed of the Cr-, Ce- and La-mordanted hay, respectively; the corresponding values for the goats were 3.4 ± 2.1, 5.6 ± 4.3 and 5.6 ± 4.2 g as fed.

Faecal sampling, wet sieving and marker analysis

All faeces were defecated naturally by the animals, and faeces were collected from the floor. Samples for further analyses were taken from the inside of faecal balls and faeces not touching the ground, to avoid any contamination. Three faecal samples prior to marker feeding were taken from each animal to determine marker baselines. Faecal samples were taken at 0, 4, 8, 12, 16, 20, 24, 28, 32, 38, 44, 50, 56, 62, 68, 74, 80, 86, 92 and 98 h after marker feeding. Additionally, samples were taken at 104, 112, 120, 128, 136, 144, 152, 160, 172, 184, 196, 208 and 216 h in goats; in ponies, these sampling times were one hour later due to the shift to winter time during the experiment, i.e. with the same intervals from 105 to 197 h after marker feeding. Faeces were always collected completely, but because faeces were collected from the floor, data on total faecal output and digestibility need to be interpreted with the fact in mind that some inaccuracy in actually collecting all faeces may have occurred. The DM concentration of each sample was determined by drying a representative subsample at 103°C to calculate faecal DM excretion. For wet sieving, samples were submitted to a standardized wet sieving method (Fritz et al., 2012). The sieve cascade (Retsch, Haan, Germany) contained 9 sieves with pore sizes (linear dimension of holes) of 0.063 mm, 0.125 mm, 0.25 mm, 0.5 mm, 1.0 mm, 2.0 mm, 4.0 mm, 8.0 mm, and 16.0 mm. Faecal samples were left in beakers of water overnight with magnetic stirrers to achieve a disintegration of the sample without changing particle sizes. Subsequently, the sample was poured onto the sieve cascade that was placed on a sieving machine (Retsch® AS 200 digit, Haan, Germany) set to a vibration amplitude of approximately 2 mm, with a water throughput of 2 litres per minute, and sieved for 10 minutes. Material retained on the sieves was collected as 'large

particles' (sieves 2.0 mm - 16 mm), 'intermediate particles' (sieves 0.5 mm and 1.0 mm) and 'small particles' (sieves 0.063 mm - 0.25 mm), which were then dried and prepared for marker analysis. Additionally, a pooled faecal sample from each animal was used to determine the faecal particle size (FPS) as the weighted average (dMEAN) as described in Fritz et al. (2012). For this, the remains on each sieve were transferred onto pre-weighed petri dishes, dried at 103°C for at least 15 h, and weighed after cooling to room temperature in a desiccator using an analysis balance with measuring accuracy of 1 mg. Whole faecal samples from ponies, and the large and intermediate particle fraction from both species, were ground to 2 mm using a micromill (Culatti AG, Zurich, Switzerland). To minimize marker contamination between samples, the mill was cleaned thoroughly after each sample and samples were also milled in chronological order for each animal.

Concentrations of Co, Cr, La, and Ce were analysed after wet ashing with 4 ml nitric acid and 2 ml hydrogen peroxide in a microwave oven (Frei et al., 2015). Temperature was increased over 15 min to 170°C, and over 20 min to 200°C, then held at 200°C for 5 min. Wave-length was 12·25 cm and the frequency 2·45 GHz. Determination of Co, Cr, La and Ce in the samples was performed using an inductively coupled plasma optical emission spectrometer (model Optima 8000, Perkin Elmer, Rodgau, Germany).

Calculations and statistics

The relative dry matter intake was expressed on the basis of body mass$^{0.85}$ (Müller et al., 2013) and dry matter digestibility calculated as the percentage of dry matter intake not excreted in faeces. The percentage of particles that passed the finest sieve was calculated by subtracting the DM of material retained on all sieves from the calculated total DM of the sample submitted to sieve analysis based on previous DM determination of faeces. The FPS was calculated according to the dMEAN procedure of Fritz et al. (2012) as

$$FPS = \sum_{i=1}^{n} p(i) * \frac{S(i+1) + S(i)}{2}$$

where i is the number of sieves in the respective cascade (with 1 as the number of the smallest sieve), $p(i)$ the proportion of dry matter on sieve i, and $S(i)$ the pore size of the sieve. The total amount of marker excreted was calculated by multiplying the marker concentration of a sample with its mass of DM and summing the resulting amounts per animal; for the calculation of the amount of marker excreted with a particle fraction, the percentage of particles represented by the respective particle fraction in total amount of DM of a faecal sample was used. The MRT in the whole digestive tract was calculated according to Thielemans et al. (1978) as

$$\text{MRT} = \frac{\Sigma\, t_i\, C_i\, dt_i}{\Sigma\, C_i\, dt_i}$$

with C_i = marker concentration in the faecal samples from the interval represented by time t_i (h after marker administration, using the midpoint of the sampling interval) and dt_i = the interval (h) of the respective sample

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

Marker excretion was assumed complete once faecal marker concentrations had returned to the background-levels determined in pre-dose faecal samples. Additionally, MRT were also determined using a multi-compartmental model (Dhanoa et al., 1985); due to the close similarity of the MRT values thus obtained to those calculated using the Thielemans method, only the latter are presented.

Statistical comparisons between species were made by t-test; comparisons within species were made by repeated measurements ANOVA with Sidak post hoc comparisons. Additionally, within each species, a General Linear Model (GLM) was used to test simultaneously for an effect of marker and particle size category and their interaction, including individual as a random factor. Finally, to test whether

chewing efficiency affected the retention of different-sized particles, the correlation between FPS and the difference in MRT between large and small particles was analysed sing Pearson's correlation coefficient. Analyses were performed in SPSS 22.0 (IBM, Armonk, NY), with the significance level set to $P < 0.05$.

Results

While the relative dry matter intake did not differ significantly between goats and ponies, ponies excreted significantly more faecal dry matter, had lower dry matter digestibilities, greater mean faecal particle size and shorter MRTs than goats (Table 1). Faecal particle size distribution showed a larger proportion of particles > 2 mm, and a more even distribution of 0.5 mm to 2 mm particles in ponies (Fig. 3). Goats had a significantly higher proportion of particles that passed through the finest sieve (0.063 mm; Table 1).

Generally, marker results were similar for the three different particle markers. Faecal marker concentrations returned to pre-dose levels at 148-202 h after marker application in goats and at 47-71 h after marker application in ponies. A higher percentage of the total marker was excreted via large and medium particles in ponies than in goats, whereas the opposite was the case for small particles (Table 2). In both species, a large percentage of the particle marker must have been associated with the particle fraction that passed through the finest sieve, and this was similar for all three particle markers. The solute marker had a significantly shorter MRT than the particle markers in goats, but not in ponies (Table 1, Fig. 4).

In goats, larger particles were consistently excreted earlier than small particles for each marker, with medium particles in between; the difference was most distinct for the Cr marker (Table 2, Fig. 4A). The difference between large and small particles was approximately 8.6 h in goats. The marker excreted as large particles represented less than 2% of total marker excretion (Table 2).

In ponies, larger particles were excreted later than small particles at a difference of less than one hour; this difference was significant only for the Cr marker (Table 2, Fig. 4B). The marker excreted as large particles represented nearly 5% of the total marker excretion (Table 2).

Comparing, within species, individual, marker and particle size, the effect of individual on MRT was significant in both goats and ponies (Table 3). In goats, there was no effect of marker, whereas for ponies, Cr was associated with significantly longer MRT than Ce or La (Table 3). Particle size had a significant effect in both species, with small particles having significantly longer MRT than other particle fractions in goats, and large particles having significantly longer MRT than other particle fractions in ponies (Table 3).

There was no significant correlation between FPS and the difference in MRT between small and large particles for any marker in goats ($P \geq 0.204$ for all markers). In ponies, there was no correlation for the Ce and La marker ($P \geq 0.783$), but for Cr, the correlation approached significance ($R = 0.788$; $P = 0.063$; Fig. 5).

Discussion

The results of this study corroborate known differences in the selective retention of digesta particles in ruminants that lead to different-sized faecal particles: large particles are rare in ruminant faeces and represent digesta fractions that escape the forestomach comparatively soon after ingestion; most particles are retained and submitted to repeated comminution via rumination (Udén, 1978; Lauper et al., 2013). Differences in MRT between large and small particles in the ruminant species were not only significant, but also of a magnitude (8-9 h) that is relevant in terms of fermentative digestion (Hummel et al., 2006). In contrast, the difference between large and small particle excretion in the ponies of less than one hour, though statistically significant for the Cr marker,

cannot be considered relevant in terms of fermentative digestion. Thus, the results demonstrate that in contrast to previous findings (Argenzio et al., 1974; Björnhag et al., 1984; Sperber et al., 1992) and resulting graphical reviews (Drogoul et al., 2000; Van Weyenberg et al., 2006), net differences in the passage of different-sized particles through the gastrointestinal tract are not a decisive part of the digestive physiology of horses. Notably, previous findings on a selective large particle retention in the dorsal colon of horses were observed with polyethylene markers of 10 and 20 mm length, which is not representative of an important fraction of the horse's digesta when compared to the sieve results of the present study in Fig. 3, or to reported mean FPS in equids of 0.5-1.9 mm (Carmalt et al., 2005; Fritz et al., 2009; Zwirglmaier et al., 2013; Clauss et al., 2014; Gunnarsdottir et al., 2014). Given these data on physiological mean particle sizes in the digesta of horses, the 10-20 mm particles used by Argenzio et al. (1974) might even be more comparable to the foreign bodies, the coarse hay or 'lawnmower grass' typically associated with cecal or colonic impaction (Collatos and Romano, 1993; Dabareiner and White, 1995).

However, between the six ponies of the present study, the selective retention of larger particles tended to increase, on the Cr marker, with reduced chewing efficiency, being largest (1.6 hours) in the animal with the largest mean faecal particle size (Fig. 5). This might be a tentative indication for a mechanism that might compensate for reduced chewing efficiency within a physiological range. Such compensation has been described across nonruminant herbivores, where species with lower chewing efficiency have longer digesta retention times (Clauss et al., 2009b). At high age, with distinctively reduced chewing efficiency due to dental wear and pathology, this mechanism might lead to an increased frequency of impactions of the caecum or colon and hence colic (Brosnahan and Paradis, 2003; Cox et al., 2007; Du Toit et al., 2009). Whether such a compensatory mechanism is really a relevant part of the horse's digestive physiology, that possibly aims at maintaining digestive efficiency at reduced dental efficiency and hence increasing longevity, remains to be investigated in further studies.

In the present study, marker recovery rates could not be estimated because the amount of marker ingested was not quantified. However, given that the typical gradual marker concentration decrease occurred in both species (Fig. 4), and that in ponies, faecal samples taken between 80-197 hours after marker feeding consistently yielded no remaining passage marker concentrations, it appears unlikely that non-recovered marker had remained in the digestive tract. Another methodological aspect of this study relates to those very fine particles that passed through the smallest sieve. Results referring to this very fine particle fraction indicate that (i) on the regular hay, the goats produced a higher proportion of these particles than horses (Table 1); (ii) the proportion of marker putatively excreted with this particle fraction (44-53%, the difference to 100 in Table 2) is larger than the proportion of these particles in the faeces and (iii) the proportion of marker putatively excreted with this particle fraction was larger in ponies (51-53%) than in goats (44-48%). If these observations only applied to the cerium and lanthanum markers, one might suspect these markers to have separated from the particulate matter they were bound to, and migrated to either other very fine particles or the liquid phase. It has been reported that the binding of these rare earth markers to particles is acceptable (especially if applied by mordanting, as in the present study, rather than by spraying) (Owens and Hanson, 1992), but not as tight as that of mordanted chromium (Udén et al., 1980; Van Soest et al., 1988). However, as this pattern also applied to the chromium marker, for which a very tight binding to particulate matter has been demonstrated, another explanation is required. We

consider it most likely that the fracture properties of the marked whole hay differs from that of normal hay, due to the washing in detergent solution, and the repeated drying it was subjected to. Assuming that these processes rendered the marker hay more brittle, a higher proportion of very fine particles, and a particularly high proportion during the intensive ingestive chewing in the ponies, appear understandable. If one wanted to avoid such effects, marker hays would have to be grown to result in a special isotope signature (Huhtanen and Hristov, 2001).

It should be noted that the conditions of our experiment do not reflect feeding practices for most pleasure or sport horses. The ponies and goats of the present study received a hay-only diet *ad libitum*, which could be considered close to the natural diet of equids (Ellis and Hill, 2005). In contrast, previous results on selective particle retention were gained in equids on two pelleted diets fed *ad libitum* during two 1-h intervals per day (Argenzio et al., 1974; estimated dry matter intake 29-43 g $kg^{-0.85}$ d^{-1}), or on restricted amounts of hay and crushed oats with oat straw *ad libitum* (Björnhag et al., 1984; Sperber et al., 1992; food intake not reported). Under practical feeding conditions, horses often receive diets that include cereals or pectin-rich substances as additional energy substrates. A side effect of these feeds is an increase in viscosity of the chyme (Lopes et al., 2004) and a higher water-binding capacity of the digesta (Zeyner et al., 2004; Jensen et al., 2014), which would even further reduce any propensity of the chyme to separate into different fractions of fluid or (different-sized) particles. The addition of such energy-dense feeds might also lead to a reduced overall intake level. Because intake is closely linked to digesta passage, lower intake levels are associated with longer retention times and lower gut fill (Clauss et al., 2014). Under such conditions, a selective particle size retention would be less relevant, because at low intakes, neither gut capacity nor time available for digestion are as constrained as at high intakes.

The results for ponies are in line with those for other large, nonruminant foregut fermenters including macropods, peccaries, hippos, and colobine monkeys, in which no selective retention of a certain particle size fraction was observed (Schwarm et al., 2008; Schwarm et al., 2009b; Munn et al., 2012; Matsuda et al., 2015). Anyhow, a selective retention of large particles in a nonruminant herbivore appears unlikely. Even though any putative retention mechanism for large particles (Argenzio et al., 1974; Sellers et al., 1982) might be considered beneficial in terms of additional time available for the fermentation of fibre, it would need to be reversible at some point to minimize blockage of the gut and avoid a constraint on intake. In ruminating animals, the secondary chewing action of rumination represents the mechanism to reverse selective retention. In animals without this option, the parallel movement of particles within the gut is the most likely and parsimonious scenario.

A different scenario involves the selective retention of small particles by a colonic separation mechanism, as reported in small herbivores (Björnhag, 1987) and suggested for horses (Björnhag et al., 1984; Sperber et al., 1992). Such a mechanism would require a periodical emptying of the organ fine particles are directed to (typically, the caecum), which is typically linked to the production of a peculiar kind of faeces such as caecotrophs in rabbits and rodents (Björnhag and Snipes, 1999), or liquid faeces in herbivorous birds (Björnhag, 1989). Such peculiar faeces are not part of the horse's normal physiology. Faecal water running out of the anus at the same or at other times as defecations are not part of the normal repertoire of healthy horses but considered a pathological condition (Kienzle et al., 2016). The selective retention of fine particles in a colonic separation mechanism is either linked to a retrograde transport of fluid, which is then reflected in comparatively long MRT for a solute marker (Franz et al., 2011) - something not typically observed in horses, including in the present study.

Alternatively, solute and small particle markers may move more or less in parallel in animals with a colonic separation mechanism - as in the ponies of the present study, but then particular anatomical structures for the retention of microbes are required, such as the colonic groove of hystricomorph rodents (Björnhag and Snipes, 1999), which are absent in horses.

Even though differences in the retention of fluids and particles may occur at the level of the caecum (Drogoul et al., 2000) or the boundary of the large dorsal colon and the colon transversum (Sperber et al., 1992), no net selective retention of any investigated particle size class occurs in horses. Furthermore, differences in the retention of fluid and particles that indicate 'digesta washing' are small in equids compared to many other herbivores (Müller et al., 2011). Evidence for a low degree mixing of digesta in the horse gastrointestinal tract, such as stemming from observations of sequential changes in volatile fatty acid concentration in the caecum or faeces depending on the sequence of feeding individual ration ingredients (Schwabenbauer et al., 1982; Zeyner et al., 2004), or intra-day differences in the recovery of an alkane marker applied as daily pulse-doses (Bachmann et al., 2016), matches our finding of uniform digesta passage in terms of particle size classes. These findings raise the question about the physiological relevance of the two prominent isthmi in the horse's large intestine. These anatomical features, typically interpreted as delay structures, have been documented in domestic and wild equids (Clauss et al., 2008), including donkeys (Jerbi et al., 2014), and also in tapirs (Hagen et al., 2015). In contrast, they are absent in the third group of the perissodactyls, the rhinoceroses, and also in elephants (Clauss et al., 2003). If these structures would really enhance a delay of digesta passage, one would expect a higher food throughput in animals that lack them. However, food intake levels are generally high in horses and elephants, and lower in donkeys, tapirs and rhinos (Meyer et al., 2010). The veterinary relevance of the isthmi as major predilection sites for obstipations and thus colic are undisputed (Decker et al., 1975; Campbell et al., 1984; Rakestraw and Hardy, 2006), and obstipation actually represents an extreme, pathological case of particle retention. However, given the absence of an evident link between the presence of the isthmi and food intake level in large hindgut fermenting mammals, and the absence of a net selective retention of both fluids and different-sized particles in the present study, the physiological function of the isthmi remains to be fully explored. The hypothesis resulting from a weakly-supported finding of the present study is that they may serve to cause a distinctively lower degree of selective large particle than hitherto assumed, in order to compensate for losses in chewing efficiency with age.

In summary, the most parsimonious concept of digesta movement patterns in the equine digestive tract is that of a parallel movement of different-sized particles, with only a minor degree of digesta washing indicated by slightly shorter solute than particle marker MRT.

Acknowledgements

We thank Prof. Wolfgang Holtz for support in the goat trial, Heidrun Barleben for the marker analyses and Nathalie Heinje and Henrike Schwanhold for sieve analyses. This study was supported by a grant of the Foundation for Research in Science and the Humanities at the University of Zurich to MC (F-53111-04-01), and granted an experimental licence by the cantonal veterinary office as experiment no. 17/2014. Two anonymous reviewers made significant contributions towards the improvement of this manuscript.

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Table 1. Mean (±SD) body mass, intake, faecal excretion, apparent digestibility of dry matter (DM), mean faecal particle size, and mean retention time (MRT, Thielemans) determined in whole faeces for a solute (Co) and three particle markers (Cr, Ce, La; bound to whole hay) determined in goats and ponies fed grass hay *ad libitum*

Measure	**Goat**		**Pony**		***P***
	mean	SD	mean	SD	(species)
Body mass (kg)	56	7	164	31	<0.001
DM intake (kg d^{-1})	1.34	0.09	3.13	0.60	n.a.
DM intake (g $kg^{-0.85}$ d^{-1})	44	5	41	4	0.226
DM excretion (g $kg^{-0.85}$ d^{-1})	20	2	23	3	0.026
DM apparent digestibility (%)	55	3	43	4	<0.001
Hay as offered (g/kg DM)					
Crude protein	69.8		59.5		n.a.
Total ash	74.1		86.3		n.a.
Ether extracts	15.7		13.6		n.a.
Crude fibre	337.1		346.6		n.a.
Neutral detergent fibre (om)	620.9		624.9		n.a.
Acid detergent fibre (om)	372.4		390.5		n.a.
ME[1]	8.68		6.28		n.a.
Hay as ingested (g/kg DM)					
Crude protein	76.4	3.3	62.7	1.4	n.a.
Acid detergent fibre	358.8	8.7	387.6	3.8	n.a.
Faecal mean particle size (mm)	0.49	0.09	1.22	0.34	0.003
Dry matter passing the finest sieve (% of all DM)	35.1	2.9	23.4	2.6	<0.001
MRT Co	34.7[a]	3.7	24.1	3.3	<0.001
MRT Cr	49.9[b]	3.7	24.6	4.3	<0.001
MRT Ce	52.6[b]	4.2	24.0	3.8	<0.001
MRT La	52.4[b]	5.2	24.3	3.8	<0.001

[1]metabolizable energy ME estimated and for goats according to GfE (2008) and for ponies according to Kienzle and Zeyner (2010)

different superscripts in the goat column indicate significant differences in MRT between markers (repeated measurements ANOVA with Sidak post hoc tests); no such differences were significant in ponies

Table 2. Mean (±SD) mean retention time (MRT, Thielemans) determined for faecal particle fractions (large > 2 mm, 2 mm > medium > 0.5 mm, 0.5 mm > small > 0.063 mm) for three particle markers (Cr, Ce, La) determined in goats and ponies fed grass hay *ad libitum*

Marker (particle size)	**MRT (h)**					**% of total marker excretion**				
	Goat		**Pony**		***P***	**Goat**		**Pony**		***P***
	mean	SD	mean	SD	(species)	mean	SD	mean	SD	(species)
Cr (large)	44.1[a]	4.7	25.5[a]	5.4	<0.001	1.7[a]	1.8	4.5[a]	2.0	0.029
Cr (medium)	48.1[b]	4.2	25.1[b]	5.3	<0.001	9.9[b]	1.0	11.5[b]	4.5	0.429
Cr (small)	53.5[c]	4.0	24.6[b]	5.0	<0.001	43.7[c]	9.2	33.0[c]	4.9	0.031
Ce (large)	44.7[a]	6.8	24.5	5.0	<0.001	1.6[a]	1.5	4.9[a]	2.1	0.009
Ce (medium)	46.9[a]	5.6	24.3	4.8	0.003	9.8[b]	1.2	12.9[b]	5.9	0.251
Ce (small)	52.9[b]	5.1	23.8	4.4	<0.001	40.5[c]	5.7	29.8[c]	7.3	0.018
La (large)	44.7[a]	8.7	24.7	4.9	<0.001	1.7[a]	1.7	4.9[a]	1.8	0.010
La (medium)	46.0[a]	8.1	24.2	5.0	<0.001	11.3[b]	1.2	13.1[b]	4.5	0.383
La (small)	53.1[b]	6.1	24.1	4.5	<0.001	43.1[c]	6.0	28.8[c]	6.6	0.003

different superscripts in columns indicate significant differences between particle size categories for a marker (repeated measurements ANOVA with Sidak post hoc tests); no such differences were significant in ponies for MRT of Ce or La

Table 3. Results of General Linear Model for measurements of mean retention times using three different faecal particle size categories (large, medium, small) and three different particle markers (Cr, Ce, La) and considering the individual animal in goats and ponies fed grass hay ad libitum

Species	Particle size	Marker	Individuum	Size x marker interaction
Goats	$F_{2,40} = 38.77$	$F_{2,40} = 0.22$	$F_{5,40} = 28.85$	$F_{4,40} = 0.31$
	$P < 0.001$	$P = 0.804$	$P < 0.001$	$P = 0.872$
	(large = medium[1] < small)*			
Ponies	$F_{2,40} = 11.71$	$F_{2,40} = 19.43$	$F_{5,40} = 1107.4$	$F_{4,40} = 0.54$
	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.707$
	(large > medium = small[2])*	(Cr > Ce = La)*		

*Sidak post hoc tests

[1]difference between medium and large particles tending towards significance at $P = 0.059$

[2]difference between small and medium particles tending towards significance at $P = 0.072$

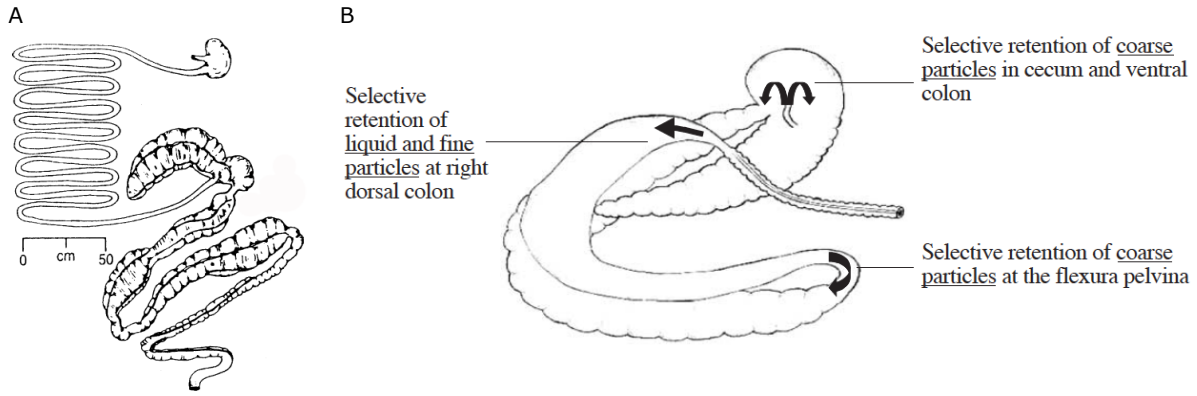

Figure 1. (A) Digestive tract of equids, with isthmi/narrow points between the caecum and colon, and after both of the large colon layers (from Clauss et al., 2008); (B) summary of functional hypotheses relating to these isthmi (from Van Weyenberg et al., 2006).

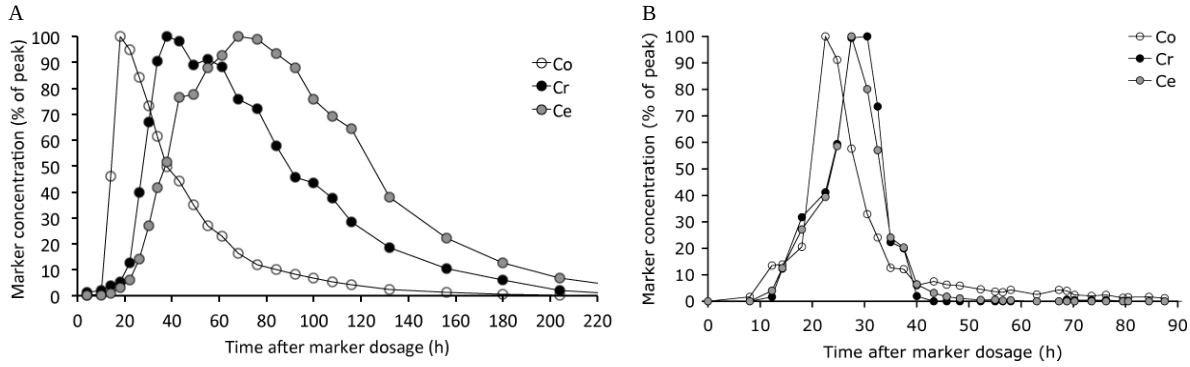

Figure 2. Marker excretion patterns in (A) a banteng (*Bos javanicus*, a cattle-type ruminant)(from Schwarm et al., 2008) and (B) a pony (K. Schiele, I. Lechner and M. Clauss, pers. obs.). Animals ingested a marker bolus comprising a solute marker (cobalt-EDTA, Co), a small particle (< 2 mm) marker (chromium-mordanted fibre, Cr) and a large particle (10 mm) marker (cerium-mordanted fibre, Ce) fed as a pulse dose with normal ingestive mastication. Note the difference in the excretion pattern of the different-sized particles in the ruminant and the similarity of the excretion of the different-sized particles in the pony. Changes in marker particle size due to ingestive mastication could not be excluded in this setup.

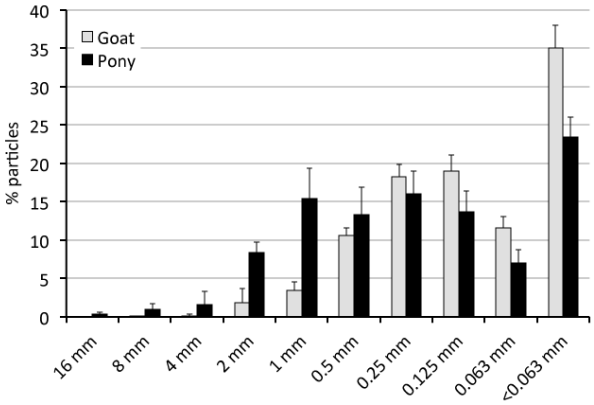

Figure 3. Mean (±SD) percentage faecal dry matter distribution on the different sieves after sieve analysis of faeces in goats (n=6) and ponies (n=6) fed grass hay *ad libitum*.

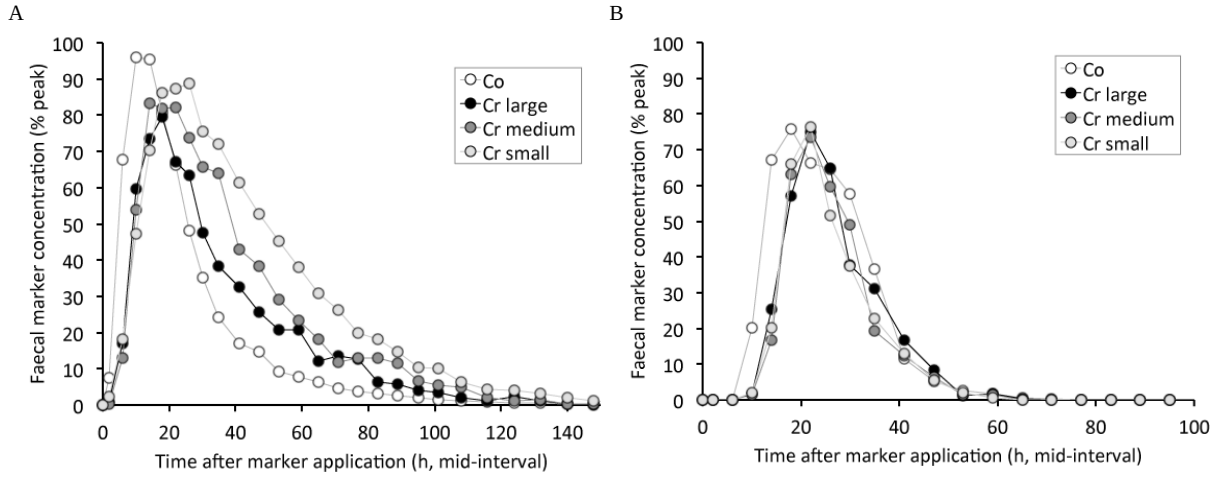

Figure 4. Mean marker excretion patterns in (A) goats (n=6) and (B) ponies (n=6) fed a solute marker (Co) and whole Cr-marked hay with analysis of markers in large (>2 mm), medium (0.5-2 mm) and small (0.063-0.5 mm) faecal particles. Note that values do not reach 100% because they are averaged across the six study animals. Note the general similarity to Fig. 2, and the inverse pattern in particle sizes in the ruminant. This is because particle sizes do not denote different ingested markers in this experiment, but different fractions of the same marker escaping the forestomach; in ruminants, the minor fraction of large particles that escapes rumination is typically excreted very soon after ingestion.

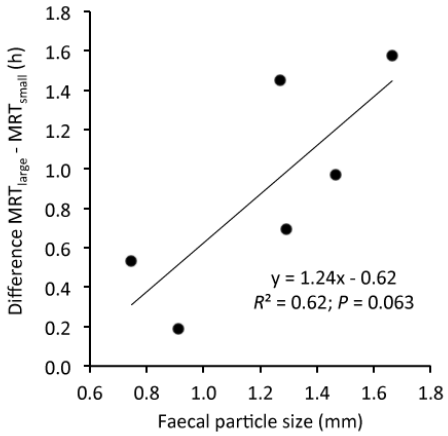

Figure 5. Relationship between the mean faecal particle size and the difference between the mean retention time for large and for small particles in the six ponies of this study.