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# No size-dependent net particle retention in the hindgut of horses

Angela Schwarm[1] | Marcus Clauss[2] | Sylvia Ortmann[3] | Rasmus B. Jensen[1]

[1]Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Ås, Norway

[2]Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

[3]Leibniz Institute for Zoo and Wildlife Research (IZW) Berlin, Berlin, Germany

**Correspondence**

Rasmus B. Jensen, Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, PO Box 5003, 1432 Ås, Norway.
Email: rasmus.bovbjerg.jensen@nmbu.no

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## Abstract

Sieve analyses of hindgut contents of horses as well as observations in horses where plastic markers had been applied to a caecal cannula suggested that there may be a discrimination by particle size in the passage or retention of digesta. Here, we performed a similar experiment with five caecum-cannulated horses (562 ± 31 kg) fed a constant amount (6.81 kg dry matter/day) of grass hay. Passage markers representing the liquid (Co-EDTA) as well as the particulate digesta phase (Yb—undefined; Cr mordanted fibre 1–2 mm; Ce-mordanted fibre 8 mm) were given as a pulse-dose into the cannula to measure their mean retention times (MRT). The MRTs were compared by repeated-measurements analysis of variance. The MRT in the hindgut was 22.2 ± 2.4 h for Co, 25.0 ± 3.4 h for Yb, 26.2 ± 1.6 h for Cr and 26.3 ± 1.5 h for Ce. Whereas differences between the particle marker MRTs were not significant ($p_{adj.} > 0.05$), significant differences were observed between the solute marker Co and each of the particle markers Cr and Ce ($p_{adj.} < 0.009$). The results confirm the well-known significant, albeit small, difference in MRT in horses between the fluid and the particle digesta phase, and corroborate another recent study that used a combination of whole, marked hay and individual marker analysis in different particle size fractions of the faeces, which also did not detect a selective retention of any particle size class.

**KEYWORDS**

digestive physiology, equus, hindgut fermenter, particle size, passage, sorting mechanism, markers, mean retention time

## 1 | INTRODUCTION

Horses (*Equus caballus*) are hindgut fermenters with microbial fermentation occurring mainly in the caecum and the proximal colon (Argenzio, Southworth et al., 1974). The proximal colon consists of two layers, a ventral one that originates from the caecum, leading into the dorsal layer, which finally joins the *Colon transversum*, in which the formation of faecal boli begins. The macroscopic appearance of these fermentation sites is not only dominated by extensive haustration and the pelvic flexure of the proximal colon linking its ventral to its dorsal layer, but also by 'narrow points'—at the transition of the caecum to the colon, and at the transition of the proximal colon to the *Colon tranversum* (Clauss et al., 2008). These anatomical peculiarities beg for a functional explanation, with selective retention of particles of a certain size—that is, small versus large particles—an evident candidate. Reports on an accumulation of small (as opposed to large) particles at the boundary of the dorsal proximal and the distal colon (Björnhag et al., 1984; Sperber et al., 1992), and of selective retention of large (as opposed to small) particles at the junction

of ventral and dorsal colon (Argenzio, Lowe et al., 1974) appear contradictory with respect to functional relevance. Nevertheless, these two mechanisms might occur in parallel and result in no net difference in the passage of large and small particles. Both postulated mechanisms support a concept of selective retention of a certain particle size class, which was summarized graphically in Drogoul et al. (2000) and Van Weyenberg et al. (2006). With respect to a differential retention of particulate digesta per se as compared to fluid digesta components, it is well-known that particulate markers in general are retained longer in the horse digestive tract than solute markers (reviewed in Clauss et al., 2014).

Studying digesta passage in horses is challenging insofar as simply feeding passage markers of different particle size is unlikely to yield representative results, due to the intensive ingestive mastication of horses (reviewed in Hummel et al., 2018). Other methods include the application of markers via oesophageal tube or via caecal or colonic cannulae (Argenzio, Lowe et al., 1974; Udén et al., 1980). Alternatively, marked forage can be fed whole, and retention assessed for the different size classes in faeces that are separated by sieving prior to passage marker analysis in the respective samples (Hummel et al., 2018).

The latter approach recently indicated that there is no distinct net size-discriminating particle retention in the digestive tract of horses (Hummel et al., 2018), contradicting a previous study based on markers applied into caecal cannula (Argenzio, Lowe et al., 1974). In order to corroborate this recent result, we applied passage markers of different particle size as well as a solute marker directly into the hindgut of caecum-cannulated horses.

## 2 | MATERIALS AND METHODS

### 2.1 | Study design, animals and diet

The protocol and procedures employed were in line with the Norwegian Animal Research Authority (i.e., Regulations on the Use of Animals in Experiments, July 2015), and the experiment was performed in accordance with relevant institutional and national guidelines for the care and use of laboratory animals.

The experiment was designed as a longitudinal study lasting 15 days with a 10-day adaptation period and 5 days of data collection. Five Norwegian cold-blooded trotters (geldings) with a body weight of 530–610 kg (Table 1) and caecum cannula (custom made by Nordic 3D, Oslo, Norway, using a mould provided by NMBU; approximately 15 cm barrel length and 3 cm internal and 4 cm external diameter), surgically applied more than 10 years prior to the study, were used in the experiment. The horses were kept individually in stalls (3 × 3 m, with rubber mats and wood shavings as bedding material), with access to a common gravel paddock (25 × 45 m) for 4 h each day after the morning and afternoon meals. They were fed daily with 6.81 kg dry matter (DM) of timothy-dominated, late-harvested hay, resulting in a narrow range of relative daily DM intake (11–13 g/kg BW or 55–62 g/kg$^{0.75}$). The hay was fed in portions of 2.27 kg DM each at 0600, 1400 and 2200 h, and was always consumed completely by all animals. The contents of DM, organic matter (OM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) (expressed without residual ash) and water-soluble carbohydrates (WSC) were 908, 927, 151, 592, 331 and 75 g/kg DM respectively (analysis methods are described in Section 2.3). The morning meal included 30 g/day sodium chloride and 100 g/day of a commercial mineral and vitamin supplement (Champion Multitiskud; Fellesskjøpet Forutvikling) consisting, per kg original matter, of Ca, 100 g; P, 70 g; Mg, 32 g; NaCl, 50 g; Cu, 840 mg; Zn, 2830 mg; Mn, 1530 mg; Fe, 2460 mg; I, 18 mg; Co, 6 mg; Se, 10.2 mg; vitamin A, 107,000 I.U.; vitamin D, 11,300 I.U.; vitamin E, 9600 mg; vitamin B1, 260 mg; vitamin B2, 120 mg; vitamin B6, 100 mg: vitamin B12, 0.8 mg; niacin, 270 mg; folic acid, 150 mg; biotine, 15 mg and vitamin C, 270 mg. Water was available for ad libitum consumption from automatic water troughs in the individual stalls, and from buckets in the gravel paddock, and was not quantified. Body mass was measured with an electronic scale at the end of the experimental period.

### 2.2 | Marker preparation, administration and sample collection

The retention time was determined with the solute marker cobalt ethylenediaminetetraacetate (Co-EDTA), and three particle markers, ytterbium (Yb) acetate and grass hay cut to pass 2- and 8-mm screens mordanted with chromium (Cr) and cerium (Ce) respectively. The grass hay was from a different batch than the one fed to the animals, and particle size was approximated by using particles that passed a 2 and 8 mm sieve screen but was retained on a 1 and 4 mm sieve screen respectively. The preparation of the Co, Cr and Ce markers followed Udén et al. (1980) and details of the chemicals and the marker preparation are described in Grandl et al. (2018). Distilled water was used to dissolve Yb(III) acetate tetra-hydrate (Sigma-Aldrich/Merck Life Sciences) (3 g/500 ml) and Co-EDTA (4 g/50 ml). Note that Yb is considered to bind particularly to small particles both in vitro and in vivo by some authors (e.g., Erdman & Smith, 1985), even when added to the fermentation chamber as a liquid (e.g., Siddons et al., 1985).

**TABLE 1** Details of the horses and nutrient digestibility; dry matter intake was equal for all animals at 6.81 kg/day

| Horse | Age (years) | Body mass (kg) | Apparent digestibility (%) | | | | |
|---|---|---|---|---|---|---|---|
| | | | DM | OM | CP | NDF | ADF |
| 1 | 15 | 613 | 59 | 59 | 75 | 54 | 49 |
| 2 | 26 | 565 | 60 | 60 | 79 | 55 | 50 |
| 3 | 23 | 557 | 60 | 60 | 77 | 55 | 50 |
| 4 | 20 | 549 | 58 | 57 | 78 | 51 | 44 |
| 5 | 14 | 528 | 59 | 59 | 74 | 55 | 51 |
| mean | 20 | 562 | 59 | 59 | 77 | 54 | 49 |
| SD | 5 | 31 | 1 | 1 | 2 | 2 | 3 |

Abbreviations: ADF, acid detergent fibre; CP, crude protein; DM, dry matter; NDF, neutral detergent fibre; OM, organic matter.

Particles mordanted with different markers (40 g Cr-2 mm + 40 g Ce-8 mm) were mixed, soaked in water and administered through the caecum cannula using a plunger from a 20 ml syringe to push the marker through the cannula into the caecum. This was immediately followed by administration of the solutions of ytterbium acetate (500 ml) and Co-EDTA (50 ml) into the caecum by using a syringe. The markers were administered after the morning meal on day 12 of the experiment (day 2 of the sampling period).

Feed samples were collected daily from day 8 to 14 and pooled to one sample. Faecal samples were collected on day 11 (blank) and days 12–15. Four consecutive days of total collection of faeces from each horse were performed using a collection harness (Stablemaid). Each collection harness was emptied every fourth hour, faeces were weighed and a subsample (~200 g) was stored at −18°C for marker analysis. Faecal excretions from each horse were compiled over 24 h and stored in containers at 3°C, mixed thoroughly and a subsample of 10% was stored from each day at −18°C. The daily faecal subsamples were thawed and pooled into two composite subsamples (approximately 500 g each) per horse for DM determination and for nutrient analysis. Feed and faecal samples were freeze dried and then milled to pass a 1 mm screen (Cutting mill SM 200; Retsch GmbH).

## 2.3 | Chemical analyses and calculations

Feeds and faeces were analyzed for DM, ash, NDF, ADF and nitrogen, and feeds for WSC. DM content was determined by drying to a constant weight (24 h at 105°C), and samples were incinerated at 550°C for approximately 16 h for crude ash determination. The NDF and ADF content were analyzed with the filter bag technique (ANKOM, 2017a, 2017b) using heat-stable amylase and expressed without residual ash. Nitrogen was determined by the Kjeldahl technique using a Kjeltec TM 8400 (FOSS) and CP calculated as 6.25 × N. WSC were determined as described by Randby et al. (2010).

The marker concentrations in the mordanted hay particles and in the faeces were analyzed after wet ashing using inductively coupled plasma optical emission spectrometry (ICP-OES; Optima 8000; Perkin Elmer). For wet ashing we heated 0.2 g sample with 2 ml hydrogen peroxide and 4 ml nitric acid with the microwave MLS 'START 1500' (MLS GmbH). Temperature was raised to 170°C within 15 min and to 200°C within 20 min, then held for 5 min at 200°C. The frequency was 2.45 GHz and the wave length 12.25 cm. Samples were introduced into the ICP-OES by means of a peristaltic pump connected to a Meinhard nebulizer with a cyclon spray chamber. The measured spectral element lines were Co: 228.616 nm, Cr: 267.716 nm, Yb: 328.937 nm and Ce: 413.764 nm. The nebulizer gas was 0.6 L argon $min^{-1}$, the plasma gas was 8 L argon $min^{-1}$ and the radio frequency power was set to 1400 W. Certified single element calibration standards (Perkin Elmer) were used for the calibration curves and control measurements which were run after every tenth sample. The mordanted particles of 2 and 8 mm size and the EDTA contained, per kg DM, 32.8 g Cr, 41.5 g Ce and 151 g Co respectively. The baseline concentrations measured in samples before the marker application were used to correct for faecal background levels in each individual horse. In cases where marker concentration did not decline to the baseline level, marker concentrations below 1% of peak concentration were set to zero (modified correction from Bruining & Bosch, 1992). In depicting the marker excretion patterns, we followed the recommendation of Matsuda et al. (2015) by expressing marker concentrations in % of the peak concentration, to focus the reader's attention on the shape of the excretion curves and not differences between them due to different concentration magnitudes; for readers preferring other visualizations, the raw data is provided in the online supplement.

The apparent total tract digestibility (ATTD) of DM, OM, CP, NDF and ADF was calculated as

$$\text{ATTD} = \frac{[\text{Intake(g)} - \text{Faecalexcretion(g)}]}{\text{Intake(g)}} \times 100$$

The faecal sample with the first appearance of the respective marker was used to indicate the transit time (TT), which was given as the midpoint of the sampling interval of the respective faecal sample (i.e., if the sample taken 8 h after marker application had contained no marker, but the sample after 12 h had, the TT was indicated as 10 h). The mean retention time (MRT) in the hindgut 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 $C_i$ is the 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 $dt_i$ is the interval (h) of the respective sample

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

## 2.4 | Statistical analysis

MRTs of passage markers (Co, Yb, Cr, Ce) were compared using repeated-measurements analysis of variance and followed by Bonferroni post-hoc pairwise comparisons using R (R Core Team, 2017). A deviation of the ratios of the different marker MRTs from unity (a ratio of 1) was assessed by one-sample *t*-test. A level of $p_{adj.} < 0.05$ was considered significant.

# 3 | RESULTS

All horses tolerated the experimental procedures well. The ATTD (mean ± SD) for DM, OM, CP, NDF and ADF was 59 ± 1%, 59 ± 1%, 77 ± 2%, 54 ± 2% and 49 ± 3% respectively (Table 1).

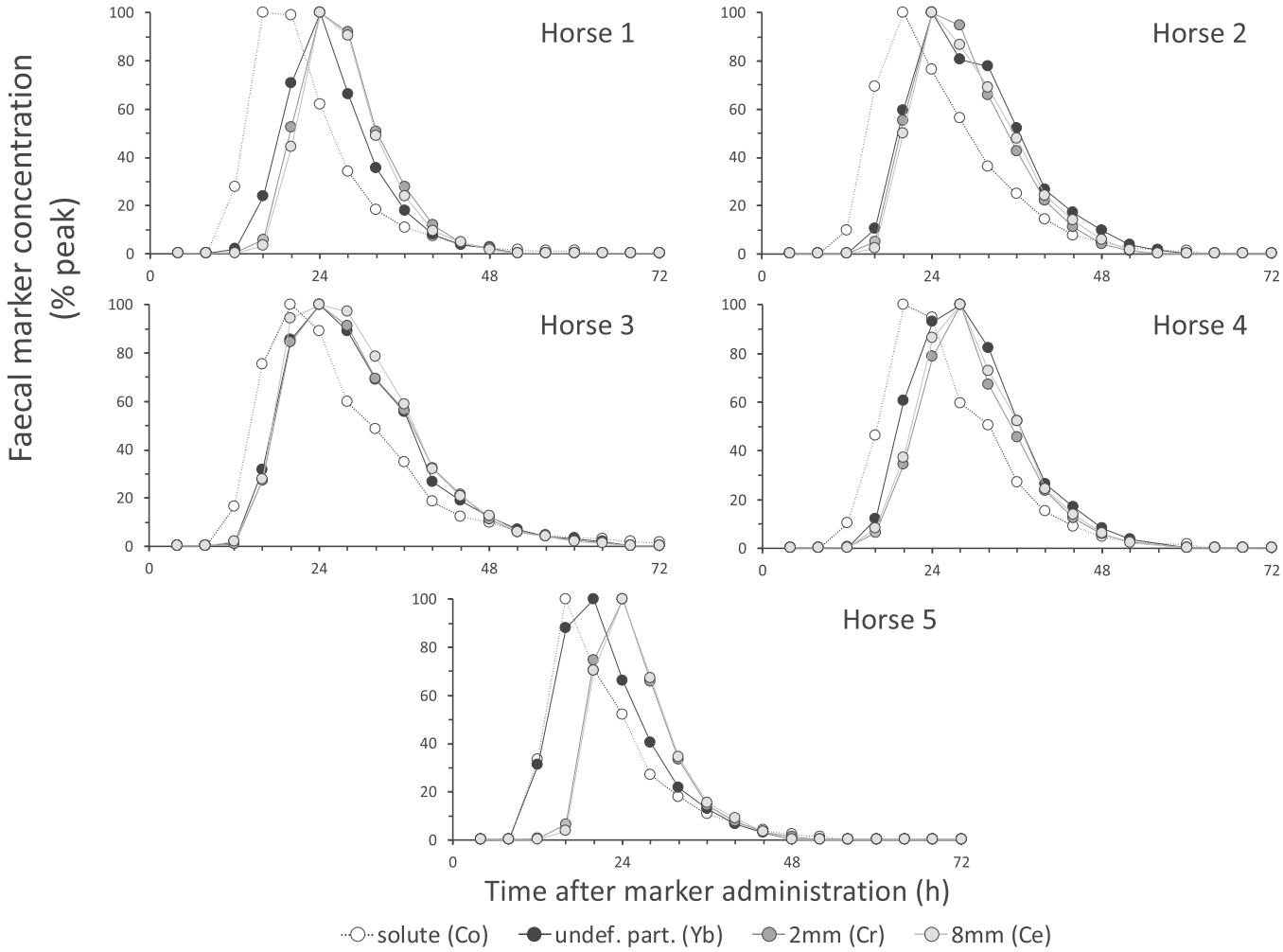


**FIGURE 1** Passage marker excretion patterns in five caecum-cannulated horses; four markers (solute: cobalt-EDTA; undefined small particles: Yb-acetate; 2 mm particles: Cr-mordanted fibre; 8 mm particles: Ce-mordanted fibre) were applied to the cannula in each animal

Marker excretion curves (Figure 1) showed that in all animals, the peak for the solute marker occurred distinctively earlier than that of the particle markers. In all animals, the 2 and the 8 mm particle marker moved in parallel. In four of the five animals, the Yb marker also moved in parallel to the other particle markers, only in horse no. 5, the Yb peaked in between the solute and the other particle markers. The mean TT from the fistula through the hindgut was 10± 0 h for Co (solute marker representing fluids), 11 ± 2 h for Yb (unspecified) and Cr (small particles, 2 mm) and 12 ± 2 h for Ce (large particles, 8 mm), with no significant differences between any markers (Table 2). The MRT in the hindgut was 22.2± 2.4 h for Co, 25.0 ± 3.4 h for Yb, 26.2 ± 1.6 h for Cr and 26.3 ± 1.5 h for Ce (Table 3). Differences between the particle marker MRTs were not significant ($p_{adj.} > 0.05$). The only significant differences were between the solute marker Co and each of the two particle markers Cr and Ce ($p_{adj.} < 0.009$).

Correspondingly, the ratio of a particle marker MRT to that of the solute marker was always higher than 1 (Yb/Co: 1.13 ± 0.07; Cr/Co: 1.18 ± 0.07; Ce/Co: 1.19 ± 0.07). By contrast, the ratio of the two particle markers of defined size to the one of undefined size was not different from unity (Cr/Yb: 1.05 ± 0.09; Ce/Yb: 1.09 ± 0.09), as was the ratio of the former two (Ce/Cr: 1.01 ± 0.01) (Table 4). The original data measured in this study are available as an electronic supplement linked to this article.

**TABLE 2** Transit time of markers applied to the caecum (Co: solutes; Yb: undefined fine particles; Cr: 2 mm particles; Ce: 8 mm particles) of horses

| | Transit time (h) | | | |
|---|---|---|---|---|
| Horse | Co | Yb | Cr | Ce |
| 1 | 10 | 10 | 10 | 14 |
| 2 | 10 | 14 | 14 | 14 |
| 3 | 10 | 10 | 10 | 10 |
| 4 | 10 | 10 | 10 | 10 |
| 5 | 10 | 10 | 10 | 14 |
| mean | 10.0 | 10.8 | 10.8 | 12.4 |
| SD | 0.0 | 1.8 | 1.8 | 2.2 |

**TABLE 3** Mean retention time of markers applied to the caecum (Co: solutes; Yb: undefined fine particles; Cr: 2 mm particles; Ce: 8 mm particles) of horses

| Horse | Mean retention time (h) | | | |
|---|---|---|---|---|
| | Co | Yb | Cr | Ce |
| 1 | 20.0 | 23.6 | 25.4 | 25.4 |
| 2 | 23.0 | 27.5 | 26.6 | 27.2 |
| 3 | 24.8 | 27.0 | 27.3 | 27.1 |
| 4 | 23.9 | 27.4 | 27.7 | 27.7 |
| 5 | 19.5 | 19.8 | 23.8 | 24.1 |
| mean | **22.2**[a] | **25.0**[ab] | **26.2**[b] | **26.3**[b] |
| SD | **2.4** | **3.4** | **1.6** | **1.5** |

*Note*: [a,b]means with no common superscripts differ significantly ($p_{adj.} < 0.05$, repeated-measurements analysis of variance and Bonferroni post-hoc).

**TABLE 4** Ratios of the mean retention times of markers applied to the caecum (Co: solutes; Yb: undefined fine particles; Cr: 2 mm particles; Ce: 8 mm particles) of horses

| Horse | Ratio | | | | | |
|---|---|---|---|---|---|---|
| | Yb/Co | Cr/Co | Ce/Co | Cr/Yb | Ce/Yb | Ce/Cr |
| 1 | 1.18 | 1.27 | 1.27 | 1.08 | 1.08 | 1.00 |
| 2 | 1.19 | 1.16 | 1.18 | 0.97 | 0.99 | 1.02 |
| 3 | 1.09 | 1.10 | 1.09 | 1.01 | 1.00 | 0.99 |
| 4 | 1.15 | 1.16 | 1.16 | 1.01 | 1.01 | 1.00 |
| 5 | 1.01 | 1.22 | 1.23 | 1.20 | 1.22 | 1.01 |
| mean | **1.13** | **1.18** | **1.19** | **1.05** | **1.06** | **1.01** |
| SD | **0.07** | **0.07** | **0.07** | **0.09** | **0.09** | **0.01** |
| *P*[a] | 0.021 | 0.003 | 0.004 | 0.254 | 0.235 | 0.477 |

*Note*: Bold values are statistically significant at $p < 0.05$.

[a]one-sample *t*-test comparison against unity (1.00).

## 4 | DISCUSSION

The results complement those of another study on selective particle retention in the digestive tract of the horse that used a different method but came to the same conclusion (Hummel et al., 2018). In that study, the passage markers were fed as whole mordanted forages that were chewed during ingestion by the animals, all faecal samples were fractionated for particle size classes by wet sieving, and the marker excretion patterns were analyzed separately for the different particle size classes. Thus, there is no evidence for a physiological discrimination by particle size in the total digestive tract, and also not in the total hindgut, of the horse, as investigated with forage-based particle markers. Previous findings to the contrary, which had been found in a study also using caecum-cannulated horses (Argenzio, Lowe et al., 1974), are most likely an effect of the plastic particles (and their very large size) used in that study. The finding of no discrimination by particle size also matches observations that in the equid hindgut, there is no difference in the particle retention time for hay fed whole and a barley-based pelleted concentrate fed simultaneously (Austbø & Volden, 2006), even though it cannot be excluded that ingestive mastication reduced all material to the same particle size. Note that this similar retention of hay and concentrate does not contradict findings that if the same feed is fed at the same intake level at different particle sizes, horses have different overall retention times between these treatments (Drogoul et al., 2000), even though this effect is also often limited or absent (Miyaji et al., 2011; Moore-Colyer et al., 2003; Silva et al., 2014). This difference in overall retention time observed by Drogoul et al. (2000) does not imply a discrimination by particle size in the digestive tract, but different effects of the same diet at different particle size on overall gut motility. Although we did not observe a size-dependent particle retention, we cannot exclude the existence of a selective retention of coarse particles at the junction of ventral and dorsal colon and a selective retention of fine particles at the boundary of the dorsal proximal and the distal colon (Drogoul et al., 2000) with total hindgut retention times finally leveling each other out in our and other studies. The adaptive value of such a compensating mechanism remains to be demonstrated both empirically and theoretically.

In ruminating foregut fermenters (ruminants and camelids), the selective retention of large particles in the forestomach serves their subsequent resubmission to repeated mastication after regurgitation into the oral cavity (Dittmann et al., 2015). In animals that do not ruminate, a selective retention of large particles would not be expected, and so far, large non-ruminant herbivores—whether foregut or hindgut fermenters—have not been found to discriminate passage markers by particle size (Clauss et al., 2004; Matsuda et al., 2019; Munn et al., 2012; Schwarm et al., 2008, 2009), similar to our findings in horses. In small herbivore that practice coprophagy ('caecotrophy') as part of their digestive strategy, a selective retention of small particles—including the microbes that are a major component of the 'caecotrophs'—has been suggested repeatedly (Cork et al., 1999). This latter strategy has also been linked to excreting the more-difficult-to-digest larger particles sooner, to rid the large intestine of this putatively intake-limiting ballast. We cannot exclude the possibility that such a selective excretion of larger particles could also make sense in equids; yet, evidence for such a mechanism is lacking. Observations that on more finely ground or chopped diets, MRT are typically slightly longer in horses compared to coarser equivalents fed at the same intake level, are parsimoniously explained by the more distinct stimulation of gut motility by the coarser material (Drogoul et al., 2000).

The main limitation of the present study was that the particles marked by the Yb marker remained undefined. Ytterbium was applied as a liquid, yet it is known to bind to particles, particularly to fine ones (Erdman & Smith, 1985; Siddons et al., 1985). Our results suggest that this is how the marker behaved, with the exceptions of horse no. 5 and, to a lesser degree, horse no. 1, in which a certain proportion of the Yb may have moved with the fluid digesta phase. Typically, when

preparing Yb-labelled particles, the marker material is washed as the last step of marker preparation, to eliminate unbound marker (Austbø & Volden, 2006). In future studies, it is recommended to reduce this uncertainty by applying Yb to defined (ideally, fine) particles and use this material after appropriate washing, rather than injecting Yb acetate.

Another limitation of the present study was that we could only assess the horses on one level of intake. When expressing the relative daily DM intake on the basis of body weight, the 11–13 g/kg BW used in the present study are at the 12.5 g/kg BW that Harris et al. (2017) consider 'the absolute minimum' in a feeding regime that also contains compound feeds; these authors state that lower amounts should only be given if no other feeds are part of the diet, and only under special circumstances. When expressing the relative DM intake on the basis of metabolic body weight, the 55–62 g/kg$^{0.75}$ used in the present study still within, but at the lower part of, the range of intakes previously reported for passage studies in horses (reviewed in Clauss et al., 2014). In the present study, the feed restriction followed the typical regime used in the experimental animals to prevent weight gain. Evidently, if would be desirable to also include higher relative intake levels, and thus be more representative for the range of intake observed in horses, in future studies. Although the ratio of small particle versus fluid MRT is not affected by intake level in horses (reviewed in Clauss et al., 2014), no studies on an effect of different intake levels on a putative change in the retention patterns of small versus large particles exist. In ruminants, particle size separation is typically least pronounced at higher gut fill, with large particle escape from the rumen being more likely immediately during or after feeding (Hummel et al., 2018). In combination with Hummel et al. (2018), one cannot claim with evidence that at daily DM intake levels below 55 g/kg$^{0.75}$ or above 68 g/kg$^{0.75}$, a differentiation of particle retention by size does not occur in horses. However, given the findings of the present study and of Hummel et al. (2018), it appears prudent to not claim that such a differentiation occurs until evidence is provided, rather than waiting for evidence of absence.

Retention times measured in horses with a caecal cannula may be longer than those measured in the same individuals prior to cannulation surgery (Austbø & Volden, 2006; Pulse et al., 1973), but on the other hand, Drogoul et al. (2000) found shorter MRT in cannulated ponies as compared to non-cannulated animals fed the same diets at the same intake level. However, because we did not aim to determine the absolute retention time of the diet in the present experiment but only the difference between the different markers, this is not a concern regarding our results.

An important question is whether the markers applied are actually representative for material found in the digesta. In horses, we expect roughly about a quarter of all digesta particles to be smaller than 0.125 mm, and about 80% to be smaller than 2 mm (Hummel et al., 2018). Nevertheless, particles of a size of 8 mm do occur in the faeces. In cattle, in which size discrimination is well-described as in all ruminants investigated so far (Dittmann et al., 2015), particle markers from the same batch of defined sizes as those used in the present study yielded a clear signal of differential retention, with larger particles being retained longer for 5–6 h (Grandl et al., 2018). Therefore, the absence of size discrimination in our horses is not due to an absence of susceptibility to such discrimination in these markers.

Our findings replicate the well-known fact that there are significant, albeit small, differences in the MRT between the fluid and the particulate digesta phase in horses. The magnitude of this difference has been described by the ratio of the particle/solute marker retention, and the 1.13–1.19 measured in the present study in the hindgut match both, previously determined magnitudes between 1 and 1.5 measured in the total digestive tract (reviewed in Clauss et al., 2014), even though individual reports of values below 1 or values up to 1.68 have been reported (Drogoul et al., 2000; Pearson et al., 2006). Magnitudes measured in the hindgut in other studies with caecum-cannulated horses were as high as 1.24 (Udén et al., 1982) or 1.43 (Drogoul et al., 2000). However, the low ratios of 1 observed in two individuals of Udén et al. (1982), in caecum-cannulated donkeys at 1.02–1.04 (Ouedraougo, 1998) and in horses on ground forage material at 1.06 (Drogoul et al., 2000) indicate that a simultaneous movement of fluid and particles can also occur. A major effect of a faster liquid compared to particulate passage is the washing of very small particles, including microbes, out of the digesta in the direction of the fluid flow (reviewed in Hristov et al., 2019; Müller et al., 2011). For hindgut fermenters like horses, which do not normally use gastrointestinal microbes via coprophagy as part of their digestive strategy, the value in such digesta washing has been suggested to lie in maintaining a microbial population in the active growth rather than in the putatively less active maintenance stage. Whether this plays a role for horses remains to be investigated.

## 5 | CONCLUSION

To conclude, pending further investigations, possibly into the fate of very fine marked particles representing microbes, narratives explaining the digestive physiology of horses should not build on a selective retention of a certain particle size class in their hindgut.

### AUTHOR CONTRIBUTIONS

All authors designed the study. Angela Schwarm and Rasmus B. Jensen performed the animal experiment. Sylvia Ortmann provided the analyses of the markers. Angela Schwarm, Marcus Clauss and Rasmus B. Jensen wrote the manuscript with input from Sylvia Ortmann.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

The original data measured in this study are available as an electronic supplement linked to this article.

## ORCID

*Angela Schwarm* http://orcid.org/0000-0002-5750-2111
*Marcus Clauss* http://orcid.org/0000-0003-3841-6207
*Sylvia Ortmann* http://orcid.org/0000-0003-2520-6251
*Rasmus B. Jensen* http://orcid.org/0000-0001-6108-0233

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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