Passage marker excretion in red kangaroo (Macropus rufus), collared peccary (Pecari tajacu) and colobine monkeys (Colobus angolensis, C. polykomos, Trachypithecus johnii)

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Key words: Mean retention time, fluids, particles, forestomach, foregut fermenter
Abstract

Ruminants are characterised by an efficient particle sorting mechanism in the forestomach followed by selective rechewing of large food particles. For the non-ruminating foregut fermenter pygmy hippo it was demonstrated that large particles are excreted as fast as, or faster than, the small particles. The same has been suggested for other non-ruminating foregut fermenters. We determined the mean retention time of fluids and different sized particles in six red kangaroos (*Macropus rufus*), seven collared peccaries (*Pecari tajacu*) and three colobine monkeys (*Colobus angolensis, C. polykomos, Trachypithecus johnii*). We fed Co-EDTA as fluid and mordanted fibre as particle markers (Cr, Ce). Mean (± SD) total tract retention time for fluids, small and large particles was 14±2 h, 29±10 h and 30±9 h in red kangaroos, 26±2 h, 34±5 h and 32±3 h in collared peccaries and 57±17 h, 55±19 h and 54±19 h in colobine monkeys, respectively. Large and small particles were excreted simultaneously in all species. There was no difference in the excretion of fluids and particles in the colobine monkeys, in contrast to the other foregut fermenters. In the non-primate, non-ruminant foregut fermenters, the difference in the excretion of fluids and small particles decreases with increasing food intake. In contrast, ruminants keep this differential excretion constant at different intake levels. This may be a prerequisite for the sorting of particles in their forestomach and enable them to achieve higher food intake rates. The functional significance of differential excretion of fluids and particles from the forestomach requires further investigations.
Introduction

Foregut fermentation has evolved at least four times independently in mammals, namely in
kangaroos (Marsupialia, Macropodidae), in tree sloths (Bradypodidae), among primates in the
Colobinae and Cercopithecidae, and within the large monophyletic group that includes the
camels, pigs, peccaries, whales, hippos and ruminants (Cetartiodactyla) (Karasov and
Martínez del Rio, 2007).

Foregut fermenters can be divided into those that ruminate and those that do not. Ruminating
foregut fermenters such as cattle chew their food repeatedly, during ingestion and
rumination. They clear the gut of the digested small particle fraction and rechew only large
particles, thereby achieving high proportions of small digesta particles and thus increasing the
area exposed to microbial attack per quantity of food (Pond et al., 1984; Bjorndal et al., '90).
In contrast, some hindgut fermenters such as horse and rabbit maintain high food intake by
clearing the gut from large particles and retaining the smaller, easier-to-digest particles
(Björnhag et al., '84; Cork et al., '99). Based on these observations in hindgut fermenters,
Hume ('99, p 240) and Clauss et al. (2004) suggested that large particles might be excreted
faster than small particles from the forestomach of non-ruminants such as macropods and
sloths, respectively, as a means of clearing less digestible bulk from the forestomach. In
feeding trials with non-ruminating foregut fermenting hippos, Clauss et al. (2004) and
Schwarm et al. (2008) observed that large particles were either excreted faster than small
particles or together with the small particles. The faster excretion of large particles was,
however, not accompanied by a high food intake but even occurred at the low food intake
typical for hippos (Schwarm et al., 2006). Actually, in interspecific comparisons of
mammalian herbivores, non-ruminanting foregut fermenters generally had a lower food intake
than hindgut fermenters or ruminants (Clauss et al., 2007). This general pattern suggests that
regardless of the reported findings on particle retention, efficient intake-enhancing sorting
mechanisms are absent from the forestomach of non-ruminating foregut fermenters; yet, comparative data on their physiology are missing.

Another general feature of all non-ruminating foregut fermenters could be the differential excretion pattern of fluids and particles. Kangaroos and hippos excrete particles distinctively later than fluids, resulting in an excretion pattern characterised by two well-separated peaks (Dellow, ’82; Hume, ‘99; Clauss et al., 2004). In contrast, ruminants are characterised by a more convergent excretion of fluids and particles (e.g. Dellow, ‘82; Schwarm et al., 2008).

The delay in particle excretion in kangaroos and hippos, and possibly in other non-ruminating foregut fermenters, might be related to food intake (kangaroo: Munn and Dawson, 2006; Munn et al., 2008, hippo: Schwarm et al., 2006, 2008), or to the morphology of the fermentation chamber.

In general, forestomachs consists of compartments which are primarily sacciform and others that are primarily tubiform. In terms of chemical reactor theory, sacciform compartments are analogue to ‘batch reactors’ or ‘continuous-flow stirred-tank reactors’, with well-mixed contents. Tubiform compartments are analogue to ‘plug-flow reactors’ (Caton and Hume, 2000), with no or little axial mixing of digesta. In sacciform compartments digesta flow is directed ‘off-set’ and in tubiform ‘in-line’ (Langer ’88). In foregut fermenters the proportions of these different compartments vary; according to Langer (’88) the proportions of the tubiform compartment decreases, and that of the sacciform increases, from grazing kangaroos to hippos, peccaries, colobine monkeys, ruminants/camelids, and sloths.

Martinez del Rio et al. (’94) and Jumars (2000) explained how passage marker excretion patterns can be used to understand the function of animal guts in terms of reactor theory. An immediate, steep increase in marker excretion and a smooth decrease is typical for a single continuous-flow stirred-tank reactor (e.g. ruminant), whereas in a tubiform compartment (which is understood as a series of many continuous-flow stirred-tank reactors),
the increase in marker excretion is more gradual and the decrease steeper and more similar to
the increase (e.g. kangaroo).

Data on the pattern of fluid and particle passage in other non-ruminating foregut
fermenting are currently scarce (colobine monkeys: Caton, '99; Nijboer et al., 2007; sloth:
Foley et al. '95) or missing (peccaries). We investigated passage time of fluids, small and
large particles in three non-ruminating foregut fermenters, the red kangaroo (*Macropus rufus*),
the collared peccary (*Pecari tajacu*) and colobine monkeys (*Colobus angolensis spec.; C.
polykomos; Trachypithecus johnii*) (for taxonomic classification see Wilson and Reeder,
2005). We predicted that non-ruminating foregut fermenters excrete large and small particles
simultaneously, and thus lack efficient sorting mechanisms that are characteristic for
ruminants. Additionally, we predicted that the degree of fluid-particle-separation decreases
with an increasing proportion of the sacciform forestomach compartment.

**Materials and Methods**

The trials were performed with six red kangaroos, seven collared peccaries and three colobine
monkeys at the Zoological Gardens of Berlin (ZGB), Stuttgart (ZGS), Kaiserslautern (ZGK),
Erfurt (ZGE) and Dortmund (ZGD) in 2004, 2005, 2006 and 2007. Body mass (BM) of four
peccaries and the colobine monkeys were measured at the end of the trial, whereas BM of the
kangaroos and the other three peccaries was estimated by the keepers by visual judgement
(height and width) and age and sex as reference parameters. Details of the animals are
summarized in Table 1.

Kangaroos were fed with forage-only diets consisting of grass or a grass-lucerne mix; dried
forage was used as bedding. The peccaries and colobine monkeys were fed a mixed diet, in
peccaries consisting of rabbit concentrate (Holstenstolz, 23685 Pansdorf, Germany; 15%
crude fibre in dry matter), apples, green vegetables (salad, curly kale, chard, stem cabbage
leaves) and carrots. Of the colobine monkeys, the Angolan black and white colobus (*Colobus angolensis adolfi-frederici*) was fed with cooked and peeled potato, cooked rice, apple, peeled banana, leaves (plum, cherry, ash, lilac; 9 % of the ingested dry matter), salad and peeled egg. The Nilgiri langur (*Trachypithecus johnii*) and the Southern black and white colobus (*Colobus polykomos*) were fed with leaves (45 % and 47 % of the ingested dry matter, respectively), apples, vegetables (sweet potato, leek, carrot, stem cabbage, pepper, chicory, stem celery) and salad. Leave species fed were mainly ash, cherry and plum, and additionally lilac, Norway maple, forsythia, rose and ash leave maple, which were plucked from the twigs before feeding. The foliage was stored in a freezer and thawed overnight. An adaptation period of 14 days was allowed to pass before the trials started. Each trial lasted 7 days in peccary 1-4 and colobine monkeys, 6 days in peccary 5-7 and 4 days in kangaroos.

Three kangaroos (1-3) and four peccaries (1-4) could be kept and fed separately. The other three kangaroos (4-6) could only be kept and fed separately during the night and three peccaries (5-7) could not be separated at all. Faeces of animals that were kept together could be ascribed to the individuals due to different coloring agents in their food ration (kangaroo 4, peccary 6: titanium dioxide 4-6 g/d; kangaroo 5, peccary 7: brilliant blue food colour, Sensient Food Colors, Geesthacht, Germany, approx. 0.5 g/d; both fed twice daily; the colors were mixed with a small peace of apple or peeled banana for better acceptance).

All animals had ad libitum access to food until the next meal was offered. Feeding frequency differed between species due to husbandry conditions at the respective zoo. Three kangaroos (1-3) received food once daily, approximately at 09:00 hours. The other kangaroos (4-6) received another meal in the afternoon, at approximately 18:00 hours. Peccaries were fed twice daily, at approximately 08:00 and 14:30 hours. Colobine monkeys received food three times a day, at 07:00, 11:30 and 15:30 hours. Food items offered and leftovers were quantified on the individual or group level on a daily basis by weighing, and representative
samples (for each individual or for the group) of food offered and leftovers were stored frozen (-20°C) until analysis. Drinking water was always provided ad libitum to all animals.

The mean retention time (MRT) of particles and fluids through the forestomach and the gastrointestinal tract was determined with chromium-mordanted fibre (2 mm) and cerium-mordanted fibre (10 mm) as particle marker and cobalt ethylenediaminetetraacetate (Co-EDTA) as fluid marker. Marker were prepared according to Udén et al. ('80), Heller et al. ('86a) and Schwarm et al. (2008) and fed as a pulse dose (together with a portion of fruits for better acceptance) at approximately 15:00 to max. 16:00 hours on the day before the trial started.

Time of individual defecations was recorded during the day, feces voided at night were treated as one defecation unit, and an average time between the last check in the evening and the first check in the morning was calculated (circa 01:00 h). Individual defecations were collected in toto, had sand removed from them, weighed, and the whole sample or an aliquot (after thoroughly mixing; max. 300 g) was stored frozen.

Food and faecal samples were dried at 40 °C and 60 °C, respectively. Dry matter (DM) content of food and faecal subsamples was determined by drying at 103 °C to constant weight. Faecal samples were ground with a ‘Nossener mill’ (Gebrüder Jehmlich, Nossen, Germany, 1 mm round perforated screen). Individual dry matter intake in kangaroo 4-6 and peccary 5-7 was extrapolated from faecal (acid detergent) lignin content, an indigestible compound of plant cell walls. Acid detergent lignin was analysed according to Van Soest (1967) and Van Soest et al. (1991) using the Ankom Fibre Analyser. Faecal subsamples were microwave digested and analysed for Cr, Ce and Co concentration by mass spectrometry following Schwarm et al. (2008). MRT was calculated for both the gastro-intestinal tract (GIT, Thielemans et al., '78) and the forestomach (FRST, Hungate, '66; Grovum and Williams, '73). The latter calculation is based on the decrease of the faecal liquid marker
concentration $c_i$ at time $t$ (mg/kg DM) with time after marker application $t_i$ (h) according to the equation:

$$\ln c_i = -k t_i + b$$

with $k = \text{rate constant (h}^{-1})$ and $b = \text{intercept}$. The reciprocal of $k$ represents the fluid MRT in the FRST. Because fluid and particles do not differ in passage characteristics distal to the FRST (empirically confirmed for ruminants by Grovum and Williams 1973; Kaske and Groth 1997; Mambrini and Peyraud 1997 and for kangaroos by Dellow 1982), particle MRT in the FRST ($\text{MRT}^{\text{particle FRST}}$) is calculated as follows:

$$\text{MRT}^{\text{particle FRST}} = \text{MRT}^{\text{particle GIT}} - (\text{MRT}^{\text{fluid GIT}} - \text{MRT}^{\text{fluid FRST}}).$$

The “selectivity factor” – the quotient of particle over fluid MRT – was calculated according to Lechner-Doll et al. (’90).

The Kruskal-Wallis-Test (ANOVA) with *a posteriori* multiple comparisons was used for comparisons between species. The colobine monkey species (*Colobus angolensis*, C. *polykomos* and *Trachypithecus johnii*) were treated as one unit (n=3). Within species, monotonous associations between pairs of variables were assessed by calculating Spearman’s rank correlation coefficients (SCC). Power estimations for the SCC test were based on the respective calculations for the Pearson Coefficient, after assigning ranks to the data. Repeated Measures ANOVA and linear contrasts (referring to the 2 mm results as the reference category) of fluids, small (2 mm) and large particles (10 mm) served to compare fluid and particle mean retention times (MRT) within each species. The significance level was set to $\alpha=0.05$. Statistical calculations were performed with SPSS 16.0 (SPSS, Chicago, IL) or Statistica 8.0 (StatSoft Inc., Hamburg, Germany) (only multiple comparisons after Kruskal-Wallis-Test), and nQery 7.0 (Statistical Solutions Ltd., Cork, Ireland).
Results

The average relative dry matter intake (rDMI, mean ± SD) in red kangaroos and colobine monkeys did not differ significantly from each other, constituting 34±14 g/(kg$^{0.75}$d) (n=6) and 27±1 g/(kg$^{0.75}$d) (n=3), respectively, and was significantly lower in both species than in collared peccary (53±4 g/(kg$^{0.75}$d)) (n=7) (Table 2).

The mean MRT$_{\text{fluid GIT}}$, MRT$_{2 \text{ mm particle GIT}}$ and MRT$_{10 \text{ mm particle GIT}}$ in red kangaroos were 14±2 h, 29±10 h and 30±9 h and in collared peccaries 26±2 h, 34±5 h and 32±3 h. In colobine monkeys, the respective MRTs were 57±17 h, 55±19 h and 54±19 h (Table 2). Large and small particles were excreted simultaneously in all species (representative excretion curves in Figs. 1 a-c; statistics in Table 3). One peccary differed in the particle marker excretion pattern from the other peccaries, because the peak concentration and the baseline level for large particles were reached earlier than those for small particles (animal 5). Accordingly, in that animal large particles were excreted, on average, 11 hours earlier than small particles. Fluids and particles were excreted consecutively with two distinct peaks in the kangaroos, whereas fluid and particle curves were less separated in the peccaries, and colobine monkeys excreted fluids and particles simultaneously (one-peak excretion) (Fig. 1 a-c). In kangaroo and peccary, fluids were excreted significantly faster than particles (both from the gastrointestinal tract (GIT) and the forestomach (FRST)), in contrast to colobine monkeys (Table 3).

Colobine monkeys had on average longer MRT$_{\text{fluid GIT}}$, MRT$_{2 \text{ mm particle GIT}}$ and MRT$_{10 \text{ mm particle GIT}}$ than kangaroos and peccaries, and the differences to the kangaroos were significant (Table 2). The selectivity factor for 2 mm particles compared to fluids (GIT) was, on average, higher in kangaroo and peccary than in colobine monkeys, and again the difference between kangaroo and monkeys was significant (Table 4). The selectivity factor for
10 mm particles as compared to 2 mm particles (GIT) did not differ significantly between species (Table 4).

Neither in kangaroo, nor in peccary was the MRT$_{2\text{mm particle}}$GIT correlated to the rDMI (kangaroo: n=6, SCC=-0.41, p=0.425, power=0.11; peccary: n=7, SCC=0.50, p=0.253, power=0.19; monkeys were not considered for this analysis because of the small sample size of 3). Similarly, the selectivity factors (for 2 mm particles compared to fluids, 10 mm particles compared to fluids and 10 mm compared to 2 mm particles - GIT) were not associated with rDMI in kangaroo and peccary (kangaroo: n=6, SCC=-0.26, p=0.623, power=0.06; SCC=-0.26, p=0.623, power=0.06; SCC=-0.15, p=0.784, power=0.04 and peccary: n=7, SCC=0.33, p=0.465, power=0.10; SCC=0.26, p=0.574, power=0.07; SCC=-0.22, p=0.629, power=0.06, respectively). Insignificant correlations might be due to the very small power for the small data sets. However, the majority of the correlation coefficients are very small and do not indicate a meaningful monotonous relationship, such that a significant result could eventually be suspected only for the correlations between MRT$_{2\text{mm particle}}$GIT and rDMI (both species).

The FRST MRTs calculated in kangaroo 6 for the different markers are overestimated. The fluid marker concentration did not decline to the baseline level before marker feeding, but declined in small steps at a slight elevated level, resulting in a fluid MRT distal to the stomach of 1 hour, which is not physiological. Even a correction as applied by Schwarm et al. (2008, fluid marker concentration set to zero when it dropped below 1% of the peak concentration) did not amend the result.

**Discussion**

This study describes patterns of digesta excretion from the digestive tract of three groups of nonruminant foregut fermenters. As predicted, the results do not indicate a particle sorting
mechanism in the forestomach of these animals. As predicted, the degree of fluid-particle-separation decreased with the increasing proportion of the sacciform forestomach compartment from kangaroo to peccary to colobus monkey. The data collected in this study allow some interesting speculations about functional differences between ruminating and non-ruminating foregut fermenters.

Passage times of small vs. large particles

The results of this study are consistent with the hypothesis of a functional difference in the excretion mechanism of different sized particles from the digestive tract of non-ruminating and ruminating foregut fermenters. As predicted, kangaroos, peccaries and colobine monkeys on average excreted large particles not later than small particles from the digestive tract like ruminants do. Instead, large particles were excreted simultaneously with small particles in kangaroos, peccaries and colobine monkeys. This is in accordance with another non-ruminating foregut fermenter, the hippo, which excreted large particles at the same time as small particles or earlier (Clauss et al., 2004; Schwarm et al., 2008). Apparently, hippos, kangaroos, peccaries and colobine monkeys lack a sorting mechanism for ingesta particles. In this study, passage times were assessed with an orally-fed particle marker and ingestive mastication could not be prevented. Although the faeces of kangaroos, peccaries and colobine monkeys contained particles larger than 10 mm (the size of the large particle marker), the difference in size between the two markers was possibly less distinct than if the markers had been introduced directly into the forestomach of kangaroo, peccary or colobine monkeys, respectively. Kangaroos, peccaries and monkeys have a lower body mass than pygmy hippos and banteng cattle (kangaroos: 17-100 kg, peccaries: 19-23 kg, colobine monkeys: 8-12 kg, pygmy hippos: 196-248 kg, banteng cattle 200-700 kg). Since body mass and food (faecal) particle size are linked – with smaller species having smaller faecal particles (Udén and Van
Soest, '82; Clauss et al., 2002) –, kangaroos, peccaries and colobine monkeys should chew the food - and the marker - to smaller particles than the heavier pygmy hippos. Kangaroos are sexually dimorph; however, both the light and the heavy kangaroos (20 kg vs. 60 and 100 kg) excreted small and large particles simultaneously, i.e. body mass (and thus surface area of tooth row; Lanyon and Sanson, 2006) did not impact differential particle size excretion in this species in this study. When the same markers were fed to pygmy hippos and banteng cattle, a difference in particle excretion was observed (Schwarm et al., 2008), demonstrating that oral feeding of particle marker per se did not prevent detection of differences in their excretion pattern. One peccary of this study (peccary 5) excreted large particles 11 hours earlier than small particles (Table 2), similarly to one pygmy hippo in the study of Schwarm et al. (2008, Fig. 1, animal 2 LI). It may not be a coincidence that in both species differential particle excretion occurred sporadically, since both species are not able to chew their food particularly efficiently because of their interlocking canines, and thus may produce particularly large food particles at times.

With the occasional faster excretion of large particles, the peccary and hippo resemble the (non-cecotrophic) hindgut fermenter horse and the (cecotrophic) hindgut fermenter rabbit. The selective excretion of large particles in horses and rabbits is achieved by the colonic separation mechanism (Björnhag, '87). This mechanism is usually interpreted as a trait that facilitates high food intake by quickly clearing the gut of the more difficult-to-digest larger particles (Cork et al., '99). However, the one peccary and one hippo in which large particles were excreted faster than small particles had a similar relative dry matter intake as the other animals of the same species. These observations suggest that any possible separation mechanism, even if operating in the sense of a selective retention of smaller particles in the (ventral tip of the) forestomach blindsacs of these species (Clauss et al., 2004; Wings et al., 2008) is not as effective as the separation mechanism in ruminants or hindgut fermenters.
However, the same experiment should be performed with many different individuals, not just to fulfil the absolute minimum requirements for statistical analyses in terms of sample sizes, but also to illustrate and understand physiological functions and their intraspecific variation.

To corroborate the results of this study, the distribution of small and large particles between dorsal and ventral regions of the forestomach of non-ruminating foregut fermenters should be investigated. In grazing ruminants the faster excretion of small than large particles is reflected in their distribution in the reticulorumen. Particles are sorted according to their density with large particles flotating in the dorsal rumen and with small particles sedimenting in the ventral rumen (Grau, '55; Sutherland ‘88; Kaske et al. ’92; Hummel et al., 2008b, Clauss et al., 2009). For other foregut fermenters, according studies are rare. They indicate that particles in the peccary forestomach are not sorted according to different size or density (Langer, ’79); in hippos there is some evidence that this could be the case (Langer, ’76; Wings et al., 2008). In other foregut fermenters, foregut sections were only sampled as a whole and not in a well differentiated way (e.g. dorsal vs. ventral or central vs. peripheral content), so that conclusions on separation mechanisms within these sections are not possible (kangaroo: Freudenberger, ’92; Lentle et al., 2002; sloth: Langer, ’88; Foley et al., ’95). For kangaroos it has been suggested that digesta passage may be regulated by temporary sacculations (haustractions) in the forestomach which also prevent that microbes are washed out in great quantities (Langer, ’88; p 363).

Passage times of fluids vs. particles

The separate excretion of fluids and particles is not a general feature of non-ruminant forestomachs and appears to be related to the digestive anatomy, with more sacciform forestomach tending towards a more simultaneous excretion of the two digesta phases (Table 3, Fig. 1). In colobine monkeys, fluids and particles were simultaneously excreted, similar to
previous findings (Caton, '99; Nijboer et al., 2007 with a different marker system; note that
the level of dry matter intake was not assessed in these studies). Additionally, Foley et al.
(‘95) demonstrated – albeit with a different marker system – that there was no difference in
the excretion of fluid and particles in the three-toed sloth (Bradypus tridactylus).

We compared the results of this study with data from the literature where passage time
was assessed with the same markers, cobalt-EDTA and chromium mordanted fibre. The
cangoos and colobine monkeys had shorter particle MRTs than pygmy and common hippo
and the Bactrian camel, *Camelus bactrianus*, on similar rDMIs (Fig. 2). Peccaries achieved
similar particle MRTs as kangaroos and colobine monkeys but at higher rDMIs. Ruminants
achieved longer MRTs than peccaries at similar rDMIs. Among ruminants, domestic cattle
maintain particularly long particle MRTs. rDMI and particle MRT were not correlated if data
from all species were used (n=19, SCC=-0.15, p=0.539; Fig. 2), which is due to the
comparatively short particle MRTs on the comparatively low rDMIs in colobines and
macropods. These groups are characterized by comparatively low capacities of their
forestomachs (see below). If these groups are excluded, particle MRT decreases with
increasing rDMI (n=12, SCC=-0.67, p=0.017).

Concerning the passage time of fluids, colobine monkeys and camels had
comparatively long fluid MRTs whereas kangaroos, peccaries, hippos and ruminants are
characterised by short fluid MRTs and thus a high fluid throughput (Fig. 3). In an analysis
with all species fluid MRT decreased with increasing rDMI (n=19, SCC=-0.53, p=0.019; Fig.
3). Ruminants achieved these MRTs for fluids at notably higher rDMIs than kangaroos and
hippos. Accordingly, the ratio of particle over fluid MRT is low in monkeys, high in hippos
and comparatively high in ruminants for their high rDMI (Fig. 4 a).
Fluid and particle passage in relation to gut volume, salivary flow rate, dry matter intake, forestomach morphology and diet

The excretion of fluids and particles is, amongst others, likely to be a consequence of the interplay of gut volume, salivary flow rate, dry matter intake, forestomach morphology, and diet. Assuming that MRT, DMI and gut volume are linked, shorter particle MRTs in kangaroos and monkeys than in hippos (and camels) on similar rDMIs (Fig. 2) could be a consequence of a smaller forestomach volume (kangaroo: 6-11% of body weight – Langer, 1988; colobine monkey: 2-4% - Chivers, '94, 5-8% stomach wet content - Kay et al., '76; common hippo: 11-27% - Langer, '88; camel: 11-24% forestomach volume - Heller et al., '86b). The extremely long ingesta retention times reported by Foley et al. (’95) for sloths, at intakes similar to those measured in colobine monkeys in this study, are probably the result of a particularly high forestomach volume (up to 29% of body weight). Gut-distension is likely to be very limited in kangaroo and colobine monkeys because the hopping gait and the active arboreal mode of living necessitate a tight abdominal musculature (kangaroo: Munn et al., 2008).

Why are fluid MRTs short in ruminants (Fig. 3)? It has been suggested that short fluid MRTs may at least for grazing ruminants (bovids) be required to maintain a stratified forestomach content - the constant supply of low-viscosity fluid supposedly facilitates the separation of small and large particles into a sedimenting and a flotating fraction, respectively (Cahill and McBride, '95; Hummel et al., 2008b). Grazing ruminants have larger omasa which reabsorb fluids distal to the reticulorumen; these larger omasa have been interpreted to be indicators of a particularly high fluid throughput in the forestomach in these species (Clauss et al., 2006a). Short fluid MRTs may also be advantageous to maximise the harvest of microbial nitrogen (N) from the forestomach (Van Soest, '94; Meng et al., '99). It has been shown that microbial growth (measured in g of microbial N/kg of organic matter truly digested) and thus
the supply of microbial protein to the small intestine increases with decreasing fluid MRT (Meng et al., '99). The authors proposed that high dilution rates may select for fast growing microbial species and lead to a greater proportion of microbial flora being in the exponential phase of growth. Thereby nutrient gain may be maximised in foregut fermenters who digest microbial cells in the small intestine. Relatively short fluid MRTs in kangaroo, peccary and hippo may thus enhance bacterial protein yield from the forestomach, without having the additional effect of maintaining a stratified forestomach content as in ruminants. Colobine monkeys (and sloths) presumably have a lower salivary flow rate than other groups, leading to the comparatively long fluid MRTs. However, the saliva volume secreted by colobine monkeys (or sloths) is unknown (Kay and Davies, '94).

The data of the selectivity factor (SF) of 2 mm particles as compared to fluids in relation to rDMI (Fig. 4 b, c) suggest that differences between ruminating and non-ruminating foregut fermenters exist, as well as among the ruminating foregut fermenters themselves. In non-ruminating foregut fermenters, the SF tends to decrease with increasing rDMI (N=55, SCC=-0.26, p=0.053; Fig. 4 b), and if the colobine monkeys are excluded from the analysis because of the comparatively long fluid MRTs (Fig. 3), the SF significantly decreases with increasing rDMI (N=49, SCC=-0.46, p=0.001). In ruminating foregut fermenters, SF and rDMI are not correlated (N=43, SCC=-0.26, p=0.098; without camel: N=42, SCC=-0.26, p=0.100; Fig. 4 c). This analysis should be considered as explorative since repeated measures are included and data have not been corrected for phylogenetic relatedness. However, ruminants, especially bovids, seem to be capable of maintaining a relatively high SF despite high rDMIs. The high selectivity factor is achieved by both the long particle and the short fluid passage time. If the separation of fluids and particles pose a digestive advantage, it remains to be elucidated why colobines did not evolve this mechanism. This differential excretion might be simply a function of DMI in non-primate non-ruminants (Fig. 4 b).
In non-ruminating foregut fermenters the difference between fluid and particle retention time decreases with the increasing proportion of the sacciform forestomach compartment – from kangaroo (and hippo) to peccary to colobine monkey (and sloth). Ruminants are the exception of this pattern, with a notable difference between fluid and particle retention despite the sacciform rumen (continuous-flow stirred-tank reactor, Caton and Hume, 2000).

Martinez del Rio et al. (1994) and Jumars (2000) explained how the excretion pattern as that observed in a ruminant (Fig. 1, banteng) is typical for a single mixing compartment (the ruminoreticulum interpreted as one continuous-flow stirred-tank reactor, CSTR), with an immediate, steep increase in marker excretion indicating rapid mixing of ingesta in the compartment, followed by a smooth excretion that follows a negative exponential function shifted to the right. The authors explained that the marker excretion pattern of the kangaroo, in contrast, resembles that of a series of CSTR (which is similar to a complex or modified plug-flow reactor); such an arrangement is characterised by a more gradual increase and a steeper decrease of marker excretion, so that the increasing and the decreasing part of the excretion curve have a similar slope. Kangaroos and hippos are similar in this respect, with hippos showing a less steep, more gradual pattern. Peccaries and colobine monkeys present an intermediate pattern, with a more gradual increase, and a steeper decrease, than ruminants. Consequently, it can be assumed that the forestomachs of ruminants functions as a very short, that of peccaries and colobines as a short, and that of kangaroos and hippos as a longer series of CSTRs.

An ultimate cause for differences between taxa of non-ruminating foregut fermenters in the excretion of fluids and particles could be related to the natural diet. In ruminants, species adapted to feeding on grass show a pronounced difference between fluid and particle passage, whereas this difference is less pronounced in species consuming diets with low
proportions of monocotyledons (Clauss and Lechner-Doll, 2001; Hummel et al., 2005; Clauss et al., 2006b; Schwarm et al., 2008). Among the nonruminant foregut fermenters, colobines, sloths and peccaries are adapted to diets with no or only a low proportion of monocotyledon material (Corn and Warren, ’85; Waterman and Kool, ’94; Chiarello, ’98); in contrast, red kangaroos are grazers (Chippendale, ’62). However, the pygmy hippopotamus is an opportunistic browser and therefore does not fit this pattern (Eltringham, ’99). Why a differential fluid and particle passage should evolve particularly in grazing herbivores remains to be explained.

Differences in passage times of liquid and solid phase digesta markers reported for the gastrointestinal tract of numerous vertebrate species may be the consequence of incomplete reabsorption of the liquid phase into the digesta (Lentle et al., 2006). Liquid phase is regularly extruded from the solid elements of the digesta plug. Depending on the time interval between subsequent contractions, the concomitant reabsorption of the liquid phase into the digesta plug is limited and liquid is driven aborally (Lentle et al., 2006). The more liquid is put into the digestive tract (as drinking water, saliva, or gastrointestinal secretion), the faster the liquid will move along the tract and through the particle phase. In colobine monkeys (and sloths) the process of liquid extrusion and reabsorption might differ from the other non-ruminating foregut fermenters due to a supposed low amount of fluid supply in the digesta.

Ruminants achieve the high degree of separation of the liquid and solid phase of digesta despite their high rDMI and despite their sacciform forestomach, probably due to a higher salivary flow rate. Ruminants produce a large amount of saliva every day (sheep produce 15 l/d or more, and cattle produce 180 l/d or more), contributing to about 70% of the water entering the rumen (Van Soest, ’94). Total salivary flow is related to time spent eating and ruminating, although some basal flow constantly occurs (Van Soest, ’94). Thus, the strategy of rumination itself could not only lead to a faster forestomach clearance allowing a
comparatively high intake (Schwarm et al., 2009), but also a generally higher saliva production due to repeated chewing, with a consequence of a high SF. A high SF combined with an increased rDMI could be a prerequisite for the impressive performance of grazing ruminants which has evolved to maximise the utilisation of cellulolic carbohydrates, and from which the most productive domestic ruminants are recruited. The relevance of a differential excretion of fluids and particles from the forestomach should be further investigated.

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