# Faecal particle size: Digestive physiology meets herbivore diversity

Marcus Clauss [a], Patrick Steuer [b], Kerstin Erlinghagen-Lückerath [b], Jacques Kaandorp [c], Julia Fritz [a], Karl-Heinz Südekum [b,*], Jürgen Hummel [b,d]

[a] Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Switzerland
[b] Institute of Animal Science, University of Bonn, Endenicher Allee 15, 53115 Bonn, Germany
[c] Safari Park Beekse Bergen, Hilvarenbeek, The Netherlands
[d] Department of Animal Sciences, Georg-August-University, Göttingen, Germany

## ARTICLE INFO

## ABSTRACT

In herbivore ecophysiology, comparative chewing efficiency has only recently received increased attention. This measure is best assessed on un-processed forage-only diets; corresponding comparative datasets are missing. We measured a faecal mean particle size (MPS [mm]) in 14 large herbivore species (body mass (M) range 60–4000 kg; 8 ruminants and 6 hindgut fermenters) fed a consistent grass hay diet, in which intake, digesta mean retention times (MRT [h]) and digestive efficiency (as digestibility of faecal fibre measured by 96 h cumulative in vitro gas production GP96h [ml per 200 mg faecal fibre], and metabolic faecal nitrogen MFN [% organic faecal matter]) had been quantified simultaneously. MPS was generally lower in ruminants than in hindgut fermenters and increased with M in the total dataset, but was nearly constant among closely related taxa (e.g. within ruminants, within equids) irrespective of M. MPS (but not MRT) was significantly correlated to GP96h, whereas MRT (but not MPS) was significantly correlated to MFN, suggesting different effects of these factors on different aspects of digestibility. Combinations of measures including MPS mostly explained digestibility better than other combinations. The phylogenetic signal λ, which was mostly 1 when linking any single measure to digestibility, was estimated 0 in models that linked digestive efficiency to combinations of measures. These results support the intuitive concept that species diversification in large herbivores is tightly related to digestive physiology, and that chewing efficiency as measured by faecal particle size is an integral aspect of this scenario.

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## 1. Introduction

Large herbivores display a conspicuous diversity within and across ecosystems, with a fascinating variety that includes ruminants, camelids, hippopotamids, suids, equids, rhinocerotids and elephants, to name some prominent groups (Owen-Smith, 1988). Among the attempts to classify this diversity, to explain niche differentiation, but also to understand the substantial convergence between herbivores from different clades, digestive physiology has played a major role. Approaches that build on basic differences in anatomy and physiology have focussed on a dichotomy between hindgut and foregut fermenters (Janis, 1976; Alexander, 1991). More recently, this dichotomy has been expanded by emphasizing differences between nonruminant and ruminant foregut fermenters, and the flexibility of the hindgut fermentation system (Schwarm et al., 2009; Clauss et al., 2010a).

Another approach focuses on variation linked to body mass (M) (Demment and Van Soest, 1985; Illius and Gordon, 1992). In particular, it is assumed that larger herbivores have longer digesta mean retention times (MRT) in the gastrointestinal tract and hence can achieve higher digestive efficiencies. This approach has been criticised because of conceptual problems as well as lacking support from empirical data (reviewed in Clauss et al., 2013; Müller et al., 2013). On the contrary, empirical data suggest no difference in digestive efficiency due to a variation in M (Pérez-Barbería et al., 2004; Steuer et al., 2013, 2014). In particular, it should be noted that the M-concept included the ruminant–hindgut fermenter dichotomy (Illius and Gordon, 1992), and therefore actually allowed for a difference in digestive physiology (or phylogeny).

While MRT was included in these concepts from the very beginning as a crucial physiological factor, chewing efficiency or digesta (=faecal) mean particle size (MPS) was not (Clauss and Hummel, 2005). The relevance of MPS lies in the fact that smaller particles allow a faster microbial digestion due to an increased surface–volume ratio (e.g. Bjorndal et al., 1990), i.e., at a given MRT, smaller MPS should result in higher digestive efficiency. MPS has only been investigated more recently in a comparative approach (Fritz et al., 2009) that demonstrated both a systematic interspecific scaling with M, but also fundamental differences between different herbivore groups. MPS measurements were not included in large-scale comparative studies on the digestive

* Corresponding author. Tel.: +49 228 732287; fax: +49 228 732295.
  *E-mail address:* ksue@itw.uni-bonn.de (K.-H. Südekum).

physiology of herbivores (e.g. Foose, 1982), and hence no large-scale comparative study that recorded several digestive measurements including MPS exists so far. For example, conclusions on the compensating effects of MRT and MPS were based on data collated from different studies (Clauss et al., 2009). Here, we report MPS measurements in individuals of a larger number of mammalian herbivores from experiments during which one consistent (grass hay) diet was fed and food intake and MRT of solute and particle markers (Steuer et al., 2011) as well as proxies for digestibility were recorded simultaneously, the in vitro digestibility of faecal fibre (Steuer et al., 2013; a proxy for how thoroughly an animal digested fibre) and metabolic faecal nitrogen (MFN) (Steuer et al., 2014; a proxy for microbial nitrogen which increases with increasing digestibility).

The following hypotheses guided our approach:

1. MPS increases with increasing M (Fritz et al., 2009).
2. MPS is lower in ruminants than in nonruminants (in our sample, nonruminants were all hindgut fermenters) (Fritz et al., 2009).
3. MRT characteristics and MPS together explain digestive efficiency better than M (Clauss et al., 2009). In detail, we expect no influence of M on MRT or digestibility as already shown in these data (Steuer et al., 2011, 2013), a positive relationship between MRT and digestibility (i.e. a negative relationship with in vitro digestibility of faeces), a negative relationship between MPS and digestibility (i.e. a positive relationship with in vitro digestibility of faeces), and a clear negative relationship between the relative food intake and MRT (Clauss et al., 2007a,b).
4. Given that ruminants and hindgut fermenters in this dataset had a large range of overlap in M, that they differed clearly for in vitro digestibility of faecal fibre with no overlap between the groups (Steuer et al., 2013), but that both relative food intake and MRT characteristics, though generally different, did show some overlap (Steuer et al., 2011), we predicted that MPS is a better measure to explain the difference in digestive efficiency between the groups.

In evaluating the effects of the different variables on the digestibility proxies, we used an information–criterion based approach to select the most parsimonious models. Two important aspects were included. First, the models were tested with and without the inclusion of the general digestion type (ruminant vs. hindgut fermenter) as a cofactor. Our premise was that should models be selected that include this information, this would indicate that aspects of these digestive strategies were relevant that are not reflected in the physiological measures included in this study. Second, the models were tested with ordinary statistics and with

phylogenetic information using Phylogenetic Generalized Least Squares, which also allowed the estimation of the phylogenetic signal in the investigated models. Our premise was that if a single-factor model contained a significant phylogenetic signal but a model that included several factors in combination did not, this combination of factors likely represents a trait typical for a phylogenetic group. As an evident control example, we expected that in our dataset, the inclusion of digestion type (which largely reflected the phylogenetic composition of our sample, with elephants and warthogs as individual taxonomic outliers in the hindgut fermenter group) should lead to no significant phylogenetic signal.

## 2. Materials and methods

The experimental setup for this study was described recently (Steuer et al., 2011, 2013, 2014). In brief, 16 species (9 functional ruminants, including 8 taxonomic ruminants and one camelid, and 7 hindgut fermenters) were used on the consistent diet in captivity, with 1–5 individuals per species (Table 1). Sampling periods during winter seasons 2008 and 2009 were at locations in the Netherlands, northern Germany, and Switzerland. Faecal samples were taken after an adaptation period of 14 days during which all animals had ad libitum access to grass hay that was fed whole (i.e., not chopped). Chemical composition (in % organic matter [OM] $\pm$ standard deviation) of the grass hay was: neutral-detergent fibre (NDF) 72 $\pm$ 4, acid-detergent fibre 39 $\pm$ 4, acid-detergent lignin 5 $\pm$ 1 and crude protein 10 $\pm$ 2. Details of this part of the study can be found in Steuer et al. (2011). Body mass of the animals ranged from 58 kg (a domestic goat) up to 6500 kg (an African elephant bull). For ten of the 16 species, M was estimated (from estimations by zoo keepers, zoo veterinarians and the second author, based on literature data and personal experiences); for the rest, individuals were weighed for the experiment. It was logistically not possible to weigh animals before and after the diet transition; based on visual judgements of animal keepers, the supervising veterinarian and the second author, no animal lost body condition during the experiment. For 14 species, data were also available on intake, measured as dry matter intake (DMI [kg d$^{-1}$]), MRT of a solute (fluid) and a particle marker, and the digestibility of faecal fibre measured by 96 h cumulative in vitro gas production (GP96h [ml per 200 mg faecal NDF]), as well as the concentration of metabolic faecal nitrogen (MFN [%faecal OM]) (Steuer et al., 2011, 2013, 2014) (Table 1). Note that a higher GP96h value indicates a higher digestibility of faecal fibre in vitro, which in turn means a lower fibre digestibility achieved by

**Table 1**

Dataset (means $\pm$ standard deviations) on measures of digestive physiology in wild and domestic herbivores of different digestion type (DT, ruminants [RUM] or hindgut fermenters [HF]) used in this study, combining original results on body mass (M [kg]) and mean faecal particle size (MPS [mm]) and previously reported results on dry matter intake (DMI [kg d$^{-1}$]), particle and solute mean retention time (MRT [h]), faecal fibre digestibility (measured as cumulative in vitro gas production over 96 h, GP96h [ml per 200 mg organic matter]) and metabolic faecal nitrogen (MFN [% organic matter]) from the same experiment (Steuer et al., 2011, 2013, 2014).

| Species | n | DT | M | $\pm$SD (range) | MPS | $\pm$SD | DMI | $\pm$SD | MRT$_{part}$ | $\pm$SD | MRT$_{sol}$ | $\pm$SD | GP96h | $\pm$SD | MFN | $\pm$SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Camelus bactrianus* | 4 | RUM | 450[a] | 0 | 0.54 | 0.115 | – | – | – | – | – | – | – | – | – | – |
| *Bos taurus primigenius* | 3 | RUM | 1287 | 25 (1260–1310) | 0.38 | 0.165 | 8.02 | 1.15 | 75 | 4.9 | 35 | 0.6 | 8.5 | 1.85 | 1.60 | 0.07 |
| *Syncerus caffer nanus* | 1 | RUM | 350[a] | – | 0.31 | – | 5.52 | – | 51 | – | 21 | – | 13.9 | – | 1.68 | – |
| *Kobus ellipsiprymnus* | 2 | RUM | 210[a] | 42 (180–240) | 0.27 | 0.044 | 2.36 | 0.34 | 52 | 13.4 | 27 | 10.6 | 13.3 | 4.24 | 1.79 | 0.23 |
| *Connochaetes taurinus* | 2 | RUM | 160[a] | 0 | 0.45 | 0.028 | 3.20 | 0.31 | 42 | 2.8 | 31 | 9.2 | 13.9 | 0.64 | 1.65 | 0.02 |
| *Oryx gazella* | 2 | RUM | 175[a] | 21 (160–190) | 0.52 | 0.223 | 2.13 | 0.12 | 64 | 2.1 | 32 | 5.7 | 11.1 | 0.99 | 1.57 | 0.07 |
| *Hippotragus niger* | 2 | RUM | 175[a] | 21 (160–190) | 0.43 | 0.135 | 1.86 | 0.20 | 54 | 21.2 | 33 | 14.8 | 12.7 | 2.33 | 1.69 | 0.33 |
| *Capra aegagrus hircus* | 3 | RUM | 60 | 2 (58–62) | 0.34 | 0.071 | 1.09 | 0.16 | 51 | 6.2 | 30 | 3.8 | 13.1 | 2.41 | 1.16 | 0.06 |
| *Ovis orientalis aries* | 3 | RUM | 94 | 4 (91–99) | 0.26 | 0.027 | 1.20 | 0.31 | 54 | 4.0 | 34 | 2.1 | 19.3 | 7.60 | 1.42 | 0.24 |
| *Loxodonta africana* | 5 | HF | 4000[a] | 1458 (3000–6000) | 4.98 | 1.064 | 49.90 | 8.17 | 30 | 6.0 | 29 | 4.3 | 28.1 | 1.26 | 1.33 | 0.12 |
| *Phacochoerus africanus* | 1 | HF | 77 | – | 1.22 | – | 1.72 | – | 44 | – | 34 | – | 33.1 | – | 0.99 | – |
| *Equus grevyi* | 4 | HF | 390[a] | 20 (380–420) | 1.55 | 1.248 | 8.09 | 2.61 | 28 | 7.3 | 25 | 8.7 | 25.3 | 1.87 | 1.11 | 0.14 |
| *Equus ferus przewalskii* | 3 | HF | 250[a] | 0 | 1.20 | 0.454 | – | – | – | – | – | – | – | – | – | – |
| *Equus ferus caballus* (horse) | 5 | HF | 571 | 52 (488–629) | 1.12 | 0.115 | 9.74 | 2.517 | 26 | 3.9 | 22 | 5.4 | 26.8 | 3.86 | 0.90 | 0.14 |
| *Equus ferus caballus* (pony) | 3 | HF | 97 | 6 (90–101) | 1.07 | 0.106 | 2.24 | 0.600 | 26 | 1.0 | 20 | 1.0 | 24.8 | 0.55 | 1.10 | 0.09 |
| *Ceratotherium simum* | 1 | HF | 2000[a] | – | 5.10 | – | 20.03 | – | 50 | – | 34 | – | 27.6 | – | 1.58 | – |

[a] Estimated body masses.