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Research Article
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A Simple Approach to Monitor Faecal Particle Size in the Asian Elephant – A Proof of Concept Study

Christian Schiffmann[1]*, Linda Schiffmann[1], Javier Bonillo[2], Ineta Bulkeviciute[2], Elisa Gozalbes Aparicio[2], Jorge Paniagua[2], Gloria Ribera[2], Marta Ruiz[2], Mario Torró[2] and Marcus Clauss[3]

[1]*Tier-Erlebnispark Bell, Bell, Germany*
[2]*Terra Natura, Benidorm, Alicante, Spain*
[3]*Clinic of Zoo Animals, Exotic Pets and Wildlife, University of Zürich, Zürich, Switzerland*
**Corresponding author's e-mail: c.schiffmann.elephantproject@gmail.com*

Abstract. In contrast to most mammals, elephants express a life-long dentition change called molar progression, with a continuous change in size of the grinding surface. This may impact chewing efficiency and consequently lead to age-related variation in faecal particle size. Assessing faecal particle size relies on tedious laboratory analysis and would benefit by a more simple and practical method. We develop such a method and evaluate its reliability by comparison with the results from laboratory analysis. The method was based on measuring the 10 largest particles per sample and produced comparable ranking as laboratory analysis, in four test-elephants.

Introduction

Asian elephants are megaherbivores feeding on a variety of browse and grass species. Their digestive tract contains a voluminous stomach and small intestine followed by a voluminous large intestine (Clauss *et al*. 2007). The latter presents a fermentation chamber for the high-fibre diet of the elephant and is characteristic for the group of 'hind-gut-fermenters'. Due to their low-energy diet and relatively short mean retention time of 20–40 hours (Hackenberger 1987; Clauss *et al*. 2003; Beirne *et al*. 2019), elephants need to feed almost continuously. In herbivores, chewing to reduce food particle size is considered important for digestive efficiency. As elephants are non-ruminants, chewing is only one-step of digestion and chewing efficacy is possibly critical. In contrast to most mammalian species, elephants do not replace their deciduous with permanent teeth once. Instead, they express a life-long dentition change called molar progression, with a continuous change in size and structure of the grinding surface (Laws 1966; Roth & Shoshani 1988; Lee *et al*. 2012). Although no measurements of the grinding surface in relation to an elephant's age are available, a correlation between chewing efficacy and body mass cyclicity has been hypothesised in zoo-kept elephants under a constant diet (Schiffmann *et al*. 2019). Previous work has reported mean faecal particle size in 18 Asian and 13 African elephants without taking into account their age (Fritz *et al*. 2009), and assessed mean faecal particle size against body mass (Clauss *et al*. 2015). These studies determined faecal particle size by a wet-sieving procedure conducted in the lab.

The measurement of an average particle size of a sample of particles (such as a lump of faeces, or a cup of food) is usually performed using a combination of a sieve analysis and a subsequent calculation step. Methods may differ in the kinds of sieves used; in whether the sieving is performed 'dry' (as e.g. done for meal-type feed samples, but not applicable to pelleted feeds or faeces) or 'wet' with water being sprayed on the sieves (as e.g. done for pelleted feeds or faeces, which require soaking prior to sieving); whether the sieve column is shaken or not; whether the material passing the finest sieve is accounted for or not; and in how the results are used to calculate a certain measure of 'average particle size' (Fritz *et al*. 2012; Bertsch *et al*. 2022). For example, for samples of which some portion remains on the largest sieve, it has been recommended to measure the size of the

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largest particles on that sieve, to estimate the upper limit of particle size that is used in certain measures of 'average particle size' (Fritz *et al*. 2012). Depending on the nature of the sample and the question asked, only results from selected sieves might be used to have a closer look at a certain fraction of the overall sample (Weary *et al*. 2017; Bertsch *et al*. 2022). A major disadvantage of the wet-sieving procedure is its laborious and time-consuming nature. For each sample analysed, all material from all sieves must be manually transferred to either tared petri dishes or filter paper, which can easily take half an hour. These individual sub-samples then need to be dried to constant weight, cooled down without gathering moisture, and then weighed again. Hence, a more simple and practical monitoring method would be welcome.

We determined the faecal particle size of four Asian elephants of various age and molar status, using a simple method and compared the results to the standard wet-sieving laboratory method to assess its reliability and consistency.

Material and methods

The study was conducted with four female Asian elephants (*Elephas maximus*) at Terra Natura Benidorm in Spain. They were all wild-born individuals with a life history of several inter-zoo transfers across Europe after importation. They were unrelated and none of them had ever had any offspring. They were fed a hay-based diet and weighed between 2960 and 3700 kg at the time of the study (Table 1).

Determination faecal particle size by simple method

A sample was taken from the inside of an excreted dung bolus avoiding the outside that has bedding, soil or dirt attached to it (Fig. 1). Of the collected sample 60 ± 5 g was weighed out, put

Figure 1. Sample collection from inside the faecal bolus.

in a kitchen sieve and rinsed under cold water until the fibres were clean (usually around 1 minute). The sieve was agitated gently, taking care not to break the fibres. Then the sample was spread out on a clean surface and the 10 longest fibres taken out. They were lined up on a sheet of paper and labelled with the elephant's name and date (Fig. 2). Bent fibres were straightened, measured and the mean length of fibres calculated. The paper was photographed for reference. This procedure was conducted by the elephant keeper team at Terra Natura Benidorm, once a week for 10 continuous weeks from October 2022, providing a total of 40 samples. The diet of the elephants, consisting mainly of hay, remained identical during the course of sampling. The hay originated from one single batch.

Determination faecal particle size by wet-sieving procedure in the lab

The first two authors collected faecal samples according to the same protocol as the simple method on two consecutive days following the week of the last sampling by the keepers, from six different defecations per day from each of the four elephants. The samples were frozen at -16°C immediately after collection and shipped to the lab two days later.

Table 1. Date of birth, body mass and relevant notes for the elephants investigated here.

Elephant	Age [years]	Body mass [kg]	Notes
Female 1 (K)	40	2990	severe molar disorder in 2017
Female 2 (L)	40	3700	none
Female 3 (M)	37	3530	none
Female 4 (P)	50	2960	suffered from trunk paralysis

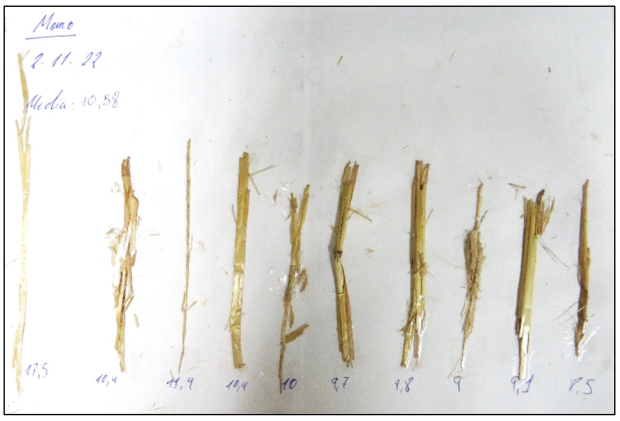

Figure 2. The longest ten fibres of one sample after sieving, straightening and measuring.

At the lab, 20 g of each sample was used for dry matter determination by drying at 103°C for 24 h, and 40 g used for sieve analysis. For wet sieving, samples were first soaked under stirring in 1 l of water until they were completely thawed, and coherent fibre masses were dissolved. Then, the material was submitted to wet sieving under vibration following the protocol of Fritz *et al*. (2012), using a series of 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.063, 0.040 and 0.025 mm sieves (linear dimensions of holes) using a Retsch AS 200 digit wet sieving machine (Retsch, Haan, Germany). The machine consists mainly of a heavy base that includes a vibrating platform on which the sieve column is placed. Water is sprayed on the top of the sieve column and flows out of it at the bottom, carrying particles that pass the finest sieve. Particles passing the finest sieve were discarded. The vibration amplitude used was approximately 2 mm, water was applied at 2 l/min, and sieving was conducted for 10 min per sample.

The particles caught on each sieve were transferred onto pre-weighed petri dishes. From the material retained on the largest sieve, the 10 largest particles were measured individually. This is not necessarily part of the routine lab wet sieve analysis protocol, although it has been recommended for samples, such as elephant or giant tortoise faeces, of which relevant portions are caught on the largest sieve; this is not done to report their length, but for inclusion in the overall calculation method of the average particle size of the sample (Fritz *et al*. 2012). All dishes were then dried at 103°C for 24 h and weighed after cooling to room temperature in a desiccator using an analysis balance with measuring accuracy of 1 mg (Model AE160; Mettler-Instrumente, Gießen, Germany).

Results are presented as the mean length of the ten largest particles, the mean particle size (MPS) calculated as the dMEAN procedure from Fritz *et al*. (2012) using the material retained on the sieves; we calculated the MPS either using the actually measured largest particle size or setting it at 40 mm for all animals. Additionally, we expressed the results in the % of all particles retained above the 4 or the 8 mm sieve, and as MPS based only on sieves up to 4, 2 or 1 mm. The particles lost through the finest sieve were estimated by subtracting the sum of dry matter retained on the sieves from the calculated amount of dry matter submitted to sieve analysis. Results are presented as means ± standard deviation.

As the sampling was non-invasive and no study-related diet adaptations were conducted, no approval for experiments involving animals was required.

Results

Animals differed in the various particle size measurements (Table 2). The longest fibres occurred in female 1 and female 3. Female 2 and female 4 showed a shorter mean fibre length (Table 2). This pattern between the four animals can be observed with considerable consistency between all methods applied here that include, or focus only on, larger particles (Table 2, Fig. 3). By contrast, MPS measures that considered only the smaller particle size classes did not show the same systematic differences between the animals (Table 3, Fig. 4). When plotting the average individual results for all individual sieves, it is evident that the main difference between the animals was in the largest particle class (Fig. 5), which is also evident when comparing sieve remains in two individual animals (Fig. 6).

Discussion

Our investigation of mean particle sizes in the faecal fibres of four female Asian elephants revealed consistency between the two methodolo-

Table 2. Faecal mean particle size (MPS) measurements (mean ± SD) that include or are based only on large particles.

Measurement	Female 1 (K)	Female 2 (L)	Female 3 (M)	Female 4 (P)
MPS of ten largest fibres - zoo [mm]	87.5 ± 20.3	70.3 ± 12.9	98.0 ± 19.9	54.0 ± 6.0
MPS of ten largest fibres - lab [mm]	66.1 ± 7.8	58.8 ± 8.7	83.7 ± 14.6	49.7 ± 3.3
MPS whole faeces (max. size set to 40 mm) [mm]	16.7 ± 1.7	9.6 ± 3.4	17.7 ± 1.3	10.7 ± 3.1
MPS whole faeces (actual maximum size) [mm]	23.6 ± 2.6	12.2 ± 5.0	31.0 ± 6.1	12.2 ± 4.4
Particle mass retained on 4 mm sieve and higher [%]	67 ± 4	45 ± 7	66 ± 4	51 ± 8
Particle mass retained on 8 mm sieve and higher [%]	61 ± 4	35 ± 11	63 ± 4	46 ± 9

gical approaches. The methods differ greatly in their simplicity and thus practicality. While the simple method requires hardly any equipment and can be conducted in the field, including at zoos, the more sophisticated one requires a laboratory.

We found consistent results, independent of the method applied to determine faecal particle size in zoo elephants, as long as the larger particle fraction of the faeces is included in the particle size calculation. By contrast, using the smaller particles in the faeces does not lead to a consistent ranking of the animals. These discrepancies underline the importance of the large particle fraction in quantifying elephant chewing efficacy: all animals produce fine particles to some degree, but it is in the amount of large particles that have undergone minimal size reduction by chewing, that individuals differ. Therefore, the 'simple method' of only measuring the top 10 large particles repeatedly over several weeks

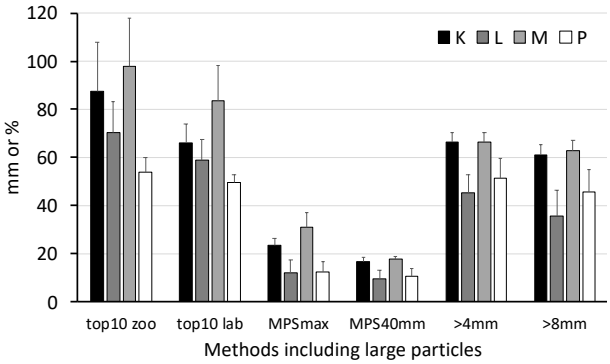

Figure 3. Different faecal particle size measures that all include large particles in four elephants: The largest 10 particles measured in faecal samples by keepers at the zoo (top10 zoo, in mm), the largest 10 particles measured in faecal samples in the lab (top10 lab, in mm), the mean particle size (MPS) calculated with the actually largest particles (MPSmax, in mm), or with assuming a standard maximum particle size of 40 mm (MPS40mm, in mm), or the percentage of particles retained on the 4 mm sieve and all sieves above (>4mm, in %) or those retained on the 8 mm sieve and the sieve above (>8mm, in %). Note that animals K and M are consistently recorded with larger particles than animals L and P across methods.

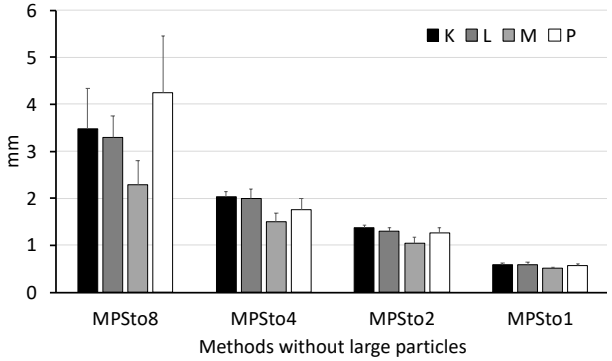

Figure 4. Different faecal particle size measures that all exclude the largest particles in four Asian elephants: The mean particle size (MPS) calculated with all sieves up to 8 mm (MPSto8), up to 4 mm (MPSto4), up to 2 mm (MPSto2) and up to 1 mm (MPSto1). Note the overall lack of consistency in the ranking of the animals.

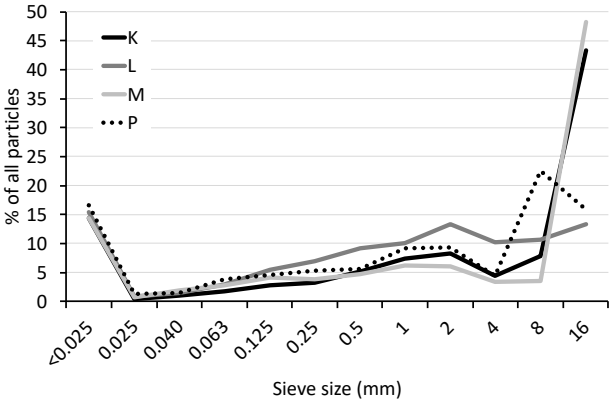

Figure 5. The average distribution of particulate mass on the sieves in four Asian elephants. Note the difference between individuals with respect to the largest sieve size.

Table 3. Faecal mean particle size (MPS) measurements (mean ± SD, in mm) that are based only on particles ≤8mm.

Measurement	Female 1 (K)	Female 2 (L)	Female 3 (M)	Female 4 (P)
MPS whole faeces (up to sieve size 8 mm)	3.48 ± 0.86	3.30 ± 0.44	2.30 ± 0.51	4.24 ± 1.22
MPS whole faeces (up to sieve size 4 mm)	2.04 ± 0.11	2.00 ± 0.20	1.50 ± 0.19	1.76 ± 0.24
MPS whole faeces (up to sieve size 2 mm)	1.37 ± 0.07	1.30 ± 0.08	1.05 ± 0.12	1.26 ± 0.11
MPS whole faeces (up to sieve size 1 mm)	0.59 ± 0.04	0.58 ± 0.05	0.51 ± 0.02	0.56 ± 0.04

per individual appears adequate to differentiate between animals, and to possibly monitor changes in chewing efficacy over time.

A limiting aspect of this research is the small sample size. Finding keeper teams motivated and able to take the additional workload of such an investigation is not that easy, hence increasing the number of individuals participating in this kind of research is challenging. The fact that one of the elephants with particularly large faecal particles had a history of cheek tooth disorder suggests that faecal particle monitoring might reflect, dental status and health.

Reports on individual, live elephants' molar grinding surface are lacking, most probably due to the challenge of taking standardised pictures and/or measurements of a living elephant's molar teeth (Weihs 2001). In addition, knowledge on mastication parameters in elephants is very scarce. von Koenigswald (2016) provides an accurate description of the biomechanical chewing pattern in the African and Asian elephants and Weihs (2001) recorded chewing frequency in captive and free-ranging Asian elephants in Sri Lanka. The latter found a negative correlation between chewing frequency and age but was not able to investigate the impact of the molar grinding surface because inspection was not possible. A general decrease in chewing frequency with body size is well-known across mammal species (Gerstner & Gerstein 2008) and is explained by the fact that larger jaws, like larger pendulums on clocks, do not move as fast as smaller jaws or smaller pendulums. However, to which degree individual elephants differ in their chewing intensity, as e.g. demonstrated for individual cows (Zhang *et al*. 2022), has so far not been investigated.

Studies investigating the individuality of chewing frequency and intensity in elephants have not been conducted so far on the level of individual elephants with known age. It is reasonable to assume that not only the size of the grinding surface itself, but also chewing intensity in terms of chews per food bolus will impact faecal particle size. Animals with a smaller chewing surface might compensate by chewing a food bolus more intensively. In addition, the impact of varying food structure on faecal particle size is also unknown. The simple method to determine faecal particle size demonstrated here can be used for conducting such studies and longitudinal monitoring of chewing efficacy in captive elephants. It provides a practical and straight-forward approach for elephant care teams to regularly check the chewing efficacy of individual elephants non-invasively,

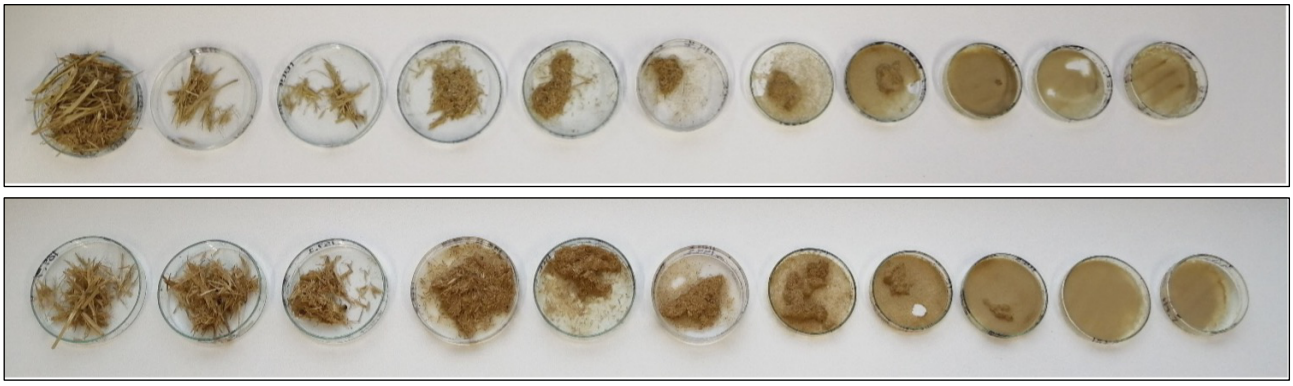

Figure 6. Wet-sieving residues in the lab from female 3 (upper row) and female 2 (lower row). Note the different distribution of the amount of fibres between the sieving stages, representing – from left to right, the 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.063, 0.040 and 0.025 mm sieves.

hence contribute to preventive health in elephant husbandry.

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