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Diet Component Estimation in Asian Elephants by Microhistological Faecal Analysis

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Abstract. We report on the gut passage times of dicot and monocot fodder in Asian elephants and the utility of microhistological faecal analysis for the quantitative evaluation of diet in elephants. A feeding trial was conducted with three domesticated elephants and faecal samples analyzed using a point quadrat method. Gut passage times in elephants were found to be comparable to that reported for ruminants, but with a shorter 'time of elimination'. The percent epithelial fragment occurrence of each forage class in the faeces accurately reflected its percent dry weight in the diet. We conclude that microhistological faecal analysis is a valid method for studying forage class variation in the diet of elephants.

Introduction

Studies on the feeding behaviour of Asian elephants (*Elephas maximus*) have employed direct observations (McKay 1973; Vancuylenberg 1977; Sukumar 1989), feeding site inspection (Ishwaran 1983; Mueller-Dombois 1971; Dhakal & Ojha 1995), isotope studies (Sukumar & Ramesh 1992; Codron *et al*. 2011), and microhistological analysis (De Boer *et al*. 2000; Steinheim *et al*. 2005; Pradhan *et al*. 2008).

Free-ranging Asian elephants mostly inhabit poor-visibility habitat, display aggressive or avoidance behaviours to close approach by human observers, and are largely nocturnal (Fernando 1997), limiting the utility of direct observations. Feeding site inspection is of limited applicability because enumeration of food items once they have been removed is difficult, allows only qualitative analysis, and can be confounded by feeding of other animals. Isotope analysis only provides a graze:browse ratio. Faecal microhistological analysis overcomes many drawbacks of the other techniques, and could provide detailed information on diet composition, and its individual, temporal, and spatial variation.

A number of studies have been conducted on gut passage times of elephants using seeds and beads as markers (Dudley 1999; Weerasinghe *et al*. 1999; Campos-Arceiz *et al*. 2008). We failed to find any studies reporting gut passage times for fodder, or assessing the applicability of microhistological faecal analysis to elephants, in the published literature. Knowledge of gut passage times and any introduced bias is important in interpreting results of microhistological faecal analysis. Elephants consume both grass and browse and their diet consists of leafy and woody components. The relative proportions of forage class and component in the diet may vary seasonally and by habitat, which has implications for digestibility and nutritional value of consumed fodder, hence habitat suitability. Knowledge of the relative contribution to diet of elephants from different forage types and components, and their individual, temporal and seasonal variation is of interest both in the study of their feeding ecology and in managing habitat for elephants.

The objectives of this study were to assess 1) the gut passage times for fodder 2) the utility of microhistological faecal analysis for the quantitative evaluation of diet composition, and

3) the 'woody' component when consuming a mainly leafy diet and its variation with major forage types.

Methods

The study was conducted at the 'Millenium Elephant Foundation' in Kegalle, Sri Lanka, using three captive elephants. The study animals were one male and two female elephants, aged 11, 30 and 50 years respectively. All three were in good health throughout the study period. Their regular day routine consisted of being tethered in place, interacting with tourists, taking them on short rides, and being taken to the river and bathed twice a day. Little food was consumed during the day. They were tethered in place at the end of the day and provisioned with coconut palm fronds supplemented with grass or dicot fodder, which they consumed through the night. This pattern of feeding and activity was preserved during the entire experiment to prevent any effect on digestion, induced by a sudden change in the feeding or activity pattern.

Feeding experiment

The three elephants were provided with an exclusive diet of coconut palm (*Cocos nucifera*) fronds for 5 days, an experimental feed of measured quantities of dicot, grass and coconut palm fronds over 3 days, and again an exclusive coconut palm frond diet for 5 days. The dicot component in the experimental feed consisted mostly of cut branches of jackfruit (*Artocarpus integrafolia*) and a few of breadfruit (*Artocarpus atilis*), of which the elephants stripped the branches and consumed mainly the leaves. A few of the smaller twigs and some pieces of bark stripped from the larger branches were also consumed. As sufficient quantities of single species stands of grass were not available, the grass component of the experimental feed consisted of about 10 graminoid species. The elephants were provided with whole coconut palm fronds, which they stripped, consuming mainly the leafy part.

The experimental feed of dicot, grass and coconut palm fronds was given at 18:00 h on all three days, and a 'day' was defined as starting at that time and extending for 24 hours. At 8:00 h each morning after an experimental feed, unconsumed fodder was weighed and the amount consumed estimated. As the interval between pre- and post-feeding forage weight determinations was relatively cool with humidity levels approaching saturation, loss of weight due to desiccation was assumed to be minimal. Only coconut palm fronds were given during the day and the amount consumed was similarly estimated and added to that day's diet. The mean daily moist weight of fodder consumed by an elephant over the three days of experimental feed was 89.1 ± 14.63 kg, with the mean percentage of grass and dicot in the diet being 21.39 ± 4.57% and 21.62 ± 5.90% respectively.

To estimate dry weight for each fodder class, a correction factor was derived by sun-drying two 1 kg samples from each type of fodder to complete desiccation. The younger female and the male were noted to have consumed a small amount of dicot fodder on one of their walks, on the day prior to the start of the experimental feed. Consequently, values for their diets on that day were excluded from the analysis.

Sample collection

The study elephants produced 4–6 dung piles during the day and approximately the same in the night. Each pile consisted of 4–7 discrete boli. In an adult, a bolus is approximately 45–55 cm in circumference and 10–15 cm in height. Samples were collected by breaking off a piece of a bolus (approximately 100 g), placing it in a screw-cap container and adding 70% ethanol. Samples were stored at ambient temperature.

Based on a pilot study, dung samples were collected from two days prior to the commencement to five days after cessation of the experimental feed. Five samples were collected per day per elephant. As the elephants were tethered in one place throughout night, all dung piles produced in the night accumulated in one heap. Therefore, samples one and two were collected at 8:00 h the next morning from a bolus at the bottom and top respectively of the overnight heap of dung. Samples three, four and five were collected from

a random bolus in the dung pile deposited closest to 10:00, 14:00 and 18:00 h respectively.

Sample preparation

A subsample of about 20 g was taken from each sample, placed in a 50 ml plastic tube and boiling water added to 40 ml. The tube was capped and agitated until the dung was broken up, and then left standing for 30 min with occasional agitation.

Removal of large items (Storr 1960) has been reported to decrease bias. Elephant dung contains a large macroscopic component with individual pieces of bark, woody fibres etc. measuring many cm in length. Therefore, the slurry was filtered through a 2.5 mm sieve to exclude the macroscopic fraction. As fragments smaller than 0.1 mm cannot be conclusively identified (Martin 1955) and sampling from a relatively homogeneous size fraction reduces bias (Chamrad & Box 1964), the filtrate was washed using a 0.2 mm sieve, obtaining a fragment size range of 0.2–2.5 mm.

The fragments were re-suspended in 3 ml of water, and an equal volume of domestic bleach solution (sodium hypochlorite) was added to clear the fragments of pigment that would impair identification (Williams 1969; Vavra & Holechek 1980). The suspension was left standing for approximately 30 min until all particulate residue was visibly bleached. The residue was again rinsed with water employing the 0.2 mm sieve to remove the bleach, and a scraping taken with a spatula for analysis.

Microscopic identification

The scraping was placed in a counting chamber with a 1 mm grid, water added, and the fragments dispersed over the counting surface at a density precluding significant overlap (Bartolome *et al*. 1995). The chamber was overlaid with a coverslip and scanned systematically at 100 X under an optical microscope. A point quadrat method was used to control for different fragmentation rates of components by counting only the fragments overlapping cross points of the grid (Takatsuki 1978). A minimum of 100 total fragments were identified and enumerated by fragment class at each count.

Reference slides of epithelium from fodder species were made to facilitate identification of epithelial fragments in dung, which were identified on the basis of density, size and shape of epidermal cells, structural peculiarities of the cell wall, and cellular inclusions (Martin 1955; Zyznar & Urness 1969). Mesophyll and fragments without a recognizable architecture were not counted. Fragments were scored as ‘grass’, ‘coconut’, ‘dicot’, and ‘woody’.

Analysis

Throughput time was estimated as the difference between the time of first ingestion of a particular item and the time of collection of the first sample with the presence of the item, as determined by microhistological analysis. Elimination time was similarly estimated as the time between the last ingestion and the last sample of dung with the item.

The actual ingestion of a food item could have taken place anytime between provision of the experimental feed at 18:00 h and its clearing the next morning at 8:00 hr. Therefore, the time of ingestion was taken as the midpoint of this period with an error of ± 7 h for calculation of the times of throughput and elimination. Although it would have been preferable to reduce the possible variation in time of ingestion by providing access to the food for only one hour, this was not done in order to preserve the regular pattern of feeding.

To test the relationship of percent dry weight of each food class in the diet to its percent epithelial fragment occurrence in the dung, the mean of the five daily samples for each of the forage classes on a given 'day' was taken to represent the value for that 'day' for a particular elephant. Correlation analyses were carried out by pairing the daily values for dung with daily values for food 24 h prior to compensate for throughput time, and to test the relationship of different forage classes to the ‘woody’ fragment component in the dung, by pairing the values for woody fragments in dung with the values for each class of food.

Results

For grasses, throughput time in the three elephants was 20–41 h and the elimination time 29–88 h. For dicots, the throughput time was 24–38 h and elimination time 78–99 h.

The variation of percent epithelial fragments of each forage class in dung reflected the variation of percent dry weight of the corresponding forage class in the diet, with the estimates from dung initially lagging behind the diet proportions, then equalling or exceeding them (Fig. 1). The major portion of both grasses and dicots appeared in the faeces approximately one day after first ingestion and was eliminated by approximately two days after last ingestion, with almost all being eliminated by the fourth day after last ingestion (Fig. 1).

The percent dry weight in the diet of each food class was significantly correlated ($p < 0.001$) with the corresponding percent fragment occurrence in dung. The coefficients of determination for the three food classes were: coconut, $r^2 = 0.836$; dicots, $r^2 = 0.826$; and grass, $r^2 = 0.897$.

The woody fraction in dung was maintained at a conserved level throughout the experiment (mean daily percentage of woody fragments in dung = 43.28% ± 6.98), and showed a weak but statistically significant correlation with food class in diet; positive with coconut and negative with dicot and grass (coconut, $r^2 = 0.248$; dicot, $r^2 = 0.264$; and grass $r^2 = 0.188$; $p < 0.05$). Therefore, although the elephants stripped both the coconut fronds and dicot branches and mainly consumed the leafy portion, more 'woody' material appears to have been ingested in the case of coconut fronds.

Discussion

Gut passage times

Grasses – Stewart (1967) reported values ranging from 20–34 h for times of throughput for different species of grass fed to two grazing ruminants, a wildebeest (*Connochaetes taurinus*) and a buffalo (*Syncerus caffer*), and a grazing non ruminant, a

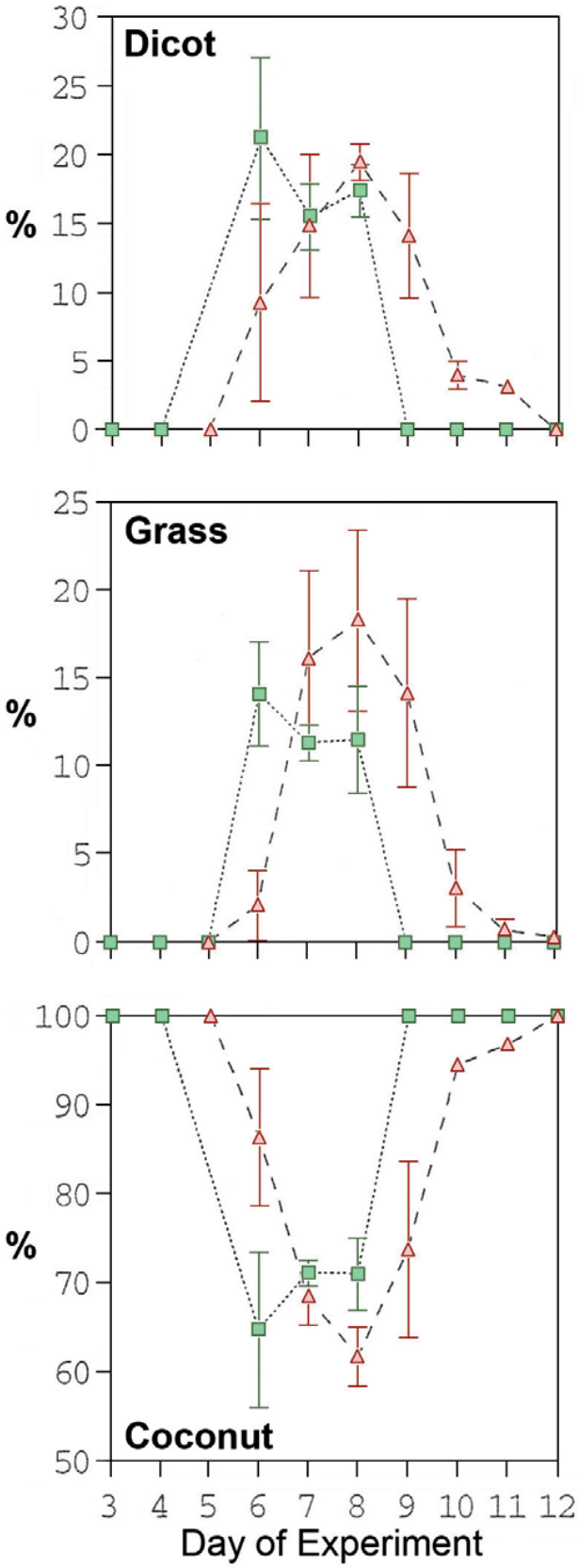

Figure 1. Variation of percentage epithelial fragments in dung (red triangles) and percentage dry weight in food (green squares) during the feeding experiment, by forage class.

zebra (*Equus burchellii*), observing no difference between the ruminants and the non-ruminant. Takatsuki (1978) reported a throughput time of "about two days" for grass in a ruminant, Sika deer (*Cervus nippon*), a 'mixed feeder' (consuming both grass and browse). Therefore, the value of 20–41 h throughput time for grass observed by us for elephants, is similar to that reported for other species. Stewart (1967) reported times of elimination of grasses in ruminants as 4.5–6 days and as 3 days for the zebra, and the range of 29–88 h observed by us for elephants is less than that for the two ruminants but similar to that of the zebra.

Dicots – Zyznar and Urness (1969) reported a throughput time of 36 h for mule and white tailed deer (*Odocoileus hemionus* and *O. virginianus*) based on the appearance of droppings stained with fuchsin dye, following feeding with an unspecified fodder species treated with dye. They used 17 forage species including grasses, browse and forbes in their study. Voth and Black (1973) fed 20 species of browse to a mountain beaver (*Aplodontia rufa*) – a non-ruminant small mammal – and reported a value of 24 h for the time of throughput and 5 days for the time of elimination. Thus, the value of 24–38 h for throughput of dicots observed by us in elephants is similar to that reported for other species. The value of 78–99 h for elimination observed by us in elephants, is less than that reported for the beaver. Although no comparative elimination times for dicots in ruminants were available, in the view of gut passage times for grasses, it could be expected to be longer than in elephants.

A study assessing gut passage times with markers in an Asian elephant, reported throughput times, times of elimination and gut retention times respectively of 14 h, 73 h and 20.2 h for melon seeds and 17 h, 72 h and 29.2 h for plastic beads (Weerasinghe *et al*. 1999). A study using tamarind seeds reported a retention time of 39.5 h (Campos-Arceiz *et al*. 2008). Gut retention time for *Acacia erioloba* seeds in African elephants was 24.5–36 h (Dudley 1999).

Observed differences in gut passage times of grasses and dicots in the present study, and that of markers (Weerasinghe *et al*. 1999; Campos-Arceiz *et al*. 2008), suggest that fodder type and species influence gut-passage time. Therefore, only gross deviations can be considered significant in comparing results of studies using different fodder species.

Our results indicate that the dung of elephants mainly reflect the diet over days one to three, with some influence from the diet of days four to five, preceding defecation. Given that there is little difference in mean retention time with variation in dry matter intake in elephants (Clauss *et al*. 2007; Campos-Arceiz *et al*. 2008), we expect our results to be robust and characteristic of the species.

Estimation of diet components

The present study indicates that the percent epithelial fragment occurrence of diet components in the faeces of elephants is a reliable estimator of the percent dry weight of each component in the diet. Thus it demonstrates a high level of accuracy in diet assessment through microhistological faecal analysis in elephants, even without the application of correction factors.

The variation between dietary components in the biomass represented by the specific epidermal fragments (epidermal weight index) and the degree of degradation of the epidermis due to digestion (epidermal erodibility factor) were considered to be important correction factors by Bartolome *et al*. (1995). Digestion in elephants is much abbreviated compared to ruminants, and may cause less degradation of the epidermis. Therefore, for diet assessment through microhistological analysis in elephants, variation in epidermal degradation between different fodder species may be of less importance than in ruminants. While the epidermal weight index correction maybe important if there is wide variation in the ratio of leaf surface to weight between fodder species, the use of dry weight of fodder species in the comparison should reduce such variation.

The level of accuracy demonstrated in this study is adequate for the quantitative study of forage

class variation in the diet of elephants. A higher level of accuracy employing correction factors may be required for the quantitative evaluation of variation in individual fodder species in the diet.

A faecal microhistological study on the diet of free ranging elephants in Nepal, found 26% of the diet to be composed of 'woody' fragments (Steinheim *et al*. 2005). We found a higher but fairly conserved fraction, possibly due to methodological differences. We suggest that standardized faecal microhistological analysis can provide useful comparative information on the proportion of leafy vegetation in the diet, and its seasonal and spatial variation in free-ranging elephants.

The present study demonstrates the robustness of microhistological analysis of dung in analyzing diet composition variation in elephants and we conclude that it is a valid, reliable, and useful technique for the purpose.

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