

Animal diets in the Waterberg based on stable isotopic composition of faeces

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Faecal analysis of diet in free-ranging mammals can provide insight into local habitat conditions by reflecting the resources actually utilized. Here we used stable light isotope analysis of faeces to qualify, as well as quantify, certain aspects of mammal food selection in a recovering, nutrient-poor, savanna habitat in the Waterberg. Stable carbon isotope ratios in faeces reflect proportions of C₃-foods (browse) to C₄-foods (grass) consumed, whereas stable nitrogen isotope ratios reflect a combination of trophic behaviour, protein intake, and water and nutritional stress. Percentage nitrogen indicates the nutritional quality of the diet, at least in terms of crude protein intake. We used these data to reconstruct and compare the diets of various mammal species from two reserves in the Waterberg: the Welgevonden Private Game Reserve and Zoetfontein Private Game Farm.

Key words: carbon isotopes, nitrogen isotopes, Welgevonden, Zoetfontein.

INTRODUCTION

Information regarding the feeding habits of free-ranging mammals provides insight into habitat conditions because the data represents the resources actually available to, and utilized by, these animals (Witt *et al.* 1998; Grant *et al.* 2000). This information, however, is seldom readily accessible to wildlife managers because of the constraints imposed by time-consuming field observations. Faecal indices of diet can overcome many of the problems associated with obtaining dietary information. For example, faecal nitrogen (N) and phosphorous (P) content are considered useful indicators of the nutritive value of available forage (*e.g.* Erasmus *et al.* 1978; Holecheck *et al.* 1982; Grant *et al.* 2000). Stable carbon and nitrogen isotope ratios in faeces can complement more well-established faecal indices, in that the proportions in which various food classes are utilized can be qualified and quantified.

¹³C/¹²C ratios ($\delta^{13}\text{C}$) in savanna mammal tissues and excreta reflect the relative proportions in which grass and browse are consumed (Vogel 1978; Tieszen *et al.* 1989; Cerling & Harris 1999). The basis of this technique is that C₃- (trees, shrubs, and forbs) and C₄- (grasses) photosynthesizing plants fractionate stable carbon isotopes in different ways, leading to distinct, non-overlapping $\delta^{13}\text{C}$ values (Smith & Epstein 1971; Vogel *et al.*

1978). This distinction is faithfully recorded in animal tissue and faecal $\delta^{13}\text{C}$ values (Tieszen *et al.* 1979; Lee-Thorp & Van der Merwe 1987; Cerling & Harris 1999).

¹⁵N/¹⁴N ratios ($\delta^{15}\text{N}$) in mammalian body tissues and excreta are related to a combination of variables. The complexity of the nitrogen cycle leads to a variety of patterns in soil and plant $\delta^{15}\text{N}$ values at the base of the food chain (*e.g.* Handley & Raven 1992; Muzuka 1999). In animals, $\delta^{15}\text{N}$ values also show a stepwise increase along trophic levels of the food chain and increase with protein intake (Schoeninger & DeNiro 1984; Sponheimer *et al.* 2003a). In addition, higher values are associated with arid environments and water stress (Ambrose & DeNiro 1986; Sealy *et al.* 1987; Ambrose 1991).

Isotopic studies of mammalian ecology have typically used hard tissues, *i.e.* bones and teeth, as sample material (*e.g.* Ambrose & DeNiro 1986; Thackeray *et al.* 1996; Smith *et al.* 2002; Cerling *et al.* 2003). Fewer studies have employed faecal isotopic data for free-ranging mammals (but see Tieszen *et al.* 1979, 1989; Van der Merwe *et al.* 1988; Vogel *et al.* 1990; Sponheimer *et al.* 2003b). The advantage of faeces as sample material is the short turnover time, in the order of only a few days, that allows for documenting ecological variability over short time periods. By contrast, hair and skeletal material aggregate dietary information over a much longer time frame, *i.e.* months, years,

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or decades (Tieszen *et al.* 1989; Cerling & Harris 1999). A potential limitation to faecal-based isotopic studies of diet is that the coarse, refractory, and undigested component of the diet might be over-represented in the faeces. Recent controlled feeding experiments of carbon isotopic fractionation have shown that large herbivore faecal $\delta^{13}\text{C}$ values correlate with dietary values in similar ways with herbivores fed C_3 browse, C_3 grass, and C_4 grass feeds, with very small variations (Sponheimer *et al.* 2003c; Codron *et al.* 2005 (in press)). In other words, the isotopic fractionation between diet and faeces is consistent regardless of the carbon isotopic content of the food and the relative digestibility (at least based on crude protein content).

Regardless of the tissue used, isotopic tools allow documentation of diets over shorter or longer time periods without time-consuming and expensive observation studies. Consistent, detailed and reliable information on mammalian feeding ecology, obtained in the absence of direct observations and/or animal slaughter, can be invaluable for monitoring change over seasonal and annual periods, and for implementing adaptive management strategies to nature conservation. Here, we examine isotopic distributions amongst faeces from mammals living in the Waterberg, an area of mountainous savanna 'bushveld' in the southwestern parts of the Limpopo Province. Annual rainfall often exceeds 600 mm, with the wet season occurring during the summer months between October and March (H. Kilian, pers. comm., 2002). The vegetation is classified as 'sour bushveld', *i.e.* a high rainfall area where leaching of soil nutrients results in poor nutritional quality of available forage, especially during the dry season (Tainton 1999). Much of the region's conservation land has been reclaimed from previously exploited and overgrazed agricultural land, further lowering the nutritional condition of the veld (A. Burger, pers. comm., 2002).

We aimed to explore the dietary preferences of mammal communities inhabiting a relatively nutrient-poor savanna habitat. We present results obtained from analysis of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and %N from 445 faecal specimens and 158 plant specimens collected during September 2002 and January 2003 from two reserves in the Waterberg. Data representing the two reserves, and the different seasons, are combined, providing a rigorous insight into dietary ecology on an inter-specific level, rather than across spatio-temporal scales.

Faecal $\delta^{13}\text{C}$ values are used to infer C_3/C_4 food choice in various mammal species, and we examine patterns of faecal $\delta^{15}\text{N}$ amongst mammals with different dietary and ecological adaptations.

METHODS

We collected plant and faecal samples from two reserves in the Waterberg: Zoetfontein Private Game Farm, a small (*c.* 7 km²) reserve located about 40 km south of the town Ellisras, and the Welgevonden Private Game Reserve, a larger reserve (*c.* 330 km²) about 60 km to the south of Zoetfontein. Samples included a variety of plant specimens, comprising tree leaves, tree fruits, forbs, grasses and succulent plants. Faeces were sampled from representative grazing, browsing, and mixed-feeding herbivore taxa. We also obtained faeces from the carnivorous brown hyaena (*Hyaena brunnea*), and a large number of specimens of mixed-feeding African elephant (*Loxodonta africana*; Welgevonden only) and chacma baboon (*Papio ursinus*). In all cases, only the most recently deposited faeces were sampled, and different dung piles were taken to represent different individuals. Whole faecal samples were collected for all species except elephants, where a random sample was taken from the bolus.

Plants and faeces were oven dried at 60°C for 24 hours, thereafter being mill-ground through a 1 mm sieve into a homogenous powder. Samples were combusted individually in an automated Elemental Analyzer (Carlo-Erba). The resultant CO₂ and N₂ gases were analysed for stable carbon and nitrogen isotope ratios, respectively, using a continuous flow-through inlet system attached to a Finnigan MAT 252 Mass Spectrometer. This method also provided us with percentage N for each sample. ¹³C/¹²C and ¹⁵N/¹⁴N ratios are presented in conventional delta (δ) notation in parts per mil (‰) relative to the PDB and N₂ air standards, respectively. Standard deviation for repeated measurements of laboratory standards was less than 0.1‰ for $\delta^{13}\text{C}$, and 0.3‰ for $\delta^{15}\text{N}$.

RESULTS AND DISCUSSION

Reconstructing diet using $\delta^{13}\text{C}$ from plants and faeces

Tree and forb $\delta^{13}\text{C}$ values were as expected for plants following the C_3 -photosynthetic pathway (combined mean = $-25.5 \pm 1.6\text{‰}$, $n = 104$). In contrast, all grass $\delta^{13}\text{C}$ values indicated use of the C_4 pathway (mean = $-11.7 \pm 1.6\text{‰}$, $n = 50$)

Table 1 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (in ‰, relative to the PDB and atmospheric N_2 standards, respectively) and %N of plants from the Waterberg, collected during September 2002 and January 2003.

Growth form	Species	Part	n	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		%N		
				Mean	S.D.	Mean	S.D.	Mean	S.D.	
Tree	<i>Acacia</i> sp.	Fruit	1	-22.8	–	4.4	–	0.7	–	
		Leaves	6	-25.8	1.5	3.0	4.6	2.6	1.3	
	<i>Burkea africana</i>	Leaves	1	-27.2	–	3.4	–	1.7	–	
	<i>Combretum</i> sp.	Fruit	1	-24.6	–	4.4	–	0.7	–	
		Leaves	8	-24.5	1.0	5.2	1.8	1.6	0.2	
	<i>Dichrostachys cinerea</i>	Leaves	1	-26.0	–	5.8	–	3.0	–	
	<i>Diplorhynchus condylocarpon</i>	Leaves	10	-23.9	1.6	3.1	1.6	1.1	0.3	
	<i>Englerophytum magalismontanum</i>	Leaves	3	-26.3	0.5	1.4	0.3	1.2	0.2	
	<i>Euclea crispa</i>	Leaves	1	-27.6	–	-0.1	–	1.6	–	
	<i>Faurea saligna</i>	Leaves	1	-25.9	–	2.6	–	0.8	–	
	<i>Ficus</i> sp.	Fruit	1	-23.1	–	11.3	–	1.6	–	
		Leaves	3	-25.3	1.3	9.6	1.0	1.2	0.4	
	<i>Grewia</i> sp.	Fruit	6	-24.8	1.1	5.9	3.9	1.1	0.3	
		Leaves	15	-26.9	0.8	5.9	2.3	1.9	0.6	
	<i>Ozoroa paniculosa</i>	Leaves	3	-24.4	1.8	6.4	2.5	1.2	0.1	
	<i>Peltophorum africanum</i>	Fruit	1	-24.5	–	2.9	–	0.7	–	
		Leaves	3	-25.0	0.3	5.1	1.1	1.0	0.2	
	<i>Pseudolachnostylis maprouneifolia</i>	Leaves	3	-25.5	0.9	4.8	0.7	1.2	0.0	
	<i>Pterocarpus rotundifolius</i>	Leaves	3	-27.9	0.5	0.6	1.8	1.7	1.2	
	<i>Strychnos pungens</i>	Leaves	1	-25.6	–	2.8	–	1.6	–	
	<i>Syzygium cordatum</i>	Leaves	4	-25.9	1.1	4.3	0.8	1.1	0.2	
	<i>Terminalia sericea</i>	Fruit	1	-23.7	–	4.1	–	1.3	–	
		Leaves	3	-24.6	0.4	2.5	0.3	0.7	0.2	
	<i>Vitex mombassae</i>	Leaves	1	-27.2	–	7.4	–	1.9	–	
	<i>Ximenia caffra</i>	Leaves	7	-28.0	0.6	4.0	1.5	1.3	0.2	
	<i>Ziziphus mucronata</i>	Leaves	3	-26.3	1.2	1.4	1.0	1.9	1.4	
		Combined		91	-25.7	1.6	4.4	2.8	1.5	0.7
Forb	Unidentified	Whole plants	6	-25.3	0.6	4.2	0.8	1.2	0.4	
	Unidentified legume	Leaves	2	-25.0	1.1	1.9	3.2	1.5	0.0	
		Whole plants	3	-27.8	1.9	2.5	2.7	2.5	0.7	
	<i>Xerophyta retinervis</i>	Leaves	1	-25.0	–	2.1	–	1.8	–	
		Stem	1	-23.7	–	-1.4	–	0.3	–	
		Combined		13	-25.7	1.6	2.9	2.2	1.5	0.8
Grass	<i>Aristida</i> sp.	Leaves	6	-12.0	2.0	2.9	3.5	0.9	0.3	
		Roots	6	-12.6	1.3	2.6	0.8	0.6	0.2	
		Seeds	6	-11.8	1.0	3.4	1.3	1.0	0.2	
	<i>Centropodia glauca</i>	Leaves	2	-10.8	0.4	3.3	0.1	1.0	0.2	
		Roots	2	-10.5	0.6	3.9	1.0	0.9	0.1	
		Seeds	2	-10.8	0.5	3.2	1.1	1.2	0.1	
	<i>Digitaria eriantha</i>	Leaves	1	-13.0	–	7.2	–	1.6	–	
		Roots	1	-15.4	–	3.7	–	0.7	–	
		Seeds	1	-12.9	–	3.7	–	1.4	–	
	<i>Enteropogon monostachys</i>	Leaves	1	-14.4	–	1.2	–	0.8	–	
		Roots	1	-11.8	–	1.3	–	1.3	–	
		Seeds	1	-12.6	–	3.1	–	2.0	–	
	<i>Eragrostis</i> sp.	Leaves	5	-11.6	2.2	2.8	0.8	0.8	0.2	
		Roots	3	-12.8	1.2	3.3	0.8	0.6	0.6	
		Seeds	3	-10.9	0.6	4.4	0.9	0.9	0.2	
	<i>Heteropogon contortus</i>	Leaves	1	-9.6	–	0.4	–	1.0	–	
		Roots	1	-10.1	–	0.5	–	1.1	–	
		Seeds	1	-9.4	–	-0.2	–	0.8	–	
	<i>Miscanthus junceus</i>	Leaves	2	-10.9	1.8	7.2	0.4	0.9	0.1	
		Seeds	2	-10.7	2.6	4.8	1.1	1.1	0.1	
	<i>Panicum maximum</i>	Leaves	1	-10.3	–	2.6	–	0.6	–	
		Roots	1	-10.2	–	1.7	–	0.9	–	
		Combined		50	-11.7	1.6	3.2	1.9	0.9	0.3
	Succulent	<i>Agave</i> sp.	Leaves	1	-8.9	–	5.7	–	1.4	–
			Roots	1	-11.9	–	13.3	–	0.7	–
		<i>Euphorbia ingens</i>	Cladode	2	-13.7	1.4	6.0	1.7	1.1	0.6
			Combined		4	-12.1	2.4	7.7	3.8	1.1

n = number of samples.

S.D. = standard deviation.

Table 2. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (in ‰, relative to the PDB and atmospheric N_2 standards, respectively) and %N of mammal faeces from the Waterberg.

Order/family	Species	n	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		%N	
			Mean	S.D.	Mean	S.D.	Mean	S.D.
Primates	<i>Papio ursinus</i>	185	-21.5	1.9	3.9	1.1	2.6	0.7
Rodentia	<i>Hystrix africaeaustralis</i>	7	-24.2	1.0	5.1	0.6	1.9	0.1
Carnivora	<i>Hyaena brunnea</i>	7	-15.9	2.3	7.3	1.0	0.9	0.4
Perissodactyla	<i>Ceratotherium simum</i>	4	-14.5	0.3	4.7	1.0	0.9	0.1
	<i>Equus burchellii</i>	21	-13.9	0.7	4.1	0.8	0.8	0.1
Proboscidea	<i>Loxodonta africana</i>	139	-24.4	1.0	3.4	0.6	1.0	0.2
Artiodactyla								
Suidae	<i>Phacochoerus aethiopicus</i>	2	-13.0	0.5	4.0	0.4	1.0	0.1
	<i>Potamochoerus porcus</i>	3	-21.3	2.7	3.7	0.1	1.8	0.4
Giraffidae	<i>Giraffa camelopardalis</i>	9	-26.0	0.5	4.9	0.8	2.0	0.3
Bovidae	<i>Syncerus caffer</i>	6	-15.0	0.6	4.8	1.2	1.2	0.1
	<i>Taurotragus oryx</i>	4	-26.9	0.2	5.7	0.6	2.1	0.1
	<i>Tragelaphus strepsiceros</i>	17	-25.7	1.0	5.1	0.7	1.9	0.3
	<i>Oryx gazella</i>	4	-14.4	0.7	4.5	0.8	0.9	0.2
	<i>Kobus ellipsiprymnus</i>	2	-14.2	0.3	4.4	0.3	1.1	0.3
	<i>Connochaetes taurinus</i>	19	-14.5	0.8	4.9	0.7	1.0	0.2
	<i>Aepyceros melampus</i>	14	-19.4	4.0	5.3	0.8	1.4	0.3
	<i>Oreotragus oreotragus</i>	2	-25.2	0.9	5.7	0.3	1.7	0.1

n = number of samples; S.D. = standard deviation.

(Table 1). CAM-photosynthesizing (Crassulacean Acid Metabolism) succulent plants (*Euphorbia ingens* and *Agave* sp.) had $\delta^{13}\text{C}$ values indistinguishable from that of C_4 grasses (mean = $-12.1 \pm 2.4\text{‰}$, $n = 4$), signifying obligate CAM pathways (see O'Leary 1988; Keeley & Rundel 2003).

Variations in $\delta^{13}\text{C}$ values within C_3 - and C_4 -photosynthetic groups can influence dietary interpretations significantly, because animal tissues record variations in the carbon isotopic composition of plants. Amongst C_4 grasses, species following different photosynthetic sub-pathways (NAD-ME, PCK, or NADP-ME) reportedly differ significantly in mean $\delta^{13}\text{C}$ values, albeit that these differences are small (Hattersley 1982). In Kenya, Cerling & Harris (1999) found that mean $\delta^{13}\text{C}$ values of NADP-ME grasses was $-11.8 \pm 0.7\text{‰}$, while that for NAD-ME and PCK grasses combined was $-12.8 \pm 0.8\text{‰}$. They further asserted that utilization of these different grass types amongst grazing taxa is discernible in tooth enamel $\delta^{13}\text{C}$ values. We tested for C_4 -subpathway variations in $\delta^{13}\text{C}$ values, using the taxonomic assignments provided by Sage *et al.* (1999). No difference (one-way ANOVA, $P = 0.97$) was found between NADP-ME (mean = $-11.8 \pm 1.8\text{‰}$, $n = 26$) and NAD-ME (mean = $-11.6 \pm 1.5\text{‰}$, $n = 20$) C_4 grass subtypes. This discordance with the findings of Cerling & Harris (1999) is probably because their study

included both mesic and xeric environments, the former predominated by NADP-ME grasses, while the NAD-ME and PCK subtypes were prominent in the latter. The implication is that carbon isotopic differences between grasses are more significant across habitats of different climate regimes, and thus more likely to significantly affect dietary reconstructions when comparing animals from very different environs.

Analysis of $\delta^{13}\text{C}$ variations between different plant parts revealed that mean $\delta^{13}\text{C}$ values of grass parts (leaves, roots, and seeds) did not vary significantly ($P = 0.56$). However, we found that tree fruits were significantly ^{13}C -enriched, by about 1.5 to 2‰ on average, compared to tree leaves (one-way ANOVA, $P < 0.004$). Cerling *et al.* (2004) reported that canopy fruits and seeds of trees in Ituri Forest, Democratic Republic of Congo, were ^{13}C -enriched compared to subcanopy plant plants. The difference in $\delta^{13}\text{C}$ between tree leaves and fruits in the Waterberg has potential implications for dietary interpretations from faecal $\delta^{13}\text{C}$ values, particularly for species such as kudu, giraffe, elephants, and baboons, which regularly consume significant amounts of fruits, seeds, and pods (e.g. Skinner & Smithers 1990).

Faecal $\delta^{13}\text{C}$ values conformed, for the most part, to dietary expectations for browsing (C_3 -feeding), grazing (C_4 -feeding), and mixed-feeding taxa

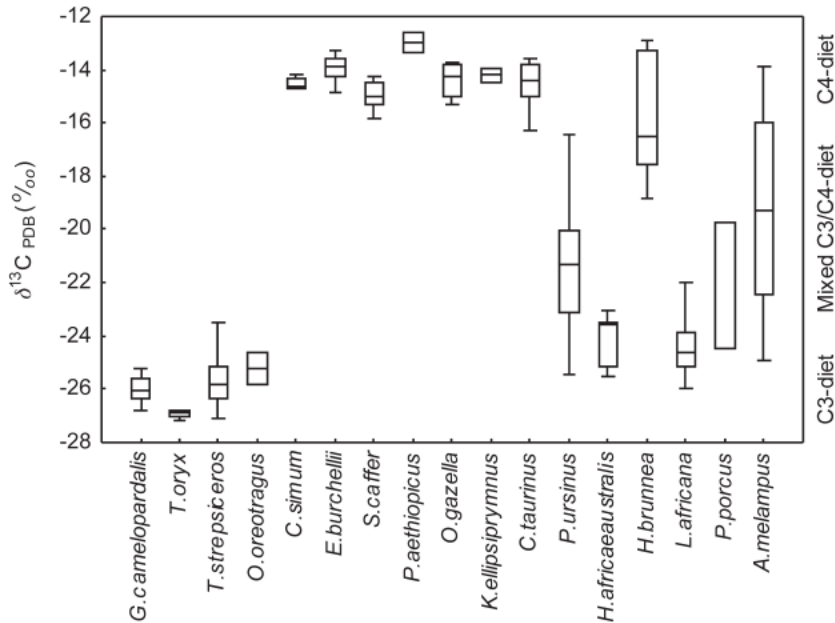


Fig. 1. Distribution of C₃-, C₄-, or mixed C₃/C₄-feeding amongst animals from the Waterberg, as reflected by $\delta^{13}\text{C}$ values of faeces. In the graph, the median for each group is represented by a line within the boxes, the upper and lower limits of each box are the 25th and 75th percentiles, and the whiskers show the non-outlier range.

(Table 2). Giraffe (*Giraffa camelopardalis*), greater kudu (*Tragelaphus strepsiceros*), klipspringer (*Oreotragus oreotragus*), and eland (*Taurotragus oryx*) all have faecal $\delta^{13}\text{C}$ values reflecting C₃-based diets (Fig. 1). This finding is expected, at least for giraffe, kudu and klipspringer, which are predominantly, and often exclusively, browsers (see Skinner & Smithers 1990). Eland are usually classified as mixed-feeders with a preference for browse (*e.g.* Buys 1990; Skinner & Smithers 1990). However, our eland specimens were strongly C₃ (mean = $-26.9 \pm 0.2\text{‰}$, $n = 4$), and thus appear to eat mainly browse-based foods in the Waterberg. Previously, eland diets were shown to be browse-based (dominated by forbs) in another South African reserve, the Mountain Zebra National Park (Watson & Owen-Smith 2000).

Faeces from species classified as grazers had $\delta^{13}\text{C}$ values strongly indicative of C₄-based diets (Fig. 1). The combined mean $\delta^{13}\text{C}$ value for faeces of the square-lipped rhinoceros (*Ceratotherium simum*), Burchell's zebra (*Equus burchellii*), warthog (*Phacochoerus aethiopicus*), gemsbok (*Oryx gazella*), waterbuck (*Kobus ellipsiprymnus*) and blue wildebeest (*Connochaetes taurinus*) was $-14.2 \pm 0.7\text{‰}$ ($n = 52$). Buffalo (*Syncerus caffer*) had slightly lower faecal $\delta^{13}\text{C}$ values than other grazers (mean = $-15.0 \pm 0.6\text{‰}$, $n = 6$, one-way

ANOVA, $P < 0.02$). These results suggest that buffalo in the Waterberg include a small amount of C₃ foods into their diet. However, the buffalo on Welgevonden are sporadically provided with a supplementary feed (A. Burger, pers. comm., 2002). We found that the feed had a mixed C₃/C₄ carbon isotopic content (mean $\delta^{13}\text{C} = -18.1 \pm 0.9\text{‰}$, $n = 3$), thus inferred inputs of C₃ foods to buffalo diets may be complicated by consumption of the artificial food source.

The isotopic distinction between faeces of C₃-feeding browsing taxa (mean = $-25.9 \pm 0.9\text{‰}$, $n = 32$) and C₄-feeding grazers (mean = $-14.3 \pm 0.8\text{‰}$, $n = 58$) was clear and non-overlapping (one-way ANOVA, $P < 0.000001$). In addition, faeces from taxa known to have more mixed diets were isotopically distinct from faeces of both grazers and browsers, and the difference between each mixed-feeding species and the browsing and grazing groups was significant for all cases (Tukey's HSD, $P < 0.05$). This supports that $\delta^{13}\text{C}$ values in faeces are reliable indicators of food intake not only amongst species with pure C₃ or C₄ diets, but amongst mixed-feeding species as well.

The carbon isotopic composition of faeces of porcupine (*Hystrix africaeaustralis*) and bushpig (*Potamochoerus porcus*) reflected varied, but predominantly C₃-based, diets. Even though

sample sizes for these species were small ($n = 7$ and $n = 3$, respectively), a mixed-diet is consistent with expectations for both (see Skinner & Smithers 1990).

Impala (*Aepyceros melampus*) are mixed-feeders that consume varying proportions of browse/grass depending on environmental and demographic factors (Dunham 1982; Skinner & Smithers 1990; Meissner, *et al.* 1996). $\delta^{13}\text{C}$ values of impala faeces from the Waterberg averaged -19.4‰ ($n = 14$), but analysis of 25th and 75th percentiles revealed values ranging from -16.0 to -22.4‰ (Fig. 1). Assuming a diet-faeces carbon isotopic offset of -0.9‰ (Sponheimer *et al.* 2003b,c), and using mean $\delta^{13}\text{C}$ values for C_3 (-25.7‰) and C_4 (-11.7‰) plants from the Waterberg (Table 1) to define isotopic endpoints, these values indicate that impala diets in the Waterberg ranged to constitute between roughly 30 and 75% C_4 grass.

African elephant are known to be mixed-feeders, but may have near-pure grass- or browse-based diets in some regions (Wing & Buss 1970; Laws *et al.* 1974; Owen-Smith 1988). Elephant faecal $\delta^{13}\text{C}$ values show that, in the Waterberg, their diets are predominantly C_3 (mean = $-24.4 \pm 1.0\text{‰}$, 25 to 75% interquartile range = -23.9 to -25.1‰ , $n = 139$), suggesting that grasses make up between about 10 and 20% of the bulk diet in this region (see also Codron 2004). Thus, while impala diets varied considerably, elephants displayed a more concentrated feeding behaviour, at least in terms of browse/grass selection.

Chacma baboon faeces also had a carbon isotopic composition denoting predominantly C_3 -based diets (mean = $-21.5 \pm 1.9\text{‰}$, 25 to 75% interquartile range = -20.1 to -23.1‰ , $n = 185$). These results reflect a diet comprising between about 50 and 70% C_3 -foods for baboons in the Waterberg. The balance of the diet may have included grasses, but CAM-photosynthesizing *Euphorbia ingens*, with a $\delta^{13}\text{C}$ value indistinguishable from that of C_4 grasses, appears to contribute largely to baboon diets at least on Welgevonden (Codron 2003).

Quantifying the diets of carnivores using $\delta^{13}\text{C}$ values is more complex, because different organs and tissues of prey species have different diet-tissue fractionation factors (Tieszen *et al.* 1983; Lee-Thorp *et al.* 1989; Sponheimer *et al.* 2003c). For instance an animal with a 100% browse-based diet may have hair with a $\delta^{13}\text{C}$ value of $\sim -23\text{‰}$, while its bone collagen would tend towards $\sim -21\text{‰}$. The isotopic composition of the tissues of

a carnivore that fed on this animal would depend on the degree to which different body parts were consumed. Nevertheless, carnivore $\delta^{13}\text{C}$ values are consistent with the isotopic composition of their diet, thus it is possible to quantify dependence on C_3 , C_4 , or mixed-feeding prey species. The mean faecal $\delta^{13}\text{C}$ value of brown hyaena (*Hyaena brunnea*) in the Waterberg was $-15.9 \pm 2.3\text{‰}$ ($n = 7$), varying at the 25th and 75th percentile from -17.6 to -13.3‰ , placing them near the C_4 end of the scale (see Fig. 1). Thus, isotopic evidence from faeces suggests that hyaena in the Waterberg feed mainly on the remains of grass-dependent herbivores, although mixed-feeding species make significant contributions to the overall diet.

Patterns of $\delta^{15}\text{N}$ in plants and faeces

Amongst plant groups, the highest $\delta^{15}\text{N}$ values were observed in succulents (mean = $7.7 \pm 3.8\text{‰}$, $n = 4$) (Table 1). A similar ^{15}N -enrichment has previously been reported for succulent plants from East Africa (Koch *et al.* 1991; Muzuka 1999). Inter-group statistical comparisons, using one-way ANOVAs revealed no significant differences ($P = 0.16$) between tree, forb, and grass mean $\delta^{15}\text{N}$ values (tree mean = $4.4 \pm 2.8\text{‰}$, $n = 91$; forb mean = $2.9 \pm 2.2\text{‰}$, $n = 13$; and grass mean = $3.2 \pm 1.9\text{‰}$, $n = 50$). Nitrogen-fixing plants (mainly legumes) may display lower $\delta^{15}\text{N}$ values than non-fixing plants, although this pattern is not consistent. Schmidt & Stewart (2003) observed that ^{15}N -depletion is only consistent amongst nitrogen-fixing plants that have mycorrhizal root associations. In the Waterberg, leguminous trees and forbs (mean = $3.0 \pm 3.0\text{‰}$, $n = 21$) were generally ^{15}N depleted compared to non-leguminous plants (mean = $4.6 \pm 2.7\text{‰}$, $n = 83$) but this difference was not significant ($P = 0.22$), and the ranges overlap considerably.

Percentage nitrogen (%N) of plants followed expected trends for savanna vegetation (*e.g.* Boutton *et al.* 1988). Available browse (trees and forbs) had higher N content (mean = $1.5 \pm 0.7\%$, $n = 104$) than grasses (mean = $0.9 \pm 0.3\%$, $n = 50$), and the difference is significant ($P < 0.01$) (Table 1).

For inter- and intra-specific comparisons of faecal $\delta^{15}\text{N}$ values, all grazing and browsing (including eland) taxa were combined into two groups, respectively, so as to improve the sample sizes for each (Fig. 2). Faecal $\delta^{15}\text{N}$ values are expected to display similar trends to those previously

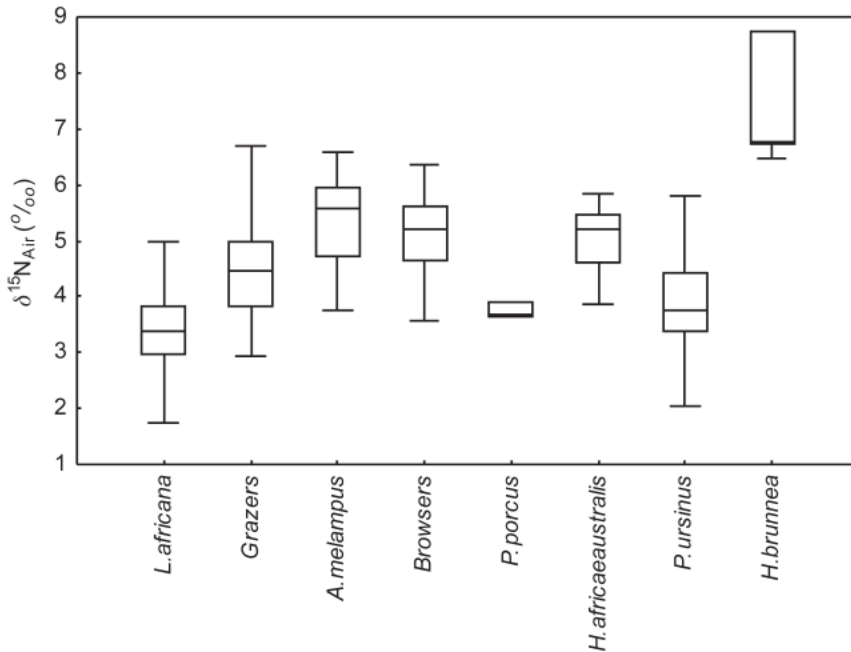


Fig. 2. $\delta^{15}\text{N}$ values of faeces from animals with different dietary habits living in the Waterberg. The median for each group is represented by a line within the boxes, the upper and lower limits of each box are the 25th and 75th percentiles, and the whiskers show the non-outlier range.

observed in mammalian bone collagen, because the nitrogen source of each of these materials is the ^{15}N -enriched protein pool that remains in the body following excretion of ^{15}N -depleted urea (see Ambrose 1991; Sponheimer *et al.* 2003a). Thus, we examined inter-specific differences in $\delta^{15}\text{N}$ of faeces in terms of the predictions based on bone collagen data.

Authors have generally agreed that animal $\delta^{15}\text{N}$ displays a stepwise enrichment of 3 to 4‰ along different trophic levels of the food chain, in terrestrial (Sealy *et al.* 1987; Ambrose 1991), freshwater (Post 2002), and marine environments (Minagawa & Wada 1984). There is a clear distinction between faecal $\delta^{15}\text{N}$ values of herbivores and carnivores. Hyaena faeces were significantly ^{15}N -enriched (mean = $7.3 \pm 1.0\text{‰}$, $n = 7$) compared to herbivore taxa ($P < 0.0001$) (Fig. 2). The trophic enrichment, however, is not consistent in the case of some omnivores. Bushpig (mean = $3.7 \pm 0.1\text{‰}$, $n = 3$) and baboons (mean = $3.9 \pm 1.1\text{‰}$, $n = 185$), both partially omnivorous species, had lower mean faecal $\delta^{15}\text{N}$ values than all other herbivores except for elephants (mean = $3.4 \pm 0.6\text{‰}$, $n = 139$). It might be that omnivores generally have lower $\delta^{15}\text{N}$ values compared to both carnivores and herbivores, but the degree of omnivory in baboons and

bushpigs, particularly in the Waterberg, has not been well documented, thus at this stage it is difficult to resolve their trophic behaviour based on $\delta^{15}\text{N}$ data alone.

According to the urea mass-balance model for ^{15}N -abundances in mammals, drought tolerant species (generally browsers) are expected to be ^{15}N -enriched compared to obligate drinkers (mostly grazers) (Ambrose 1991). This is because drought tolerant species generally retain water for longer periods, and thus produce more concentrated urine than obligate drinkers. The former thereby lose greater amounts of ^{15}N -depleted nitrogen in the form of urea, leading to more ^{15}N -enriched nitrogen remaining in the body nutrient pool. We found that faeces from both browsers (mean = $5.1 \pm 0.8\text{‰}$, $n = 32$) and impala (mean = $5.3 \pm 0.8\text{‰}$, $n = 14$) were significantly ^{15}N -enriched compared to those of grazers (mean = $4.5 \pm 0.8\text{‰}$, $n = 58$) ($P < 0.05$). Interestingly, mixed-feeding impala showed higher mean $\delta^{15}\text{N}$ values than even the browsers, although this difference is not significant ($P = 0.99$). However, the explanation for inter-specific $\delta^{15}\text{N}$ differences as predicted by Ambrose (1991) appears to be an over-simplification. Some drought tolerant species, such as gemsbok, had similar faecal $\delta^{15}\text{N}$

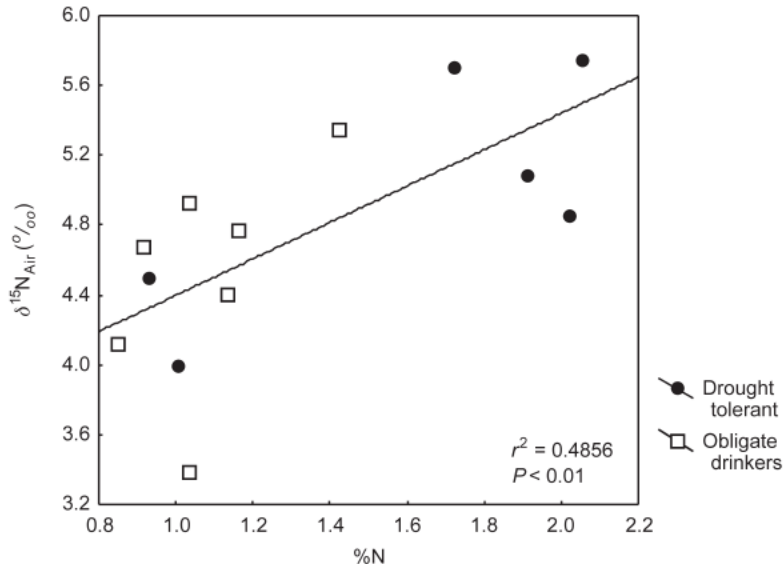


Fig. 3. Relationship between faecal $\delta^{15}\text{N}$ and %N for herbivore species included in this study. Linear regression analysis revealed a positive, significant relationship between these two variables. Species could not be consistently separated into drought tolerant and obligate drinking groups based on $\delta^{15}\text{N}$ values.

compared to other grazer species (Table 2). Ambrose (1991) further predicted that because higher protein intake increases urea loss, species with more protein-rich diets should have higher body tissue (and hence faecal) $\delta^{15}\text{N}$. We examined this scenario further, through analysis of faecal N levels across taxa.

Faecal N content (reflecting crude protein content) provides an indication of the nutritional value of an animal's diet (*e.g.* Holecheck *et al.* 1982; Grant *et al.* 2000). Browsers displayed higher faecal N levels (mean = $1.9 \pm 0.3\%$, $n = 32$) than mixed-feeding impala (mean = $1.4 \pm 0.3\%$, $n = 14$), and grazers had the lowest faecal %N (mean = $1.0 \pm 0.2\%$, $n = 58$) amongst these herbivore groups. Differences in mean faecal %N are significant for browsers compared to impala ($P < 0.05$) and to grazers ($P < 0.0001$), and for impala compared to grazers ($P < 0.05$), and these trends mirror the patterns observed in plants (*i.e.* browse had higher %N than grass). Elephant faeces displayed N levels as low as grazer faeces (mean = $1.0 \pm 0.2\%$, $n = 139$), even though they appear to consume almost only browse-foods in Welgevonden. The most likely explanation is that low %N in elephant faeces is the result of bulk-feeding – elephants seem to compensate for their digestive inefficiencies by consuming very large quantities (Owen-Smith 1988). The implication is that elephants will *per force* consume a wide

variety of food items, many being of poor nutritive value or high fibre content, which contribute to their low faecal %N. The lowest faecal %N values were observed in hyaena (mean = $0.9 \pm 0.4\%$, $n = 7$); this result is undoubtedly related to osteophagy and the resultant high concentrations of calcium in their faeces. Hence, in this case, undigested material strongly influences the result and is not a good reflection of dietary intake quality. Conversely, baboon faeces had the highest N concentrations of all mammals we examined (mean = $2.6 \pm 0.7\%$, $n = 185$; $P < 0.0001$), advocating highly selective, protein-rich diets.

Sponheimer *et al.* (2003a) reported a positive correlation between dietary protein intake and $\delta^{15}\text{N}$, for mammals fed diets of known crude protein and isotopic composition. Excluding carnivores and omnivores to control for trophic level effects on ^{15}N -abundances in mammals, we found that faecal $\delta^{15}\text{N}$ correlated positively with faecal N (Fig. 3). Linear regression analysis revealed that this correlation is significant ($P < 0.01$), although the relationship is fairly weak ($r^2 = 0.4856$), suggesting that other factors are also influencing herbivore $\delta^{15}\text{N}$ values. We then divided species into obligate drinkers and drought tolerant groups, based on whether surface water is considered an essential habitat requirement or not (from Skinner & Smithers 1990). Obligate drinkers and drought tolerant species could not be consistently sepa-

rated after the inferred effect of faecal N content on $\delta^{15}\text{N}$ was controlled for (see Fig. 3). Hence, protein intake appears to have the primary influence on herbivore $\delta^{15}\text{N}$ values, although one or more other environmental variable(s) likely controls further inter- and inter-specific variability, and future studies will need to provide qualitative and quantitative evidence for these factors.

Stable isotopic data from faeces provide a useful means for quantifying mammalian diets over a relatively short time scale. Stable carbon isotope ratios reliably and consistently distinguish between the diets of C_3 -feeders (browsers), C_4 -feeders (grazers), and mixed-feeders. The data can be used to qualify and quantify gross dietary differences across different species. In this study for instance, we observed that eland, known to consume large quantities of grass in some habitats, are predominantly browsers in the Waterberg. We also showed dietary differences between various mixed-feeding taxa, notably that in the Waterberg elephants concentrated largely on browse-based foods, while impala ate a far wider range of C_3/C_4 food items. Faecal $\delta^{15}\text{N}$ values offer some insight into trophic behaviour and dietary crude protein levels. As contemporary understandings of ^{15}N -abundances in mammals improve, the importance of using and applying this isotope as an ecological indicator will increase.

Faecal analysis has the advantage of being a short-term indicator of diet. With increased temporal resolution of field collections, dietary changes could be assessed over biweekly, monthly, or seasonal scales. For example, the Waterberg is a nutrient-poor, recovering sourveld savanna environment, yet results showed that grazing taxa were able to subsist on almost purely grass-based diets. Analysis of grazer faeces collected during the dry winter months, when productivity and nutritional content of the grass layer would be at its lowest, might be expected to reveal a higher intake of C_3 foods by grazers. Monitoring resource utilization through time, especially during critical feeding periods such as at the height of the dry season, would provide information necessary to constantly adapt management policies to suit the needs of resident biota.

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