Functional Ecology
British Ecological Society

Functional Ecology 2014 doi: 10.1111/1365-2435.12275

Does body mass convey a digestive advantage for large herbivores?

Patrick Steuer[1], Karl-Heinz Südekum[1], Thomas Tütken[2,†], Dennis W. H. Müller[3,4], Jacques Kaandorp[5], Martin Bucher[6], Marcus Clauss[3] and Jürgen Hummel*[1,7]

[1]Institute of Animal Science, University of Bonn, Bonn, Germany; [2]Steinmann Institute for Geology, Mineralogy and Palaeontology, University of Bonn, Bonn, Germany; [3]Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland; [4]National Park 'Bavarian Forest', Grafenau, Germany; [5]Safari Beekse Bergen, Hilvarenbeek, The Netherlands; [6]Zoo Zurich, Zurich, Switzerland; and [7]Department of Animal Sciences, Georg-August-University Göttingen, Göttingen, Germany

Summary

1. A key concept of body mass (BM) in niche separation of large herbivores assumes that the decrease in diet quality inherent to increasing BM (due to less selective feeding behaviour) is balanced by a simultaneous increase in digestive ability (due to longer retention times), resulting in no or less-than-expected reduction in digestibility (as measured in the animal as a result of diet quality and digestive ability). However, the second part of this concept has been challenged recently due to theoretical problems and mismatch with empirical data.

2. A proxy for digestibility, such as metabolic faecal nitrogen (MFN), will comprise both information on diet quality and digestive ability in free-ranging animals. In captive animals, if diet is kept constant, such a proxy can exclusively indicate digestive ability. Comparing free-ranging and captive animals under such conditions, one would expect an increase in MFN with BM in captive animals and no relationship between these measures in free-ranging animals if BM was related to digestive ability.

3. We compared captive ungulates on a consistent grass hay diet (17 species; 30–4000 kg BM) to a sample of free-ranging East African ungulates (19 species; 12–4000 kg BM). MFN was used as the major proxy for digestibility.

4. In captive animals, there was no influence of BM on MFN ($P = 0{\cdot}466$); for free-ranging animals, a significant decreasing effect of body mass on MFN ($P = 0{\cdot}002$) and therefore diet quality was found at a scaling of $\mathrm{BM}^{-0{\cdot}15}$.

5. In conclusion, scenarios that assume a compensation of the evident decrease in diet quality with BM via an increased digestive ability are not supported by this study. This does not rule out other feeding-related factors in facilitating large BM, such as compensation by an increased diet intake.

Key-words: African ungulates, Diet quality, faecal nitrogen, feeding ecology, Jarman–Bell principle

Introduction

Body size is an important biological characteristic that determines many anatomical, physiological, ecological and life-history characteristics of animals and hence represents an important feature in evolutionary scenarios (Case 1979; Clutton-Brock & Harvey 1983; Peters 1983; Schmidt-Nielsen 1984; Sibly, Brown & Kodric-Brown 2012). According to Cope′s rule, an increase in body mass (BM) is a typical feature in many lineages, implying a general advantage, which is likely to be composed of a variety of factors related directly to reproductive success or predation avoidance (Hone & Benton 2005). As a particularity of herbivores and especially ungulates, large BM has also been assumed to have an advantageous effect on the species-specific digestibility an animal can achieve on a given food source (digestive ability), influenced, for example, by food retention time (Demment & Van Soest 1985). The latter consideration is based on an influential concept of herbivore nutritional ecology, the Jarman–Bell principle (Bell 1970; Geist 1974; Jarman

---

*Correspondence author. E-mail: jhummel@gwdg.de

[†]Present address. Institute for Geosciences, University of Mainz, Mainz, Germany.

1974), stating that diet quality is negatively correlated with BM in herbivores. Demment and Van Soest (1985) suggested that the scaling of metabolic requirements and food intake ($BM^{0{\cdot}75}$) is opposed by a scaling of gut capacity with $BM^{1{\cdot}0}$, which leads to less energy requirement (or food intake) per unit gut capacity with increasing BM. Longer retention times are therefore considered a consequence of larger BM, which putatively leads to a higher digestive ability in large animals, and therefore, a higher digestibility realized on a given diet compared with smaller herbivores (Demment & Van Soest 1985). This concept is often accepted in its original form, but it has recently been challenged conceptually (Müller *et al.* 2013). Notably, tests using empirical data from the literature (Pérez-Barbería *et al.* 2004; Clauss *et al.* 2007; Müller *et al.* 2013) or our own trials (Steuer *et al.* 2011, 2013) did not support essential parts of this concept (reviewed in Clauss *et al.* 2013).

To avoid conceptual confusion, we define *digestibility* as the realized digestibility, a measure obtained from a specific animal. *Digestibility* can be regarded to be the consequence of the 'animal factor' *digestive ability* (which can be seen as the product of species-specific retention time, food comminution and other animal-specific characteristics) and the 'diet factor' *diet quality* (which means the degradability of the selected forage, which varies due to selected plant species and plant parts). Any measures of *digestibility* will automatically represent the result of the combination of both *digestive ability* and *diet quality*. Differences in *digestive ability* between species have, for example, been extensively documented between ruminant and nonruminant herbivores (Foose 1982; Steuer *et al.* 2013). Differences in *diet quality* due to different nutrient and fibre levels in forage plants have been extensively demonstrated by *in vitro* and *in vivo* experiments (e.g. Hummel *et al.* 2006).

To separate the effects of *digestive ability* and *diet quality* on *digestibility* in free-ranging animals, a comparison of measurements on a consistent test diet fed to various herbivore species with measurements on a comparable sample of species foraging freely in the wild can be used. If *digestive ability* increases significantly with BM, this should be evident on the consistent diet as an increase in *digestibility* with BM (Fig. 1a,c); conversely, if *digestive ability* is not linked to BM, then no scaling between *digestibility* of the consistent diet with BM should be evident (Fig. 1b,d), as indicated by Foose (1982). Additionally, if *diet quality* in the wild does not decrease with BM, then no difference in the scaling of *digestibility* on the consistent diet and the free-range diets should occur (Fig. 1a,b). If, however, *diet quality* in the wild decreases with BM (as suggested theoretically by the relationship between forage quality and abundance – Demment & Van Soest 1985, and empirically by various data collections – Clauss *et al.* 2013), then the scaling exponent for *digestibility* on the free-range diet should be lower than that on the consistent diet (Fig. 1c, d). When assuming an increase in the gut capacity/energy requirements ratio, the hypothetical increase in *digestive ability* in larger herbivores has been assumed to compensate largely for the unavoidable decrease in *diet quality*, resulting in a less severe or no drop (or even an increase) in *digestibility* (Demment & Van Soest 1985). However, as mentioned above, important aspects of this hypotheses have been challenged in a way that would make such compensation less likely.

Given this situation, an evaluation of *digestibility* (as realized by herbivores of varying BM) appears highly desirable. However, measuring *digestibility* directly (which is traditionally carried out in digestion studies that require some manipulation or even confinement of animals to quantify food intake and faecal elimination) cannot be considered a viable option in free-ranging conditions; therefore, any such evaluation basically depends on the availability of an appropriate proxy that can quantify, or at least rank, digestibility under free-range conditions. The most common method in this respect is (total) faecal nitrogen (TFN), which was established first by Lancaster 1949, but has been used routinely since, mostly in ruminants (Lambourne & Reardon 1963; Wallace & Van Dyne 1970; Hofmann & Musangi 1973; Boval *et al.* 2003; Lukas *et al.* 2005; Wang *et al.* 2009), but also in equids (Mésochina *et al.* 1998). Although regularly misinterpreted as a measure for the protein content or protein digestibility of the diet, it should be basically regarded as a proxy for organic matter (OM) digestibility. The major proportion of TFN is based on microbial N (either as residues of microbial cell wall or intact microbial cells). The growth of microbes in the gut will be largely triggered by their energy supply, which is closely linked to the OM digestibility of the diet (Lukas *et al.* 2005). To improve the proxy TFN, it is generally related to OM (and not dry matter) to integrate the effect of the digestion of OM (the majority of which is carbohydrates) into the proxy (as nitrogen concentration in faeces will obviously be increased by more nitrogen first, but to some degree also by less OM). An approach to further increase the correlation between faecal N and OM digestibility is the elimination of N not digested in the gut. Following the elimination of undigested N from TFN, the remaining N fraction in the faeces is defined as metabolic faecal N (MFN). Metabolic faecal N is based predominantly on microbial growth and to a much lower degree on endogenous N losses like sloughed gut cells. A separation between these TFN and MFN fractions is feasible via neutral-detergent fibre analysis, which solubilizes, for example, all microbial matter (Van Soest 1994); the neutral-detergent insoluble N (NDIN) therefore largely represents indigestible N bound to plant cell wall, and its difference to TFN thus represents MFN (Mason 1969; Schwarm *et al.* 2009). The use of MFN facilitates the integration of data from animals feeding on browse. In contrast, the use of TFN as a diet quality or OM digestibility proxy for animals feeding on browse is problematic (Wehausen 1995; Holechek, Pieper & Herbel 2004; Schlecht & Susenbeth 2006), because tannins from such

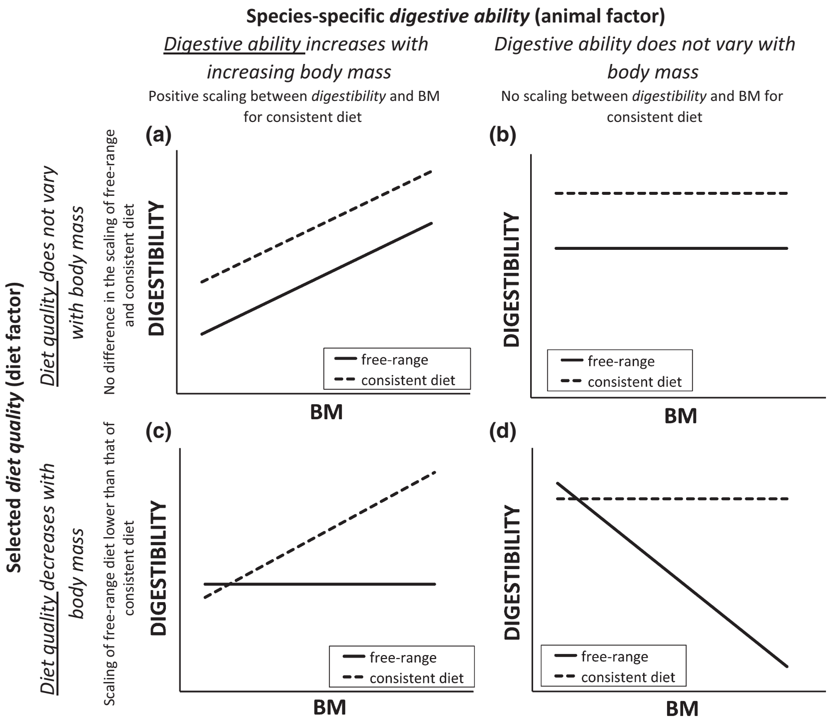

Fig. 1. Schematic presentation of predicted patterns of the scaling of the realized digestibility (*digestibility*) with body mass (BM) on a consistent diet fed in captivity, and diets selected by free-ranging animals, depending on different hypotheses related to the scaling of species-specific *digestive ability* (animal factor contributing to the *digestibility* measurement) and the scaling of selected *diet quality* (diet factor contributing to the *digestibility* measurement) (all axes log-scaled).

diets form insoluble complexes with proteins, which are excreted via faeces and hence inflate the TFN value (Robbins *et al.* 1987; Carulla *et al.* 2005). At least part of the effect of such tannins is eliminated by the use of MFN (Van Soest 1994 page 206).

This study aimed to re-evaluate the connection between BM and *digestibility* in free-ranging herbivores using MFN and TFN as measures of OM digestibility of the diet. To separate the effect of *digestive ability* (animal factor) and *diet quality* (diet factor) (Fig. 1), this was approached with two samples of ungulate herbivores of varying BM, either free-ranging and thus selecting their natural diets, or fed *ad libitum* on a uniform grass hay diet in captivity. While the former ranks the species according to the *digestibility* of their diets in their natural habitat, the latter allows separation of potential effects of body mass (via *digestive ability*) on *digestibility*.

Materials and methods

Seventeen species (10 ruminants, including one camelid, and 7 hindgut fermenters) were used on the consistent diet in captivity (Table 1). Sampling periods were during winter seasons 2008 and 2009; wild animals were investigated at Safari Park Beekse Bergen, The Netherlands, and domestic animals at University of Bonn, Germany (steers and goats), ETH Zurich, Switzerland (ponies, sheep) and a private riding stable close to Wuppertal, Germany (riding horses). Faecal samples were taken after an adaptation period of 14 days during which all animals had *ad libitum* access to a ration of meadow grass hay (unchopped). Chemical composition (in% ± SD organic matter, OM) of the grass hay was neutral-detergent fibre (NDF): 72 ± 3·6%, acid detergent fibre (ADF): 39 ± 3·7%, acid detergent lignin (ADL): 5 ± 1·4%, and crude protein (CP): 10 ± 1·8%. Details of this part of the study can be found in Steuer *et al.* (2011, 2013). The BM of the animals ranged from 49 kg (a domestic goat) up to 6500 kg (an African elephant bull).

Table 1. Body mass (BM), total faecal nitrogen (TFN), metabolic faecal nitrogen (MFN), faecal NDF (FNDF) of herbivores on a consistent (captive) diet

	N	BM Kg	TFN % OM	MFN % OM	FNDF % DM
Antidorcas marsupialis	2	30	2·35	1·90	50·9
Capra aegagrus hircus	6	58	1·56	1·20	64·1
Ovis orientalis aries	3	94	1·72	1·42	54·8
Connochaetes taurinus	5	160	2·18	1·76	52·9
Oryx gazella	3	170	2·05	1·60	56·1
Hippotragus niger	3	170	2·10	1·73	52·7
Kobus ellipsiprymnus	2	210	2·13	1·79	48·4
Syncerus caffer nanus	2	350	2·13	1·73	57·0
Camelus bactrianus	4	450	2·04	1·64	47·7
Bos primigenius taurus	3	1287	1·63	1·32	56·1
Phacochoerus africanus	1	77	1·35	0·99	67·1
*Equus ferus caballus**	3	97	1·40	1·10	68·7
Equus ferus przewalski	4	250	1·49	1·17	70·7
Equus grevyi	4	390	1·39	1·11	66·8
Equus ferus caballus†	6	564	1·24	0·91	70·2
Ceratotherium simum	7	1800	2·06	1·62	55·8
Loxodonta africana	6	4000	1·66	1·31	63·8

*Shetland pony.
†German riding horse.

Samples from free-ranging animals were collected during the dry season in a private sanctuary for wild animals (Lewa Wildlife Conservancy) in Kenya (October/November 2006). Nineteen species of wild ungulates were used including 13 ruminants and 6 hindgut fermenters (Table 1). Faecal samples were collected immediately after observed defecations and dried in a well-ventilated tent placed in the shade. The body mass of all animals was determined using literature data (Robinette 1963; Ledger 1968; Owen-Smith 1988; Estes 1991; Grand 1997).

Dried samples from both trials were ground through a 1-mm sieve. Ash was measured by combustion at 550 °C (VDLUFA 2007; method 8.1), and OM calculated as 100-ash. TFN was measured in dried samples. The undigested N from the diet was quantified by analysing NDIN. Samples were boiled with neutral-detergent solution (Van Soest & Robertson 1985) using the NDF analysis (Van Soest, Robertson & Lewis 1991) with the Gerhardt fibre-bag system (Gerhardt, Königswinter, Germany). For both, whole faeces and faecal NDF residue, N was analysed by the Dumas method (VDLUFA 2007; method 4.1.2; Dumas method) (instrument: FP-328, Leco Inc., St. Joseph, MI, USA). Metabolic faecal N was calculated as MFN = TFN - NDIN (Mason & Frederiksen 1979). This fraction includes mainly microbial debris, but also some sloughed-off animal cells, mucus and gut enzymes (Mason 1969). N concentrations are expressed as related to OM. Faecal NDF (FNDF) values were corrected for ash in NDF residues. FNDF values for captive animals have been reported in Steuer *et al.* (2013).

For the free-ranging animals, actual composition of food ingested during the sampling period (% of grass in diet) was estimated from the faecal stable C-isotope signature (Codron *et al.* 2005). In environments where all grasses are C4 plants, isotope signature can be used as a proxy for grass intake. Estimation was carried out by setting the highest δ^{13}C as a 100% grazer (*Equus grevyi*) and the lowest δ^{13}C as 100% browser (*Tragelaphus scriptus*). For C-isotope analysis, powdered (Retsch ZM1, Retsch GmbH, Haan, Germany) faecal samples were combusted in an automated Carlo Erba Elemental Analyser (NC 2500) and the resultant CO_2 gas was measured in a Thermo Delta Plus XL gas isotope ratio mass spectrometer in the stable isotope laboratory of the Department of Geosciences at the University of Tübingen. The reproducibility of δ^{13}C measurements was better than 0·1‰.

All statistical comparisons were performed with species' means. Relationships between the variables were tested by correlation analysis and general linear models that included digestion type (DT, hindgut fermenter or ruminant) and origin (free-range or captivity) as cofactors (see Supporting information). To account for ancestry-based correlations in the data sets (Felsenstein 1985; Pagel 1999), the data were controlled for phylogenetic influences using phylogenetic leneralized least squares (PGLS) (Martins & Hansen 1997; Rohlf 2001), estimating a covariance matrix of the species due to their ancestral roots and including it in a generalized least squares algorithm to determine model parameters. The phylogenetic trees for the two data sets were derived by pruning the mammalian supertree (Bininda-Emonds *et al.* 2007, 2008) to include only the species of concern for our study, using mesquite (Maddison & Maddison 2008). In the data set on captive animals, the two different domestic horse breeds were represented as direct relatives in the tree. Because the resulting trees were not based on our own calculations of branch lengths with consistently the same characters, we used trees with all branch lengths set to one. Statistical analyses were performed using ordinary least squares (OLS), which do not account for phylogeny, and PGLS. Statistical data evaluations (dependent variables: TFN, MFN and FNDF) were performed with general linear models with log-transformed BM and the% of grass in the natural diet as covariables and digestion type (DT, hindgut fermenter or ruminant) as a cofactor. Even though hay from a single batch was used in captivity, differences between parts of the hay batch fed were controlled for using the gas production after 24 h in the Hohenheim gas test, an *in vitro* fermentation procedure (Menke & Steingass 1988), as a covariable.

In the sample from free-ranging animals, allometric regressions ($y = aBM^b$) were derived for TFN, MFN and FNDF for all ungulates with log-transformed data. The statistical calculations were performed with PASW 18.0 (SPSS Inc., Chicago, IL, USA) and COMPARE 4·6 (Martins 2004). The significance level was set to $\alpha = 0{\cdot}05$.

Results

On the consistent diet, TFN was 1·99 ± 0·26% OM (mean ± SD), MFN was 1·61 ± 0·23% OM, and FNDF was 54·1 ± 4·8% DM in ruminants and 1·51 ± 0·27% OM, 1·17 ± 0·23% OM and 66·2 ± 5·1% DM in hindgut fermenters, respectively (Table 1, Fig 2). A significant effect of BM was not found for TFN or MFN (PGLS: $P = 0{\cdot}531$ and $P = 0{\cdot}466$; Table 2). In contrast, digestion type generally had an effect on both TFN ($P = 0{\cdot}004$) and MFN ($P = 0{\cdot}003$), indicating a higher *digestive ability* in ruminants; similarly, FNDF was lower in ruminants (Steuer *et al.* 2013). Notably, 24 h gas production, the proxy for diet quality of the different parts of the grass hay batch used in the trial, never had a significant effect on the results (Table 2).

In free-ranging animals, TFN ranged from 1·68% OM (hartebeest) to 3·78% OM (gerenuk) in ruminants and from 1·05% OM (elephant) to 1·82% OM (warthog) in hindgut fermenters (Table 3). In the GLM, BM and DT both had a significant effect on TFN, while% grass only had an influence in the OLS data set (Table 4). MFN ranged from 0·79% OM (giraffe) to 1·85% OM (Grant's gazelle) in ruminants and 0·46% OM (black rhino) to 1·06% OM (warthog) in hindgut fermenters (Table 3; Fig 2). In the GLM, both BM and DT had an influence on MFN values, while% grass did not (Table 4). FNDF values ranged from 36·4% DM (hartebeest) to 67·9% DM (giraffe) in ruminants and from 59·2% DM (warthog) to 79·8% DM (black rhinoceros) in hindgut fermenters (Table 3). BM, DT and % grass all had significant effects on FNDF (Table 4).

Allometric regressions for data of free-ranging animals (all PGLS) were

$TFN = 2{\cdot}9\ (95\%\ CI: 1{\cdot}6; 5{\cdot}4) * BM^{-0{\cdot}09\ (95\%\ CI: -0{\cdot}17; -0{\cdot}01)}$; $P = 0{\cdot}054$, $R^2 = 0{\cdot}20$,

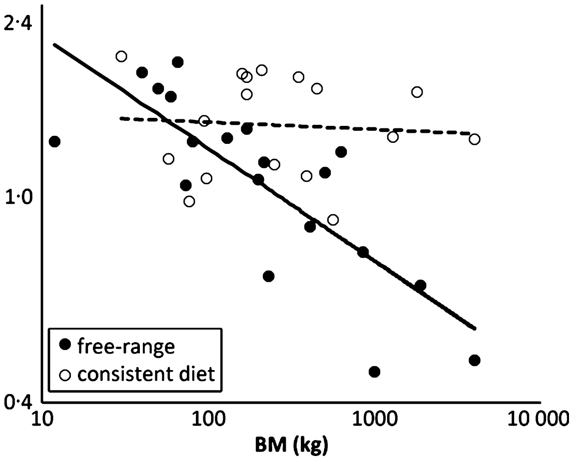

Fig. 2. Original data from this study on the relation of metabolic faecal nitrogen (MFN, in% organic matter; a proxy for the realized *digestibility*) and body mass (BM) in herbivores fed a consistent diet in captivity and feeding on their natural diets as selected by wild, free-ranging animals (axes log-scaled).

Table 2. Results of the statistical analysis of the data set of captive animals using OLS and PGLS (Dependent variables: TFN, MFN, FNDF; independent variables/factors: BM, DT, 24 h GP).

Dependent Variables	Independent Variables	OLS F	*P*	R^2	PGLS t	*P*	R^2
TFN	BM	0·450	0·514	0·49	0·64	0·531	0·47
	DT	11·958	0·004		3·40	0·004	
	24 h GP	0·000	0·987		0·00	1·00	
MFN	BM	0·604	0·451	0·52	0·75	0·466	0·51
	DT	13·470	0·003		3·62	0·003	
	24 h GP	0·005	0·942		0·00	1·00	
FNDF*	BM	1·909	0·190	0·68	1·29	0·221	0·65
	DT	26·500	<0·001		4·93	<0·001	
	24 h GP	0·006	0·941		0·19	0·850	

TFN, total faecal nitrogen; MFN, metabolic faecal nitrogen; FNDF, faecal neutral-detergent fibre; BM, body mass; DT, digestion type (ruminant foregut fermenter or hindgut fermenter); 24-h GP, gas production of hay in Hohenheim gas test (24 h); OLS, ordinary least squares; PGLS, phylogenetic generalized least squares.
*Data from Steuer *et al.* (2013).

Table 3. Body mass (BM), total faecal nitrogen (TFN), metabolic faecal nitrogen (MFN), faecal NDF (FNDF) and % grass in the natural diet as estimated from faecal stable C-isotope composition for free-ranging herbivores on a natural diet

	N	BM Kg	TFN % OM	MFN % OM	FNDF % DM	Grass % in diet
Tragelaphus spekii	3	80	2·96	1·30	58·4	92·1
Alcelaphus buselaphus	1	130	1·68	1·31	36·4	93·4
Oryx beisa	10	170	1·77	1·37	39·8	92·8
Kobus ellipsiprymnus	8	215	2·06	1·18	46·3	86·7
Syncerus caffer	10	630	2·05	1·23	59·7	89·3
Aepyceros melampus	10	50	2·77	1·65	50·6	38·9
Oreotragus oreotragus	9	12	2·81	1·29	42·9	19·0
Lithocranius walleri	4	40	3·78	1·76	53·4	13·8
Tragelaphus scriptus	12	60	2·65	1·58	44·8	0·0
Nanger granti	13	65	2·99	1·85	41·9	19·5
Tragelaphus strepsiceros	10	200	2·29	1·09	61·3	13·0
Taurotragus oryx	12	500	2·12	1·13	58·8	7·3
Giraffa camelopardalis	9	850	2·87	0·79	67·9	8·9
Phacochoerus africanus	11	73	1·82	1·06	59·2	94·8
Equus quagga	12	230	1·19	0·71	64·1	97·0
Equus grevyi	11	410	1·35	0·89	64·0	100·0
Ceratotherium simum	10	1900	1·12	0·68	65·3	95·8
Diceros bicornis	10	1000	1·19	0·46	79·8	11·4
Loxodonta africana	11	4000	1·05	0·48	79·7	33·6

$\mathrm{MFN} = 2{\cdot}3\ (95\%\ \mathrm{CI}{:}\ 1{\cdot}4;\ 4{\cdot}1) * \mathrm{BM}^{-0{\cdot}15\ (95\%\ \mathrm{CI}:\ -0{\cdot}23;\ -0{\cdot}07)}$; $P = 0{\cdot}004$, $R^2 = 0{\cdot}40$, and

$\mathrm{FNDF} = 36{\cdot}3\ (95\%\ \mathrm{CI}{:}\ 26{\cdot}3;\ 50{\cdot}1) * \mathrm{BM}^{0{\cdot}08\ (95\%\ \mathrm{CI}:\ 0{\cdot}02;\ 0{\cdot}14)}$; $P = 0{\cdot}014$, $R^2 = 0{\cdot}31$.

Relationships between the variables are presented in the Supplement. Notably, FNDF did not significantly relate to TFN, but to MFN, in general linear models, emphasizing that variability in TFN most likely introduced by dietary tannins is less when using MFN as digestibility proxy.

Discussion

Questions on resource partitioning and coexistence of large herbivores in diversity hotspots such as East Africa have triggered many investigations and considerations on this topic (Jarman 1974; Hofmann 1989; Duncan *et al.* 1990). Body mass plays an important role in most explanations (Owen-Smith 1988; Gordon & Illius 1994, 1996; Cromsigt, Prins & Olff 2010; Sensenig, Demment & Laca 2010), with potential effects of body size on both an animal′s ability to select forage of a certain degradability (*diet quality*) and to digest food with a species-specific *digestive ability*, the combination of which being the realized *digestibility*. A clear differentiation between these three variables appears essential to the understanding of the problem, and different scenarios can be imagined concerning the combination of the factors (summarized in Fig 1). It was the major goal of this study to test which of these scenarios were best reflected by data.

Data clearly supported scenario 1d (Fig 2): there is no indication for a significant influence of BM on MFN values on a consistent diet, which is in line with results on mean retention times and fibre digestion from the same trial (Steuer *et al.* 2011, 2013). In contrast, MFN data from free-ranging animals decreased significantly with BM. Due to the absence of a significant effect of BM on a consistent diet on *digestibility* and hence *digestive ability* in our captive sample, it may be assumed that the decrease in *digestibility* with BM in the free-ranging animals is attributable to the effect of a decreasing *diet quality*.

A direct evaluation of a variable such as *digestibility* may be unrealistic under free-ranging conditions, so any attempt to establish and quantify relations as outlined above will

Table 4. Results of the statistical analysis of the data set on free-ranging animals using OLS and PGLS (Dependent variables: TFN, MFN, FNDF; independent variables/factors: BM, DT,% grass in diet)

Dependent Variables	Independent Variables	OLS F	*P*	R^2	PGLS t	*P*	R^2
TFN	BM	8·09	0·012	0·76	2·56	0·022	0·72
	DT	8·55	0·010		3·04	0·008	
	% grass	4·51	0·051		1·62	0·127	
MFN	BM	14·2	0·002	0·78	3·78	0·002	0·77
	DT	10·3	0·006		3·15	0·007	
	% grass	0·143	0·711		0·380	0·706	
FNDF	BM	11·9	0·004	0·76	3·42	0·004	0·76
	DT	11·5	0·004		3·33	0·005	
	% grass	6·17	0·025		2·42	0·028	

TFN, total faecal nitrogen; MFN, metabolic faecal nitrogen; FNDF, faecal neutral-detergent fibre; BM, body mass; DT, digestion type (ruminant foregut fermenter or hindgut fermenter); OLS, ordinary least squares; PGLS, phylogenetic generalized least squares.

ultimately depend on the suitability of the digestibility proxies used. These proxies generally each have specific assumptions and potential shortcomings (Barboza, Parker & Hume 2009). Of the three measures used in this study – TFN, MFN, FNDF –, the latter has the disadvantage that it can be influenced in a misleading way by digestibility of the non-NDF fraction: increasing digestibility of this fraction (clearly an indication of higher diet quality) increases FNDF, which is regarded as being negatively correlated to diet quality (in addition, FNDF was most distinctively affected by the botanical composition of the diet). This shortcoming is not present in TFN and MFN where, besides the increase in faecal N due to the digestibility induced increased microbial growth, an increase in OM digestibility itself is reinforcing the increasing effect on the proxy. While TFN is the value used in most studies, we consider subtracting indigestible N (NDIN) as a logical way to exclude this interference factor and to improve the relation. This approach has been suggested and applied by several studies (e.g. Mason 1969; Schwarm *et al.* 2009). In a sample of animals ingesting some tannin-rich forage, the use of MFN instead of TFN also has the capacity to exclude this potentially interfering factor at least partly from analyses (see a detailed discussion of the solubility of tannins in detergent solutions in Van Soest 1994). This is strongly supported by the fact that botanical composition of the diet had much less influence on MFN than on TFN or FNDF (Table 4). MFN is therefore clearly the proxy of choice for DIGESTIBILITY in this study; however, reference to TFN or FNDF would not change conclusions (although generally putting them on a slightly lower statistical significance level than for MFN). While the approach of subtracting undegradable fractions to increase validity of a proxy for diet quality is straightforward, further detailed validations of MFN as a digestibility proxy superior to TFN are encouraged for different feeding situations.

Indications for a negative correlation of BM and diet quality have been found in several studies on East African herbivores. Depending on the proxy used for diet quality, they may represent more the level of *digestibility,* for example, TFN or FNDF (as used in Codron *et al.* 2007) or more the level of *diet quality,* for example, protein content of gut contents or the proportion of non-stem material in gut contents (Owen-Smith 1988). Owen-Smith (1988) also gives estimations for allometric scaling of these diet quality proxies: The proportion of non-stem material in gut contents was related to $BM^{-0{\cdot}12}$, while the protein content of gut contents was related to $BM^{-0{\cdot}23}$ (see Illius & Gordon 1999; and Clauss *et al.* 2013 for comprehensive summaries and discussions of estimated allometries). In the allometric equation of our free-range sample, a decrease in MFN proportional to $BM^{-0{\cdot}15}$ was found. It is tempting to make direct comparisons of allometric exponents of different proxies for one trait; however, the shortcomings linked to such comparison can best be demonstrated for the pair ‘faecal N’ and ‘digestibility’, both proxies for the trait ‘diet quality’: For these variables (that have an identical biological meaning), significantly different allometric exponents will be derived in a data set as the values of these variables fall within a different range of numerical magnitudes (for DM digestibility, assumed range is between 40 and 70%, resulting in a multiplying factor of 1·75; corresponding MFN values range from 0·4 to 1·8, resulting in a multiplying factor of 4·5). Allometries based on the proxy with the larger numerical range/multiplying factor will inevitably result in numerically larger exponents. For the example of MFN and digestibility, this means that the effect of BM on digestibility is overestimated by the use of MFN as a proxy.

A decrease in *diet quality* with BM can be regarded as a safe assumption in a sample of large herbivores; however, it was not clear to which extent its reflection in *digestibility* could have been masked by an increased *digestive ability* in larger animals. Such an increase in *digestive ability* (via a prolonging of retention time) with BM is a major postulate in descriptions of differentiation of nutritional niches of large herbivores. However, given the lack of evidence for an effect of BM on MFN in animals on a consistent diet, this relation is not supported by data of this study. It has been implied that an increase in retention time always has the capacity to balance a decrease in diet quality; however, this assumes a decrease in forage degradability mainly as a decrease in degradation rate (as a consequence of an increase in content of slower-fermenting cell wall), and not due to a decrease in overall potential degradability (more lignin in the cell walls). In the latter case, which is present in any decrease in diet quality, an increase in fermentation time would not improve digestibility, as lignin and lignified cellulose can be considered completely indigestible in the anaerobic condition of the gut irrespective of how long they are retained (Van Soest 1994). Therefore, an increased retention time may be considered much less of an advantage (perhaps even a disadvantage) if the decrease in diet quality is due to lignin contents (Hummel *et al*. 2006).

The results of this study provoke the question whether differences in BM of herbivores represent any advantage for their capacity to handle high fibre diets. Is the presence of large animals in low-quality diet niches a result of the fact that this is the only niche representing enough biomass? Do small herbivores actually not *require* high-quality forage, but, in contrast to larger species, may they simply be able *to be as selective as to get it* (Clauss *et al*. 2013), for example, due to smaller muzzles? At this stage, other advantages of large body size related to digestive physiology must not be discounted, for example, the postulate of a more favourable relation of gut fill to energy requirements with increasing body mass has been confirmed, allowing large animals a higher intake per unit of energy requirement (Hackmann & Spain 2010; Müller *et al*. 2013). In addition, the ability to harvest physically more resistant parts of plants should be considered a potential positive outcome on the capability to handle high fibre food in large herbivores that contributes to high biomass availability for larger animals.

To conclude, while an increase in BM in herbivores results in a significant decrease in the quality of forage generally ingested, this is apparently not balanced by a higher digestive ability of larger animals. The latter result clearly contradicts former views on herbivore nutritional physiology. Data do not indicate the existence of an advantage of large BM for chemical digestion; however, such an advantage should not be ruled out for more physical aspects of ingestion. Other concepts of large herbivore species diversification and niche differentiation than those based on digestive physiology should be further explored.

Acknowledgements

We thank Lewa Wildlife Conservancy for providing the free-range samples and several institutions (Safari Park Beekse Bergen, Prof. Dr. U. Braun (University Zurich), Prof. Dr. M. Kreuzer (ETH Zurich), riding stable Lückerath) for supporting the collection of samples on the grass hay diet. This is contribution no. 162 of the DFG Research Unit 533 "Biology of the Sauropod Dinosaurs".

References

Barboza, P.S., Parker, K.L. & Hume, I.D. (2009) *Integrative Wildlife Nutrition*. Springer-Verlag, Berlin Heidelberg.

Bell, R.H.V. (1970) The use of the herb layer by grazing ungulates in the Serengeti. *Animal Populations in Relation to their Food Resources* (ed. A. Watson), pp. 111–124. Blackwell Scientific Publications, Oxford.

Bininda-Emonds, O.R.P., Cardillo, M., Jones, K.E., Macphee, R.D.E., Beck, R.M.D., Grenyer, R. *et al*. (2007) The delayed rise of present-day mammals. *Nature*, **446**, 507–512.

Bininda-Emonds, O.R.P., Cardillo, M., Jones, K.E., Mac Phee, R.D.E., Beck, R.M.D., Grenyer, R. *et al*. (2008) Corrigendum: the delayed rise of present-day mammals. *Nature*, **456**, 274.

Boval, M., Archimède, H., Fleury, J. & Xandé, A. (2003) The ability of faecal nitrogen to predict digestibility for goats and sheep fed with tropical herbage. *Journal of Agricultural Science (Cambridge)*, **140**, 443–450.

Carulla, J.E., Kreuzer, M., Machmüller, A. & Hess, H.D. (2005) Supplementation of *Acacia mearnsii* tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. *Australian Journal of Agricultural Research*, **56**, 961–970.

Case, T.J. (1979) Optimal body size and an animal's diet. *Acta Biotheoretica*, **28**, 54–69.

Clauss, M., Schwarm, A., Ortmann, S., Streich, W.J. & Hummel, J. (2007) A case of non-scaling in mammalian physiology? Body size, digestive capacity, food intake, and ingesta passage in mammalian herbivores. *Comparative Biochemistry and Physiology A*, **148**, 249–265.

Clauss, M., Steuer, P., Müller, D.W.H., Codron, D. & Hummel, J. (2013) Herbivory and body size: allometries of diet quality and gastrointestinal physiology, and implications for herbivore ecology and dinosaur gigantism. *PLoS One*, **8**, e68714.

Clutton-Brock, T.H. & Harvey, P.H. (1983) The functional significance of variation in body size among mammals. *Advances in the Study of Mammalian Behaviour. Special Publication American Society Mammalogy 7* (eds J. F. Eisenberg & D. G. Kleimann), pp. 632–663. Shippenburg State College, Stillwater, OK, USA.

Codron, D., Codron, J., Sponheimer, M., Lee-Thorp, J.A., Robinson, T., Grant, C.C. *et al*. (2005) Assessing diet in savannah herbivores using stable carbon isotope ratios of faeces. *Koedoe*, **48**, 115–124.

Codron, D., Lee-Thorpe, J.A., Sponheimer, M., Codron, J., De Ruiter, D. & Brink, J.S. (2007) Significance of diet type and diet quality for ecological diversity of African ungulates. *Journal of Animal Ecology*, **76**, 526–537.

Cromsigt, J.P.G.M., Prins, H.H.T. & Olff, H. (2010) Habitat heterogeneity as a driver of ungulate diversity and distribution patterns: interaction of body mass and digestive strategy. *Diversity and Distributions*, **15**, 513–522.

Demment, M.W. & Van Soest, P.J. (1985) A nutritional explanation for body-size patterns of ruminant and nonruminant herbivores. *American Naturalist*, **125**, 641–672.

Duncan, P., Foose, T.J., Gordon, I.J., Gakahu, C.G. & Lloyd, M. (1990) Comparative nutrient extraction from forages by grazing bovids and equids: a test of the nutritional model of equid/bovid competition and coexistence. *Oecologia*, **84**, 411–418.

Estes, R.D. (1991) *The Behaviour Guide to African Mammals*. University of California Press, Berkeley, CA, USA.

Felsenstein, J. (1985) Phylogenies and the comparative method. *The American Naturalist*, **125**, 1–15.

Foose, T.J. (1982) *Trophic Strategies of Ruminant versus Nonruminant Ungulates*. PhD thesis University of Chicago, Chicago, IL, USA

Geist, V. (1974) On the relationship of social evolution and ecology in ungulates. *American Zoologist*, **14**, 205–220.

Gordon, I.J. & Illius, A.W. (1994) The functional significance of the browser-grazer dichotomy in African ruminants. *Oecologia*, **98**, 167–175.

Gordon, I.J. & Illius, A.W. (1996) The nutritional ecology of African ruminants: a reinterpretation. *Journal of Animal Ecology*, **65**, 18–28.

Grand, T.I. (1997) How muscle mass is part of the fabric of behavioural ecology in East African bovids (*Madoqua, Gazella, Damaliscus, Hippotragus*). *Anatomy and Embryology*, **195**, 375–386.

Hackmann, T.J. & Spain, J.N. (2010) Ruminant ecology and evolution: perspectives useful to ruminant livestock research and production. *Journal of Dairy Science*, **93**, 1320–1334.

Hofmann, R.R. (1989) Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. *Oecologia*, **78**, 443–457.

Hofmann, R.R. & Musangi, R.S. (1973) Comparative digestibility coefficients of domestic and game ruminants from marginal land in East Africa. *Bulletin of Epizootic Diseases of Africa*, **21**, 385–388.

Holechek, J.L., Pieper, R.D. & Herbel, C.H. (2004) *Range Management Principles and Practices*, 5th edn. Pearson Prentice Hall, Upper Saddle River, NJ, USA.

Hone, D.W.E. & Benton, M.J. (2005) The evolution of large size: how does Cope's Rule work? *Trends in Ecology and Evolution*, **20**, 4–6.

Hummel, J., Südekum, K.-H., Streich, W.J. & Clauss, M. (2006) Forage fermentation patterns and their implications for herbivore ingesta retention times. *Functional Ecology*, **20**, 989–1002.

Illius, A.W. & Gordon, I.J. (1999) The physiological ecology of mammalian herbivores. *Nutritional Ecology of Herbivores. Proceedings of the 4th International Symposium on the Nutrition of Herbivores*. (eds H.J.G. Jung & G.C. Fahey), pp. 71–96. American Society of Animal Science.

Jarman, P.J. (1974) The social organization of antelope in relation to their ecology. *Behaviour*, **48**, 215–268.

Lambourne, L. & Reardon, T.F. (1963) The use of chromium oxide and faecal nitrogen concentration to estimate the pasture intake of merino wethers. *Australian Journal of Agricultural Research*, **14**, 257–271.

Lancaster, R.J. (1949) Estimation of digestibility of grazed pasture from faeces nitrogen. *Nature*, **163**, 330–331.

Ledger, H.P. (1968) Body composition as a basis for a comparative study of some East African mammals. *Symposium of the Zoological Society London*, **21**, 289–310.

Lukas, M., Südekum, K.-H., Rave, G., Friedel, K. & Susenbeth, A. (2005) Relationship between fecal crude protein concentration and diet organic matter digestibility in cattle. *Journal of Animal Science*, **83**, 1332–1344.

Maddison, W.P. & Maddison, D.R. (2008) Mesquite: a modular system for evolutionary analysis. http://mesquiteproject.org.

Martins, E. (2004) COMPARE, version 4.6. *Computer Program for the Statistical Analysis of Comparative Data*. Available at: http://compare.bio.indiana.edu/. Department of Biology, Indiana University, Bloomington, IN, USA.

Martins, E. & Hansen, T. (1997) Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into analysis of interspecific data. *American Naturalist*, **149**, 646–667.

Mason, V.C. (1969) Some observations on the distribution and origin of nitrogen in sheep faeces. *Journal of Agricultural Science (Cambridge)*, **73**, 99–111.

Mason, V.C. & Frederiksen, J.H. (1979) Partition of the nitrogen in sheep faeces with detergent solutions, and its application to the estimation of the true digestibility of dietary nitrogen and the excretion of non dietary nitrogen. *Journal of Animal Physiology and Animal Nutrition*, **41**, 121–131.

Menke, K.H. & Steingass, H. (1988) Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Animal Research and Development*, **28**, 7–55.

Mésochina, P., Martin-Rosset, W., Peyraud, J.L., Duncan, P., Micol, D. & Boulot, S. (1998) Prediction of the digestibility of the diet of horses: evaluation of faecal indices. *Grass and Forage Science*, **53**, 189–196.

Müller, D.W.H., Codron, D., Meloro, C., Munn, A., Schwarm, A., Hummel, J. *et al*. (2013) Assessing the Jarman-Bell principle: scaling of intake, digestibility, retention time and gut fill with body mass in mammalian herbivores. *Comparative Biochemistry and Physiology A*, **164**, 129–140.

Owen-Smith, N. (1988) *Megaherbivores*. Cambridge University Press, Cambridge.

Pagel, M. (1999) Inferring the historical patterns of biological evolution. *Nature*, **401**, 877–884.

Pérez-Barbería, F.J., Elston, D.A., Gordon, I.J. & Illius, A.W. (2004) The evolution of phylogenetic differences in the efficiency of digestion in ruminants. *Proceedings Royal Society London B*, **271**, 1081–1090.

Peters, R. (1983) *The Ecological Implications of Body Size*. Cambridge University Press, Cambridge.

Robbins, C.T., Hanley, T.A., Hagerman, A.E., Hjeljord, O., Baker, D.L., Schwartz, C.C. *et al*. (1987) Role of tannins in defending plants against ruminants: reduction in protein availability. *Ecology*, **68**, 98–107.

Robinette, W.L. (1963) Weights of some of the larger mammals of Northern Rhodesia. *The Puku*, **1**, 207–215.

Rohlf, F. (2001) Comparative methods for the analysis of continuous variables: geometric interpretations. *Evolution*, **55**, 2143–2160.

Schlecht, E. & Susenbeth, A. (2006) Estimating the digestibility of Sahelian roughages from faecal crude protein concentration of cattle and small ruminants. *Journal of Animal Physiology and Animal Nutrition*, **90**, 369–379.

Schmidt-Nielsen, K. (1984) *Scaling*. Cambridge University Press, Cambridge.

Schwarm, A., Schweigert, M., Ortmann, S., Hummel, J., Janssens, G.P.J., Streich, W.J. *et al*. (2009) No easy solution for the fractionation of faecal nitrogen in captive wild herbivores: results of a pilot study. *Journal Animal Physiology and Animal Nutrition*, **93**, 596–605.

Sensenig, R.L., Demment, M.W. & Laca, E.A. (2010) Allometric scaling predicts preferences for burned patches in a guild of East African grazers. *Ecology*, **91**, 2898–2907.

Sibly, R.M., Brown, J.H. & Kodric-Brown, A. (2012) *Metabolic Ecology: A Scaling Approach*. John Wiley and Sons, Hoboken, NJ, USA.

Steuer, P., Südekum, K.-H., Müller, D.W.H., Franz, R., Kaandorp, J., Clauss, M. *et al*. (2011) Is there an influence of body mass on digesta mean retention time in herbivores? A comparative study on ungulates. *Comparative Biochemistry and Physiology A*, **160**, 355–364.

Steuer, P., Südekum, K.-H., Müller, D.W.H., Kaandorp, J., Clauss, M. & Hummel, J. (2013) Fibre digestibility in large herbivores as related to digestion type and body mass - an *in vitro* approach. *Comparative Biochemistry and Physiology A*, **164**, 319–326.

Van Soest, P.J. (1994) *Nutritional Ecology of the Ruminant*, 2nd edn. Cornell University Press, Ithaca, NY, USA.

Van Soest, P.J. & Robertson, J.B. (1985) *Analysis of Forages and Fibrous Foods. A Laboratory Manual for Animal Science*. Cornell University, Ithaca, NY, USA.

Van Soest, P.J., Robertson, J.B. & Lewis, B.A. (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, **74**, 3583–3597.

VDLUFA (2007) *Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten. Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch), Bd. III. Die Chemische Untersuchung von Futtermitteln*. VDLUFA-Verlag, Darmstadt.

Wallace, G. & Van Dyne, G.M. (1970) Precision of indirect methods for estimating digestibility of forage consumed by grazing cattle. *Journal of Range Management*, **23**, 424–430.

Wang, C.J., Tas, B.M., Glindemann, T., Rave, G., Schmidt, L., Weißbach, F. *et al*. (2009) Fecal crude protein content as an estimate of the digestibility of forage in grazing sheep. *Animal Feed Science and Technology*, **149**, 199–208.

Wehausen, J.D. (1995) Fecal measures of diet quality in wild and domestic ruminants. *Journal of Wildlife Management*, **59**, 816–823.

Received 12 September 2013; accepted 10 March 2014
Handling Editor: Clare McArthur

Supporting Information

Additional Supporting information may be found in the online version of this article:

Fig. S1. Relationships between the variables of this study (species averages): FNDF faecal neutral-detergent fibre (in % faecal dry matter DM), TFN total faecal nitrogen (in % faecal organic matter OM), NDIN neutral-detergent insoluble nitrogen (%OM), MFN metabolic faecal nitrogen (%OM).

Table S1. Bivariate correlations between the variables of this study (species averages, OLS only, all data combined, free-ranging and captive animals separately, and divided according to digestion type into ruminants RUM and hindgut fermenters HF; all data normally distributed): FNDF faecal neutral-detergent fibre (in % faecal dry matter DM), TFN total faecal nitrogen (in % faecal organic matter OM), NDIN neutral-detergent insoluble nitrogen (%OM), MFN metabolic faecal nitrogen (%OM).

Table S2. General Linear Model results for the variables of this study (species averages, OLS only, all data as well as all model residuals normally distributed an): FNDF faecal neutral-detergent fibre (in % faecal dry matter DM), TFN total faecal nitrogen (in % faecal organic matter OM), NDIN neutral-detergent insoluble nitrogen (%OM), MFN metabolic faecal nitrogen (%OM).

Table S3. Original data used in the study: captive animals.

Table S4. Original data used in the study: free-ranging animals.