Comparative Biochemistry and Physiology, Part A 156 (2010) 380–388

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Comparative investigations on digestion in grazing (*Ceratotherium simum*) and browsing (*Diceros bicornis*) rhinoceroses

P. Steuer [a], M. Clauss [b], K.-H. Südekum [a], J.-M. Hatt [b], S. Silinski [c], S. Klomburg [d], W. Zimmermann [e], J. Fickel [f], W.J. Streich [f], J. Hummel [a,*]

[a] *Institute of Animal Science, University of Bonn, 53115 Bonn, Germany*
[b] *Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, 8057 Zurich, Switzerland*
[c] *Westfälischer Zoologischer Garten Münster, 48161 Munster, Germany*
[d] *Zoo Osnabrück, 49082 Osnabruck, Germany*
[e] *Zoologischer Garten Köln, 50735 Cologne, Germany*
[f] *Leibniz-Institute of Zoo and Wildlife Research (IZW), 10315 Berlin, Germany*

ARTICLE INFO

Article history:
Received 18 December 2009
Received in revised form 8 March 2010
Accepted 9 March 2010
Available online 12 March 2010

Keywords:
Black rhino
White rhino
Fiber digestion
Mean retention time
Fecal particle size

ABSTRACT

Rhinoceroses represent the largest extant herbivores with extensive dietary specialization for plant groups like browse (black rhino *Diceros bicornis*) or grass (white rhino *Ceratotherium simum*). However, it is not clear to what extent such diet selection patterns are reflected in adaptations of digestive physiology of the respective feeding types. In this study, feeding trials with four black and five white rhinos were conducted in four zoos. The animals had ad libitum access to the same batch of grass hay (second cut; neutral detergent fiber (NDF) 63% dry matter (DM), crude protein 10.2% DM). Total intake, fecal N content, in vitro digestibility of NDF residues of feces, fecal particle size and mean retention time (MRT) of particles (Cr-mordanted fiber; 1–2 mm) and fluid (Co-EDTA) were quantified. The average daily DM intake was 70 ± 12 g/kg $BW^{0.75}$ for white and 73 ± 10 g/kg $BW^{0.75}$ for black rhinos. In the in vitro fermentation test fecal NDF residues of black rhinos resulted in higher gas productions at fermentation times of 12 to 24 h, indicating that white rhinos have a superior capacity to digest NDF. Average MRT for fluids and particles was 28 ± 4 h and 43 ± 5 h in white and 34 ± 4 h and 39 ± 4 h in black rhinos. The selectivity factor ($SF = MRT_{particle}/MRT_{fluid}$) was higher for white ($1.5 \pm 0.2$) than for black rhinos (1.2 ± 0.1) ($p = 0.016$). In a comparison of 12 ruminant and 3 rhino species, SF was correlated to percentage of grass in diet ($R = 0.75$). Mean fecal particle size was higher in white (9.1 ± 1.94 mm) than in black rhinos (6.1 ± 0.79 mm) ($p = 0.016$). The results demonstrate differences between white and black rhinos in terms of retention times and fiber digestibility. The more selective retention of particles by the white rhino corresponds with the higher digestion of fiber measured indirectly. Furthermore there is indication for a general pattern of high SF in grazing ruminants and rhinos. The difference in fecal particle size between both rhino species might be due to the considerable difference in body weight.

1. Introduction

1.1. Digestive physiology of browsers and grazers

Among extant vertebrates, mammals have developed the largest diversity of herbivores. In accordance with their selection of food plants, they have been classified as grazing (focusing on leaves and stems of grass), browsing (focusing on leaves and stems of trees, shrubs or herbs) or intermediate feeding types (the latter switching between the two extremes). The respective feeding niche can be reflected in various aspects of biology (see Gordon and Prins, 2008 for reviews). Morphological adaptations of feeding types have received most attention in ruminants (Hofmann, 1973, 1989), and to some extent in macropods (Sanson, 1989; Hume, 1999). On a physiological level, an effective particle retention was postulated to be a particularly adaptive evolutionary feature in grazers (Kay et al., 1980; Foose, 1982). This is explained by the higher proportion of slow fermenting fiber in grass compared to browse (Short et al., 1974; Foose, 1982; Hummel et al., 2006), and has been described for ruminants (Clauss and Lechner-Doll, 2001; Hummel et al., 2005; Clauss et al., 2002b). Furthermore, differences in tooth morphology can potentially lead to a decrease in food comminution in browsing herbivores leading to larger fecal particles in browsing ruminants (Clauss et al., 2002b) and macropods (Lentle et al., 2003).

Comparable specialization has been reported for other herbivores such as hyraxes (Deniro and Epstein, 1978) and rodents (Williams

* Corresponding author. University of Bonn, Institute of Animal Science, Endenicher Allee 15, 53115 Bonn, Germany. Tel.: +49 228 732281; fax: +49 228 732295.
E-mail address: jhum@itw.uni-bonn.de (J. Hummel).

1095-6433/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2010.03.006

and Kay, 2001). Grazers and browsers are well documented in perissodactyls in the fossil record with browsing taxa like *Sinohippus* occurring well into the late Miocene (MacFadden, 2005). However, rhinoceroses are the only group with extant representatives of both feeding types.

1.2. Grazing and browsing rhinos

The white rhinoceros (*Ceratotherium simum*) is classified as a typical grazing species, with a dietary proportion of herbs as low as 1% and no intake of browse at all, while the black rhinoceros (*Diceros bicornis*) has a proportion >95% of dicot material in its diet (Owen-Smith, 1988). Separation of the genera took place in the late Miocene to early Pleistocene (6 to 2 million years ago) (Hooijer, 1969; Hooijer and Pattersson, 1972; Hooijer, 1976; Groves, 1997). Both species can be considered to represent the largest extant herbivores truly specialized for a forage type (Owen-Smith, 1988; Shrader et al., 2006), with only the common hippopotamus (*Hippopotamus amphibius*) rivaling the white rhino as the largest specialized grazer. In accordance with their feeding habit, adaptations of the chewing apparatus have been described for rhinos. *D. bicornis* has a two-phased chewing activity with a cutting ectoloph and more grinding lophs on the lingual side, while *C. simum* has more hypsodont teeth and shows a flat grinding occlusal surface in the upper molars with closely packed shearing blades and more cementum (Schaurte, 1966; Fortelius, 1982; Thenius, 1989). *Ceratotherium* is also described to have more pronounced lateral jaw movements, a longer relative premolar row length, and a lower degree of blade sharpness (Thenius, 1989; Popowics and Fortelius, 1997; Palmqvist et al., 2003). Based on his comprehensive comparative investigations on digestion in ungulates, Foose (1982; page 130–133) postulated differing trophic strategies for grazing and browsing rhinos: The latter are expected to have a shorter retention time and a lower digestibility. Based on data collected from various feeding trials, these assumptions seem to be confirmed (Clauss et al., 2005a, 2006a). Potentially related to that, experience indicates that the black rhino can be considered a more challenging herbivore to feed in captivity compared to its grazing relative (Dierenfeld, 1995, 1999; Clauss and Hatt 2006).

In comparative physiological studies, the aim generally is to test for adaptations to certain environmental factors, e.g. characteristics of food plants. In this respect, a two-species approach inherently has shortcomings: The most important is that differences between species always are very likely, but need not be interpreted as adaptations but simply as by-chance results of genetic separation, as outlined in detail by Garland and Adolph (1994). Recommendations of the aforementioned latter paper on strategies to circumvent the shortcomings of a two-species comparative study were followed as closely as possible and are outlined in the discussion.

1.3. Aims of the study

In this study we intended to investigate whether the differences in aspects of digestive physiology described for browsing and grazing ruminants can also be found in rhinos. In detail, for the white rhino (grazer) we expected a longer mean retention time of particles ($MRT_{particle}$), a higher selectivity factor ($SF = MRT_{particle}/MRT_{fluid}$), higher fiber digestibility and smaller average fecal particle size (better chewing efficiency).

2. Material and methods

Five white and four black rhinos from four different zoological institutions were available for the study (Table 1). Body weights were estimated based on the known weight of one black rhino (not included in this study), plus information from experienced zoo staff. The animals were kept separately during the trials to allow individual recording and sampling of food and feces, except for rhinos W3, W4 and W5, which were kept together for 3–4 h a day on the outside enclosure. Color markers (beetroot and betanin) were fed to distinguish between individuals in this case.

Table 1
Study animals.

Zoo	Animal	Sex	Age at trial [years]	Body weight estimated [kg]
Black rhinoceros				
Köln	B1	F	11.7	1300
	B2	M	11.0	1300
Zürich	B3	F	9.7	1200
	B4	F	5.0	1200
White rhinoceros				
Osnabrück	W1	F	35.2	2200
	W2	M	29.4	2200
Münster	W3	F	15.1	1900
	W4	F	18.9	2200
	W5	M	14.9	2400

F = female; M = male.

For an adaptation period of 14 days and a collection period of a minimum of 6 days, all animals had ad libitum access to a mixed hay of temperate grasses (second cut). Hay from one identical batch was used in all four facilities. Additionally black rhinos received 500 g and white rhinos 600 g of a pelleted compound (crude protein (CP): 18% dry matter (DM); neutral detergent fiber (NDF): 22% DM) per day and animal for management purposes.

During the collection period, food intake was quantified, and representative samples were taken from the diet (every second day) and the feces (every day, representing app. 10% of daily fecal output, the outer layer of each dung ball being removed to avoid contamination of the sample). The fecal samples were frozen and freeze dried. For chemical analysis, hay and dried feces were ground through a 1 mm sieve. Both the feed and fecal samples were analyzed for DM, ash and CP (Dumas method). Feed samples were analyzed additionally for ether extract (EE) according to Bassler (1976), and for NDF, acid detergent fiber (ADF) and acid detergent lignin (ADL) according to Van Soest et al. (1991). All fiber fractions are expressed as ash-corrected values. In vitro fermentation of the hay was evaluated with the Hohenheim Gas Test (HGT; Menke et al., 1979), using standardized sheep rumen fluid as the inoculum source. Metabolizable energy (ME) and apparent organic matter digestibility (aD OM) for ruminants were estimated from 24 h in vitro gas production (GP) (plus nutrient composition) according to the following regression equations: ME [MJ/kg DM] = 0.72 + 0.1559 GP_{24h} [ml/200 mg DM] + 0.0068 CP [g/kg DM] + 0.0249 EE [g/kg DM] (Menke and Steingass, 1988); aD OM [%] = 0.889 GP_{24h} [ml/200 mg DM] + 0.0448 CP [g/kg DM] + 0.0651 ash [g/kg DM] + 14.88 (Menke and Huss, 1987).

Cell wall degradation was quantified using an approach comparable to Prins et al. (1981) and Prins et al. (1983). NDF residues of hay and feces were fermented in vitro in the HGT, with GP quantified at 4, 8, 12, 18, 24, 32, 48, 56, 72, 80 and 96 h (GP related to ash-corrected NDF residue, expressed as ml/200 mg NDF).

From the undried fecal samples, fecal particle size was quantified in triplicates using a wet sieving machine (Vibrotronic Type VE 1, Retsch Technology, Haan, Germany). Samples were sieved for 10 min (water flow 2 L/min) over a cascade of sieves with apertures of 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 and 0.063 mm. The mean fecal particle size was expressed as weighted average of particle size (WAPS), calculated as the modulus of fineness according to Poppi et al. (1980), but by using sieve aperture size instead of consecutive numbers for sieves.

Cobalt EDTA and chromium-mordanted fiber (1–2 mm) were used to quantify retention times for the fluid and the particle phase, respectively (Udén et al., 1980). Markers were given in a pulse dose mixed with two bananas or two small bread rolls, all ingested within less than 10 min. Samples were taken from each defecation. One

overnight sample was taken (the middle of this interval being used as sampling time). The samples were dried at 103 °C and ground through a 1 mm sieve. Marker concentration was measured after wet ashing according to Behrend et al. (2004) with atomic absorption spectroscopy (Perkin-Elmer 1100 B, Perkin Elmer, Wellesley, Massachusetts, USA). MRT was calculated according to Thielemans et al. (1978). The selectivity-factor (SF) was calculated as $MRT_{particle}/MRT_{fluid}$ (Lechner-Doll et al., 1990).

To test for the generality of the relation of feeding type and SF, data of 12 ruminant and 3 rhino species was compiled (for data see Electronic appendix), all using Co-EDTA and Cr mordanted fibre as markers and allowing animals ad libitum diet access. Data were analyzed by phylogenetically controlled regression analysis. The subjects of the analysis were species. Relationships among them due to the evolutionary process were inferred from a phylogenetic tree based on the complete mitochondrial cytochrome *b* gene. Respective DNA sequences were available from GenBank (http://www.ncbi.nlm.nih.gov). Sequences were aligned using CLUSTALX (Thompson et al., 1997), visually controlled and trimmed to identical length (1.143 bp). To select the best-fitting nucleotide substitution model for the data, a combination of the software packages PAUP* (v.4.b10; Swofford, 2002) and MODELTEST (v.3.7; Posada and Crandall, 1998) was used. Analysis was based on a hierarchical likelihood ratio test approach implemented in MODELTEST. The model selected was the general time-reversible (GTR) model (Lanave et al., 1984; Tavaré, 1986) with an allowance both for invariant sites (I) and a gamma (G) distribution shape parameter (α) for among-site rate variation ($GTR+I+G$) (Rodriguez et al., 1990). The nucleotide substitution rate matrix for the $GTR+I+G$ model was similarly calculated using MODELTEST. Parameter values for the model selected were: $-\ln L=x$, $I=xy$, and $\alpha=xyz$. The phylogenetic reconstruction based on these parameters was then performed using the maximum likelihood (ML) method implemented in TREEPUZZLE (v.5.2; Schmidt et al., 2002). Support for nodes was assessed by a reliability percentage after 100,000 quartet puzzling steps; only nodes with more than 50% support were retained. The basal polytomy for familial relationships was resolved assuming it to be soft polytomy (Purvis and Garland, 1993). To meet the input requirements for the phylogenetic analysis implemented in the COMPARE 4.6 program (Martins, 2004), we resolved the remaining polytomies to full tree dichotomy by introducing extreme short branch length ($l=0.00001$) at multifurcating nodes.

We used the Phylogenetic Generalized Least Squares approach (Martins and Hansen, 1997; Rohlf, 2001) in which a well established method was extended to enable the inclusion of interdependencies among species due to the evolutionary process. To test the robustness of the results, the comparative analysis was performed for both a set of phylogenetic trees involving branch length and another set with equal branch length. As there were no relevant differences in the results, only the tests using the former tree are given here. The COMPARE 4.6 program (Martins, 2004) served for phylogenetically controlled calculations. Other statistical calculations including a nonparametric test (Mann–Whitney) to test for differences between the two species were performed using SPSS 16 software (SPSS, Chicago, IL, USA). The significance level was set to $\alpha=0.05$.

3. Results

The DM content of the hay used in the study (one mixed sample per institution) was 89.9 ± 0.9%, the nutrient composition (DM basis) was 63.4 ± 0.8% for NDF, 32.8 ± 0.8% for ADF, 3.1 ± 0.7% for ADL, 10.2 ± 0.5% for CP, 2.0 ± 0.5% for EE and 8.2 ± 0.7% for ash. Standardized 24 h in vitro GP was 44.6 ± 1.4 ml/200 mg DM. Metabolizable energy and apparent organic matter digestibility of the hay were estimated to be 8.8 ± 0.3 MJ/kg DM and 65 ± 1.3% respectively.

Daily DM intake (DMI) was variable between rhinos (Table 2) and ranged from 60 to 84 g/kg $BW^{0.75}$ for black rhinos. For white rhinos DMI ranged from 56 to 90 g/kg $BW^{0.75}$. In the in vitro fermentation test, GP of NDF residues of rhino feces was significantly higher for black compared to white rhinos at the time intervals of 12–18 and 18–24 h ($p=0.016$), while no difference was apparent for the earlier or later time intervals (Fig. 1).

Table 2
Means (± standard deviation SD) of daily dry matter intake (DMI) and fecal nitrogen (N) content (OM = organic matter).

Animal	DMI			Fecal N
	[kg]	[g/kg $BW^{0.75}$]	[g/kg BW]	[g/kg OM]
Black rhinoceros				
B1	16.3 ± 3.14	75 ± 14	13 ± 2.4	1.98
B2	18.1 ± 1.83	84 ± 8	14 ± 1.4	2.03
B3	12.1 ± 1.06	60 ± 5	10 ± 0.9	2.21
B4	14.9 ± 1.19	73 ± 6	12 ± 1.0	2.77
Mean ± SD	15.4 ± 2.53	73 ± 10	12 ± 1.6	2.25 ± 0.362
White rhinoceros				
W1	22.6 ± 4.29	70 ± 13	10 ± 2.0	2.98
W2	28.9 ± 3.00	90 ± 10	13 ± 1.4	2.72
W3	19.8 ± 2.96	69 ± 11	10 ± 1.6	2.74
W4	21.2 ± 1.96	66 ± 6	10 ± 0.9	2.21
W5	19.2 ± 2.55	56 ± 7	8.0 ± 1.1	2.14
Mean ± SD	22.3 ± 3.90	70 ± 14	10 ± 1.8	2.56 ± 0.365
p (*U*-test)	Not tested	0.556	0.191	0.286

The fluid marker was excreted faster than the particle marker in both species (see Figs. 2 and 3 for excretion curves). Mean retention time for fluid (MRT_{fluid}) ranged from 29 to 38 h for black rhinos (34 ± 4 h) and from 22 to 31 h for white rhinos (28 ± 4 h) (Table 3). $MRT_{particle}$ ranged from 34 to 43 h for black (39 ± 4 h) and from 38 to 49 h for white rhinoceroses (43 ± 5 h). While MRT_{fluid} and $MRT_{particle}$ did not differ between the two species ($p=0.111$ MRT_{fluid}, $p=0.286$ $MRT_{particle}$), SF for black rhinos (1.2 ± 0.1) was significantly lower than for white rhinos (1.5 ± 0.2) ($p=0.016$). In the phylogenetic regression analysis, literature data on SF and percentage of grass in diet revealed a significant relationship between these traits ($R=0.75$; $R^2=0.57$; $F_{1,13}=16.995$; $p=0.001$) (Fig. 4).

Average fecal particle size quantified via WAPS ranged from 5.1 to 6.8 mm for the black rhinoceroses (6.1 ± 0.79 mm) and from 7.4 to 11.5 mm for the white rhinoceroses (9.1 ± 1.94 mm) (Table 3); differences between the two species were significant ($p=0.016$).

4. Discussion

4.1. Inferring on adaptation from comparative studies

A comparative study using a small sample size has to be careful in its interpretation of differences as adaptations to environmental factors (Garland and Adolph, 1994). The most important point of criticism is that interspecific differences in any character are very likely to be present, but need not necessarily be interpreted as adaptations. A misinterpretation of random differences as adaptations, or confounding reasons for characteristics (e.g. body weight vs. feeding style) are possible in an approach using only a limited amount of species. Establishing a correlation between the respective trait and the environmental factor is a way to cope with this problem, but obviously has a statistical requirement of at least 3 species.

Among the strategies to enhance the value of an approach using a limited amount of species is a) to make explicit predictions on the traits of interest which should be as independent as possible from each other (see the aims section for a list of predictions for the variables of our study); b) to choose species which evolved in environments that differ as little as possible except for the environmental factor of interest (a requirement satisfactorily met in the rhino taxa investigated, since they can occur sympatrically); and c) to give an indication of the quantity of the difference (see Hulbert,

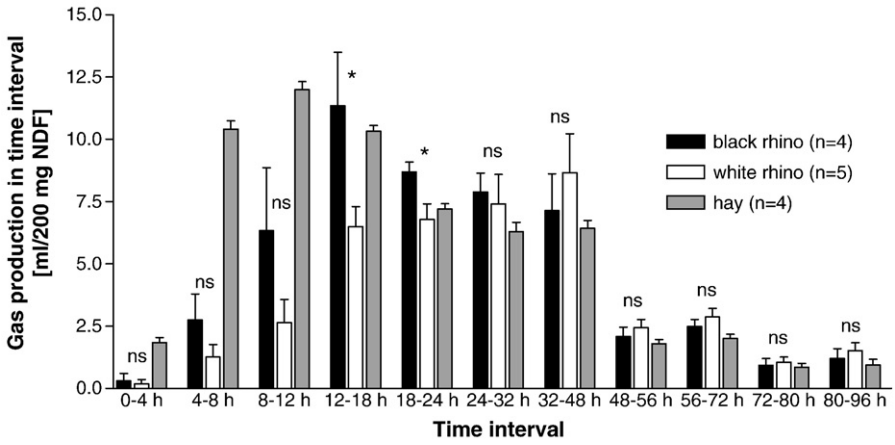

Fig. 1. In vitro fermentation of NDF preparations (rhino feces and grass hay).

1984), and therefore its relevance for species performance (attempts for quantifying relevance are made for each trait investigated).

4.2. Intake and digestibility

For browsing rhinos on a diet comparable to that in the wild, a strategy of high intake/low digestibility can be expected (Foose, 1982; Clauss et al., 2005a). Browse material contains considerably higher amounts of lignin, rendering a larger part of this forage completely indigestible, than in grass (Foose, 1982; Hummel et al., 2006). The question would be if such a strategy of high intake can be considered as 'fixed' for feeding types to an extent that makes it detectable even when the diet is identical for both. While the intraspecific variability in intake was considerable in our study, a comparison between the two rhino taxa does not support the view of a strict interspecific difference in relation to feeding type. This is true for intake related to body weight, a measure which relates intake to gut capacity (which scales to $BW^{1.0}$ according to Parra (1978) and Demment and Van Soest (1985)), or intake related to metabolic body size ($BW^{0.75}$), which puts intake more in relation to energy requirements. A lack of a difference between the rhino taxa is in accordance with the results of Foose (1982; Table 4).

In literature, different concepts of regulation of food intake are reported. For ruminants, Conrad (1966) described diet intakes to be regulated via energy dominantly in well digestible/high concentrate diets, and by gut fill dominantly in diets low in digestibility/high in forage. For the giraffe, another large browsing herbivore, considerable intake limitation has been described on a grass hay diet in comparison to grazing bovids (Foose, 1982), probably due to intake limitation related to gut fill (Clauss et al., 2002a). No indication for a lower intake in the browsing species was found for rhinos on a grass hay diet in this study.

It should be added here that our results are only valid for hay of the quality used in this study (second cut, estimated OM digestibility for ruminants 65%). A differing hay quality (e.g. a first cut hay rich in stems) would probably have challenged the intake capacity of the species to a larger extent. If the quality of the study hay is put into relation with the natural food resources, food quality in terms of NDF and CP seems to be lower in the wild for white rhinos ($n=6$; NDF $74.6 \pm 1.0\%$ DM; CP $4.7 \pm 1.1\%$ DM; (Kiefer et al., 2003), while in black rhinos NDF values of a level comparable to the study hay are generally found in their natural forage ($n=24$; $58 \pm 9\%$ NDF; $12 \pm 4\%$ CP; Dierenfeld et al., 1995).

Fecal N values are regarded to be an indicator of the production of microbial biomass in the fermentation chambers, and therefore to reflect the digestion of the diet (Mésochina et al., 1998 for horses; Lukas et al., 2005 for ruminants). This method can be regarded as a potential tool in the evaluation of diet quality in rhinos under free-ranging conditions, especially in grazing taxa (see Leslie et al., 2008 for a recent review). Validity of the approach has also been shown for browsing taxa such as the greater kudu (*Tragelaphus strepsiceros*) (van der Waal et al., 2003). In this study, no significant difference in fecal N values was found between the rhino species, therefore giving no indication for a difference in OM digestibility.

The studies of Ullrey et al. (1979) and Foose (1982) indicated a higher fiber digestibility in white compared to black rhinos (Table 4).

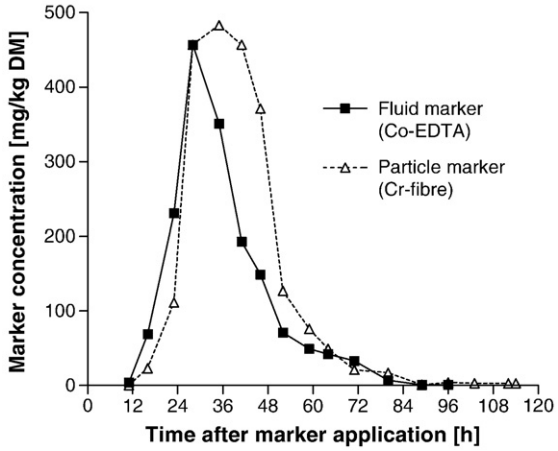

Fig. 2. Marker excretion pattern of a black rhinoceros (B3).

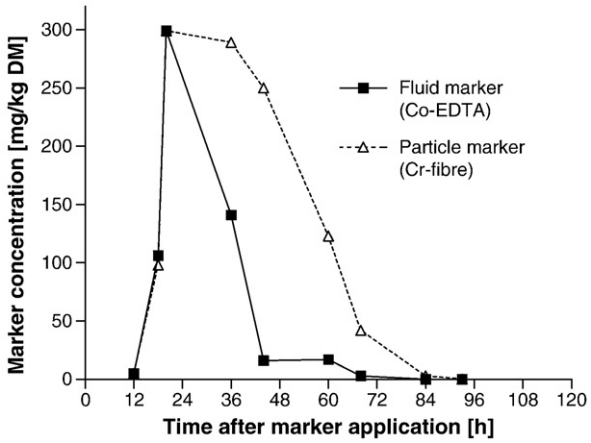

Fig. 3. Marker excretion pattern of a white rhinoceros (W4).

Table 3
Means (± standard deviation) of defecation rate, mean retention time of fluid and particles (MRT_{fluid} and $MRT_{particle}$) and selectivity factor ($SF = MRT_{particle}/MRT_{fluid}$) in the whole gastrointestinal tract, and average fecal particle size (WAPS = weighted average of particle size).

	Defecations [d^{-1}]	MRT_{fluid} [h]	$MRT_{particle}$ [h]	SF	WAPS [mm]
Black rhinoceros					
B1	3.7 ± 1.2	29	34	1.2	5.1 ± 0.49
B2	3.6 ± 1.3	38	43	1.1	5.9 ± 0.38
B3	3.0 ± 1.1	36	40	1.1	6.6 ± 0.55[a]
B4	3.0 ± 0.9	31	38	1.2	6.8 ± 0.98[a]
Mean ± SD	3.3 ± 0.4	34 ± 4	39 ± 4	1.2 ± 0.1	6.1 ± 0.79
White rhinoceros					
W1	2.6 ± 0.5	30	49	1.6	11.5 ± 0.82
W2	3.4 ± 0.9	30	41	1.4	10.8 ± 0.64
W3[b]	2.1 ± 0.6	22	40	1.8	8.4 ± 1.12
W4[b]	2.9 ± 0.4	28	38	1.4	7.4 ± 0.73
W5[b]	2.7 ± 1.0	31	48	1.6	7.4 ± 0.54
Mean ± SD	2.7 ± 0.5	28 ± 4	43 ± 5	1.5 ± 0.2	9.1 ± 1.94
p (*U*-test)	0.064	0.111	0.286	0.016	0.016

[a] On a diet based on browse leaves, WAPS was 5.9 ± 0.65 for B3 and 8.1 ± 2.29 for B4.
[b] Retention times in white rhinoceroses 3–5 were measured on a different occasion than the rest of the data for these animals; W3 and W4 had an average daily intake of 17.6 kg DM or 58 g/kg $BW^{0.75}$, and W5 of 18.9 kg DM or 55 g DM/kg $BW^{0.75}$.

The results of the in vitro fermentation of the NDF residues from rhino feces and hay are therefore of particular interest. Our expectation that the in vitro gas production from the fiber fraction of white rhino feces would be lower than that of black rhinos (indicating a more comprehensive fiber digestion already having taken place in the animal gut) was indeed met for fermentation times of 12–24 h. If this result is interpreted considering potential differences in retention times between the species, it can be assumed that the better digestion of the 12–24 h fraction of the in vitro test by white rhinos indicates a superior retention capacity in this species. Both rhinos seem to digest little of the slow fermenting NDF-fractions (in vitro fermentation times >24 h). The higher in vitro gas production in fecal compared to grass hay NDF-residues for the slow-fermenting fractions can be explained by the fact that the distribution of the faster (0–12 h), intermediate (12–24 h) and slower (>24 h) fermenting NDF fractions is changed in the rhino feces in the direction of the slower fermenting fraction, resulting in a higher proportion of slow fermenting fiber. It should be emphasized here that while the ranking of the samples will not be influenced by the in vitro conditions, these conditions will have some influence on the degradation kinetics of the NDF samples. For example, a factor accelerating fermentation in the in vitro system significantly is the necessary milling of the samples before the analysis, while the use of dried material may delay the onset of fermentation to some degree. Given our estimations for the retention times in the part of the digestive tract where fiber fermentation takes place (see below), fermentation seems to be rather faster under the in vitro conditions compared to the GIT of the animal. The use of in vitro fermentation of the fecal fiber fraction can be regarded as a useful tool for investigations on differences in the digestive physiology of herbivores.

4.3. Ingesta retention

Due to the slow fermentation rate of fiber, which is on a comparable level with the passage rate from the fermentation chamber of larger herbivores (Mertens, 1993), mean retention time of food in the digestive tract can be considered a key parameter in herbivores. Compared to other data on grass diets (≥75% grass in the diet on a dry matter basis) (Table 4), the $MRT_{particle}$ of the grazing white rhino appears to be rather short. Data from studies with comparable markers indicate longer retention times in Indian rhinos (Clauss et al., 2005b: $MRT_{particle}$ 57 h for an animal on a 100% grass forage diet). The study of Foose (1982) using Fuchsin stained particles arrives at MRT of 61/71 h (Indian), 63/65 h (white) and 60 h (black) for rhinos. Data on equids at ad libitum intake indicate $MRT_{particle}$ of 32–34 h for ponies and 29–32 h for donkeys (Pearson et al., 2006).

In studies on differences in digestive/fermentative capacity of herbivores, the major site of interest is generally the fermentative chamber. Attempts have been made to give estimations for the retention time in the fermentation chamber of perissodactyls (Moore-Colyer et al., 2003). In this study, the approach of Udén et al. (1982a) was followed (which in the latter study was applied to fecal marker excretion curves after administering the markers into the caecum), backed by additional considerations (Grovum and Williams, 1973; Martínez del Rio et al., 1994; Caton and Hume, 2000): In exponential marker excretion models in ruminants, the time of first marker appearance in the feces has been interpreted as the retention time in the tubular, non-mixing portions of the digestive tract, largely the small intestine and portions of the large intestine. In an attempt to translate this concept to the digestive tract of the rhino, the small

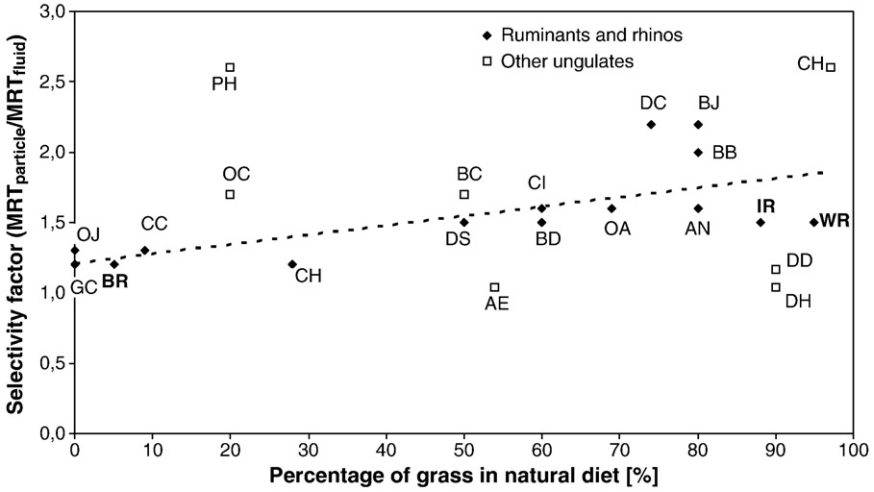

Fig. 4. Relation of selectivity factor and percentage of grass in diet ($R = 0.75$; $R^2 = 0.57$; $F_{1,13} = 16.995$; $p = 0.001$); dotted line represents the linear regression for ruminants and rhinos. Other ungulates like equids, the African elephant and particularly hippos do not seem to follow the pattern of the former two groups. (Ruminants: GC = *Giraffa camelopardis*, OJ = *Okapia johnstoni*, CC = *Capreolus capreolus*, CH = *Capra hircus*, DS = Domestic sheep, BD = *Bubalus depressicornis*, CI = Capra ibex, OA = *Ovis ammon musimon*, AN = Addax nasomaculatus, BB = *Bubalus bubalis*, BJ = *Bos javanicus*, DC = Domestic cattle; Camelids: BC = Bactrian camel; OC = one-humped camel; Hippos: PH = pygmy hippo; CH = common hippo; Rhinos: BR = black rhino, IR = Indian rhino, WR = white rhino; Equids: DD = Domestic donkey, DH = Domestic horse; AE = African elephant).

Table 4
Comparison of data of feeding studies on rhinos on grass hay based diets (>75% grass); NDF = neutral detergent fiber; CP = crude protein; DM = dry matter; aD = apparent digestibility; $MRT_{particle/fluid}$ = mean retention time of particles/fluid in the gastrointestinal tract.

N	Grass in diet	Diet composition		Daily DM intake		aD NDF	$MRT_{particle}$	MRT_{fluid}	
	[%]	NDF, [% DM]	CP, [% DM]	[kg]	[g/kg $BW^{0.75}$]	[%]	[h]	[h]	
White rhinoceros									
5	100	72	4.8	19.7 ± 3.44	70 ± 3	48 ± 1	63/65[a,b]	–	Foose (1982)
1	100	75.4	5.6	25.0	70	38	–	–	Foose (1982)
2	100	62	7	–	–	67	–	–	Ullrey et al. (1979)
3	100	65.5	13.2	–	–	57 ± 2	49/53[a,c]	–	Kiefer (2002)
3	100 (fresh)	65.5	7.5	–	–	43 ± 1	–	–	Kiefer (2002)
5	95	63.4	10.2	22.3 ± 3.90	70 ± 12	–	43 ± 5	28 ± 4	This study
Black rhinoceros									
3	100	75	4.5	15.7 ± 3.72	69 ± 12	41 ± 3	60[d]	–	Foose (1982)
2	100	62	7	–	–	33	–	–	Ullrey et al. (1979)
2	76	46.1	8.9	19.1	94	45 ± 2	28–41	25–34	Clauss et al. (2005a), Fröschle and Clauss unpubl.[e]
4	95	63.6	10.2	15.4 ± 2.53	73 ± 10	–	39 ± 4	34 ± 4	This study

[a] $n = 2$.
[b] Marker: fuchsin-stained particles.
[c] Marker: Cr_2O_3; MRT calculated in Clauss et al. (2005a).
[d] $n = 1$.
[e] As cited in Clauss and Hatt (2006) and Castell (2005).

intestine plus the distal large intestine are interpreted as a plug flow reactor and the caecocolon as a mixing chamber. The subtraction of the transit time from MRT can be regarded to result in a proxy for the retention time in the mixing compartments (MC) of the GIT. In rhinos this should dominantly correspond to the caecocolon, the major site of fermentative activity. Applying this concept to the $MRT_{particle}$ of the study rhinos and subtracting the transit times by trend results in a difference in the $MRT_{particle}MC$ (black rhino: 20 ± 2.6 h; white rhino: 28 ± 8.6 h; $p = 0.0635$) and supports the idea of a higher fermentative capacity in the white rhinoceroses of the study. These calculations also suggest that MRT measurements for the whole GIT might mask differences in MRT in the fermentation chambers. However, it must be stated that one has to be careful when applying such concepts to situations in which an in situ evaluation is not possible (i.e., in non-fistulated animals): The assumption of the concept that the caecocolon actually works dominantly as a mixing chamber in rhinos must be met.

As already outlined, a difference found between species should be checked for possible omission of adaptation to different environmental factors. In our case, this means quantifying the consequence of the measured longer retention time of food in the hindgut of the white rhino in terms of fiber digestion and in terms of its energy budget. Using the reciprocal values of the retention times in the hindgut as passage rates (Hungate, 1966), and using the fermentation rate calculated from our in vitro fermentations of the cell wall fraction of the study hay, one may apply the approach of Ørskov and McDonald (1979) and McDonald (1981), in estimating the proportion of feed actually degraded in the hindgut. In our case, this means multiplication of the maximal gas production with the factor $c/(c+k)$ (c being the fermentation rate, and k being the passage rate, both expressed as %/h). Assuming 20 h as retention time for the black rhino and 28 h for the white rhino results in actually realized gas productions of 16.1 ml from the NDF fraction of 200 mg DM of the study hay in the black and 18.9 ml in the white rhino species. Assuming that around 50% of the gas comes from CO_2 developing from the buffer and that one mol of CO_2 corresponds to one mol of short chain fatty acids produced (Blümmel et al., 1999) and assuming proportions of 65% acetate, 20% propionate and 15% butyrate (Wolin, 1960), this indicates a difference of 0.38 MJ ME/kg DM of hay. In conclusion, the difference in retention time results in a higher energy extraction on the size of 5% for a white rhino per unit of ingested dry matter — without doubt a difference relevant for the animal.

4.4. Selectivity factor

Lechner-Doll et al. (1990) first introduced the selectivity factor (SF, the quotient between $MRT_{particle}$ and MRT_{fluid}), as a measure to quantify differences in digestive strategies of ruminant feeding types. Clauss and Lechner-Doll (2001) and Hummel et al. (2005) followed this approach and arrived at the conclusion of generally lower SF in browsing compared to grazing ruminants. The SF is considered a useful tool to compare animals, since fluids and particles will be influenced in the same way by factors such as DMI or husbandry and even social components (since feces play some role in marking behavior of rhinos, daily defecation patterns can be influenced).

In this study, a significant difference in SF between white (1.5 ± 0.2) and black rhinos (1.2 ± 0.1) was found. Data from other studies on black (Clauss et al., 2005a: 1.1–1.3) or Indian rhinos (Polster, 2004: 1.4–1.6) fit into this pattern, and the clear distinction between the rhinos can be considered to be a major result of this study.

The significant positive correlation between SF and the percentage of dietary grass in a sample of 12 ruminant and 3 rhino species makes an interpretation of SF as an adaptation to a diet high in grass warranted. What could be the causes for, or the adaptive value of, the observed differences in SF? A longer $MRT_{particle}$ allows more extensive use of the slowly digestible dietary fiber — a fraction that has been stated to be far more prominent in grass compared to browse. The black rhino represents a species with a very high intake of woody twigs in its diet — a potentially almost completely indigestible food item, which is of little energetic benefit for the animal (Foose, 1982; Hummel et al., 2006) and therefore has to be cleared from the digestive tract relatively fast.

A longer MRT_{fluid} in browsing species may be more difficult to explain. Clauss et al. (2006b) interpreted the shorter MRT_{fluid} in grazing ruminants as a consequence of a higher fluid throughput, necessary to achieve the physical mechanisms for the flotation and sedimentation described to be important for the functioning of the fermentation chamber of grazing ruminants. In terms of energy metabolism, it could be due to a higher relevance of the soluble digesta fraction in browse; in fact, the soluble fiber fraction (e.g. pectins) is generally regarded to be more important in browse than in grass (see Robbins, 1993; page 248). However, soluble fiber fractions like pectins are generally regarded to have a high fermentation rate (Van Soest et al., 1991; Hall et al., 1998), which diminishes the beneficial effect of longer retention times.

The longer MRT_{fluid} could also be due to the fact that the high fraction of soluble fiber (e.g. pectins), which have a water-binding effect, increase the viscosity of the fluid phase and hence ultimately slow down its passage; a physiological adaptation to a higher fluid throughput (e.g. in the form of increased saliva production) might therefore not have an advantageous effect in browsers. A considerable soluble fiber fraction will also occur in grazing hindgut fermenting species, since a significant fraction of dietary hemicelluloses — which are generally found to be particularly prominent in grasses (Robbins, 1993; Hummel et al., 2006) — is probably turned soluble in the proximal sections of the gut (Keys et al., 1969; Parra, 1978); in addition, hemicellulose might have a lesser effect on the viscosity of the digesta compared to pectins. Thus the fiber composition of the diet might have facilitated an adaptation to a higher fluid flow through the GIT, which improves washing of soluble, absorbable nutrients out of the digesta plug towards the absorptive gut surface (Lentle et al., 1996).

An alternative explanation attempt may be that water is absorbed more completely in browsing compared to grazing species, therefore slowing down the movement of a fluid phase marker in the distal parts of the GIT, the major site of water absorption. However, a lower fecal dry matter content was not found for *C. simum* compared to *D. bicornis* in this study (20.2 ± 0.8 vs. $18 \pm 1.9\%$).

While for ruminants and rhinos, the pattern of a positive correlation of percentage of grass in the diet and SF can be regarded as given (Fig. 4), this correlation is less evident when all further ungulate data available (horse, donkey, African elephant, Bactrian camel, one-humped camel, common hippo and pygmy hippo) are added to the data set, resulting in 22 species altogether (Fig. 4). Although the correlation of percentage of grass in diet and SF stays significant when applying phylogenetic control, the level of the correlation and its significance is considerably lower ($R = 0.48$ instead of 0.75; $p = 0.022$ instead of $p = 0.001$), and at visual inspection, the relationship is far less evident than in the dataset of ruminants and rhinos only, indicating that factors other than botanical dietary niche (grazers and browsers) most likely play a role. Remarkably, the ungulate groups not fitting the pattern of ruminants and rhinos are following either a strategy of considerably higher intake (equids, elephants, with particularly low SF) or lower intake (camelids, hippos, with particularly high SF; both latter groups additionally characterized by a relatively low metabolic rate). The hypothesis relating SF to feeding type would fit into this pattern insofar as browsing ruminants (showing low SF) can be expected to realize a higher food intake/lower digestibility than their grazing relatives, at least when feeding on their natural diets. However, summing up this discussion, in contrast to rhinos and ruminants, for ungulates as a whole no safe conclusion on a potential relation of selective retention of particles in the gut and feeding type can be drawn.

4.5. Fecal particle size

Studies like Lentle et al. (2003) on wallabies or Clauss et al. (2002b) on ruminants found larger fecal particle sizes in browsing compared to grazing herbivores. This is coherent with characteristics of teeth structure and the chewing apparatus in grazers and browsers, like the tendency to have more enamel crests vertical to the direction of mastication on the flat occlusal surface in the former (Fortelius, 1982). In contrast to this, Fritz et al. (2007) found no difference in fecal particle size between *D. bicornis* and *C. simum*, despite their different feeding type (smaller fecal particle size was only found in *R. unicornis*). These animals were fed their regular zoo diets, to some extent reflecting the natural feeding habits of the rhinos. From the background of these studies, the results of our study are unexpected, since the black rhinos were found to have smaller fecal particle sizes than white rhinos when being fed an identical diet of grass hay — in contrast to the three studies mentioned above. While the particularly high values in the two older white rhinos may indicate an impact of age-related tooth wear in these animals, the difference between the taxa holds true even after correction for this influence.

Fecal particle size can be regarded as a good measure to quantify the degree of food comminution in the oral cavity. To allow the comparison of the rhino feeding types under this latter perspective, body size differences between the taxa need to be considered, since body weight is discussed to be of relevant influence on different parameters of digestive physiology. While a recent data collection could not find an influence on retention time in ungulates (Clauss et al., 2007), fecal particle size has in fact been found to increase with body weight (Udén and Van Soest, 1982; Clauss et al., 2002b; Fritz et al., 2009). Based on the data collection of Udén (1978), Pérez-Barbería and Gordon (1998) estimate a scaling of fecal particle size to $BW^{0.19}$, while Fritz et al. (2009) found a scaling to $BW^{0.22}$. The latter data collection includes all guilds of mammalian herbivores, while the former includes 3 ruminants, 2 equids and one lagomorph. Correcting black rhino fecal particle size accordingly results in a fecal particle size of app. 6.9 mm on average (range of the individual black rhinos 5.7–7.7), the upper range overlapping with the values of white rhinos of this study (7.4–11.5 mm). While the difference in fecal particle size between the rhinos gets somewhat smaller when correcting for body weights, it can be safely stated for the hay used in this study that there was no indication at all for conspicuously larger fecal particle size in the browsing rhino compared to its grazing relative.

5. Conclusions

- The higher selectivity factors ($MRT_{particle}/MRT_{fluid}$) of white rhinos are consistent with data available for ruminants, and indicate a more selective retention of particles compared to fluid in the digestive tract of the grazing rhino. While this relation seems to hold true for ruminants and rhinos, the situation is more complicated when all ungulate groups (e.g. hippos, camelids, equids and elephants) are included, potentially due to a much larger range of food intake levels within the whole group than within ruminants and rhinos only.
- Based on in vitro fermentation of the fecal NDF fraction, the white rhino is a more comprehensive digester of fiber.
- The black rhino was found to have smaller fecal particle sizes in this study; at least a part of this difference might be explained to be an effect of body size.

Acknowledgements

We would like to sincerely thank the staff at the different rhino facilities for their kind cooperation and help in this study. This research was supported by the German Research Foundation (DFG, HU 1308/4-1) and is publication no. 17 of the DFG Research Unit 771 "Function and enhanced efficiency in the mammalian dentition — phylogenetic and ontogenetic impact on the masticatory apparatus".

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cbpa.2010.03.006.

References

Bassler, R., 1976. VDLUFA-Methodenbuch, Band III. Die chemische Untersuchung von Futtermitteln. VDLUFA-Verlag, Darmstadt.

Behrend, A., Lechner-Doll, M., Streich, W.J., Clauss, M., 2004. Seasonal faecal excretion, gut fill, liquid and particle marker retention in mouflon *Ovis ammon musimon*, and a comparison with roe deer *Capreolus capreolus*. Acta Theriol. 49, 503–515.

Blümmel, M., Aiple, K.-P., Steingaß, H., Becker, K., 1999. A note on the stoichiometrical relationship of short chain fatty acid production in vitro in feedstuffs of widely differing quality. J. Anim. Physiol. Anim. Nutr. 81, 157–167.

Castell, J., 2005. Untersuchungen zu Fütterung und Verdauungsphysiologie am Spitzmaulnashorn (Diceros bicornis). Dissertation thesis, Veterinary faculty LMU München, München, p. 200.
Caton, J.M., Hume, I.D., 2000. Chemical reactors of the mammalin gastro-intestinal tract. Z. Säugetierk. 65, 33–50.
Clauss, M., Hatt, J.-M., 2006. The feeding of rhinoceros in captivity. Int. Zoo Yearb. 40, 197–209.
Clauss, M., Lechner-Doll, M., 2001. Differences in selective reticulo-ruminal particle retention as a key factor in ruminant diversification. Oecologia 129, 321–327.
Clauss, M., Lechner-Doll, M., Streich, W.J., 2002a. Faecal particle size distribution in captive wild ruminants: an approach to the browser/grazer dichotomy from the other end. Oecologia 131, 343–349.
Clauss, M., Lechner-Doll, M., Flach, E.J., Wisser, J., Hatt, J.-M., 2002b. Digestive tract pathology of captive giraffe (*Giraffa camelopardalis*): an unifying hypothesis. European Association of Zoo- and Wildlife Veterinarians 4th scientific meeting, Heidelberg, pp. 99–107.
Clauss, M., Froeschle, T., Castell, J., Hatt, J.-M., Ortmann, S., Streich, W.J., Hummel, J., 2005a. Fluid and particle retention times in the black rhinoceros *Diceros bicornis*, a large hindgut-fermenting browser. Acta Theriol. 50, 367–376.
Clauss, M., Polster, C., Kienzle, E., Wiesner, H., Baumgartner, K., von Houwald, F., Ortmann, S., Streich, W.J., Dierenfeld, E.S., 2005b. Studies on digestive physiology and feed digestibilities in captive Indian rhinoceros (*Rhinoceros unicornis*). J. Anim. Physiol. Anim. Nutr. 89, 229–237.
Clauss, M., Castell, J.C., Kienzle, E., Dierenfeld, E.S., Flach, E.J., Behlert, O., Ortmann, S., Streich, W.J., Hummel, J., Hatt, J.-M., 2006a. Digestion coefficients achieved by the black rhinoceros (*Diceros bicornis*), a large browsing hindgut fermenter. J. Anim. Physiol. Anim. Nutr. 90, 325–334.
Clauss, M., Hummel, J., Streich, W.J., 2006b. The dissociation of the fluid and particle phase in the forestomach as a physiological characteristic of large grazing ruminants: an evaluation of available, comparable ruminant passage data. Eur. J. Wildlife Res. 52, 88–98.
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. Comp. Biochem. Physiol. 148, 249–265.
Conrad, H.R., 1966. Symposium on factors influencing the voluntary intake of herbage by ruminants: physiological and physical factors limiting feed intake. J. Anim. Sci. 25, 227–235.
Demment, M.W., Van Soest, P.J., 1985. A nutritional explanation for body-size patterns of ruminant and nonruminant herbivores. Am. Nat. 125, 641–672.
Deniro, M.J., Epstein, S., 1978. Carbon isotopic evidence for different feeding patterns in two hyrax species occupying the same habitat. Science 201, 906–908.
Dierenfeld, E., 1995. Rhinoceros nutrition: an overview with special reference to browsers. Verhandlungsb. Erkrank. Zootiere 37, 7–14.
Dierenfeld, E., 1999. Rhinoceros feeding and nutrition. In: Fowler, M., Miller, R. (Eds.), Zoo and Wild Animal Medicine. WB Saunders, Philadelphia, PA, pp. 568–571.
Dierenfeld, E., du Toit, R., Braselton, W.E., 1995. Nutrient composition of selected browses consumed by black rhinoceros (*Diceros bicornis*) in the Zambesi valley, Zimbabwe. J. Zoo. Wildlife Med. 26, 220–230.
Foose, T.J., 1982. Trophic Strategies of Ruminant Versus Nonruminant Ungulates. PhD thesis, University of Chicago, Chicago, p. 337.
Fortelius, M., 1982. Ecological aspects of dental functional morphology in the Plio-Pleistocene rhinoceroses of Europe. In: Kurtén, B. (Ed.), Teeth: Form, Function, and Evolution. Columbia University Press, New York, pp. 163–181.
Fritz, J., Hummel, J., Kienzle, E., Streich, W., Clauss, M., 2007. Faecal particle size in captive rhinoceroses. In: East, M., Hofer, H. (Eds.), Contributions to the 6th International Zoo and Wildlife Research Conference on Behaviour, Physiology and Genetics, 07.-10.10.2007, Berlin, Leibniz Institute for Zoo and Wildlife Research (IZW), p. 87.
Fritz, J., Hummel, J., Kienzle, E., Arnold, C., Nunn, C., Clauss, M., 2009. Chewing efficiency in mammalian herbivores. Oikos 118, 1623–1632.
Garland, T., Adolph, S., 1994. Why not to do two-species comparative studies: limitations on inferring adaptation. Physiol. Biochem. Zool. 67, 797–828.
Gordon, I.J., Prins, H.H.T., 2008. The Ecology of Browsing and Grazing. Springer, Berlin.
Groves, C., 1997. Die Nashörner — Stammesgeschichte und Verwandtschaft. Die Nashörner, Filander, Fürth, pp. 14–32.
Grovum, W.L., Williams, V.J., 1973. Rate of passage of digesta in sheep. 3. Differential rates of passage of water and dry matter from the reticulo-rumen, abomasum and caecum and proximal colon. Br. J. Nutr. 30, 231–240.
Hall, M.B., Pell, A.N., Chase, L.E., 1998. Characteristics of neutral detergent-soluble fiber fermentation by mixed ruminal microbes. Anim. Feed Sci. Technol. 70, 23–29.
Hofmann, R.R., 1973. The ruminant stomach. Stomach Structure and Feeding Habits of East African Game Ruminants. East African Literature Bureau, Nairobi.
Hofmann, R.R., 1989. Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. Oecologia 78, 443–457.
Hooijer, D., 1969. Pleistocene East African rhinos. Fossil Vert. Africa 1, 71–98.
Hooijer, D., 1976. Phylogeny of the rhinocerotids of Africa. Ann. South Afric. Mus. 71, 167–168.
Hooijer, D., Pattersson, B., 1972. Rhinoceroses from the Pliocene of Northwestern Kenya. Harvard University, Cambridge, Massachussetts. Museum of Comparative Zoology 144, 1–26.
Hulbert, S., 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. 54, 187–211.
Hume, I., 1999. Marsupial Nutrition. Cambridge University Press, Cambridge.
Hummel, J., Clauss, M., Zimmermann, W., Johanson, K., Nørgaard, C., Pfeffer, E., 2005. Fluid and particle retention in captive okapi (*Okapia johnstoni*). Comp. Biochem. Physiol. 140, 436–444.
Hummel, J., Südekum, K.H., Streich, W.J., Clauss, M., 2006. Forage fermentation patterns and their implications for herbivore ingesta retention times. Funct. Ecol. 20, 989–1002.
Hungate, R., 1966. The Rumen and its Microbes. Academic Press, New York.
Kay, R.N.B., von Engelhardt, W., White, R.G., 1980. The digestive physiology of wild ruminants. In: Ruckebusch, Y., Thivend, P. (Eds.), 5. International Symposium on Ruminant Physiology, Clermont-Ferrand, pp. 743–761.
Keys, J.E., van Soest, P.J., Young, E.P., 1969. Comparative study of forage cellulose and hemicellulose in ruminants and nonruminants. J. Anim. Sci. 29, 11–15.
Kiefer, B., 2002. Qualität und Verdaulichkeit der vom Breitmaulnashorn (Ceratotherium s. simum) aufgenommenen Nahrung. Dissertation thesis, Tierärztliche Fakultät, LMU München, München, p. 129.
Kiefer, B., Gansloßer, U., Kretzschmar, P., Kienzle, E., 2003. Food selection and food quality in territorial males of a free-ranging population of white rhinoceros (*Ceratotherium simum*) in South Africa. In: Fidgett, A., Clauss, M., Gansloßer, U., Hatt, J.-M., Nijboer, J. (Eds.), Zoo Animal Nutrition II, Filander, Fürth, pp. 199–208.
Lanave, C., Preparata, G., Sacone, C., Serio, G., 1984. A new method for calculating evolutionary substitution rates. J. Mol. Evol. 20, 86–93.
Lechner-Doll, M., Rutagwenda, T., Schwartz, H.J., Schultka, W., v. Engelhardt, W., 1990. Seasonal changes of ingesta mean retention time and forestomach fluid volume in indigenous camels, cattle, sheep and goats grazing in a thornbush savannah pasture in Kenya. J. Agric. Sci. 115, 409–420.
Lentle, R., Hemar, Y., Hall, C., 1996. Viscoelastic behaviour aids extrusion from and reabsorption of the liquid phase into the digesta plug: creep rheometry of hindgut digesta in the common brushtail possum *Trichosurus vulpecula*. J. Comp. Physiol. B 176, 469–475.
Lentle, R.G., Hume, I.D., Stafford, K.J., Kennedy, M., Springett, B.P., Haslett, S., 2003. Observations on fresh forage intake, ingesta particle size and nutrient digestibility in four species of macropod. Aust. J. Zool. 51, 627–636.
Leslie, D.M., Bowyer, R.T., Jenks, J.A., 2008. Facts from feces: nitrogen still measures up as a nutritional index for mammalian herbivores. J. Wildlife Manag. 72, 1420–1433.
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. J. Anim. Sci. 83, 1332–1344.
MacFadden, B.J., 2005. Fossil horses — evidence for evolution. Science 307, 1728–1730.
Martínez del Rio, C., Cork, S.J., Karasov, W.H., 1994. Modelling gut function: an introduction. In: Chivers, D.J., Langer, P. (Eds.), The Digestive System in Mammals. Cambridge University Press, Cambridge, pp. 25–53.
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. Am. Nat. 149, 646–667.
McDonald, I., 1981. A revised model for the estimation of protein degradability in the rumen. J. Agric. Sci. 96, 251–252.
Menke, K.H., Huss, W., 1987. Tierernährung und Futtermittelkunde. UTB Ulmer, Stuttgart.
Menke, K.H., Steingass, H., 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Anim. Res. Dev. 28, 7–55.
Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D., Schneider, W., 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor in vitro. J. Agric. Sci. 93, 217–222.
Mertens, D.R., 1993. Kinetics of cell wall digestion and passage in ruminants. In: Jung, H.G., Buxton, D.R., Hatfield, R.D., Ralph, J. (Eds.), Forage Cell Wall Structure and Digestibility. American Society of Agronomy, Madison, Wisconsin, pp. 535–570.
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 Forage Sci. 53, 189–196.
Moore-Colyer, M.J.S., Morrow, H.J., Longland, A.C., 2003. Mathematical modelling of digesta passage rate, mean retention time and in vitro apparent digestibility of two different lengths of hay and big-bale grass silage in ponies. Br. J. Nutr. 90, 109–118.
Ørskov, E.R., McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J Agric. Sci. 92, 499–503.
Owen-Smith, N., 1988. Megaherbivores. Cambridge University Press, Cambridge.
Palmqvist, P., Gröcke, D.R., Arribas, A., Farina, R.A., 2003. Paleoecological reconstruction of a lower Pleistocene large mammal community using biogeochemical ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, Sr:Zn) and ecomorphological approaches. Paleobiology 29, 205–229.
Parra, R., 1978. Comparison of foregut and hindgut fermentation in herbivores. In: Montgomery, G.G. (Ed.), The Ecology of Arboreal Folivores. Smithonian Institute Press, Washington DC, pp. 205–230.
Pearson, R.A., Archibald, R.F., Muirhead, R.H., 2006. A comparison of the effect of forage type and level of feeding on the digestibility and gastrointestinal mean retention time of dry forages given to cattle, sheep, ponies and donkeys. Br. J. Nutr. 95, 88–98.
Pérez-Barbería, F.J., Gordon, I.J., 1998. Factors affecting food comminution during chewing in ruminants: a review. Biol. J. Linnean Soc. 63, 233–256.
Polster, C., 2004. Untersuchungen zur Fütterung und Verdauungsphysiologie am Indischen Panzernashorn (*Rhinoceros unicornis*). Dissertation thesis, Veterinary Faculty, LMU München, München, p. 182.
Popowics, T.E., Fortelius, M., 1997. On the cutting edge: tooth blade sharpness in herbivorous and faunivorous mammals. Ann. Zool. Fenn. 34, 73–88.
Poppi, D., Norton, B., DJ, M., Hendricksen, R., 1980. The validity of the critical size theory for particles leaving the rumen. J. Agric. Sci. 94, 275–280.
Posada, D., Crandall, K., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Prins, R.A., Cliné-Theil, W.C., Van ´t Klooster, A.T., 1981. An in vitro procedure for the estimation of in vivo digestibility of roughage plant cell wall components in herbivores using mixed rumen microorganisms. Agricult. Environ. 6, 183–194.
Prins, R.A., Rooymans, T.P., Veldhuizen, M., Domhof, M.A., Cliné-Theil, W., 1983. Extent of plant cell wall digestion in several species of wild ruminants kept in the zoo. Zool. Garten N.F. 53, 393–403.
Purvis, A., Garland, T., 1993. Polytomies in comparative analyses of continuous characters. System Biol. 42, 569–575.
Robbins, C.T., 1993. Wildlife Feeding and Nutrition. Academic Press, San Diego.
Rodriguez, F., Oliver, J., Marin, A., Medina, J., 1990. The general stochastic model of nucleotide substitution. J. Theor. Biol. 142, 485–501.
Rohlf, F., 2001. Comparative methods for the analysis of continuous variables: geometric interpretations. Evolution 55, 2143–2160.
Sanson, G., 1989. Morphological adaptations of teeth to diets and feeding in the Macropodoidea. In: Grigg, G., Jarman, P., Hume, I.A. (Eds.), Kangaroos, Wallabies and Rat-kangaroos. Surrey Beatty and Sons, Sydney, pp. 151–168.
Schaurte, W., 1966. Beiträge zur Kenntnis des Gebisses und Zahnbaus der afrikanischen Nashörner. Säugetierkund. Mitteil. 14, 327–341.
Schmidt, H., Strimmer, K., Vingron, M., von Haeseler, A., 2002. TREE-PUZZLE: maximum likelihood phylogenetic analysis using quartets and parallel computing. Bioinformatics 18, 502–504.
Short, H., Blair, R., Segelquist, C., 1974. Fiber composition and forage digestibility by small ruminants. J. Wildlife Manag. 38, 197–209.
Shrader, A., Owen-Smith, N., Ogutu, J., 2006. How a mega-grazer copes with the dry season: food and nutrient intake rates by white rhinoceros in the wild. Funct. Ecol. 20, 376–384.
Swofford, D., 2002. PAUP*: Phylogenetic Analyses Using Parsimony (and Other Methods), Version 4.0 Beta. Smithsonian Institution, Washington DC.
Tavaré, S., 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. Lect. Math Life Sci. 17, 57–86.
Thenius, E., 1989. Zähne und Gebiß der Säugetiere. Walter de Gruyter, Berlin.
Thielemans, M.F., Francois, E., Bodart, C., Thewis, A., 1978. Mesure du transit gastrointestinal chez le porc à l´aide des radiolanthanides. Comparaison avec le mouton. Ann. Biol. Anim. Biochim. Biophys. 18, 237–247.
Thompson, J., Gibson, T., Plewniak, F., Jeanmougin, F., Higgins, D., 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignement aided by quality analysis tools. Nucleic Acids Res. 15, 4876–4882.
Udén, P., 1978. Comparative Studies on Rate of Passage, Particle Size and Rate of Digestion in Ruminants, Equines, Rabbits and Man. Cornell University, Ithaca, NY, p. 242.
Udén, P., Van Soest, P.J., 1982. The determination of digesta particle size in some herbivores. Anim. Feed Sci. Technol. 7, 35–44.
Udén, P., Colucci, P., Van Soest, P.J., 1980. Investigation of chromium, cerium, and cobalt as markers in digesta. Rate of passage studies. J. Sci. Food Agricult. 31, 625–632.
Udén, P., Rounsaville, T.R., Wiggans, G.R., Van Soest, P.J., 1982. The measurement of liquid and solid digesta retention in ruminants, equines and rabbits given timothy (*Phleum pratense*) hay. Br. J. Nutr. 48, 329–339.
Ullrey, D.E., Robinson, P.T., Whetter, P.A., 1979. Comparative digestibility studies with zoo herbivores. American Association Zoo Veterinarians Annual Proceedings, pp. 120–121a.
van der Waal, C., Smit, G.N., Grant, C.C., 2003. Faecal nitrogen as an indicator of the nutritional status of kudu in a semi-arid savanna. South Afric. J. Wildlife Res. 33, 33–41.
Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fibert, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.
Williams, S.H., Kay, R.F., 2001. A comparative test of adaptive explanations for hypsodonty in ungulates and rodents. J. Mammal. Evol. 8, 207–229.
Wolin, M., 1960. A theoretical rumen fermentation balance. J. Dairy Sci. 43, 1452–1459.