Limitation of body mass of herbivores
-
Allometry of food quality and of digestive aspects

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General abbreviations

aD	apparent digestibility
ADF	acid detergent fibre
ADL	acid detergent lignin
BM	body mass
CI	confidence interval
CP	crude protein
DM	dry matter
FNDF	faecal neutral detergent fibre
GIT	gastrointestinal tract
HGT	Hohenheim gas test
MFN	metabolic faecal nitrogen
MBS	metabolic body size
N	nitrogen
NDF	neutral detergent fibre
NDIN	neutral detergent insoluble nitrogen
OM	organic matter
SD	standard deviation
TFN	total faecal nitrogen
VFA	volatile fatty acid
VPDB	Vienna-Pee-Dee Belemnit

Animal abbreviations

AB	African buffalo
AE	African elephant
BB	Bushbuck
BR	Black rhinoceros
DC	Domestic cattle
DG	Domestic goat
DH	Domestic horse
DP	Domestic pony
DS	Domestic sheep
EL	Eland antelope
FB	Forest buffalo
GE	Gerenuk
GG	Grant's gazelle
GI	Giraffe
GK	Greater kudu
GZ	Grevy's zebra
HB	Hartebeest
IM	Impala
KS	Klipspringer
OY	Oryx antelope
PZ	Plains zebra
SA	Sable antelope
SI	Sitatunga
WA	Waterbuck
WH	Warthog
WI	Blue Wildebeest
WR	White rhinoceros

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General introduction

The largest land living animals like nowadays African elephants or sauropod dinosaurs (the largest living terrestrial animals ever) in the Mesozoic, are herbivores. The low trophic level these animals feed on facilitates the development of large body masses. A certain number of individuals is necessary to sustain a stable population which is able to survive droughts or other disaster. These animals need a certain amount of food. With each trophic level 90% of the energy is lost. Accordingly only 10% of the stored plant energy is available for the next trophic level. Finally herbivores have access to much more food than carnivores, so they are able to build up large populations and/or large body masses.

Since many years palaeontologists search for the reason of gigantism in sauropods. One aspect of their research focused on the nutrition of these herbivorous animals. Beside the possible feeding plants of the animals also the kind of digestion plays an important role in this context. With extant descendants of potential sauropod feeding plants in vitro digestion trials were conducted (Hummel et al. 2008) and experiments showed that the digestibility of these plants is comparable with food plants of extant herbivores.

A possibility to estimate the influence of body mass on physiological parameters of digestion is to conduct digestibility trials with extant animal species of differing body masses. The results of such studies make it possible to extrapolate the findings up to body masses which were estimated for sauropods. This study was conducted within a research group which is interested in the biology of sauropod dinosaurs with the scope on the evolution of the gigantism in these animals. The group of sauropods included some species which exceed the body mass of extant animals clearly. *Brachiosaurus brancai* for example reached a body length of 23 metres and an estimated body mass of about 16 - 38 t. The largest extant land living animal, the African elephant, reaches maximum body masses of about 6 - 10 t (the 10 t which can be found in literature are probably to high, 8 t as the upper limit seems to be more realistic). In the group of mammalian herbivores two major strategies to digest plant material can be found (Fig. 1), foregut fermenters like ruminants, camelids, hippopotami and kangaroos and hindgut fermenters like elephants, rhinoceroses and horses. Vertebrates are not able to digest plant fibre with their own set of enzymes. They are dependent on microbes in their gut to digest plant fibre. These microbes are generally aggregated in a fermentation chamber as a perfect habitat. Hindgut and foregut fermenters differ in the position of the fermentation chamber. In foregut fermenters the fermentation takes place prior of the main stomach and the small intestine, which means in consequence that food is degraded first by

the microbes. Then the host animal absorbs the microbial waste products (short chain fatty acids: acetate, butyrate and propionate) and also the microbes themselves which are washed out of the fermentation chamber. In hindgut fermenters the food enters first the stomach as well as the small intestine of the host animal and the easily digestible nutrients (like soluble carbohydrates and protein) can be digested by the host animal itself directly. The fermentation chamber is located in the hindgut of the animals and the microbes mainly digest the fibrous part of the digesta. Due to the position of the fermentation chamber the host cannot use the washed out microbes but only their waste products.

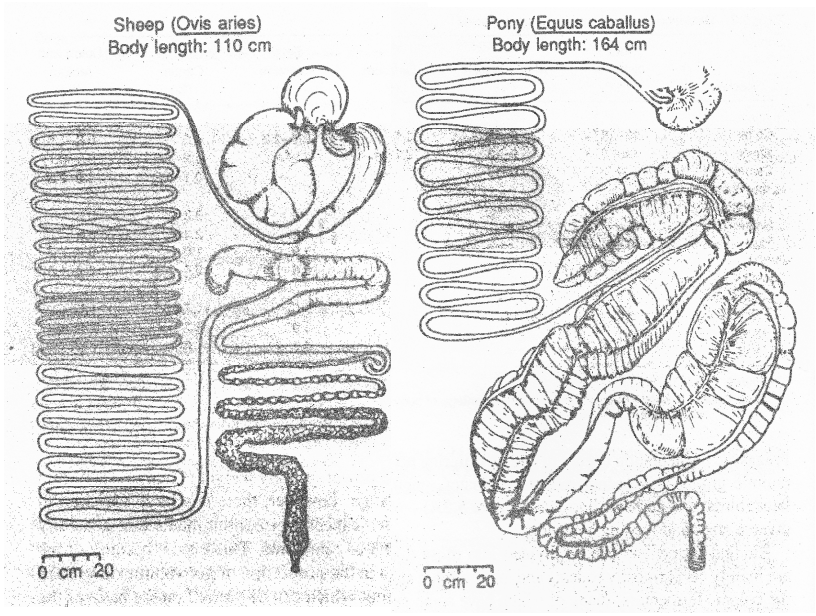

Fig. 1: Gut anatomy of a sheep (ruminant) and a horse (hindgut fermenter). Figures from Stevens and Hume (1998)

In extant herbivore populations a partition of species along the body mass spectrum takes place: Small and large sized mammalian herbivores are generally non-ruminants, whereas middle sized herbivores are generally ruminants (Janis 1976; Demment and Van Soest 1983). Demment and Van Soest (1983) postulated that the reason for this is the differing metabolic requirements requirements to gut capacity (MR/GC) ratio. Small herbivores with a high MR/GC ratio are restricted to high rates of passage because of their high energy requirements. With these short food retention times they have to ingest highly digestible food to fulfil their daily energy needs. Highly digestible food does not have to be degraded like fibrous food. Furthermore the hindgut fermenting animal can use the digestible parts of the food fast and directly (Janis 1976). This supports the non-ruminant species which were specialized on high throughput rates. Medium sized herbivores, generally ruminants, are able to digest food longer because of their lower MR/GC ratio. Accordingly they are able to feed on fibrous foods which have to be digested longer to degrade the plant cell walls. As an adaptation to these diets, ruminants evolve a selective retention of large particles in the fermentation chamber to increase fibre degradation. The regurgitation increases particle size reduction and the outflow of food particles. For ruminants there seems to be an upper body size limit caused by some restrictions based on their kind of feeding and digestion. The largest ruminants have

been postulated to have such a low MR/GC ratio that they are able to digest the food nearly completely. Fibre digestibility is an important parameter in herbivore digestion and the influence of a wide range of parameters (body mass, kind of digestive system, food particle size, fibre content/composition of the food, duration of digestion, adaptation of the gut microbes on the ingested food) on this variable were reviewed recently. Two of these factors were determined as very important in this context: first the particle size of the food and second the duration of digestion. The smaller a food particle is the higher is the degree of digestion caused by the gut microbes. The longer a food particle is retained in the fermentation chamber the longer the microbes can break up the cell walls and the higher is the digestion of the food particle. From a certain point of digestion onwards there is little further benefit for the animal to digest the food longer. For highly lignified plant material this point is reached at an early time of digestion. Ruminants are limited in food intake because of the selective particle retention in the rumen. Hindgut fermenters do have such restrictions to a lesser degree and with high food intakes and short digesta retention times they are able to feed on plants of lower digestibility and are able to increase their body mass above the ruminant spectrum. Several studies had their focus on the influence of body mass on the digesta retention time. The results of these studies are differing. Some have identified large influences of body mass on retention times, others only found little influences. Not only the digestive physiology itself is or might be influenced by body mass. Also the food choice is relevant. Free ranging herbivores show an intra- and interspecific decrease of food quality with increasing body mass (Owen-Smith 1988; Woolley et al. 2009). There are indications that larger animals are not able to feed selective on plants or plant parts with moderate fibre contents, because of the large amounts of food they have to ingest per day, additionally their larger muzzles restrict their selectivity. Another factor is that no large amounts of high quality food exist; medium to low quality roughage is much more abundant. It was shown for elephants that they reached a maximum of feeding time of approximately 18 - 20 h a day in the dry season, due to the large amounts of food they have to ingest per day. So larger animals are also time restricted regarding the food intake. Accordingly large herbivores have to feed on plants with a higher fibre content and lower quality than smaller ones (Owen-Smith 1988). This is proofed by investigations which found more stem material in the faeces and the stomachs of larger animals than in smaller ones. Accordingly the lower food selectivity of larger animals leads to a food of lower quality and a higher portion of hardly digestible fibre in it.

Topic of the thesis

In this study a number of wild and domestic ungulate herbivores were used to investigate the influence of body mass on food intake, digesta mean retention time and several chemical parameters in the faeces of the animals. The choice of the species followed their body mass and digestive strategy (Fig. 2).

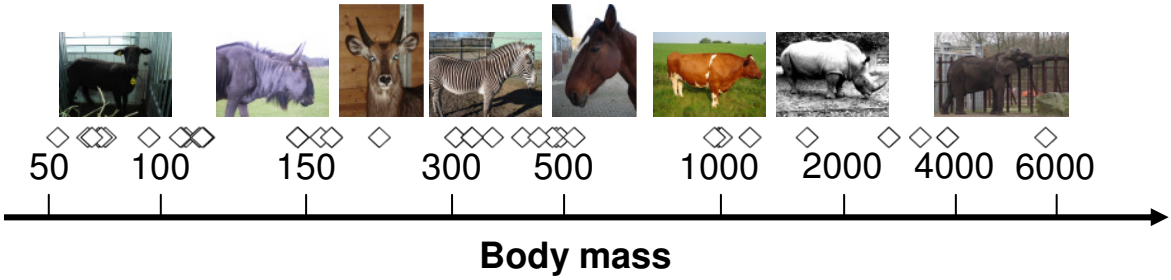

Fig. 2: Range of the body masses of the animals used in this study.

Two groups of animals were distinguished (grass eating ruminants/hindgut fermenters) with an almost equal number of species per group. The different digestive strategies must be taken into account because ruminants and hindgut fermenters are considered different in some points of interest, like food intake and mean retention time as mentioned before. Free ranging animals (Northern Kenya) were used to estimate the influence of body mass on the food quality/selectivity. To estimate food quality the nitrogen in faecal samples was measured. With this method it is possible to estimate the quantity of microbes in the gut of the animals. High faecal nitrogen contents indicate a large number of microbes in the fermentation chamber caused by high quality food. Accordingly it was possible to estimate food quality indirectly by measuring the nitrogen in the faeces. This method was approved and accepted for grazing mammals. In this study a modified faecal nitrogen analysis was used which made it possible to estimate food quality of browsing mammals, too.

This thesis is divided into three major chapters. The first chapter discusses the relation between food intake, digesta mean retention time and the body mass of the animals.

Major questions for the **first chapter** are:

1. Is there an influence of body mass on the digesta mean retention time in ungulates?
2. How is the scaling of daily dry matter intake related to body mass?
3. To what extent do hindgut fermenters have shorter digesta mean retention times and higher intake levels than ruminants?

Chapter two deals with the fibre digestion in ruminants and hindgut fermenters estimated with the Hohenheim gas test (HGT).

The questions for the **second chapter** are:

1. Do hindgut fermenters have higher HGT gas productions (GP) for faecal NDF than foregut fermenters?
2. Is there a correlation between metabolic faecal nitrogen contents and GP from faecal NDF?
3. Is there a correlation between the mean retention time of the digesta (MRT) and the GP of the faecal NDF in the HGT?
4. Is there a correlation between BM and GP of the faecal NDF?

The **third chapter** focuses on the nitrogen content in the faeces of free ranging herbivores. As mentioned before nitrogen content in faeces was used to get information about the food quality of the animals. This method was used to test the hypothesis that large herbivores are less selective feeders than smaller ones.

The hypotheses for the third chapter are:

1. Total faecal nitrogen and metabolic faecal nitrogen decrease with body mass
2. Neutral detergent insoluble nitrogen and faecal neutral detergent fibre increase with body mass
3. Neutral detergent insoluble nitrogen is higher in browsing than in grazing animals

At the end of these three chapters a synthesis will summarise the results.

Chapter 1

Is there an influence of body mass on digesta mean retention time in herbivores? A comparative study on ungulates

Abstract

The relation between body mass (BM) and digesta mean retention time (MRT) was in the focus of several studies in the last years. Because of the accepted linear scaling between gut capacity and BM in herbivorous mammals, and the fact that the energy intake of animals scales to BM to the power of 0.75 ($BM^{0.75}$) it was assumed that MRT scales with $BM^{0.25}$. Literature studies that tested this hypothesis produced differing results. This study was conducted with 8 ruminating species (n = 2 - 6) and 6 hindgut fermenting species/breeds (n = 2 - 6, warthog n = 1) of an average BM range of 60 - 4000 kg. All animals received a ration of 100% grass hay with ad libitum access. Dry matter intake was measured and MRT was estimated by the use of a fluid and a particle (<2 mm) marker. There was no significant scaling of $MRT_{particle}$ with BM for hindgut fermenters (30.97 $BM^{0.01}$, p = 0.9120) and only a trend for ruminants (29.11 $BM^{0.12}$, p = 0.0730). Ruminants on average had a $MRT_{particle}$ 1.61 fold longer than that of the hindgut fermenters. Whereas an exponent of 0.25 is reasonable from theoretical considerations on the scaling of MRT with BM much lower exponents were found in this and other studies. The energetic benefit of increasing MRT is by no means continuously increasing since the energy released from a given food unit via digestion is decreasing continuously over time. The low and non-significant scaling factors for both digestion types suggest that MRT is largely independent of BM or at least considerably less influenced than often reported, with a scaling exponent of not more than 0.1.

1. Introduction

1.1. Mean retention time, food intake and body mass

Due to the low degradation rates (%/h) of cell walls - prominent components of herbivore diets - mean retention time (MRT) of food in the digestive tract is a relevant factor that determines the digestive efficiency of herbivores. In connection with intake capacity, it may reflect nutritional niche separation within herbivore communities. Besides other variables determining the nutritional ecology of a herbivore, retention time is considered to be influenced significantly by body mass (BM), and a significant positive correlation of MRT and BM has been proposed repeatedly. This is based on the reasoning that the volume of the gastrointestinal tract (GIT) in herbivorous animals increases in proportion to $BM^{1.0}$ (Parra 1978; Demment and Van Soest 1985) while the energy requirements of an animal scale to $BM^{0.75}$ only (Kleiber 1932). As a result larger animals have larger fermentation capacities than smaller animals in relation to their energy needs. This effect is at the core of the so-called Jarman-Bell principle (Geist 1974). Accordingly it has been proposed that the MRT of the ingesta should scale to $BM^{0.25}$, and that larger animals have capacities to digest food longer and more extensively and can therefore handle food of lower quality (i. e. forage with a high lignified fibre content) (Owen-Smith 1988).

Demment (1983) ($MRT[h] = 0.69 \times aD[\%\ DM] \times BM^{0.30}$) and Demment and Van Soest (1983) ($MRT[h] = 0.60 \times aD[\%\ DM] \times BM^{0.28}$) were the first to propose this based on theoretical considerations. Based on literature data on MRT, Illius and Gordon (1992) in fact found a comparable scaling of BM and MRT for both digestion types of MRT = 9.4 $BM^{0.26}$ for hindgut fermenters and MRT = 15.3 $BM^{0.25}$ for ruminants. Gordon and Illius (1994) show in a data collection on ruminants a correlation of MRT to $BM^{0.22}$. Gross et al. (1996) also reported a strong positive correlation of BM and MRT in Nubian ibex, where males (60 kg BM) had a longer MRT of 57 h compared to females (23 kg BM) with 35 h (both sexes being fed identical diets). Robbins (1993) found for ruminants and macropods exponents of $BM^{0.28}$ up to $BM^{0.31}$. Because of the assumed positive scaling of retention time and BM, Demment and Van Soest (1983) argued that BM in ruminants is limited at a point where any further corresponding increase in MRT does not pay any longer or would even become a constraint due to excessive methane losses.

However, the scaling factor of 0.25 has not been generally accepted: Other studies found considerably lower scaling factors for groups like hindgut fermenters (32.0 $BM^{0.08}$) or

perissodactyls (22.8 $BM^{0.14}$) (Owen-Smith 1988), or even no significant scaling in ruminants (Duncan et al. 1990) or in a data collection on all available ungulate species (Owen-Smith 1988). These evaluations were all based mainly on the data set of Foose (1982). In a recent re-evaluation of the question based on a comprehensive literature review Clauss et al. (2007a) found a non-significant scaling of MRT in colon fermenters ($BM^{0.04}$), non-ruminant foregut fermenters ($BM^{0.08}$) and in browsing ($BM^{0.06}$) and grazing ($BM^{0.04}$) ruminants. Only for caecum fermenters they found a significant scaling of MRT with $BM^{0.24}$, implying that in mammalian herbivores, the assumed $BM^{0.25}$ scaling applied to the low end of the BM spectrum below a certain threshold only.

1.2. Different digestive tracts

Animals ingesting forage with high fibre contents can follow two different strategies. For maximizing digestion of high fibre forage it is necessary that the MRT is long enough for the gut microbes to digest the cell walls (Udén et al. 1982; Owen-Smith 1988; Van Soest 1994; Hummel et al. 2006). Long MRT are the typical strategy of ruminants. Their effective particle retention in the forestomach holds back particles, and consequently slows down the MRT of particles (Foose 1982; Udén et al. 1982; Duncan et al. 1990). However, the advantage of high digestibility combined with longer MRT comes at the price of some restriction of food intake (Lechner-Doll et al. 1991). This already implies a variation of this strategy, which is to maximize intake: this means to ingest larger amounts of high fibre forage and to have shorter MRT, associated with lower degrees of digestibility in consequence (Udén et al. 1982; Owen-Smith 1988). This strategy has been described as typical for hindgut fermenters like equids and elephants, both having a strategy of shorter MRT, but higher food intake compared to ruminants (Foose 1982; Duncan et al. 1990) resulting in the lower nutrient digestibility found for these species (Owen-Smith 1988; Duncan et al. 1990).

Aims of this chapter

To date, results on the influence of BM on MRT can be considered equivocal to some extent. Since they are mainly based on the data set of Foose (1982) and/or a summary of results of different trials from literature, our study aimed at evaluating the influence of BM (and of the digestive system) on food intake and particularly MRT with an independent data set created under relatively uniform conditions. By measuring intake and MRT in different ungulate species ranging in average BM from 60 - 4000 kg and all equally fed, the following questions should be answered:

1. Is there an influence of BM on the MRT in ungulates?

2. How scales dry matter intake (DMI) with BM?
3. To what extent do hindgut fermenters have shorter MRT and higher intake levels than ruminants?

2. Materials and Methods

2.1. Animals and feeding

While a plethora of mammalian herbivores belong to the group of hindgut fermenters, ungulates are at the centre of interest of this contribution. For the sake of simplicity, the term hindgut fermenters means ungulate hindgut fermenters (such as equids, rhinoceroses and elephants) in this study.

Tab. 1: Body mass (BM) [kg] of the study animals (± standard deviation (SD) or both individual values when n = 2)

		n	BM [kg]	SD
Ruminant species				
Domestic goat[1]	(*Capra aegagrus hircus)*	6	58	4.7
Domestic sheep[2]	(*Ovis orientalis aries)*	3	94	4.2
Blue wildebeest[3]	(*Connochaetes taurinus)*	4	160*	0.0
Oryx antelope[3]	*(Oryx gazella)*	3	170*	17.3
Sable antelope[3]	*(Hippotragus niger)*	3	170*	17.3
Waterbuck[3]	(*Kobus ellipsiprymnus)*	2	210*	180/240
Forest buffalo[3]	(*Syncerus caffer nanus)*	2	350*	350/350
Domestic cattle[1]	*(Bos primigenius taurus)*	3	1287	25.2
Hindgut fermenting species				
Warthog[3]	*(Phacochoerus africanus)*	1	77	-
Shetland pony[2]	(*Equus ferus caballus)*	3	97	6.1
Grevy´s zebra[3]	(*Equus grevyi)*	4	390*	20.0
Domestic horse[4]	*(Equus ferus caballus)*	6	564	49.2
White rhinoceros[3]	*(Ceratotherium simum)*	2	1750*	1500/2000
African elephant[3]	*(Loxodonta africana)*	6	4000*	1300

(n = number of sampled animals per species) *weights were estimated; [1]University of Bonn, Germany; [2]University and ETH Zurich, Switzerland; [3]Safari Park Beekse Bergen, Netherlands; [4]Riding stable Lückerath, Germany

Food intake and MRT were estimated for 8 ruminant species and 6 hindgut fermenting species/breeds (Tab. 1). Species were chosen that were known to readily accept a high percentage of grass hay in their ration. All animals were kept separately during the collection period. Exceptions were the elephants which as a group had access to an outside enclosure for 4-6 hours a day, where they were monitored all the time to be able to attribute defecations to individuals. The BM of the animals ranged from 49 kg of the smallest goat up to 6500 kg of an African elephant bull. Cattle, goats, sheep, horses, ponies and the warthog were weighed; BM of the other animals were estimated by zoo keepers, zoo veterinarians and the conductor of this study. For an adaptation period of 14 days and a collection period of minimum 6 days

for zoo animals (African elephants: 5 days) and 8 days for farm animals, all animals had ad libitum access to a 100% grass hay ration.

The range of the NDF content of the grass hay fed at different feeding places was 64.2 - 75.8% organic matter (OM), for ADF 30.0 - 43.1% OM, for ADL 3.1 - 7.8% OM and for crude protein 6.83 - 12.13% OM (Tab. 2).

Tab. 2: Organic matter (OM) [%] composition of the fed grass hay of the different trials (± standard deviation (SD))

Species	NDF_{seq}	ADF_{seq}	ADL_{seq}	CP
		[% OM]		
Warthog	75.8	41.6	4.6	12.13
Oryx antelope and blue wildebeest	70.7	39.1	4.1	11.83
African elephant	71.0	39.5	4.6	10.41
Forest buffalo and waterbuck	73.4	42.0	7.8	10.85
Grevy´s zebra and sable antelope	74.6	39.5	6.4	11.26
White rhinoceros	64.2	34.3	5.9	11.67
Domestic sheep and Shetland pony	71.0	39.4	5.7	6.95
Domestic horse	66.9	30.0	3.1	9.51
Domestic cattle	73.6	38.9	3.9	9.54
Domestic goat	74.6	43.1	6.9	7.70
Mean ± SD	**71.5 ± 3.55**	**38.8 ± 3.71**	**5.3 ± 1.42**	**10.19 ± 1.759**

(NDF_{seq} = neutral detergent fibre, ADF_{seq} = acid detergent fibre, ADL_{seq} = acid detergent lignin, CP = crude protein, NDF and ADF were analyzed sequentially and were ADL_{seq}-ash corrected)

Because of the huge amount of grass hay that was needed, three batches of hay were delivered by the same company. All boxes and stables were covered with material the animals could not feed on (saw dust, rubber mats or bare floor). For all animals daily food intake was measured during the collection period. In the morning of each day the rest of the fed grass hay was quantified and fresh hay was offered. Several times a day the animals received additional hay to ensure ad libitum access at all times.

2.2. Mean retention time

To estimate the MRT two passage markers were fed in a single pulse dose at the beginning of the collection period. Cobalt-EDTA was used as a marker for the fluid phase of the ingesta and chromium-mordanted fibre (1 - 2 mm particle size, made of grass hay) as a marker for the particle phase. The preparation was conducted according to Udén et al. (1980). Chromium content of the chromium-mordanted fibre was 1.9% DM. Faecal samples from zoo animals were collected twice during the day, and one pool-sample was taken for the night for minimum 6 days. In case of the African elephants, each dropping was sampled (5 days long) because there was access to a video control over night. From cattle, sheep, goats, horses and ponies samples were taken every 4 h (day 1 - 2), every 6 h (day 3 - 4), every 8 h (day 5 - 6) and every 12 h (day 7 - 8).

2.3. Chemical analysis

Grass hay samples were analyzed for dry matter (DM) during the sampling periods. For further analysis food samples were ground through a 1 mm sieve. The DM and ash were analyzed according to the VDLUFA-Method 8.1 (2007). Neutral detergent fibre (NDF_{seq}), acid detergent fibre (ADF_{seq}) and acid detergent lignin (ADL_{seq}) were analyzed sequentially for the grass hay and faeces according to Van Soest et al. (1991) with the Gerhardt fibre bag system (C. Gerhardt GmbH & Co. KG, Koenigswinter, Germany). NDF_{seq} and ADF_{seq} were ADL_{seq}-ash corrected. Solutions were produced according to Van Soest et al. (1991). Crude protein content of the fed grass hay was analyzed by the Dumas burning method (Instrument FP-328, LecoEnterprise, St. Joseph, Michigan, USA). The faecal samples for the MRT analysis 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 inc., Wellesley, USA).

2.4. Calculations

The MRT for the whole gastrointestinal tract (GIT) was calculated according to Thielemans et al. (1978):

$$MRT = \sum(ci * dt * ti) / \sum(ci * dt)$$

(MRT = mean retention time [h]; ci = marker concentration in the faeces at time i [mg/kg DM]; dt = length of time interval which represents the marker concentration ci [h]; ti = time after marker application (middle of time interval which represents the marker concentration ci) [h])

As an estimate of the ability to retain particles selectively in the GIT the selectivity-factor (SF) was calculated as $MRT_{particle}/MRT_{fluid}$ (Lechner-Doll et al. 1990).

2.5. Statistics

Statistical comparisons were performed with means for each species. Analysis combining ruminants and hindgut fermenters were not made because of the differences of digestive physiology between these two groups. Allometric regressions (linear regression between the logarithmic values) between BM (as the independent variable) and MRT, relative dry matter intake (rDMI) were performed for both digestion groups separately. The resulting equations are always given with the confidence intervals (CI) of 95% for the exponent. Regression analysis was performed between DMI/rDMI and MRT (as the independent variable), also given with confidence intervals of 95%. Differences between hindgut fermenters and ruminants in rDMI and MRT were tested with the Mann-Whitney test. The level of statistical

significance was set at $p < 0.05$, while for $0.1 > p > 0.05$, differences are regarded as a trend. All statistical calculations were performed with GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego, California, USA.

3. Results

3.1. Food intake

The DMI for the species are shown in Tab. 3. The rDMI were calculated related to body mass [g DM/(kg $BM^{1.0}$*d)] and related to metabolic body size [g DM/(kg $BM^{0.75}$*d)]. There was a significant positive correlation for BM and DMI [kg DM/d] for ruminants (CI = 0.42 - 0.95, p = 0.0007) and hindgut fermenters (CI = 0.70 - 0.98, p = 0.0001) (Tab. 3, Fig. 3).

Tab. 3: Means of dry matter intake (DMI) [DMI/d] and DMI related to body mass (BM) [g DM/ (kg $BM^{1.0}$*d)] and to metabolic body size (MBS) [g DM/(kg $BM^{0.75}$*d)] (± standard deviation (SD) or both individual values when n = 2)

	DMI	**SD**	**$rDMI_{BM}$**	**SD**	**$rDMI_{MBS}$**	**SD**
	[kg DM/d]		[g DM/(kg $BM^{1.0}$*d)]		[g DM/(kg $BM^{0.75}$*d)]	
Ruminant species						
Domestic goat	1.1	0.15	18.7	1.91	51.5	5.50
Domestic sheep	1.2	0.31	12.7	3.00	39.7	9.54
Blue wildebeest	3.4	0.25	21.0	1.57	74.5	5.59
Oryx antelope	2.0	0.20	12.0	1.62	43.3	5.17
Sable antelope	2.0	0.22	11.7	2.30	41.9	7.38
Waterbuck	2.4	2.1/2.6	11.6	14.4/8.9	43.9	52.9/34.9
Forest buffalo	5.1	4.7/5.5	14.6	15.8/13.5	63.3	68.3/58.3
Domestic cattle	8.0	1.15	6.2	0.81	37.3	4.99
Mean			**13.6**	**4.57**	**49.4**	**13.05**
Hindgut fermenting species						
Warthog	1.5	-	19.5	-	57.0	-
Shetland pony	2.2	0.60	22.9	5.00	71.7	15.95
Grevy´s zebra	8.1	2.61	20.6	5.52	91.5	25.77
Domestic horse	9.8	2.26	17.3	3.27	82.8	16.75
White rhinoceros	18.6	17.2/20.0	10.7	10.0/11.5	68.4	67.0/69.8
African elephant	51.0	13.33	13.1	3.44	103.1	24.94
Mean			**17.3**	**4.62**	**79.1**	**16.79**

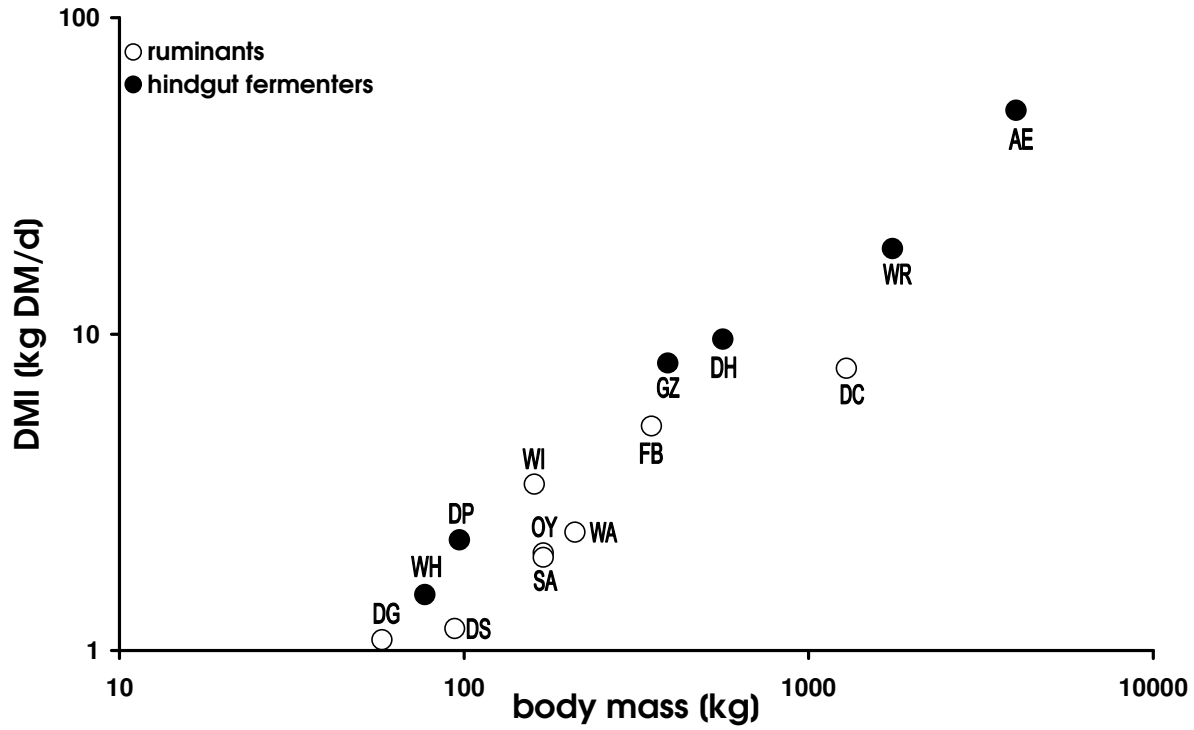

Fig. 3: Relationship between dry matter intake (DMI) [g DM/d] and body mass (BM) [kg] of all species of this study. r*uminants:* DMI = 0.06 $BM^{0.69}$, *hindgut fermenters:* DMI = 0.05 $BM^{0.84}$ (**Abbreviations** are the same for all figures: AE = African elephant, DC = domestic cattle, DG = domestic goat, DH = domestic horse, DP = domestic pony, DS = domestic sheep, FB = forest buffalo, GZ = Grevy's zebra, OY = oryx antelope, SA = sable antelope, WA = waterbuck, WH = warthog, WI = blue wildebeest, WR = white rhinoceros)

3.2. Mean retention time

In figure 4 typical marker excretion curves for ruminants (forest buffalo (a)) and hindgut fermenters (horse (b), African elephant (c), warthog (d)) are shown. The range of $MRT_{particle}$ for ruminants was between 43 h (blue wildebeest) and 75 h (domestic cattle). For the hindgut fermenters the range was between 26 h (Shetland pony) and 47 h (white rhinoceros). The range for the MRT_{fluid} was between 23 h (forest buffalo) and 37 h (sable antelope) for ruminants and between 20 h (Shetland pony) and 34 h (warthog) for hindgut fermenters. Hindgut fermenters had significantly shorter $MRT_{particle}$ than ruminants ($p = 0.0055$) while there was no significant difference for the MRT_{fluid} ($p = 0.1337$) (Tab. 4). Ruminants had always higher SF than hindgut fermenters ($p = 0.0013$) (Tab. 4).

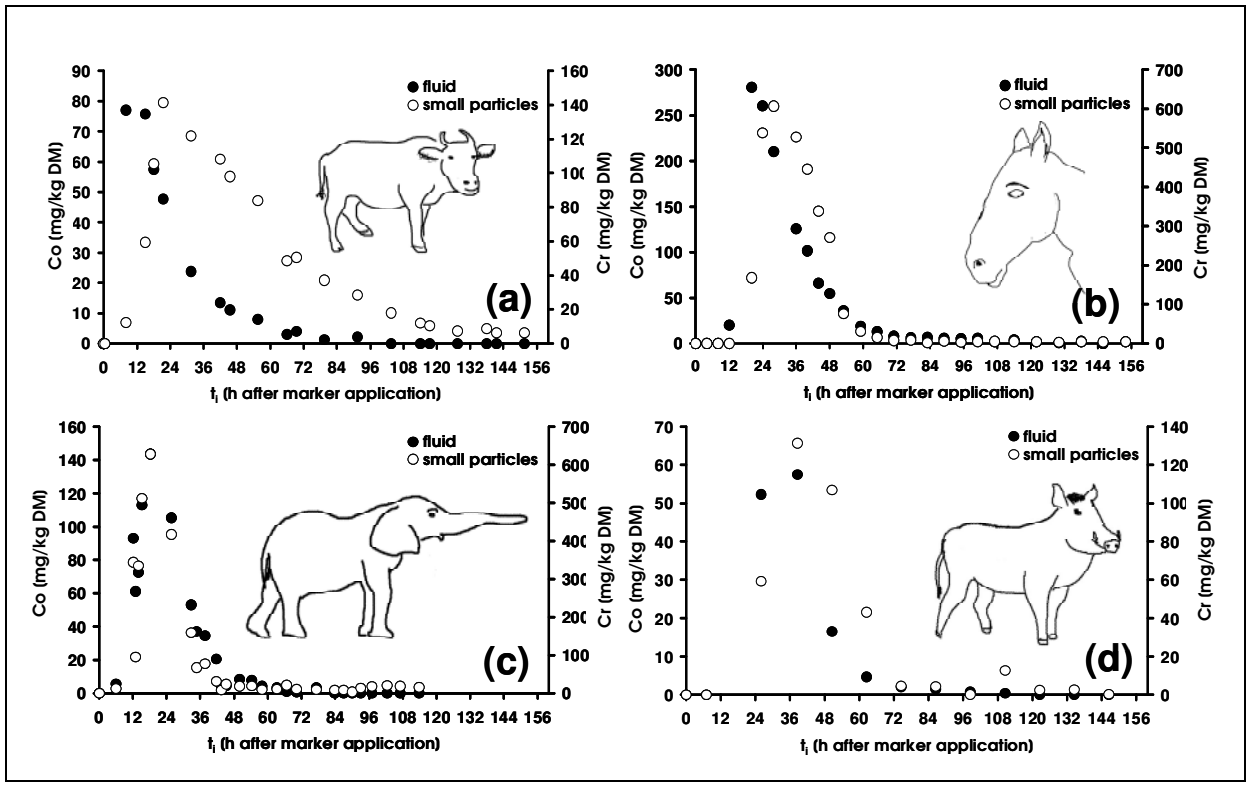

Fig. 4: Marker excretion pattern of a forest buffalo (a), domestic horse (b), African elephant (c) and warthog (d). Fluid marker (Co-EDTA), small particles (Cr-mordanted fibre, < 2 mm) (DM = dry matter)

There was no significant correlation, but a trend, between BM and $MRT_{particle}$ for ruminants ($p = 0.0730$) and no correlation for hindgut fermenters ($p = 0.9120$) (Tab. 5, Fig. 5). No significant correlation was found between BM and MRT_{fluid} for both digestion types (Tab. 5). In figure 6 the relation between $MRT_{particle}$ [h] and $rDMI_{MBS}$ [g DM/(kg $BM^{0.75}$*d)] is shown; there was no significant relation, but a trend, between the $MRT_{particle}$ and $rDMI_{MBS}$ for ruminants ($y = 77.80 - 0.46x$, CI = -0.94 - 0.01, $r^2 = 0.4889$, $p = 0.0536$) and no correlation was found between $rDMI_{MBS}$ and $MRT_{particle}$ for hindgut fermenters ($y = 62.06 - 0.35x$, CI = -0.92 - 0.22, $r^2 = 0.4256$, $p = 0.1603$). There was also no correlation between MRT_{fluid} and $rDMI_{MBS}$ for both groups (ruminants: $y = 43.23 - 0.21x$, CI = -0.54 - 0.11, $r^2 = 0.2999$, $p = 0.1600$; hindgut fermenters: $y = 34.39 - 0.09x$, CI = -0.50 - 0.33, $r^2 = 0.0729$, $p = 0.6049$).

Tab. 4: Mean retention times of particles ($MRT_{particle}$) and fluid (MRT_{fluid}) and selectivity factor (SF) ($MRT_{particle}/MRT_{fluid}$) for the whole gastrointestinal tract (± standard deviation (SD) or both individual values when n = 2)

	$MRT_{particle}$	SD	MRT_{fluid}	SD	SF	SD
	[h]				($MRT_{particle}/MRT_{fluid}$)	
Ruminants						
Domestic goat	50	5.2	32	3.3	1.6	0.21
Domestic sheep	54	4.2	34	1.8	1.6	0.19
Blue wildebeest	43	4.9	32	8.7	1.4	0.33
Oryx antelope	59	7.8	30	4.5	2.0	0.21
Sable antelope	54	15.0	37	13.1	1.5	0.33
Waterbuck	52	42/61	27	19/34	2.0	1.8/2.2
Forest buffalo	49	48/51	23	21/24	2.2	2.0/2.4
Domestic cattle	75	5.0	34	0.6	2.2	0.17
Mean	**55**	**9.5**	**31**	**4.5**	**1.8**	**0.31**
Hindgut fermenters						
Warthog	44	-	34	-	1.3	-
Shetland pony	26	1.0	20	1.2	1.3	0.11
Grevy´s zebra	28	7.2	25	8.5	1.2	0.20
Domestic horse	29	5.6	25	6.5	1.2	0.15
White rhinoceros	47	43/50	32	30/34	1.5	1.4/1.5
African elephant	30	5.2	30	4.0	1.0	0.10
Mean	**34**	**9.1**	**28**	**5.2**	**1.3**	**0.16**

Tab. 5: Allometric regressions for DMI, $MRT_{particle}$ and MRT_{fluid}.

	Equation	95% CI	r^2	p-value
DMI and BM				
ruminants	**$0.06\ BM^{0.69}$**	0.42 - 0.95	0.8702	0.0007
hindgut fermenters	**$0.05\ BM^{0.84}$**	0.70 - 0.98	0.9856	0.0001
$MRT_{particle}$ and BM				
ruminants	**$29.11\ BM^{0.12}$**	-0.02 - 0.25	0.4399	0.0730
hindgut fermenters	**$30.97\ BM^{0.01}$**	-0.22 - 0.24	0.0030	0.9120
MRT_{fluid} and BM				
ruminants	**$34.36\ BM^{-0.02}$**	-0.19 - 0.15	0.0160	0.7653
hindgut fermenters	**$21.09\ BM^{0.04}$**	-0.13 - 0.21	0.1002	0.5410

(With: the formula of the regression line, the 95% confidence interval (CI) for the exponent, the coefficient of determination (r^2) and the p-value.) DMI = dry matter intake, BM = body mass, $MRT_{particle}$ = mean retention time of particles, MRT_{fluid} = mean retention time of fluid)

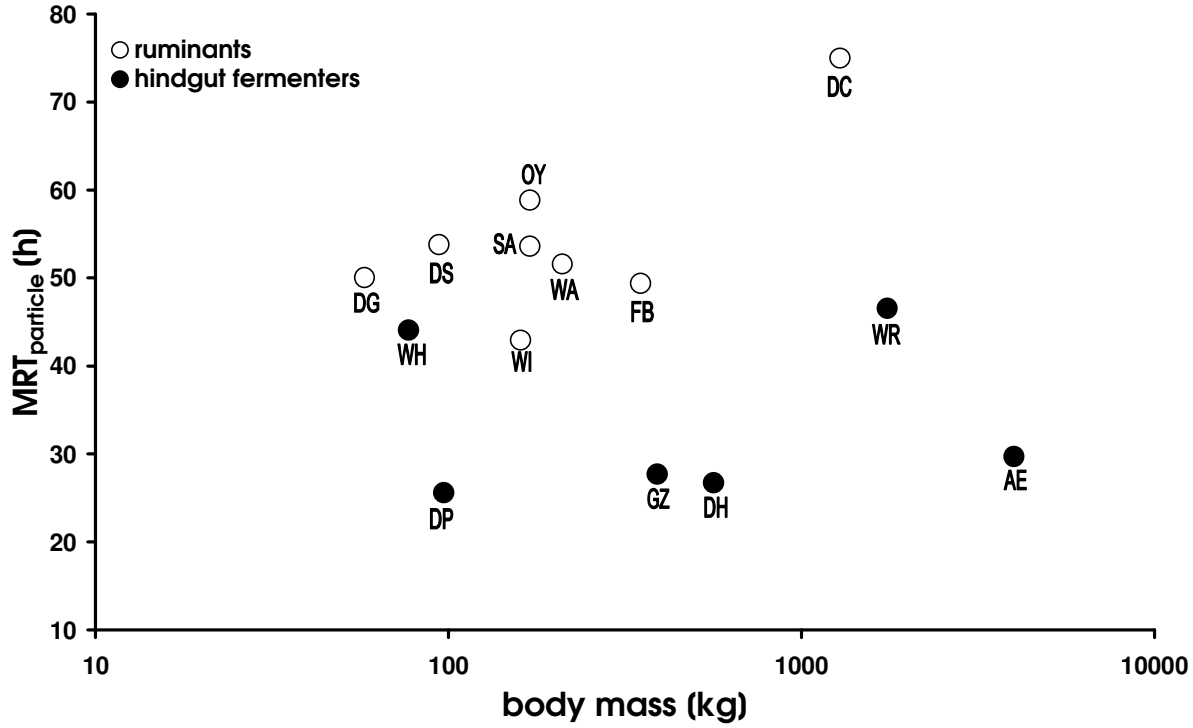

Fig. 5: Relationship between $MRT_{particle}$ [h] and body mass (BM) [kg] of all species of this study. (MRT = mean retention time). *ruminants:* MRT = 29.11 $BM^{0.12}$, *hindgut fermenters:* MRT = 30.97 $BM^{0.01}$ (For species abbreviations see Fig. 3)

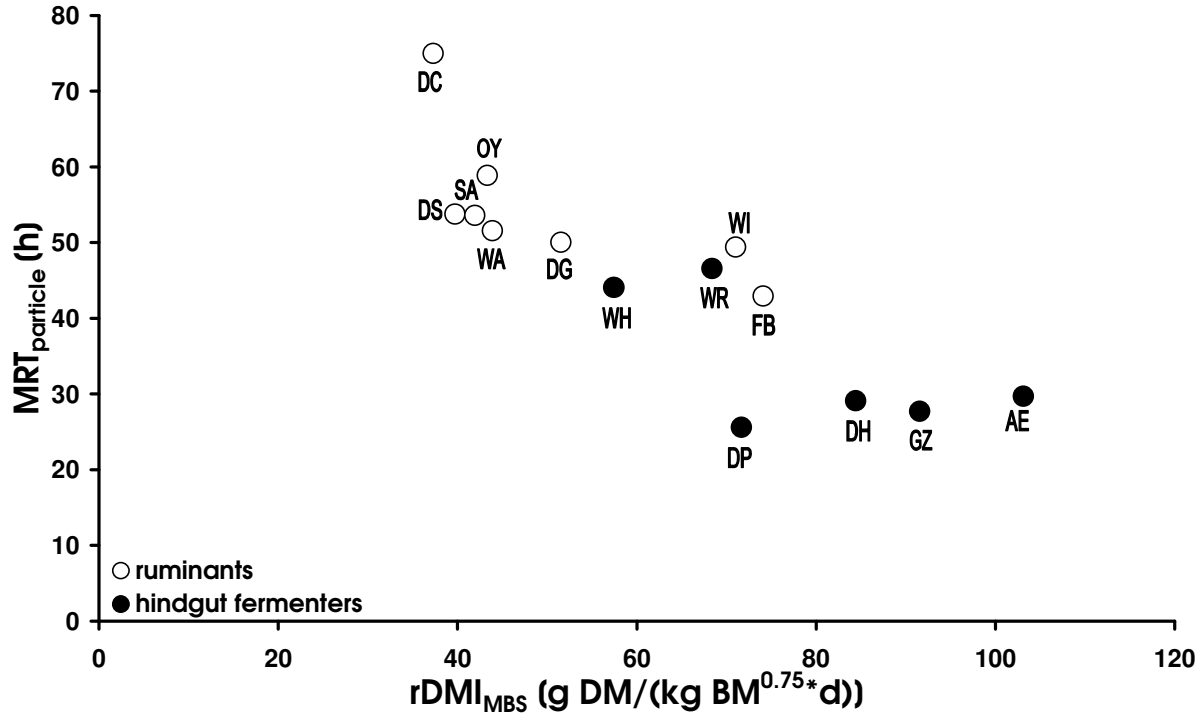

Fig. 6: Relationship between $MRT_{particle}$ [h] and $rDMI_{MBS}$ [g DM/(kg $BM^{0.75}$*d)] of all species of this study. (MRT = mean retention time, rDMI = relative dry matter intake, MBS = metabolic body size, BM = body mass, DM = dry matter). *ruminants:* MRT = 77.80 - 0.46 $rDMI_{MBS}$ *hindgut fermenters:* MRT = 62.06 - 0.35 $rDMI_{MBS}$ (For species abbreviations see Fig. 3)

4. Discussion

4.1. Method validation

For any inter-species comparison of physiological data, all other factors should ideally be as constant as possible. Concerning MRT, feeding regime and food quality are factors that can have a significant influence on the results of a trial (Varga and Prigge 1982; McCollum and Galyean 1985; Shaver et al. 1988; Tatman et al. 1991). In this study, feeding regime was ad libitum access to grass hay in all trials, allowing for expected species-differences in food intake to occur. While a single provider of the hay was chosen, the quality of the hay was found to vary between trials (see Tab. 2 for differences). The composition of the grass hay is shown as % OM because of its varying ash contents. Since no unidirectional distribution of hay quality with BM was evident (like larger species or ruminants systematically receiving hay of a higher quality) and since the focus of this study is on the establishment of regressions over a range of BM rather than comparing individual species, this is considered acceptable.

Another relevant factor is the passage marker used (Udén et al. 1982; Poncet and Al-Abd 1984; Clauss et al. 2006; Clauss et al. 2007a). For example, in the comprehensive data set of Foose (1982) on ruminant and hindgut fermenting herbivores a different passage marker (fuchsin stained unchopped hay) was used than in this study. Since the marker particles of the animals in the study of Foose (1982) were mostly longer than the 1-2 mm of the Cr-mordanted fibre in this study, systematically higher (15 - 38%) MRT values were estimated in the Foose (1982) study compared to this study. Clauss et al. (2007a) stated that also the sampling intervals can influence the MRT calculation (with increasing sampling frequency the MRT also increase) depending on the equation used to estimate MRT. In contrast to the study of Foose (1982) ($T = \sum P_x t_x$; T = mean retention time, P_x = percentage excreted in sample collected at time x, t_x = time x), the equation by Thielemans et al. (1978) used in this study is not prone to variation in estimated MRT at different sampling intervals (Van Weyenberg et al. 2006). But while these factors will significantly influence any comparison of individual values between the data sets, a comparison of the scaling factors between the studies (which are based on within-study comparisons) should not be hampered. The BM is a central topic in the approach of this paper; as it is true for almost all other cited studies dealing with wild animals, in the zoo animals of this study this variable had to be estimated for practical reasons. While this was done by experienced zoo staff and the investigator, based on opportunistic knowledge of weights of individuals and literature data, the accuracy has to be considered less exact than in farm animals, which could all be actually weighed. Again the argument applies that results based on a regression over several taxa will be less influenced

by this fact than two-species comparisons. As desirable as any actual weighing may be, this appears to stay very unlikely in zoo studies for logistic and animal temperament reasons, at least as long as no permanent scale is available in each enclosure.

4.2. General influence of BM on different aspects of digestion

Various aspects of the biology of a species can be influenced by BM (Owen-Smith 1988; Clauss et al. 2008). As far as variables related to digestion are concerned, absolute food intake [kg DM/d] may be among the most obvious: Intuitively any increase in BM is related to an increase in the daily amount of food consumed by an animal (reviewed in Clauss et al. 2007a). Based on the exponent of the allometric regression of the DMI it seems feasible to state that food intake of the animals was restricted by energy needs in this study. It was mentioned above that the energy requirements of an animal scale to BM with the exponent of 0.75. The 95% confidence interval of the regression of both groups included the 0.75 but do not reach or exceed the 1.00. If the latter exponent was reached this can be interpreted as an indication that the animals were gut fill limited because the gut of the animals increases in proportion to $BM^{1.0}$. Conrad (1966) considers food intake to be limited by gut fill in high fibre diets, and by energy requirements in diets of higher digestibility; accordingly, the animals of this study do not appear to have been limited by gut fill, but rather were able to regulate intake according to their energy requirements. In the wild situation, another variable changing significantly with BM in herbivores is the quality of the ingested food (Owen-Smith 1988). For a group of African elephants (so an intraspecific comparison) Woolley et al. (2009) found that there is an influence of BM on food quality. Large herbivores may eat the same amount of metabolizable energy per kg $BM^{0.75}$ but due to lower diet quality they require larger amounts of food. In the wild this factor can also be considered to influence the relation of BM and MRT, which is at the core of interest of this contribution.

4.3. Influence of BM on MRT

As mentioned before, the Jarman-Bell principle is based on the assumption that gut capacity increases with a scaling factor of 1.0 with BM and energy requirements only with 0.75. Based on this relation MRT was hypothesised to scale to $BM^{0.25}$ - almost identical to the generally agreed scaling of time-related physiological variables to $BM^{0.27}$ (Taylor 1980; Peters 1983). Actually some studies have found positive correlations between MRT and BM for animals as diverse as carnivores or birds (Robbins 1993). Data collections on mammalian herbivores have also resulted in scaling exponents close to 0.25 (see introduction and Tab. 6).

Tab. 6: Literature data about allometric exponents for the relationship between BM and $MRT_{particle}$, including: exponents, sample size (n), p-value, 95% confidence interval (CI) and digestion type of the sampled animals

Equation	n	p-value	95% CI	digestion type	Source
$BM^{0.30}$		-	-	all herbivores (based on theoretical calculations)	Demment (1983)
$BM^{0.28}$		-	-	all herbivores (based on theoretical calculations)	Demment and Van Soest (1983)
9.4 $BM^{0.26}$	40	-	-	hindgut fermenters	Illius and Gordon (1992)
15.3 $BM^{0.25}$	40	-	-	ruminants	
$BM^{0.22}$	45	-	-	ruminants	Gordon and Illius (1994)
15.9 $BM^{0.31}$	12	-	-	ruminants and macropods	Robbins (1993)
43.9 $BM^{0.41}$	5	-	-	hindgut fermenters (marsupials)	
15.4 $BM^{0.13}$	14	-	-	hindgut fermenters (eutherians)	
3.3 $BM^{0.24}$	6	-	-	carnivores and insects	
1.6 $BM^{0.33}$	13	-	-	birds	
32.0 $BM^{0.08}$	11	< 0.05	-	hindgut fermenters	Owen-Smith (1988)
22.8 $BM^{0.14}$	9	< 0.01	-	perissodactyls	
46.1 $BM^{0.05}$	26	n.s.	-	ungulates	
7.3 $BM^{0.17}$	60	-	-	foregut, hindgut and caecum fermenters	White and Seymour (2005)
23.6 $BM^{0.24}$	29	< 0.001	0.16 - 0.33	caecum fermenters	Clauss et al. (2007a)
34.2 $BM^{0.04}$	20	0.455	-0.07 - 0.14	colon fermenters	
34.7 $BM^{0.08}$	19	0.137	-0.03 - 0.19	non-ruminant foregut fermenters	
24.7 $BM^{0.13}$	25	0.001	0.06 - 0.21	ruminant foregut fermenters	
32.8 $BM^{0.07}$	81	0.001	0.03 - 0.10	all herbivores > 0.5 kg	
24.4 $BM^{0.14}$	93	< 0.001	0.10 - 0.17	all herbivores	
29.1 $BM^{0.12}$	8	0.0730	-0.02 - 0.25	ruminants	this study
31.0 $BM^{0.01}$	6	0.9120	-0.22 - 0.24	hindgut fermenters	

(BM = body mass)

Physiologically an increase of MRT with BM is beneficial if one assumes an increase of dietary (fermentable) cell wall content with BM (= lower diet quality), or the fact that there is an increase of digesta particle size with BM (Fritz et al. 2009). Some tradeoff between the latter two characteristics has been demonstrated for different groups of herbivores (Clauss et al. 2009). However, several other studies find scaling exponents considerably lower than the postulated 0.25 (Tab. 6). The data fits with the idea that MRT is less depending on BM than assumed from theoretical considerations: No significant increase was found at all for hindgut fermenters and for ruminants the correlation only approached significance. Regarding the low, but not significant p-value for the ruminants in this context it is arguable to state a tendency of an influence of BM on MRT in ruminants. When excluding the large cattle in the allometric regression for the ruminants the exponent is $BM^{-0.009}$ and the p-value is 0.8969. So there is a large influence of the cattle in this study on the relation between BM and MRT for

ruminants, while for ruminants of an average BM between 58 kg and 350 kg there is no influence of BM on the MRT visible. Interestingly, for ruminants and hindgut fermenters (caeco-colon fermenters in Clauss et al. (2007a)) Clauss et al. (2007a) found in their study nearly the same scaling factor for BM and MRT ($BM^{0.13}$, $BM^{0.04}$) (the calculation for the ruminants was significant) as in this study ($BM^{0.11}$, $BM^{0.03}$). However splitting the ruminant group in grazers and browsers resulted in lower (non-significant) scaling exponents for MRT and BM (grazers: $BM^{0.04}$; browsers: $BM^{0.06}$) in the study of Clauss et al. (2007a), implying that the inherently inhomogeneous BM distribution of ruminant feeding types (grazing species being considerably heavier than browsers on average) in connection with a significant difference in retention times between these feeding types has some potential to influence the estimated scaling factor.

Considerations explaining a relation of MRT to $BM^{0.25}$ explicitly do not take into account the significantly lower degree in selectivity that can be safely assumed for larger animals (see Owen-Smith (1988) and chapter 3 of this thesis for a review on allometry of some variables describing selectivity). In the wild situation one should expect at least a part of the "spare gut capacity" of large animals to be used up by the lower quality of a less digestible diet (Hummel and Clauss in press). Presumably, such differences in diet selectivity and therefore quality are also reflected in regular zoo diets: The amounts of coarse forage are regularly higher in diets of large herbivores well adapted to grass like wild cattle, white rhinos or elephants than in those of small antelopes. The larger the differences in diet quality are, the lower a potential increase in MRT with BM can be expected therefore. On the other hand, if one assumes an allometric increase of MRT with BM, this should be maximal if the diet of all animals is comparable. Therefore our approach should have resulted in an over- rather than an underestimation of the scaling factor compared to the wild situation.

Clauss et al. (2007a) found a significant increase of MRT with $BM^{0.24}$ for caecum fermenters. This implies the conclusion that an increase of MRT coinciding with an increase in BM is only beneficial for efficiency of the digestive process up to certain limit. Demment and Van Soest (1985) argument in this way, stating that disadvantages will dominate advantages above a certain threshold for retention times. An endless prolongation of the MRT also makes little sense because energy gained from a given amount of food is getting less over digestion time and the probability of excessive methane losses is considered to increase especially for ruminants (Van Soest 1994). It may be noted that the degree how much prolonged retention pays for a herbivore ingesting a diet higher in fibre will finally depend to what extent this means lignified or unlignified fibre: While the former will not be degradable irrespective of

the duration of exposure to microbial fermentation, the latter will be digested to a higher degree the longer it is retained in the fermentation chamber.

Most investigations on a potential influence of BM on MRT have focused on $MRT_{particle}$. However, if such an influence exists for $MRT_{particle}$, it could be expected to be less influenced by other disrupting factors (like feeding type) and therefore to be even more visible in MRT_{fluid}. In fact, Robbins (1993) reports a scaling of $MRT_{fluid} = 18.3\ BM^{0.18}$ for hindgut fermenting mammals. Fluid retention times do not represent an actual passage of fluid from ingestion to excretion/mouth to anus, but represent the amount of fluid secretion and absorption that is occurring in the GIT (Clauss et al. 2010). In contrast to Robbins (1993) there is no indication that these mechanisms scale with BM in this study. MRT_{fluid}, or the passage of unabsorbable solutes through the GIT, was not associated with BM in large herbivores.

4.4. Herbivores going to extremes – implications for herbivorous dinosaurs

Considerations on the type of the relation between BM and MRT are also of interest in a fascinating chapter of herbivore digestive physiology: How should we speculate on the digestive physiology of extraordinarily large herbivores like sauropod dinosaurs, which push the BM envelope to 50 t or even more, and for which extrapolations based on high scaling factors simply result in "an improbability" (Van Soest 1994)? Based on the results of this study and other recent studies (Clauss et al. 2007a), the reconstruction of retention times for these animals causes less problems as may have been expected, because an increase of BM is by no means inherent with a continuous increase in MRT beyond the scope of the reasonable. Besides this elephants are the best example for an animal contradicting any automatism of an increase of MRT with BM dramatically (Foose 1982; Clauss et al. 2003).

Reconstructing retention times of sauropods via calculations based on estimations of gut capacity, DMI and diet digestibility is another option to give estimations for retention times of sauropods (Franz et al. 2009). From the latter contribution, and as already stated by Farlow (1987), it can be seen that a potential characteristic of some dinosaurs would go in line with long retention times: Assuming a metabolism lower than that of an average ungulate almost inevitably implies a relatively low intake and long MRT, just like in extant hippos, sloths or -most extreme- herbivorous reptiles.

4.5. Differences between MRT of ruminants and hindgut fermenters

While BM was shown to have only limited - if any - influence on MRT, digestive strategy can be considered as a category influencing MRT. In this respect, the difference between ungulate hindgut fermenters and ruminants can be considered as established since the seminal

contributions of Janis (1976) and Foose (1982). The opportunity to use the present data set to re-quantify some of the established relations will be used.

It was found in Foose (1982), Sponheimer et al. (2003) and also in this study that hindgut fermenters have significantly higher $rDMI_{MBS}$ than ruminants. In line with the ability of ruminants to hold back particles in their fermentation chamber to elongate the digestion time for the rumen microbes (Udén et al. 1982; Demment and Van Soest 1985; Renecker and Hudson 1990; Gordon and Illius 1994) are the findings in Foose (1982), Udén et al. (1982), Parra (1978) and the present study that ruminants always have longer $MRT_{particle}$ than hindgut-fermenting species, but it has to be kept in mind, that only ungulates were tested in this study. In the present study ruminants on average had a $MRT_{particle}$ 1.61 fold longer than hindgut fermenters, a value close to the 1.50 found in Foose (1982) for grazing ruminants compared to grazing hindgut fermenters. Similarly the $rDMI_{MBS}$ was 1.58 fold higher for hindgut fermenters than for ruminants in this study and 1.55 fold higher in Foose (1982) (calculated with $rOMI_{MBS}$). Lechner-Doll et al. (1990) introduced the selectivity factor (SF) ($MRT_{particle}/MRT_{fluid}$) which is evidently a good measure to compare the selective retention of food particles within different feeding and digestion types. The higher the SF of an animal is the better is its ability to hold back particles in the mixing chamber. Because of the fact that the SF is less influenced by the feeding regime or type of food, it is also a factor which facilitates comparisons among studies with different feeding trial conditions (Hummel et al. 2005). In accordance with the difference in $MRT_{particle}$, the different digestive strategies of the two digestion types are also reflected in the means of the SF in this study for grazing ruminants (1.80 ± 0.312) and grazing hindgut fermenters (1.23 ± 0.151). MRT_{fluid} was not found to be significantly different ($p = 0.24$) between ruminants and hindgut fermenters. This implies no different strategy of ruminants and hindgut fermenters in this respect, and that the significant difference of the SF between the two groups is based on the difference between $MRT_{particle}$ basically.

In general, a negative correlation can be expected for $rDMI_{MBS}$ and MRT. Interestingly, a significant negative correlation between MRT and $rDMI_{MBS}$ was only found for ruminants, but not for hindgut fermenters. In Foose (1982) results were the other way round, while Lechner-Doll et al. (1990) and Pearson et al. (2001) found negative correlations for ruminants and equids, respectively. Clauss et al. (2007a) found for their entire data set (caecum, caeco-colon, non-ruminant foregut and ruminant foregut fermenters) a low but significant, negative correlation between $rDMI_{MBS}$ and MRT. An insensitivity of MRT to an increase in intake has been considered as a major trait in digestive strategies of herbivores (Clauss et al. 2007b).

The result would be in line with the general view of hindgut fermenters as being able to keep DMI high more easily than ruminants when diet quality decreases; if MRT is less influenced by DMI in hindgut fermenters, this would facilitate a strategy of high intakes by attenuating the negative effects of increased intake.

4.6. "The" hindgut fermenters - equids, rhinos, elephants and warthog

While grazing ruminants can be considered as relatively uniform in their digestive strategy (as far as intakes and MRT are concerned), in accordance with older literature this is the case to a far lesser degree for the more variable (and phylogenetically much more heterogeneous) group of hindgut fermenters (e.g. Foose 1982): While both equids and elephants follow a strategy of high rDMI/low MRT, the white rhino and the warthog appear closer to ruminants in some traits. This also points to some potential difficulties in the establishment of allometric relations: Either is the increase of MRT with BM from equids to rhinos considered as according to a significant allometry (and elephants as outliers), or no increase of MRT with BM is concluded from the data on equids and elephants, and rhinos are considered as deviating from this rule. What can generally be stated is that the phylogenetically fixed feeding strategy seems to overrule any effects of BM on MRT, like increasing gut volume.

5. Conclusions

- The results of our study give little indication for a significant influence of BM on MRT in hindgut fermenters and ruminants.
- The influence of the BM on DMI was in the expected range and indicates that the food intake of the animals in this study was restricted by energy needs and not by gut fill.
- Ruminants had longer $MRT_{particle}$ than hindgut fermenters (factor 1.61), and a lower $rDMI_{MBS}$ (factor 1.58).

Chapter 2

Measuring differences in fibre degradability realized by large herbivores - using an in vitro test

Abstract

Digestion trials are difficult to conduct or are sometimes even not possible for wild animals kept in zoos. Limited access to stables and nervous animals complicate trials with food intake recording and total faecal collection. To get information about the fibre digestion realized by these animals a method with non-invasive sampling is desirable. In this study long term in vitro digestibility trials with faecal neutral detergent fibre (NDF) were used to estimate fibre degradability in ruminants. Therefore only spot samples of food and faeces are necessary, hence a non invasive method also for captive wild animals. The study has been conducted with 10 foregut fermenting (9 ruminants/1 camelid) species (n = 2-6) and 7 hindgut fermenting species/breeds (n = 3-7, warthog n = 1). All animals received a ration of 100% grass hay with ad libitum access. Neutral detergent fibre of food and faeces were fermented in vitro in a long term Hohenheim gas test (HGT) (96 h). In addition, faecal nitrogen and faecal NDF content were measured. For the time intervals up to 16 h after incubation no significant differences in HGT gas production (GP) of faecal NDF between foregut and hindgut fermenters were observed. For all following time intervals there were significantly higher GP values for faecal NDF of hindgut compared to foregut fermenters. Accordingly foregut fermenters were more effective in fibre degradation up to a certain threshold of fermentation than hindgut fermenters. No relation was found for foregut and hindgut fermenters between the metabolic faecal nitrogen (MFN) (indicator for microbial growth in the fermentation chamber) and the cumulative GP after 24, 48 and 96 h. For ruminants a significant negative relation was found between $MRT_{particle}$ and GP. It was shown that the foregut fermenters in this study are able to digest the fibre fraction of the food to a higher degree than the hindgut fermenters.

1. Introduction

In most digestion trials with herbivorous animals food digestibility in general and especially fibre digestibility is in the focus. The differences in fibre digestibility of foregut and hindgut fermenters can be evaluated by several methods. One classic method is a feeding trial with the documentation of food intake and total collection of faeces. With these two variables it is possible to estimate the apparent digestibility (aD) of the food the animals ingested. This method generally involves some restrictions for the studied animal (individual housing, food restrictions). For captive wild animals a method would be useful which can be conducted less invasive and without total faecal collection and daily food intake documentation. Feeding animals external digestion markers, for example titanium dioxide (TiO_2) is one alternative to total faecal collection (Jagger et al. 1992; Kavanagh et al. 2001; Titgemeyer et al. 2001; Glindemann et al. 2009). For reliable results of this method the animals have to ingest the marker completely for the duration of several days. As a result this method would be practicable under circumstances allowing controlled feeding. In case of zoo animals this method is difficult to conduct, for some animal species it would be impossible. It is also possible to use an internal marker like acid insoluble ash (AIA) to estimate digestibility. In this case it is important, that the animal does not ingest other sources of AIA than that contained in the food and it is necessary that the food is ingested nearly unselective or the food residues have to be sampled in a representative way. When animals are fed by using hay racks, the food enclosed soil can fall through the grates and is consequently not ingested by the animal. Accordingly this method cannot be used in studies using hay racks. A rarely used possibility, which is not relying on quantitative conclusions on faecal output or intake, is to use in vitro tests for estimating differences in fibre digestion between animals or species. Faecal NDF can be used for in vitro digestion. The principle is that the higher the gas production is the more potentially digestible fibre has been left undigested in the faeces. The Hohenheim gas test (HGT) was developed for in vitro evaluation of food digestibility (Menke et al. 1979). Prins et al. (1981) used results of long term in vitro digestion trials (336 h) to quantify cell wall degradation in the faeces of animals. This method, which only needs spot samples of food and faeces of an animal, will fit with the circumstances of estimating differences in fibre degradability in wild animals in zoos.

Aims of this chapter

Based on other studies which have shown that the fibre digestibility in foregut fermenters is higher than in hindgut fermenters, the following questions are investigated in this chapter:

5. Do hindgut fermenters have higher HGT gas productions (GP) for faecal NDF than foregut fermenters?
6. Is there a correlation between metabolic faecal nitrogen contents and GP from faecal NDF?
7. Is there a correlation between the mean retention time of the digesta (MRT) and the GP of the faecal NDF in the HGT?
8. Is there a correlation between BM and GP of the faecal NDF?

2. Materials and Methods

2.1. Methods

In this study an approach comparable to the method of Prins et al. (1981) has been conducted, by using a long term HGT (96 h), to achieve information about the differences of neutral detergent fibre (NDF) degradation realized by several wild and some farm animals. The faecal nitrogen content was also measured because it is an indicator for microbial growth in the fermentation chamber of animals (Lancaster 1949; Mésochina et al. 1998; Robinson et al. 2001; Lukas et al. 2005). Faecal NDF has been analyzed for additional information. In the first chapter of this thesis the digesta mean retention time (MRT) was estimated. The time of fermentation is an important factor for fibre degradation. The longer the MRT was the longer was the time the microbes in the fermentation chambers had for fibre degradation. Accordingly it can be hypothesized that there is a correlation between the MRT and the results of the HGT.

2.2. Animals and feeding

Food and faecal samples of 10 foregut fermenting species (ungulate and camelid species, n per species = 2-6) and 7 hindgut fermenting species/breeds (n per species = 3-7, warthog n = 1) were collected (Tab. 7).

Cattle, goats, sheep, horses and ponies (farm animals) were weighed; body mass (BM) of zoo animals was estimated by zoo keepers, zoo veterinarians and the conductor of this study (except the warthog which was weighed). All animals got a ration of 100% grass hay with ad libitum access for an adaptation period of 14 days. Most animals were kept separately during the collection period. Exceptions were the elephants which as group had access to an outside

enclosure for 4-6 hours a day, where they were monitored to be able to attribute defecations to individuals. Food and faecal samples were collected daily for a period of minimum 6 days (African elephants 5 days) after the adaptation period. All boxes and stables were covered with material the animals do not feed on (saw dust, rubber mats). Most of the animals were fed by using hay racks. The warthog, white rhinoceroses, domestic ponies, domestic cattle and domestic horses were fed using feeding troughs. For all animals daily food intake was measured during the collection period. Exceptions for all points mentioned above were the Przewalski horses and Bactrian camels. They were permanently kept on large outside enclosures and their faeces were sampled after 14 days of feeding with a 100% grass hay ration. Because the collection period was conducted during winter, Przewalski horses and Bactrian camels did not have significant food available from their outside pastures.

Tab. 7: Body mass [kg] of the studied animals

		n	Body mass [kg]
Foregut fermenters			
Springbok[3]	(*Antidorcas marsupialis)*	2	30*
Domestic goat[1]	(*Capra aegagrus hircus)*	6	58
Domestic sheep[2]	(*Ovis orientalis aries)*	3	94
Blue wildebeest[3]	(*Connochaetes taurinus)*	5	160*
Oryx antelope[3]	*(Oryx gazella)*	3	170*
Sable antelope[3]	*(Hippotragus niger)*	3	170*
Waterbuck[3]	(*Kobus ellipsiprymnus)*	2	210*
Forest buffalo[3]	(*Syncerus caffer nanus)*	2	350*
Bactrian camel[3]	(*Camelus bactrianus)*	4	450*
Domestic cattle[1]	*(Bos primigenius taurus)*	3	1287
Hindgut fermenters			
Warthog[3]	*(Phacochoerus africanus)*	1	77
Shetland pony[2]	(*Equus ferus caballus)*	3	97
Przewalski horse[3]	(*Equus ferus przewalskii)*	4	250*
Grevy´s zebra[3]	(*Equus grevyi)*	4	390*
Domestic horse[4]	*(Equus ferus caballus)*	6	564
White rhinoceros[3]	*(Ceratotherium simum)*	7	1800*
African elephant[3]	*(Loxodonta africana)*	6	4000*

*weights were estimated; [1]University of Bonn, Germany; [2]University and ETH Zurich, Switzerland; [3]Safari Park Beekse Bergen, Netherlands;[4]Riding stable Lückerath, Germany (n = number of sampled animals per species)

2.3. Chemical analysis

Faecal samples for nutrient analysis were taken every day of the collection period, pooled at the end of the trial, stored at -20 °C for further analysis and were later freeze dried. Grass hay and all faecal samples were ground through a 1 mm sieve and the ash content was measured. Neutral detergent fibre (NDF_{seq}), acid detergent fibre (ADF_{seq}) and acid detergent lignin (ADL_{seq}) were analyzed sequentially for the grass hay and faeces according to Van Soest et al. (1991). NDF_{seq} and ADF_{seq} were ash corrected. Solutions were produced according to Van

Soest et al. (1991), for the analysis the Gerhardt-fibre bag system (Gerhardt, Königswinter, Germany) was used. The nitrogen content in the food was measured with the Dumas method (instrument: FP-328, Leco, St. Joseph, Michigan, USA).

For estimating the fibre degradability of food and faecal NDF the samples were washed with ND solution according to Van Soest (1991). In vitro fermentation of the NDF was evaluated with the HGT (Menke et al. 1979), using standardized sheep rumen fluid as inoculum source for food and faecal NDF comparisons. To quantify the degree of cell wall degradation, an approach comparable to Prins et al. (1981) and Prins et al. (1983) was used. GP of NDF of the fed grass hay and faeces was quantified at 4, 8, 12, 16, 24, 32, 48, 56, 72, 80 and 96 h (GP related to NDF residue, expressed as ml/200 mg NDF). Because there was some variability in the GP for the grass hay NDF (GP_{GNDF}) in the latter data evaluation two intervals of the HGT were always combined to minimize the influence of the varying grass hay on the GP measured for the faecal NDF (GP_{FNDF}) (0-4 + 4-8, 8-12 + 12-16, 16-20 + 20-24, 24-32 + 32-48, 48-56 + 56-72, 72-80 + 80-96). The GP measured during the HGT gives indications of the digestibility of the foodstuff or the residual fibre in faeces. The higher the GP is the more digestible material was in the NDF residue. For splitting faecal nitrogen into fractions the method of Mason (1969) was used. Therefore the samples were washed with neutral detergent (ND) solution. This method separates the total faecal nitrogen (TFN) into two fractions:

1. Neutral detergent insoluble nitrogen (NDIN): Neutral detergent (ND) washed faecal samples contained the undigested food nitrogen which was included in the undigested fibre in the faeces.
2. Metabolic faecal nitrogen (MFN): This nitrogen fraction was calculated by subtracting the NDIN value from the TFN value following an approach of Van Soest (1994). This fraction included: microbial debris (main part of the fraction), sloughed-off animal cells, mucus and gut enzymes.

The nitrogen content of all three fractions (TFN, NDIN and MFN) was measured with the Dumas method.

The mean NDF_{seq} content of the fed grass hay was 72% in organic matter (OM); ADF_{seq} content was 39% OM and for nitrogen 1.64% OM was measured. The mean 24 h GP for the grass hay was 33.1 ml/200 mg OM. There were no significant differences between the grass hay fed to foregut and hindgut fermenters in the NDF_{seq}, ADF_{seq}, ADL_{seq}, nitrogen content and the 24 h GP (measured with the HGT) (Tab. 8).

Tab. 8: Fibre (NDF_{seq}, ADF_{seq}, ADL_{seq}) and nitrogen (N) content [% OM] and 24 h gas production (GP) [ml/200 mg OM] measured with the Hohenheim gas test of the fed grass hay.

	NDF_{seq}	ADF_{seq}	ADL_{seq}	N	24 h GP
		[% OM]			[ml/200 mg OM]
Foregut fermenters					
Springbok	71.0	39.5	4.6	1.67	30.5
Domestic goat	76.6	43.1	6.9	1.23	34.9
Domestic sheep	71.0	39.4	5.7	1.11	36.5
Blue wildebeest	70.7	39.1	4.1	1.89	34.1
Oryx antelope	70.7	39.1	4.1	1.89	34.1
Sable antelope	74.6	39.5	6.4	1.80	31.7
Waterbuck	73.4	42.0	7.8	1.74	26.2
Forest buffalo	73.4	42.0	7.8	1.74	26.2
Bactrian camel	71.0	39.5	4.6	1.68	34.2
Domestic cattle	73.6	38.9	3.9	1.53	33.7
Mean ± SD	**72.6 ± 2.03**	**40.2 ± 1.53**	**5.6 ± 1.54**	**1.63 ± 0.265**	**32.2 ± 3.57**
Hindgut fermenters					
Warthog	75.8	41.6	4.6	1.94	24.8
Shetland pony	71.0	39.4	5.7	1.11	37.4
Przewalski horse	71.0	39.5	4.6	1.67	34.1
Grevy´s zebra	74.6	39.5	6.4	1.80	31.7
Domestic horse	66.9	30.0	3.1	1.52	34.0
White rhinoceros	64.2	34.3	5.9	1.87	41.8
African elephant	71.0	39.5	4.6	1.67	34.2
Mean ± SD	**70.6 ± 4.05**	**37.7 ± 4.05**	**5.0 ± 1.11**	**1.65 ± 0.278**	**34.0 ± 5.20**
P-value	**0.5159**	**0.4535**	**0.6560**	**0.9609**	**0.5241**

(p-values were calculated with the Mann-Whitney test; means ± standard deviation (SD), NDF_{seq} = neutral detergent fibre, ADF_{seq} = acid detergent fibre, ADL_{seq} = acid detergent lignin (fibre fractions were analyzed sequentially, NDF_{seq} and ADF_{seq} were ADL-ash corrected), OM = organic matter)

2.4. Statistics

Allometric regressions (linear regression between the logarithmic values) between BM (as the independent variable) and 24, 48 and 96 h GP_{FNDF} and the regression between MFN (as the independent variable) and 24, 48 and 96 h GP_{FNDF} were performed for both digestion groups separately. The regression between the MRT (as the independent variable) and the 48 h GP_{FNDF} (foregut fermenters) and 24 h GP_{FNDF} (hindgut fermenters) was calculated equally. The resulting equations are always given with the 95% confidence intervals (CI) for the exponent. The level of statistical significance was set at $p < 0.05$, while for $0.1 > p > 0.05$, differences are regarded as a trend. For differences between foregut and hindgut fermenters in food (Tab. 8) (NDF_{seq}, ADF_{seq}, ADL_{seq}, N and 24h GP), faecal parameter (TFN, NDIN, MFN, faecal NDF) and GP per time interval (0-8 h, 8-16 h, 16-24 h, 24-48 h, 48-72 h, 72-96 h) the Mann-Whitney test was used. For all calculation the species means were used. All statistical calculations were performed with GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, California, USA).

3. Results

3.1. Gas production

The mean GP_{FNDF} of foregut and hindgut fermenters was not significantly different for the time intervals 0-8 h ($p = 0.5912$) and 8-16 h ($p = 0.4182$). In the following time intervals (16-24, $p = 0.0015$; 24-48, $p = 0.0001$; 48-72, $p = 0.0001$ and 72-96 h, $p = 0.0002$) GP_{FNDF} was significantly higher for hindgut fermenter samples than for the foregut fermenter samples (Fig. 7). For the cumulative 24 h, 48 h and 96 h GP_{FNDF} of foregut and hindgut fermenters a tendency for higher GP_{FNDF} for hindgut fermenters was measured (Tab. 9).

3.2. Faecal nitrogen and faecal NDF

For foregut fermenters the springbok had the highest (2.35% OM) and the goat the lowest (1.56% OM) TFN values. For hindgut fermenters the white rhinoceros had the highest (2.06% OM) and the horse the lowest (1.24% OM) TFN values. The springbok had the highest MFN values for foregut fermenters (1.90% OM) and the goat the lowest (1.20% OM). For hindgut fermenters the white rhinoceros had the highest (1.62% OM) and the horse had the lowest (0.91% OM) MFN values. The TFN and MFN contents were significantly higher in foregut fermenters faeces than in hindgut fermenters faeces (TFN $p = 0.0046$; MFN $p = 0.0040$) (Tab. 9). There is no significant difference between the NDIN values of foregut and hindgut fermenters in this study ($p = 0.1423$). The faecal NDF_{seq} values of the foregut fermenters were significantly lower than that of the hindgut fermenters ($p = 0.0020$) (Tab. 9).

3.3. Relation between gas production and body mass

No significant correlation was observed between BM and 24, 48 and 96 h GP_{FNDF} for foregut and hindgut fermenters (Tab. 10). For foregut fermenters there is a trend of decreasing GP with increasing BM after 48 h of incubation.

3.4. Relation between gas production and metabolic faecal nitrogen

There were also no significant correlations between the MFN content and the 24, 48 and 96 h GP_{FNDF} for foregut and hindgut fermenters (Tab. 11).

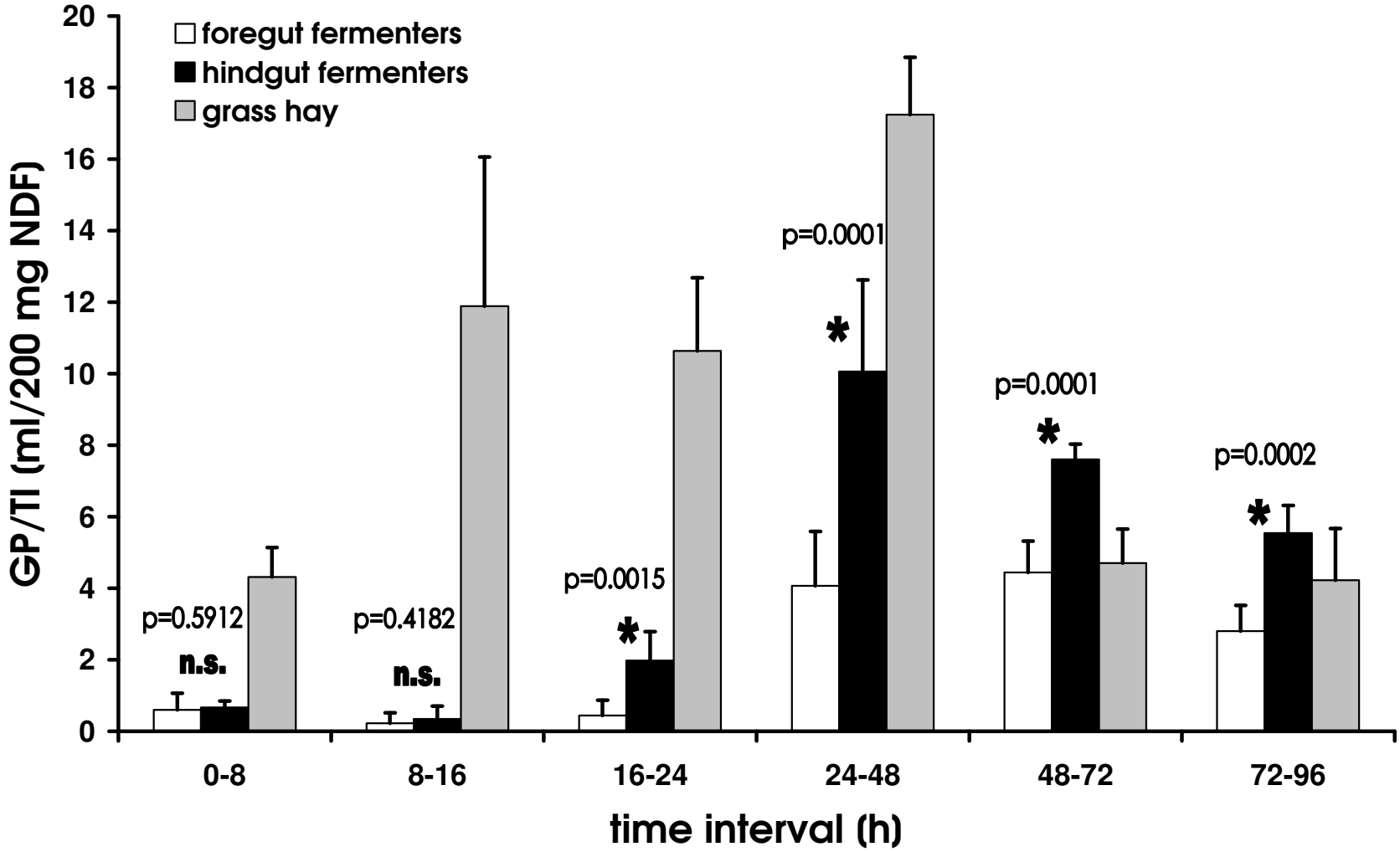

Fig. 7: The gas production (GP) per time interval (TI) [ml/200 mg NDF] for neutral detergent fibre (NDF) of foregut and hindgut fermenter faeces and grass hay (p-values between foregut and hindgut fermenters were calculated with the Mann-Whitney test, n.s. = not significantly different, * = significantly different, DM = dry matter)

Tab. 9: Total faecal nitrogen [TFN], neutral detergent insoluble nitrogen [NDIN] and metabolic faecal nitrogen [MFN] for study animals [% OM], faecal NDF_{seq} content [% DM] and cumulative 24 h/48 h/96 h gas production (GP) for faecal NDF [ml/200 mg NDF] (± standard deviation (SD) or both individual values when n = 2)

	TFN	SD	NDIN	SD	MFN	SD	NDF_{seq}	SD	24 h GP	SD	48 h GP	SD	96 h GP	SD
			[% OM]				[% DM]				[ml/200 mg NDF]			
Foregut fermenters														
Springbok	2.35	2.22/2.49	0.46	0.45/0.46	1.90	1.76/2.04	50.9	50.5/51.2	0.9	0.9/0.9	4.2	3.6/4.8	10.3	9.0/11.5
Domestic goat	1.56	0.076	0.36	0.029	1.20	0.090	64.1	2.73	1.7	0.54	5.9	1.25	13.4	2.02
Domestic sheep	1.72	0.271	0.30	0.034	1.42	0.237	54.8	7.20	2.0	1.58	8.6	4.98	19.3	7.56
Blue wildebeest	2.18	0.194	0.42	0.057	1.76	0.242	52.9	3.61	1.7	0.66	6.8	1.24	14.8	1.51
Oryx antelope	2.05	0.076	0.45	0.013	1.60	0.065	56.1	2.71	0.4	0.19	3.8	0.51	11.2	0.77
Sable antelope	2.10	0.273	0.36	0.048	1.73	0.248	52.7	5.40	1.6	0.42	6.1	1.21	12.4	1.71
Waterbuck	2.13	1.95/2.30	0.34	0.33/0.35	1.79	1.62/1.95	48.4	45.0/51.7	1.6	1.1/2.2	6.9	4.7/9.1	13.2	10.2/16.2
Forest buffalo	2.13	2.07/2.18	0.40	0.39/0.40	1.73	1.68/1.78	57.0	56.2/57.7	1.6	1.5/1.7	6.1	5.8/6.4	14.5	13.9/15.2
Bactrian camel	2.04	0.128	0.40	0.039	1.64	0.164	47.7	4.99	1.2	0.50	2.6	1.39	7.9	1.29
Domestic cattle	1.63	0.141	0.31	0.027	1.32	0.118	56.1	3.07	0.4	0.05	2.3	1.10	8.5	1.83
Mean	**1.99**	**0.260**	**0.38**	**0.054**	**1.61**	**0.225**	**54.0**	**4.77**	**1.3**	**0.56**	**5.3**	**2.03**	**12.6**	**3.34**
Hindgut fermenters														
Warthog	1.35	-	0.35	-	0.99	-	67.1	-	4.2	-	19.6	-	33.0	-
Shetland pony	1.40	0.077	0.31	0.025	1.10	0.091	68.7	1.82	3.7	0.59	13.9	0.80	24.8	0.54
Przewalski horse	1.49	0.047	0.32	0.023	1.17	0.049	70.7	2.82	2.4	0.92	10.0	3.48	24.4	4.91
Grevy´s zebra	1.39	0.111	0.28	0.025	1.11	0.136	66.8	1.68	3.1	1.30	11.4	2.68	25.0	1.99
Domestic horse	1.24	0.121	0.33	0.026	0.91	0.124	70.2	1.71	4.1	1.45	14.4	3.45	27.3	3.62
White rhinoceros	2.06	0.232	0.44	0.028	1.62	0.224	55.6	6.36	2.5	0.63	12.4	1.12	25.6	2.97
African elephant	1.66	0.117	0.35	0.017	1.31	0.118	63.8	6.56	5.4	1.70	14.1	1.99	27.4	1.83
Mean	**1.51**	**0.271**	**0.34**	**0.050**	**1.17**	**0.234**	**66.1**	**5.16**	**3.6**	**1.06**	**13.7**	**3.06**	**26.8**	**2.99**
P-value	**0.005**		**0.143**		**0.002**		**0.002**							

(p-values were calculated between foregut and hindgut fermenters with the Mann-Whitney test; SD = standard deviation, NDF_{seq} = Neutral detergent fibre analyzed sequentially (ADL-ash corrected), OM = organic matter, DM = dry matter)

Tab. 10: Allometric regressions for 24 h, 48 h and 96 h gas production (GP) and body mass (BM).

	Equation	95% CI	r^2	p-value
24 h GP and BM				
Foregut fermenters	**$2.02\ BM^{-0.10}$**	-0.40 - 0.20	0.0667	0.4713
Hindgut fermenters	**$2.36\ BM^{0.05}$**	-0.20 - 0.29	0.0474	0.6392
48 h GP and BM				
Foregut fermenters	**$15.24\ BM^{-0.22}$**	-0.48 - 0.03	0.3379	0.0780
Hindgut fermenters	**$11.48\ BM^{0.01}$**	-0.10 - 0.12	0.0074	0.8545
96 h GP and BM				
Foregut fermenters	**$22.96\ BM^{-0.13}$**	-0.31 - 0.05	0.2517	0.1396
Hindgut fermenters	**$24.04\ BM^{0.01}$**	-0.03 - 0.04	0.0260	0.7299

(With: formula for the regression line, 95% confidence interval for the exponent, the coefficient of determination (r^2) and the p-value)

Tab. 11: Relationship between 24 h, 48 h and 96 h gas production (GP) and metabolic faecal nitrogen (MFN).

	Equation	95% CI	r^2	p-value
24 h GP and MFN				
Foregut fermenters	**$1.30\ MFN^{-0.13}$**	-2.41 - 2.15	0.0022	0.8971
Hindgut fermenters	**$3.44\ MFN^{-0.60}$**	-2.40 - 1.21	0.1255	0.4357
48 h GP and MFN				
Foregut fermenters	**$2.61\ MFN^{0.42}$**	-1.85 - 2.68	0.0220	0.6824
Hindgut fermenters	**$12.53\ MFN^{-0.26}$**	-1.05 - 0.54	0.1192	0.4481
96 h GP and MFN				
Foregut fermenters	**$12.25\ MFN^{-0.09}$**	-1.64 - 1.47	0.0020	0.9024
Hindgut fermenters	**$25.30\ MFN^{-0.14}$**	-0.38 - 0.11	0.2881	0.2141

(With: formula for the regression line, 95% confidence interval for the exponent, the coefficient of determination (r^2) and the p-value)

3.5. Relation between gas production and $MRT_{particle}$

There is a significant relation between $MRT_{particle}$ and 48 h GP for the foregut fermenters, no significant relation was found for the hindgut fermenters (Tab. 12).

Tab. 12: Relationship between 48 h gas production (GP) and mean retention time for particles ($MRT_{particle}$) for foregut fermenters and 24h GP and $MRT_{particle}$ for hindgut fermenters.

	Equation	95% CI	r^2	p-value
48 h GP and $MRT_{particle}$				
Foregut fermenters	**$4.15\ MRT^{-2.00}$**	-3.37 - (-0.56)	0.6622	0.0140
24 h GP and $MRT_{particle}$				
Hindgut fermenters	**$1.13\ MRT^{-0.40}$**	-1.78 - 1.03	0.1207	0.4999

(With: formula for the regression line, 95% confidence interval for the exponent, the coefficient of determination (r^2) and the p-value)

4. Discussion

4.1. Method evaluation

The HGT was developed to estimate food quality for herbivores in vitro. In this study the HGT was used to estimate differences in fibre degradability between foregut and hindgut fermenters using their faecal fibre following the ideas of Prins et al. (1981). As mentioned in the introduction a high GP in the HGT indicates high proportions of undigested, but potentially digestible fibre left in the faecal NDF and therefore a lower digestion of fibre realized by the animal. Time of fermentation is an important factor in fibre degradability. In chapter one the MRT for the animals of this study were estimated. Regarding the time for total clearance of the chromium mordanted fibres, which were used as particle passage marker (foregut fermenters = 172 h; hindgut fermenters = 97 h), it could be useful in further studies to elongate the duration of the in vitro digestion especially for the foregut fermenters samples maybe up to 240 h. It has to be kept in mind that digestion times of 172 h are probably not physiologically important for the animal; most of the digestible fibre will be digested much earlier. The longer HGT-trials with faecal NDF will mostly give additional information about the ingested food. On a time scale the GP measured with the HGT and the estimated MRT cannot be compared one-to-one. The HGT is an in vitro simulation of the fermentation chamber of an animal and the MRT was estimated in vivo for the whole gastrointestinal tract (GIT). For the in vitro method the food and faecal samples were ground trough a 1 mm sieve. Animals chew their food but they do not have such a homogenous ingesta in their fermentation chamber. This is one influencing factor and the fermentation of the in vitro samples might be faster than in vivo. Small particles (< 2 mm), like the chromium marker, left the fermentation chamber and the GIT of foregut and hindgut fermenters earlier than larger particles so MRT for smaller particles are always measured shorter. However the in vitro method is very useful to give indications of differences in fibre degradability between species but can be compared to the in vivo estimated MRT with some restrictions only. The long average MRT for particles for foregut fermenters (55 h, see chapter one Tab. 4) and especially the time of total marker excretion (172 h) indicates that for larger food particles digestion is still relevant after 96 h in the ruminant GIT. That digestible fibre was left in the faeces, which was degraded after 96 h of incubation, is shown for both digestion types by figure 8: No plateau in cumulative GP_{FNDF} was reached for faeces after the measured 96 h.

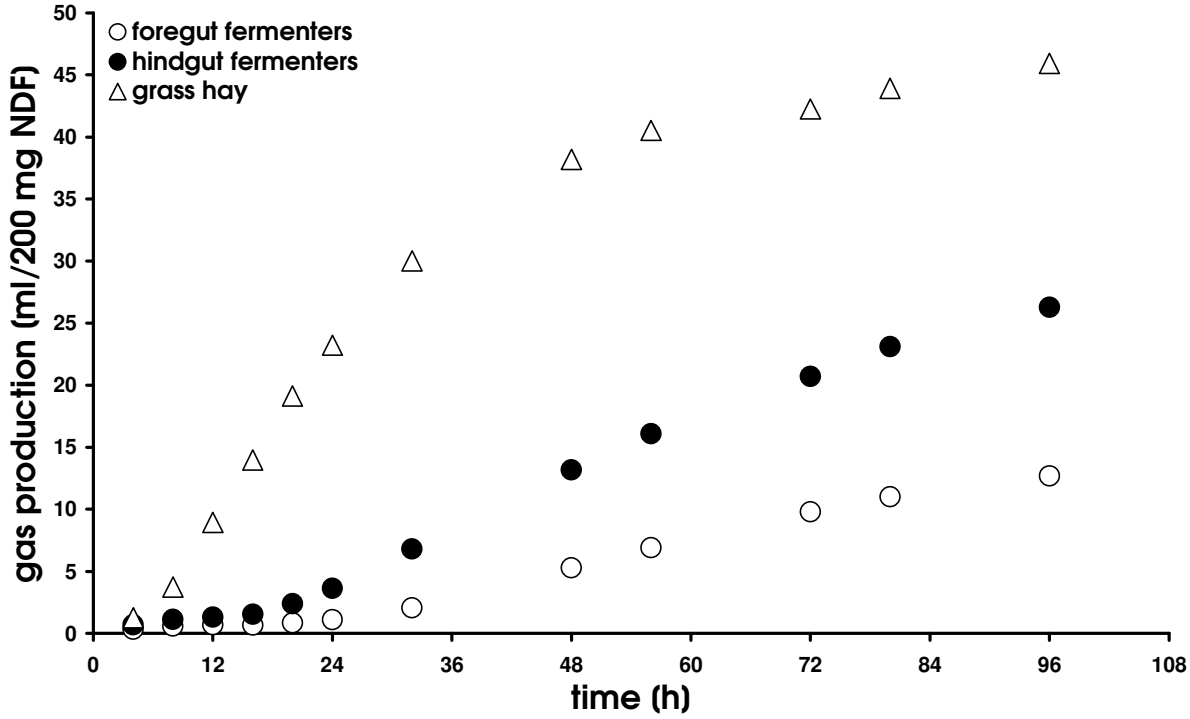

Fig. 8: Cumulative gas production [ml/200 mg NDF] for NDF of foregut and hindgut fermenter faeces and grass hay. (NDF = neutral detergent fibre)

For this reason a longer HGT-trial (200 h) was conducted with NDF residue samples of sheep (representative for foregut fermenters), Shetland ponies (representative for hindgut fermenters) and the hay the animals were fed with, to get information about the GP kinetics after 96 h (Fig. 9). 70% of the faecal NDF and 85% of the food NDF GP was covered with the 96 h HGT compared to the GP after 200 h. The animal-relevant first part of the GP-kinetic was covered with the 96 h HGT and so a comparison of both digestion types was feasible. Remarkable is the time of incubation where no further differences between foregut fermenters (sheep) and hindgut fermenters (pony) were measured. This was the fact after 56 h of incubation. Above this threshold the GP_{FNDF} was the same for the samples of sheep and pony. Before this time of incubation faecal samples of the pony had always higher GP_{FNDF} rates. The average $MRT_{particle}$ for sheep was 54 h (chapter one). Even though it was not possible compare MRT and the time scale of the GP directly, this fact is interesting and shows that it is possible to get accurate results also for the time response of fibre degradation. The comparison of the GP_{FNDF} and GP_{GNDF} values shows that both groups digested most of the available NDF fraction up to 24 h of fermentation (Fig. 7). As a result for the first two intervals no significant differences between foregut and hindgut fermenters were measured (0-8 h, $p = 0.5912$; 8-16 h, $p = 0.4182$) (Fig. 7).

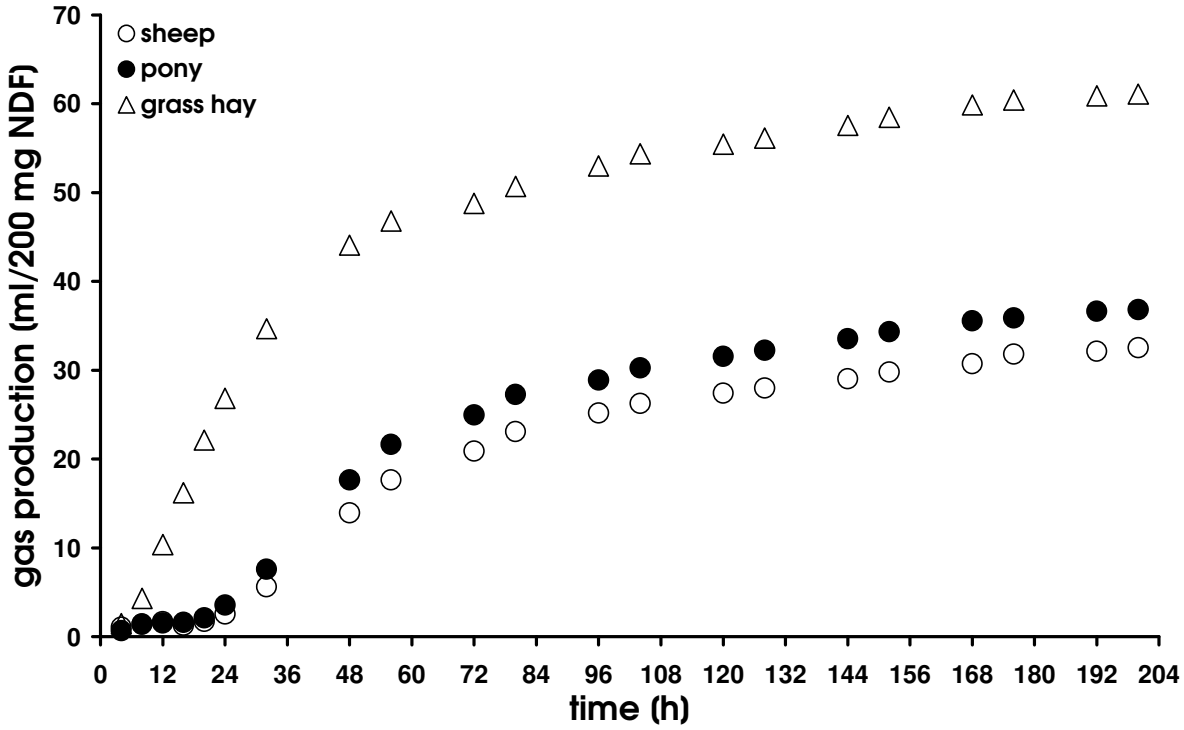

Fig. 9: Cumulative gas production [ml/200 mg NDF] for NDF of foregut (sheep) and hindgut fermenters (Shetland pony) faeces and of grass hay. (NDF = neutral detergent fibre)

After 16 h of incubation foregut fermenters samples always had significantly lower GP than hindgut fermenters samples until the HGT was stopped after 96 h (16-24 h, p = 0.0015; 24-48 h, p = 0.0001; 48-72 h, p = 0.0001, 72-96 h, p = 0.0002). This indicates a better fibre degradation in foregut than in hindgut fermenters. The faecal NDF_{seq}-values support this. The animals were fed with grass hay which was not significantly different in the NDF_{seq}-content and hindgut fermenters showed higher faecal NDF_{seq}-contents than foregut fermenters. So a better NDF degradability in foregut than in hindgut fermenters can be assumed. These findings are in accordance with the results of other studies which compared foregut and hindgut fermenters regarding their digestive capacity (Duncan et al. 1990; Menard et al. 2002; Sponheimer et al. 2003; Pearson et al. 2006). It was stated that hindgut fermenters generally had higher food intakes than foregut fermenters, shorter MRT and hence a lower fibre digestion. Because of the close relationship between fibre digestion and MRT as well as the supposed relation of BM and MRT (which was not confirmed in chapter one), the relation between BM and GP was evaluated. No significant relationship was found which corresponds with the non existing relation between MRT and BM found in chapter one, only for the foregut fermenters there is a trend between the 48 h GP and body mass (Tab. 9).

4.2. Differences in faecal NDF composition

The ADF content in the NDF fraction was significantly higher in the faeces ($p < 0.0001$; data not shown) than in the food of the animals, but between foregut and hindgut fermenters there were no significant differences ($p = 0.4747$). This indicates that foregut and hindgut fermenters digest NDF and ADF to the same relation, both fractions were reduced equally, but not to the same degree. Ruminants had a higher ADL content in the faecal NDF ($p = 0.0020$), accordingly they digest more of the NDF and ADF fraction than the hindgut fermenters because they were fed with grass hay of equal ADL concentrations. This is in accordance with the HGT results. The significantly higher GP of the faecal NDF of the hindgut fermenters during the HGT showed that the foregut fermenters digest more of the digestible fibre. The food NDF contains the complete potentially digestible fibre fraction, faeces contains only the rest of the fibre fraction which was hardly digestible or indigestible. However to evaluate differences in NDF degradability between foregut and hindgut fermenters this data is helpful.

4.3. Faecal nitrogen and mean retention time

In this study also the faecal nitrogen content was measured. According to the fact that high nitrogen contents in faeces are related to a high microbial growth in the fermentation chamber of the host animal (= large fermentation capacity) it was hypothesized that faecal nitrogen contents were negatively related to the GP_{FNDF}. The higher MFN values of foregut compared to hindgut fermenters are based on the higher microbial growth in their fermentation chamber. The relation between the MFN and the cumulative GP_{FNDF} after 24, 48 and 96 h of the foregut and hindgut fermenters was not significant (Tab. 11), so the hypothesized relationship between GP_{FNDF} values and MFN contents could not be affirmed in this study. Comparing the $MRT_{particle}$ (chapter one) and the 48 h GP_{FNDF} of foregut fermenters, no significant negative relation between these two parameters was observed but the p value indicated a trend to decreasing GP_{FNDF} with increasing $MRT_{particle}$ for foregut fermenters (Tab. 12). In hindgut fermenters no relation of $MRT_{particle}$ and 24 GP_{FNDF} was found (Tab. 12). In this case the 24 h GP_{FNDF} was chosen, because of average MRT of 34 h and the 24 h GP_{FNDF} were more related to the MRT than the 48 h GP_{FNDF} used for foregut fermenters with an average MRT of 54 h.

4.4. Apparent digestibilities

For some of the farm and zoo animals food intake and total faecal output could be recorded. Accordingly it was possible to calculate the dry matter aD with these two parameters. With the knowledge about the NDF contents of the food and faeces the NDF aD was calculated. Reliable results for domestic sheep, domestic goats, domestic cattle, Shetland ponies and two

of the domestic horses were generated. Also for two of the white rhinoceroses and for two of the African elephants values for dry matter aD were calculated (Tab. 13). The resultant dry matter aD were in accordance with the findings of the HGT trials that the foregut fermenters always had higher aD than the hindgut fermenters. Also for the NDF digestibility differences were found. Most of the dry matter aD calculated in this study were lower than in other studies (Tab. 13) which is in agreement with the quality of the grass hay used in this study. For some of the animals the low nitrogen content in the food was at the lower end of food quality, comparable with the food the animals find at the end of the dry season in the wild.

Tab. 13: Apparent dry matter digestibility (aD DM) [%] and neutral detergent fibre (NDF) aD [%] of some of the sampled species (± SD)

	aD DM [%]	aD NDF	food	Source
Domestic goat	47 ± 4	50 ± 5	grass hay	this study
	61 ± 7	-	grass hay	Sponheimer et al. (2003)
	59	60	grass hay	Gihad (1976)
Domestic sheep	48 ± 3	50 ± 9	grass hay	this study
	59	57	grass hay	Gihad (1976)
	57	66	grass hay	Pearson et al. (2006)
Domestic cattle	63	71	grass hay	this study
	63	70	grass hay	Pearson et al. (2006)
Shetland pony	37 ± 3	38 ± 9	grass hay	this study
	43	47	oat straw	Pearson et al. (2001)
	58	38	alfalfa hay	Pearson et al. (2001)
	53	60	grass hay	Pearson et al. (2006)
	59	43	alfalfa	Cuddeford et al. (1995)
	46	47	oat straw	Cuddeford et al. (1995)
Domestic horse	46 (54/37)	30 (40/20)	grass hay	this study
	44 ± 11	-	grass hay	Sponheimer et al. (2003)
	51	41	alfalfa cubes + alfalfa-grass hay	Pagan et al. (1998)
	69	44	alfalfa	Cuddeford et al. (1995)
	48	41	oat straw	Cuddeford et al. (1995)
African elephant	28/25	-	grass hay	this study
	45	43	grass hay	Foose (1982)
	39	35	timothy hay	Roehrs et al. (1989)
White rhinoceros	34/44	-	grass hay	this study
	45	-	grass hay	Steuer et al. (2010)
	51	48	grass hay	Foose (1982)

(DM = dry matter, SD = standard deviation)

It was possible to calculate the organic matter aD by using the 24 h GP, the crude protein content and the ash content of the offered food (Menke and Huss 1987) by using the calculation:

$$aD\ OM\ [\%] = 0.889\ GP_{24h}\ [ml / 200mg\ DM] + 0.0448\ CP\ [g / kg\ DM] + 0.0651\ ash\ [g / kg\ DM] + 14.88\ .$$

An organic matter aD of 53% for cattle, 56% for sheep and 53% for goats was calculated with this formula. For goats (46%) and sheep (50%) lower organic matter digestibilities were estimated in this study than calculated with the formula of Menke and Huss (1987). This

might be based on the fact that their formula was generated with roughage of higher digestibility. The calculated organic matter aD was lower than the estimated organic matter aD for cattle in this study (63%). But the cattle had very long MRT in this study (75 h see chapter 1, Tab. 4). Pearson et al. (2006) got comparable digestibilities for dry matter and the NDF fraction when feeding cattle with a 100% grass hay ration.

It was also used an equation of Lukas et al. (2005) (OM digestibility, % = $72.86\text{-}107.7^{(-0.0151 * \text{faecal CP, g/kg OM})}$) for estimating the aD of OM of the foregut fermenters in this study. Therefore the faecal nitrogen values in fresh faeces (Kjeldahl method) (see appendix, Tab. 14) were used. No significant differences between the nitrogen contents measured with the Dumas and Kjeldahl method (for foregut fermenters p = 0.6225, for the hindgut fermenters p = 0.6089) were found. An equation of Mésochina et al. (1998) (OM digestibility, % = 0.734-(17.872 / faecal CP, g /kg OM) was used for the estimation of the aD of OM for the hindgut fermenters in this study (Tab. 13). For goats and sheep comparable digestibilities were calculated with the formula of Lukas et al. (2005) which were comparable to those estimated in this study (48 ± 1.7 % OM for goats (46 ± 4.2 % OM in this study) and 51 ± 5.1 % OM for sheep (50 ± 2.9 % OM in this study). Lower aD were calculated for cattle with 50 ± 3.1 % OM (63 ± 3.5 % OM). The aD for the hindgut fermenters calculated with the formula of Mésochina et al. (1998) were always higher than those estimated in this study. This could also be caused by the fact that the grass hay fed in this study was probably of lower quality than that used in the study of Mésochina et al (1998) to generate the formula.

4.5. The "special" white rhinoceros

As shown in a previous study (Steuer et al. 2010) and chapter one, white rhinoceroses had low dry matter intakes and long MRT compared to other hindgut fermenters. Because of this it was expected that they also differ in 96 h GP_{FNDF} in HGT-results, but this was not the case. The white rhinos (mean 96 h GP_{FNDF}: 25.6 ± 2.97 [ml/200 mg DM]) did not have significantly lower 96 h GP_{FNDF} values than for example the equids (mean 96 h GP_{FNDF}: 25.4 ± 1.30 [ml/200 mg DM]; p = 0.8489) or the African elephants (mean 96 h GP_{FNDF}: 27.4 ± 1.83 [ml/200 mg DM]; p = 0.2518) in this study. Maybe their low defecation rate of 2-3 times per day (horse: 6-8 times, African elephant: 8-10 time; pers. observation) and potentially long retention in the rectum were some reasons for the long MRT. No further digestion takes place in the rectum (Van Soest 1994) accordingly the HGT results did not differ to the other hindgut fermenters. Rhinoceroses use defecation places which are used for information exchange between animals and groups. Potentially they store their faeces for such places and the retention of faeces in the rectum is determined by social factors.

5. Conclusions

- The results of this study gave evidence for differences in fibre digestion capacity between foregut and hindgut fermenters. The low GP_{FNDF} indicated more comprehensive NDF digestion in foregut than in hindgut fermenters.
- There were no significant influences measured for BM on GP.
- No relation between the MFN and the HGT GP_{FNDF} (24 h, 48 h and 96 h) was found; there are indices for a negative effect of long MRT on the HGT GP_{FNDF} for foregut fermenters but not for hindgut fermenters.

Appendix

Tab. 14: Faecal crude protein (CP) for the all animals in this study measured with Dumas-method (dried material) and with the Kjeldahl-method (fresh material) (± standard deviation (SD) or both individual values when n = 2)

	CP	SD	CP	SD
			[% OM]	
	Dumas-method		**Kjeldahl-method**	
Foregut fermenters				
Springbok	14.7	13.9/15.5	13.5	12.7/14.4
Domestic goat	9.8	0.48	9.1	1.01
Domestic sheep	10.8	1.70	11.1	1.89
Blue wildebeest	13.6	1.21	13.3	1.34
Oryx antelope	12.8	0.47	12.1	1.29
Sable antelope	13.1	1.71	14.1	2.64
Waterbuck	13.3	12.2/14.4	13.0	11.9/14.1
Forest buffalo	13.3	12.9/13.6	12.3	12.3/12.4
Bactrian camel	12.8	0.80	12.9	0.31
Domestic cattle	10.2	0.88	9.0	0.63
Mean	**12.4**	**1.62**	**12.1**	**1.76**
Hindgut fermenters				
Warthog	8.4		9.2	
Shetland pony	8.8	0.48	7.9	0.97
Przewalski horse	9.3	0.29	7.7	0.64
Grevy´s zebra	8.7	0.69	10.1	1.19
Domestic horse	7.8	0.75	7.6	0.89
White rhinoceros	12.8	1.45	11.4	1.36
African elephant	10.4	0.73	10.1	1.45
Mean	**9.5**	**1.70**	**9.1**	**1.47**

(OM = organic matter)

Chapter 3

Allometry of diet quality - quantification via faecal nitrogen and faecal neutral detergent fibre

Abstract

As a non-invasive method to estimate the quality of food of free ranging herbivores analyzing the faecal nitrogen is often used. It makes an estimation of the influence of the body mass on food choice and diet quality feasible. In this study faecal samples from 19 species of free ranging animals in a private sanctuary for wild animals in Northern Kenya were collected at the end of the dry season. The allocation to different feeding groups (5 grass feeding ruminants (n = 1 - 10), 7 browse feeding ruminants (n = 4 - 13), 4 grass feeding hindgut fermenters (n = 10 - 12), 1 browse feeding hindgut fermenter (n = 10), 1 mixed feeding hindgut fermenter (n = 11) and one mixed feeding ruminant (n = 10)) follows the relation of browse to grass in their diet estimated via C-isotopes (< 20% browse in the diet = grazers, < 20% grass in the diet = browsers, > 20% browse and > 20% grass in the diet = mixed feeders) and their kind of digestive system (ruminants/hindgut fermenters). Faecal samples were analyzed for nitrogen and neutral detergent fibre (FNDF) content. The total faecal nitrogen (TFN) was splitted in two different fractions: the neutral detergent insoluble nitrogen (NDIN) and the metabolic faecal nitrogen (MFN). For TFN, MFN and FNDF, at least a trend of decreasing diet quality with BM was found in all cases, with allometric scaling exponents significantly different from 0 for MFN in ruminants and TFN in hindgut fermenters, the latter even irrespective of the limited sample size (n = 6). These results give strong indications for the inability of larger herbivorous ungulates to feed selectively on high quality forage.

1. Introduction

In the course of evolution different terrestrial herbivores developed very high body masses (BM), like e.g. sauropod dinosaurs in Mesozoic or elephants, rhinos, hippos and large ruminants in extant ecosystems. While in general, an increase in BM is seen as evolutionary advantage and has been a regular observation in systematic lineages (reviewed by Alroy 1998), feeding on a low trophic level apparently facilitates large BM (see general introduction of the thesis for further information). For herbivores, particular advantages of an increase in BM on digestive capacity have been postulated (Bell 1971; Demment and Van Soest 1985). However, any increase in BM may also result in additional constraints for herbivores (Clauss and Hummel 2005), e.g. in terms of food quality. Obviously the amount of food an animal and its population require increases with BM (Owen-Smith 1988), and available biomass of plant food is negatively correlated to its nutritional quality (Owen-Smith and Novellie 1982; Demment 1983; Demment and Van Soest 1985). While this alone implies a tendency for the food of large animals to shift towards lower quality, an additional decrease in selective capacity with BM can be expected due to increasing muzzle width (Jarman 1974). In fact, the latter variable has been shown repeatedly to increase with BM (Tab. 15), mostly close to isometric scaling ($BM^{0.33}$). While organs like prehensile lips (e.g. black rhino, horse), tongues (e.g. giraffe) or a trunk (elephants) may partly overcome this limitation, any herbivore will finally end up in a trade-off between being selective and realising sufficient intake. In fact a tendency of foraging time to increase with BM has been found for large herbivores (Owen-Smith 1988) (Foraging budget [%] = 24.2 $BM^{0.12}$). Among the longest daily foraging times are probably those of African elephants with up to 20 hours (Wyatt and Eltringham 1973). While another data collection for ungulates did not arrive at the conclusion of an increase of foraging time with BM (Bunnell and Gillingham 1985), it stated that there is a decrease of foraging time per food unit with BM – which also implies less selectivity in large animals.

Tab. 15: Relation of body mass (BM) [kg] to muzzle width and incisor breadth [mm]

Muzzle width [mm] = 4.4 $BM^{0.46}$	n = 27 (ungulates)	(Christiansen 1999)
Muzzle width [mm] ~ $BM^{0.34}$	n = 12 (ungulates)	(Paul 1998)
Incisor breadth [mm] = 8.6 $BM^{0.36}$	n = 32 (ruminants)	(Illius and Gordon 1987)
Incisor breadth [mm] = 6.36 $BM^{0.40}$	n = 89 (ruminants)	(Gordon and Illius 1988)

In consequence, these factors should lead to an overall decrease of diet quality with BM (Demment and Van Soest 1985), e.g. in terms of fibrousness or for browsing animals also in

terms of the ingested amounts of secondary plant compounds (van Hoven and Furstenburg 1992; Van Soest 1994), both resulting in a lower digestibility of the diet.

Based on theoretical considerations (relation of gut capacity and energy requirements), the acceptable diet quality for herbivores in terms of digestibility was estimated to scale to $BM^{-0.1}$ (Owen-Smith 1988) or $BM^{-0.14}$ (Illius and Gordon 1999). Any empirical test of the existence of a negative correlation of BM and diet quality is demanding. The major challenge in this context is the collection of a sample representative for the food selected by a free-ranging herbivore. Simulating animal feeding preferences via continuous observation and hand clipping of representative samples is an option, but does not exclude subjective selection by the researcher entirely, is very time consuming and impossible for animals with a large flight distance. Estimations of different food quality variables from gut contents have been made for ruminants (crude protein (CP) in rumen [%] = 21.6 $BM^{-0.23}$, n = 9 (Owen-Smith 1988); ruminal fermentation rate [mol VFA/kg DM*d] = 945 $BM^{-0.22}$, n = 11 (Hoppe 1977)) or ungulates (non-stem material in GIT [%] = 116 $BM^{-0.118}$, n = 21 (Owen-Smith 1988)).

Quantifications of diet quality are mostly done during evaluations of habitat quality, e.g. in reintroduction programs (Leslie et al. 1989; Grant et al. 1995; Bleich et al. 1997). Therefore a non invasive procedure not relying on oesophagally fistulated animals or gut contents of shot animals and which can be conducted with limited efforts would be desirable. The concept to use the nitrogen content of animal faeces to estimate the quality of their food was already formed by Lancaster (1949). Following his ideas several studies used faecal nitrogen as a proxy for the quality of the ingested food (Bredon et al. 1963; Sinclair 1977; Mould and Robbins 1981; Sinclair et al. 1982; Loeb and Schwab 1989; Howery and Pfister 1990; Leite and Stuth 1990; Bleich et al. 1997; Robinson et al. 2001; Chapman et al. 2005).

While several studies, dominantly on wildlife, interpreted and used faecal nitrogen as an estimation of dietary nitrogen content (Erasmus et al. 1978; Mould and Robbins 1981; Leslie and Starkey 1985; 1987; Leite and Stuth 1990; Irwin et al. 1993; Kamler and Homolka 2005; Woolley et al. 2009), many other studies aimed to estimate digestibility of the diet via this variable (Lancaster 1949; Lambourne and Reardon 1963; Wallace and Van Dyne 1970; Wilson et al. 1971; Hofmann and Musangi 1973; Scales et al. 1974; Holloway et al. 1981; Leslie and Starkey 1985; Bartiaux-Thill and Oger 1986; Schmidt and Jentsch 1994; Wehausen 1995; Mésochina et al. 1998; Robinson et al. 2001; Boval et al. 2003; Lukas et al. 2005; Wang et al. 2009). The latter approach is based on the fact that with increasing digestibility of the diet, microbial growth and in consequence also the proportion of undigested microbial nitrogen in faeces is increased, while at the same time the proportion of

undigested food residues decreases (Schlecht and Susenbeth 2006). While most studies were conducted with ruminants, there are also some studies in which faecal nitrogen was determined as a good indicator for estimating food digestibility in equids (Vander Noot and Trout 1971; Chenost and Martin-Rosset 1985; Chenost 1986; Mésochina et al. 1998).

Microbial growth (and therefore faecal-N content) can be influenced by both nitrogen and energy supply to microbes in the digestive tract and therefore the respective concentrations in the diet. The limitation by energy can be considered to be relevant on pastures of all qualities, while dietary nitrogen will have a significant influence on microbial growth mainly at nitrogen levels low enough to limit growth. So the microbial growth in the fermentation chamber is regarded as determined dominantly by the food factor energy digestibility (Lukas et al. 2005), closely related to organic matter digestibility in vegetative plant material.

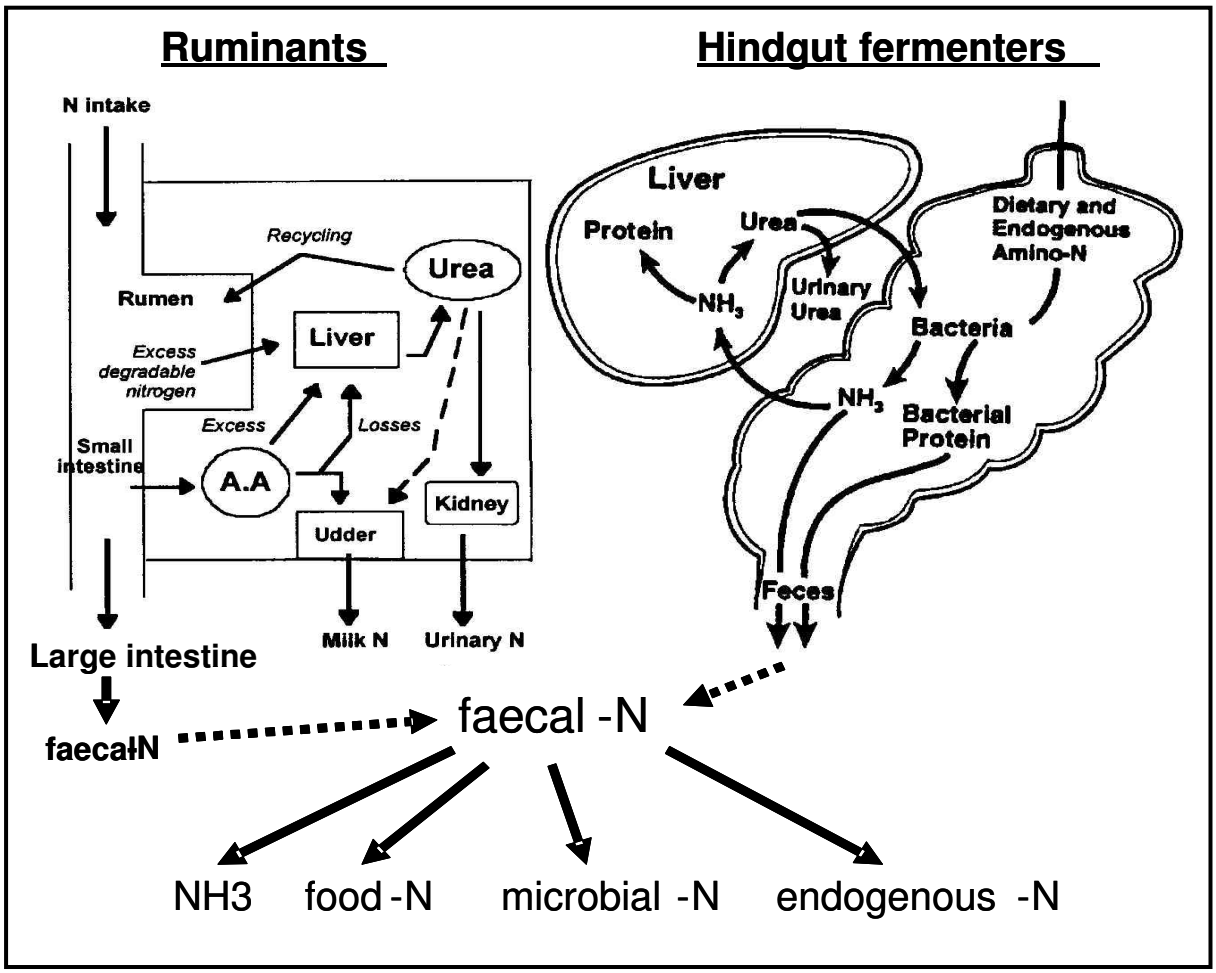

Fig. 10: Modified model of the nitrogen cycle of ruminants according to Vérité and Delaby (2000) (left) and the nitrogen cycle in the hindgut of hindgut fermenters according to Stevens and Hume (1998) (right).

Most of the nitrogen which can be measured in faeces of animals is based on gut microbes (Mason 1969; Stevens and Hume 1998) (Fig. 10). In ruminants, the majority of this microbial

N is considered to be undigested microbial cell wall stemming from microbial growth in the rumen while in hindgut fermenters most microbial N can be considered to be whole microbial cells probably (Van Soest 1994). Besides this faecal nitrogen also consists of ammonia originating from microbial activity in the colon of an animal (Mason 1969; Van Soest 1994) and also has some endogenous part (gut enzymes, mucus, sloughed cells). The endogenous part is related to food intake and rather constant per food unit (Lukas et al. 2005). These fractions (microbial-N, ammonia, endogenous-N) are referred to as metabolic faecal nitrogen (MFN). The further faecal-N represents the comparatively insoluble part (e.g. insoluble in neutral detergent (ND) solution), is often considerably smaller and origins from undigested plant material (Mason 1969; Ørskov et al. 1969). For browsing animals also tannin-protein complexes are included in this fraction (Van Soest 1994).

While faecal-N has often been applied successfully as a measure of diet quality, critics like Hobbs (1987) mention various dietary and environmental influences as limitations of this technique. Hobbs (1987) concluded that the faecal-N technique does not offer results which were reliable enough to give estimations on food quality of herbivores. Leslie et al. (2008) criticized non-discrimination of different digestive systems, different habitats and different seasons and stated that with these crossover comparisons no substantial results are possible. Loeb and Schwab (1989) also concluded from their study that faecal nitrogen is not a good predictor for organic matter digestibility, but found a positive correlation between faecal and dietary nitrogen. To estimate food digestibility with the nitrogen content of faeces apparently can be problematic for animal having considerable amounts of browse in their diet. Browse contains more crude protein than grass (Hummel et al. 2006), but also significant amounts of secondary plant compounds like tannins, which can build complexes with food protein and cause some flow of undigested, tannin bound crude protein into faeces (Chapman et al. 2005). This results in a higher crude protein content in the faeces not correlated at all with higher microbial growth (Mould and Robbins 1981; Robbins et al. 1987; Osbourn and Ginnett 2001). Consequently some studies stated that digestibility estimations from faecal nitrogen were difficult or not possible for browsers (Holechek et al. 1982; Wofford et al. 1985; Hodgman et al. 1996; Kucera 1997; Schlecht and Susenbeth 2006).

Another faecal factor giving information on food quality is faecal NDF (FNDF). The FNDF content could be used as an index of hardly or indigestible fibre in the food. Large amounts of high fibre contents in diets will also cause high FNDF values because of the inability of the animal to digest large amounts of lignified fibre (Van Soest 1994).

Aims of this chapter

In this chapter the diet of different African herbivores will be ranked according to several faecal variables, reflecting diet quality and selectivity of the respective animals. Besides total faecal nitrogen (TFN) a fractionation into neutral detergent insoluble nitrogen (NDIN), including dominantly unavailable nitrogen like cell wall bound nitrogen or tannin-protein complexes (Van Soest 1994), and in metabolic faecal nitrogen (MFN) is done. The latter should be influenced much less by tannins, and reflect most directly microbial growth in the digestive tract.

It is hypothesised that:

1. TFN and MFN decrease with BM
2. NDIN and FNDF increase with BM
3. NDIN is higher in browsing than in grazing animals

2. Materials and Methods

2.1. Animals and sampling

Faecal samples from 19 species of free ranging animals (animals per species = 1 - 13, total = 176 faecal samples) were collected at the end of the dry season in a private sanctuary for wild animals (Lewa Wildlife Conservancy) in Northern Kenya. The animals were observed and faecal samples were collected immediately after dropping. Samples were dried in a well ventilated tent placed in the shade.

The BM of all animals was estimated using literature data (Robinette 1963; Ledger 1968; Mason 1985; Owen-Smith 1988; Estes 1991; Grand 1997). The different species could be separated in six groups: 5 grass feeding ruminants (n = 1 - 10), 4 grass feeding hindgut fermenters (n = 10 - 12), 7 browse feeding ruminants (n = 4 - 13), 1 browse feeding hindgut fermenter (n = 10), 1 mixed feeding ruminant (n = 10) and 1 mixed feeding hindgut fermenter (n = 11) (Tab. 16). Differentiation was done according to the faecal isotope signature (Fig. 11). Because of the importance of the diet composition (influence of tannins) in this study the proportion of browse in the diet of the animals was estimated with the $^{12}C/^{13}C$ isotope relation in the faeces of free ranging animals (Codron 2006; Codron et al. 2007a; Codron et al. 2007b; Codron and Codron 2009). The latter studies showed that it is possible to estimate the relation of C_3- (browse) and C_4-plants (grass) of the food the animal ingested recently (~ last two weeks of feeding) from faecal isotope signature.

Tab. 16 : Number (n) and body mass of the free ranging animals (Northern Kenya); species were attributed to feeding groups according to the faecal isotope signature; body mass of

species was estimated according to literature data (Robinette 1963; Ledger 1968; Mason 1985; Owen-Smith 1988; Estes 1991; Grand 1997)

		n	Body mass [kg]
Grass feeding ruminants			
Sitatunga	(*Tragelaphus spekii)*	3	80
Hartebeest	(*Alcelaphus buselaphus)*	1	130
Oryx antelope	*(Oryx gazella)*	10	170
Waterbuck	(*Kobus ellipsiprymnus)*	8	215
African buffalo	(*Syncerus caffer)*	10	630
Mixed feeding ruminants			
Impala	(*Aepyceros melampus)*	10	50
Browse feeding ruminants			
Klipspringer	(*Oreotragus oreotragus)*	9	12
Gerenuk	(*Litocranius walleri)*	4	40
Bushbuck	(*Tragelaphus scriptus)*	12	60
Grant's gazelle	(*Nanger granti)*	13	65
Greater kudu	(*Tragelaphus strepsiceros)*	10	200
Eland	(*Taurotragus oryx)*	12	500
Giraffe	(*Giraffa camelopardalis)*	9	850
Grass feeding hindgut fermenters			
Warthog	(*Phacochoerus africanus)*	11	73
Plains zebra	(*Equus quagga)*	12	230
Grevy´s zebra	(*Equus grevyi)*	11	410
White rhinoceros	*(Ceratotherium simum)*	10	1900
Mixed feeding hindgut fermenters			
African elephant	(*Loxodonta africana)*	11	4000
Browse feeding hindgut fermenters			
Black rhinoceros	*(Diceros bicornis)*	10	1000

(n = number of faecal samples per species)

The powdered faecal samples were combusted in an automated Elemental Analyser (NC 2500) and the resultant CO_2 gas was measured in a Thermo Quest Delta + XL mass spectrometer (Finnigan, Bremen). For grass feeding animals the range of the $\delta^{13}C$ values was between -16.03 up to -14.22‰ VPDB (‰ Vienna-Pee-Dee Belemnit (VPDB); the PDB standard is fossile calciumcarbonate from South Carolina, USA. Its isotope relation ($^{13}C/^{12}C$) is 0.0112372. This value is the reference point for an international PDB-scale for the $\delta^{13}C$-values expressed as parts per mille ($^{0}/_{00}$)). For browse feeding animals the values were between -26.89 and -22.57‰ VPDB (Fig. 11). As expected there was a significant difference between the V-PDB values of the grazing and the browsing animals ($p = 0.0008$). A regression line was calculated with the Grevy´s zebra as a 100% grazer and the bushbuck as a 100% browser. Along this regression line the rest of the animal species were arranged according to their $\delta^{13}C$ values.

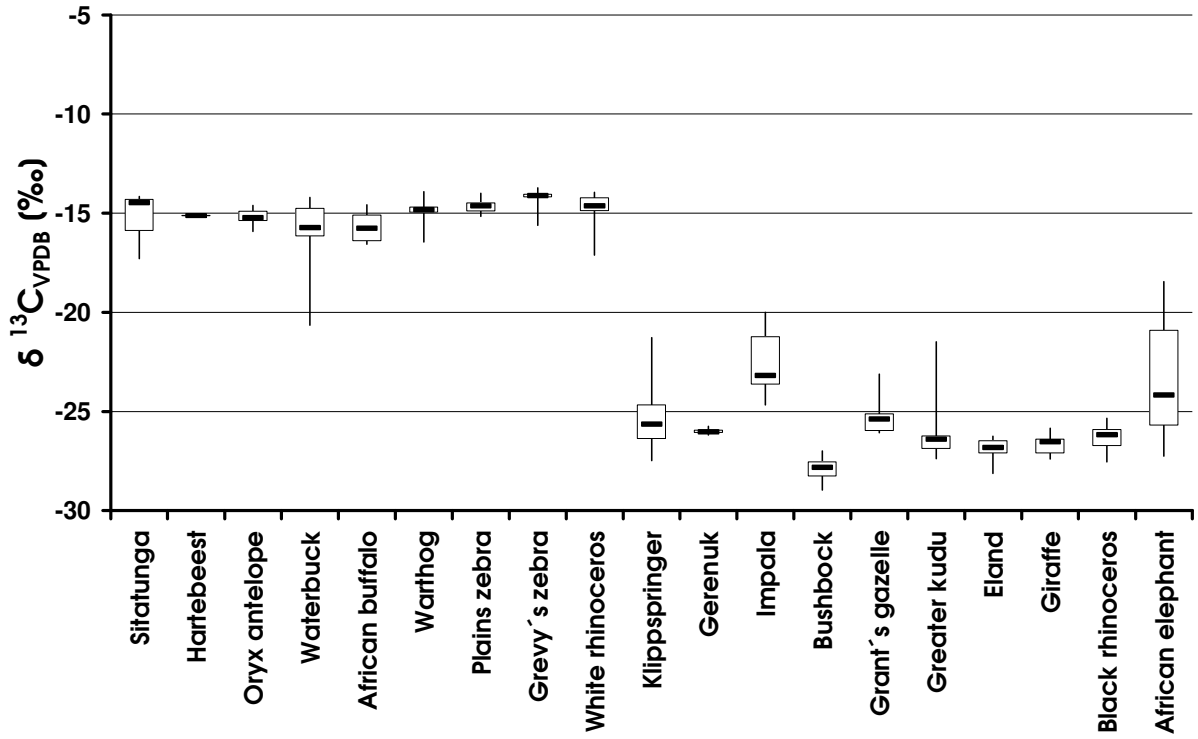

Fig. 11 : Differentiation of animal species according to their faecal $\delta^{13}C$ values [‰]. The median for each species is represented by the black line within the boxes. The upper and lower limits of the boxes are the upper and lower quartile. The minimum and the maximum of each species are represented by the whiskers. (VPDB = Vienna-Pee-Dee Belemnit)

The classification of the animal species follows the relation of browse to grass in their diet during the sampling period (> 80% grass in the diet = grazers, > 80% browse in the diet = browsers, < 80% grass and < 80% browse in the diet = mixed feeders) and their kind of digestive system. The sitatunga is described as a mixed feeder with a tendency for C4-plants in the wild (68% grass in diet (Gagnon and Chew 2000)), but according to the measured $\delta^{13}C$ values in their faeces they fed almost exclusively on C_4-plants (for example reed) during the sample period. Therefore it was allocated to the grass feeding ruminants. The impala and the African elephant were characterised as mixed feeders (39% C_4-plants in the diet of the impalas and 34% in the diet of the African elephants). For the evaluation of a relation between MFN and the BM of the two animal groups (ruminants and hindgut fermenters) data of both animal species were included. With the isotope signature it was possible to detect the assumed dietary shift of mixed feeders towards browse at the end of the dry season. For example the eland is described in literature as a mixed feeder during the wet season but the isotope signature in this study indicated a nearly pure browse diet (93% browse in diet) for this species.

2.2. Chemical analysis

The air dried samples were ground through a 1 mm sieve and ash was measured (combustion at 550 °C in a muffle furnace). Faecal neutral detergent fibre (FNDF) (expressed as NDF-ash corrected value) was analyzed according to Van Soest et al. (1991) by using the fibre bag system (C. Gerhardt GmbH & Co. KG, Koenigswinter, Germany). To estimate the nitrogen content which was unavailable/indigestible for the animal and its microbes, samples were boiled with neutral detergent (ND) solution according to Mason (1969) and the nitrogen content was analysed in the residue.

This allows differentiation of three faecal nitrogen fractions:

1. Total faecal nitrogen (TFN): The complete nitrogen content in the faeces is covered in this fraction (this is identical to the faecal crude protein in chapter 2).
2. Neutral detergent insoluble nitrogen (NDIN): ND-washed faecal samples contain the unavailable food nitrogen. For browsing animals most of the tannin-protein complexes are recovered in this fraction (Van Soest 1994).
3. Metabolic faecal nitrogen (MFN): This nitrogen fraction is calculated by subtraction of the NDIN value from TFN, following an approach of Van Soest (1994). In this fraction is included: microbial debris (main part of the fraction), sloughed-off animal cells, mucus and gut enzymes.

Nitrogen was measured with the Dumas method (instrument: FP-328, Leco Inc., St. Joseph, Michigan, USA) for all three fractions (TFN/NDIN/MFN). Nitrogen contents are given related to organic matter (OM); this can be regarded as common sense in studies on faecal-N as predictor of OM-digestibility due to the higher explanatory power of this value compared to a relation of N to dry matter (DM).

2.3. Statistics

Allometric regressions (linear regression of the logarithmic values) between BM (as the independent variable) and TFN, NDIN, MFN and FNDF were performed for both digestion types (hindgut fermenters and ruminants) separately. The resulting equations are always given with the 95% confidence intervals (CI) for the allometric exponent. For differences between hindgut fermenters and ruminants and between grazing and browsing animals for TFN, NDIN, MFN and FNDF values Mann-Whitney tests were conducted. All statistical calculations were performed with GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego, California, USA. The level of statistical significance was set at $p < 0.05$, while for $0.1 > p > 0.05$, differences are regarded as a trend.

3. Results

3.1. Faecal ash contents

The faecal ash contents of the free ranging animals are given in Tab. 17. In this study the ruminants had higher faecal ash contents than the hindgut fermenters ($p = 0.0225$).

3.2. TFN

The TFN values for the grass feeding ruminants ranged from 1.68% OM (hartebeest) to 2.96% OM for the sitatunga, for grass feeding hindgut fermenters the range was from 1.12% OM (white rhinoceros) to 1.82% OM (warthog). For browse feeding ruminants the range for TFN was 2.12% OM (eland) to 3.78% OM (gerenuk), for the black rhinoceros as the only browse feeding hindgut fermenter the value was 1.19% OM. The TFN values of the browse feeding ruminants were significantly higher than those of the grass feeding ruminants ($p = 0.0451$) and ruminants (all species) had significantly higher TFN values than hindgut fermenters (all species) ($p = 0.0014$) (Tab. 17).

3.3. NDIN

The NDIN values of grass feeding ruminants were between 0.37% OM for the hartebeest and 1.66% OM for the sitatunga. For grass feeding hindgut fermenters the range was 0.44% OM (white rhinoceros) to 0.76% OM (warthog). For browse feeding ruminants the NDIN values ranged between 0.99% OM (eland) and 2.08% OM (giraffe). Browse feeding ruminants had significantly higher NDIN values than grass feeding ruminants ($p = 0.0451$) and ruminants had significantly higher NDIN values than hindgut fermenters ($p = 0.0201$) (Tab. 17). Calculating the proportion of NDIN of the TFN ruminants there is no significant difference between these two groups ($p = 0.9650$) but grazers had a significantly lower NDIN proportion than browsers ($p = 0.0360$).

3.4. MFN

The range of MFN for grass feeding ruminants was 1.18% OM (waterbuck) up to 1.37% OM (oryx antelope). For browse feeding ruminants the MFN values ranged between 0.79% OM for the giraffe and 1.85% OM for Grant's gazelle. There were no significant differences between grass and browse feeding ruminants ($p = 0.8329$). Ruminants had significantly higher MFN values than hindgut fermenters ($p = 0.0014$) (Tab. 17).

Tab. 17: Total faecal nitrogen (TFN), neutral detergent insoluble nitrogen (NDIN), metabolic faecal nitrogen (MFN) in % organic matter (OM), faecal NDF (FNDF) and faecal ash of free ranging animals in % dry matter (DM)

	TFN [% OM]	SD	NDIN [% OM]	SD	MFN [% OM]	SD	FNDF [% DM]	SD	ash [% DM]	SD
Grass feeding ruminants										
Sitatunga	2.96	0.094	1.66	0.042	1.30	0.133	58.4	0.54	20.0	0.54
Hartebeest	1.68	-	0.37	-	1.31	-	36.4	-	16.0	-
Oryx antelope	1.77	0.114	0.39	0.043	1.37	0.125	39.8	4.11	23.9	1.49
Waterbuck	2.06	0.238	0.88	0.208	1.18	0.256	46.3	20.17	21.9	4.82
African buffalo	2.05	0.634	0.81	0.289	1.23	0.625	59.7	13.53	22.2	4.21
Mean	**2.10**	**0.506**	**0.82**	**0.523**	**1.28**	**0.075**	**45.6**	**10.27**	**20.8**	**3.02**
Mixed feeding ruminants										
Impala	2.77	0.220	1.12	0.106	1.65	0.195	50.6	5.00	18.1	1.91
Browse feeding ruminants										
Klipspringer	2.81	0.196	1.52	0.198	1.29	0.167	42.9	14.36	34.4	19.65
Gerenuk	3.78	0.218	2.01	0.123	1.76	0.272	53.4	3.86	15.9	2.20
Bushbuck	2.65	0.328	1.07	0.512	1.58	0.644	44.8	10.60	21.3	16.35
Grant's gazelle	2.99	0.574	1.14	0.405	1.85	0.365	41.9	10.07	27.0	10.48
Greater kudu	2.29	0.316	1.20	0.427	1.09	0.300	61.3	7.83	14.8	4.14
Eland	2.12	0.277	0.99	0.078	1.13	0.237	58.8	5.01	19.8	2.29
Giraffe	2.87	0.397	2.08	0.220	0.79	0.237	67.9	10.15	11.9	3.99
Mean	**2.79**	**0.498**	**1.39**	**0.432**	**1.39**	**0.376**	**52.7**	**9.40**	**20.4**	**7.27**
Grass feeding hindgut fermenters										
Warthog	1.82	0.181	0.76	0.121	1.06	0.185	59.2	3.45	18.8	2.15
Plains zebra	1.19	0.129	0.48	0.051	0.71	0.112	64.1	6.73	17.2	3.23
Grevy´s zebra	1.35	0.399	0.47	0.061	0.89	0.362	64.0	6.94	15.8	1.93
White rhinoceros	1.12	0.182	0.44	0.082	0.68	0.134	65.3	4.19	14.8	2.14
Mean	**1.37**	**0.315**	**0.54**	**0.149**	**0.84**	**0.176**	**63.2**	**2.68**	**16.7**	**1.74**
Mixed feeding hindgut fermenters										
African elephant	1.05	0.185	0.57	0.187	0.48	0.080	79.7	2.35	8.3	2.16
Browse feeding hindgut fermenters										
Black rhinoceros	1.19	0.261	0.73	0.149	0.46	0.189	79.8	3.54	7.1	0.58

(NDF = neutral detergent fibre NDF-ash corrected, SD = standard deviation)

3.5. Relation between TFN, NDIN, MFN and BM

There is no significant relation between TFN and BM for all ruminants, for all hindgut fermenters there is a significant decrease of TFN with increasing BM (Fig. 12). For the NDIN there is no significant relation for both groups. For the MFN there is a significant negative relation between BM and MFN for ruminants. For hindgut fermenters the relation between these two parameters only approached significance (Fig. 13) (Tab. 18).

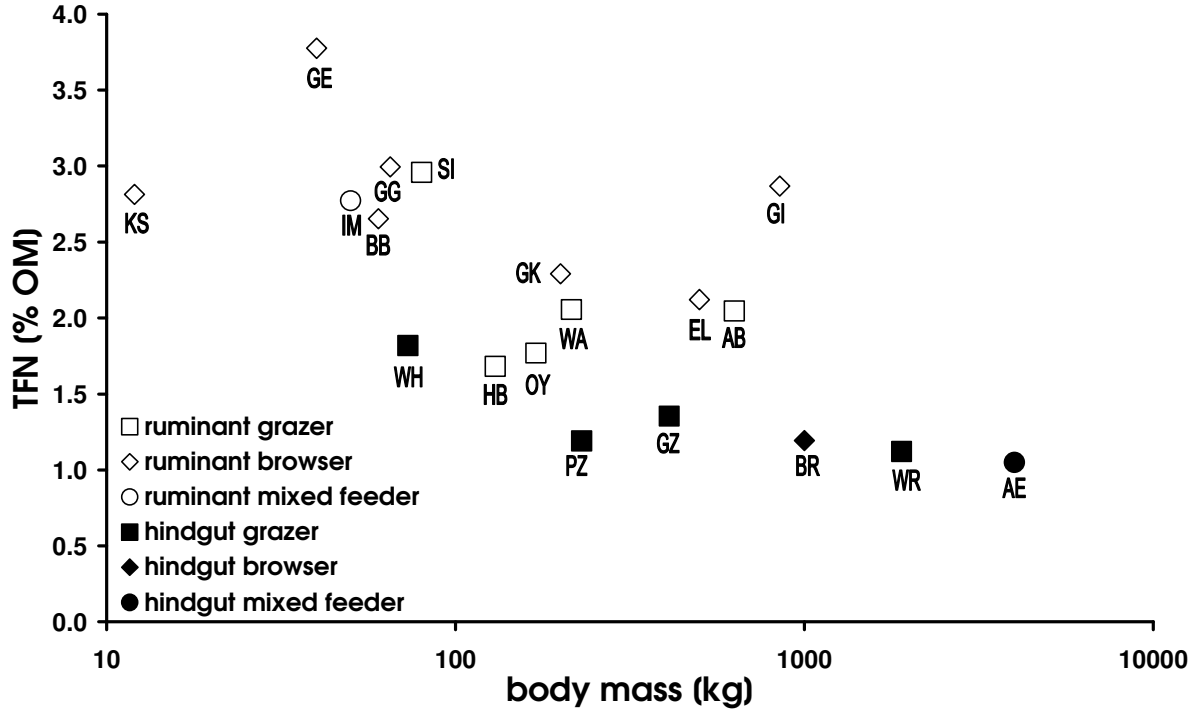

Fig. 12: Relation of total faecal nitrogen (TFN) and body mass (BM) of free ranging animals (OM = organic matter) *all ruminants:* TFN = 3.84 $BM^{-0.09}$, r^2 = 0.2252, p = 0.1013 that the exponent of BM was different from zero; *all hindgut fermenters:* TFN = 2.71 $BM^{-0.12}$, r^2 = 0.7817, p = 0.0194

Abbreviations: AB = African buffalo, AE = African elephant, BB = bushbuck, BR = black rhinoceros, EL = eland antelope, GE = gerenuk, GG = Grant's gazelle, GI = giraffe, GK = greater kudu, GZ = Grevy's zebra, HB = hartebeest, IM = impala, KS = klipspringer, OY = oryx antelope, PZ = plain zebra, SI = sitatunga, WA = waterbuck, WH = warthog, WR = white rhinoceros

Tab. 18: Table of allometric regressions for TFN, NDIN, MFN and FNDF for the wild animals.

	Equation	95% CI	r^2	p-value
TFN and BM				
All ruminants	**3.84 $BM^{-0.09}$**	-0.21 - 0.02	0.2252	0.1013
All hindgut fermenters	**2.71 $BM^{-0.12}$**	-0.21 - (-0.03)	0.7817	0.0194
NDIN and BM				
All ruminants	**1.56 $BM^{-0.08}$**	-0.37 - 0.21	0.0353	0.5388
All hindgut fermenters	**0.77 $BM^{-0.05}$**	-0.26 - 0.16	0.0943	0.5537
MFN and BM				
All ruminants	**2.47 $BM^{-0.13}$**	-0.22 - 0.04	0.4704	0.0096
All hindgut fermenters	**2.18 $BM^{-0.18}$**	-0.37 - 0.01	0.6392	0.0563
FNDF and BM				
All ruminants	**33.50 $BM^{0.08}$**	-0.01 - 0.17	0.2754	0.0656
All hindgut fermenters	**44.67 $BM^{0.07}$**	-0.01 - 0.14	0.5921	0.0736

(With: formula for the regression line, 95% confidence interval of the exponent, the coefficient of determination (r^2) and the p-value; TFN = total faecal nitrogen, NDIN = neutral detergent insoluble nitrogen, MFN = metabolic faecal nitrogen, FNDF = faecal neutral detergent fibre)

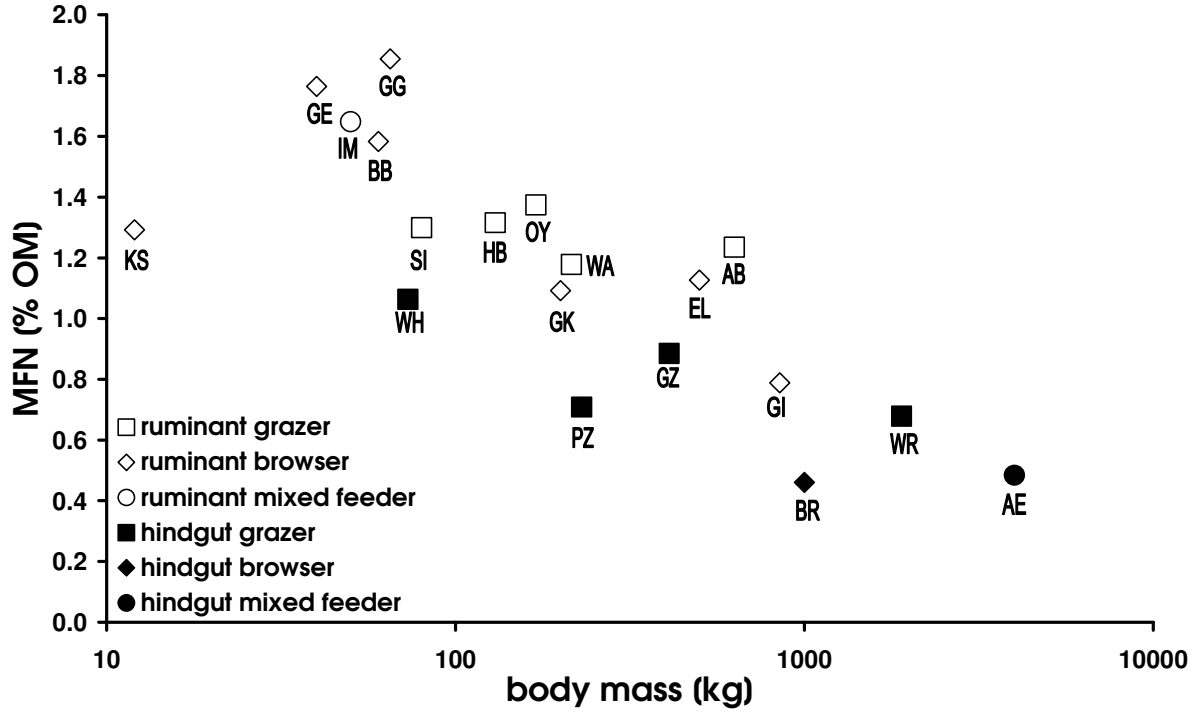

Fig. 13: Relation of metabolic faecal nitrogen (MFN) and body mass (BM) of free ranging animals. (For animal abbreviations see Fig. 12) (OM = organic matter)
all ruminants: MFN = 2.47 $BM^{-0.13}$, r^2 = 0.4704, p = 0.0096 that the exponent of BM was different from zero; *all hindgut fermenters:* MFN = 2.18 $BM^{-0.18}$, r^2 = 0.6392, p = 0.0563

3.6. FNDF

The FNDF values for the grass feeding ruminants ranged between 36.4 (hartebeest) and 59.7 (African buffalo) % dry matter (DM) (Tab. 3). For the grass feeding hindgut fermenters the range was 59.2 (warthog) up to 65.3 (white rhinoceros) % DM, the browse feeding ruminants had a range of 41.9 (Grant's gazelle) up to 67.9% DM (giraffe). The browse feeding black rhinoceros had a value of 79.8% DM. Ruminants had significantly lower FNDF values than

hindgut fermenters (p = 0.0025). There was no significant relation, but a trend, between FNDF and BM for ruminants (Tab. 18, Fig. 14).

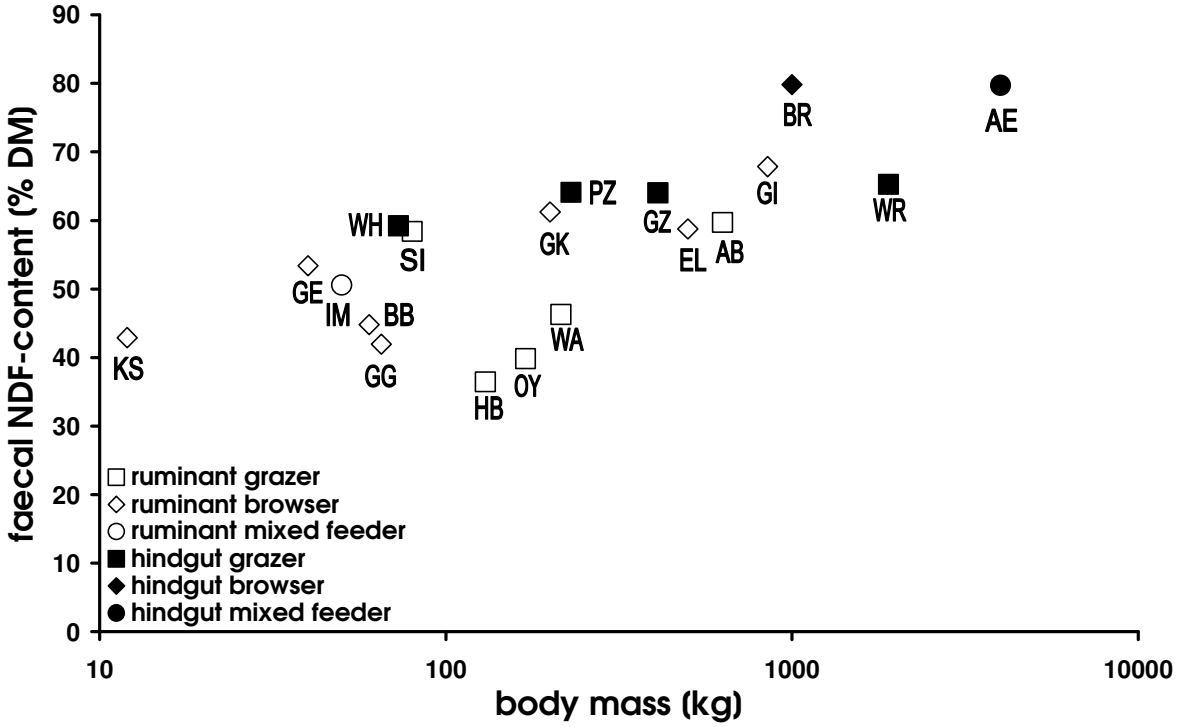

Fig. 14: Relation of faecal neutral detergent fibre (FNDF) and body mass (BM) of free ranging animals. (DM = dry matter) (For animal abbreviations see Fig. 12) *all ruminants:* FNDF = 33.50 $BM^{0.08}$, r^2 = 0.2754, p = 0.0656 that the exponent of BM was different from zero; *all hindgut fermenters:* FNDF = 44.67 $BM^{0.07}$, r^2 = 0.5921, p = 0.0736

4. Discussion

4.1 Methods

4.1.1. Faecal nitrogen

Nitrogen in the faeces of animals can be of different origin (Fig. 10). To separate TFN in non-available and available (and therefore already metabolised) N, different approaches have been applied. Using N in acid detergent (AD) residues as a measure for non-available N has the disadvantage that with this method also some cell wall proteins are washed out (Mason 1969; Mason and Frederiksen 1979; Van Soest 1994). Boiling with ND-solution should not remove any cell-wall bound nitrogen, and the residues also contain most of the tannin-protein complexes (Van Soest 1994). This latter factor can be of importance for browse diets. The N fraction resulting from the subtraction of NDIN from TFN contains mainly the metabolic losses of the animal, microbial debris being the main part (additionally mucus, gut enzymes and sloughed-off animal cells). Accordingly it appears possible to exclude or at least to reduce the disturbing influence of the tannins which facilitates a quantification of diet quality in browsing animals.

In a study on dogs, Hesta et al. (2003) suggested that it is possible to split the MFN fraction further into bacterial N and endogenous N. Schwarm et al. (2009) used the method of Hesta et al. (2003) for splitting up MFN in herbivore faeces. However they arrived at the conclusion that this method will not work here, since the estimation of endogenous faecal nitrogen was always higher than the bacterial nitrogen content which is in contrast to the general opinion on herbivore faecal composition.

4.1.2. Carbon isotopes

To validate the separation of the different wild ranging African animals in grazers and browsers the stable carbon isotope ($^{13}C/^{12}C$) signature was used (Tieszen et al. 1979; Cerling and Harris 1999; Cerling et al. 1999; Codron and Codron 2009). As studies show, it is possible to detect differences in food choice between animal species according to their $\delta^{13}C$ values in the faeces (Codron et al. 2005b). With this method the food composition of an animal for the recent past (~ last 2 weeks) can be measured. This method was used to give some broad estimation of the diet of the animals, and the results for the area and season of this study are comparable with findings in Codron et al. (2005a). In consequence, the animals could be allocated to six groups in this study.

4.1.3 Faecal neutral detergent fibre

In addition to the nitrogen content in the faeces the FNDF content was used to get further information on food quality. High fibre contents in the faeces of herbivores could be seen as an indicator of high amounts of hardly or indigestible fibre and therefore low food quality (Owen-Smith 1988). In the FNDF fraction of browse feeding animals also tannin-protein complexes are included in significant amounts. Since high contents of tannins are indicators of low quality food, this does not contradict the use of FNDF as an indicator of low diet quality. However, it should be kept in mind that the concentration of a substance in faeces is not only changed by its own presence but obviously also by the amounts of other substances. This effect increases the accuracy of indicators positively correlated to diet digestibility/quality like TFN or MFN, but would have the opposite effect in an indicator correlated negatively to diet quality like FNDF. However, due to the dominant contribution of NDF in the diets of the animals this effect can be considered to be less than in other substances.

4.2. Control study

The study with captive animals fed grass hay (chapter 1+2) can be used to control for potential direct influences of BM or digestive system on faecal indices. The results in chapter 2 show that the TFN and MFN values are significantly higher in ruminants than in hindgut fermenters (TFN p = 0.0046; MFN p = 0.040) when they are fed a comparable diet, which would be in agreement with a higher microbial production in ruminants.

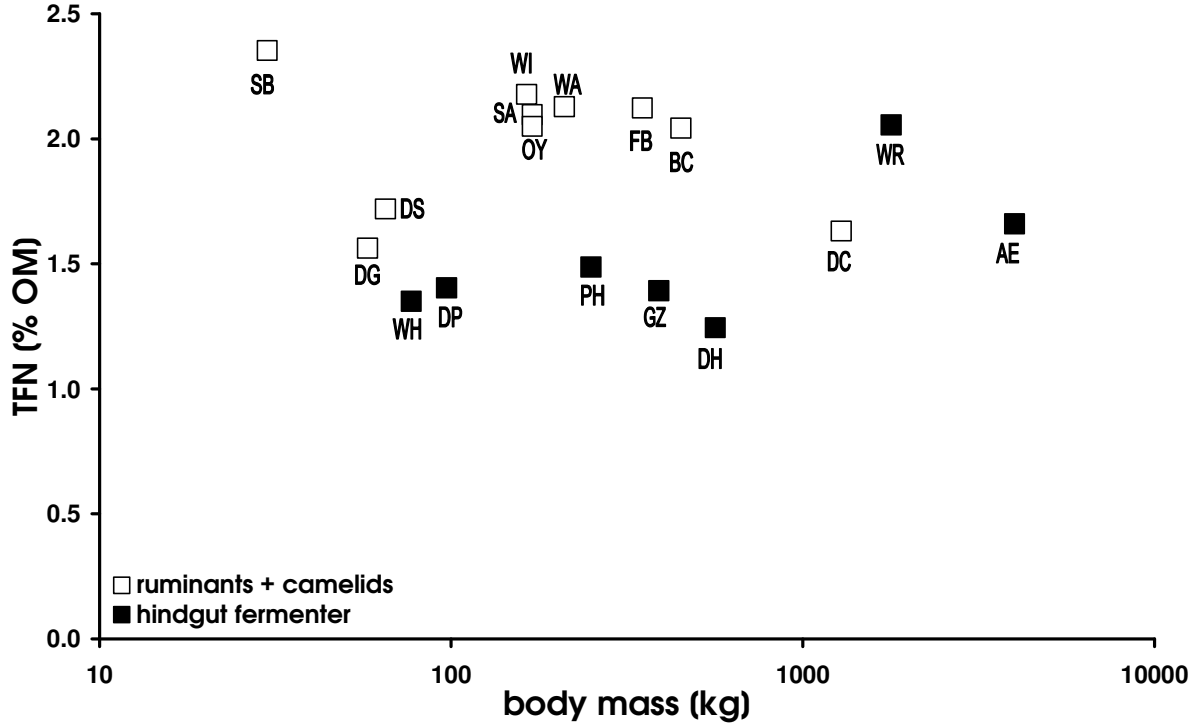

Fig. 15: Relation of metabolic faecal nitrogen (MFN) and body mass (BM) of captive animals on a grass hay diet. (OM = organic matter) *ruminants + camelids*: TFN = 1.73 $BM^{-0.02}$, r^2 = 0.0143, p = 0.7418 that the exponent of BM was different from zero; *hindgut fermenters*: TFN = 0.70 $BM^{0.08}$, r^2 = 0.3995, p = 0.1278. **Abbreviations:** AE = African elephant, BC = Bactrian camel, DC = domestic cattle, DG = domestic goat, DH = domestic horse, DP = domestic pony, DS = domestic sheep, FB = forest buffalo, GZ = Grevy's zebra, OY = oryx antelope, PH = Przewalski horse, SA = sable antelope, SB = springbok, WA = waterbuck, WH = warthog, WI = blue wildebeest, WR = white rhinoceros

In consequence, groups have to be separated for comparisons on the basis of faecal nitrogen. The NDIN [% OM] values were not significantly different between captive ruminants and hindgut fermenters (p = 0.1423). But the proportion of NDIN [%] in TFN was significantly higher in hindgut fermenters [19.3%] than in ruminants [22.7%] (p = 0.0167), a higher fibre digestibility of ruminants probably contributing to this effect. The proportion of NDIN in TFN is much higher in free ranging than in captive animals, potentially based on higher proportions of indigestible fibre in the food of the free ranging animals. At the end of the dry season the proportion of indigestible fibre in the food would be higher than in the grass hay used in the control study. The differences in MFN values were based on higher microbial

contents in the fermentation chambers of the ruminants which cause higher MFN values in the faeces. The results of animals fed with a 100% grass hay diet could be interpreted in the way that the difference in MFN values is more based on the different digestive strategies of ruminants and hindgut fermenters while the difference in NDIN values is more influenced by food parameters.

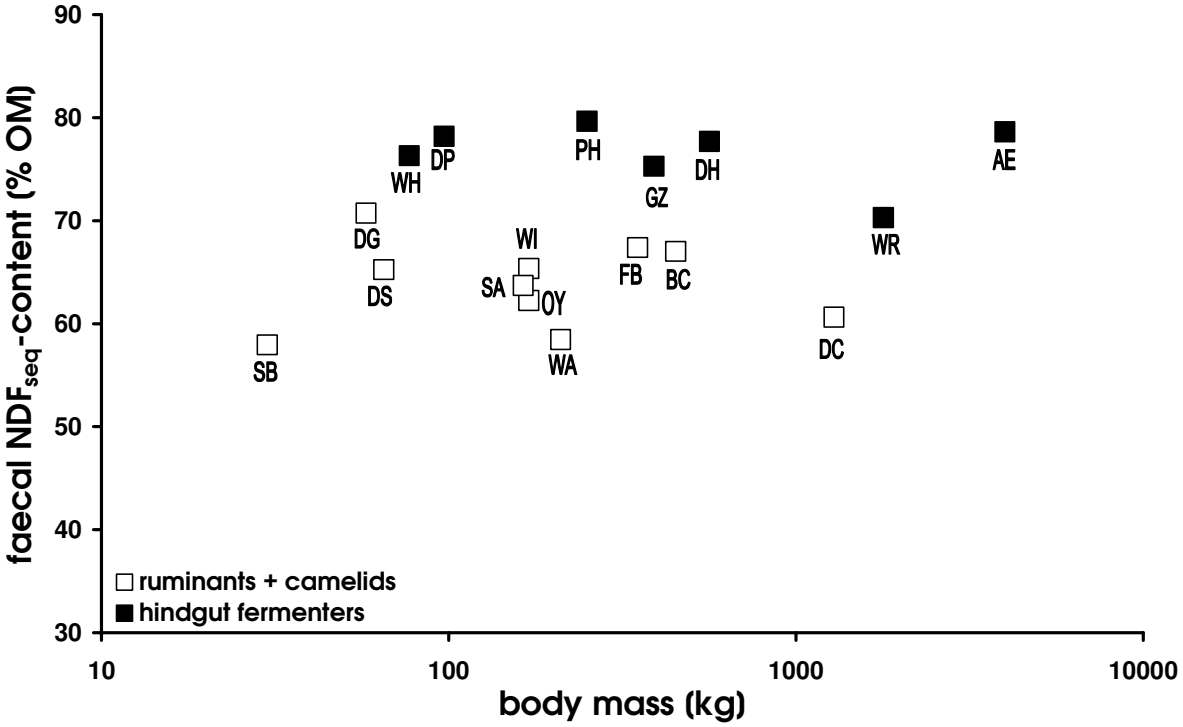

Fig. 16: Relation of faecal NDF_{seq} and body mass (BM) of captive animals (DM = dry matter, NDF_{seq} = neutral detergent fibre ADL-ash corrected, sequential analyzed). (For animal abbreviations see Fig. 15) *ruminants*: FNDF = 57.54 $BM^{-0.01}$, r^2 = 0.0231, p = 0.6754 that the exponent of BM was different from zero; *hindgut fermenters*: FNDF = 82.60 $BM^{-0.04}$, r^2 = 0.4168, p = 0.1173

There is no significant relation between TFN/NDIN/MFN and BM for ruminants and hindgut fermenters when they were fed equally (Fig. 15, Tab. 19). The FNDF [% DM] was significantly higher for captive hindgut fermenters than for ruminants in this study (chapter 2) (p = 0.0020) (Fig. 16). At least part of the explanation should be the better fibre digestibility in ruminants (see above) (Van Soest 1994). For both groups there is no significant relation between BM and FNDF [% DM] (Fig. 16, Tab. 19). As conclusion, significant systematic changes of the faecal indicators used in this study should be due to the quality of food selected basically, and not due to allometric effects in the digestion of the animal.

Tab. 19 : Table of allometric regressions for TFN, NDIN, MFN and FNDF for the captive animals.

	Equation	95% CI	r^2	p-value
TFN and BM				
All ruminants + camelids	**2.18 $BM^{-0.02}$**	-0.12 - 0.08	0.0235	0.6724
All hindgut fermenters	**0.96 $BM^{0.07}$**	-0.03 - 0.18	0.3882	0.1350
NDIN and BM				
All ruminants + camelids	**0.45 $BM^{-0.04}$**	-0.14 - 0.07	0.0677	0.4680
All hindgut fermenters	**0.26 $BM^{0.04}$**	-0.06 - 0.14	0.2067	0.3054
MFN and BM				
All ruminants + camelids	**1.73 $BM^{-0.02}$**	-0.13 - 0.09	0.0143	0.7418
All hindgut fermenters	**0.70 $BM^{0.08}$**	-0.03 - 0.20	0.3995	0.1278
FNDF and BM				
All ruminants + camelids	**57.54 $BM^{-0.01}$**	-0.08 - 0.05	0.0231	0.6754
All hindgut fermenters	**82.60 $BM^{-0.04}$**	-0.09 - 0.01	0.4168	0.1173

(With: formula for the regression line, 95% confidence interval of the exponent, the coefficient of determination (r^2) and the p-value; TFN = total faecal nitrogen, NDIN = neutral detergent insoluble nitrogen, MFN = metabolic faecal nitrogen, FNDF = faecal neutral detergent fibre)

4.3. Faecal indices of diet quality

As a measure of diet quality, faecal-N (in most instances TFN) has shown considerable potential during the 60 years since Lancaster (1949). Its non-invasive sampling and relatively simple, commonly available analysis method makes it attractive not only for projects on basic research but also in situations where regular monitoring is the final goal. While often interpreted as a measure of dietary nitrogen, it should be stated here that the diet quality trait probably best represented by faecal nitrogen is OM-digestibility (via its influence on microbial growth in the digestive tract, and the corresponding production of microbial nitrogen in the GIT), and not dietary crude protein (CP). Most available dietary CP is degraded in the digestive tract (average CP digestibility assumed is app. 86-90%). Ironically primarily those parts will be recovered in faeces that are indicators of low quality like fibre bound CP and tannin-protein complexes. A way in which dietary nitrogen amount may influence faecal-N beyond the level of recovery of indigestible N would be at very low nitrogen levels via a limiting influence on microbial protein production in the fermentation chambers. Since large herbivores have developed elaborate ways of nitrogen recycling to minimize such situations of excessive nitrogen deprivation (and to save water via the resulting reduction of urinary volume) (Schmidt-Nielsen et al. 1957; Simmonet et al. 1957), this may rather apply at very low dietary CP levels only - which must not be considered unrealistic in a wild situation, however. But since in this situation OM-digestibility will also be compromised, it can be added directly that this would not at all be in conflict with a definition of faecal-N as a valid indicator of diet digestibility.

For most of the investigated animals the TFN values were comparable to data found in literature (Tab. 20). The TFN values are here presented as % of DM because in most cases this value was given in literature. Codron et al. (2007b) indicated the season in which the samples were collected (the dry season values were chosen) while the other studies pooled samples from dry and wet seasons (Clemens and Maloiy 1982; Grant et al. 1995; Codron et al. 2005a; Codron et al. 2005b; Codron 2006; Codron et al. 2006; Codron and Codron 2009).

Tab. 20: Total faecal nitrogen values (TFN) [% DM] for free ranging African species from literature and this study

	TFN [% DM]	Source		TFN [% DM]	Source
Grass feeding animals			**Browse feeding animals**		
Oryx antelope	0.9	(Codron et al. 2005b)	**Klipspringer**	1.7	(Codron et al. 2005a)
	1.4	this study		1.9	this study
Waterbuck	1.5	(Codron and Codron 2009)	**Impala**	2.0	(Codron and Codron 2009)
	1.9	(Codron et al. 2007b)		1.9	(Codron et al. 2007b)
	1.5	(Codron 2006)		2.1	(Codron et al. 2006)
	1.1	(Codron et al. 2005a)		2.0	(Codron 2006)
	1.6	this study		1.4	(Codron et al. 2005a)
				1.9	(Grant et al. 1995)
				2.3	this study
African buffalo	1.5	(Codron and Codron 2009)	**Bushbuck**	2.5	(Codron et al. 2007b)
	1.4	(Codron et al. 2007b)		2.5	(Codron 2006)
	1.5	(Codron 2006)		2.1	this study
	1.2	(Codron et al. 2005a)			
	1.3	(Grant et al. 1995)			
	1.7	this study			
Warthog	1.7	(Codron and Codron 2009)	**Greater kudu**	2.8	(Codron and Codron 2009)
	2.0	(Codron et al. 2007b)		2.6	(Codron et al. 2007b)
	1.7	(Codron 2006)		2.8	(Codron 2006)
	1.5	this study		1.9	(Codron et al. 2005a)
				2.0	(Grant et al. 1995)
				1.9	this study
Plains zebra	1.2	(Codron and Codron 2009)	**Eland**	1.8	(Codron et al. 2007b)
	1.2	(Codron et al. 2007b)		2.6	(Codron 2006)
	1.3	(Codron et al. 2006)		2.1	(Codron et al. 2005a)
	1.2	(Codron 2006)		1.7	this study
	0.8	(Codron et al. 2005a)			
	1.0	this study			
White rhinoceros	1.2	(Codron and Codron 2009)	**Giraffe**	2.6	(Codron and Codron 2009)
	1.3	(Codron et al. 2007b)		2.6	(Codron et al. 2007b)
	1.3	(Codron 2006)		2.7	(Codron et al. 2006)
	0.9	(Codron et al. 2005a)		2.6	(Codron 2006)
	1.0	this study		2.0	(Codron et al. 2005a)
				2.0	(Grant et al. 1995)
				2.4	this study
			Black rhinoceros	1.3	(Codron et al. 2007b)
				1.3	(Codron 2006)
				1.8	(Clemens and Maloiy 1982)
				1.1	this study
			African elephant	1.0	(Codron et al. 2005a)
				1.3	(Clemens and Maloiy 1982)
				1.0	this study

(DM = dry matter)

Literature data generally was within the range of the standard deviation of the values in this study. Most of the data in Tab. 20 was collected in South Africa (Kruger National Park and Zoetfontein) but also in Namibia (Waterberg). It has to be noted that most of the literature faecal nitrogen data (Codron et al. 2005a; Codron et al. 2005b; Codron 2006; Codron et al. 2006; Codron et al. 2007b; Codron and Codron 2009) was measured by using a mass

spectrometer and not the Dumas method as in this study. However, the influence of this can be considered negligible since both methods measure the whole nitrogen content of the sample. The trend that browse feeding animals had higher TFN values was confirmed by the literature data set. According to Owen-Smith (1988), OM-digestibility represents the most desirable estimate of diet quality. Klaassen and Nolet (2008) propose a shift in the major limiting dietary trait from N (representing protein content) to C (representing energy content) for endotherms feeding on green plant material. For large, endotherm herbivores OM-digestibility may therefore be a more critical dietary trait than e.g. for arthropod herbivores, for which nitrogen appears more limiting. MFN can be considered to represent an even better (but analytically slightly more laborious) indicator of digestibility than TFN, and is definitely advisable in browsing herbivores. The latter is a reasonable indicator in grazers, and has the advantage of allowing the use of regression equations actually estimating digestibility. In the grazers of this study, the use of regressions established for forage-based diets or even pasture like situations does not result in unreasonable results (Tab. 21) (note that crude protein (the same like TFN multiplied with the factor 6.25) values were corrected by a multiplication factor of 1.12 for nitrogen losses during drying, since the equations were established with faecal crude protein values from fresh faeces). The second formula of Lukas et al. (2005) was chosen because it is based on trials where animals were fed with forages only.

Tab. 21: Estimations of organic matter digestibility (aD OM) [%] from regression equations based on crude protein (CP) content in organic matter

	Lukas et al. (2005)[1]	Wang et al. (2009)[2]	Mésochina et al. (1998)[3]
		aD OM [%]	
Sitatunga	68	70	-
Hartebeest	55	57	-
Oryx	56	58	-
Waterbuck	61	62	-
African buffalo	61	62	-
Plains zebra	-	-	52
Grevy´s zebra	-	-	53
White rhino	-	-	51

[1]OM digestibility [%]=72.86−107.7e$^{(-0.01515\times\text{fecal CP (g/kg OM)})}$;
[2]OM digestibility [%]=0.899−0.644e$^{(-0.5774\times\text{fecal CP (g/kg OM)}/100)}$;
[3]OM digestibility [%]=0.433 + 0.001×fecal CP (g/kg OM)

As intended, the exclusion of the NDIN fraction may allow a clearer view on the microbial growth in the animal, facilitating the combination of browsing and grazing ruminants in one analysis. In fact, in the ruminant data set no significant difference in the MFN content ($p = 0.8329$) can be found between browsers and grazers, while in the same data set a significant

difference for NDIN is present ($p = 0.0451$). As reasons for the higher NDIN values in browse feeding ruminants and hindgut fermenters, the tannins in the forage of the animals have to be considered (Van Soest 1994). Leslie et al. (2008) stated that tannins in the food do not automatically increase the faecal nitrogen of ruminants, but the significant difference between the grass and browse feeding animals regarding the NDIN content indicates such an effect. Another reason for higher NDIN values might be the higher lignin contents in browse. Since the digestibility of NDF in browse can be considered to be lower than that of grass due to its high lignin content, any nitrogen bound in the cell wall is also more likely to be recovered in the NDIN fraction.

The same explanation (lignified fibre) may also apply to the trend of increasing FNDF contents with increasing BM for ruminants and hindgut fermenters and is an indication for a decrease of food quality with increasing BM. Twigs contain high amounts of lignified fibre and low nitrogen contents compared to leaves (Hummel et al. 2006). Accordingly large amounts of wood in the diet can cause high faecal NDF and low MFN values attributable to the low digestibility of highly lignified fibre which causes a low microbial growth in the rumen. As mentioned before also the tannin-protein complexes were included in this fraction. The results point out that a decrease of diet quality in terms of digestibility with BM can be quantified using these parameters. This trend is confirmed by the very high faecal NDF and low MFN contents of the largest browse feeding animal in this study, the black rhinoceros. Also the African elephant, classified as a mixed feeder during the dry season, has very high FNDF values.

When using faecal-N as an indicator of food quality, some conditions need to be met. Because faecal samples were collected within a short period and were compared within digestion groups, the points of criticism of Hobbs (1987) and Leslie et al. (2008) regarding faecal nitrogen studies were taken into account. The fact that the ruminants in this study had always higher TFN, NDIN and MFN values than hindgut fermenters is based, as mentioned before, on their differing digestive systems and different amounts of microbes in their fermentation chambers (Van Soest 1994). A combination of data of both digestive systems therefore would inevitably include considerable shortcomings.

4.4. Diet quality and body mass

It was the major goal of this study to quantify the relation of BM and food quality in free ranging herbivores. For TFN, MFN and FNDF, at least a trend of decreasing diet quality with BM was found in all cases, with allometric coefficients significantly different from 0 for MFN in ruminants and TFN in hindgut fermenters, the latter even irrespective of the low

sample size (n = 6). To correct for the confounding effect of indigestible faecal-N compounds, MFN was used as a measure of diet quality. The major and most variable part of the MFN is the microbial debris, while the other parts of the MFN, mucus and sloughed cells are rather constant, so this fraction should be the most robust variable for estimating microbial growth in the animal. In fact, for the ruminants there is a significant decrease of MFN with increasing BM ($BM^{-0.13}$). Somewhat surprisingly, in contrast to TFN data, for MFN and BM no significant relation was found for hindgut fermenters, although a strong trend was still present ($p = 0.0563$).

In free ranging animals of both digestion types no significant correlation was found between FNDF and BM but both groups had p-values which were close to the level of significance (ruminants $p = 0.0656$, hindgut fermenters $p = 0.0736$), so a trend of increasing FNDF values with increasing BM was visible in both groups. This which would be in accordance with the findings for the MFN contents. With the trend of increasing FNDF and the significant decrease of MFN for ruminants and trends for hindgut fermenters in the same direction, some indication for a decrease of food quality with BM was confirmed for ruminants and indications were shown for hindgut fermenters. Obviously a larger sample size would be desirable for hindgut fermenters, but for this digestive system all truly herbivorous ungulates of the area (and of East Africa) were already used in this study.

The relation of diet quality and BM has inspired several contributions on this topic dealing with large terrestrial herbivores. Some authors consider low diet quality as a trigger of large BM (Midgley et al. 2002). Others predict even an increase of BM with increasing food quality based on model calculations (Case 1979), however directly adding that empirical evidence strongly suggests a negative correlation of BM and diet quality. Obviously, one has to distinguish between the ontogenetic and phylogenetic level: While during ontogeny, a higher diet quality will allow the individual to use its growth potential to a large extent, on a phylogenetic scale large BM is generally associated with low food quality.

It is generally agreed that BM and diet quality are negatively correlated (Owen-Smith 1988; Codron et al. 2007b). Studies like that of Woolley et al. (2009) on free ranging African elephants show that this concept is also valid on an intraspecific level, the larger individuals of the group having lower faecal nitrogen (and phosphorus) values than the smaller ones.

However, there are different opinions on the degree of correlation of diet quality with BM on an interspecific level. The scaling with $BM^{-0.1}$ based on theoretical considerations by Owen-Smith (1988) represents a starting point. Empirical evidence pointed to higher values like $945\ BM^{-0.22}$ for ruminal fermentation rates [µmoles gas/(g DM*d)] (Hoppe 1977), or like 21.6

$BM^{-0.23}$ for ruminal crude protein content [%] (Owen-Smith 1988). Other authors arrived at much lower estimations of a decrease of diet digestibility proportional to $BM^{-0.019}$ (based on data of non-stem material in the digestive tract) and assumptions on the digestibility of these plant parts according to Illius (1997), or on the level of 0.86 $BM^{-0.049}$ as modelled by Gordon and Illius (1996) for the decline of potential digestibility of diets supporting the fermentation rates given in Hoppe (1977). Gordon and Illius (1996) consider the rates given by Hoppe (1977) as being influenced in part by the longer retention of slowly fermenting, refractory material in large taxa (which however can be considered to have an effect on the diet quality as experienced by the animal actually). Interestingly, the present data set (ruminants: MFN ~ $BM^{-0.13}$; hindgut fermenters: MFN ~ $BM^{-0.18}$) seems to resemble most the exponents estimated for the minimum acceptable DM digestibility over a longer period (considering the larger compensation potential of large animals for low digestibility via stored energy depots) of 0.88 $BM^{-0.143}$ (Illius and Gordon 1999), or the exponents estimated for non-stem material in the GIT = 116 $BM^{-0.118}$ (Owen-Smith 1988). Since the allometric decrease in diet quality has been shown to be largest during dry season (Illius and Gordon 1999), based on the data set of Zeeman et al. (1983)) – the exponents represent the maximum rather than the minimum for the Lewa site.

5. Conclusions

- There is a significant decrease of TFN with increasing BM for hindgut fermenters and a significant decrease of MFN for ruminants in this study and also trends of increasing FNDF with BM - strong indications for an intake of low quality forage for large ungulates (ruminants and hindgut fermenters).
- The high NDIN values for browsing animals are one hint for the presence of the tannin-protein complexes in this fraction

Synthesis

This study was conducted to investigate the influence of BM on digestive parameters of ungulates. This was done by using captive and free-ranging animals of a wide BM range, the captive large herbivores representing an average BM range of 50 kg up to 4000 kg. Animal species were chosen regarding their acceptance of grass hay to ensure that it would be possible to increase the portion of grass hay in their ration up to 100%. The faecal samples of all animals (captive and free ranging) were analyzed for different parameters: TFN/NDIN/MFN and faecal NDF. In addition, faecal NDF were analysed for in vitro digestibility. For the captive animals food intake and MRT were analyzed to investigate correlations between BM and these parameters. The free ranging animal data set also included samples from browsing animals. An adapted faecal nitrogen analysis was used to exclude the influence of tannins on the results.

Methodological considerations

Animals

Studies using wild animals always have difficulties to get high sample sizes for quantifications. This study has to cope with this fact too. The number of species and of individuals per species was restricted by several factors like the acceptance of a grass hay diet or the acceptance of the husbandry practices during the sampling period. Other husbandry constraints like animals in therapy also can play a role in individual cases. All these factors lead to the fact that in some species only one or two animals were used in this study. For most of the species at least 3 individuals were sampled which was considered enough to get general indications for these species. Moreover, in this study the focus was on the scaling of variables over a large body mass range and on the differences between groups like ruminants and hindgut fermenters. For such evaluations the number of individuals per species can be considered to be a less important factor than e.g. in two-species comparisons.

For the free ranging animals it was possible to sample a very comprehensive spectrum of ungulate species living in Northern Kenya. But also here it was difficult to get an equal sample size for all of the species. The effort to collect and prepare all the samples was great regarding time and man power.

Feeding

The feeding of the animals represented no major challenge in this study. All animals accepted the 100% grass hay diet well after a short habituation period, and ingested it well during the adaptation period of 14 days and the sampling period.

Body mass of wild animals

All farm animals and the captive warthog were weighed, BM of all other animals were estimated. For weight estimations, literature data was collected and was combined with the estimations of experienced zoo veterinarians, zoo keepers and the conductors of this study. In case of the captive wild animals, the quantification of the food intake as related to MBS and BM was influenced by these estimations. Due to the large range of taxa and BM and the fact that the comparisons were mostly made on the level of groups (ruminants vs. hindgut fermenters or browser vs. grazer), errors which were eventually made in BM estimations will not influence the general outcomes of this study.

Grass hay quality

The intention of this study was to feed all captive animals with grass hay as uniform as possible. The grass hay which was used was ordered at the same company but because of the large amounts of hay which were needed in this study at different places three different deliveries of grass hay were necessary. The delivered grass hay was a second cut but of slightly differing composition as nutrient composition indicates. Especially the ash content of the hay differed. Because of this, most nutrient concentrations were related to organic matter. As shown, the differences between ruminants and hindgut fermenters regarding the fed grass hay composition were not significant. Accordingly it was feasible to compare these groups without taking the food factor into consideration.

Faecal total collection

As speculated the total collection of faeces for the wild animals in the zoo was not practicable for most of the species. Due to the fact that it was not possible to collect in short time intervals, the faecal collection could not be conducted with the accuracy necessary for digestibility trials. As mentioned before in several studies titanium dioxide (TiO_2) was used as a marker to determine faecal output for estimating apparent digestibility (Titgemeyer et al. 2001; Glindemann et al. 2009). For this method it is necessary that the animal ingests the TiO_2 marker completely, ideally two times a day for the duration of an adaptation period (minimum two weeks) and the trial itself (6 - 8 days). In this study the TiO_2 was fed included in food pellets to raise the acceptability. The horses, sheep, cattle and also the African

elephants ingested the marker properly. The African elephants were trained to open the mouth, so it was possible to feed TiO_2 pellets directly. For the other animals, ingestion of the daily portion of TiO_2 was not reliable enough for several reasons, like limited acceptance or unacceptable spillage of the pellets.

Another approach to determine the digestibility of the food was to determine the acid insoluble ash (AIA) in the food and faeces (Van Keulen and Young 1977) (*organic matter apparent digestibility* $(\%) = 100 - (food\ AIA / faecal\ AIA * 100)$).

The AIA was measured for all animals but did not provide reliable results. The digestibilities were always underestimated. One reason could be the proportion of soil in the fed grass hay. In the case that the animals ingest all of the soil which was in the grass hay there would be no problem in determining food digestibility. However, if this was ingested slightly disproportional to the rest of the hay, the use of AIA as an internal marker is obviously impaired. In those animals (especially zoo animals) for which the grass hay was presented in a rack for fodder, most of the soil fell down through the grate and was not ingested. In conclusion the AIA method was not applicable in the circumstances of this study.

Retention time marker (chapter 1)

The retention time markers, chromium mordanted fibre and Co-EDTA, were fed as a pulse dose at the beginning of each trial mixed with food pellets. Food pellets were added to increase the acceptance of the markers. In addition to the food pellets and chromium mordanted fibres some water was added to get the fibres to stick on the pellets. The Co-EDTA was dissolved in water and was added to this mixture. Except three individuals (they needed app. 30 min to ingest the marker) this mixture was accepted very well and was ingested within 15 to 20 minutes. The dosage of approximately 1 g/kg $BM^{0.75}$ of chromium mordanted fibre and 0.1 g/kg $BM^{0.75}$ of Co-EDTA resulted in sufficiently high marker concentrations in faeces and caused no problems in the animals.

Hohenheim gas test (chapter 2)

To estimate differences in fibre degradation between the sampled animals the HGT was used. As indicated by the results in chapter 2 the measured differences between the two groups (ruminants and hindgut fermenters) were considerable. Just one point could be improved in future: the duration of the HGT. No plateau in GP was reached for the NDF of the faeces and the food after 96 h, but could be shown for the longer HGT (200 h) in chapter 2. This can deliver information for additional interpretations of results. However, for the comparison of

different animal species, the time of 96 h was sufficient, since it is within the range of the retention times that can be expected for the investigated taxa.

Modified faecal nitrogen analysis (chapter 3)

The differentiation of the NDIN and the MFN was made to isolate the part of faecal N potentially most relevant for an evaluation of diet digestibility/quality (microbial debris). This differentiation is expected to comprehensively reduce the influence of tannins in the food of the browsing animals on an estimation of digestibility from faecal N. In future studies it has to be tested if the additional separation of NDIN (dominantly cell-wall bound nitrogen and tannin-protein complexes) and MFN (dominantly microbial debris) could generally increase the accuracy of the faecal nitrogen method.

Results

The influence of BM on MRT was investigated in chapter one. Because of the contradictory results of previous studies on this topic (chapter 1), this study had a close look on this relation by feeding all animals with one diet (100% grass hay) and by using the same trial setup for all animals. In this study no significant relation for ruminants and hindgut fermenters was found between $MRT_{particle}$ and BM (chapter 1), which confirms the findings of Clauss et al. (2007a) and Owen-Smith (1988). From a physiological point of view an endless prolongation of the MRT makes little sense: Energy gained from a given amount of food is getting less over digestion time and the probability of excessive methane losses is considered to increase especially for ruminants.

The longer $MRT_{particle}$ for ruminants (chapter 1) is in line with the results of the HGT which was conducted with the faecal NDF of the animals in chapter 2. The GP of the faecal NDF of the ruminants were significantly lower than of those of the hindgut fermenters. Low GP indicates that there was less digestible fibre left in the faecal NDF and that most of it was digested by the microbes hosted by the animal. This was also indicated by the significantly lower NDF contents in the faeces of the captive ruminants (chapter 2). Accordingly more fibre was digested by ruminants than by hindgut fermenters.

The intention of chapter 3 was to investigate the relation between BM and food quality. As method for evaluating this relation an extended faecal nitrogen analysis was applied on a set of samples of free ranging African herbivores. By excluding the influence of tannins on the results it was possible to compare herbivores regardless if they were browse, grass or mixed feeders. With the MFN content of the animals it was possible to show for ruminants a decrease of food quality with increasing BM, and a strong tendency for hindgut fermenters.

As a second faecal parameter the FNDF content was measured and a trend of an increase in NDF (indicator for low food quality) in the faeces was shown for ruminants and hindgut fermenters. These findings confirm the result of a lower food quality of large ruminants as estimated with MFN. Because of the close relationship between food quality and food selectivity it is feasible to state that large free ranging ungulate ruminants were less selective than smaller ones and that there is a trend that this is also fact for ungulate hindgut fermenters.

Perspectives

A rewarding target for future studies could be further investigations on the use of MFN as an estimation of food quality. Among the first and most interesting steps, as a follow-up of the study on faecal samples from dry season, samples collected during or at the end of the rainy season in Northern Kenya should be investigated. Some parameters will be different in the rainy season like the inclusion of browse in the diets (generally lower tendency in the rainy season), or the differences between grasses and grass parts (considered to be more comprehensive during the rainy season). It would be also interesting to get more information about the individual that dropped the faeces (sex, age, estimated weight, habitat of sampling: savannah, bush savannah, open forest) and to include these information into considerations.

It might be possible to fractionate the MFN in two fractions: microbial debris and endogenous losses of the animal. Hesta et al. (2003) showed this for dog faeces. This method did not work with herbivore faeces as Schwarm et al. (2009) showed in their study. Diaminopimelic acid analysis could be an alternative. This amino acid is found exclusively in bacterial cell walls and not in plant material or mammal cells (Work and Dewey 1953; Purser and Buechler 1966). This would allow to estimate the microbial content in the MFN fraction and to recalculate the endogenous losses of the animal. Gaps in the BM range of this study could be further reduced by conducting studies using a trial design and grass hay as comparable as possible to this study.

Implications for the digestive strategy of the Sauropod dinosaurs

This study was conducted within a DFG research unit (FOR 533 “Biology of the Sauropod Dinosaurs”) which investigates the evolution of gigantism in this group of dinosaurs; conclusions for sauropods can be formed with the results of this study. Sander and Clauss (2008) concluded that due to the lack of mastication/tooth batteries, the head of sauropods is rather small (short), which in consequence allows the development of a long neck with all its advantages in terms of food harvesting. Despite the smallness of the head, high intake rates appear feasible; Hummel and Clauss (in press) stated (based on the study of Christiansen

(1999)) that the skulls are short but the widths of them scale with those of extant animals. Accordingly it appears to have been no problem to ingest the large amounts of forage they needed every day. Because of high growth rates in sauropods high basal metabolic rates for growing sauropods were assumed. However in giant adult sauropods heat dissipating problems could develop if they had high basal metabolic rates. Accordingly Sander and Clauss (2008) supposed high basal metabolic rates for the offspring and later a shift to lower (but still higher than in extant reptiles) basal metabolic rates when sauropods reached a certain BM. Hummel et al. (2008) conducted in vitro digestion trials with plants which were descendents of potential sauropod food plants. The results showed that several of them were nearly as digestible as food plants of extant herbivores. Franz et al. (2009) calculated organ sizes for sauropods with regression lines of extant animals and concluded that from the aspect of organismal reconstructions there were no restrictions that sauropods had very large gastrointestinal tracts. The fact that sauropods did not chew their food makes longer MRT necessary to achieve a comparable digestion of plant cell walls. The results of this study do not necessarily imply a continuous increase of MRT with BM; but while we would exclude high BM as a safe predictor of very long retention times, basal metabolic rates lower than mammals would probably induce some prolongation in MRT (Hummel and Clauss in press). In contrast to MRT, diet quality actually seems to be related to BM, and can therefore be hypothesized to be a continuously increasing constraint in large herbivores like sauropods. It has to be mentioned that an extrapolation from a regression for animals of maximal 4000 kg up to dinosaur body masses is speculative. However, the steepness of the increase decreases, and the decreasing effect of an increase in body mass on food quality will become smaller the larger the body sizes are: When extrapolating with the allometric equation set up for hindgut fermenters, an increase of BM from 1000 kg to 11000 kg results in a decrease in MFN of 35 % (0.63 to 0.41 g/kg DM), while a further increase from 11000 to 21000 kg BM only results in a decrease of MFN of 13 % (to 0.36 g/kg DM), and only a decrease of 5 % for an increase from 41000 to 51000 kg. In conclusion, despite their many particularities, reconstructions of the feeding ecology and digestive physiology of sauropods based on data from extant animals seem to arrive at conclusions which do not appear too unrealistic and which are less beyond the existing models and concepts of large herbivore digestive physiology than maybe thought on the first instance, and as sometimes suggested in literature.

Zusammenfassung

Ein Einfluss der Körpermasse auf die Länge der Passagezeit der aufgenommenen Nahrung wurde und wird für große herbivore Säuger kontrovers diskutiert. In Rechnungen, die mit immer größer werdenden Datensätzen angestellt werden, zeigt sich eine Tendenz zu nicht vorhandenen oder zumindest deutlich geringeren als bisher unterstellten Einflüssen der Körpermasse auf die Retentionszeiten bei großen herbivoren Säugern. Zu diesem Ergebnis kommt auch die vorliegende Studie.

In Verdaulichkeitsstudien mit Wiederkäuern und Dickdarmfermentierern wurde gezeigt, dass die Faserverdaulichkeit bei Wiederkäuern, vor allem bedingt durch ihre längeren Passagezeiten, höher ist als bei Dickdarmfermentierern. Verdaulichkeitsstudien sind jedoch bei manchen Zootieren aufgrund der nötigen Restriktionen in der Haltung schwierig. In dieser Studie wurde versucht die Unterschiede zwischen diesen beiden Verdauungstypen mittels eines *in vitro* Testes abzuschätzen. Basierend auf der Restverdaulichkeit der Kotrückstände an pflanzlicher Zellwand im Hohenheimer Futterwerttest wurde ein signifikant höherer Faserabbau für die Wiederkäuer ermittelt.

Studien haben gezeigt, dass es möglich ist, mittels Bestimmung der Stickstoffgehalte im Kot von Pflanzenfressern Rückschlüsse auf die Qualität des aufgenommenen Futters zu ziehen. Anhand von Proben, die im Norden von Kenia von diversen Laub und Gras fressenden Huftieren gesammelt wurden, wurde in dieser Studie versucht den Einfluss der Körpergröße auf die Futterqualität bzw. -wahl abzuschätzen. Da in Laub enthaltene Tannine in früheren Studien immer wieder die Aussagekraft und Beurteilung der Ergebnisse negativ beeinflussten, wurde dieser Einfluss mittels einer modifizierten Kot-Stickstoff-Analyse gemindert. Für die Wiederkäuer konnte, unter Verwendung des Faktors „metabolischer Kot-Stickstoff“ eine mit zunehmender Körpermasse abnehmende Futterqualität/Selektivität nachgewiesen werden. Für die Nicht-Wiederkäuer konnte ein solcher Einfluss sowohl mit dem Faktor „gesamter Kot-Stickstoff“ (signifikant) als auch mit dem metabolischen Kot-Stickstoff (Trend) ermittelt werden. Es ist jedoch möglich, dass dies mit der Jahreszeit in Verbindung steht zu der die Proben gesammelt wurden und sollte in einer Kontrollstudie, deren Proben in der Regenzeit gesammelt wurden, überprüft werde.

Summary

The potential influence of body mass on digesta passage time of large herbivores was discussed controversially in the past and present. Recent studies with increasing data sets show tendencies to very low or not existent influences of animal BM on mean retention times. The results of this study support the findings that there is no significant relation between these two factors.

Digestibility trials comparing ruminants and hindgut fermenters show a higher fibre digestibility in ruminants than in hindgut fermenters. However, digestibility trials are often difficult to conduct for some wild animal species. This study tried to show differences between these groups by using an in vitro test. It was possible to show higher fibre digestibility in ruminants by incubating the faecal fibre of the animals in the Hohenheim gas test.

Studies showed that it is possible to get information about the ingested food quality by analysing the faecal nitrogen of the animals. With samples of free ranging wild herbivores (Northern Kenya) this study tried to draw conclusions about the relation between body mass and food quality. In previous studies tannins, which can be included in browse in considerable amounts, disturbed the analysis and negatively influenced the validity of results. Hence in this study a modified faecal nitrogen analysis was used, and it was possible to show a decrease in food quality/selectivity with increasing BM for ruminants by using the metabolic faecal nitrogen data. For the hindgut fermenters a trend of decreasing food quality was found using the metabolic faecal nitrogen and a significant negative influence by using the total faecal nitrogen data. However, these relations might be influenced by the season in which the samples were collected. This fact should be controlled for in a future study conducted during or at the end of the rainy season.

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