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Digesta kinetics in gazelles in comparison to other ruminants: Evidence for taxon-specific rumen fluid throughput to adjust digesta washing to the natural diet

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ABSTRACT

Digesta flow plays an important role in ruminant digestive physiology. We measured the mean retention time (MRT) of a solute and a particle marker in the gastrointestinal tract (GIT) and the reticulorumen (RR) of five gazelles and one dikdik species. Species-specific differences were independent from body mass (BM) or food intake. Comparative evaluations (including up to 31 other ruminant species) indicate that MRT GIT relate positively to BM, and are less related to feeding type (the percentage of grass in the natural diet, %grass) than MRT RR. The $MRT_{particle}$RR is related to BM and (as a trend) %grass, matching a higher RR capacity with increasing BM in grazers compared to browsers. MRT_{solute}RR is neither linked to BM nor to %grass but shows a consistent phylogenetic signal. Selectivity factors (SF; $MRT_{particle}/MRT_{solute}$, proxies for the degree of digesta washing) are positively related to %grass, with a threshold effect, where species with >20% grass have higher SF. These findings suggest that in different ruminant taxa, morphophysiological adaptations controlling MRT_{solute}RR evolved to achieve a similar SF RR in relation to a %grass threshold. A high SF could facilitate an increased microbial yield from the forestomach. Reasons for variation in SF above the %grass threshold might represent important drivers of ruminant diversification and await closer investigation.

1. Introduction

Ruminants have traditionally been classified into different feeding types, in particular browsers, grazers and intermediate feeders, which vary in the amount of grass in their natural diet (*e.g.* Hofmann and Stewart, 1972; Gagnon and Chew, 2000). Dietary diversification is usually considered a major driver of ruminant speciation (*e.g.* Hofmann, 1989; Pérez-Barbería et al., 2001; Codron et al., 2008a). The different dietary resources pose different challenges on various levels of organismal organization, from distribution patterns at landscape and at plant scale to physical characteristics and biochemical composition; therefore, a large number of particular adaptations to these feeding niches have been suggested and demonstrated in the scientific literature (reviewed in Clauss et al., 2008). In particular, because the time required by microbes to digest grass or browse material was found to differ distinctively, it has been suggested that variation in digesta retention times reflects a set of such adaptations (Hummel et al., 2006): grazers should have longer retention times for digesta particles than browsers. Although comparisons between individual species support this concept (Clauss and Lechner-Doll, 2001; Lechner et al., 2010), comparative statistical evaluations have been hampered so far by methodological differences in retention time measurements between the individual studies used for data compilations (Gordon and Illius, 1994; Hummel et al., 2006).

Additionally, it has been suggested that the difference between the retention time for fluid and particles – as measured by solute and particulate markers – is a physiological characteristic linked to feeding type (Clauss et al., 2006b). This difference has been named as 'selectivity factor' (SF, calculated as the ratio of particle to solute retention) by Lechner-Doll et al. (1990), emphasizing a concept that particles are

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selectively retained longer than fluid in the ruminant forestomach in order to maximize particle digestion. Focusing on another aspect, this difference was described more recently as 'digesta washing' (Müller et al., 2011), thus emphasizing the process by which particulate matter is 'washed' by fluid, which removes solutes and very fine particles such as bacteria from the digesta plug. In the former concept, a high SF is considered indicative of a particularly pronounced particle retention, and emphasis is placed on mechanisms that might enhance such a particle retention. In the latter concept, a high SF is considered indicative of a particularly high fluid throughput, facilitated by particularly short retention times of solutes (fluid) due to a high saliva flow.

While browsers usually have a comparatively low SF, the SF is higher in grazing species (Hummel et al., 2005; Clauss et al., 2006b). The current explanation of this difference invokes a constraint in saliva production in browsing ruminants, which may have to secrete tannin-binding proteins in their saliva as a protection against the secondary plant compounds present in browse. Therefore, browsers may be limited in the amount of saliva they can produce, and hence may have a lower fluid flow through the forestomach and hence less 'digesta washing' (Hofmann et al., 2008; Codron and Clauss, 2010). Grazers, not requiring salivary protection against secondary plant compounds, may be able to have higher saliva flows with the advantage of increased microbial harvest from the forestomach (Clauss et al., 2010b; Hummel et al., 2015). Notably, the transition between the feeding types in this respect does not appear to follow a linear pattern of increasing SF with increasing proportion of grass in the natural diet, but a threshold pattern with a certain proportion of grass above which an increased SF can be observed (Codron and Clauss, 2010).

The statistical evaluation of these concepts is constrained by the number of species for which the corresponding data is available. In particular, many bovid and cervid species have not been assessed for their digestive physiology. Here, we increased the existing dataset by performing passage studies in various gazelle and one dikdik species (Bovidae) covering a range of body masses, using markers, sampling protocols and methods of calculation that ensure comparability with published data that have been assessed under similar conditions. Additionally, we compiled such published data on MRT in the GIT and the RR, determined using the same markers and the percentage of grass in their natural diet, for ruminant species for comparative evaluations. Our approach was guided by four hypotheses:

1. Feeding type (as represented by the percentage of grass in the natural diet of a species) has an influence on particle retention time (Hummel et al., 2006), with longer retention times found in species ingesting diets with higher proportions of grass.
2. Feeding type has an influence on the difference between solute and particle marker retention times across ruminant species (Clauss and Lechner-Doll, 2001; Hummel et al., 2005; Clauss et al., 2006b), with a larger difference in ruminant species that have higher proportions of grass in their natural diet.
3. There is no relationship between body mass and digesta retention time (Wenninger and Shipley, 2000); alternatively, a putative relationship disappears if feeding type is taken into account (Clauss et al., 2007a). Retention times do not scale with body mass at an exponent of 0.25 (reviewed in Clauss et al., 2013).
4. Digesta retention is negatively related to food intake (intra-specific examples in Clauss et al., 2007b; Munn et al., 2008; Hebel et al., 2011).

2. Methods

2.1. Experiments

The experiment was carried out with captive individuals of five gazelle and one dikdik species at Al Wabra Wildlife Preservation (AWWP), Qatar, similar to previous studies performed at this institution (Hummel et al., 2008; Hebel et al., 2011; Hummel et al., 2015). All individuals were adult, male and healthy according to the veterinarian in charge, and all procedures were approved by the internal ethics committee of AWWP. The experiment was carried out in three separate sessions: one in 2009 with four Dama gazelles (*Nanger dama*) and five Soemmerring's gazelles (*Nanger soemmerringii*), one in 2010 with seven Rheem gazelles (*Gazella subgutturosa marica*), and one in 2012 with five Idmi gazelles (*Gazella gazella*), five Speke's gazelles (*Gazella spekei*) and another six Soemmerring's gazelles, as well as seven Phillip's dikdiks (*Madoqua saltiana phillipsi*).

Prior to the measurements, all animals underwent a two-week adaptation period to the experimental diet, either in their usual outdoor pens where males were kept together, or in the experimental pens. Animals were then housed individually in separate indoor pens of 2.4 × 1.0 m size (Soemmerring's gazelles had access to two of these pens each in 2012); Dama gazelles were housed in their outdoor enclosures. Indoor and outdoor enclosures were arranged in such a way that animals could be moved to a second, similar enclosure during cleaning, feeding, and fecal collection. Outdoor pens were covered with natural sandy soil; the epoxide floors of the indoor pens were covered with a carpet on a bed of woodchips. For indoor pens, artificial light was provided from 6.00 to 18.00 and temperatures were kept between 19 and 25 °C. Throughout the adaptation phase and the experiments animals had *ad libitum* access to food and water. The animals investigated in 2009 and 2010 received limited amounts of fresh lucerne (*Medicago sativa*), grass hay (Rhodes grass *Chloris gayana*) *ad libitum* and, in the case of Dama and Soemmerring's gazelles, a pelleted compound feed (Dama: 18 to 22%, Soemmerring's: 12 to 21% of total dry matter (DM) intake). Subjects studied in 2012 received fresh lucerne *ad libitum* only (Table 1). Pens were cleaned on a daily basis. Animals were weighed prior to the experiments and food intake was determined by weighing diet items offered and the corresponding refusals for 6 to 7 days, accounting for drying losses for the fresh lucerne. Representative samples of food, refusals and feces were taken from all individuals and dried immediately at 50 °C. Samples were later analyzed by standard nutrient analyses (AOAC, 1995) for DM (AOAC no. 942.05), crude protein (AOAC no. 977.02) as well as neutral detergent fiber (NDF) (after treatment with α-amylase) (Van Soest et al., 1991) and acid detergent fiber (ADF; AOAC 973.18). Fiber values are expressed without residual ash. Analyses were performed in duplicate. The analysed nutrient contents of the diets offered to the animals are given in Table 1.

To measure retention times of particles and solute, two markers were used: chromium (Cr)-mordanted fiber from grass hay (<2 mm) and the water-soluble cobalt ethylene diaminetetracetic acid (Co-EDTA). Dikdiks received a smaller-sized Cr-marker ground to 0.5 mm as in a previous experiment in this species (Hebel et al., 2011). Markers were prepared according to Udén et al. (1980). Individuals were then fed with Cr-marker at 0.1 g/kg body mass (0.2 g/kg for dikdiks) and Co-EDTA at 0.01 g/kg (0.03 g/kg for dikdiks) dissolved in water. Markers were either fed to the animals after being mixed with a small amount of wheat bran, which was consumed within approximately 30 min, or applied directly *via* syringe into the oral cavity of manually restrained individuals (Idmi and Speke's gazelle, dikdik). Subsequent to the intake of the markers, feces were sampled on regular basis several times a day using different protocols between species due to husbandry specifics such as accessibility during daylight hours only *vs.* round-the-clock accessibility (Table S1).

All samples were immediately dried at 50 °C and later ground to 0.75 mm. Samples were analysed for marker concentration according to Behrend et al. (2004). After wet ashing with sulphuric acid (72%), Cr and Co concentrations were determined *via* atomic absorption spectroscopy. Values were corrected for the highest baseline concentrations of Cr and Co in the feces collected prior to the marker application. To avoid an artificial increase in retention measures by infinite excretion curves due to natural variation in baseline concentrations, values below a concentration of 1.5% (Cr) or 2.5% (Co) of the maximum

concentration in the excretion curve were set to 0 (adapted from Bruining and Bosch, 1992).

Following Thielemans et al. (1978), mean retention time (MRT) in the gastrointestinal tract (GIT) was calculated by weighting the sampling time by marker concentration and sampling interval duration:

$$MRT\ \ GIT = \sum(C_i \times dt \times t_i)/\sum(C_i \times dt)$$

where t_i = time after marker application in hours determined as the middle between two sampling intervals; dt = time interval represented between marker concentration calculated as $((t_{i+1} - t_i) + (t_i - t_{i-1}))/2$; C_i = fecal marker concentration at t_i in mg/kg DM.

Mean retention time of the solute marker in the reticulorumen ($MRT_{solute}RR$) was calculated according to Lechner-Doll et al. (1990) by estimating the rate constant of the descending part of the marker excretion curve *via* an exponential equation:

$$y = A \times e^{-k \times ti}$$

(y = fecal marker concentration at time (t_i) in mg/kg CM; A = a constant, k = the rate constant of the descending part of the excretion curve in h^{-1}; t_i = time after marker application in hour).

According to Hungate (1966), the reciprocal of k represents the MRT within the compartment characterized by k.

$MRT_{particle}RR$ is calculated as follows, based on the assumption that fluid and particles do not differ in passage characteristics distal to the RR (empirically confirmed by Grovum and Williams, 1973; Kaske and Groth, 1997; Mambrini and Peyraud, 1997):

$$MRT_{particle}RR = MRT_{particle}GIT - (MRT_{solute}GIT - MRT_{solute}RR).$$

The 'selectivity factor' (SF), defined as the quotient of particle over solute MRT, was calculated for both the total GIT and the RR by dividing the $MRT_{particle}$ by the corresponding MRT_{solute}.

2.2. Literature review

For a broader comparison, we carried out a literature review on retention times reported from other ruminant species. We selected those data on MRT in the GIT and the RR that were determined using the same markers (Cr-mordanted fiber and Co-EDTA or, if only a fluid marker was used, also Cr-EDTA), and where body mass (BM) and/or DM intake were reported. The references used are listed in the Supplementary material (Table S2), and the complete comparative datasets are available from the last author. Additionally, we compiled literature data on the natural diet of each species for which MRT data were available to determine the percentage of grass in the species natural diet as a proxy for feeding type; these sources are also listed in the Supplementary material (Table S3). Species means for all measures were first calculated as an average per source, and then as mean of all source averages. In total, we collated data from 40 different wild and domestic ruminant species.

2.3. Statistics

The relative DM intake (rDMI) was calculated using a higher exponent of $BM^{0.85}$ instead of the conventional 'metabolic body weight' ($BM^{0.75}$), following recent reports on mammalian herbivores (Hackmann and Spain, 2010; Müller et al., 2013; Riaz et al., 2014). In the experimental data from gazelles and dikdik, DMI scaled at $BM^{0.93(95\%\ CI\ 0.87;\ 0.99)}$, supporting a factor higher than 0.75. All data were checked for normal distribution by applying a Shapiro–Wilk test. Based on this we used ANOVA followed by Tukey HSD post hoc tests or Kruskal–Wallis tests followed by pairwise tests corrected for multiple comparison (R function: kruskalmc) for comparisons of SF, MRT and rDMI between the species investigated. In order to investigate statistical relationships, we applied general linear models using MRT or SF as dependent variable and BM, rDMI or the percentage of grass in the natural diet as independent variables. While analyses of the gazelle and dikdik data from the experiment were carried out using data of all individuals, the comparative analyses including literature data were performed on species means. To characterize feeding types, we used literature values for the %grass in the natural diet, independent of the diet animals actually received in experiments. Various studies have indicated that the main differences in digesta kinetics between ruminant species are evident irrespective of the diets fed (*e.g.* Renecker and Hudson, 1990; Lechner et al., 2010).

Not all publications used reported all MRT and SF measurements, hence the number of species varied between datasets (*e.g.*, $MRT_{particle}GIT$ had a higher species number than $MRT_{particle}RR$). Analyses of the larger comparative datasets were performed using both Ordinary Least Squares (OLS) and Phylogenetic Generalized Least Squares (PGLS); BM and the different retention parameters were log-transformed when related to each other in order to yield allometric relationships, whereas %grass and rDMI, and retention measures related to them only, were not. For PGLS analyses, data were linked to a supertree of extant mammals (Bininda-Emonds et al., 2007, 2008) and additionally to a more recent tree for cetartiodactyls (Hassanin et al., 2012). The value of the phylogenetic signal (λ) (Pagel, 1999) was estimated with maximum likelihood (Revell, 2010), using the PGLS command from the package caper (Orme et al., 2010). Generally, λ varies between 0 (no phylogenetic signal) and 1 (the observed pattern is predicted by the phylogeny; similarity among species scales in proportion to their shared evolutionary time) (Pagel, 1999; Freckleton et al., 2002). In interpreting the results of the PGLS analyses, we followed the scheme outlined in the Supplementary material (Fig. S1). An estimated λ of 0 indicates that there is no phylogenetic pattern in the dataset (Fig. S1AB), irrespective of whether (Fig. S1A) or not (Fig. S1B) the relationship between the two traits in question is significant. Correspondingly, an estimated λ of 1 indicates a clear phylogenetic structure in the dataset (Fig. S1C–F), again irrespective of whether (Fig. S1C–D) or not (Fig. S1F) the relationship between the two traits is significant. If the traits on the axes are changed (*e.g.* when plotting a retention measure on the y-axis against either BM or % grass on the x-axis), the data pattern will change and different values for λ will result. The absence of a phylogenetic signal in a significant relationship (Fig. S1B) is interpreted as evidence that the relationship represents a (mechanistic or functional) pattern that is not influenced by phylogeny, and could represent a convergence. All statistical tests were carried out in R 2.15.0 (R Core Development Team) using the package ape (Paradis et al., 2004), caper (Orme et al., 2010) and nlme (Pinheiro et al., 2011). In contrast to a common recommendation (Freckleton, 2009), we display results of both OLS and PGLS analyses, to elucidate which digestive parameters are tightly related to phylogeny as explained above. Significance levels were set to $\alpha = 0.05$, with values between 0.05 and 0.10 considered as trends.

3. Results

3.1. Measurements in the gazelle species and the dikdiks

Except for $MRT_{solute}RR$ ($F_{5,33} = 1.71$, $P = 0.161$), species had an effect on all retention time traits and selectivity factors ($MRT_{particle}GIT$: $F_{5,33} = 11.44$, $P < 0.001$; $MRT_{solute}GIT$: $F_{5,33} = 4.62$, $P = 0.003$; $MRT_{particle}RR$: $F_{5,33} = 11.64$, $P < 0.001$; SF GIT: $F_{5,33} = 7.83$, $P < 0.001$; SF RR: $F_{5,33} = 7.42$, $P < 0.001$) (Table 1, excretion curves displayed in Fig. 1 A–F).

As rDMI did not differ between species ($F_{5,33} = 1.87$, $P = 0.127$) and there was no significant relationship between rDMI and MRT or SF ($MRT_{particle}GIT$: $R^2 = 0.01$, $P = 0.575$; $MRT_{solute}GIT$: $R^2 = 0.02$, $P = 0.369$; $MRT_{particle}RR$: $R^2 = 0.01$, $P = 0.653$; $MRT_{solute}RR$: $R^2 = 0.03$, $P = 0.314$; SF GIT: $R^2 = 0.001$, $P = 0.829$; SF RR: $R^2 = 0.01$, $P = 0.515$), the observed differences in MRT between species cannot be explained by quantitative differences in intake.

Table 1
Body mass, food intake and retention measurements in the gazelle and dikdik species of this study.

Species	*Nanger dama*	*Nanger soemmerringii*		*Gazella subgutturosa marica*	*Gazella gazella*	*Gazella spekei*	*Madoqua saltiana phillipsi*
Common name	Dama gazelle	Soemmerring's gazelle[1]		Rheem gazelle	Idmi gazelle	Speke's gazelle	Phillip's dikdik
Year of experiment (number of animals)	2009 (4)	2009 (5)	2012 (6)	2009 (7)	2012 (5)	2012 (5)	2012 (7)
Diet	L, GH, P	L, GH, P	L	L, GH	L	L	L (leaves)
Crude protein (g kg DM^{-1})	169 ± 3	177 ± 7	201 ± 1	199 ± 9	234 ± 4	214 ± 3	295 ± 3
NDF (g kg DM^{-1})	483 ± 17	468 ± 51	411 ± 5	452 ± 24	389 ± 9	374 ± 14	206 ± 1
ADF (g kg DM^{-1})	275 ± 4	280 ± 16	309 ± 4	316 ± 2	304 ± 9	296 ± 12	151 ± 3
Body mass (kg)	61.1 ± 7.9^{a}	35.6 ± 3.7^{b}		17.6 ± 1.1^{c}	16.4 ± 1.3^{c}	13.0 ± 1.3^{c}	2.1 ± 0.1^{d}
DMI (kg d^{-1})	1.15 ± 0.08^{a}	0.80 ± 0.22^{b}		0.40 ± 0.09^{c}	0.36 ± 0.06^{c}	0.29 ± 0.02^{c}	0.05 ± 0.01^{d}
rDMI (g $kg^{-0.85\ d-1}$)	35.3 ± 5.0	38.2 ± 9.6		35.0 ± 7.1	32.8 ± 3.3	32.6 ± 4.7	28.6 ± 4.7
$MRT_{particle}GIT$ (h)	31.9 ± 5.3^{ab}	31.5 ± 6.2^{b}		40.7 ± 4.9^{a}	32.5 ± 3.4^{ab}	21.8 ± 3.7^{c}	25.3 ± 2.9^{bc}
$MRT_{solute}GIT$ (h)	20.5 ± 3.9^{abc}	24.7 ± 4.5^{b}		26.3 ± 3.6^{ab}	25.9 ± 2.2^{ab}	17.9 ± 2.3^{c}	21.8 ± 3.7^{abc}
SF GIT	1.58 ± 0.24^{a}	1.28 ± 0.18^{b}		1.55 ± 0.10^{a}	1.25 ± 0.08^{b}	1.22 ± 0.10^{b}	1.17 ± 0.13^{b}
$MRT_{particle}RR$ (h)	20.2 ± 3.6^{ab}	17.8 ± 3.8^{bc}		25.5 ± 3.1^{a}	17.6 ± 3.2^{bd}	13.0 ± 4.1^{cd}	13.7 ± 2.3^{cd}
$MRT_{solute}RR$ (h)	8.8 ± 2.2	11.1 ± 1.9		11.1 ± 1.2	11.1 ± 1.9	8.1 ± 3.1	10.2 ± 3.3
SF RR	2.41 ± 0.76^{a}	1.64 ± 0.39^{b}		2.31 ± 0.22^{a}	1.60 ± 0.21^{b}	1.63 ± 0.17^{b}	1.41 ± 0.31^{a}

ADF, acid detergent fiber; DM, dry matter; DMI, dry matter intake; GH, grass hay; GIT, gastrointestinal tract; L, fresh Lucerne; MRT, mean retention time; NDF, neutral detergent fiber; P, pelleted feed; RR, reticulorumen; rDMI, relative dry matter intake calculated as DMI divided by $BM^{0.85}$; SF, selectivity factor calculated as the ratio of $MRT_{particle}$ to MRT_{solute}.
Different superscript letters indicate significant differences between species within one row.
[1] See Table 2 for results of each group of Soemmerring's gazelles.

The species in this experiment received qualitatively slightly different diets (lucerne *vs.* lucerne + hay + pellets), which might have influenced MRT. In Soemmerring's gazelles, the only species that received both diet types, we found shorter $MRT_{particle}$ and lower SF when lucerne was the only feed than when lucerne, grass hay and pellets were offered ($MRT_{particle}GIT$: $T = 2.50$, $P = 0.041$; $MRT_{particle}RR$: $T = 4.06$, $P = 0.004$; SF GIT: $T = 2.98$, $P = 0.028$; SF RR: $T = 4.02$, $P = 0.007$). However, there were no differences in MRT_{solute} between sessions ($MRT_{solute}GIT$: $T = 0.50$, $P = 0.631$; $MRT_{solute}RR$: $T = -0.38$, $P = 0.715$) (Table 2). rDMI was similar in the two sessions ($T = -1.66$, $P = 0.145$). Together, this implies a difference in retention time due to different diets. The NDF content of the complex diet (46.8% in DM) tended to be slightly higher than in the pure lucerne diet (41.1% in DM) ($W = 26$, $P = 0.052$) (Table 1). However, even on the same diet some species differed in MRT (*e.g.* Speke's gazelle *vs.* Idmi, both on lucerne, Table 1), indicating that, despite similar intakes and diets, there must be other, possibly species-related factors causing differences between the species. In principle this could be BM. However, BM did not differ between Idmi and Speke's gazelle (Table 1). In addition, there was no significant relationship of any MRT with BM in the gazelle and dikdik data ($MRT_{particle}GIT$: $R^2 = 0.05$; $P = 0.182$; $MRT_{solute}GIT$: $R^2 < 0.001$; $P = 0.979$; $MRT_{particle}RR$: $R^2 = 0.07$, $P = 0.102$; $MRT_{solute}RR$: $R^2 = 0.002$, $P = 0.780$). SF values were significantly related with BM (SF GIT: $R^2 = 0.17$; $P = 0.009$; SF RR: $R^2 = 0.16$, $P = 0.011$). Still, the low coefficients of determination and the small slopes of the regression lines (SF GIT: 0.005; SF RR: 0.011) do not indicate a strong relationship.

3.2. Literature data

In OLS BM was not related ($P > 0.1$) with %grass, except for the largest dataset ($n = 37$) in the case of $MRT_{particle}GIT$ ($R^2 = 0.16$, $P = 0.014$). This positive relationship probably existed because species with a low %grass occurred across the whole BM range (1.5–800 kg), whereas species with a high %grass only occurred from a certain BM upwards (>12 kg) (Fig. 2). In PGLS, BM was weakly related with %grass in the same dataset and the dataset for $MRT_{particle}RR$ ($R^2 = 0.13$–0.14, $P = 0.03$–0.04), and there were trends ($P = 0.05$–0.08) towards a relationship in all other datasets. High λ values (0.93–1.00) found in the PGLS analysis indicate a strong phylogenetic structure in the relationship between BM and %grass.

There were significant allometric relationships between most retention measurements and BM, with scaling exponents of 0.08 to 0.13 in OLS; the 95% CI of these exponents did not go up to 0.25 (Table S4, Fig. 3). Only $MRT_{solute}RR$ did not scale with BM. Both SF GIT and SF RR also scaled with BM at 0.05 to 0.07 (Table S4) in OLS and PGLS. The λ was estimated as 0 for both $MRT_{particle}$ and for $MRT_{solute}GIT$, indicating that there is no phylogenetic structure in the relationship of these measures with BM. For SF GIT and SF RR, λ was numerically (albeit not significantly) higher than 0, suggesting some phylogenetic structure in the relationships of these traits with BM. For the relationship of BM and $MRT_{solute}RR$, λ was significantly different from 0. Together with the absence of a significant effect of BM, this indicates that, irrespective of BM, species groups differed systematically in this measure.

There was a trend ($P = 0.054$ to 0.106) for an inverse relationship between rDMI and $MRT_{particle}GIT$ in OLS and PGLS (Table S5; Fig. 4). For all other retention and SF measures, rDMI had no influence ($P > 0.1$); hence it was excluded from the subsequent analyses. The λ varied from 0 ($MRT_{particle}GIT$ and SF RR) to 1 ($MRT_{particle}RR$), with intermediate values for all other measures, indicating the presence of some phylogenetic structure in these relationships.

There was a positive relationship of %grass with $MRT_{particle}GIT$ and $MRT_{particle}RR$ in OLS and PGLS but not with any MRT_{solute}, and %grass was closely related with both SF measures (Table S6, Fig. 5A). The λ was again estimated as 0 for both $MRT_{particle}$, for $MRT_{solute}GIT$, and for SF RR, indicating the absence of phylogenetic structure in the relationship of these measures with %grass. For SF GIT, λ was numerically (albeit not significantly) higher than 0. For $MRT_{solute}RR$, λ was significantly different from 0; together with the absence of a significant effect of %grass, this indicates that, irrespective of %grass in their diet, ruminant groups differ systematically in this measure. The λ of 0 and the highly significant effect of %grass on SF RR indicate that across ruminant species (whether closely related or not) a high SF RR in mainly grazing species could be a convergence.

When relating BM and %grass simultaneously to retention measurements, BM was significantly related with both $MRT_{particle}GIT$ and to $MRT_{solute}GIT$, whereas %grass was not (Table 3). In both cases, λ was estimated as 0, suggesting that the MRT GIT traits are influenced by BM in a similar manner across taxonomic ruminant groups. For $MRT_{particle}RR$, the effect of BM was significant ($P = 0.010$–0.013) and that of %grass showed a tendency ($P = 0.081$–0.095), again with λ estimated as 0, suggesting that both BM and %grass have some influence on this measure, in a similar manner across taxonomic ruminant groups. In contrast, $MRT_{solute}RR$ was not related with either BM or %grass, yet showed a significant phylogenetic signal with λ at 0.95–0.98, indicating that ruminant groups differ in $MRT_{solute}RR$ irrespective of their BM or %grass. In other words, $MRT_{solute}RR$ emerges as a physiological

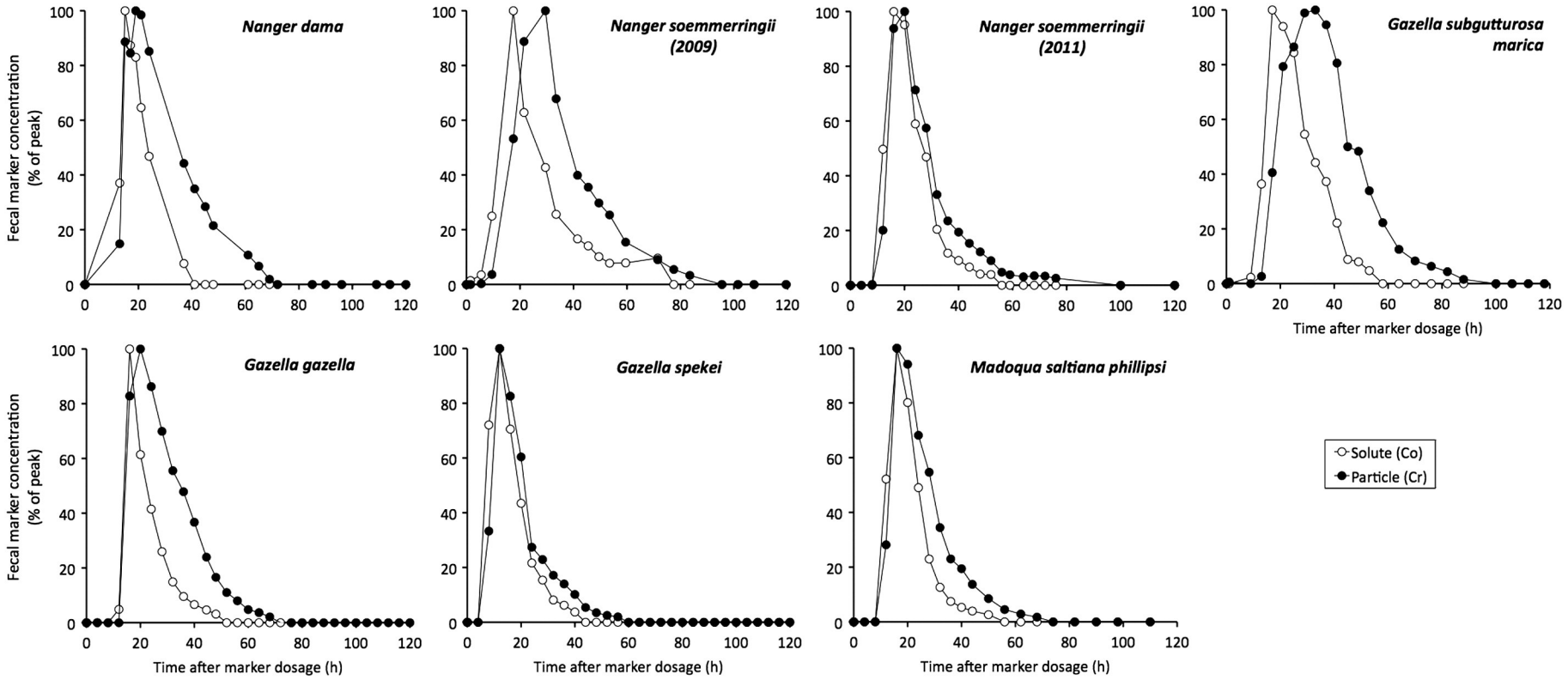

Fig. 1. Examples of excretion curves of a solute (Co-EDTA) and a particle (Cr-mordanted fiber, <2 mm) marker of one individual of Dama gazelle (*Nanger dama*), Soemmerring's gazelle (*Nanger soemmerringii*) in 2009 and in 2012, Rheem gazelle (*Gazella subgutturosa marica*), Idmi gazelle (*Gazella gazella*), Speke's gazelle (*Gazella spekei*), and Phillip's dikdik (*Madoqua saltiana phillipsi*).

Table 2
Body mass, food intake and retention measurements in the two different groups of Soemmerring's gazelles (*Nanger soemmerringii*) of this study.

Year of experiment (n animals)	2009 (5)	2012 (6)
Diet	L, GH, P	L
Body mass (kg)	36.7 ± 3.0	34.6 ± 4.2
DMI (kg d^{-1})	0.71 ± 0.21	0.87 ± 0.22
rDMI (g $kg^{-0.85}$ d^{-1})	33.4 ± 10.6	42.2 ± 7.0
$MRT_{particle}$GIT (h)	35.7 ± 5.81[a]	28.0 ± 4.1[b]
MRT_{solute}GIT (h)	25.5 ± 5.5	24.0 ± 3.8
SF GIT	1.42 ± 0.18[a]	1.17 ± 0.08[b]
$MRT_{particles}$RR (h)	21.0 ± 2.6[a]	15.2 ± 2.1[b]
MRT_{solute}RR (h)	10.8 ± 1.7	11.3 ± 2.1
SF RR	1.97 ± 0.30[a]	1.37 ± 0.17[b]

DMI, dry matter intake; rDMI, relative dry matter intake calculated as DMI divided by $BM^{0.85}$; MRT, mean retention time; GIT, gastrointestinal tract; SF, selectivity factor (ratio of $MRT_{particle}$ to MRT_{solute}); RR, reticulorumen; diet: L, fresh lucerne, GH, grass hay, P, pelleted feed.
Different superscript letters indicate significant differences between species within a row.

adaptation in which ruminant taxa differ, independent of size or diet. For SF RR, only %grass was significant, again with λ estimated as 0. This indicates that the variation in MRT_{solute}RR leads to a systematic tuning of SF RR to diet in a similar manner across ruminant groups, in a way probably best explained as a threshold effect (Fig. 5A), with general difference between species ingesting less and more than a threshold value of approximately 20% grass in the natural diet.

When relating MRT_{solute} with $MRT_{particle}$ for species in which both measures were available, MRT_{solute} was highly related to $MRT_{particle}$, with %grass having a significant additional, negative influence, but no effect of BM (Table 4, Fig. 5B). The λ was estimated to be significantly different from 0 for this relationship in the RR, again supporting the concept that MRT_{solute}RR is a taxon-specific characteristic.

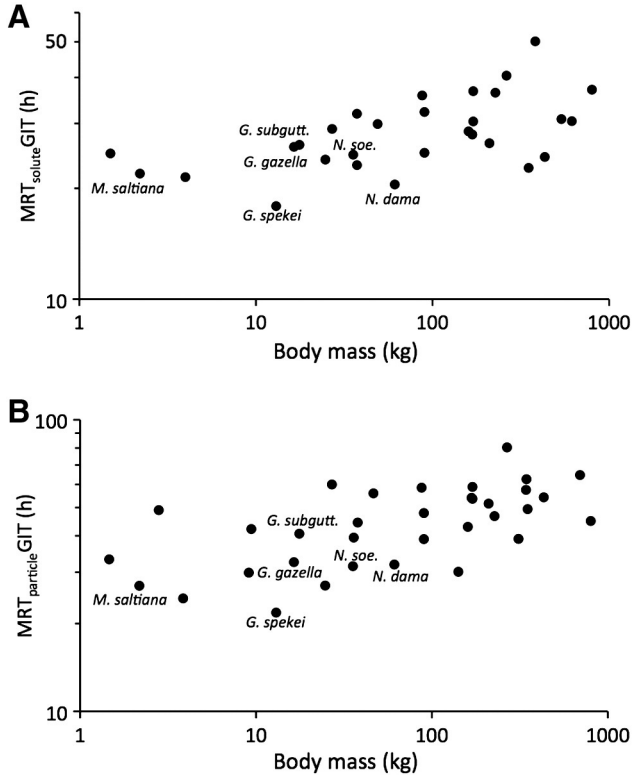

Fig. 3. Relationship between body mass and the mean retention time (MRT) of solute (A) and particle (B) (<2 mm) markers in different ruminant species. The species investigated in this study are indicated.

4. Discussion

This study is part of a continuing endeavor to understand rumen physiology by a comparative investigations of digesta retention in an ever-increasing number of ruminant species (Behrend et al., 2004; Flores-Miyamoto et al., 2005; Hummel et al., 2005; Hummel et al., 2008; Lechner et al., 2010; Clauss et al., 2011b; Hebel et al., 2011; Darlis et al., 2012; Hummel et al., 2015). The present results expand the existing dataset by five new species, and thus allow a further comprehensive evaluation of basic factors influencing ruminant digesta retention. They reveal complex relationships between digestive physiology, BM and putative adaptations of ruminant species to their natural diet.

4.1. Gazelle and dikdik results

The dataset based on the dikdik and gazelle species revealed clear differences in MRT between species, which can neither be conclusively explained by BM nor by food intake. In another study with dikdiks (Hebel et al., 2011), no relationship between BM and MRT was found, but an experimental reduction of intake increased MRT, indicating that the lack of relationship of MRT with rDMI in the present study could be explained by the low variation in rDMI as all animals were fed *ad libitum*. Wenninger and Shipley (2000) suggested in a study on blue duikers that the relationship between intake and retention time was masked by variations in fiber content of the diet, supporting that not only the amount but also the composition of food influences MRT.

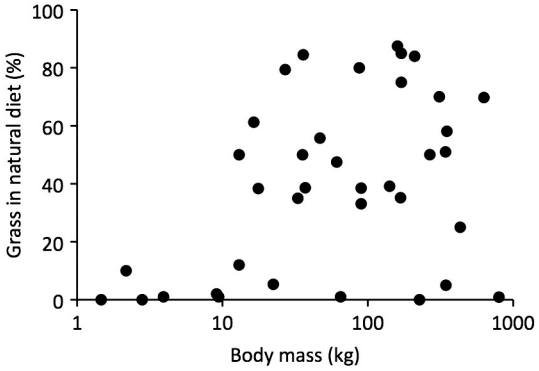

Fig. 2. Relationship between the percentage of grass in the natural diet (%grass) and body mass (BM) in 37 ruminant species for which data on the mean retention time of particles in the gastrointestinal tract was available.

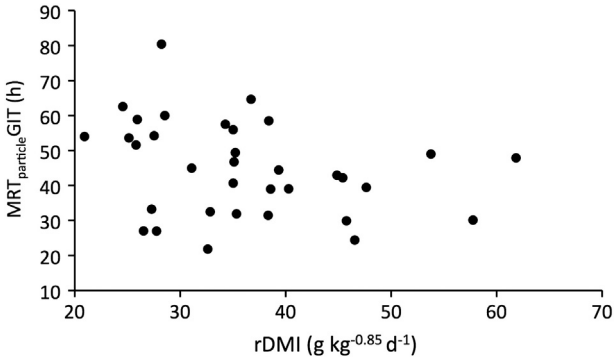

Fig. 4. Relationship between the relative dry matter intake (rDMI) and the mean retention time (MRT) of particle marker (<2 mm) in the gastrointestinal tract (GIT) in different ruminant species.

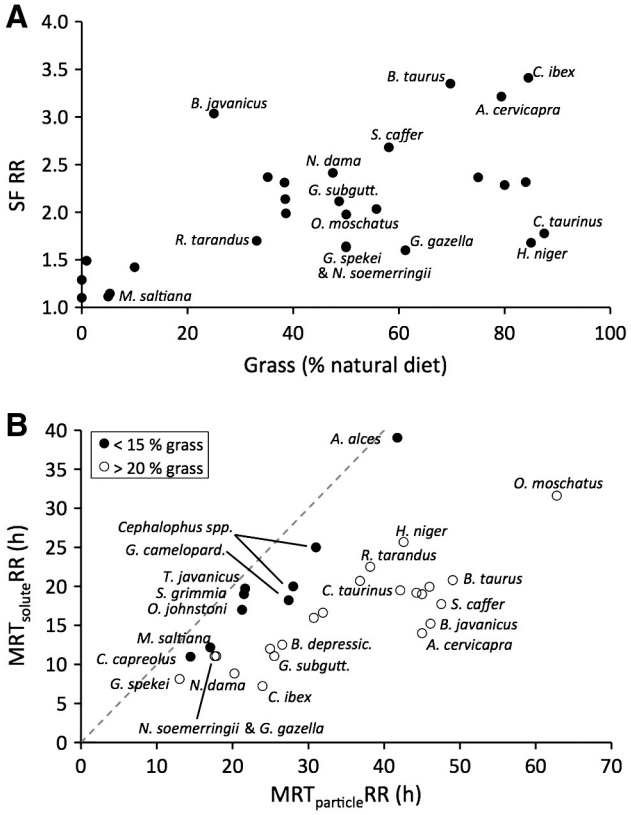

Fig. 5. Relationship between the percentage of grass in the natural diet (%grass) and the selectivity factor (SF) for the reticulorumen (RR) in different ruminant species (A), and between the mean retention time (MRT) of solutes and particles in the RR (visually separated by the %grass threshold; statistics performed with %grass as continuous variable) (B). The dotted line represents y = x.

Only Soemmerring's gazelles could be measured on two different diets for logistical reasons. These results indicate shorter MRT at similar DMI on the pure lucerne diet compared to a mixed diet consisting of grass hay, fresh lucerne and pellets. This finding could be explained by fibrous components being digested at a slower rate with the mixed diet. Additionally, the inclusion of grass hay could have increased the 'filter bed effect' that leads to a prolonged retention of small particles in various ruminant species of different feeding types when receiving a grass diet (Clauss et al., 2011a; Lauper et al., 2013). It remains to be determined whether such a difference in MRT between diets could also be found in the other species.

The results obtained from the dikdik and gazelle species support the concept that the difference between solute and particle retention is a species-specific, and hence a heritable, characteristic. This concept is supported by comparative analyses of this difference among ruminants (Clauss and Lechner-Doll, 2001; Clauss et al., 2006b), perissodactyls (Clauss et al., 2010c; Steuer et al., 2010), or mammals in general (Müller et al., 2011). In domestic ruminants, it has also been demonstrated that retention time characteristics are heritable traits: In cattle, 'frothy bloat' (a disease where rumen contents become extremely frothy) is linked to low saliva production (Mendel and Boda, 1961; Gurnsey et al., 1980) and long fluid retention in the rumen (Majak et al., 1986; Okine et al., 1989). Selective breeding against bloat susceptibility can be successful (Morris et al., 1997), a finding that indicates the potential for selective breeding for increased saliva production and hence increased rumen fluid throughput. Sheep selected for high wool growth (Thompson et al., 1989; Smuts et al., 1995) and for low methane production (Goopy et al., 2014) were found to have shorter MRT. As early as in 1966, it has been suggested that rumen retention characteristics offer a phenotypic trait for selective breeding in domestic ruminants (Hungate, 1966; Hegarty, 2004). The question about the biological relevance of the heritable retention characteristics remains. In order to further address this question, we performed the comparative analyses in >30 wild and domestic ruminant species.

4.2. Comparison across ruminant species

One finding that might complicate the interpretation of our results is the relationship between BM and %grass in the natural diet, which makes it difficult to unravel which one is the better predictor of MRT traits. But given the nature of this relationship, which depends in particular on the inclusion of small species and is not apparent above a BM of 10 kg, the simultaneous inclusion of both BM and %grass in GLM analyses is justified.

Table 3
Linear regression equations corresponding to log y = a + b logBM + c %grass for comparative datasets of ruminant species.

Dependent variable	n	Model	λ	BM b (95% CI)	t	P	%grass c (95% CI)	t	P
$MRT_{particle}$GIT	37	OLS	–	0.11 (0.06; 0.17)	3.96	<0.001	0.000 (−0.001; 0.002)	0.63	0.534
	36	PGLS[a]	0[c]	0.12 (0.06; 0.17)	4.09	<0.001	0.001 (−0.001; 0.002)	0.75	0.456
	35	PGLS[b]	0[c]	0.13 (0.08: 0.18)	4.77	<0.001	0.001 (−0.001; 0.002)	0.94	0.352
MRT_{solute}GIT	32	OLS	–	0.08 (0.04; 0.13)	3.68	0.001	0.000 (−0.001; 0.001)	0.06	0.955
	32	PGLS[a]	0[c]	0.08 (0.04; 0.13)	3.68	0.001	0.000 (−0.001; 0.001)	0.06	0.955
	31	PGLS[b]	0[c]	0.09 (0.04; 0.13)	3.66	0.001	0.000 (−0.001; 0.001)	0.00	0.999
SF GIT	32	OLS	–	0.05 (0.01; 0.09)	2.39	0.024	0.002 (0.001; 0.003)	3.11	0.004
	32	PGLS[a]	0[c]	0.05 (0.01; 0.09)	2.39	0.024	0.002 (0.001; 0.003)	3.11	0.004
	31	PGLS[b]	0[c]	0.05 (0.01; 0.09)	2.24	0.033	0.002 (0.001; 0.003)	3.17	0.004
$MRT_{particle}$RR	33	OLS	–	0.11 (0.03; 0.20)	2.74	0.010	0.002 (−0.000; 0.004)	1.73	0.095
	33	PGLS[a]	0[c]	0.11 (0.03; 0.20)	2.74	0.010	0.002 (−0.000; 0.004)	1.73	0.095
	32	PGLS[b]	0[c]	0.11 (0.03; 0.20)	2.66	0.013	0.002 (−0.000; 0.004)	1.81	0.081
MRT_{solute}RR	34	OLS	–	0.06 (−0.04; 0.16)	1.20	0.239	0.000 (−0.003; 0.002)	−0.26	0.798
	34	PGLS[a]	0.95[d]	0.10 (−0.02; 0.21)	1.63	0.113	0.000 (−0.003; 0.002)	−0.04	0.722
	33	PGLS[b]	0.98[d]	0.10 (−0.02; 0.22)	1.68	0.104	−0.001 (−0.004; 0.001)	−1.18	0.247
SF RR	32	OLS	–	0.04 (−0.02; 0.09)	1.20	0.240	0.003 (0.002; 0.004)	4.13	<0.001
	32	PGLS[a]	0[c]	0.04 (−0.02; 0.09)	1.20	0.240	0.003 (0.002; 0.004)	4.13	<0.001
	31	PGLS[b]	0[c]	0.04 (−0.02; 0.09)	1.17	0.251	0.003 (0.002; 0.004)	4.17	<0.001

GIT, gastrointestinal tract; MRT, mean retention time; n, number of species; OLS ordinary least squares; RR, reticulorumen; SF, selectivity factor calculated as the ratio of $MRT_{particle}$ to MRT_{solute}.

[a] Phylogenetic generalized least squares using the tree of Bininda-Emonds et al. (2007, 2008).

[b] Phylogenetic generalized least squares using the tree of Hassanin et al. (2012).

[c] λ significantly different from 1.

[d] λ significantly different from 0.

Table 4
Linear regression equations corresponding to log MRT_{solute} = a + b log $MRT_{particle}$ + c logBM + d %grass for a comparative datasets of ruminant species.

	n	Model	λ	Variable	Factor (95% CI)	t	P
MRT_{solute}GIT	32	OLS	–	$MRT_{particle}$GIT	0.70 (0.40; 1.00)	4.51	<0.001
				BM	−0.01 (−0.08; 0.05)	−0.46	0.648
				%grass	−0.001 (−0.002; 0.000)	−2.36	0.026
	28	PGLS[a]	0[c]	$MRT_{particle}$GIT	0.70 (0.40; 1.00)	4.51	<0.001
				BM	−0.01 (−0.08; 0.05)	3.94	0.648
				%grass	−0.001 (−0.002; 0.000)	2.55	0.026
	27	PGLS[b]	0[c]	$MRT_{particle}$GIT	0.71 (0.40; 1.03)	4.43	<0.001
				BM	−0.02 (−0.08; 0.05)	−0.49	0.626
				%grass	−0.001 (−0.002; 0.000)	−2.45	0.021
MRT_{solute}RR	32	OLS	–	$MRT_{particle}$RR	0.79 (0.47; 1.10)	4.93	<0.001
				BM	−0.001 (−0.08; 0.08)	−0.01	0.988
				%grass	−0.003 (−0.004; −0.001)	−3.75	<0.001
	28	PGLS[a]	0.70[c]	$MRT_{particle}$RR	0.63 (0.33; 0.92)	4.20	<0.001
				BM	0.04 (−0.05; 0.13)	0.82	0.417
				%grass	−0.002 (−0.004; −0.001)	−2.63	0.014
	27	PGLS[b]	0.69[c]	$MRT_{particle}$RR	0.64 (0.35; 0.94)	4.26	<0.001
				BM	0.03 (−0.06; 0.12)	0.67	0.506
				%grass	−0.002 (−0.004; −0.001)	−2.83	0.009

GIT, gastrointestinal tract; MRT, mean retention time; n, number of species; OLS, ordinary least squares.
[a] Phylogenetic generalized least squares using the tree of Bininda-Emonds et al. (2007, 2008).
[b] Phylogenetic generalized least squares using the tree of Hassanin et al. (2012).
[c] λ significantly different from 1.

Another limitation consists in the procedure of estimating MRT RR. This was done *via* equations based on the MRT GIT for many of the data used for the present evaluation and not measured directly by sampling through a rumen cannula. However, mathematical concepts applied to calculate MRTs in the RR were tested and approved by former studies (*e.g.* Hungate, 1966; Mambrini and Peyraud, 1997), and the estimates may therefore be considered reliable.

Assigning a natural diet to distinct ruminant species carries uncertainties. It is remarkable how little literature exists on the natural feeding habits of certain ruminant species, including most gazelles. Food preferences obviously vary over seasons and regions, which makes it even harder to classify them by a single number, *i.e.* the percentage of grass in the diet. It is particularly notable that some members of the bovini, such as the banteng (*Bos javanicus*), may have a much higher proportion of browse in their natural diet (Clauss and Hofmann, 2014) than previously suspected (*e.g.* by Clauss et al., 2006a).

Given the results of the present and previous evaluations, it appears important to clearly define the kind of retention measurement is referred to, *i.e.* total tract retention time (GIT) or retention time in the foregut (RR). Also the digesta phase in question, *i.e.* the excretion patterns of solute or particle markers, has to be specified. It should also be considered that the SF is calculated by two variables ($MRT_{particle}$ divided by MRT_{solute}). In cases where one of these variables alone is not significantly related to another factor, but SF is, this could result from two different issues. Either the sample size was not large enough to demonstrate an effect of the individual variables but was sufficient for detection of such an effect in their ratio, or the individual measures might not vary systematically across species yet in combination produce a consistent result. Alternatively, if one of the variables is significant and SF is as well, this likely indicates which variable is the one that mainly influences the SF estimate. In PGLS analyses, it was surprising that in several cases we found a phylogenetic signal for the MRT_{solute}RR but none in the respective $MRT_{particle}$RR and SF RR. This indeed suggests that a convergence in SF RR is achieved by a taxon-specific adjustment of MRT_{solute}RR.

A first factor of influence on taxon-specific adjustments could be food intake level. In contrast to our assumption, the relative food intake did not exert a major influence on retention parameters. This is surprising as digesta retention can be expected to be directly affected by the gut fill rate, as shown within individual ruminant species (Clauss et al., 2007b; Munn et al., 2008; Hebel et al., 2011). We only found a trend towards a negative relationship of $MRT_{particle}$GIT and rDMI, while MRT_{solute} appeared to be unaffected by intake. This corresponds to results of Müller et al. (2011), who interpreted that $MRT_{particle}$ traits are mainly dependent on BM and intake, which mirrors mere physical effects such as volume and throughput. In contrast, MRT_{solute} are determined by digestion type and therefore represent a specific physiological characteristic that may even be linked to an ecological adaptation. The finding that SF measures were independent of food intake levels within and across ruminant species corresponds to those reported earlier (Schwarm et al., 2009); this also applies to several nonruminant foregut fermenters, hindgut fermenters, and mammals in general (Schwarm et al., 2009; Müller et al., 2011; Clauss et al., 2014b).

We further investigated body mass as another factor of influence. As opposed to our initial hypothesis based on our previous findings (Clauss et al., 2007a), the literature dataset revealed a scaling with BM of all retention measures in the GIT and $MRT_{particle}$RR, with scaling exponents of both $MRT_{particle}$ measures at 0.12 to 0.14. Some former studies found exponents of 0.22 to 0.25 (Illius and Gordon, 1992; Robbins, 1993; Gordon and Illius, 1994). More recent evaluations with stricter data selection did not find strong allometric relationships in ruminants, with exponents ranging from 0 to 0.18 (Clauss et al., 2007a; Steuer et al., 2011; Müller et al., 2013). Digesta retention times in ruminants, hence, share the characteristic of several other temporal traits that have a lower BM scaling than expected by quarter-power scaling, such as generation time (Gaillard et al., 2005), age at first reproduction (Duncan et al., 2007), gestation period (Clauss et al., 2014a), longevity (Lemaître et al., 2014), herbivore chewing cycle duration (Gerstner and Gerstein, 2008) or chewing frequency (Shipley et al., 1994). Differences in scaling exponents might arise not only from different data selection criteria (*e.g.*, using only data from experiments with comparable methods, *cf.* Clauss et al., 2010c) and the accounting of phylogenetic dependence, but also from different species selections in the datasets. Larger BM appears to actually facilitate, to a certain degree, a longer MRT and also a higher SF in ruminants. A potential reason for this could be a disproportionately higher increase in RR volume with increasing BM (Clauss et al., 2003). In contrast to all other retention measurements, MRT_{solute}RR was independent of BM yet contained a strong phylogenetic signal, indicating that species vary in this measure irrespective of their size, but due to taxon-specific characteristics.

A third factor of influence may be the natural diet. As hypothesized, $MRT_{particle}$ and SFs were found to be related to %grass in ruminant diets; this is most likely due to a general positive relationship between %grass and RR volume (Clauss et al., 2003). The findings correspond to the

expectation derived from the finding that the fermentation of grasses, with their higher proportion of slowly degradable cell walls as compared to browse, takes more time (Hummel et al., 2006). In contrast, the MRT_{solute} traits were found to be independent from the natural diet, and $MRT_{solute}RR$ again contained a strong phylogenetic signal. Yet the relationship with the SF RR contained no phylogenetic signal, which indicates that ruminants of different taxonomic groups adjust their $MRT_{solute}RR$ in different ways to achieve a similar SF RR in relation to their natural diet.

The pattern of the relationship between %grass and the SF (Fig. 5A) deserves closer scrutiny. Ruminants vary in the degree that their RR contents are stratified. A high stratification can be accompanied by a higher difference in the amount of fluid present between the dorsal and the ventral RR contents (Clauss et al., 2009a; Clauss et al., 2009b) or a higher heterogeneity of the intraruminal papillation pattern (Clauss et al., 2009c). We previously found significant relationships between the intraruminal papillation pattern and the SF RR (Clauss et al., 2011b; Hebel et al., 2011), as well as between the intraruminal papillation pattern and the distribution of fluid in rumen contents (Codron and Clauss, 2010). This suggests that all three measures – SF RR, distribution of fluid in rumen contents, and intraruminal papillation pattern – are indicators of the same mechanism. While our explanatory focus was first on potentially beneficial effects of a stratification of the rumen content as such, more recent experimental work led us to hypothesize that this stratification was rather a by-product of another ultimate aim – the maximization of fluid throughput (Lechner et al., 2010; Clauss et al., 2011a; Lauper et al., 2013). All three measures are linked to %grass in a way that suggests a threshold effect: At very low %grass, SF RR is low (Fig. 5A), fluid is distributed homogenously across the rumen content, and the intraruminal papillation pattern is homogenous (Codron and Clauss, 2010). This was interpreted as a consequence of the necessity to produce saliva rich in tannin-binding proteins, which is therefore rich in protein, viscous, and of restricted volume (Hofmann et al., 2008; Clauss et al., 2009c; Codron and Clauss, 2010). However, when either side of the threshold is considered alone, the SF RR, the rumen contents' fluid distribution and the intraruminal papillation pattern do not necessarily change monotonously at increasing %grass as one would expect if RR physiology was a consistent mirror of natural feeding behavior. Rather, the measured ranges are simply unsystematically narrow below, and increase more or less unsystematically above the threshold, indicating that in particular above the threshold, 'anything goes' (Fig. 5A). In other words, above this threshold, other reasons for differences in RR physiology than %grass need to be invoked, which remain to be explored.

Finally, we evaluated the effects of body mass and diet simultaneously. This suggested that for MRT in the whole GIT of either particles or solutes, BM is the more important factor of influence. This in turn indicates that potential direct adaptations to feeding type are rather focused on the forestomach than on the whole GIT. Actually, although the original concept of feeding type adaptations of the ruminant GIT included also the intestines themselves (Hofmann, 1989), statistical demonstration of such adaptations has so far been provided only for the forestomach (Clauss et al., 2003; 2006a; 2009c; 2010a). Differences in the anatomy of the intestines have so far only been related to varying necessity to conserve water in different habitats (Woodall and Skinner, 1993; Clauss et al., 2004). Even though both $MRT_{particle}GIT$ and $MRT_{solute}GIT$ were positively related to BM, their ratio (SF GIT) also showed such a significant, positive relationship. Therefore, $MRT_{particle}GIT$ and $MRT_{solute}GIT$ must differ in their scaling with BM (with a steeper scaling for particles, as documented in Table S4). Because %grass was also significantly related to the SF GIT, it appears likely that this difference in scaling is an adaptation to feeding type. The finding that in the model that included both covariables, the relationship of MRT_{solute} and $MRT_{particle}$ was not influenced by BM but by %grass (Table 4), supports this interpretation. But rather than interpreting measurements related to the entire GIT in their relevance to dietary adaptation, we suggest that they should be considered as mere consequences of adaptations in the forestomach. The finding that $MRT_{particle}RR$ was not only related to BM, but also (though only as trend) to %grass matches the observation that RR capacity increases with both BM and %grass across ruminant species (Clauss et al., 2003). Given these determinants for $MRT_{particle}RR$, ruminant taxa on the other hand evolved morphophysiological adaptations controlling $MRT_{solute}RR$ so that it is independent from BM or %grass. The significant phylogenetic signal suggests that this heritable trait that is similar among more closely related species. As a result of such adaptations, the SF RR is tuned to correspond to the feeding type and, as stated above, possibly to other factors above a certain %grass threshold.

4.3. Implications for the relevance of difference in solute and particle retention time

A large number of older and recent *in vitro* studies with ruminant inoculum (*e.g.* Isaacson et al., 1975; Meng et al., 1999; Eun et al., 2004; Fondevila and Pérez-Espés, 2008; Martínez et al., 2009) or other *in vitro* work (Herbert et al., 1956; Tempest and Herbert, 1965), and more limited *in vivo* evidence in domestic cattle (*e.g.* Kropp et al., 1977; Bird et al., 1993) or sheep (Harrison et al., 1975) suggest that an increased fluid throughput will enhance microbial yield from fermentation chambers, and potentially also microbial fiber digestion. A likely explanation is that a high rumen fluid throughput, *i.e.* an intensive washout of microbes, keeps microbial populations in a stage of growth rather than maintenance (Isaacson et al., 1975), and that particularly fast-growing, and hence efficient, microbe strains are selected. In human subjects in whom digesta passage had been manipulated by medication, fecal inoculum from shorter retention time treatments actually yielded higher *in vitro* fermentation rates on a standard substrate than inoculum from longer MRT (El Oufir et al., 2000). This supports the concept that by shorter MRT, microbes of higher metabolic activity are selected. Increases in both, microbial yield and fiber digestion, represent important selective advantages in foregut fermenters, and thus could explain convergent evolution towards higher fluid throughput in various ruminant lineages above the relevant %grass threshold.

Yet, in spite of this theoretical and comparative evidence, proof that increased fluid throughput is relevant for animal growth or productivity is scarce. In sheep, the association of shorter MRT with higher wool growth was interpreted as an adaptation for a higher delivery rate of amino acids to the lower digestive tract (Thompson et al., 1989; Smuts et al., 1995). A higher rumen fluid throughput (*via* increased saliva production) could not only increase the microbial biomass harvest from the forestomach, or potentially increase the microbial digestive efficiency in the rumen, but due to the buffering effect of the saliva also reduce the susceptibility to acidosis and hence increase the capacity to use high-energy diets typical for modern livestock production systems (Hibbard et al., 1995).

Quite obviously, there must be an optimum fluid throughput above which microbes are washed out at a higher rate than they grow. This optimum will evidently vary with the rate at which particles themselves are moved from the fermentation system. Therefore, to achieve an optimal digesta washing, fluid throughput must be adjusted to the distinct conditions of particle retention. Hence, analyses aiming to explain $MRT_{solute}RR$ without considering $MRT_{particle}RR$ (as done in Table 3) indicate that $MRT_{solute}RR$ is a taxon-specific characteristic with strong phylogenetic signal and no influence of BM and natural diet. In contrast, when analyzing $MRT_{solute}RR$ in its dependence from $MRT_{particle}RR$ (as done in Table 4), the phylogenetic signal is reduced, but the natural diet contributes significantly, confirming that the taxon-specific $MRT_{solute}RR$ serves to achieve a specific degree of digesta washing in a way that is convergent across ruminant taxa. Investigating potential factors of influence on digesta washing beyond the %grass threshold, such as differences in grass quality (Codron et al., 2008b), and exploring degrees by which digesta washing could be optimized in domestic

ruminants (Clauss et al., 2010b), appear as promising areas of future research.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.cbpa.2015.01.013.

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