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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A B S T R A C T
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.
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 retention in browsing ruminants, which may have to secrete tannin-binding proteins in their saliva as a protection against the secondary plant for comparative evaluations. Our conclusions are based on data that have been assessed under similar conditions. Additional examples in Clauss et al., 2007b; Munn et al., 2008; Hebel et al., 2007a). Retention times do not scale with body mass at an exponent of 0.25 (reviewed in Clauss et al., 2013).

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 Gf 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 phillipsii).

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 indoor 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 diaminetetraacetic 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:

$$MRT_{GIT} = \frac{\sum(Ci \times dt \times ti)}{\sum(Ci \times dt)}$$

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 $((ti + 1 - ti) + (ti - ti - 1))/2$; $C_i =$ 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:

$$y = A \times e^{k \times t_i}$$

($y =$ 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 $k$.

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

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 BM0.85 instead of the conventional ‘metabolic body weight’ (BM0.75), 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, BMI scaled at BM0.85 (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. Remeke 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 ($\lambda$) (Pagel, 1999) was estimated with maximum likelihood (Revell, 2010), using the PGLS command from the package caper (Orme et al., 2010). Generally, $\lambda$ 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 $\lambda$ of 0 indicates that there is no phylogenetic pattern in the dataset (Fig. S1A), irrespective of whether (Fig. S1A) or not (Fig. S1B) the relationship between the two traits in question is significant. Correspondingly, an estimated $\lambda$ 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 $\lambda$ 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.
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 MRTparticle and lower SF when lucerne was the only feed than when lucerne, grass hay and pellets were offered. MRTparticleGIT was the only feed than when lucerne, grass hay and pellets were offered. 

MRTparticleGIT (h) 31.9 ± 5.3a 31.5 ± 6.2a 40.7 ± 4.9a 26.3 ± 3.6ab 17.6 ± 3.1b 17.6 ± 3.1b

MRTparticleRR (h) 8.8 ± 2.2a 11.1 ± 1.9a 11.1 ± 1.2a 11.1 ± 1.2a 8.1 ± 3.1b 10.2 ± 3.3b

SF RR 2.41 ± 0.76a 1.64 ± 0.39a 2.31 ± 0.22a 1.60 ± 0.21b 1.63 ± 0.17b 1.41 ± 0.31b

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 BM0.85; SF, selectivity factor calculated as the ratio of MRTparticle to MRTsolute.

See Table 2 for results of each group of Soemmerring’s gazelles.

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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 phillipsii).
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 MRTsoluteRR 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 MRTsolute with MRTparticle for species in which both measures were available, MRTsolute was highly related to MRTparticle, 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 MRTsoluteRR is a taxon-specific characteristic.

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.
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 \( MRT_{\text{particle}} \) to \( MRT_{\text{solute}} \).

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 (Hugate, 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 \( \log y = a + b \log BM + c \%\text{grass} \) for comparative datasets of ruminant species.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>n</th>
<th>Model</th>
<th>( \lambda )</th>
<th>BM (95% CI)</th>
<th>( t )</th>
<th>( P )</th>
<th>%grass (95% CI)</th>
<th>( t )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( MRT_{\text{par}} )</td>
<td>37</td>
<td>OLS</td>
<td>–</td>
<td>0.11 (0.06; 0.17)</td>
<td>3.96</td>
<td>&lt;0.001</td>
<td>0.000 (−0.001; 0.002)</td>
<td>0.63</td>
<td>0.534</td>
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<tr>
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<td>PGLS</td>
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<td>0.12 (0.06; 0.17)</td>
<td>4.09</td>
<td>&lt;0.001</td>
<td>0.001 (−0.001; 0.002)</td>
<td>0.75</td>
<td>0.456</td>
<td></td>
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<tr>
<td>35</td>
<td>PGLS</td>
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<td>0.13 (0.08; 0.18)</td>
<td>4.77</td>
<td>&lt;0.001</td>
<td>0.001 (−0.001; 0.002)</td>
<td>0.94</td>
<td>0.352</td>
<td></td>
</tr>
<tr>
<td>( MRT_{\text{sol}} )</td>
<td>32</td>
<td>OLS</td>
<td>–</td>
<td>0.08 (0.04; 0.13)</td>
<td>3.68</td>
<td>0.001</td>
<td>0.000 (−0.001; 0.001)</td>
<td>0.06</td>
<td>0.955</td>
</tr>
<tr>
<td>32</td>
<td>PGLS</td>
<td>0.01</td>
<td>0.08 (0.04; 0.13)</td>
<td>3.68</td>
<td>0.001</td>
<td>0.000 (−0.001; 0.001)</td>
<td>0.06</td>
<td>0.955</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>PGLS</td>
<td>0.01</td>
<td>0.09 (0.04; 0.13)</td>
<td>3.66</td>
<td>0.001</td>
<td>0.000 (−0.001; 0.001)</td>
<td>0.00</td>
<td>0.999</td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td>32</td>
<td>OLS</td>
<td>–</td>
<td>0.05 (0.01; 0.09)</td>
<td>2.39</td>
<td>0.024</td>
<td>0.002 (0.001; 0.003)</td>
<td>3.11</td>
<td>0.004</td>
</tr>
<tr>
<td>31</td>
<td>PGLS</td>
<td>0.01</td>
<td>0.05 (0.01; 0.09)</td>
<td>2.39</td>
<td>0.024</td>
<td>0.002 (0.001; 0.003)</td>
<td>3.11</td>
<td>0.004</td>
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</tr>
<tr>
<td>( MRT_{\text{par}} )</td>
<td>33</td>
<td>OLS</td>
<td>–</td>
<td>0.11 (0.03; 0.20)</td>
<td>2.74</td>
<td>0.010</td>
<td>0.002 (0.000; 0.004)</td>
<td>1.73</td>
<td>0.095</td>
</tr>
<tr>
<td>33</td>
<td>PGLS</td>
<td>0.01</td>
<td>0.11 (0.03; 0.20)</td>
<td>2.74</td>
<td>0.010</td>
<td>0.002 (0.000; 0.004)</td>
<td>1.73</td>
<td>0.095</td>
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<tr>
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<td>PGLS</td>
<td>0.01</td>
<td>0.11 (0.03; 0.20)</td>
<td>2.66</td>
<td>0.013</td>
<td>0.002 (0.000; 0.004)</td>
<td>1.81</td>
<td>0.081</td>
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<tr>
<td>( MRT_{\text{sol}} )</td>
<td>34</td>
<td>OLS</td>
<td>–</td>
<td>0.06 (−0.04; 0.16)</td>
<td>1.20</td>
<td>0.239</td>
<td>0.000 (−0.003; 0.002)</td>
<td>−0.26</td>
<td>0.708</td>
</tr>
<tr>
<td>34</td>
<td>PGLS</td>
<td>0.95</td>
<td>0.10 (−0.02; 0.21)</td>
<td>1.63</td>
<td>0.113</td>
<td>0.000 (−0.003; 0.002)</td>
<td>−0.04</td>
<td>0.722</td>
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</tr>
<tr>
<td>33</td>
<td>PGLS</td>
<td>0.98</td>
<td>0.10 (−0.02; 0.22)</td>
<td>1.68</td>
<td>0.104</td>
<td>−0.001 (−0.004; 0.001)</td>
<td>−1.18</td>
<td>0.247</td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td>32</td>
<td>OLS</td>
<td>–</td>
<td>0.04 (−0.02; 0.09)</td>
<td>1.20</td>
<td>0.240</td>
<td>0.003 (0.002; 0.004)</td>
<td>4.13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>32</td>
<td>PGLS</td>
<td>0.01</td>
<td>0.04 (−0.02; 0.09)</td>
<td>1.20</td>
<td>0.240</td>
<td>0.003 (0.002; 0.004)</td>
<td>4.13</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

\( \text{GIT, gastrointestinal tract; MRT, mean retention time; n, number of species; OLS ordinary least squares; RR, reticulorumen; SF, selectivity factor calculated as the ratio of } MRT_{\text{par}} \text{ to } MRT_{\text{sol}} \text{.} \)

\( a \) 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).

\( c \) \( \lambda \) significantly different from 1.

\( d \) \( \lambda \) significantly different from 0.
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 feed preferences and that a convergence in SF RR is achieved by a taxon-specific 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 MRTparticleRR with scaling exponents of both MRTparticle 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 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 MRTparticleGIT and rDMI, while MRTsolute appeared to be unaffected by intake. This corresponds to results of Müller et al. (2011), who interpreted that MRTparticle traits are mainly dependent on BM and intake, which mirrors mere physical effects such as volume and throughput. In contrast, MRTsolute 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 forest feeders, 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 MRTparticleRR with scaling exponents of both MRTparticle 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 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 MRTparticleGIT and rDMI, while MRTsolute appeared to be unaffected by intake. This corresponds to results of Müller et al. (2011), who interpreted that MRTparticle traits are mainly dependent on BM and intake, which mirrors mere physical effects such as volume and throughput. In contrast, MRTsolute 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 forest feeders, hindgut fermenters, and mammals in general (Schwarm et al., 2009; Müller et al., 2011; Clauss et al., 2014b).
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\textsubscript{solute} traits were found to be independent from the natural diet, and MRT\textsubscript{solute} 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\textsubscript{particle}, ruminant taxa on the other hand evolved morphophysiological adaptions controlling MRT\textsubscript{solute} 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 adaptions, 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 in-oculum (e.g. Isaacs 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 (Isaacs et al., 1975), and that particularly fast-growing, and hence efficient, microbial strains are selected. In human subjects in whom digesta passage had been manipulated by medication, fecal in unction 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 foreguard 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 salvia 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\textsubscript{solute} without considering MRT\textsubscript{particle} (as done in Table 3) indicate that MRT\textsubscript{solute} is a taxon-specific characteristic with strong phylogenetic signal and no influence of BM and natural diet. In contrast, when analyzing MRT\textsubscript{solute} in its dependence from MRT\textsubscript{particle} (as done in Table 4), the phylogenetic signal is reduced, but the natural diet contributes significantly, confirming that the taxon-specific MRT\textsubscript{solute} 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.

Acknowledgments

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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.cjbspa.2015.01.013.

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