Physical characteristics of rumen contents in two small ruminants of different feeding type, the mouflon (Ovis ammon musimon) and the roe deer (Capreolus capreolus)

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Abstract

In domestic ruminants, the stratification of forestomach contents – the results of flotation and sedimentation processes - is an important prerequisite for the selective particle retention in this organ. A series of anatomical and physiological measurements suggests that the degree of this stratification varies between browsing and grazing wild ruminants. We investigated the forestomach contents of free-ranging mouflon and roe deer shot during regular hunting procedures. There was no difference between the species in the degree by which forestomach ingesta separated according to size due to buoyancy characteristics in vitro. However, forestomach fluid of roe deer was more viscous than that of mouflon, and no difference in moisture content was evident between the dorsal and the ventral rumen in roe deer, in contrast to mouflon. Hence, the forestomach milieu in roe deer appears less favourable for gas or particle separation due to buoyancy characteristics. These findings are in accord with notable differences in forestomach papillation between the species. In roe deer, particle separation is most likely restricted to the reticulum, whereas in mouflon, the whole rumen may pre-sort particles to a higher degree. The results suggest that differences in forestomach physiology may occur across ruminant species.
Introduction

In domestic ruminants, the stratification of the ingesta in the reticulorumen (RR) represents an important, acknowledged mechanism contributing to the selective retention of particles in the rumen and, hence, to the high digestive efficiency in this organ (Lechner-Doll et al., 1991). This stratification is characterised by a dorsal gas dome, a “fibre mat” of particulate matter floating on a fluid phase, in which, at the very bottom, very dense, small particles form a “sludge” layer (Grau, 1955; Capote and Hentges, 1967). It should be noted that it may be especially difficult to detect the transition from the particle to the fluid layer in live or recently killed animals, if the RR volume is nearly completely used up by the particle phase (Kovács et al., 1997; Ahvenjärvi et al., 2001; Hummel et al., 2008a). Buoyancy characteristics of ingested forage particles (i.e., their propensity to float or sediment) help to establish this stratification (Sutherland, 1988). The stratification has various anatomical and physiological consequences, like the mentioned selective retention of particles, as assessed by the comparative passage of fluids and particles from the RR (Lechner-Doll et al., 1990), or regional differences in the short-chained fatty acid concentration in the RR (Smith et al., 1956; Tafaj et al., 2004), which actually lead to regional differences in papillary surface enlargement, with only small papillae in the dorsal (gas dome) or ventral (sludge layer) area of the RR, but large papillae in the region of the fibre mat (Hofmann and Schnorr, 1982).

While such a stratification has also been observed in grazing wild ruminants (Hofmann, 1973), it has been noted that wild ruminants of other feeding types, particularly browsers, do not display a stratified RR content but a rather homogenous, “frothy” mass, without a distinct separation of gas, particles, fluids, and sludge (Westerling, 1970; Hofmann, 1973; Hobson et al., 1976; Nygren and Hofmann, 1990; Renecker and Hudson, 1990). This absence of RR contents stratification in browsers has been at the core of a new explanatory hypothesis for the difference in RR physiology between grazing and browsing ruminants (Clauss and Lechner-Doll, 2001; Clauss et al., 2003; Clauss et al., 2006b; Clauss et al., 2006a; Clauss et al., 2008).
However, in contrast to fistulated domestic ruminants, the presence or absence of RR contents stratification is difficult to quantify in free-ranging wild ruminants.

In this study, we investigated the RR contents of free-ranging mouflon (*Ovis ammon musimon*) and roe deer (*Capreolus capreolus*) with respect to measurements related to the RR contents stratification. Within the ruminant feeding type classification (Hofmann, 1973; 1988; 1989), mouflon have been classified as grazers (Geiger et al., 1977) and roe deer as browsers (Hofmann et al., 1976). These species show anatomical and physiological differences considered typical for grazers and browsers, respectively, such as smaller salivary glands, larger RR, stronger rumen pillars, higher reticular crests, larger omasa, an uneven rumen papillation, a more diverse protozoal fauna, and a pronounced difference in fluid and particle passage from the RR, in mouflon, with opposite findings in roe deer (Drescher-Kaaden, 1976; Hofmann et al., 1976; Drescher-Kaden and Seifelnasr, 1977; Geiger et al., 1977; Enzinger and Hartfiel, 1998; Kamler, 2001; Behrend et al., 2004; Clauss et al., 2006b; Hofmann et al., 2008). Many reports on the diet of free-ranging mouflon emphasize the importance of grass for this species (Türcke and Schmincke, 1965; Stubbe, 1971; Onderscheka and Jordan, 1976; Garcia-Gonzalez and Cuartas, 1989; Faliu et al., 1990; Homolka, 1993; Hadjisterkotis, 1996; Cransac et al., 1997). In contrast, a comprehensive review on the natural diet of roe deer confirmed that this species consumes mainly browse and herbs, with grasses comprising only an annual average of about 5% of the diet (Cornelis et al., 1999). However, it has been observed that mouflon can also include a comparatively high proportion of browse in their natural autumn and winter diet (Homolka, 1991; Heroldova et al., 2007); therefore, one would more readily accept a classification of this species as an intermediate feeder (with a tendency towards grazing). For the purpose of this study, it is particularly important to notice that whenever mouflon and roe deer were compared in the same habitat, their diet was very dissimilar (Homolka, 1993; 1996).
We hypothesized that mouflon would have a higher degree of rumen contents stratification than roe deer. In particular, from our primary investigation in domestic cattle (Hummel et al., 2008a), we assumed that differences in stratification should be reflected by differences in the dry matter (DM) concentration in the respective regions (RR content being less moist dorsally than ventrally), and maybe also in particle size (larger particles in the dorsal than in the ventral RR contents). In order to investigate whether RR contents stratification could be explained by characteristics of the particulate or fluid RR contents, we investigated the flotation/sedimentation characteristics of the RR contents following the setup of Sutherland (1988), and determined the viscosity of RR fluid. We predicted that the forage ingested by roe deer, in contrast to that ingested by mouflon, shows no difference in particle size in the floating and sedimenting fraction (Clauss et al., 2001), and that the RR fluid of roe deer is more viscous than that of mouflon (Clauss et al., 2008).

**Materials and Methods**

*Animals*

Nineteen mouflon and 23 roe deer were available for this study. Mouflon were sampled during two consecutive hunting days at Seesen/Harz, Germany (51°53’N, 10°10’E), in the autumn of 2004. Roe deer were sampled during four different hunting days at Ebersberg, Germany (48°04’N, 11°58’E), in the autumn of the same year. Dead animals were brought to a central collection site by hunters within approximately 30 minutes after the shot. The animals were weighed and dissected immediately, and the complete gastrointestinal tract was transferred into isolated thermo boxes, placed in the physiological (upright) position. Subsequently, the RR were processed within the next hour. There was no systematic difference in the handling of carcasses and RR between the two species; all sampling procedures were performed by the same investigator.
RR dissection and sampling

The RR was placed on its left side; after opening a particular RR region, its contents were immediately sampled and processed before another region was opened. All incisions were placed in such a way that RR contents would not spill out of the opening. First, the reticulum was cut open at its cranial side, then the dorsal rumen close to its roof, then the ventral rumen close to its floor, and finally the omasum along its lesser curvature. Sampling generally took less than ten minutes. In order to gain material representative for any stratification, the sample from the dorsal rumen was taken from the material directly underneath the organ wall facing the investigator, and from the ventral rumen from material above the organ wall lying on the ground. In the case of the omasum, the material between the omasal leaves was sampled.

First, a sample for DM and particle size determination was taken, either by hand or – if the material contained separate liquid (which sometimes occurred in the reticulum and ventral rumen), by allowing the material to drop out of the reticulum or ventral rumen by gently massaging the outside of the organ. These samples were sealed watertight, and stored at -20°C until DM and wet sieving analysis.

Subsequently, the rumen was opened completely, the contents mixed, and, following the method described by Sutherland (1988) and Clauss et al. (2001), a subsample (volume of approximately 100 ml) was immediately placed into a longish plastic bag (10 x 35 cm, opening at the small end), and 1 l of warmed (37°C) buffer solution according to McDougall (1948) was added, containing 9.80 g NaHCO₃, 7.00 g Na₂HPO·7H₂O, 0.57 g KCl, 0.47 g NaCl and 0.12 g MgSO₄·7H₂O per l. The plastic bag was turned several times to achieve complete mixing of contents, and then placed vertically into a water bath (37 °C) by securing the top with a clothespin. After exactly two minutes, during which the particles of the RR contents subsample could float or sediment according to their density, the two fractions were separated by folding the bag in the middle across a stiff wire. The two resulting fractions
(designated as floating – f, and sedimenting – s) were put into individual sample containers and stored in a cooler at 4 °C until sieve analysis.

From the remaining RR contents, approximately 200 ml of rumen fluid were gained, by gently squeezing the ingesta through four layers of cheesecloth and catching the extruded fluid. This fluid was immediately centrifuged for 10 minutes at 3421 g (6000 rpm in the EBA 20 Tischzentrifuge, Hettich, Tuttlingen, Germany). The supernatant was sampled, carefully avoiding contamination with any underlying layers of debris. Since, similar to findings by Dardillat and Baumont (1992), preliminary comparisons had shown no effect of freezing and thawing on viscosity measurements in this fluid these samples were stored at -20°C until analysis. Rumen fluid of cattle has been subjected to a variety of viscosity measurements for comparative purposes, e.g. the comparison between diets or animals of varying susceptibility to bloat (e.g. Dardillat and Baumont, 1992; Stanford et al., 2001; Yabuuchi et al., 2007); there appears to be no common standard for this procedure. Therefore, we chose a method that allowed sample preparation in the field and comparison between species (cf. Hummel et al., 2008a).

Finally, mucosa samples of the dorsal and ventral rumen and the Atrium ruminis were cut out and stored in 10% formalin; these samples were later photographed for the illustration of differences in rumen papillation.

Analysis

Rumen samples were analysed for DM concentration by drying a representative subsample to constant weight at 103°C.

Particle size analysis was performed by a wet sieving technique (AS 200 digit, Retsch, Haan, Germany) using a column of seven sieves (square apertures of 4 mm, 2 mm, 1 mm, 0.5 mm, 0.25 mm, 0.125 mm, 0.063 mm); samples – either the complete sample from the flotation experiment, or a single contents sample of app. 7-9 g (less for omasal contents) -
were put on the column and sieved for 10 minutes at a water throughput of 2 litres per minute and a constant vibration (oscillation 2 mm). The particles of each sieve were transferred onto pre-weighed petri dishes, dried at 80 °C for a minimum of 24 hours, and weighed after cooling to room temperature in an exsiccator. The water and particles passing the finest sieve were lost. Results were expressed as cumulative proportions of the total weight of retained particles (starting at the largest sieve, with 100 % of particles retained on the smallest sieve).

As in Hummel et al. (2008c), the mean particle size (MPS, mm) of each sample was calculated numerically after fitting a suitable function to the respective sample data using the software TableCurve 2D v5.01 (Systat Software UK Ltd., London, UK). It should be noted that the MPS is a parameter calculated on the basis of the linear dimensions of the sieve holes. Rather than actually describing the mean particle size of the feces, it describes the average sieve hole size through which the particles passed.

After thawing, the viscosity of the rumen fluid supernatant was measured at 37 °C using 16 ml in a Brookfield LVDVE230 viscosimeter (Serial Number E6536, Brookfield Engineering Laboratories, Middleboro, MA, USA) with a UL/Y adapter and spindle (rotational system with concentric cylinder as recommended by Lentle and Janssen, 2008). With this system, the shear rate is calculated as 1.29 x revolutions s\(^{-1}\); the producer recommends not to measure a water-like substance at more than 1.29 s\(^{-1}\). Preliminary investigations indicated that rumen fluid was pseudoplastic or shear-thinning (cf. the decrease in apparent viscosity in roe deer rumen fluid with increasing shear rate, Fig. 1). As the aim of this study was not to completely characterise the viscosity of fluid (or RR contents) but generate comparative data for the two species, it was decided to measure the apparent viscosity at the highest possible shear rate with our system, i.e. 1.29-2.15 s\(^{-1}\).

Afterwards, the DM concentration of the supernatant was determined by drying at 103°C to constant weight. Finally, several samples of the remaining DM were pooled for nitrogen...
analysis (n = 12 in mouflon and n = 7 in roe deer) using the Kjeldahl method (Kjeltec 2400, Foss, Hamburg, Germany) and expressed as crude protein (6.25 x N).

**Statistical analysis**

Results are expressed as means ± standard deviation. Differences between species were tested by independent t-test or Mann-Whitney U-test. Differences within a species between the forestomach regions were tested by repeated measurement ANOVA and subsequent paired t-tests. If ANOVA presumptions were not met, the Friedman test and subsequent pairwise Wilcoxon tests were used. Multiple tests, including between species-comparisons by t-tests or U-tests for a parameter, were Dunn-Sidak adjusted. All analyses were performed with SPSS 16.0 (SPSS Inc., Chicago, IL).

**Results**

**Qualitative observations: rumen mucosa**

In the mouflon, there was a marked difference in papilla size between both the dorsal and ventral rumen wall on the one, and the atrium on the other hand (Fig. 2a). In contrast, such a difference was less distinct in the roe deer (Fig. 2b). These qualitative observations illustrate the quantitative comparison of rumen mucosa between the roe deer and sheep by Enzinger and Hartfiel (1998).

**Qualitative observations: RR contents**

The RR of mouflon was always filled completely with fibrous material. The material often had a dry appearance. At the ventral RR floor, there was always more (watery) fluid than in the rest of the RR. During filtration, the RR fluid appeared watery. The RR of roe deer was always filled with fibrous plant particles mixed into seemingly homogenous, frothy contents;
there was no agglomeration of fluid visible anywhere in the RR. During filtration, the RR fluid appeared thick and often creamy.

*Dry matter concentration*

Comparing between species, mouflon had significantly drier ingesta than roe deer for any forestomach region (t-test, always p<0.001; Fig. 3). Within species, there were significant differences between the forestomach regions (RM-ANOVA, p<0.001). The pair-wise post-hoc tests revealed the following results: Omasum contents were consistently drier than the other ingesta in either species (always p<0.001). In mouflon, DM concentration decreased from the dorsal to the ventral rumen (p=0.024) to the reticulum (dorsal rumen-reticulum: p<0.001; ventral rumen-reticulum: p=0.012). In roe deer, DM concentration of dorsal and ventral rumen was similar (p=0.156), but that of the reticulum was significantly lower (p<0.001 for comparisons to both dorsal and ventral rumen).

*Mean particle size*

Comparing between species, the MPS of the ingesta of any forestomach region was always higher in mouflon than in roe deer (U-test, always p<0.001; Fig. 4). Within both species, there were significant differences between the forestomach regions (Friedman test, p<0.001). Omasum contents were consistently finer than the other ingesta (Wilcoxon test, always p<0.001). In mouflon, we observed no difference in the MPS between the dorsal and the ventral rumen (Wilcoxon test, p=0.872), and MPS only tended to decrease from the ventral rumen to the reticulum (Wilcoxon test, p=0.036; non-significant after adjustment; dorsal rumen-reticulum p=0.184). In roe deer, there was a non-significant numerical decrease of MPS from the dorsal to the ventral rumen (Wilcoxon test, p=0.274); the reticulum contained finer particles than the dorsal rumen (Wilcoxon test, p=0.011) but not the ventral rumen (Wilcoxon test, p=0.101).
**RR contents: particle characteristics**

Mouflon tended to have less floating particles (calculated as the weight percentage of floating particles of the total weight of particles [floating and sedimenting]) in the total RR contents than roe deer (mouflon: 38 ± 20 %; roe deer: 52 ± 26 %; t-test p=0.055). The MPS of the floating and the sedimenting particle fractions differed significantly in both species (mouflon: MPS floating fraction 11.0 ± 3.6 mm, MPS sedimenting fraction 5.7 ± 4.2 mm, paired t-test p<0.001; roe deer: MPS floating fraction 3.7 ± 2.2 mm, MPS sedimenting fraction 1.6 ± 1.1 mm, paired t-test p<0.001).

**RR contents: fluid characteristics**

Between the species, there were significant differences in the apparent RR fluid viscosity (mouflon: 1.83 ± 0.30 mPas; roe deer 3.20 ± 1.41 mPas; t-test p<0.001) as well as in the DM concentration of centrifuged RR fluid (mouflon: 2.1 ± 0.1 %; roe deer 2.7 ± 0.3 %; t-test p<0.001), with roe deer having a more viscous RR fluid with a higher DM concentration. RR fluid viscosity and DM concentration were not correlated in mouflon (Pearson’s R=0.14, p=0.560), but were significantly correlated in roe deer across animals (Pearson’s R=0.81, p<0.001). Additionally, roe deer RR fluid had a nearly sevenfold higher crude protein concentration (mouflon: 2.6 ± 1.9 % DM; roe deer 17.5 ± 4.1 % DM; t-test p<0.001).

**Discussion**

The results of our study allow further insight into the characteristics and potential drivers of RR contents stratification in free-ranging ruminants. While some of our predictions were confirmed by the results, others were not. In the following, we first discuss results with relevance to the characteristics of the ingested forages, and then those with relevance to the
conditions in the RR itself, trying to link our findings to generally accepted concepts of RR function.

Some evident limitations to this study need to be stated first. The fact that shot animals were used that could not be held in physiological (upright) position between death and investigation (even if forestomachs were stored in upright position prior to dissection), will make the results less reliable than those gained from living, fistulated animals. Although an exchange of contents from the different forestomach areas subjectively appeared unlikely, due to the high degree of fill (and the expected difference in moisture content of the reticulum and the omasum as compared to the rumen areas), it cannot be excluded. Additionally, differences in the time elapsed since the last foraging bout surely contributed to data scatter. Although there was no systematic difference in the way the two species were handled, and the focus of this study was comparative, the results need to be considered with caution.

Forage characteristics

Different reports on the fracture and buoyancy characteristics of monocot and dicot material suggest that systematic differences might exist between these forage groups (collated in Clauss et al., 2008); however, systematic investigations are lacking. In a previous study in roe deer, in which a similar technique for the separation of floating and sedimenting fractions of RR ingesta had been used (Clauss et al., 2001), no particle size difference in the floating and the sedimenting fraction had been measured. At the time, it had been concluded that the diet of the roe deer investigated in that study did not separate itself according to size along the density gradient. In retrospect, it cannot be decided whether this absence of density separation according to size in the earlier study was due to a different autumn diet consumed by the roe deer investigated at that time, or due to the fact that Clauss et al. (2001) subjected 300 ml of RR contents, rather than 100 ml as in the present study, to the flotation/sedimentation separation. One could argue that with less material taking up the same space in the plastic
bags, a separation according to density could occur more quickly. In the present study, the material subjected to flotation/sedimentation separation always separated easily, either assembling at the top or the bottom of the fluid column, without relevant portions in the middle; in contrast, in Clauss et al. (2001), a ‘middle layer’ had also been identified, which could have been due to a lack of space in the flotation bag. The results of this study support the concept that RR ingesta, irrespective of its origin, will separate according to buoyancy characteristics. Such general characteristics of forage (in the process of digestion) could be prerequisite for the evolution of universal, density-dependent physiological mechanisms as suggested not only for ruminants and camelids (Lechner-Doll et al., 1991) but also nonruminant foregut fermenters (Clauss, 2004; Clauss et al., 2004; Schwarm et al., 2008).

**RR contents characteristics**

The uniformity of measures performed on the ingesta contrasts with the notable differences in the stratification of forestomach contents between the two investigated species. The evident difference in the papillation pattern displayed in Fig. 2 already suggest that in roe deer, factors limiting papillary growth, such as a dorsal gas dome or a ventral sludge layer, are less pronounced than in mouflon. Although the absence of a dorsal gas dome could not be demonstrated directly in the present study, this has been suggested in another browser, the moose (*Alces alces*), based on an ultrasonographic investigation of a live specimen, and which contrasted to the clearly visible gas domes found in domestic cattle (Tschuor and Clauss, 2008). The absence of both, a distinct gas dome and a distinct sludge layer, could theoretically be explained by a higher viscosity of both the rumen contents and the rumen fluid in the browsing ruminants, which leaves both gas bubbles and fine, high-density particles suspended in the fluid rather than allowing them to rise or sink within the RR. The subjective impression regarding the consistency of the rumen fluid, as well as the quantitative comparison of the RR fluid viscosity, corroborate this hypothesis. In a similar way, Jones et
al. (2001) observed that the RR fluid of several free-ranging, wild African ruminants subjectively appeared thicker in browsers as compared to grazers.

The findings on the centrifuged RR fluid suggest that this increased viscosity in roe deer was associated with elevated DM and protein concentrations. Jones et al. (2001) also found a comparatively high DM concentration in the RR fluid of African browsing ruminants. In the RR fluid of domestic cattle suffering from frothy bloat (a condition that has been compared to the frothy RR contents in browsing ruminants, Clauss et al., 2006a), the viscosity-inducing substances are either proteinaceous or mucopolyssaccharides of either plant or microbial origin (Cheng et al., 1998). In browsing ruminants such as roe deer, an additional viscosity-inducing factor might be the saliva. Robbins et al. (1987; 1995) observed that the saliva of a browsing cervid, the mule deer, was of egg albumen-like consistency, in contrast to the watery saliva of domestic ruminants, and the difference in salivary gland size between grazing and browsing ruminants has been hypothesized to be related to salivary protein concentration (Hofmann et al., 2008). Which of the mentioned factors – plant or animal characteristics – are responsible for the difference in RR fluid viscosity demonstrated in the present study cannot be decided.

The fact that in a captive moose, Renecker and Hudson (1990) did not observe differences in the relative excretion pattern of fluids and particles from the RR, irrespective of the different forages fed, could be an indication that animal factors may be important determinants of RR fluid viscosity and hence RR contents stratification.

Even though, as discussed above, ingesta from both species separated according to particle size due to its buoyancy characteristics in vitro, the situation in the densely packed suspension of the RR is different (Lentle and Janssen, 2008). Buoyancy and sedimentation will probably occur at much slower rates in this medium. The higher viscosity of the roe deer RR fluid, and the (yet to be quantified) potentially higher viscosity of the whole RR contents in this species, will make particle separation additionally less expeditious, and complex particle aggregation mechanisms will lead to the formation of a particle mat (Lentle and Janssen, 2008). A
stratification of rumen contents in terms of particle size distribution could not be demonstrated in this study, neither in roe deer, nor in mouflon (Fig. 3). Similarly, studies in domestic cattle showed that – especially in RR that are packed with contents due to recent forage ingestion – differences in the particle size pattern between the dorsal and the ventral rumen may be difficult to demonstrate (Hummel et al., 2008a). The only reliable indicator of a RR contents stratification that was independent from the time since the last meal and forage type identified in domestic cattle was the DM concentration of the different forestomach regions (Hummel et al., 2008a). This variable showed a difference between the dorsal and the ventral rumen in mouflon but not in roe deer, indicating a saturation of the ingesta with a low-viscosity fluid in the former but not in the latter species. In roe deer, fluid and particles appear to be in a funicular state (Lentle and Janssen, 2008), which means that a liquid surface film of the particles forms bridges in which surface tension and capillary suction hold the particles together; such a state is enhanced by an increased viscosity of the fluid. This cohesion or funicular state will lead to a comparatively simultaneous passage of fluids and particles from the RR in roe deer (Behrend et al., 2004). Conversely, the saturation of RR ingesta with low-viscosity fluid in mouflon explains the distinct separation of fluid and particle passage in this species (Behrend et al., 2004).

RR physiology

The most intriguing question about the physiology of the ruminant forestomach is how the separation of large particles (that need to be ruminated) and small particles (that can be passed on to the lower digestive tract) is achieved. The answer lies in the processes of flotation and sedimentation (Lechner-Doll et al., 1991): from the reticulum, larger, floating particles are rejected into the rumen, whereas the small, dense particles are passed on into the omasum; if the motility of the reticulum is impaired, then larger than usual particles can also escape into the lower digestive tract (Kaske and Midasch, 1997). This current understanding focuses on
the reticulum as the main site of particle separation; accordingly, the DM concentration in the reticulum is lower than in the rumen (Fig. 3), as for this separation, a more fluid medium is prerequisite.

This interpretation of the reticulum as the only site of particle separation appears to apply directly to the roe deer of this study: in the roe deer, the main step of particle separation (size reduction from 0.95 to 0.24 mm, i.e. 0.71 mm) in the forestomach occurs between the reticulum and the omasum (Fig. 4). In mouflon, however, particle size does not only decrease drastically between the reticulum and the omasum (by 1.97 mm), but also already between the ventral rumen and the reticulum (by 0.75 mm, Fig. 4; the difference between the ventral rumen and the reticulum was not significant due to adjustment for multiple comparisons). In cattle, finally, Hummel et al. (2008a) found a significant decrease in particle size already between the dorsal and the ventral rumen, and between the ventral rumen and the reticulum as well. These findings suggest that among ruminant species, there may be differences in the extent to which not only the reticulum, but also the rumen itself is involved in the process of particle separation. In the three examined species, only those that offer a low-viscosity rumen fluid (mouflon, cattle) may achieve a particle size separation already in the rumen itself; note that other factors than just fluid viscosity, such as the yet-to-be-described physical properties of whole ingesta (Lentle and Janssen, 2008) will play the major role here. This could be a reason for the claim of Hofmann (1989; note that this observation still has to be quantified) that browsing ruminants have a comparatively larger reticulum. If, in grazers such as mouflon or cattle, there is a higher degree of ‘pre-sorting’ of the ingesta entering the reticulum, this organ might not need to be of the same size as in browsers. However, until more ruminant species are investigated, we cannot rule out that the ‘pre-sorting’ effect of the rumen is mainly a question of organ size and, hence, body mass of the species, as the roe deer is the smallest of the three mentioned species. Model calculations will have to elucidate the benefit of a more efficient sorting mechanism in the rumen, which is linked to a low-viscosity fluid and most
likely a high fluid throughput (Clauss et al., 2006a). One potential advantage has recently been proposed by Hummel et al. (2008b) – that, according to literature on domestic ruminants, efficiency of microbial growth and supply of valuable microbial protein to the small intestine, increases with increasing fluid throughput in the forestomach. The concomitant stratification of contents in the rumen will lead to a reduction of the total surface available for absorption – due to less papillation in the dorsal and ventral areas. But as the natural diet of grazers shows comparatively slower fermentation rates (Hummel et al., 2006) this loss may not be prohibitive for grazers. In contrast, strict browsers with a diet of high fermentation rates (Hummel et al., 2006), might need to prevent a distinct contents stratification in the rumen in order to retain the full integrity of the papillary absorptive surface.

Foraging ecology

Finally, the significantly higher proportion of floating material in the roe deer forestomachs is striking. On the one hand, the forage presumably ingested by roe deer (especially herbs) should show a faster fermentation than that ingested by mouflon (grass) (Hummel et al., 2006). On the other hand, it has been suggested that browsers feed more frequently than grazers in general (Hofmann, 1989), and with respect to the species investigated here, the data reviewed in Hummel et al. (2006) actually indicate a higher feeding frequency in roe deer as compared to mouflon. The presumed rapid fermentation of roe deer forage, together with more frequent feeding bouts in this species, could explain that on average, roe deer forestomach contents contained more floating material, i.e. material at the peak of fermentative activity.

Conclusion
The data presented in this study support the concept that differences in the physiology of the forestomach exist between ruminant species. Whether these differences are due to feeding niche or phylogenetic descent would have to be tested by applying similar techniques to a larger set of species. The findings open the possibility that the evolution of conditions favouring a low-viscosity RR fluid and a more distinct stratification of RR contents could be a step characteristic for more recently evolved ruminants. Further studies should aim at determining the viscosity and physical properties of whole RR ingesta in different ruminant species, and the effects of different forages.

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Literature


Clauss, M., 2004. The potential interplay of posture, digestive anatomy, ingesta density and gravity in mammalian herbivores, or why sloths do not rest hanging upside down. Mammal Rev. 34, 241-245.


Acta Theriol. 46, 103-107.

Clauss, M., Hofmann, R.R., Hummel, J., Adamczewski, J., Nygren, K., Pitra, C., Reese, S., 2006b. The macroscopic anatomy of the omasum of free-ranging moose (Alces alces) and muskoxen (Ovibos moschatus) and a comparison of the omasal laminal surface area in 34 ruminant species. J. Zool. 270, 346-358.


Säugetierzool. Mitt. 25, 7-21.


Fig. 1. Decreasing apparent viscosity of the rumen fluid of four different individual roe deer (*Capreolus capreolus*) with increasing shear rate.
Fig. 2. Typical appearance of the rumen mucosa of a) mouflon (*Ovis ammon musimon*) and b) roe deer (*Capreolus capreolus*) at the dorsal rumen wall (above), the atrium ruminis (middle) and the ventral rumen wall (below) in three individuals of each species. Note the difference in papillation in the mouflon, reflecting a rumen contents stratification, and the uniformity in roe deer, reflecting less stratified rumen contents. One rectangle represents a basal area of 9.4 cm².
Fig. 3. Average (± standard deviation) dry matter concentration (in % of wet weight) of the ingesta at different regions in the forestomach of mouflon (Ovis ammon musimon) and roe deer (Capreolus capreolus). Different superscripts within a species indicate significant differences between regions.
Fig. 4. Average (± standard deviation) mean particle size (mm) of the ingesta at different regions in the forestomach of mouflon (*Ovis ammon musimon*) and roe deer (*Capreolus capreolus*). Different superscripts within a species indicate significant differences between regions. Note that in mouflon, the difference between the ventral rumen and the reticulum significance was lost due to adjustment for multiple comparisons.