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Abstract

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The intraruminal papillation gradient in wild ruminants of different feeding types: implications for rumen physiology

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Running head: Papillation patterns in ruminants

Abstract

Browsing and grazing ruminants are thought to differ in the degree their rumen contents are stratified – which may be due to different characteristics of their respective forages, to particular adaptations of the animals, or both. However, this stratification is difficult to measure in live animals. The papillation of the rumen has been suggested as an anatomical proxy for stratification – with even papillation indicating homogenous contents, and uneven papillation (with few and small dorsal and ventral papillae, and prominent papillae in the atrium ruminis) stratified contents. Using the surface enlargement factor (SEF, indicating how basal mucosa surface is increased by papillae) of over 55 ruminant species we demonstrate that differences between the $\mathrm{SEF}_{\mathrm{dorsal}}$ or $\mathrm{SEF}_{\mathrm{ventral}}$ and the $\mathrm{SEF}_{\mathrm{atrium}}$ are significantly related to the percentage of grass in the natural diet. The more a species is adapted to grass, the more distinct this difference, with extreme grazers having unpapillated dorsal and ventral mucosa. The relative $\mathrm{SEF}_{\mathrm{dorsal}}$ as *anatomical* proxy for stratification, and the difference in particle and fluid retention in the rumen as *physiological* proxy for stratification, are highly correlated in species (n=9) for which both kind of data are available. The results support the concept that the stratification of rumen contents varies among ruminants, with more homogenous contents in the more browsing and more stratified contents in the more grazing species.

Keywords: grazer, browser, rumen, rumen papillae, surface enlargement factor

Introduction

In ruminants (Hofmann, 1969; Langer, 1973), hippopotamids (Langer, 1975), and several rodent species (Vorontsov, 2003), the portion of the forestomach where bacterial fermentation occurs, and hence volatile fatty acids (VFA) are produced, is characterized by an absorptive mucosa whose surface is considerably enlarged by papillae. In ruminants, it was demonstrated that the development of these papillae is stimulated by the presence of VFA (Warner et al., 1956; Sander et al., 1959; Sakata and Tamate, 1978; 1979). Because VFA production is also a function of diet quality, the number and size of forestomach papillae reflect variation in diet quality, e.g. within a species between seasons (Hofmann, 1973; Langer, 1974; König et al., 1976; Hofmann, 1982; Hofmann and Schnorr, 1982; Hofmann, 1984; 1985; Smolle-Wieszniewski, 1987; Hofmann et al., 1988a; Hofmann and Nygren, 1992; Josefsen et al., 1996; Forsyth and Fraser, 1999; Mathiesen et al., 2000; Kamler, 2001), or between free-ranging and captive individuals (Hofmann and Matern, 1988; Marholdt, 1991; Hofmann and Nygren, 1992; Lentle et al., 1996).

Differences in the degree of papillation among different rumen regions in the same animal have been recognized for a long time in cattle, where especially the dorsal rumen wall completely lacks papillae. In contrast, Martin and Schauder (1938) noted that the rumen of some deer species is evenly papillated. Differences in the papillation among different rumen regions in different ruminant species were noted repeatedly (Garrod, 1877; Langer, 1973), and put into a systematic perspective by Hofmann (1973), with extensive photographic documentation. Examples of papillation extremes are given in Fig. 1, where the rumina of roe deer (*Capreolus capreolus*), steenbok (*Raphicerus campestris*) and gerenuk (*Litocranius walleri*) display a comparatively even papillation in all regions, whereas the rumina of hartebeest (*Alcelaphus buselaphus*), gemsbok (*Oryx gazella*) and reedbuck (*Redunca redunca*) show a papillation that is distinct in the *Atrium ruminis* and around the *Ostium* *intraruminale* (i.e., in the middle rumen layer), less distinct towards the more dorsal and ventral layers, and even completely absent in the most dorsal and ventral regions. It has been proposed that differences in the papillation of specific ruminal areas, such as the dorsal rumen wall, the atrium, or the ventral rumen wall, reflect differences in the degree of ingesta stratification in the rumen (Hofmann, 1973; Langer, 1974; Hofmann, 1989; Josefsen et al., 1996; Mathiesen et al., 2000). Actually, differences the stratification of rumen contents have been found in parallel to differences in papillation in several wild ruminant species (Clauss et al., 2008a; Clauss et al., 2008b).

In cattle and other grazing ruminants, the rumen contents are stratified, with a distinct gas dome of CO_2 and methane above the “fibre mat”, which itself floats on the liquid layer, at the very bottom of which very dense, fine sedimented particles form a “sludge” layer (Grau, 1955; Capote and Hentges, 1967; Hofmann, 1973; Hummel et al., 2008b; Tschuor and Clauss, 2008). In contrast, the rumen contents of several browsing species anecdotally appeared rather homogenous, “frothy”, without a distinct separation of gas, particles, fluids, and sludge (Hofmann, 1969; 1973; Nygren and Hofmann, 1990; Renecker and Hudson, 1990; Clauss et al., 2001). As papillation growth is induced by VFA, the presence of a dorsal gas dome and a ventral sludge layer could prevent papillae formation in these locations, because gas and sludge will displace any VFA that could accumulate in these regions. Actually, differences in VFA content between different rumen ingesta layers have been measured in domestic cattle (Smith et al., 1956; Tafaj et al., 2004). In contrast, in homogenous rumen contents, VFA can be assumed to be relatively evenly distributed throughout the ingesta, leading to an even ruminal papillation.

Hofmann (1969, 1973, 1989) first proposed that the difference in rumen contents stratification was one of many differences between grazing and browsing ruminants. When comparing different ruminant species according to the macroscopic appearance of their mucosa of ruminal regions such as the dorsal rumen, the atrium and the ventral rumen (Fig. 2), it is evident that in species increasingly specialized in grass forage, the differences among the rumen regions become more pronounced. The stratification of rumen contents according to updrift and sedimentation into a ‘fibre mat’ with the larger, lighter particles above a fluid phase containing the smaller, denser particles, is regarded as the main mechanism responsible for the sorting of particles in the reticulorumen in domestic ruminants (Sutherland, 1988; Beaumont and Deswysen, 1991; Lechner-Doll et al., 1991b; Dardillat and Baumont, 1992; Kaske et al., 1992). Additionally, the continuous mixing of the rumen contents, which forces small particles through the fibre mat, where they may also be retained (the ‘filter bed effect’) (Poppi et al., 2001; Faichney, 2006) is also considered an important mechanism responsible for the selective particle retention in this organ. A well-developed stratification (with an according filter-bed effect) was suggested to cause the differential outflow of fluids and particles in grazing wild ruminant species (Clauss and Lechner-Doll, 2001; Behrend et al., 2004; Hummel et al., 2005; Clauss et al., 2006b; Hummel et al., 2008a; Schwarm et al., 2008), and has been proposed to be a major driver of particular anatomical adaptations in grazers, such as strong rumen pillars (Clauss et al., 2003) and large omasa (Clauss et al., 2006a).

However, the presence or absence of a rumen contents stratification is actually difficult to investigate in live animals. Recently, the use of ultrasonography has been shown to facilitate the differentiation between a gas dome and a fibre mat in the rumen of cattle, and allowed to demonstrate the absence of a gas dome in a browse-fed captive moose (Tschuor and Clauss, 2008), but this technique has obvious limitations in terms of applicability over a wide range of species; the same is true for comparative measurements of fluid and particle passage through the gastrointestinal tract. In contrast, using the rumen mucosa papillation as a surrogate measure for the stratification of the rumen contents is an attractive alternative. The papillation of a certain rumen region can be easily quantified by the use of the “surface enlargement factor” (SEF; Schnorr and Vollmerhaus, 1967), which represents the factor by which the absorptive surface of the rumen mucosa is increased due to the papillae in comparison to the basal rumen area; an unpapillated region of the rumen, thus, has a SEF of 1.0. For rumen contents stratification, three rumen regions are of particular interest: the *atrium ruminis* which usually shows the most distinct papillae development and hence the highest SEF (Hofmann, 1973; Langer, 1973), and both the dorsal and the ventral rumen wall. The difference between either the dorsal or the ventral rumen wall SEF and the SEF of the *atrium ruminis* thus becomes, in theory, a measure of the degree of rumen contents stratification, with a small difference indicating homogenous, and a large difference comparatively stratified rumen contents.

For this contribution, published SEF data for these three rumen regions were collated from the literature, and supplemented with hitherto unpublished data of the second author (RRH). We specifically predicted that

1. There is no correlation between body mass (BM) and the SEF of the *atrium ruminis*, the dorsal or the ventral rumen wall, because the SEF is a measure of diet quality, which we did not assume to be correlated to BM in such a broad-scale data collection.
2. There is a negative correlation of both the absolute SEF of the dorsal and the ventral rumen wall, as well as of the relative SEF of these regions (in % of the *Atrium ruminis* SEF), with the percentage of grass (%grass) in the natural diet of the respective species, indicating an increase in rumen contents stratification with increasing adaptation to a grass diet.
3. There is a positive correlation between the relative SEF (in % of the *Atrium* SEF) of the dorsal and the ventral rumen wall, indicating that the mechanism

responsible for rumen contents stratification is effective throughout the whole rumen.

4. There is a negative correlation between the relative SEF (in % of the *Atrium* SEF) of the dorsal rumen wall, as an anatomical measure of rumen contents stratification, and the selective particle retention (as compared to the fluid retention) in the reticulorumen (Clauss et al., 2006b) as a physiological measure; in other words, the more the rumen mucosa reflects contents stratification, the larger the difference between fluid and particle passage from the rumen in the same species.

Methods

The SEF is calculated as the sum of surface of the papillae and the basal area divided by the basal area. Data on the SEF of the dorsal rumen wall, the ventral rumen wall, and the *atrium ruminis* were collated from both the literature and hitherto unpublished observations. When data was available from both the literature and the second author, data from the second author was preferred for a maximum of data consistency. For this data collection, only measurements from free-ranging animals were used, because the diets fed in captivity may lead to important changes in the ruminal mucosa papillation (Hofmann and Matern, 1988; Marholdt, 1991). The only exception were animals kept in a semi-natural setting on large pastures from Whipsnade Wild Animal Park, such as Chinese water deer (Hofmann et al., 1988a), Père David’s deer, axis deer, hog deer, blackbuck, and nilgai. The SEF measurements were performed on formalin-fixed samples by counting the number of papillae for a given basal area, and measuring their height and width, as described in the literature (Hofmann, 1973; Langer, 1973). When data on BM were available, it was directly taken from the sources; if only SEF but no BM data was available, BM data was taken from Clauss et al. (2002). All data represent means calculated on the basis of individual measurements, or, in the case of many publications, on the basis of average values per season. The number of individuals investigated per specie is given in Table 1.

As in more recent evaluations of the influence of adaptation to the natural diet in ruminants (Clauss et al., 2008c), the percentage of grass in the natural diet (%grass) was used to characterize species on a continuous scale. The bulk of the respective data was taken from Van Wieren (1996) and from the data collection that formed the basis of Owen-Smith (1997, data kindly provided by the author), which were supplemented by several other publications (Table 1). Whenever seasonal data was available, the %grass used to characterise a species represents the mean of the values from different seasons. It should be noted that this literature data was collated using a variety of sources and methods, and does not represent the actual diet ingested by the individuals measured in this study.

Data on the ratio between the mean retention time of particles:fluids in the reticulorumen were taken from Clauss et al. (2006b). Data for both papillation and this ratio were available for cattle, sheep, goat, giraffe, moose, roe deer, wapiti, ibex, and mouflon.

Relationships among species were inferred from a phylogenetic tree based on the complete mitochondrial cytochrome *b* gene. Respective DNA sequences were available from GenBank (http://www.ncbi.nlm.nih.gov) for all ruminant species investigated (except for *Gazella thomsoni*, which was therefore omitted from the analyses). Sequences were aligned using ClustalX (Thompson et al., 1997), visually controlled and trimmed to identical lengths (1143 bp). To select the best-fitting nucleotide substitution model for the data, a combination of the software packages Paup* (v.4.b10; Swofford, 2002) and Modeltest (v.3.7; Posada and Crandall, 1998) was used. Analysis was based on a hierarchical likelihood ratio test approach implemented in Modeltest. The model selected was the general time-reversible (GTR) model (Lanave et al., 1984; Tavaré, 1986) with an allowance both for invariant sites (I) and a gamma (G) distribution shape parameter (α) for among-site rate variation (GTR+I+G) (Rodriguez et al., 1990). The nucleotide substitution rate matrix for the GTR+I+G model was likewise calculated using Modeltest. Parameter values for the model selected were: -lnL = 15642.5273, I = 0.4613, and α = 0.8093 (8 gamma rate categories). The phylogenetic reconstruction based on these parameters was then performed using the maximum likelihood (ML) method implemented in TreePuzzle (v.5.2; Schmidt et al., 2002). Support for nodes was assessed by a reliability percentage after 100000 quartet puzzling steps; only nodes with more than 50% support were retained. The resulting tree is displayed in Figure 3. The basal polytomy for familial relationships (Bovidae, Cervidae, Giraffidae and Antilocapridae) was resolved assuming it to be a soft polytomy (Purvis and Garland, 1993). In order to meet the input requirements for the phylogenetic analysis implemented in the COMPARE 4.6 program (Martins, 2004), we resolved the remaining polytomies to full tree dichotomy by introducing extreme short branch lengths (l = 0.00001) at multifurcating nodes. Taxa grouping in the bifurcating process followed the phylogenies proposed by Pitra et al. (2004) for Cervidae and by Fernandez and Vrba (2005) for all other taxa.

The subjects of the comparative analyses were individual species, each characterized by its respective SEF as described above. In order to achieve normal data distribution, data for BM, $\mathrm{SEF}_{\mathrm{dorsal}}$, $\mathrm{SEF}_{\mathrm{atrium}}$ and $\mathrm{SEF}_{\mathrm{ventral}}$ were *ln*-transformed. Statistical analyses were performed with and without accounting for phylogeny, to test for the validity of a general, functional hypothesis, and to then discriminate between convergent adaptation and adaptation by descent. Data were analysed by correlation and partial correlation analysis (controlling for the influence of body mass). In order to additionally include phylogenetic information, we used the Phylogenetic Generalized Least-Squares approach (Martins and Hansen, 1997; Rohlf, 2001) in which a well-developed standard statistical method was extended to enable the inclusion of interdependencies among species due to the evolutionary process. In order to test the robustness of the results, the comparative analysis was performed for both a set of phylogenetic trees involving branch lengths (tree 1) and another set with equal branch lengths (tree 2). As there were no relevant differences in the results, only the tests using tree 1 are given here. The COMPARE 4.6 program (Martins, 2004) served for the phylogenetically controlled calculations. The other statistical calculations were performed with the SPSS 12.0 software (SPSS, Chicago, IL). The significance level was set to $\alpha = 0.05$.

Results

In this dataset, there was a significant, positive correlation between body mass and %grass in the natural diet (Table 2). On average, grazing ruminants are larger than browsing ruminants (Bell, 1971; Case, 1979; Bodmer, 1990; Van Wieren, 1996; Pérez-Barbería and Gordon, 2001), but correlations between BM and and the proportion of grass in the natural diet are usually not found (Van Wieren, 1996; Clauss et al., 2003; Sponheimer et al., 2003) or very weak (Gagnon and Chew, 2000), because browsers are found across the body size range (Sponheimer et al., 2003). In our case, the significance was retained even if phylogeny was taken into account (Table 2).

There were significant correlations between BM and all parameters investigated (Table 2). Significant correlations were also evident for %grass with all parameters except SEF$_{\text{atrium}}$. Similarly, Van Wieren (1996) already had not found a significant correlation between %grass and the SEF$_{\text{atrium}}$. Phylogenetic control generally did not change the results (Table 2). Correlations of %grass with SEF parameters generally had markedly higher correlation coefficients than the rather weak correlations with BM (Table 2; cf. Fig. 4ab and 4cd). The reindeer (*Rangifer tarandus*) was an evident outlier to the general pattern, showing an unusually high degree of papillae uniformity in its rumen for its feeding type as intermediate feeder.

Partial correlations (controlling for BM) between %grass and SEF parameters (except SEF$_{\text{atrium}}$) were highly significant (Table 2), indicating that the correlations between %grass and the SEF parameters were not caused by a mediating effect of BM. The relative SEF_{dorsal} and the $SEF_{ventral}$ (in % of the SEF of the atrium) were positively correlated to each other (Fig. 4e), with a generally higher value (by 6 % of the SEF_{atrium}) in the relative $SEF_{ventral}$ (raw data: $R=0.909$, $p<0.001$; phylogenetically controlled: $R=0.857$, $p<0.001$; $n=56$). The three species that appeared to deviate the most from this pattern were the giraffe, with a higher relative SEF_{dorsal}, and the two dik-dik species (*Madoqua guentheri* and *M. kirki*), in which the relative SEF_{dorsal} was disproportionately lower than the relative $SEF_{ventral}$.

Among the few species (n=9) for which comparative data on both the relative SEF_{dorsal} and the passage characteristics (SF = selectivity factor, mean retention time of particles/ mean retention time of fluids in the reticulorumen) were available, there was a significant, negative correlation between these parameters both for the raw data ($R=-0.917$, $p<0.001$) and the phylogenetically controlled test ($R=-0.922$, $p<0.001$) (Fig. 4f).

Discussion

In general, the hypotheses concerning the correlation of the patterns of the ruminal papillation with the %grass in the natural diet were confirmed. If the ruminal papillation pattern is accepted as a proxy for the stratification of rumen contents, the results therefore indicate that the contents of more grazing wild ruminants tend to be more stratified than those of more browsing ruminants.

Large interspecific comparisons such as this one are, by necessity, limited by certain factors that need to be stated: The data on the natural diet composition was not generated from the same animals, or not even the same animal populations, that were used for the papillation measurements; the same limitation applies to the use of body weight data derived from other individuals. Whether the habitat from which the individuals used for this study were taken was representative for the “typical” habitat of the species could not be assessed. The fact that papillation characteristics as well as the natural diet are subject to seasonal variation could have introduced a source of inaccuracy in the results of our analysis. Finally, differences in the handling of rumen samples (measurements on fresh samples or after storage in formalin of varying concentrations) will influence the results (Lentle et al., 1997). Some of these variations may have been attenuated by the use of ratios (SEF dorsal in % of SEF atrium). However, our study was not concerned with the detailed positioning of individual species in the comparison, but with a broad general pattern that is clearly evident across the wide range of species from different taxonomic groups.

The results confirm that the rumen contents of free-ranging wild ruminants induce a more pronounced difference in the intraruminal papillation pattern in those species that are characterized by a higher proportion of grass in their natural diet. In addition to the quantitative findings collated in this study, there are qualitative observations on additional species that are in accord with the pattern observed here. For example, Agungpriyono et al. (1992) observed that the rumen of the lesser mousedeer (*Tragulus javanicus*) was completely evenly papillated, and Clauss et al. (2006c) observed the same in captive okapi (*Okapia johnstoni*). Both species are known to avoid grass consumption in the wild (Nordin, 1978; Hart and Hart, 1988). In contrast, the rumen of three more recently domesticated bovini, the gaur (*Bos frontalis*), the yak (*Bos grunniens*) and the zebu (Bos *indicus*) were reported to resemble that of domestic cattle (Sarma et al., 1996), presumably indicating an absence of papillae in the dorsal area. Similar observations were made in other bovini such as water buffalo (*Bubalus bubalis*) (Hemmoda and Berg, 1980), lowland anoa (*Bubalus depressicornis*) (Clauss et al., 2008d), banteng (*Bos javanicus*) and European bison (*Bison bonasus*) (Clauss, pers. obs.), the puku (*Kobus vardoni*) (Stafford and Stafford, 1990) and the lechwe (*Kobus leche*) (Stafford and Stafford, 1991). All these animals are assumed to ingest high proportions of grass in their natural diet. Similar to our study, Enzinger and Hartfield

(1998) demonstrated quantitatively that the rumen mucosa of captive roe deer was evenly papillated, in contrast to that of fallow deer, sheep and goats. The most prominent outlier in our data collection, the reindeer, has been reported to have homogenous rumen contents (Westerling, 1970; Hobson et al., 1976) and a very even rumen papillation (Soveri and Nieminen, 1995; Josefsen et al., 1996; Mathiesen et al., 2000; Soveri and Nieminen, 2007). Whether this is caused by the peculiar diet of reindeer, or by an exceptional rumen physiology, remains to be elucidated. In the hippopotamids, which also have a voluminous forestomach lined with a papillated mucosa, the absence of differential particle retention contrasts with the selective large particle retention in ruminants (Schwarm et al., 2008); correspondingly, no elaborate stratification of contents would be expected in this group, and reports on the papillation of the hippopotamus do not indicate a stratification as seen in grazing ruminants (Langer, 1988). We propose that it is a higher fluid content in the grazing ruminant forestomach as compared to the hippopotamids (Thurston et al., 1968) that facilitates the stratification of the contents by allowing particle movements according to density.

One obvious explanation for differences in papillation between ruminant species is the sheer effect of body size and hence rumen size – the larger a rumen, the more distinct the characteristics of its contents' different layers could be. The significant correlations between BM and SEF parameters, which had not been expected, would support this hypothesis. However, these findings can probably be explained by the fact that BM was correlated to %grass in this dataset. Low proportions of grass in the natural diet, and SEF parameters indicating unstratified rumen contents, do occur across the whole body size range (up to the largest ruminant, the giraffe), indicating that the consistency of the rumen contents itself, irrespective of its volume, must be considered responsible for the demonstrated effects. Similarly, the physiological measure of rumen contents stratification, which is the difference between particle and fluid retention in the rumen, also does not follow a strict pattern with body size, either (Clauss and Lechner-Doll, 2001).

But is this consistency of the rumen contents an effect of the ingested forage, or of physiological adaptations of the different animal species? Intraspecific comparisons of SEF data, from individuals of different intermediate feeder species ingesting a browse- or a grass-dominated diet (Table 3) suggest a diet component in the formation of a stratified rumen. In addition, Hofmann (1973) observed that a group of zebus – a species with an usually unpapillated dorsal area – had a completely papillated rumen when kept on lucerne pasture; similarly, he observed that an oribi kept on a diet of concentrate and occasional browse had a completely papillated rumen, in contrast to free-ranging specimens. Therefore, it can be suspected that certain characteristics of dicotyledonous forage, which need to be further elucidated, induce less stratification in the rumen than monocotyledonous forage. Actually, in domestic ruminants, certain secondary plant compounds in some dicotyledonous forages (lucerne, clover) have been identified as the cause of "frothy bloat" or "legume bloat", a disease of domestic cattle which is characterized by an absence of rumen contents stratification (Clarke and Reid, 1970). On the other hand, passage trials in a moose did not show a difference in the pattern of particle and fluid retention between diets including browse, legume, or grass hay (Renecker and Hudson, 1990), and thus suggest independence of rumen contents stratification from dietary factors at least in this species. **Maybe this is an indication that at least moose have evolved physiological adaptations that, directly or indirectly, prevent the formation of rumen contents stratification.**

The fact that both, the dorsal as well as the ventral rumen region show a difference in SEF as compared with the atrium (Fig. 4e) supports our hypothesis that a mechanism leading to this stratified papillation must be operative throughout the whole rumen. The most probable mechanism appears to be rumen content and rumen fluid viscosity (Clauss et al., 2006b). Actually, rumen fluid of roe deer or moose was found to be of a higher viscosity than that of mouflon, bison (Clauss et al., 2008a; Clauss et al., 2008b) or that of cattle (Hummel et al., 2008b). However, this hypothesis has to be tested in a larger number of species. Reasons for the deviation of giraffe (with an unusually high $\mathrm{SEF}_{\mathrm{dorsal}}$) and dikdik (with unusually low $\mathrm{SEF}_{\mathrm{dorsal}}$ as compared to the $\mathrm{SEF}_{\mathrm{ventral}}$) cannot be given here.

One of the consequences of a higher fluid viscosity would be the entrapment of gas bubbles in the rumen contents, rather than the formation of a distinct dorsal gas dome. This would explain the "frothy" appearance of browsers' rumen contents (see Introduction), and the absence of a distinct dorsal gas dome observed in a moose (Tschuor and Clauss, 2008). McCauley and Dziuk (1965) found that in resting goats only little gas accumulated in the dorsal rumen, and suggested that the gas was trapped as "numerous bubbles" in the rumen contents instead. The frothy appearance of the rumen contents of browsers suggests that the pathologic process of "frothy bloat", seen in domestic ruminants fed fresh legume forages (Clarke and Reid, 1970), may offer some comparative potential for the understanding of the differences in rumen physiology between browsing and grazing ruminants (Clauss et al., 2006b). Interestingly, domestic sheep are less susceptible to legume-caused frothy bloat than cattle (Olson, 1940; Colvin and Backus, 1988). Given the papillation pattern (Table 1) and the differential passage of fluid and particles from the rumen (Clauss et al. 2006b; Fig 3f), sheep rumen contents are naturally less distinctively stratified than those of cattle. Additionally, in direct comparisons of animals feeding on the same pastures, sheep ingest a higher proportion of browse than cattle (Sanon et al., 2007). Given these observations, we hypothesize that sheep – and the zebu cattle mentioned above - are better adapted to less stratified rumen contents, and that cattle, as extreme grazers with a very distinct rumen content stratification and a distinct difference in particle and fluid passage from the rumen, have lost the ability to cope with frothy rumen contents, most likely due to less flexible rumen motility patterns

(Colvin and Backus, 1988).

The question arising from these observations is about the adaptive value of the described differences. Evidently, given the proportion of areas that are un-papillated or only lined with small papillae, the total absorptive surface of the whole rumen is less in grazers than in browsers of similar rumen size. This trend is most likely not countered by an increase in SEF in the papillated rumen areas of grazers, as there was no significant increase in $\text{SEF}_{\text{atrium}}$ with increasing %grass in the natural diet (Table 2). This difference in absorptive area in the rumen puts measurements on the concentration of volatile fatty acids (VFA) (Hoppe, 1977; Clemens and Maloiy, 1983; Hoppe, 1984; Prins et al., 1984) and on VFA production rates (Gordon and Illius, 1994) in the rumen contents of wild ruminants into a new perspective. Most of these studies had not found a statistical difference in their measurements between grazers and browsers. The concentration of VFA depends on production and absorption rate across the epithelium of the fermentation chamber (Lechner-Doll et al., 1991a). Since a rise in VFA concentration triggers an increase in the epithelial growth in the rumen (Nocek and Kesler, 1980; Goodlad, 1981; Dirksen et al., 1984), the absorption capacity for VFA will increase and hence actual VFA concentrations measured after this adaptation period will be levelled. Thus, large differences in VFA production rates may be masked and reflected by only very small differences in VFA concentrations (Lechner-Doll et al., 1991a). Higher VFA production rates, which may be demonstrated in natural forages of browsing ruminants (Hummel et al., 2006), therefore need not necessarily translate into higher VFA concentrations in the rumen of these animals because of their larger absorptive surface.

Ruminal VFA concentrations also depend on rumen fluid volume and fluid outflow rate (Lechner-Doll et al., 1991a). Some large grazing ruminants, such as cattle (Clauss et al., 2006b; Schwarm et al., 2008), are characterised by a particularly fast fluid passage through the rumen, which might reduce VFA concentrations. These animals might compensate for their comparatively lower absorptive surface in the rumen by an increased absorption of VFA in their comparatively larger omasum (Clauss et al., 2006a). This fast throughput might additionally have the advantage of maximal use of microbial protein produced in the rumen (as suggested by Hummel et al., 2008a). The distinct stratification induced by this high fluid throughput could also render the rumination process more efficient, so that only such material from the fibre mat is regurgitated that needs further comminution. In two white-tailed deer (*Odocoileus virginianus*) (a browser, with a presumed lack of a distinct rumen contents stratification), Dziuk et al. (1963) observed regurgitation and re-swallowing of ingesta without rumination and suspected that further mastication had been unnecessary for the respective regurgitated bolus. The authors noted that such observations (of 'vain' regurgitations) had not been made in cattle or sheep. However, detailed studies on differences in regurgitation frequency and rumination in different ruminant species are mostly lacking.

At least two different extremes of rumen physiology are thus proposed by the observed differences in rumen mucosa papillation and hence rumen contents stratification:

1. Browsers, ingesting a faster-fermenting forage, have more viscous rumen contents and fluid, either due to forage or saliva characteristics, and a comparatively slow fluid throughput; the whole rumen acts as an absorptive organ, and shows no particular adaptations to a contents stratification with a fibre mat. This physiology type is a consequence of the fact that the forage induces high viscosity contents, or necessitates a high-protein and hence high-viscosity saliva due to the forage's content of secondary compounds (Hofmann et al., 2008).
2. Grazers, ingesting a slower-fermenting forage, have a less viscous rumen fluid, also due to either forage or saliva characteristics, probably supported by a

comparatively high fluid throughput; the whole rumen acts as a storage organ, with absorption occurring at more limited areas, and shows particular adaptations to a contents stratification with a fibre mat. This physiology type is a consequence of the fact that monocot forage induces less viscous contents, does not necessitate high-protein saliva, and might tend to form a more efficient fibre mat.

These findings are independent of phylogenetic relatedness between ruminant species; **e.g., parallel observations were made in cervids and bovids of different feeding type**. **The more pronounced rumen contents stratification could be a reason why grazing ruminants digest fibre more efficiently than browsing ruminants (Pérez-Barberìa et al., 2004).** But whether stratification is actually a relevant adaptation in itself (in the sense of a convergent evolution) would have to be studied in in vitro digestion models in which important characteristics of the modelled ingesta – such as its viscosity – are manipulated. Note that our study does not claim that the stratification of rumen contents itself is an adaptation; it merely describes a notable difference in the degree of stratification between the ruminant feeding types that happens, that is reflected in the rumen papillation pattern, and to which the animals adapt by various anatomical and physiological means. **To which degree these adaptations are genetically fixed or subject to modification due to the ingested forage remains to be demonstrated.** Current evidence suggests that both extremes – the extreme grazers as well as the extreme browsers – are derived from more rudimentary intermediate feeding types (Codron et al., 2008; DeMiguel et al., 2008). The extremes in rumen contents stratification and papillation therefore describe opposing rumen physiologies and emphasize the general flexibility of the ruminant digestive system.

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References

Table 1. Mean body mass (BM) and surface enlargement factors of ruminant species of different feeding type collated from different sources, and an estimation of the percentage of grass (%grass) in the natural diet of the species according to literature sources.

Species		BM		SEF		SEF in % atrium		Source	%grass	Source
		(kg)	dorsal	ventral	atrium	dorsal	ventral			
Impala	*Aepyceros melampus*	55	3.9	5.0	15.3	25	32	A (25)	60.0	1
Hartebeest	*Alcelaphus buselaphus*	174	1.0	1.8	19.0	5	9	A (9)	96.7	2
Moose	*Alces alces*	258	9.6	9.5	15.3	63	62	J (25)	2.0	1
Springbok	*Antidorcas marsupialis*	41	2.6	3.0	14.8	17	20	K (7)	30.0	4
Pronghorn	*Antilocapra americana*	40	4.5	5.2	9.0	50	58	B (2)	15.0	1
Blackbuck	*Antilope cervicapra*	33	1.7	2.3	6.3	27	37	B (1)	75.0	1
Axis deer	*Axis axis*	85	1.5	1.9	7.0	22	27	B (2)	70.0	1
Hog deer	*Axis porcinus*	45	1.9	3.1	6.0	32	52	B (1)	50.0	5
Bison	*Bison bison*	335	1.0	2.1	15.9	6	13	B (1)	84.0	1
Cattle	*Bos taurus*	600	1.0	1.5	14.2	7	11	L (1)	79.0	2
Nilgai	*Boselaphus tragocamelus*	220	2.4	2.6	8.8	28	30	B (3)	59.5	2
Domestic goat	*Capra hircus*	31	2.5	2.6	6.7	37	38	H (53)	28.0	1
Alpine ibex	*Capra ibex*	60	3.1	3.7	14.7	21	25	B (1)	60.0	1
Roe deer	*Capreolus capreolus*	25	6.2	6.5	9.0	69	72	C, D, E, F (58)	9.0	1
Serau	*Capricornis crispus*	37	3.6	3.6	12.5	29	29	O (10)	70.0	1
Red duiker	*Cephalophus harveyi*	16	4.5	7.5	11.0	41	68	A (2)	1	3
Red deer	*Cervus elaphus*	170	3.7	3.7	19.0	19	19	C (12)	47.0	1
Wapiti	*Cervus elaphus canadensis*	300	2.9	3.4	8.8	33	39	B (1)	64.0	1
Sika deer	*Cervus nippon*	70	1.9	3.3	12.6	15	26	B (14)	50.0	1
Sambar	*Cervus unicolor*	200	4.0	4.7	14.8	27	32	M (12)	45.0	1
Wildebeest	*Connochaetes taurinus*	182	1.0	7.5	36.5	3	21	A (5)	90.0	1
Fallow deer	*Dama dama*	60	2.4	4.3	16.2	15	27	G (11)	46.0	1
Tsessebe	*Damaliscus lunatus*	119	1.0	1.0	16.0	6	6	A (5)	99.3	2
Pere Davids Deer	*Elaphurus davidianus*	120	1.2	2.3	13.3	9	17	B (3)	75.0	7
Grant's gazelle	*Gazella granti*	55	3.7	5.5	20.0	19	28	A (13)	50.0	1
Thomson's gazelle	*Gazella thomsoni*	21	2.5	2.5	16.5	15	15	A (15)	85.5	2
Giraffe	*Giraffa camelopardalis*	750	24.0	18.0	30.0	80	60	A (4)	0.2	2
Himalayan tahr	*Hemitragus jemlahicus*	55	2.5	3.0	9.9	25	30	P (39)	75.0	8
Chinese water deer	*Hydropotes inermis*	11	3.3	3.7	6.7	50	55	I (58)	50.0	7
Waterbuck	*Kobus ellipsiprymnus*	201	1.0	1.0	32.0	3	3	A (5)	80.0	2
Uganda kob	*Kobus kob*	79	1.0	1.0	19.0	5	5	A (5)	95.0	9
Gerenuk	*Litocranius walleri*	43	7.5	12.0	19.0	39	63	A (8)	0.0	2
Günther's dikdik	*Madoqua guentheri*	4	7.5	16.5	19.0	39	87	A (6)	5.0	10
Kirk's dikdik	*Madoqua kirki*	5	6.5	12.5	17.5	37	71	A (6)	17.0	3
Muntjac	*Muntiacus reveesi*	15	4.5	4.8	6.9	65	69	N (33)	10	1
Suni	*Neotragus moschatus*	6	7.3	9.0	11.5	63	78	A (4)	0.0	6
Mule deer	*Odocoileus hemionus*	80	5.1	6.9	10.7	48	65	B (4)	11.0	1
White-tailed deer	*Odocoileus virginianus*	70	4.0	4.6	7.0	57	65	B (3)	9.0	1
Klipspringer	*Oreotragus oreotragus*	11	10.5	10.0	17.5	60	57	A (3)	5.0	1
Beisa oryx	*Oryx beisa*	145	1.7	2.5	11.0	16	23	B (2)	83.0	12
Gemsbok	*Oryx gazella*	182	1.0	1.5	12.5	8	12	A (3)	82.0	2
Oribi	*Ourebia ourebi*	16	1.0	-	15.0	7	-	A (5)	48.5	2
Sheep	*Ovis ammon domesticus*	31	2.1	2.2	6.5	33	34	H (14)	50.0	1
Mouflon	*Ovis ammon musimon*	40	2.6	3.5	8.6	30	41	G (10)	69.0	11
Dall’s sheep	*Ovis dalli dalli*	56	2.2	6.1	19.4	11	32	B (2)	56.0	1

Mongolian gazelle	*Procapra gutturosa*	29	4.2	4.5	15.5	27	29	R (38)	28.0	13
Reindeer	*Rangifer tarandus*	62	9.5	11.6	9.5	99	122	Q (36)	36.0	1
Steenbok	*Raphicerus campestris*	11	6.0	6.0	18.0	33	33	A (18)	10.0	1
Mountain reedbuck	*Redunca fulvolufula*	24	1.0	1.0	16.0	6	6	A (6)	99.0	2
Bohor Reedbuck	*Redunca redunca*	45	1.0	1.0	20.0	5	5	A (3)	80.0	1
Barasingha	*Rucervus duvauceli*	200	1.7	2.4	9.0	19	26	B (2)	80.0	1
Chamois	*Rupicapra rupicapra*	50	3.5	4.3	15.5	22	28	B (16)	74.0	1
Grey duiker	*Sylvicapra grimmia*	14	11.0	11.0	23.5	47	47	A (6)	5.0	2
African buffalo	*Syncerus caffer*	599	1.0	2.0	35.0	3	6	A (4)	90.0	1
Eland	*Taurotragus oryx*	465	3.8	8.0	35.0	11	23	A (4)	50.0	3
Bongo	*Tragelaphus euryceros*	250	4.1	-	11.1	37	-	B (3)	20.0	14
Lesser Kudu	*Tragelaphus imberbis*	91	8.0	9.5	31.0	26	31	A (4)	10.0	2
Bushbuck	*Tragelaphus scriptus*	50	7.0	9.0	24.5	29	37	A (4)	10.0	1
Greater Kudu	*Tragelaphus strepsiceros*	214	9.0	13.5	40.0	23	34	A (3)	5.0	1

Sources for SEF data (number of individuals investigated in parentheses): A (Hofmann, 1973), B (Hofmann, unpubl.), C (Hofmann et al., 1976), D (König et al., 1976), E (Hofmann et al., 1988b), F (Wen-Jun and Hofmann, 1991), G (Geiger et al., 1977); H (Hofmann et al., 1987), I (Hofmann et al., 1988a), J (Hofmann and Nygren, 1992), K (Hofmann et al., 1995), L (Schnorr and Vollmerhaus, 1967), M (Stafford, 1995), N (Pfeiffer, 1993), O (Jiang, 1998), P (Forsyth and Fraser, 1999), Q (Mathiesen et al., 2000), R (Jiang et al., 2003)

Sources for %grass data: 1 (Van Wieren, 1996), 2 (Owen-Smith, 1997), 3 (Gagnon and Chew, 2000), 4 (Bigalke, 1972), 5 (Dhungel and O'Gara, 1991), 6 (Heinichen, 1972), 7 (Geist, 1999), 8 (Schaller, 1973), 9 (Field, 1972), 10 (Hofmann and Stewart, 1972), 11 (Stubbe, 1971), 12 (Skinner and Chimimba, 2005), 13 (Campos-Arceiz et al., 2004), 14 (Kingdon, 1982)

Table 2. Results of statistical analyses for correlations and partial correlations (controlling for body mass) between the surface enlargement factors (SEF) of different areas of the rumen mucosa, the proportion of grass (%grass) in the natural diet, and body mass, using either raw data from Table 1 or phylogenetically controlled statistics.

		%grass	$\text{SEF}_{\text{dorsal}}$	$\text{SEF}_{\text{ventral}}$	$\text{SEF}_{\text{atrium}}$	$\text{SEF}_{\text{dorsal}}$ (% $\text{SEF}_{\text{atrium}}$)	$\text{SEF}_{\text{ventral}}$ (% $\text{SEF}_{\text{atrium}}$)
	n	58	58	56	58	58	56
Raw data							
Body mass	R	0.401	-0.281	-0.281	0.289	-0.323	-0.487
	p	0.002	0.032	0.036	0.028	0.013	<0.001
%grass	R	-	-0.872	-0.818	-0.006	-0.726	-0.737
	p		<0.001	<0.001	0.966	<0.001	0.001
%grass (partial correlation)	R	-	-0.864	-0.802	-0.138	-0.688	-0.677
	p		<0.001	<0.001	0.305	<0.001	<0.001
Phylogeny-controlled							
Body mass	R	0.469	-0.277	-0.210	-0.294	-0.328	-0.471
	p	<0.001	0.030	0.115	0.023	0.012	<0.001
%grass	R	-	-0.871	-0.649	0.010	-0.689	-0.626
	p		<0.001	<0.001	0.929	<0.001	0.001
%grass (partial correlation)	R	-	-0.861	-0.643	-0.158	-0.639	-0.576
	p		<0.001	<0.001	0.245	<0.001	<0.001

R = correlation coefficient

Table 3. Surface enlargement factor in the rumen of several wild intermediate feeding ruminants in relation to the predominant composition of their actual diet. Data from Hofmann (1973)

Species		Diet		SEF		SEF in % SEF atrium	
			dorsal	ventral	atrium	dorsal	ventral
Impala	*Aepyceros melampus*	browse	6.0	6.5	18.0	33	36
		grass	1.8	3.4	12.5	14	27
Thomson's gazelle	*Gazella thomsoni*	browse	3.5	3.5	16.5	21	21
		grass	1.5	1.5	16.5	9	9
Eland	*Taurotragus oryx*	browse	6.0	12.0	35.0	17	34
		grass	1.7	4.0	35.0	5	11
Grant's gazelle	*Gazella granti*	browse	5.5	7.0	20.0	28	35
		grass	1.9	4.0	20.0	10	20