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Original investigation

The rumen washes off abrasives before heavy-duty chewing in ruminants

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ABSTRACT

Based on comparative mandibular anatomy, observations of chewing behaviour, chewing forces and dental microwear, it has been suggested that an additional effect of the ruminant digestive strategy could be a reduction of both the required chewing load and tooth wear ruminants are exposed to. This effect is hypothesized to be the result of digestion, mixing, and digesta sorting prior to regurgitation for rumination, which might both soften the material and wash off external abrasives such as sand, grit and dust. Putatively, these external abrasives would thus be trapped in the (fore)stomach and excreted via the faeces. We investigated the location of sand in the stomach of goats fed diets containing phytoliths and sand for several months. The contents of the stomach section from where rumination material is recruited were comparatively depleted of sand. Sand mainly accumulated in another stomach section, the abomasum, without causing clinical problems. A certain phytolith content should hence affect ruminants and non-ruminant herbivores somewhat alike; however, a certain external abrasives content should affect ruminants less than non-ruminants. Results from feeding experiments as well as tooth wear studies support this hypothesis, and caution against the default use of dental anatomy and wear as taxon-free environmental proxies in paleobiology.

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Introduction

It is well-known that the ruminant forestomach serves simultaneously as a site of microbial fermentation for plant material (Van Soest, 1994) and as a growth environment for microbiota later harvested by the host (Clauss and Hummel, 2017). Additionally, it comprises a sorting mechanism (Lechner-Doll et al., 1991) that facilitates a particle size reduction of the ingested food via rumination unrivalled by other herbivores (Clauss et al., 2015). This sorting mechanism is based on the interplay between a liquid medium and the floating and sedimenting properties of the digesta (Lechner-Doll et al., 1991).

Based on comparative mandibular anatomy (Fletcher et al., 2010; Zhou et al., 2019), observations of chewing behaviour (Janis et al., 2010; Dittmann et al., 2017), chewing forces (Williams et al., 2011) and dental microwear (Mihlbachler et al., 2016), it has been suggested that an additional effect of the ruminant digestive strategy could be a reduction of both the required chewing load and tooth wear ruminants are exposed to. This effect is hypothesized to be the result of digestion, mixing, and digesta sorting prior to regurgitation for rumination, which might both soften the material and wash off external abrasives such as sand, grit and dust. Putatively, these external abrasives would thus be trapped in the (fore)stomach and excreted via the faeces. Because the majority of particle size reduction occurs in ruminants not during ingestive masticaton but during rumination (McLeod and Minson, 1988), and because the chewing pattern during rumination is much more regular and systematic than during ingestion (Dittmann et al., 2017), rumination can be considered 'heavy-duty' chewing in contrast to ingestive mastication.

Here, we trace silica content in the digestive tract of goats (*Capra aegagrus hircus*) fed pelleted diets of different abrasives content (Ackermans et al., 2018, 2019). Three diets increased gradually in phytolith content (from lucerne [L] to grass [G] to a mix of grass and rice hulls [GR]), and the fourth diet added sand to the highest level of phytoliths [GRS] and therefore also represented external abrasives.

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The location of sand in the gastrointestinal tract (GIT) was traced in live animals via computed tomography (CT), and in slaughtered animals by analysing different sections of the GIT contents.

Analyses were performed for dry matter (DM; a measure how moist the contents are), for acid detergent fibre (ADFom; a measure for fibre content), for acid detergent insoluble ash (ADIA, a proxy for silica, regardless of whether derived from phytoliths or sand; Hummel et al., 2011), and for total ash (a proxy for all inorganic elements, including ADIA and minerals). Given common knowledge about the changes of digesta along the GIT in ruminants, we expected contents of the dorsal rumen to be drier than those of the ventral rumen and the reticulum, contents of the omasum to be particularly dry, and dry matter to increase gradually from the abomasum to the faeces (Clauss et al., 2016, 2017). Because fibre, silica or total ash are less digestible than non-fibrous organic substrates, we expected a general increase in GIT contents as compared to the diets (due to the disappearance of other substrates in the course of digestion). We were particularly interested in whether the dorsal (upper) contents of the rumen – the location from where digesta is recruited for regurgitation and re-mastication during rumination – resembles the ingested diet in terms of silica concentration, or whether it is depeleted in silica compared to the diet, indicating a washing effect in the rumen.

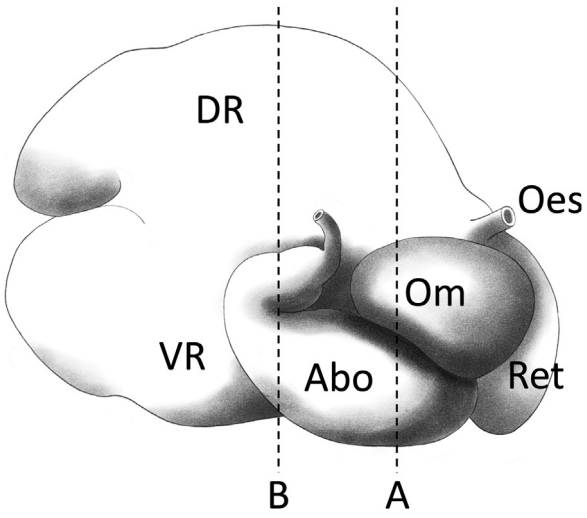

Fig. 1. Schematic representation of the ruminant forestomach, viewed from its right side. The cranial part is to the right, with the oesophagus (Oes), and the caudal part to the left. While the forestomach is positioned next to the left abdominal wall of the animal, on its right side, between the scheme and the viewer, the intestines are placed in the abdominal cavity, which are not shown here. DR dorsal rumen (from where contents are regurgitated for rumination), VR ventral rumen, Ret reticulum, Om omasum, Abo abomasum. The dotted lines indicate the planes of the CT images shown in Fig. 2A and B. Plane A also intersects the dorsal part of the thoracal cavity with the dorsal part of the lungs, not shown here. Drawing modified from Nickel et al. (2004).

Methods

Experiments were performed with approval of the Swiss Cantonal Animal Care and Use Committee Zurich (animal experiment licences 69/2008 and 115/2009). As previously described in detail (Ackermans et al., 2018, 2019), 28 goats were divided into four groups that received, for six months, either one of three pelleted diets of increasing phytolith (internal abrasives) content (lucerne L: 9.2 g ADIA/kg dry matter; grass G: 16.4 g/kg; grass and rice hulls GR: 29.0 g/kg) or a diet of high internal and external abrasive content (grass, rice hulls and sand GRS: 92.8 g/kg). To ensure all pelleted diets were isocaloric and hence ingested in similar amounts, they were designed so that the proportion of indigestible silica abrasives in the GRS diet was matched by a similar proportion of indigestible, non-abrasive filler (pure lignocellulose, Arbocel, JRS Pharma, Rosenberg, Switzerland) in the other diets. Grass hay was provided to all groups except for the lucerne group, which was fed lucerne hay. Each animal received 1500 g of pelleted food and 100 g of hay daily. Note that in this experiment the diets were designed to mainly comprise pellets, and the provided proportion of hay was therefore lower than the normal forage ration for ruminants.

After three months, CT images were acquired using a helical multislice Siemens scanner (Siemens Medical Solutions, Erlangen, Germany) to image the abdomen of the GRS goats (tube voltage at 120 kVp, image matrix of 512×512 pixels, field of view of 980×332 pixels, slice thickness of 0.6 mm, B60 s convolution kernel) in a natural (sternal) resting position under general anaesthesia (ketamine, 10 mg/kg (Ketonarkon®, Streuli Pharma AG, Uznach, Switzerland), xylazine 0.1 mg/kg (Xylazin Streuli, Streuli Pharma AG, Uznach, Switzerland) intramuscularly, maintained with isoflurane (Attane®, Provet AG, Lyssach, Switzerland) mixed in oxygen via facemask. CT data sets were converted to DICOM medical imaging format and evaluated in Horos v3.0.1 (Horos Project 2015). Sand volumes (cm^3) were calculated by manually defining regions of interest (ROIs) on every 6^{th} slice and automated generation of missing ROIs. To guide the interpretation of the CT images, a schematic visualisation of the ruminant forestomach is given as Fig. 1.

After six months, the goats were slaughtered within two hours after their last feeding, and the GIT was dissected following a standard protocol (Clauss et al., 2016) while carefully avoiding deviations from the natural position or mixing of the contents (described in Sauer et al., 2017). Samples were taken from the dorsal rumen (from where material is regurgitated for rumination), ventral rumen, reticulum (sorting forestomach), omasum (fluid reabsorption forestomach), abomasum (stomach, initiation of auto-enzymatic digestion), small intestine, caecum, proximal colon, spiral colon, and rectum (faeces). Standard nutrient analyses (AOAC, 1995) were applied. Samples were analysed for the concentration of dry matter (DM, dried at 103 °C) and total ash (i.e., including not only silica but also minerals; AOAC no. 942.05), for analyses for acid detergent fibre (ADFom, AOAC no. 973.18) and ADIA (Hummel et al., 2011). Analyses for ADFom, ADIA and total ash were limited to the (fore)stomach contents and faeces in L, G and GR animals but done on all GIT sections in GRS animals.

Differences across diets and GIT sections were assessed using Mixed Effects Linear Models in R 3.4.3 (R_Core_Team, 2015), incorporating individual as a random factor. Each variable (DM, ADFom, ADIA, ash) was tested in models nesting GIT section within diet, with Tukey's HSD post hoc test for multiple comparisons (significance level at 0.05).

For additional corroboration, we analysed GIT contents of goats from another experiment not designed to answer questions about tooth wear and external abrasives, using the same methods. Samples generated in the course of that study on the physical characteristics of the contents of the GIT of goats fed grass hay (n = 5) or dried browse (n = 5), which had been analysed for their particle size and dry matter content (Clauss et al., 2017) as well as microbial and fermentation traits (Zeitz et al., 2016), were now additionally analysed for ADIA and total ash. In that study, which had originally been initiated to investigate effects of diagnostic imaging (Braun et al., 2011a,b; Braun et al., 2011c; Ohlert et al., 2012), animals were euthanized and frozen in a natural resting position, and subsequently cut into transversal slices, which allowed a detailed sampling of different regions of the GIT. In that experiment, the dried browse had been purchased from a commercial supplier, and had been subjectively found to contain a higher number of contaminants such as stones, pieces of plastic or wool, than typical for a dried forage feed (M Clauss, pers. obs.). For the present ADIA investigation,

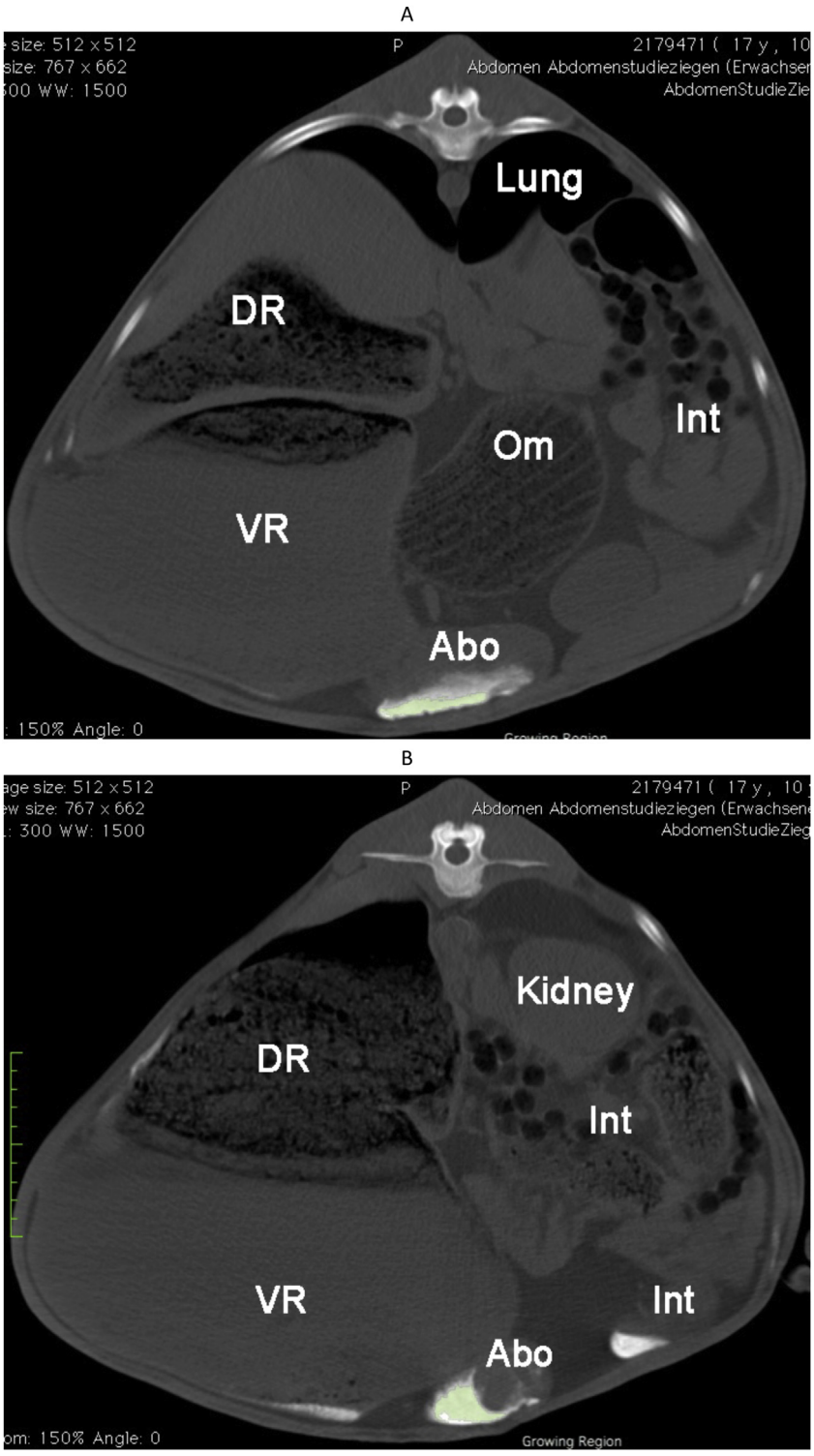

Fig. 2. Computed tomographic scans of a goat (*Capra aegagrus hircus*) fed a pelleted diet of grass and rice husks containing sand (GRS) for six months. Animal in sternal recumbency. Images represent vertical slices through the thorax and abdomen (A) or, more caudally, through the abdomen only (B), as indicated in Fig. 1. Images are from a caudal point of view, with the dorsal rumen (DR) and ventral rumen (VR) filling the left body cavity. Sand accumulation is visible as hyperattenuating (white) agglomerations in the abomasum (Abo), and in the lower scan at the bottom of the ventral rumen and in a part of the intestines (Int). Other radiodense structures correspond to vertebrae and ribs. Sand is not visible at other locations. Note the typical stratification of rumen contents, with larger particles and air entrapment in the dorsal rumen as opposed to more homogenous, fluid contents of the ventral rumen, and the lamellar structure of the omasum (Om).

samples of the following GIT regions were analysed: dorsal part of dorsal rumen (Rdd), ventral part of dorsal rumen (Rdv), dorsal part of ventral rumen (Rvd), ventral part of ventral rumen (Rvv), reticulum (Ret), omasum (Om), abomasum (Abo), faeces (Fec), and submitted to the same statistical approach.

Results

Inspection of the CT scans indicated severe accumulation of sand in the abomasum in all GRS goats (Fig. 2), representing on average, 48.1 ± (SD) 32.0 cm^3. Note that this did not cause clinical problems

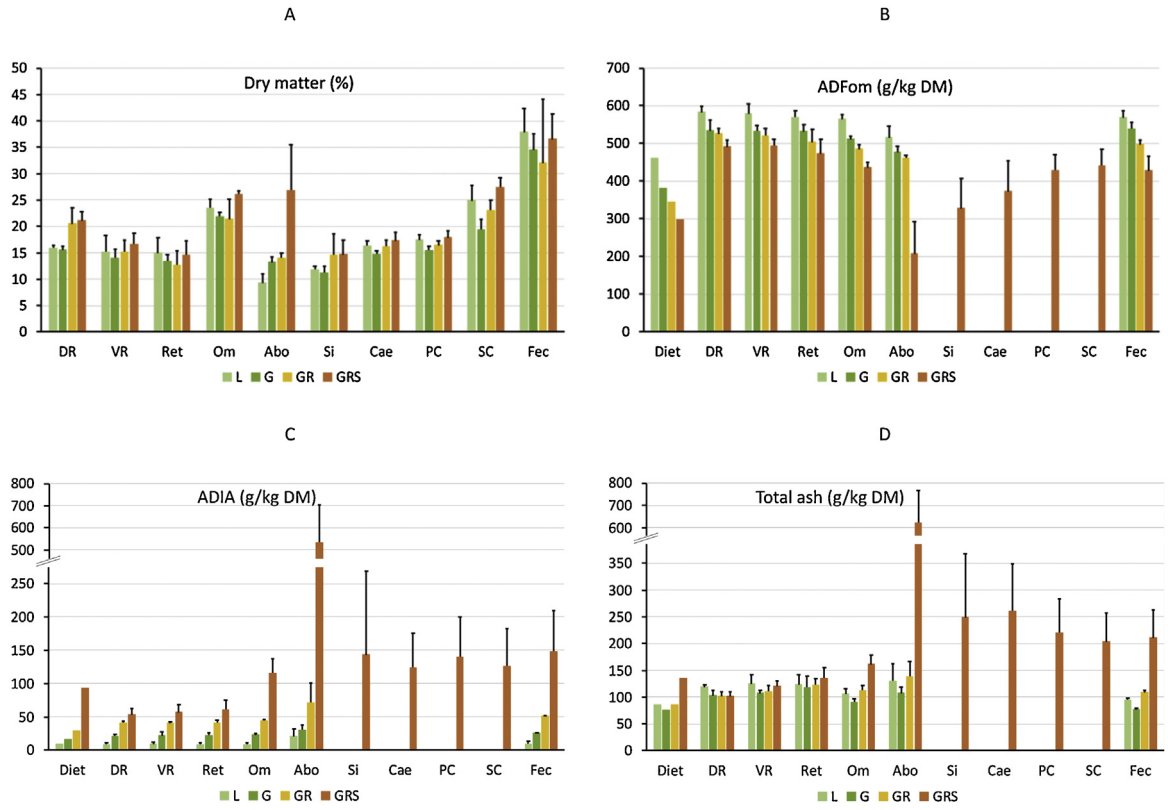

Fig. 3. The (mean + SD) concentration of (A) dry matter (DM), (B) acid detergent fibre (ADFom), (C) acid detergent insoluble ash (ADIA; a measure for silica) and (D) total ash (including insoluble ash as well as acid detergent soluble ash, i.e. minerals) in the diet and digestive tract of goats (*Capra aegagrus hircus*) fed diets of different abrasive content (lucerne L; grass G; grass and rice husks GR; grass, rice husks and sand GRS). DR dorsal rumen (from where contents are regurgitated for rumination), VR ventral rumen, Ret reticulum, Om omasum, Abo abomasum, Si small intestine, Cae caecum, PC proximal colon, SC spiral colon, Fec faeces. Note that ADFom, ADIA and total ash were not analysed in the Si, Cae, PC and SC of groups L, G and GR. For a more detailed look at (C) see Fig. 4.

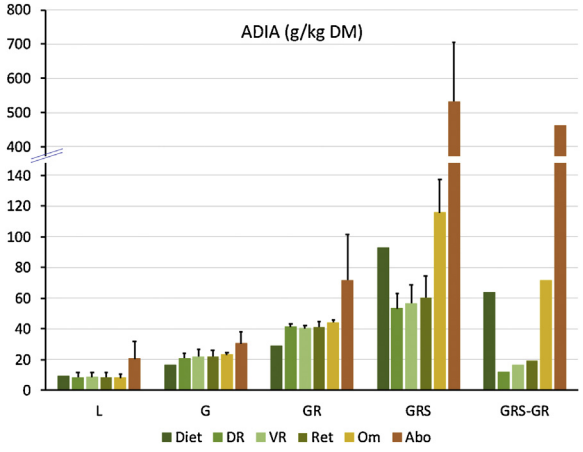

Fig. 4. Concentration of acid-detergent insoluble ash (ADIA, in dry matter; means + SD) in the diet and different sections of the (fore)stomach in goats (*Capra aegagrus hircus*) fed diets of different abrasive content (lucerne L; grass G; grass and rice husks GR; grass, rice husks and sand GRS). DR dorsal rumen (from where contents are regurgitated for rumination), VR ventral rumen, Ret reticulum, Om omasum, Abo abomasum (values outside of depicted range). GRS-GR represents the difference of the respective mean measures between the two diets that only differed in the content of sand.

(and animals lived for another 3 months after the CT, when they were finally slaughtered). Sand was also visible, with large variation between individuals, at $14.2 \pm 12.0\ cm^3$ in the ventral rumen, and in intestinal sections, in all animals. Sand was not visible in the dorsal rumen or the omasum in any animal, and in only one animal in the reticulum.

The complete findings for DM, ADFom, ADIA and total ash are displayed in Fig. 2. Diet had a significant effect on all variables, with DM, ADIA and ash higher, and ADFom lower on GRS than on all other diets (Table 1). The GIT effect was also always significant, and since it was nested within diet, this indicated that GIT sections differed in different ways across diets.

Many differences were evident for dry matter content (Table 1). Faeces were always drier than content of any GIT section; and omasum and spiral colon contents were generally drier than other GIT sections. On diet GRS, there was the exceptional situation that (most probably due to the high amount of sand) the abomasum had contents that were as dry as those of the omasum and spiral colon (Fig. 3). ADFom, ADIA and ash only varied across GIT sections on diet GRS, where the abomasum had higher (ADIA, ash) or lower (ADFom) values than other sections. For ash, the dorsal rumen was significantly lower than several other sections.

Most notably, animals on GRS had significantly higher ADIA levels in all GIT sections than animals on other diets (post hoc $P < 0.001$; Fig. 4), and animals on GR had higher levels than animals on L ($P = 0.015$). Differences between GIT sections in ADIA were only significant on GRS, where levels in the abomasum were higher than in all other GIT sections ($P < 0.001$). Most importantly, ADIA levels of the rumen samples were higher than those of the diet itself on diets G and GR, reflecting a concentration effect due to the onset of digestive processes that remove some of the digestible components but leave the phytoliths that are embedded in the cell wall matrix. In contrast, rumen samples had distinctively lower ADIA levels than the diet itself on diet GRS (Fig. 4), suggesting a removal of the major ADIA component on this diet (sand) from rumen digesta. When plotting the difference in mean ADIA levels between GRS (which was GR with added sand) and GR animals (Fig. 4), it is evident

Table 1
Results of statistical analyses for differences between diets and gastrointestinal tract (GIT) sections of goats (*Capra aegagrus hircus*) fed diets of different abrasive content (lucerne L; grass G; grass and rice husks GR; grass, rice husks and sand GRS).

Effect	df	effect *F*	effect *P*	post hoc
Dry matter				
individual		0.115	0.735	
diet	3,17	22.108	<0.001	GRS > L/G/GR; GR > G
diet/GIT	36,169	31.492	<0.001	all diets: Fec > all L: Om/SC > DR/VR/Ret/Abo/Si/Cae/PC; Cae/PC > Abo G: Om > VR/Ret/Abo/Si/Cae; SC > Si GR: SC > VR/Ret/Abo/Si GRS: Abo/Om/SC > VR/Ret/Si/Cae/PC
ADFom				
individual		0.060	0.807	
diet	3,33	151.613	<0.001	GRS < GR; GRS < L
diet/GIT	25,123	14.868	<0.001	GRS: Abo < all; Cae < DR/VR/Ret; Si < DR/VR/Ret/Om/PC/SC/Fec
ADIA				
individual		5.527	0.019	
diet	3,32	53.479	<0.001	GRS > L/G/GR; GR > L
diet/GIT	25,123	21.457	<0.001	GRS: Abo > all
Total ash				
individual		16.051	<0.001	
diet	3,28	37.610	<0.001	GRS > L/G/GR
diet/GIT	25,121	29.833	<0.001	GRS: Abo > all; Cae/Si > DR/VR/Ret; DR < PC/Fec

DR dorsal rumen, VR ventral rumen, Ret reticulum, Om omasum, Abo abomasum, Si small intestine, Cae caecum.
PC proximal colon, SC spiral colon, Fec faeces.

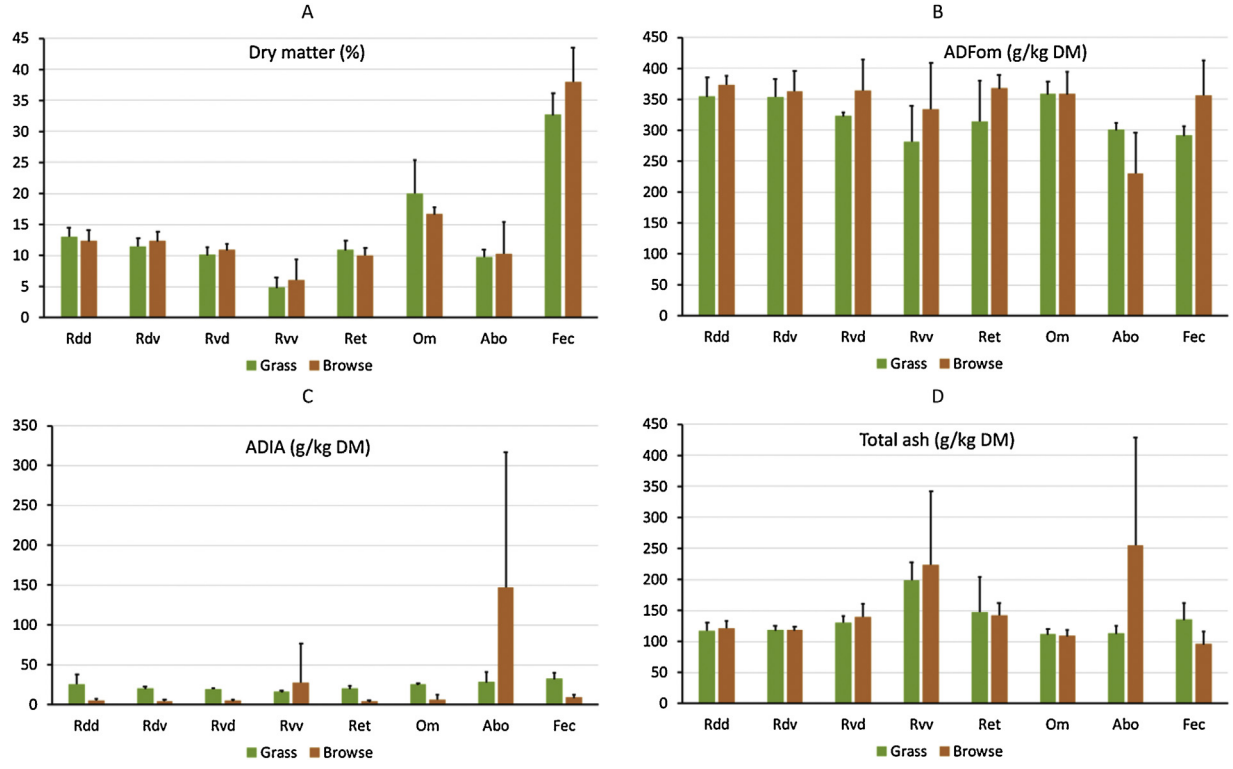

Fig. 5. The (mean + SD) (A) concentration of dry matter (DM), (B) acid detergent fibre (ADFom), (C) acid detergent insoluble ash (ADIA) and (D) total ash in the digestive tract of goats (*Capra aegagrus hircus*) fed either grass hay or dried browse. Rdd dorsal part of dorsal rumen, Rdv ventral part of dorsal rumen, Rvv ventral part of ventral rumen, Ret reticulum, Om omasum, Abo abomasum, Fec faeces. For a more detailed look at (C) see Fig. 6.

that diet differences are reflected proportionally in the difference between the omasum samples, but the difference in ADIA of rumen samples was distinctively less than expected from that of the diet samples, with the least mean difference for the dorsal rumen.

The results of the additional samples from another feeding experiments with goats are displayed in Figs. 5 and 6 and Table 2. For both diets, faeces were drier than contents of other GIT sections, and omasum contents were drier than those of preceding forestomach sections. On grass, the stratification of rumen contents was reflected in drier dorsal than ventral contents. On browse, the contents of the abomasum were higher in ADIA and total ash, and lower in ADFom, than those of other GIT sections. In this experiment, it had not been intended to introduce external abrasives with the browse diet, but there was an evident accumulation of ADIA in the ventral rumen, and significantly so in the abomasum, which suggests that similar to the main experiment, external abrasives

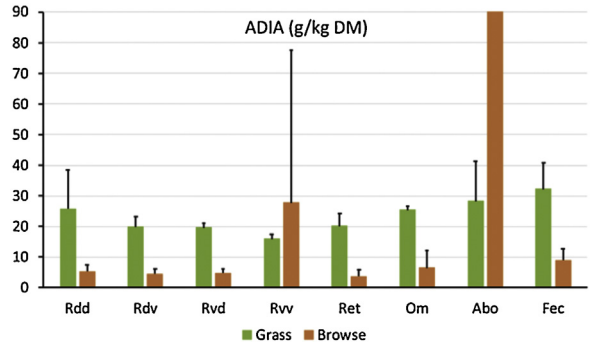

Fig. 6. The (mean + SD) concentration of acid detergent insoluble ash (ADIA) in the digestive tract of goats (*Capra aegagrus hircus*) fed either grass hay or dried browse. Rdd dorsal part of dorsal rumen, Rdv ventral part of dorsal rumen, Rvv ventral part of ventral rumen, Ret reticulum, Om omasum, Abo abomasum, Fec faeces. Note that orange column for which no whisker is visible goes beyond the scale of the y-axis (cf. Fig. 5).

Table 2
Results of statistical analyses for differences between diets and gastrointestinal tract (GIT) sections of goats (*Capra aegagrus hircus*) fed grass hay (G) or dried browse (B).

Effect	df	effect *F*	effect *P*	post hoc
Dry matter				
individual		12.974	<0.001	
diet	1,8	0.218	0.653	
diet/GIT	14,54	80.469	<0.001	both diets: Fec > all B: Om > Rvv/Ret G: Om > all; Rdd/Rdv > Rvv
ADFom				
individual		2.961	0.085	
diet	1,8	2.234	0.174	
diet/GIT	14,54	4.875	<0.001	B: Abo < all
ADIA				
individual		0.141	0.707	
diet	1,8	0.060	0.812	
diet/GIT	14,55	3.131	<0.001	B: Abo > all
Total ash				
individual		0.352	0.553	
diet	1,8	1.247	0.296	
diet/GIT	14,55	3.243	<0.001	B: Abo > Rdd/Rdv/Om/Fec

Rdd dorsal part of dorsal rumen, Rdv ventral part of dorsal rumen, Rvv ventral part of ventral rumen, Ret reticulum, Om omasum, Abo abomasum, Fec faeces.

were washed out of the ingesta, sedimented in the ventral rumen and accumulated in the abomasum.

Discussion

Our study shows that external abrasives first accumulate, due to their density, in the ventral rumen and are passed on from this site via the reticulum and the omasum to their main accumulation site in the ruminant gastrointestinal tract – the abomasum. This finding corroborates the presumption that abrasives are washed off the digesta, leading to dorsal rumen contents that are depleted in abrasives when compared to the diet, reducing overall wear loads on teeth when masticating the regurgitate. With their digestive tract, ruminants thus achieve what primates (Nakamichi et al., 1998; van de Waal et al., 2012v; Allritz et al., 2013; Neadle et al., 2017) or suids (Sommer et al., 2016; Ito et al., 2017) reportedly achieve by proactive washing of diet items: a removal of grit prior to heavy-duty mastication.

The implication is that ruminants process internal abrasives (phytoliths embedded in plant material) and external abrasives (dust, grit, sand) very differently in their digestive tract. Phytoliths are not separated from the digesta during the process of digestion and rumination, and are possibly enriched in regurgitate submitted to rumination (cf. the higher ADIA concentrations of dorsal rumen content when compared to the diet for treatments G and GR in Fig. 3). The continuous increase in ADIA from reticulum to faeces in the supplementary experiment corresponds to an increase in concentration due to the removal of digestible components of the digesta, similar to the main experiment. In the supplementary experiment, the apparent decrease in ADIA from the dorsal to the ventral rumen contents on grass hay also indicates that on whole hay, the more silica-rich stems are likely to represent the upper layer in the stratified contents, whereas the finer, more ventral contents consist of the more easily comminuted, less silica-rich leaves. By contrast, external abrasives are not trapped or contained in any part of the dietary material, and can therefore be washed off easily. Our results explain why wear features on ruminant cheek teeth should be more strictly related to diet characteristics related to features, content and distribution of phytoliths (and hence of their diet), and less to dust- and grit-related environmental factors (Kubo and Yamada, 2014; Mihlbachler et al., 2016).

The supplementary experiment had not been designed with the rumen washing mechanism in mind, and the feeds used had not been quantitatively assessed for their ADIA content or the degree of contamination. While the results indicate that the grass hay had been produced with care, avoiding evident amounts of external abrasives, they indicate that the commercially available dried browse contained relevant amounts of external abrasives, most likely due to the (unknown) harvesting method. This was an unexpected, incidental finding. In a similar way, acid insoluble ash concentrations have been described, as incidental findings, in the digestive tracts of reindeer (*Rangifer tarandus*) and muskoxen (*Ovibos moschatus*), where clear peak concentrations in the abomasum were documented without associated clinical problems (Staaland et al., 1986; Staaland and Thing, 1991). Our findings also match reports that the abomasum is the predilection site for sand impaction in ruminants (Melendez et al., 2007; Erickson and Hendrick, 2011). Due to its anatomical position, with a dorsally-oriented outlet from the pylorus to the small intestine (Fig. 1), the abomasum represents a trap for dense material. However, because the goats in our study survived without clinical signs, and without loss of appetite, and because of the reports in reindeer and moose mentioned above, we conclude that the ruminant digestive tract is able to process significant, radiographically prominent amounts of sand, and trapped material is passed continuously from the abomasum towards the lower digestive tract. This possibly contrasts with forestomach morphologies of hippopotamuses or peccaries that comprise ventrally-oriented blindsacs, from which it seems more difficult to expel dense material such as grit or sand (Wings et al., 2008; Schwarm et al., 2010). It is tempting to speculate that in the evolution of a fluid-dependent sorting mechanism that facilitates rumination, which has occurred in both ruminants and camelids (Lechner-Doll et al., 1991), and in which external abrasives will be inadvertently washed off the ingesta, the concomitant evolution of a digestive system able to process these abrasives is a prerequisite.

Our findings, together with the general predicted pattern in ruminants, explain the seeming contradiction: When using the identical diets, the sand-enriched GRS diet caused the most distinct wear-related changes *in vivo* in rabbits (Müller et al., 2014), guinea pigs (Müller et al., 2015) and in an *in vitro* chewing system on horse teeth (Karme et al., 2016), but not *in vivo* in goats (Ackermans et al., 2018, 2019). Similarly, our findings explain the results from another tooth wear-related feeding experiment using sheep, which came to the conclusion that "*dust does not matter*" (Merceron et al., 2016). The majority of the particle size-reducing mastication in ruminants occurs during rumination (Trudell-Moore and White, 1983; McLeod and Minson, 1988), on washed material; in contrast, non-ruminants cannot avoid masticating putatively contaminated food thoroughly if they aim at achieving a high

degree of food comminution. If digestive physiology protects ruminant teeth against wear effects of external abrasives, this could also be a reason contributing to the observation that ruminants are generally less hypsodont than equids of a similar diet niche (Kaiser et al., 2013). These findings contradict the concept of using dental morphology and wear features in 'taxon-free' approaches (Andrews and Hixson, 2014) because the same ingested material might not leave the same wear trace in different species. In the current debate on whether phylogeny-related peculiarities need to be considered when analysing dental wear features (DeSantis et al., 2018; Fraser et al., 2018; Clauss, 2019), our results and help understand why dental wear features of ruminants are not representative for all ungulates (Mihlbachler et al., 2016). Therefore, they suggest that it is prudent to consider functional ruminants – the Ruminantia and the camelids – separately from nonruminant herbivores, or to apply statistical methods to large tooth wear data compliations that account for the phylogenetic structure of the datasets.

To what degree ruminant taxa themselves differ in the effect of the washing mechanism remains unknown so far. To date, the fate of sand in the camelid forestomach has not been investigated to our knowledge. Among the Ruminantia, there are different types in terms of forestomach physiology (the 'cattle-type' and the 'moose-type' ruminants), which differ in the degree of fluid throughput that the forestomach contents are exposed to (Clauss et al., 2010; Dittmann et al., 2015). Whether these different forestomach physiologies lead to differences in the efficiency of the washing mechanism, making 'moose-type' ruminants, with their lower degree of digesta washing, more susceptible to external abrasives and hence limiting them in their habitat choices, remains to be explored.

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