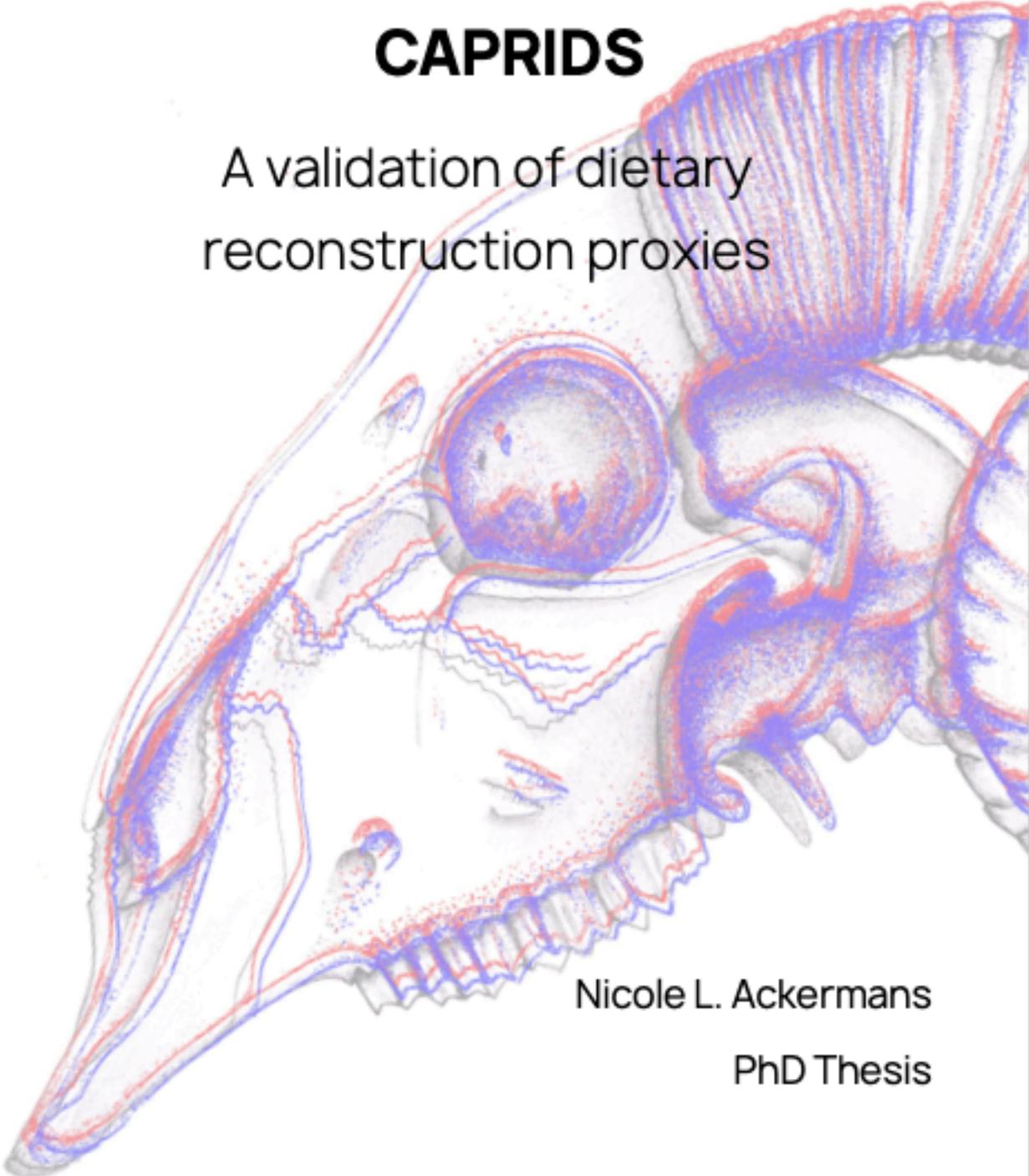


EXPLORING TOOTH WEAR IN EXPERIMENTALLY FED CAPRIDS

A validation of dietary
reconstruction proxies



Nicole L. Ackermans

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Nicole L. Ackermans

aus
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Promotionskommission

Prof. Dr. Marcus Clauss (Vorsitz)

Prof. Dr. Marcelo R. Sánchez-Villagra

Prof. Dr. Jean-Michel Hatt (Leitung der Dissertation)

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“They DO move in herds!”

- Dr. Alan Grant

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Summary

The effects created by diet on an animal's teeth can be very informative to palaeontologists and neontologists alike. Herbivores have been the main focus of tooth wear research, as the abrasive components of their diet can create changes on their teeth at macroscopic or microscopic scales, which can in turn be used to reconstruct palaeodiets and palaeoenvironments of extinct species. Grazing herbivores, for example, sustain high amounts of tooth abrasion caused by grit, or phytoliths internal to plants; while browsers have a more attrition-dominated tooth signature caused by tooth on tooth contact when chewing a softer diet. The main proxies used to infer diet from dental wear are microwear and mesowear. However, a full understanding of the timespan, resolution and correlation of these proxies can only be achieved by controlled feeding experiments. This body of work focuses on two long-term feeding experiments conducted on goats and sheep. By adding various sizes and concentrations of abrasives to experimental diets, we were able to test the effect of each diet with several dental wear proxies, allowing us to observe the development of wear over time by means of CT scans taken over the course of the experiments. Through this body of work, we determined mesowear to be much more of a general lifetime signal than previously thought, excluding the use of this type of proxy for seasonal dietary reconstruction, at least in small ruminants. Furthermore, comparing microscopic wear to the same dataset leads us to believe that these two proxies do not measure the same process, but rather different processes on different scales, the link between which remains to be explored. Following ingested abrasives through the gastrointestinal system on CT scans allowed for the description of the ruminant washing mechanism, used to mitigate tooth wear by washing off external abrasives in the rumen and providing a less abrasive food bolus for regurgitation. Finally, we described the phenomena of root growth compensating for crown wear, through volumetric absolute wear measurements. Systems such as the washing mechanism or high-crowned teeth in herbivores emphasize tooth abrasion as one of the main pressures for herbivore evolution and diversification.

Part A

Framework and Overview of the Research

1 | Introduction

1.1 Tooth wear, from coarse to fine effects

Mammals show the highest degree of convergent evolution in herbivory, with one-third of all mammalian orders containing strictly herbivores (Janis and Fortelius, 1988). Plant matter being less nutritious than animal matter, it must be ingested in greater volumes in order to sustain metabolic needs, especially for large herbivores. Problematically, the abrasive properties of plant matter create considerably higher dental wear than animal-based foods, thus driving herbivorous mammals to morphological adaptations in their dentition, in pursuit of higher functional durability.

Hypsodonty

From an evolutionary point of view, several solutions address the problem of dental wear. In herbivores, a particularly effective adaptation has been the evolution of hypsodont- or high-crowned-teeth. Hypsodonty describes teeth in which the crown is higher than the tooth is long (von Koenigswald, 2011). From a developmental viewpoint, hypsodonty is achieved by delaying the formation of roots during ontogeny. The tooth crown is retained in the jaw bone as a result, and continues to erupt during an animal's lifetime, as the tooth surface is worn down (Damuth and Janis, 2011). This is the case for horses (Fig. 1), and in some modern ruminants to a much lesser extent, in which case it can be called incipient hypsodonty (Webb and Hulbert, 1986; Witzel et al., 2018). Euhypsodont rodents and lagomorphs take this principle to the extreme, as root ontogeny is extended past the length of a lifetime in these animals, resulting in "ever-growing" teeth, constantly renewed as chewing wears away dental tissue. Having ever-growing teeth, is also called euhypsodonty or hypselodonty, and evolved in sequence after the emergence of hypsodonty in small mammals (Tapaltsyan et al., 2015).

Hypsodonty evolved independently multiple times across various herbivorous clades, at least 17 times in artiodactyls and perissodactyls (Damuth and Janis, 2011), and in as many as 26 South American mammal clades, including marsupials, rodents, extinct ungulates, and xenarthrans (Madden, 2014). High-crowned teeth must therefore represent a clear advantage for the evolving herbivore. The precise conditions of this advantage have been a subject of intensive study (Strömberg, 2004; Semprebon et al., 2019). Feeding in open, dust-swept habitats where abrasive grasses are most prevalent, is thought to be the main cause of hypsodont evolution (Williams and Kay, 2001), especially in South America with the accumulation of volcanic ash (Madden, 2014) which can be particularly

abrasive, though recent phytolith research has shown grass existing 6 million years before the apparition of the first hypsodont *Merychippus* (Strömberg et al., 2016).

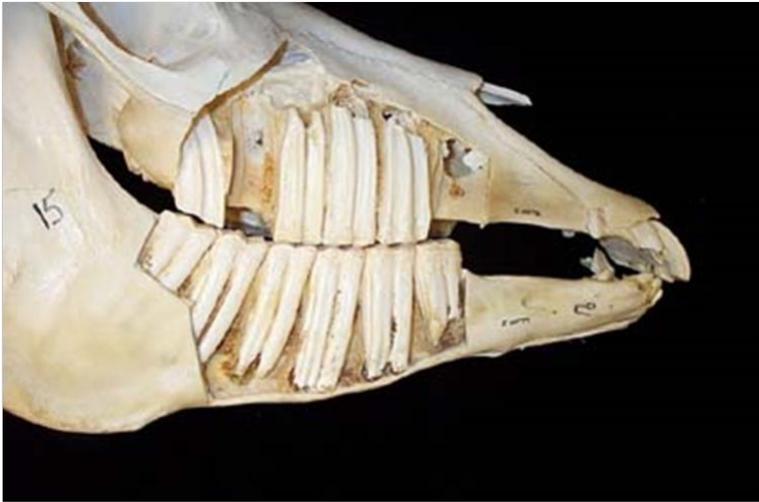


Figure 1. Sagittal view of hypsodont horse molars with the root exposed. Image credit: Wayne Dale

Abrasives out and in the herbivore diet

Some herbivorous diets cause more tooth wear than others. Indeed, plants can contain varying amounts of hard opaline silicates called phytoliths. These silica particulates are nested within the plant matrix, providing structural support. When phytoliths come into contact with tooth material during chewing, they abrade away the tooth's outer layers. Because of this abrasion effect, it is hypothesised that phytoliths are a defence-mechanism, deterring herbivores from consuming plants (Strömberg et al., 2016), a theory which has been tested experimentally in voles (Massey and Hartley, 2006) and sheep (Massey et al., 2009). Grasses in particular contain large amounts of phytoliths, compared to more browse-like vegetation such as leaves and shrubs. Thus, when browsing animals experience wear, it is mostly caused by attrition, i.e. teeth coming in contact with each other as they pass through the soft food without resistance.

In addition to mainly consuming tough grass as part of their diets, grazers feed close to the ground in open habitats, where grasses act as a “sediment trap” (Madden, 2014), leading to passive ingestion of windblown sediment, and aggravated tooth wear once consumed. External abrasives encompass all sizes of dust, sand, and grit that adhere to-, and are ingested along with- plant matter. These abrasives may have a different effect on teeth depending on their size, with fine dust thought to cause uniform wear that does not affect the tooth profile (Kaiser et al., 2013), while extreme cases of large grit ingestion have been seen to create pathological-like wear over time. The combination of these abrasives can wear down the tooth dentine and enamel, which risks exposure of the pulp, followed by decay, and even tooth loss. This concept was first explored by Healey and Ludwig in a series of experiments on sheep in New Zealand (Cutress and Healy, 1965; Healy and Ludwig, 1965;

Ludwig et al., 1966; Healy, 1967), where ewes feeding on over-stocked pastures showed high-amounts of tooth wear due to soil contamination, which then led to premature culling. These primary experiments provided a first insight into the greater scale of tooth wear in relation to seasonal climate variations. In New Zealand specifically, extreme years of excess tooth wear were caused by the combination of high stocking rates and the El Niño Southern Oscillation. Landslide erosions and mobilised abrasive soil minerals in excess and contaminated pastures (reviewed in detail by Madden, 2014). A large variety of studies have explored the effects and associations of endogenous phytoliths and exogenous grit in localities across the globe (Sanson et al., 2007; Hummel et al., 2011; Lucas et al., 2013), though the exact relationship between different types of abrasives and the evolution of hypsodonty remains a subject of current debate (Semprebon et al., 2019).

Food washing and picky eating

Evolving longer teeth is not the only adaptation against abrasives. Based on digestion, herbivores can be separated into two categories: hindgut fermenters with single-chamber stomachs (equids, rhinoceroses, lagomorphs etc.) and foregut fermenters with multi-chambered stomachs, most of which are ruminants (Fig. 2). Hindgut fermentation is typically considered less efficient, requiring the ingestion of higher volumes of plant matter. It also requires more intense processing in the oral cavity, which can result in more wear, especially if ingesta contains exogenous grit, thus requiring high-crowned molars (Madden, 2014). In comparison, foregut-fermentation shows an evolutionary advantage, in the sense that most of the mastication occurs on pre-digested cud (Fortelius, 1985), and the liquid in the rumen may “wash off” external abrasives before rumination (Schulz et al., 2010), resulting in less tooth wear (Mihlbachler et al., 2016). Ruminants therefore do not require such extreme tooth morphology as some hindgut fermenters (Figs. 1, 3).

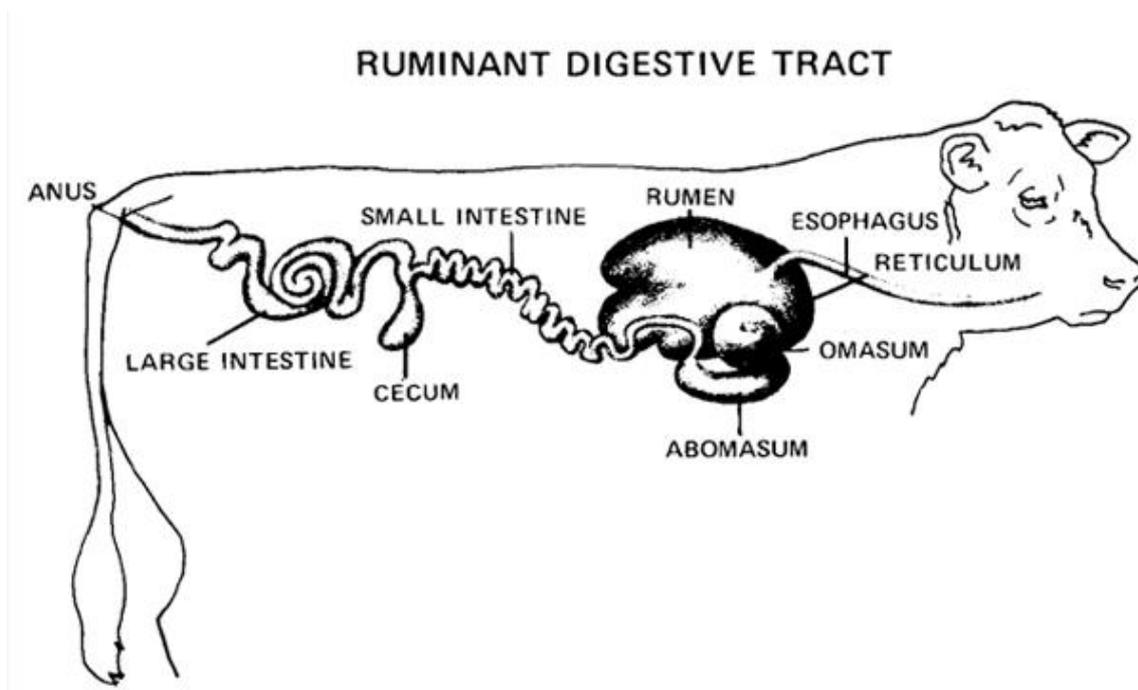


Figure 2. The ruminant digestive system. Adapted from (Rounds and Herd, 1987)

Though some herbivores have evolved these mechanisms to mitigate wear, they still may not be capable of avoiding food contaminated with abrasives upon first ingestion. An aversion to grit consumption has been observed in a variety of taxa such as suids (Sommer et al., 2016; Ito et al., 2017), otters (Neunteufel, 2007), and primates (Visalberghi and Frigaszy, 1990; Allritz et al., 2013; Schulz-Kornas et al., 2019), including humans (Prinz, 2004). However, studies investigating this phenomenon experimentally are rare (Gali-Muhtasib et al., 1992; Massey and Hartley, 2006). Abrasive avoidance and its related behaviours are a strong indicator of abrasion being a source of evolutionary pressure on dental morphology.

Teeth just keep growing

Hypsodonty is a major advantage for herbivores on the evolutionary scale, but high-crowned teeth are also subject to complex physiological regulations on the scale of a lifetime. Very little research is done to this capacity on ruminants, as mice and rats are the main models for dental research. In these animals, the tip and base of their euhyposodont incisors are separately controlled by different genetic pathways during ontogeny (Tummers and Thesleff, 2009), indicating that growth in either of these parts may happen independently. For these teeth to grow continuously, a stem cell bundle at the apex of the tooth, called the cervical loop, responds to occlusal pressure by regulating tooth material secretion (Madden, 2014) and encouraging tooth growth when dental material is lost by wear (Harada et al., 1999). Periodontal ligaments attach the teeth to their alveolar socket and are proprioceptors that protect the teeth. They provide feedback on tooth load, helping to avoid breakage, and may also detect

wear, at least in humans (Hughes, 2015). These receptors may play a part in triggering cellular proliferation in the cervical loop, most likely in the form of cementum. Such a mechanism, where tooth feedback on wear would induce the production of more tooth matter in the roots, has been shown experimentally in rabbits (Ness, 1956; Müller et al., 2014; Meredith et al., 2015) and guinea pigs (Müller et al., 2015), but its existence in other taxa is uncertain. In addition, animals with euhypodont teeth appear to show faster tooth wear than those with hypsodont teeth, which could be related to softer enamel (Table 3.1 in Berkovitz and Shellis, 2018).

High-crowned teeth with closed roots are physiologically different from ever-growing rootless teeth in many ways (Fig. 3), including the arrangement of periodontal ligaments, the placement of the pulp cavity, the physiology of the enamel- and dentin-forming organs, the enamel-to-dentin ratio, and the cementum distribution (Madden, 2014). However, cellular proliferation related to dietary hardness or crown height has also been discussed as occurring in the form of in the form of cemental-apposition in hypsodont ruminants (Lieberman, 1993; Sanson et al., 2017), suggesting that cementum thickness might be related to abrasive diets to some extent.

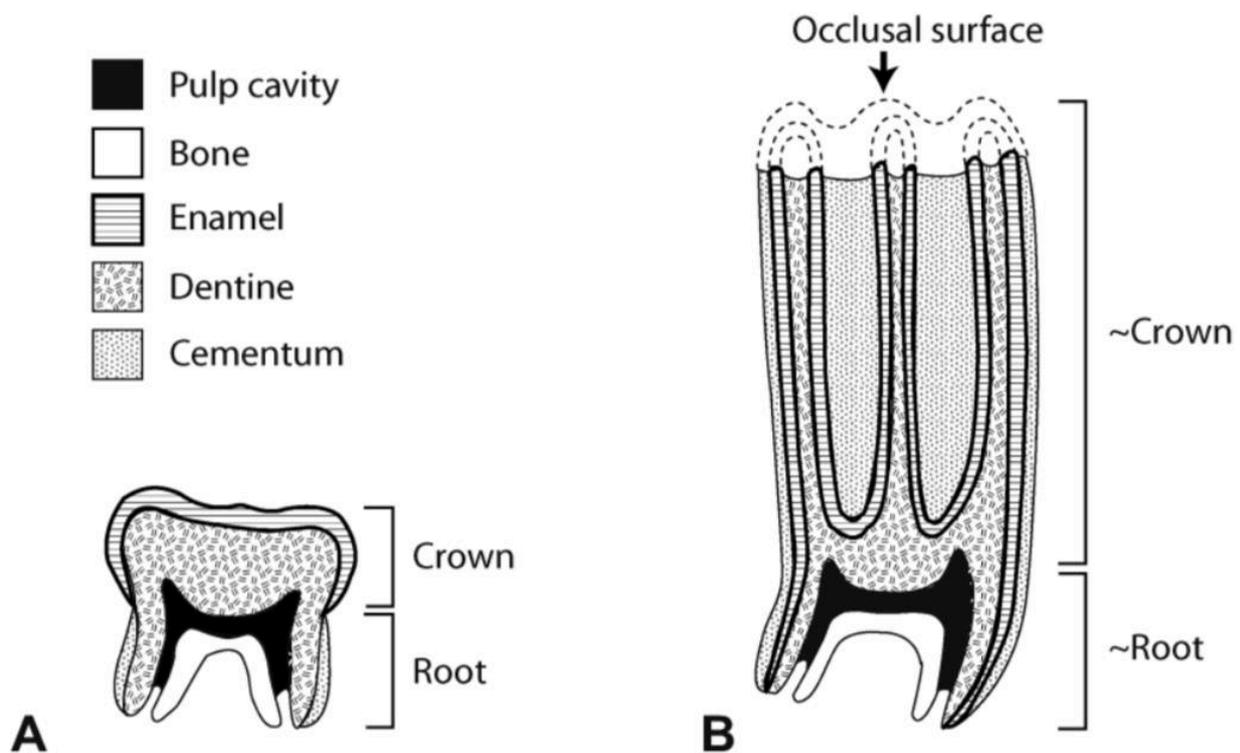


Figure 3. Comparative anatomy of a brachydont (A) and hypsodont tooth (B). Adapted from Janis and Fortelius (1988)

Short review on the loss of dental tissue over time in mammals

Macroscopic tooth wear has often been used as an accurate measure to detect an animal's age. In this literature review, we provide an overview of tooth wear rates across different mammalian species, and the methods used to measure these rates, using the review by Damuth and Janis (2014a) as a primary source. Comparing wear rates across taxa can offer insight on the relationship between hypsodonty and the rate at which tooth material is lost, by highlighting patterns and raising questions about complex dietary interactions. Data gathered from the literature is presented here as an overview of the loss of dental tissue over time in a large range of mammalian taxa.

In the existing literature, tooth wear is generally observed by measuring the cusp height of a molar tooth, using a calliper. However, dental tissue loss has also been recorded by evaluating teeth with a visual scoring system, often those developed by Payne (1987) or Grant (1982). These scoring systems were originally developed to determine the age of an animal, and are often used in combination with other techniques such as cementum-ring counting. In this review, Table 1 presents a summary of wear rates for a number of herbivorous taxa. Table 2 then illustrates the different methods used to calculate these rates across a range of references.

In Table 1, the wear rates measurements marked with an asterisk could not be found explicitly in the sources cited by Damuth and Janis (2014a); it is therefore assumed that these rates are calculated from graphs plotting crown height against age. For wear rate measurements marked as "growth rates" with a double asterisk, it is possible that Damuth and Janis (2014a) used these two terms as equal in the case of hypselodont rodents. Upper-case letters "M", "P", or "I", indicate maxillary molars, premolars, and incisors respectively; and lower-case letters indicate mandibular teeth.

Table 1. Observed molar wear rates in herbivorous mammals, based on (Damuth and Janis, 2014a)

Species	Tooth	Wear rate (mm/y)	Origin	Diet	Reference
Ungulata - Hypsodont					
EQUIDAE <i>Equus burchelli</i>	M1	3.25*	Wild		(Spinage, 1972)
RHINOCEROTIDAE <i>Ceratotherium simum</i>	M1	2.80*	Wild		(Hillman-Smith et al., 1986)
BOVIDAE <i>Aepyceros melampus</i>	M1	2.62*	Wild		(Spinage, 1971)
	m1	2.50*			
	M2	4.36*			
	m2	3.32*			
	M3	4.12*			
	m3	3.60*			
<i>Antilocras marsupialis</i>	M2	1.41	Wild		(Solounias et al., 1994)
<i>Antilocarpa americana</i>	m1	2.50*	Wild		(Lubinski, 2001)
<i>Bison bison</i>	m1	1.70	Wild		(Haynes, 1984)
	m1	3.65			(Solounias et al., 1994)
<i>Capra pyrenaica</i>	M1	0.30	Wild	Alpine and Mediterranean vegetation pooled	(Fandos et al., 1993)
	M3	0.24			
<i>Connochaetes taurinus</i>	M2	2.03	Wild		(Solounias et al., 1994)
<i>Damaliscus korrigum</i>	M1	8.06*	Wild		(Mertens, 1984)
	m1	6.82*			
<i>Gazella granti</i>	M1	1.40	Wild		(Spinage, 1976)
<i>Gazella thomsonii</i>	M1	0.49	Wild		(Robinette and Archer, 1971)
<i>Kobus ellipsiprymnus</i>	i1	1.62	Wild		(Spinage, 1973)
<i>Ovis aries</i>	i	0.83	Captive		(Healy and Ludwig, 1965)
<i>Ovibos moschatus</i>	M2	3.51	Wild & captive		(Solounias et al., 1994)
<i>Sylvicapra grimmia</i>	M1-3	0.59	Captive		(Wilson et al., 1984)
<i>Syncerus caffer</i>	m1	2.77	Wild & captive		(Taylor, 1988)
		2.17	Wild		(Sanson et al., 2017)
<i>Taurotragus oryx</i>	m3	0.27 F	Wild & captive		(Jeffery and Hanks, 1981)
	II	0.29 M			
		0.64 F			
		0.65 M			
<i>Tragelaphus angasii</i>	M1	0.71	Wild		(Anderson, 1986)
		1.15*			
CERVIDAE <i>Alces alces</i>	M1	0.39*	Wild		(Ericsson et al., 2001)
		1.56			
<i>Capreolus capreolus</i>	m1	0.33	Wild		(Aitken, 1975; Solounias et al., 1994)
	m1	0.49	Wild		(Hewison et al., 1999) for three populations
		0.48			
		0.29			
<i>Cervus canadensis</i>	M1	1.54*	Wild		(Klein, 1983)
<i>Cervus elaphus</i>	m1	0.92* M	Wild		(Lowe, 1967) starting at 2 years old
		0.47* F			
	m1	0.52* M	Wild		(Loe et al., 2003)
		0.45* F			
	m1	1.08 M	Wild		(Carranza et al., 2004)
		0.62 F			
<i>Cervus nippon</i>	m1	0.05* (avg)	Wild		(Takahashi et al., 1999)
	M1	0.107 F			
	M2	0.084 F	Wild		(Ozaki et al., 2010; Kubo et al., 2011)
	M3	0.067 F			
<i>Muntiacus reevesi</i>	m1	0.87	Wild?		(Pei and Wang, 1991)
<i>Odocoileus hemionus</i>	m1	0.48*	Wild		(Robinette et al., 1957) starting at 2 years old
	m2	0.43*			
	m3	0.02*			
<i>Odocoileus virginianus</i>	m1	0.30*	Wild & captive		(Severinghaus, 1949)
	m2	0.57*			
	m3	0.51*			
<i>Rangifer tarandus</i>	M1	0.92	Wild		(Solounias et al., 1994)
	m2	1.19* M	Wild		(Morrison and Whitridge, 1997)
		0.82* F			
<i>Giraffa camelopardalis</i>	M1	0.96	Wild		(Hall-Martin, 1976)

**Rodentia – Non-
Hypselodont**

CRICETIDAE	<i>Akodon longipilis</i>	M	0.37*	Wild		(Pearson, 1983)
	<i>Akodon olivaceus</i>	M	0.38*	Wild		(Pearson, 1983)
	<i>Auliscomys micropus</i>	M	1.16*	Wild		(Pearson, 1983)
	<i>Cavia porcellus</i>	IR	98.80 F**	Experimental	Grain, hay & grass	(Shadle et al., 1938)
		IL	99.00 F**			
		iR	125.9 F**			
		iL	125.2 F**			
		IR	87.15 M**			
		IL	86.84 M**			
		iR	105.92M**			
		iL	108.45M**			
		I, i	85.25**			
		I, i	77.22**			
		I	56.68	Experimental	Lucerne	(Kamphues, 2001)
			57.20		Grass	(Harkness et al., 2010)
			59.80		Grass & rice hulls	(Müller et al., 2015a)
			62.04		GRS	
			106.08		Grass hay	
		i	88.92		Lucerne	
			91.00		Grass	
			102.44		Grass & rice hulls	
			108.16		GRS	
			91.00		Grass hay	
	<i>Chelemys macronyx</i>	M	0.85*	Wild		(Pearson, 1983)
	<i>Geoxus valdivianus</i>	M	0.31*	Wild		(Pearson, 1983)
	<i>Oryzomys longicaudatus</i>	M	0.29*	Wild		(Pearson, 1983)
	<i>Phyllotis darwini</i>	M	1.26*	Wild		(Pearson, 1983)
	<i>Rhabdomys pumilio</i>	mI	0.56*	Wild		(Henschel et al., 1982)
	<i>Clethrionomys glareolus</i>	M	2.60**			(von Koenigswald and Golenishev, 1979)

**Rodentia & Lagomorpha –
Hypselodont**

ARVICOLIDAE	<i>Dicrostonyx torquatus</i>	M	44.20**			(von Koenigswald and Golenishev, 1979)
	<i>Lagurus lagurus</i>	M	23.40**			(von Koenigswald and Golenishev, 1979)
	<i>Microtus mandarinus</i>	M	33.80**			(von Koenigswald and Golenishev, 1979)
	<i>Microtus fortis</i>	M	28.60**			(von Koenigswald and Golenishev, 1979)
LEPORIDAE	<i>Oryctolagus cuniculus</i>	IR	104.00	Domestic		(Shadle, 1936)
		IL	106.00			
		iR	128.00			
		iL	122.00			
		I, i	114.40**			(Spannbrucker et al., 1977)
		m	62.40**			(von Koenigswald and Golenishev, 1979)
		I	104.00**			(Lobprise and Wiggs, 1991)
		i	124.80**			
		I	83.72		Carrots	(Wolf and Kamphues, 1995)
		I	85.80		Grass hay	
		I	62.92		Grain mix	
		I	61.36		Pellets	
		i	75.40		Carrots	
		i	83.72		Grass hay	
		i	59.28		Grain mix	
		i	53.04		Pellets	
		I, i	156.00			(Meredith, 2007)
		m	36.40			(Harcourt-Brown, 2009)
		I, i	114.40**	Domestic		(Jekl and Redrobe, 2013)
		I, i	117.00			(Schumacher, 2011)
		m	31.20**			Jekl and Redrobe (2013)
		I, i	156.00			(Müller et al., 2014a)
		I	66.04	Experimental	Lucerne	
		i	81.64			
		m	63.96			
		I	68.12		Grass	
		i	86.32			
		m	69.16			
		I	69.68		Grass & rice hulls	
		i	95.68			
		m	69.16			
		I	87.36		GRS	
		i	98.80			
		m	232.44			
		I	131.04		Grass hay	
		i	105.04			
		m	66.04			(Wyss et al., 2016)
		I	99.84	Experimental	GRS	
			99.84		Grass hay	
		I	118.56		GRS	
			113.88		Grass hay	
			111.28		GRS	
		pm	48.36		Grass hay	

Marsupialia - Hypsodont

MACROPODIDAE	<i>Dendrolagus lumholtzi</i>	M1	0.49*	Wild	(Coombes, 2005)
	<i>Macropus agilis</i>	M2-3	0.18	Wild	(Damuth and Janis, 2014a)
	<i>Macropus eugenii</i>	M2-3	0.51	Wild	(Damuth and Janis, 2014a)
	<i>Macropus giganteus</i>	M2-3	0.43	Wild	(Damuth and Janis, 2014a)
	<i>Macropus giganteus</i> (fossil)	M2-3	0.62	Wild	(Damuth and Janis, 2014a)
	<i>Macropus robustus</i>	M2-4	0.56	Wild	(Damuth and Janis, 2014a)
	<i>Setonix brachyurus</i>	M2	0.33	Wild	(Damuth and Janis, 2014a)
	<i>Thylgale stigmatica</i>	M2-3	0.13	Wild	(Damuth and Janis, 2014a)

† Semifossorial species

* Deduced from graph

** Measurements for growth rates, not wear rates

GRS = Grass hay, rice hulls & sand

avg = average

In this review, methodology is not uniform across species, and the diets consumed are varied and rarely reported, making this dataset complex to interpret. We only speculate on trends visible between taxa, as a general interpretation of the relationship between hypsodonty and dental wear rate.

Glires (rodents and lagomorphs) show impressive wear rates, up to 156 mm of dental tissue lost per year on rabbit incisors (*i.e.* roughly the width of one line of text in this thesis). This can be partially explained by their ever-growing hypselodont teeth, which are known to react to dietary hardness, *i.e.* they grow faster in instances of more wear (Müller et al., 2014a; Müller et al., 2015a). Lagomorphs and rodents have also been shown to have slightly softer enamel than other mammals (Table 3.1 in Berkovitz and Shellis, 2018), another factor that may explain their extreme rate of tooth wear.

The highest rate of wear, after the glires, is seen for hypsodont ungulates, specifically grazing and mixed feeding species. The grass component in these animals' diet is highly abrasive as outlined above, due to its intrinsic phytoliths and contamination by external abrasives. Cervids generally tend to show lower wear rates, being that most are browsers or mixed feeders with generally lower level of hypsodonty. Surprisingly, although they are hypsodont, the marsupials show relatively low wear rates. Possibly in these cases, hypsodonty is a retained phylogenetic trait, despite browsing or mixed feeding preferences (Butler et al., 2014).

Even though these molar wear rates do not cover the entire spectrum of mammals, some trends are visible and seem to reflect dental morphology, prompting further research on these trends and their relationship with diet.

Table 2. Tooth wear measurement methods

	Species	Method	Reference
Ungulata -hypsodont			
	EQUIDAE <i>Equus burchelli</i>	Crown height	(Spinage, 1972)
	RHINOCEROTIDAE <i>Ceratotherium simum</i>	Crown height + Scoring system	(Hillman-Smith et al., 1986)
	BOVIDAE <i>Aepyceros melampus</i>	Crown height	(Spinage 1971)
	<i>Antilocapas marsupialis</i>	Crown height	(Solounias et al., 1994)
	<i>Antilocarpa americana</i>	Crown height	(Lubinski, 2001)
	<i>Bison bison</i>	Crown height	(Haynes, 1984)
		Crown height	Reher and Frison (1980) in Solounias et al. (1994)
	<i>Capra pyrenaica</i>	Crown height + Scoring system (Payne, 1987)	(Fandos et al., 1993)
	<i>Capra aegagrus</i>	Crown height	(McGregor and Butler, 2015)
	<i>Connochaetes taurinus</i>	Crown height	Attwell (1980) in Solounias et al. (1994)
	<i>Damaliscus korrigum</i>	Scoring system	(Mertens, 1984)
	<i>Gazella granti</i>	Crown height	(Spinage, 1976)
	<i>Gazella thomsonii</i>	Crown height	(Robinette and Archer, 1971)
	<i>Ovibos moschatus</i>	Crown height	(Solounias et al., 1994)
	<i>Sylvicapra grimmia</i>	Crown height	(Wilson et al., 1984)
	<i>Syncerus caffer</i>	Crown height	(Taylor, 1988)
		Crown height	(Sanson et al., 2017)
	<i>Taurotragus oryx</i>	Crown height	(Jeffery and Hanks, 1981)
	<i>Tragelaphus angasii</i>	Crown height	(Anderson, 1986)
	CERVIDAE <i>Alces alces</i>	Crown height	(Ericsson et al., 2001)
	<i>Capreolus capreolus</i>	Crown height	(Hewison et al., 1999)
	<i>Cervus canadensis</i>	Crown height	(Klein, 1983; R.J., 1983)
	<i>Cervus elaphus</i>	Crown height	(Lowe, 1967)
		Crown height	(Loe et al., 2003)
		Crown height	(Carranza et al., 2004)
	<i>Cervus nippon</i>	Crown height	(Takahashi et al., 1999)
		Crown height + Crown height by X-ray CT	(Ozaki et al., 2010)
		Crown height	(Kubo et al., 2011)
	<i>Muntiacus reevesi</i>	Crown height	(Pei and Wang, 1991)
	<i>Odocoileus hemionus</i>	Crown height	(Robinette et al., 1957)
	<i>Odocoileus virginianus</i>	Crown height lingual	(Severinghaus, 1949)
	<i>Rangifer tarandus</i>	Crown height	(Morrison and Whitridge, 1997)
	GIRAFFIDAE <i>Giraffa camelopardalis</i>	Crown height	(Hall-Martin, 1976)
Rodentia – Non-Hypsodont			
	CRICETIDAE <i>Akodon longipilis</i>	Crown height by microscope	(Pearson, 1983)
	<i>Akodon olivaceus</i>	Crown height by microscope	(Pearson, 1983)
	<i>Auliscomys micropus</i>	Crown height by microscope	(Pearson, 1983)
	<i>Cavia porcellus</i>	Tooth mark	(Shadle et al., 1938)
		Tooth mark	(Müller et al., 2015a)
	<i>Chelemys macronyx</i> ¹	Crown height by microscope	(Pearson, 1983)
	<i>Geoxus valdivianus</i> ¹	Crown height by microscope	(Pearson, 1983)
	<i>Oryzomys longicaudatus</i>	Crown height by microscope	(Pearson, 1983)
	<i>Phyllotis darwini</i>	Crown height by microscope	(Pearson, 1983)
	<i>Rhodomys pumilio</i>	Crown height by X-ray	(Henschel et al., 1982)
	ARVICOLIDAE <i>Clethrionomys glareolus</i>	Crown height	(von Koenigswald and Golenishev, 1979)
Rodentia & Lagomorpha – Hypselodont			
	ARVICOLIDAE <i>Dicrostonyx torquatus</i>	Fluorescent marker	(von Koenigswald and Golenishev, 1979)
	<i>Lagurus lagurus</i>	Fluorescent marker	(von Koenigswald and Golenishev, 1979)
	<i>Microtus mandarinus</i>	Fluorescent marker	(von Koenigswald and Golenishev, 1979)
	<i>Microtus fortis</i>	Fluorescent marker	(von Koenigswald and Golenishev, 1979)
	LEPORIDAE <i>Oryctolagus cuniculus</i>	Tooth mark	(Shadle, 1936)
		Fluorescent marker	(von Koenigswald and Golenishev, 1979)
		Tooth mark	(Müller et al., 2014a)
		Fluorescent marker	(Wyss et al., 2016)
Marsupialia - Hypsodont			
	MACROPODIDAE <i>Dendrolagus lumholtzi</i>	Scoring system	(Coombes, 2005)
	<i>Macropus agilis</i>	Crown height	(Damuth and Janis, 2014a)
	<i>Macropus eugenii</i>	Crown height	(Damuth and Janis, 2014a)
	<i>Macropus giganteus</i>	Crown height	(Damuth and Janis, 2014a)
	<i>Macropus giganteus</i> ²	Crown height	(Damuth and Janis, 2014a)
	<i>Macropus robustus</i>	Crown height	(Damuth and Janis, 2014a)
	<i>Setonix brachyurus</i>	Crown height	(Damuth and Janis, 2014a)
	<i>Thylogale stigmatica</i>	Crown height	(Damuth and Janis, 2014a)

¹ Semifossorial species² Fossil species

Unless mentioned otherwise, “crown height” is measured with callipers from the gum to the highest cusp point and is used in combination with age to determine a wear rate.

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1.2 Tooth wear as a dietary proxy

The dietary signal informs the diet of an animal by combining scientific evidence (teeth, morphology, ingesta, etc.) with the timespan of the diet in question. Multiple dietary proxies exist, and some can be applied in palaeontology to recreate diets of extinct species and infer their palaeoenvironment (Fig. 4). The variety of dietary proxies span different timescales, and some are more adapted to fossil specimens than others. As teeth are the most conserved elements of fossil structures, they have become the main focus of dietary reconstruction proxies. Diet reconstructions allow us not only to have a better grasp of the evolution of hypsodont teeth, but also offer a window to the past, as dental wear provides us with information for palaeodietary reconstruction of extinct species. The main proxies used to determine diet from teeth are carbon and nitrogen isotopes (used to deduce a general C3 or C4 diet), mesowear (based on the molar cusp shape and height), microwear (based on microscopic pits and scratches), and microwear texture (based on enamel surface roughness and isotropy) (Davis and Pineda-Munoz, 2016).

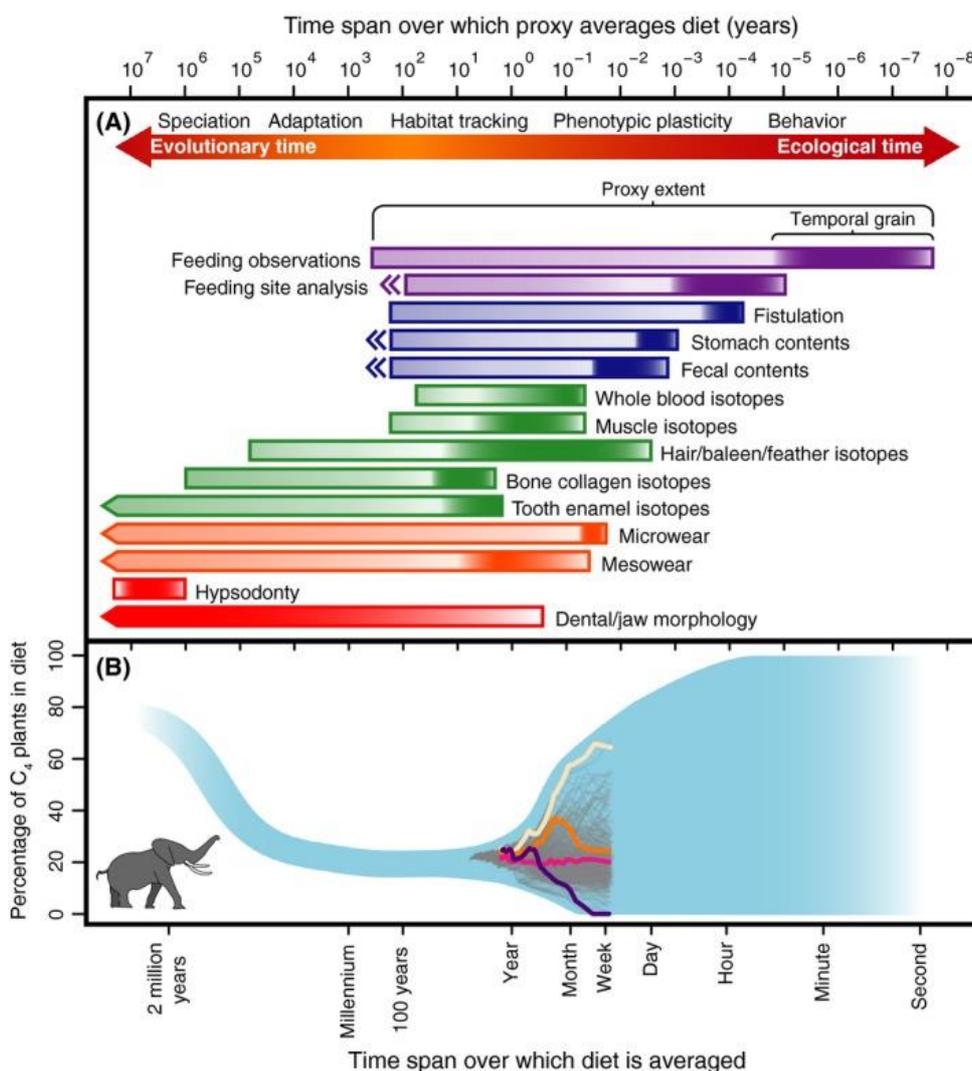


Figure 4. Range of dietary proxies and their respective timescales. From Davis and Pineda-Munoz (2016)

Microwear, a “last supper” signal

Microwear is the tooth wear proxy with the shortest timescale. For this technique, microscopic scratches and pits are observed on a defined facet of tooth enamel. In herbivores, browsers tend to show microscopic wear facets dominated by pits, whereas grazers have facets dominated by scratches. Dental microwear texture analysis (DMTA) uses 3D texture parameters to quantify the whole surface as a unit and measure surface roughness, complexity, and scratch orientation according to different diets (Clementz, 2012). With these measures, browsers tend to show a less isotropic surface texture, while grazers generally have enamel surfaces with higher isotropy. Direction of surface complexity for either diet varies depending on the microscope employed. Teaford and Oyen (1989) noted a 2D microwear signal turnover of 1-2 weeks in experimental monkeys, and DMTA applied in parallel with stomach content analysis (Merceron et al., 2010; Purnell et al., 2012; Kubo et al., 2019), rarely shows a signal correlation. These findings define 2D microwear and DMTA as average short-term signals, explaining why microscopic wear is often referred to as the “last supper” technique (Grine, 1986).

Diet at time of death has been used to describe seasonal dietary shifts (Merceron et al., 2010; Calandra et al., 2016; Berlioz et al., 2017), to distinguish between populations (Ungar et al., 2007; Merceron et al., 2010), and to detect grit vs. phytolith dominated wear (Merceron et al., 2016). Though microwear techniques may be useful at distinguishing precise diets, and DMTA can be used as a signal independent of morphology (Schulz et al., 2013), these techniques can only inform on the mechanical properties of food ingested by an animal in the last days or weeks of its life.

Long-term signals: tooth wear takes time

Long-term dietary signals are particularly useful to establish diets of extinct species and reconstruct their paleoenvironment. But characterising herbivore diets using these proxies also helps to better understand the evolution of their teeth. Stable isotope analysis is a proxy allowing for dietary interpretations independent of morphology (Clementz, 2012). Stable carbon, nitrogen and oxygen are most commonly used for dietary analysis, though calcium, strontium and magnesium isotopes are in development (Calandra and Merceron, 2016). Isotopic measurements track the proportion of C₃ vs. C₄ plants in a herbivore’s diet over different timescales depending the part of the tooth analysed (Clementz, 2012). Enamel is formed at the beginning of a specimen’s lifetime, before the teeth become metabolically inert (Davis and Pineda-Munoz, 2016); it may thus only present a signal of this short timeframe where the diet may not have been the same as that of the adult animal. However, once formed, the tooth is one of the best conserved parts of the mammal body, and can provide a record retainable for millennia.

Mesowear analysis is a series of techniques, according to which the outer profile of a herbivores' tooth row is observed with the naked eye, to determine if its diet tends more towards browser or grazer – based on its macroscopic wear. It is a rapid and cost-effective technique which has been widely employed to score large numbers of teeth across extant and extinct taxa. Since the establishment of the technique by Fortelius and Solounias (2000), many variants have been established; pertaining either more or less complexity, or specialisation to certain taxa. The original mesowear technique (Fortelius and Solounias, 2000) and its extended version (Winkler and Kaiser, 2011) – also called “mesowear I” - employ the depth of the valleys between cusps, or occlusal relief (OR), and cusp shape (CS) of a molar cusp to determine levels of wear, by scoring OR as high or low and CS as sharp, round, or blunt, and variations thereof. A more simplified version of the technique, “mesowear II”, combines OR and CS into a single ordinal score. Most recently, “mesowear III” or “inner-mesowear” was established by Solounias et al. (2014), and scores the inner enamel band of the molar, in an aim for more precise mesowear representing a shorter timeframe. Though mesowear was originally described as a rather general lifetime signal, the precise duration of the dietary signal it represents is unclear. Some have debated that it could represent seasonal diets (Kaiser and Schulz, 2006; Muhlbachler and Solounias, 2006; Rivals et al., 2013), and even - when using mesowear III - weekly changes in diet (Danowitz et al., 2016), while others defend a yearly or lifelong signal (Merceron et al., 2007; Yamada, 2012; Brent Jones and Desantis, 2017).

Mixed signals: combining proxies

Mesowear can be combined with other proxies such as microwear and microtexture (Schubert et al., 2006; Rivals and Lister, 2016; Sánchez-Hernández et al., 2016; Muhlbachler et al., 2018; Strani et al., 2018; Semprebon et al., 2019), carbon and nitrogen isotopes (Louys et al., 2012; Ecker et al., 2013; Loffredo and DeSantis, 2014; Saarinen et al., 2015), or both (Tütken et al., 2013; Rivals et al., 2015; Brent Jones and Desantis, 2017). As these proxies represent widely different timescales of an animal's diet, they rarely correlate with each other (Tütken et al., 2013; Davis and Pineda-Munoz, 2016). Discrepancies between proxies that are difficult to explain have often resulted in a categorical “mixed-feeder” diet and are said to purvey a more intricate view of diet over time, which is, more often than not, variable. Though an animal's diet is not a constant over the course of its lifetime, or even a single year of its life, one would expect a more or less stable dietary signal, especially in extant animals where observational data and stomach contents are available. If then, a stable signal cannot be produced between the different proxies, one might question their reliability. Studies addressing tooth wear experimentally are far and few between (Solounias et al., 2014; Kropacheva et al., 2017), and although

transferring experimentally-based temporal scales to field conditions may be impossible, dietary experiments remain the benchmark for testing dietary proxies.

2 | Research overview

The following projects were undertaken during the years dedicated to this thesis and are referred to in the order in which they are presented here. The first manuscript is a review on the mesowear technique and its variations (**Manuscript M1**) (Ackermans et al. in prep. -a). This technique was first established to measure the macroscopic shape change of tooth cusps in relation to diet, and has seen a slew of variations since its creation. In this dissertation alone, three variations of mesowear were applied during the goat and sheep experiments. This review was created, firstly to track and define once and for all the successive advancements and changes of the technique, frequently adapted for scoring different teeth and different species. Secondly, this review serves to identify the general type of studies involving mesowear, whether extant or extinct specimens are studied in greater proportion, and which species in particular are scored according to each version of the technique. This dataset was created to provide easy access to information regarding mesowear, to further inform future studies, to serve as a foray into the stability of the signal, and to encourage experiment reproducibility.

Following the mesowear review are a series of publications relying on data created during a pilot study on domestic goats in 2011. In order to further understand how different types of abrasives affect tooth wear, n=28 does were kept experimentally and assigned to four different groups in a controlled feeding experiment, in which they were fed pelleted experimental diets with varying levels of internal and external abrasives for 6 months. The four diets were as follows: L: lucerne based pellets, complemented with lucerne hay, very low phytolith abrasion diet acting as the control. G: grass-based pellets, completed with grass hay, medium abrasive phytolith diet. GR: grass and rice husk pellets, complemented with grass hay, high abrasion phytolith diet. GRS: the GR diet with addition of large-grained sand as a source of external abrasives. These diets were designed to reveal the effect of different types of phytolith and grit abrasives on tooth wear over the long-term. Computed tomography (CT) scans of skulls were taken at the beginning, midpoint, and end of the experiment after the skulls of the animals were skeletonized. Additionally, full-body CT scans were made of the GRS group in the midpoint of the experiment. To evaluate the development of tooth wear over the time, mesowear I and II were scored on all teeth (except incisors) using the skulls and 3D renderings of the CT scans data (**Publication P1**) (Ackermans et al., 2018). The 3D renderings were then used to evaluate the

changes in tooth volume over time or absolute wear (**Publication P2**) (Ackermans et al., 2019b). To complete the array of tooth measurements and dietary proxies, and investigate the dietary signal at different timescales, mesowear III was also applied to the goat molars (**Publication P3**) (Stauffer et al., 2019), as well as microtexture (**Manuscript M2**) (Schulz-Kornas et al., in prep.). This array of techniques allows an overview of the dietary signal formation over the full extent of the experiment. As the ingestion of sand goes beyond the teeth, we were also interested in understanding the effect of ingested abrasives on the gastrointestinal tract. We therefore measured the volume of sand in the digestive tract, using abdominal CT scans to better understand the behaviour of external abrasive particles during rumination (**Publication P4**) (Hatt et al., 2019).

As the pilot study reinforced our hypothesis regarding the manner in which abrasives affect tooth wear in small ruminants, a larger study was performed over 17 months, on n=49 sheep. The goal of this experiment was to obtain a better understanding the effect of different sizes of external abrasives on tooth wear over a longer period of time. The sheep were kept experimentally in eight groups, each fed diets with different abrasiveness. The diets consisted of a pellet base with either small (\varnothing 4 μ m), medium, (\varnothing 50 μ m) or large (\varnothing 130 μ m) abrasives, added at 0%, 4%, or 8% concentration. As a first comparison between the two studies, a food preference trial was documented in both the pilot and the large experiment (**Publication P5**) (Ackermans et al., 2019a), to investigate if either species showed an avoidance towards abrasives. As in the pilot study, CT scans were performed under anaesthesia at the beginning, midpoint, and end of the sheep experiment, and were made in conjunction with resin casts of the teeth. In order to provide a comparison to the pilot study, mesowear I and II, absolute wear, and microtexture were all applied to the sheep's teeth (**Manuscripts M3, M4, M6**) (Ackermans et al., in prep.-b; Ackermans et al., in prep.-c; Ackermans et al., in prep.-d), to follow the development of the dietary signal at different stages along the course of the experiment. As another follow-up to the pilot experiment, we also investigated the accumulation of abrasive content inside the digestive tract in the sheep (**Manuscript M5**) (Hatt et al., Submitted).

In sum, these studies provide an experimental overview of the effect of abrasive diets on teeth of small ruminants, and inform on the array of proxies with which to record these effects. Both the goat and the sheep experiments have been essential in testing fundamental tooth wear hypotheses, and providing insight into future studies concerning dietary reconstruction and the effect of different abrasives on tooth wear.

Part B

Publications

M1

The history of mesowear: A review

N. L. Ackermans

Submission pending

The history of mesowear: A review

Nicole L. Ackermans

Abstract

Here we review published mesowear data from October 2000 to June 2019 (198 publications, 688 species, 1310 data points). Mesowear is a widely applied analysis that can be used to infer a herbivore's diet by scoring the height and sharpness of molar tooth cusps. Established as a tool for palaeodiet reconstruction, the technique has seen multiple adaptations, simplifications, and extensions, which have become complex to follow. The current study provides a review of the successive changes and adaptations to mesowear, to serve as a guide for applying the appropriate technique to the research question at hand. In addition, the array of species to which mesowear has been applied, along with the equivalent recorded diets have been compiled here.

Keywords: tooth wear, diet reconstruction, herbivore, palaeoenvironmental proxy, molar wear profile

Introduction

Tooth wear and shape can be measured at different levels, informing a specimens' or a species' diet, and illustrating the evolution of entire classes of animals. Within tooth wear, attrition is caused by tooth-on tooth contact and is generally the main cause of wear in animals with a browsing diet. The soft nature of a browse diet causes opposing teeth to wear themselves upon contact, as the diet does not provide resistance. Abrasion on the other hand, is caused by internal- or external abrasives, wearing tooth material upon contact. Grasses contain large amounts of hard opaline silicates that wear tooth enamel when chewed repetitively, and, grazing animals generally to feed close to the ground in open habitats, where plants trap external abrasives, e.g. dust and grit (Janis and Fortelius, 1988). It is still debated whether phytoliths or external abrasives are the main cause of tooth wear, and therefore the main driver in the evolution of hypsodonty (Williams and Kay, 2001; Sanson et al., 2007b; Damuth and Janis, 2011b; Hummel et al., 2011b; Xia et al., 2015; Merceron et al., 2016a; Semprebon et al., 2019), though the general agreement is that both contribute at least somewhat. Historically, tooth-wear patterns have been of interest primarily for age determination, using the visual aspect of the tooth's surface (Grant, 1982), rather than dietary

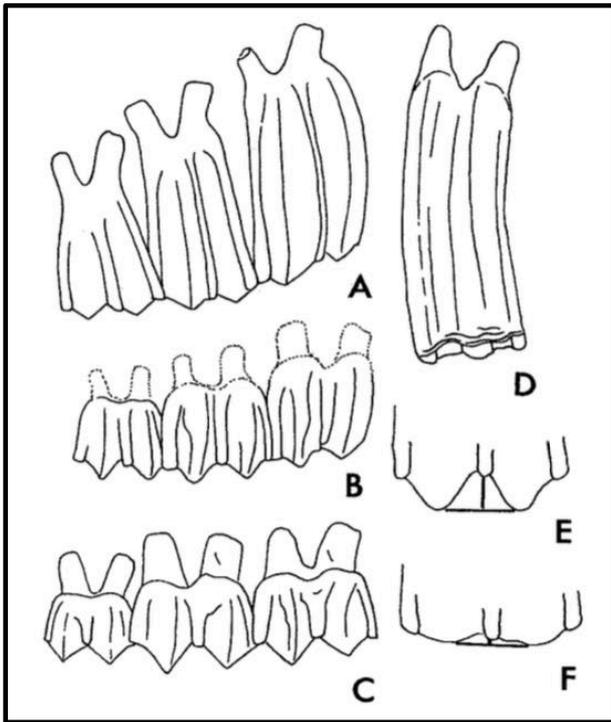
reconstruction. This technique has been called “macrowear”, and confusingly, “meso-wear” in the past, and it has the benefit of being applicable to a variety of species, from bears (Stiner, 1998) to manatees (Gonzalez-Socoloske et al., 2018)., though this type of “macrowear” requires species-specific wear charts and does not record dietary signals.

Today, mesowear is a technique used to measure the tooth wear visible on the tooth profile with the naked eye. This mesowear technique was introduced by Fortelius and Solounias (2000), (Table 1) as a method to reconstruct general paleodiets of fossil ungulates, by observing the macroscopic wear on their molars. An abrasion-attrition wear gradient is used to assign dietary categories to herbivores, with browsers generally showing a more attrition-based wear pattern, and grazers a more abrasion-dominated pattern. The original mesowear technique, also called “mesowear I”, was established to represent the average diet of a species from a certain location. As such, it serves as a midway point between the unworn shape of a tooth representing evolution on an extended timescale, and microwear, representing diet over a short amount of time (Fortelius and Solounias,

2000). Originally, mesowear analysis was designed for use on selenodont (*e.g.* bovid), or trilophodont (*e.g.* mastodon) molars, and applied by observing “*the buccal edges of the paracones and metacones of upper molars*” (Fortelius and Solounias, 2000) with the naked eye or at low magnification (Fig.1). As a direct consequence, mesowear is a fast, inexpensive technique for diet determination. Molar cusp relief or occlusal relief (OR) was defined in the original publication as “*the relative distance between cusp height and inter-cusp valleys*”, with low OR related to the high abrasion typical of the grazer diet. Cusp shape (CS) was therein defined by “*the apex of the cusp being described as sharp, rounded or blunt*”, using the maxillary M2 as the tooth of reference. Applying these variables allows dietary reconstruction based on the percentage of sharp, round, or blunt cusps; alongside the percentage of high relief. Mesowear I was developed using a database of 64 extant species (Annex 1), and was succinctly applied to six fossil species of known diet to test its strength, followed by a blind test on 20 specimens of *Hippotherium* (Kaiser, 2000) (Table1, Annex 1).

¹ Large excel file, available upon request to nlackermans@gmail.com

A



- A: *Capra hircus*, high OR, sharp CS
- B: *Cervus duvaucelli*, mesodont, high OR, round CS
- C: *Odocoliaus virginianus*, brachydont, high OR, sharp CS
- D: *Equus ferus caballus*, hyperhypsodont, low OR, blunt CS
- E: *Kobus ellipsiprymnus*, high OR, round CS
- F: *Aelaphus busephalus*, low OR, blunt CS

B

	Valley between cusps $\le 90^\circ$ $\frac{X}{Y} \ge 0.25$	Occlusal relief (OR)	CM	EM
	Valley between cusps $> 90^\circ$ $\frac{X}{Y} > 0.25 \le 0.125$		hh	hh
	$\frac{X}{Y} > 0.125 \le 0.05$		h	h
	$\frac{X}{Y} > 0.05 < 0$		hl	hl
	$\frac{X}{Y} \le 0$		l	l
	$\frac{X}{Y} \le 0$		fn	fn
	Sharp with lens 12x	Cusp shape (CS)	CM	EM
	Sharp with naked eye, at 20cm, round with lens 12x		s	s
	Sharp with naked eye, at 20cm, round with lens 12x		rs	rs
	Clearly round with naked eye, length $\le 1/2$ cusp length		r	r
	Clearly round with naked eye, length $> 1/2$ cusp length		rr	rr
	Platform present, highest point not clear, goes together with (l)		b	b

Figure 1. A: Mesowear features used for scoring in the original study from Fortelius and Solounias (2000), described in more detail in B, from Taylor et al. (2016). OR: occlusal relief, CS: cusp shape. B: CM: Description of cusp shape categories for the classical mesowear method by Fortelius and Solounias (2000); EM: enhanced mesowear method established by Winkler and Kaiser (2011).

Table 1: Additions and adaptations to the original mesowear technique - ordered by mesowear technique and date.

Technique	Reference	Description	Scores
Original mesowear - Mesowear I	Fortelius and Solounias (2000)	-Using the naked eye or x10 magnification -Scoring only sharpest buccal cusp of maxillary M2 -Last molar in occlusion and M1 shape similar to M2 -Percentage of high relief and Percentage of sharp, round and blunt cusps	OR: low, high CS: blunt, round, sharp
Mesowear I – Adapted for Equidae	Kaiser and Fortelius (2003)	Method extended to all apices on maxillary P4-M3 in equids	Original mesowear
Mesowear I	Franz-Ondendaal and Kaiser (2003)	Method extended to maxillary M3, and mandibular M2 in ruminants	Original mesowear
Mesowear I – Adapted for Lagomorpha	Fraser and Theodor (2010)	“Cusp relief” combined with “buccal shearing crush wear” on maxillary and mandibular P4-M2 – resulting in 5 dietary classes	1: 45° enamel-dentine relief with no additional wear - highly folivorous 2: 45° enamel- dentine relief with buccal shearing crush wear - leaves & woody materials 3: 45° enamel-dentine relief with buccal shearing crush & phase II wear - leaf, twig, & fruit diet 4: 90° enamel-dentine relief with no additional wear - open area grazers 5: 90° enamel-dentine relief with buccal shearing crush wear - open area browsers
Mesowear I – Adapted for Conodonta	Purnell and Jones (2012)	Scored on P1 elements	
Mesowear I – Adapted for Leporines & Murines	Ulbricht et al. (2015)	Classical mesowear on the maxillary M1, M2, and mandibular p3 in <i>leporinae</i> and distal side of the maxillary M1 and mandibular m1 in <i>murinae</i>	Original mesowear
Mesowear I – Adapted for voles	Kropacheva et al. (2017)	Maxillary M1-M2, mandibular m1	Occlusal relief 1-7 Lateral facet development 1-3
Mesowear II -“Mesowear ruler”	Mihlbachler et al. (2011)	Simplified score using gauges and a seven-point system	Combined score 0-6
Mesowear II -“Mesowear ruler”	Wolf et al. (2013)	Additional intermediate scores	Combined score 0-13, in increments of 0.5
“Mesowear angles” – Adapted for Proboscidea	Saarinen et al. (2015)	“ <i>Mean mesowear angles of three central lamellae in occlusion</i> ” on all except deciduous teeth	Mean mesowear angle < 106°: C3-plant based diet > 130°: C4-plant based diet (grazer)

“Mesowear angles” – Adapted for <i>Xenarthra</i>	Saarinen and Karme (2017)	All molariform teeth	For <i>Xenarthra, Folivora</i> : Mean mesowear angle: 60°-85°: fruit browsers 75°-100°: leaf browsers 100-132°: mixed-feeders 132°-150°: grass dominated mixed-feeders 150°-190°: grazers For <i>Xenarthra, Cingulata</i> : 60°-100°: carnivore, insectivore, omnivore, possibly browsers 100°-125°: browse-dominated mixed-feeders & herbivorous omnivores 125°-152°: grass-dominated mixed-feeders 152°-190°: grazers
Mesowear II	Mihlbachler and Solounias (2006)	Simplified score, only the proportion of sharp cusps	Proportion of sharp cusps: 40-100%: Clean browser 20-40%: Mixed feeders: 0-20%: Grazer
Mesowear II “quantitative mesowear”	Widga (2006)	Interval measurements of cusp and saddle heights to calculate cusp relief	Index of cusp relief (ICR): Low ICR: grazer High ICR: browser
Mesowear II	Rivals and Sempredon (2006)	Simplified score combining OR and CS	0: high relief & sharp cusps 1: high relief & round cusps 2: low relief & round cusps 3: low relief & blunt cusps
Mesowear II	Croft and Weinstein (2008)		0: high relief & sharp cusps 1: high relief & round cusps 2: low relief & round cusps 2.5: low relief & sharp cusps 3: high/low relief & blunt cusps
Mesowear II	Kaiser (2009)		0: high relief & sharp cusps 1: high relief & round cusps 2: low relief & sharp cusps 3: low relief & round cusps 4: low relief & blunt cusps
Mesowear II	Rivals et al. (2009)		0: high relief & sharp cusps 1: high relief & round cusps 2: low relief & round cusps 2.5: low relief & sharp cusps 3: low relief & blunt cusps
Mesowear II	Fraser et al. (2014)	Method extended to mandibular p4-m3 for ruminants	1: high relief & sharp cusps 2: high relief & round cusps 3: high relief & very round cusps 4: low relief & round-blunt cusps 5: low relief & flat-blunt cusps
Mesowear II – Adapted for Marsupialia	Butler et al. (2014)	Use of classical mesowear and a combined score on the maxillary left maxillary molars, scoring sharpest buccal cusp	Combined score as in (Kaiser et al., 2009)
Mesowear I & II – Expanded	Winkler and Kaiser (2011)	Intermediate stages added to original and combined score	OR: low, high-low, high, high-high. CS: blunt, round-round, round, round-sharp, sharp. Combined score 1-17
Mesowear I and II - Expanded, Adapted for Rhinocerotidae	Taylor et al. (2013)	Expanded version and combined score on maxillary P2-M2.	Combined score 1-11
Mesowear III – “Inner mesowear”	Solounias et al. (2014)	Scores the second enamel band, using a stereo-microscope. Mesial side, distal side and junction point are scored separately.	Enamel band wear states: 1: ideal browser 2-3: intermediate 4: ideal grazer Junction point score 1-4

In the original mesowear method described above, the sharper of the two molar cusps was scored, noting that the choice of cusp was not critical, a hypothesis confirmed by Ackermans et al. (2018). The authors also note the importance of scoring at least 10, and ideally 20-30 specimens per species and/or locality for a reasonable approximation of the score, though on palaeontological specimens, rudimentary dietary assumptions are sometimes made using a single tooth, as complete specimens are rare. Although the initial assumption was that mesowear remains relatively stable throughout an individual's life (when very young or very old specimens are excluded), Rivals et al. (2007a) later established the idea that mesowear varies based on initial crown height and is different throughout an animal's lifetime.

Further adaptations were made to the original mesowear technique (for more details, see Table 1), expanding it to more teeth (Franz-Odenaal and Kaiser, 2003; Kaiser and Fortelius, 2003), and adapting the method to specific taxa (Fraser and Theodor, 2010; Purnell and Jones, 2012; Taylor et al., 2013; Butler et al., 2014; Saarinen et al., 2015; Ulbricht et al., 2015; Kropacheva et al., 2017; Saarinen and Karne, 2017). Some, deeming OR a redundant measure, simplified mesowear by only using categories of CS (Mihlbachler and Solounias, 2006; Widga, 2006), while others simplified the technique by combining OR and CS into a single score (Rivals and Semprebon, 2006; Croft and Weinstein, 2008; Kaiser, 2009) – these simplified versions

of the original mesowear technique were deemed “mesowear II” by Solounias et al. (2014). Further simplifications include a “mesowear ruler” system (Mihlbachler et al., 2011), initially developed for horses, (Fig. 2), and a “mesowear angle” system (Saarinen et al., 2015). Mesowear also has an extended version, where intermediate stages were added to the original mesowear categories and a more complex combined score was created to provide more detail (Winkler and Kaiser, 2011) (Table 1, Fig. 1).

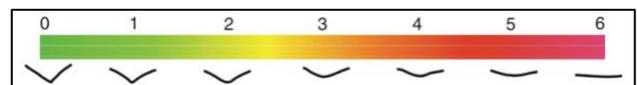


Figure 2. Wear stages of mesowear III, from Solounias et al. (2014).

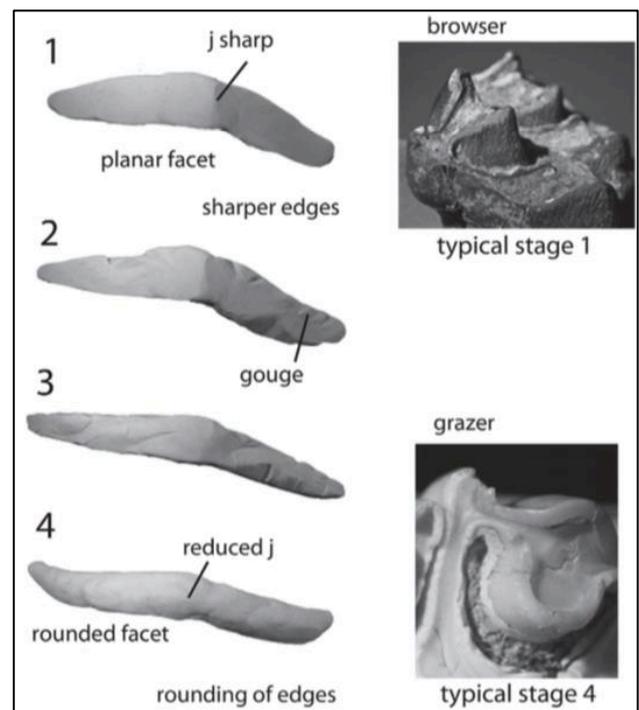


Figure 3. Wear stages of the mesowear ruler representing average mesowear score and crown height index, from Mihlbachler et al. (2011).

“Mesowear III” or “inner-mesowear” was implemented by Solounias et al. (2014) (Fig. 3), where scoring the inner enamel band of the tooth

aimed to record a more precise signal, and represent a shorter timeframe. Mesowear III has been applied in six other studies since its creation (Annex 1), but has been tested experimentally once. In that case, results did not show more precision than traditional mesowear when both techniques were applied to the same dataset (Stauffer et al., 2019).

Traditionally, mesowear has either been scored directly on the specimens' teeth, on resin casts, or on photographs of the specimen's teeth (Fortelius and Solounias, 2000). More recent studies, however, have used 3D models of wear facets (Hernesniemi et al., 2011), or scored mesowear directly onto 3D reconstructions from CT scanned skulls of live animals (Ackermans et al., 2018). Various microscopy techniques have also been used as a means of scoring mesowear on smaller specimens (Purnell and Jones, 2012; Kropacheva et al., 2017).

The many iterations and addendums to mesowear can create confusion regarding which is best applied (Viranta and Mannermaa, 2014), and the interpretation of corresponding results (Díaz-Sibaja et al., 2018). The aim of this review was to therefore create a body of reference with precise definitions and short explanations for each variation of the mesowear technique, to facilitate future applications. An overview of current dental wear techniques exists (Green and Croft, 2018), but the goal of the current study was to provide a more detailed and widely understandable overview of the history and progression of the mesowear technique in

particular. Therefore, Table 1 lists all major amendments to the original mesowear technique - including the various versions of mesowear I, II and III - along with a short description, and the scoring system used, thus hoping to ease comprehension of the available techniques and promote proper application in respect to the research question at hand. In addition, a dataset was created reuniting the dietary classifications of all species to which the mesowear technique has been applied thus far, including specimen type, phylogenetic classification, and diet, as a readily accessible resource for future research (Annex 1).

Methods

Publications were cited using the search term “mesowear” in Google Scholar, PubMed, and ResearchGate for every year from 2000 until present (30.06.2019), resulting in 197 publications analysed. Book chapters, PhD, MSc thesis, and conference proceedings were included if they contained otherwise unpublished original mesowear data. Studies using the terms “mesowear” or “macrowear” to describe wear on the macroscopic scale, without referring to the (Fortelius and Solounias, 2000) mesowear technique, were excluded. The variety of taxa explored is represented in Fig. 4. Diets in Annex 1 are indicated as shown in the corresponding references. A “various” diet indicates a diet change for the same species within the publication (different localities or time periods).

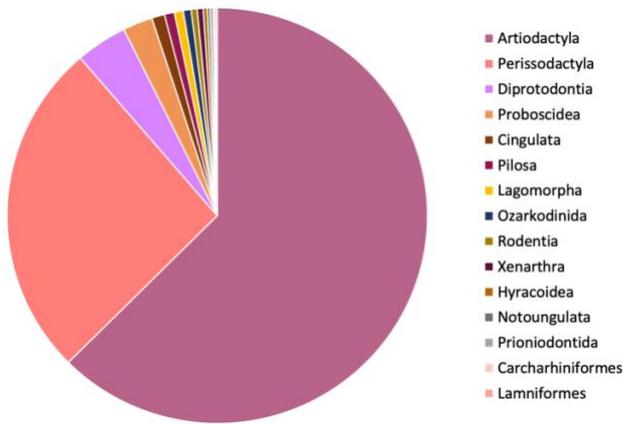


Figure 4: Percentage of taxonomic orders represented within in mesowear studies from 2000 to June 2019. Data sorted by specimen, n=1310

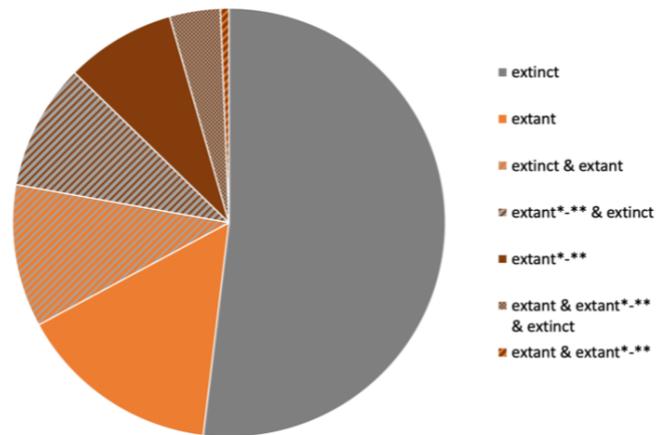


Figure 5a. Specimen status of samples represented in mesowear studies from 2000 to June 2019. Data sorted by number of publications, n=197.

A species without an assigned diet represents the lack of a diet indication or mesowear score within the text. The term “experimental” diet was used for studies in which experimental diets were fed to animals in controlled environments. When a study measured both mesowear and microwear and the indicated diets diverged, the diet indicated by mesowear was reported here. If species were listed with multiple entries, an average was made, and, if within the study mesowear was scored but the diet was not defined, a diet was assigned according to the mesowear score reported in the publication and previous research regarding the respective technique. Extant and extinct specimens were classified as either “wild”, “captive” (zoo, or experimental specimens), archaeological (excavated in an archaeological context as defined by the original publication, designated “extant*.” in Annex I, Fig. 5A) or fossil (fossil specimens of extant species designated “extant*.” in Annex 1, Fig. 5A).

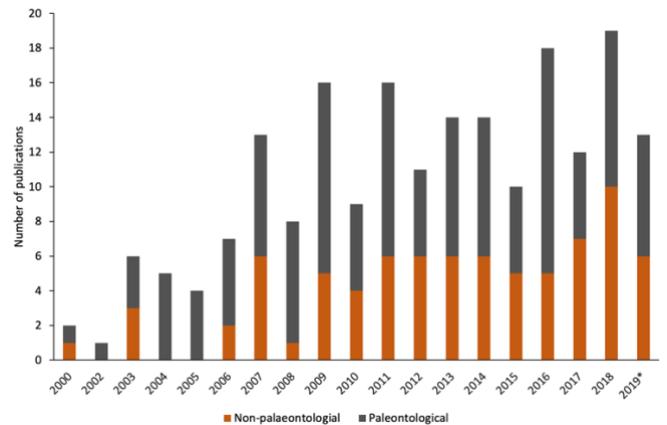


Figure 5b. Yearly amount of publications scoring mesowear on paleontological specimens versus non-paleontological specimens between 2000 and June 2019*, n=197.

When a palaeontological specimen’s identification could not be established to the species level, the specimen was designated as “fossil” in Annex 1. For simplicity of analysis, mesowear techniques are designated mesowear I, II, III, or a combination thereof in Annex 1. Extended or simplified versions are noted in the case of the “mesowear ruler”, “mesowear angle”, “mesowear I and II – extended”, and all taxon-specific techniques. Data was arranged using pivot tables in Microsoft Excel (version 16.26) for graphic representation and interpretation.

Although mesowear can vary within species at different localities or different points in time, a simple measure was established to investigate whether diets assigned using mesowear remained stable throughout multiple studies. It should be noted that the extreme variability between publications makes this a very coarse measure, however, if a species maintains the same diet throughout multiple locations and timepoints, one can assume its diet is extremely robust. When species were scored as an original measure in more than one publication, the percentage of the species' main diet across publications was plotted against the number of publications scoring the species. Thus, a higher main-diet percentage, alongside a high publication count indicates a more robust diet. This was applied to the original dataset from Annex 1, and a simplified dataset using only "grazer", "browser" and "mixed-feeder" (Fig. 7b).

Results

The data collected (Annex 1) shows that, when ordered by publication, 52% scored exclusively extinct specimens, while 15% applied mesowear to solely extant species. Eight percent of publications scored solely extant archaeological or fossil specimens; while 11% scored a mix of extinct and extant specimens, the rest scoring combinations of the above (Fig. 5a). Only four publications applied mesowear to purely captive (including experimental) animals representing about 2% of all studies. In regards to diet, the mixed diet is most highly represented among all

species (34%) as it covers a large spectrum, followed by the browser diet, at 25% (Fig. 7a).

When ordering the data by technique and publication, "mesowear I" on its own was scored in 34% of studies, followed by "mesowear II" (21%), "mesowear ruler" (13%), and "mesowear I and II" (11%), the rest using a combination thereof, or taxon-specific techniques (Fig. 6a). Most taxon-specific techniques were single-application, with the exception of "mesowear adapted for Proboscidea", used in eight publications and "mesowear adapted for Conodonta" used in four. This fits within a statement from the original mesowear study, stating that "*care should be taken not to lose the generality of the method, since restricting it to a single, morphologically uniform group will serve to limit the choice of recent species available for comparison*". Out of the 197 publications analysed, 16 studies scored over 20 species, with the highest number of species scored being 85 (Mihlbachler et al., 2011; Solounias et al., 2013).

Placental mammals were overwhelmingly scored (95%), though were surprisingly, not the only class of animals to which mesowear was applied. Butler et al. (2014) adapted mesowear to marsupials, and Purnell and Jones (2012) applied mesowear to fossil conodonts (Table 1), a technique which was also applied to elasmobranchs (McLennan, 2018).

When sorted by Order, artiodactyls were most represented (63%), followed by perissodactyls (26%) (Fig. 4).

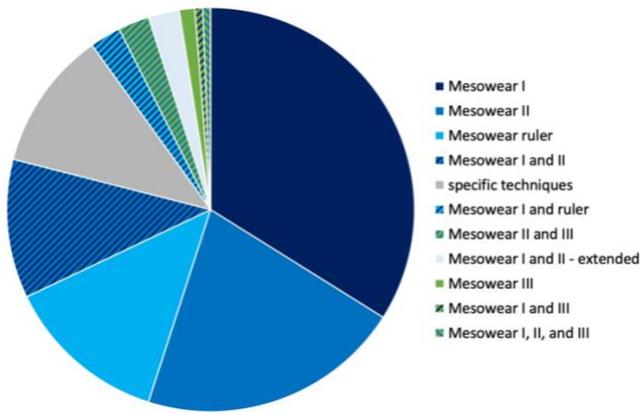


Figure 6a. Proportion of techniques employed in mesowear studies from 2000 to June 2019. Data sorted by number of publications, n=197.

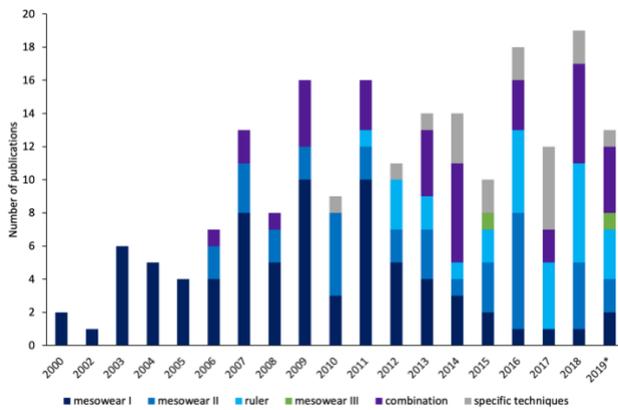


Figure 6b. Yearly amount of the different techniques used to score mesowear between 2000 and June 2019*. Data sorted by number of publications, n=197.

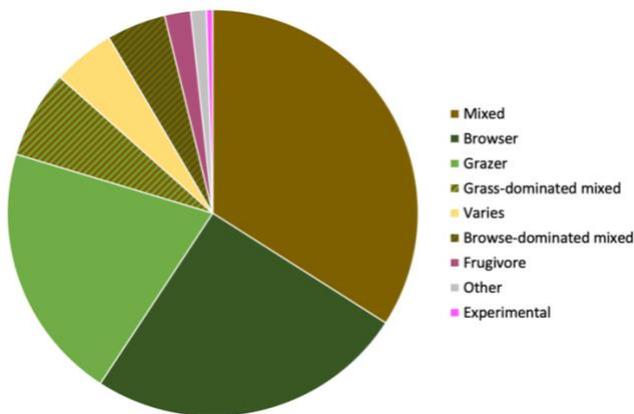


Figure 7a. Percentages of diets represented in mesowear studies from 2000 to June 2019. Data sorted by number of specimens, n=1310.

Overall, out of 688 species (excluding “sp.”), *Equus* was by far the most scored genus, with 96 counts, followed by *Bison* and *Tragelaphus* with 34 counts, and *Gazella* at 33 counts. At the species level, *Cervus elaphus* was most commonly scored, with 17 counts, followed by *Equus ferus* (15 counts). In total, 169 species were scored in more than one publication, meaning that about 75% of species were only scored once (Annex 1). In part because of the number of times it is represented in the dataset and because of its extreme hypsodonty, the species with the most robust unchanging diet is *Equus ferus*, with 100% diet robustness within 13 publications (Fig. 7b).

Discussion

Although one may envision more sophisticated or precise methods of palaeodietary reconstruction, it is important to remember that the original goal of the mesowear technique was to provide a fast and cost-effective way of determining diets for a large number of species. It has been thoroughly tested for this purpose and is extremely efficient in determining diet on a coarse scale. The “dietary robustness score” established here may be a simple measure, however, it provides a different approach in investigating the stability of a species’ diet. It also represents the number of species that are only studied in single publication, while others species have been measured over ten times.

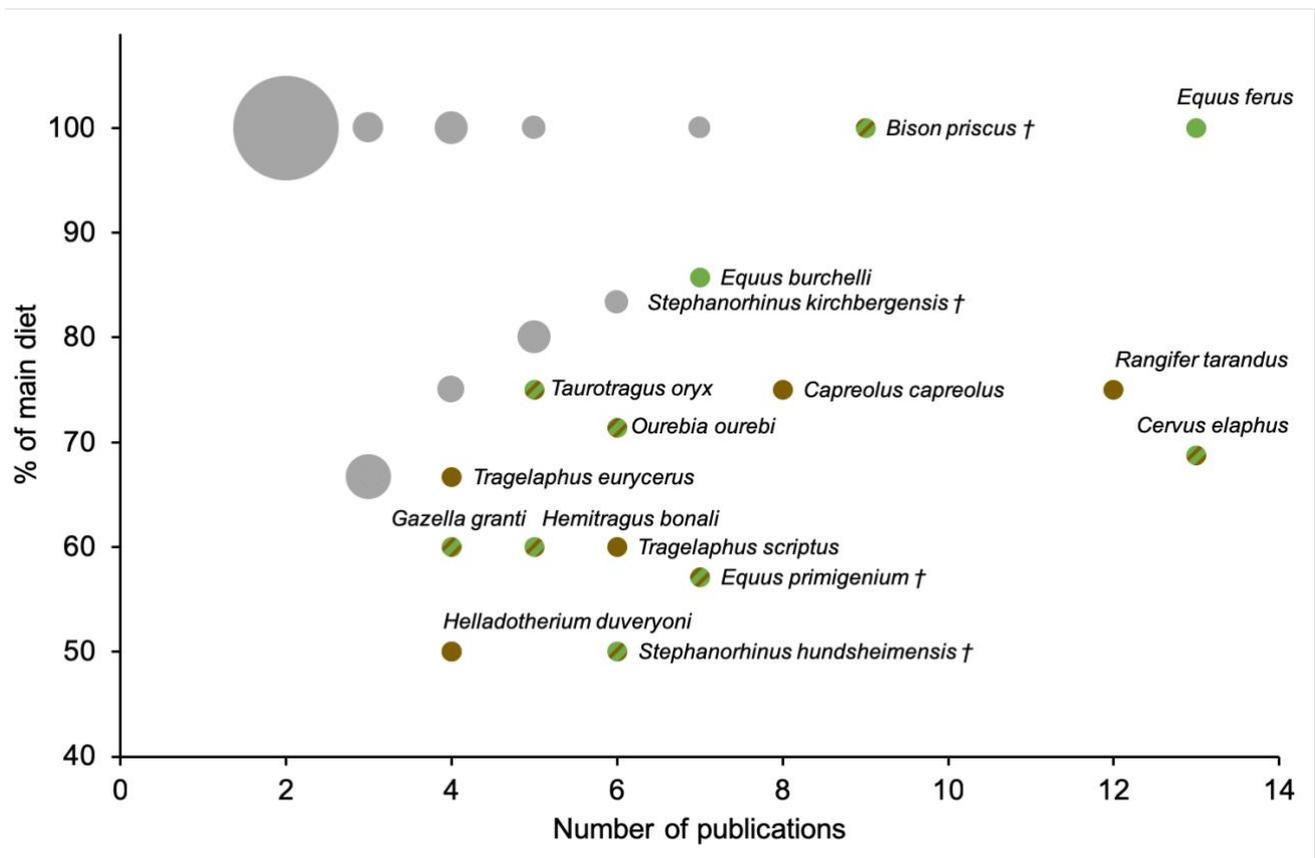


Figure 7b. Dietary robustness of species represented in in mesowear studies from 2000 to June 2019. Dietary robustness is a measure represented by the percentage of a species' main diet throughout publications, plotted against the number of publications featuring the species. Size of marker indicates the number of species per point (minimum 1, maximum 48). Grey markers indicate multiple species, green markers represent single species of grazers, brown markers represent single species of browsers, and brown and green pattern markers represent single species of mixed diets.

Providing an overview of the variability in mesowear scoring may allow for a re-balance of mesowear application in future studies, by increasing reproducibility and reducing repeated measures, e.g. on species with high dietary robustness.

Since the creation of the mesowear technique, the number of publications per year, as well as the type of publication (paleontological or not) has grown until around 2010, with a roughly even distribution between non- and purely- palaeontological publications (Fig. 5b). The type of mesowear technique applied over the years also varies, and the number of

publications applying solely “mesowear I” appears to decline over time as it becomes part of a combination of techniques, while the use of taxon-specific techniques increases (Fig. 6b). Mesowear remains an essential asset for dietary reconstruction, and has become more frequently applied in combination with other dietary proxies such as microwear or isotopic data, to provide a more accurate representation of diet over different timescales.

A precise understanding of the length of the signal represented by meso-wear requires the establishment of a baseline, to be used as a reference in defining the length of the dietary

signal. In the case of mesowear, very few experiments investigate how, or how long mesowear takes to form (Solounias et al., 2014; Kropacheva et al., 2017; Ackermans et al., 2018; Stauffer et al., 2019). Most likely due to the logistics of long-term experiments on large ungulates. These mesowear parameters therefore remain widely unknown. The few experimental tests of mesowear over the long-term seem to indicate that it is representative of a general lifetime signal, at least in small ruminants (Ackermans et al., 2018 on goats for 6 months, and; Ackermans et al., in prep.-a, on sheep for 17 months). However, it is impossible to experimentally recreate the variations of nature, and the comparison of the aforementioned results to those where mesowear shows more seasonal effects (Kaiser and Schulz, 2006; Schulz et al., 2013c; Marom et al., 2018) requires further investigation. A better understanding of the timescale represented by mesowear can only improve the precision of dietary reconstructions, all while furthering our understanding of the dental wear and dietary habits of extant species.

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P1

**Controlled feeding experiments with diets of different
abrasiveness reveal slow development of mesowear signal in
goats (*Capra aegagrus hircus*)**

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Author contributions: TMK, DWHM, PRK, MC and JMH designed the study; DWHM and MC performed the animal experiment; PRK supervised the CT scanning; JH supervised the nutritional analyses; NLA performed the mesowear scoring of CT scans and skulls under supervision of ESK, TMK and DEW; NLA and MC analysed the data and drafted the first version of the manuscript which then received input by all other co-authors.

RESEARCH ARTICLE

Controlled feeding experiments with diets of different abrasiveness reveal slow development of mesowear signal in goats (*Capra aegagrus hircus*)

Nicole L. Ackermans^{1,*}, Daniela E. Winkler^{2,3}, Ellen Schulz-Kornas^{3,4}, Thomas M. Kaiser³, Dennis W. H. Müller⁵, Patrick R. Kircher⁶, Jürgen Hummel⁷, Marcus Clauss¹ and Jean-Michel Hatt¹

ABSTRACT

Dental mesowear is applied as a proxy to determine the general diet of mammalian herbivores based on tooth-cusp shape and occlusal relief. Low, blunt cusps are considered typical of grazers and high, sharp cusps typical of browsers. However, how internal or external abrasives impact mesowear, and the time frame the wear signature takes to develop, still need to be explored. Four different pelleted diets of increasing abrasiveness (lucerne, grass, grass and rice husks, and grass, rice husks and sand) were fed to four groups of a total of 28 adult goats in a controlled feeding experiment over a 6-month period. Tooth morphology was captured by medical CT scans at the beginning and end of the experiment. These scans, as well as the crania obtained post mortem, were scored using the mesowear method. Comparisons between diet groups showed few significant differences after 6 months, irrespective of whether CT scans or the real teeth were scored. Only when assessing the difference in signal between the beginning and the end of the experiment did relevant, significant diet-specific effects emerge. Diets containing lower phytolith content caused a more pronounced change in mesowear towards sharper cusps/higher reliefs, while the feed containing sand did not result in more extreme changes in mesowear when compared with the same feed without sand. Our experiment suggests that the formation of a stable and hence reliable mesowear signal requires more time to develop than 6 months.

KEY WORDS: Dietary signal, Grit, Tooth wear, Controlled food trials, Ruminant

INTRODUCTION

In 2000, Fortelius and Solounias introduced mesowear analysis – a method to rapidly reconstruct paleodiets based on macroscopic attritive and abrasive wear on the molars of ungulate herbivores by observing cheek-tooth occlusal surfaces (Fortelius and Solounias,

2000). As a result, herbivores can quickly be classified as browsers or grazers by observation of their mesowear profile, defined by each tooth's cusp shape (CS) and occlusal relief (OR). The concept of mesowear suggests that tooth-on-tooth wear, or attrition, creates teeth with sharp, pointed cusps and high OR, which is characteristic of browsers, as their diet is hardly abrasive. For grazers, in contrast, the concept suggests that their teeth are mainly worn down by an abrasive diet, in which plant phytoliths and/or external abrasives such as grit and dust grind down the dental material, resulting in lower OR and blunter cusps (Fortelius and Solounias, 2000; Kaiser, 2000).

In the original mesowear scoring system, only the upper second molar was used, and CS was scored as sharp, round or blunt and OR as high or low (Fortelius and Solounias, 2000; Kaiser, 2000). Since then, the system was expanded to include upper and lower molars (Franz-Odenaal and Kaiser, 2003; Kaiser and Fortelius, 2003; Kaiser and Solounias, 2003), as well as a higher number of differentiated scoring states (e.g. Winkler and Kaiser, 2011). However, a more simplified version of the scoring system using a set of gauges has also been introduced (Mihlbachler et al., 2011). Owing to species- or taxon-specific adaptations and exceptions, various modified mesowear scoring systems have been developed for equids (Kaiser and Fortelius, 2003), lagomorphs (Fraser and Theodor, 2010), rhinoceroses (Taylor et al., 2013), marsupials (Butler et al., 2014) and small mammals (Kropacheva et al., 2017; Ulbricht et al., 2015). Mesowear has also been applied to fossil taxonomic lineages such as Chalicotheriidae (Schulz et al., 2007) and notoungulates (Croft and Weinstein, 2008), and in some cases alternative scoring systems using angles and gauges have been used for taxa such as proboscideans (Saarinen et al., 2015) and xenarthrans (Saarinen and Karne, 2017). Owing to the array of varying mesowear techniques, caution should be applied referring to the methodology of the respective mesowear studies, especially when comparing data from different publications.

In extant ungulates, mesowear is most commonly used to reconstruct paleodiets and paleoecology (Croft and Weinstein, 2008), under the assumption that the wear pattern 'generally reflects a substantial portion of the individual's life in ecological time' (Fortelius and Solounias, 2000). Very young individuals as well as senile individuals are generally excluded from mesowear databases, as their extreme signal is not representative of the general population. The macroscopically visible wear pattern is a guide to answer questions about the average diet of a specific species from a particular location. It has also been used to demonstrate tooth wear variability within a species, when different diets are consumed owing to various factors such as climate variables, seasonality, population-specific habitat differences or sexual segregation (Clauss et al., 2007; Kaiser et al., 2009; Kaiser and Schulz, 2006;

¹Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, 8057 Zurich, Switzerland. ²Institute for Geosciences, Johannes Gutenberg University Mainz, 55099 Mainz, Germany. ³Center of Natural History, University of Hamburg, 20146 Hamburg, Germany. ⁴Max Planck Weizmann Center for Integrative Archaeology and Anthropology, Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, 04103 Leipzig, Germany. ⁵Zoologischer Garten Halle GmbH, Fasanenstr. 5a, 06114 Halle (Saale), Germany. ⁶Division of Diagnostic Imaging, Vetsuisse Faculty, University of Zurich, 8057 Zurich, Switzerland. ⁷Department of Animal Sciences, Ruminant Nutrition, Georg-August University, 37073 Göttingen, Germany.

*Author for correspondence (nicole.ackermans@uzh.ch)

 N.L.A., 0000-0001-8336-1888; D.E.W., 0000-0001-7501-2506; E.S., 0000-0003-1657-8256; T.M.K., 0000-0002-8154-1751; D.W.H.M., 0000-0002-8064-1793; P.R.K., 0000-0002-9677-0522; M.C., 0000-0003-3841-6207; J.-M.H., 0000-0002-7043-7430

List of symbols and abbreviations

ADIA	acid-detergent insoluble ash
CS	cuspid shape
CSA	cuspid shape of the anterior cuspid
CSP	cuspid shape of the posterior cuspid
CT	computed tomography
G	grass diet
GR	grass/rice husk diet
GRS	grass/rice husk/sand diet
L	lucerne diet
m1–3	lower molar
M1–3	upper molar
OR	occlusal relief
p2–4	lower premolar
P2–4	upper premolar

Schulz et al., 2007; Taylor et al., 2016, 2014; Winkler and Kaiser, 2011; Yamada, 2012). However, it should be noted that the studies mentioned above only made assumptions about diet composition under the respective circumstances such as ‘the wild’ or ‘captive’, or referred to the literature. Very few studies correlate actual feeding observations with mesowear signals in individuals of the observed populations (Schulz et al., 2013; Wronski and Schulz-Kornas, 2015). Up to now, only a single study (Solounias et al., 2014) has measured mesowear based on a controlled experimental diet, and in this case a new scoring system was introduced, called ‘mesowear III’ [with the terms ‘mesowear I’ describing the Kaiser and Solounias (2003) method scoring more than one cuspid per tooth, and the term ‘mesowear II’ referring to the scoring system of Mihlbachler and Solounias (2006)]. For mesowear III, the shape of the inner enamel bands of molars is scored on both their occlusal-mesial and their distal side. Original mesowear scoring was not applied in that experiment, possibly because the relatively short experimental period (up to 40 days) may not have been long enough for a ‘mesowear I or II’ signal to develop (Fortelius and Solounias, 2000). Correspondingly, Danowitz et al. (2016) claim that ‘mesowear III’, designated ‘inner mesowear’, develops faster than the original or ‘outer mesowear’.

The mesowear signal changes with age within a species and at different rates across species (Rivals et al., 2007). However, the length of time this signal takes to develop has not been investigated thus far. Most likely, this period is short in lagomorphs and rodents, where a reliable signal can be obtained (Fraser and Theodor, 2010; Ulbricht et al., 2015) even though the cheek-tooth crowns are completely replaced within 1–2 weeks (Müller et al., 2014, 2015).

In order to test whether, and at which rate, a controlled diet can induce mesowear changes, goats were provided with diets of varying abrasiveness for a period of 6 months. We expected individuals fed with a phytolith-poor diet to develop a high-sharp mesowear signal and individuals fed with a phytolith-rich diet to develop a low-round to blunt mesowear signal. An even more drastic effect was anticipated when the same diet was used with added external abrasives. The change in macroscopic tooth shape was recorded using computed-tomographic (CT) imaging and mesowear scoring of CT scans at the beginning and the end of the experimental period.

MATERIALS AND METHODS**Animals**

The animal experiments were performed with approval of the Swiss Cantonal Animal Care and Use Committee Zurich (animal experiment license no. 115/2009). Twenty-eight adult female domestic goats [*Capra aegagrus hircus* (Linnaeus 1758)] of

mixed breeds and varying ages (Saanen goats, Chamois Colored goats and Toggenburger goats; mean body mass 60±8 kg, estimated age 3–10 years, exact ages unknown), with unknown previous feeding history, were randomly divided into four groups, each consisting of seven individuals, and kept in a stable with an indoor compartment (40 m² per group) consisting of a slatted concrete floor, an area covered by industrial carpet, and an outdoor compartment (12 m² per group) with a concrete floor. The duration of the feeding experiment ranged from 182 to 198 days for 24 animals and from 107 to 176 days for four other animals that were euthanized before the end of the experiment owing to reasons unrelated to the study (see the original data file in the Dryad deposit for details on individual animals: doi:10.5061/dryad.658433g). At the end of the experimental period the animals were slaughtered and the skulls were prepared by enzymatic maceration at the Center of Natural History, University of Hamburg, where they are permanently curated in the Mammals Collection.

Diets

All animals were fed lucerne hay and lucerne pellets for *ad libitum* consumption for 2 weeks before the first CT scan and the start of the experimental diet feeding period. The experimental diets differed in abrasiveness between the groups, with increasing abrasiveness from lucerne pellets (L), grass pellets (G) to grass pellets with rice husks (GR) and grass pellets with rice husks with an addition of sand (GRS; sand for playgrounds, grain size 0–1 mm, REDSUN garden products B.V., Heijen, Denmark; mean particle size 0.233 mm). These diets were of the same batch as those used in experiments with rabbits (*Oryctolagus cuniculus*) (Müller et al., 2014), guinea pigs (*Cavia porcellus*) (Müller et al., 2015) and *in vitro* with horse teeth (Karne et al., 2016). During the production of the pelleted diets, care was taken to ensure that the proportion of indigestible abrasives in the GRS diet was mimicked in the other diets by a similar proportion of indigestible, non-abrasive filler, to ensure comparable levels of energy and nutrients per amount of pellets (Müller et al., 2014). Grass hay was provided to all groups except for the lucerne group, which was fed lucerne hay. A total of 1500 g of pelleted food and 100 g of hay was provided daily per animal. It should be noted 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. Water was available for *ad libitum* consumption at all times. Samples of all diet items, as well as feces of all animals, were analyzed for acid-detergent insoluble ash (ADIA) as a measure for silica (abrasives) content (Hummel et al., 2011); a nutritional analysis of the pelleted diets is given in Müller et al. (2014). Diet samples represented a composite sample of each hay and pellet type, blended from individual weekly samples; fecal samples of all animals were collected immediately before slaughter, i.e. after the animals had been on their respective diets for 6 months.

Computed tomography

CT images were acquired from a helical multislice Siemens scanner (Siemens Medical Solutions, Erlangen, Germany) housed at University of Zurich Tierspital. The parameters kept constant throughout were: tube voltage at 120 kVp, image matrix of 512×512 pixels, field of view 980×332 pixels, slice thickness of 0.6 mm and B60s convolution kernel. The animals were scanned at the start of the experiment, while they were in groups but not yet on experimental diets. The scans took place under general anesthesia with ketamine 10 mg kg⁻¹ (Ketonarkon®, Streuli Pharma AG, Uznach, Switzerland) and xylazine 0.1 mg kg⁻¹ (Xylazin Streuli,

Streuli Pharma AG) intramuscularly. Anesthesia was maintained with isoflurane (Attane[®], Provet AG, Lyssach, Switzerland) administered in oxygen using a facemask. The first CT scan was used as baseline for the tooth condition. Another CT scan was performed post mortem.

Mesowear

The goats' skulls were scored in two ways: (1) physically, by inspection of the original skull material, with the aid of a magnifying lens (magnification $\times 12$), and (2) virtually, using the rendered 3D surface model of the CT data. Mesowear was scored on all premolars and molars excluding unworn, extremely worn or otherwise damaged specimens (Kaiser et al., 2009) using the mesowear scoring protocol from Taylor et al. (2013, 2016) adapted from Fortelius and Solounias (2000) and Winkler and Kaiser (2011). In this system, tooth CS of the anterior (CSA) and the posterior molar cusp (CSP) can be scored as sharp (CS 4), round–sharp (CS 3), round (CS 2), round–round (CS 1) and blunt (CS 0). OR was scored by observing the proportion between height and width of the anterior and posterior molar tooth cusps. Based on this, the different scores are high–high (OR 4), high (OR 3), high–low (OR 2), low (OR 1) and flat–negative (OR 0). This scoring system is not species-specific and is suitable for application on ruminants. Because it was our aim to develop a functional understanding, we investigated diet effects of the individual mesowear components (CS and OR) separately, and did not report results from a combined score (where CS and OR scores are combined into a single number, e.g. Kaiser et al., 2009; Winkler and Kaiser, 2011) here. However, a corresponding evaluation [where, to conform with the way mesowear was scored in our experiment, the combined score values are reversed from the continuous system as presented in Winkler and Kaiser (2011), with 0 being the bluntest lowest score and 17 being the sharpest and highest score] is provided in the supplementary material, and this 'ordinal score' is also given in the original data deposited in Dryad (doi:10.5061/dryad.658433g).

The CT datasets were converted to DICOM medical imaging format and rendered into 3D surface models using Amira 5.6 (Mercury Computer Systems/3D Viz group, San Diego, CA, USA) as well as Horos v3.0.1 (Horos Project 2015) for additional visualization. To allow for mesowear scoring in Amira, the view mode was set to orthographic mode and a fixed iso-surface threshold was defined such as to achieve the highest bone resolution while avoiding artefacts. Mesowear scoring of the CT images was performed using a dynamic 3D model of the data; in other words, there was no pre-set, fixed on-screen magnification. Apart from the individual scores, the score difference between the skull and final CT scan was calculated, as well as the score difference between the initial and the final CT scans for each cusp/tooth. All mesowear scoring was performed blind to diet groups, and the scoring of the skulls was performed separately from that of the CT scans. All scoring was performed by the same investigator (N.L.A.), and the descriptive scores (e.g. high, blunt, sharp; summarized in the original data in the Dryad deposit: doi:10.5061/dryad.658433g) were transferred into numerical values as described above. The difference in scores taken from skulls and CT scans was not used to adjust scoring, but were part of this investigation.

Statistical analysis

For statistical analysis, data for left and right teeth were entered separately in the dataset. The overall difference in sharpness between anterior and posterior cusps was tested, as well as the differences between mesowear results from the same time point

using different methods (virtually on CT scans and physically on skulls). Comparisons within individuals (such as between anterior and posterior cusps, between the initial and final CT scans, and between the CT and the skull scores) were made using Wilcoxon signed-rank tests for paired samples. The distributions of differences between CT and skull scores were assessed by median, mean, skewness and kurtosis. Comparisons between treatment groups were performed using ranked data and general linear models with Tukey's *post hoc* tests; data were ranked every time anew corresponding to the level of analysis (all teeth, all premolars/molars, all upper premolars/molars and all lower premolars/molars, and each individual tooth position) and compared between the four groups. Comparisons of fecal ADIA levels were made using the original data and an ANOVA with Tukey's *post hoc* test. Correlations between scores were assessed by Spearman's ρ . All analyses were performed in SPSS 22.0 (IBM, Armonk, NY, USA), with the significance level set to $P < 0.05$. Although most analyses were non-parametric (including the use of ranked data), graphs are based on means and standard deviations to allow visual interpretation of differences.

RESULTS

Dietary silica content

The ADIA content of lucerne and grass hay was very similar to that of the pelleted diets made from each respective material (Fig. 1A). As intended, the diet including rice husks had a higher ADIA concentration and the diet with added sand had a very high ADIA concentration of 92.8 g per kilogram of dry matter. The fecal ADIA levels differed significantly between the groups (ANOVA, $P < 0.001$; Fig. 1B), with a significant difference at *post hoc* testing between the GRS and the other groups ($P < 0.001$ in all cases) and no overlap in the 95% confidence intervals of any group means.

Mesowear scores from skulls

At the end of the experiment, mesowear scores from skulls and averaged across all molars show the L group as having higher OR scores compared with the other diet groups. The GRS diet group generally had significantly lower CS scores than the L group, with other diet groups in varying positions according to the level of analysis (Fig. 2, Table 1).

Combining the OR and CS scores into a single variable for measurements performed on skulls, CT1, CT2 and the difference between CT1 and CT2 resulted in equal or less differentiation between the diet groups of our experiments, but in no case in a higher level of differentiation (Tables S1–S3, Fig. S1).

Physical and digital scoring

Comparing mesowear scores from post-mortem CT scans and skulls, the percentage of score differences for the OR parameter resembled a normal distribution (albeit with positive kurtosis) around zero, indicating no systematic discrepancy between skull and CT OR scores (Fig. 3). Both CS score differences showed a similar (less kurtotic) distribution shifted (but not skewed) towards the right (Fig. 3). Statistically, no difference was found between the skull and CT scoring for OR (Table 2), and the two measures were significantly correlated ($P < 0.001$ for all teeth as well as premolars and molars separately; $\rho = 0.44$ for all teeth and premolars and 0.25 for molars). However, CS scores differed significantly in the overall comparison and in each sub-category, as well as for several individual teeth, with cusps being scored as sharper on average in the physical skulls than in the CT scans (Table 2). Nevertheless, for CSA (premolars: $P < 0.001$, $\rho = 0.28$; molars: $P = 0.004$, $\rho = 0.18$) and

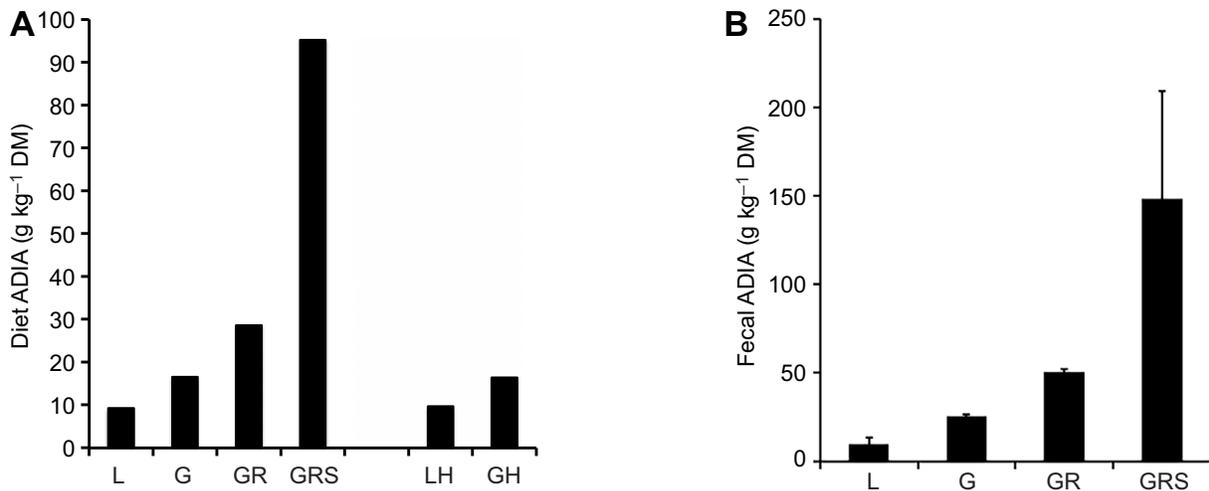


Fig. 1. Silica content of the different diet elements and fecal samples, measured by acid-detergent insoluble ash (ADIA). (A) ADIA content of different pelleted diets and lucerne (LH) and grass hay (GH), supplied for a feeding experiment where goats (*Capra aegagrus hircus*) ($n=28$) were fed four pelleted diets for 6 months. (B) ADIA (means \pm s.d.) of fecal samples produced by goats after consumption of different diets. L, lucerne; G, grass; GR, grass/rice husks; GRS, grass/rice husks/sand.

CSP (molars: $P=0.005$, $\rho=0.17$), the skull and CT data were significantly correlated as well. Diet did not have a systematic effect on the difference between skull and CT scores (Table 2).

Sharpness of anterior versus posterior cusps

Whether the physical skull or CT scans were scored had an effect on the difference in the perceived sharpness between anterior and posterior cusps. No differences between anterior and posterior cusps in CS scores were found for mesowear scored on the physical skulls (Table S4). For mesowear scored virtually on the CT scans, cusp scores differed significantly in many cases, and in nearly all molars for the second CT scan (Table S4). Diet treatment had no effect on the difference between cusps (Table S4).

Original tooth state

Before the initial CT scan and the beginning of the experimental feeding period, the animals, whose previous diets were unknown, had been kept for 2 weeks on a common diet of lucerne hay and lucerne pellets. Nevertheless, the initial CT scan indicated that significant differences in OR and CSA between the groups had been present before the start of the experiment (with the group to be fed lucerne having higher reliefs and sharper anterior cusps than the

other groups in many comparisons), even though animals had been assigned randomly to the groups (Table S5). At the end of the experiment, the results from scoring the final CT scans resembled those of the initial CT scans and those of the skulls, with animals on lucerne having higher relief and sharper cusps than the other groups (Table 3). In pair-wise comparisons, differences between the initial and final CT scores were not significant for OR, but significant differences occurred for CSA for all molars, the lower premolars, the upper M2 and the lower p2, and for CSP in the upper M1 (Table 4). Diet had a significant effect on the difference in mesowear score between the initial and final CT scans, indicating that the L diet had the most distinct (negative) score change, towards higher OR and sharper CS, while the GR diet changed towards lower OR and blunter CS (Table 4; displayed for all molars in Fig. 4 and individual teeth in Fig. 5).

DISCUSSION

The key results of this study show that documenting effects corresponding to current mesowear theory is possible in goats, but that these effects appear to be very small. Our findings further suggest that in ruminants such as goats, mesowear signals may develop at a slower rate than previously assumed.

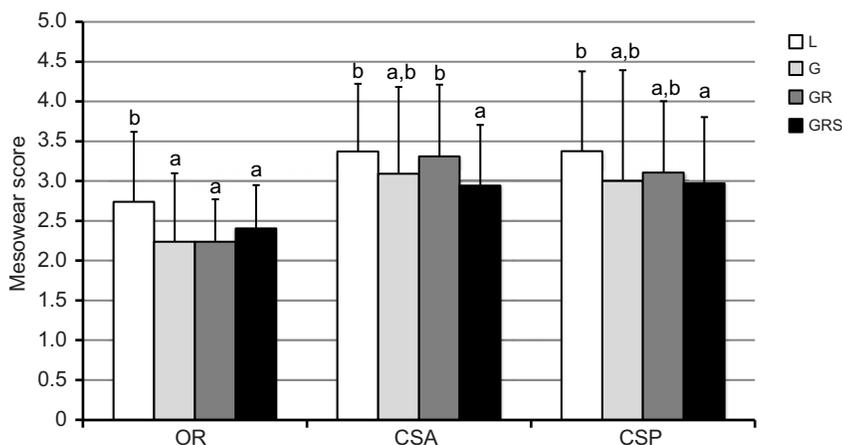


Fig. 2. Averaged mesowear score of all molars, measured on skulls of goats (*Capra aegagrus hircus*) ($n=28$) fed four pelleted diets of different abrasiveness for 6 months. A higher mesowear score represents higher and sharper teeth. Each diet is represented according to each of the three scoring parameters: occlusal relief (OR), anterior cusp shape (CSA) and posterior cusp shape (CSP). Letters above s.d. error bars represent significance between diets, within score groups. L, lucerne ($n=7$); G, grass ($n=7$); GR, grass/rice husks ($n=7$); GRS, grass/rice husks/sand ($n=7$).

Table 1. Effect of different diets on teeth of goats (*Capra aegagrus hircus*) (n=28) fed four pelleted diets of different abrasiveness for 6 months, quantified by mesowear occlusal relief and cusp shape of the anterior and posterior cusp on skulls at the end of the experiment

Tooth	Occlusal relief skull		Cusp shape anterior skull		Cusp shape posterior skull	
	<i>P</i>	<i>post hoc</i>	<i>P</i>	<i>post hoc</i>	<i>P</i>	<i>post hoc</i>
All teeth	<0.001	L>G,GR,GRS	<0.001	L>G,GRS; GR>GRS		
All premolars	<0.001	L>G,GR,GRS; GR>GRS	<0.001	L>G,GRS		
All molars	<0.001	L>G,GR,GRS	0.003	L,GR>GRS	0.034	L>GRS
Upper premolars	<0.001	L>G,GRS	0.34	–		
Lower premolars	<0.001	L,GR>GRS	<0.001	L>G,GRS		
Upper molars	0.015	L>GR	0.022	GR>GRS	0.013	L>GR,GRS
Lower molars	0.002	L>G,GR	0.057	–	0.549	–
txP2	0.021	L>GRS	0.351	–		
txP3	0.061	–	0.677	–		
txP4	0.136	–	0.587	–		
txM1	0.443	–	0.025	G<GR	0.199	–
txM2	0.413	–	0.225	–	0.069	–
txM3	0.293	–	0.750	–	0.741	–
tmP2	0.013	L>GRS	0.073	–		
tmP3	0.002	L>G,GRS	0.008	L>G,GRS		
tmP4	0.339	–	0.276	–		
tmM1	0.001	L>G,GR3	0.888	–	0.901	–
tmM2	0.064	–	0.184	–	0.586	–
tmM3	0.531	–	0.143	–	0.788	–

L, lucerne (n=7); G, grass (n=7); GR, grass/rice husks (n=7); GRS, grass/rice husks/sand (n=7); tx, maxillary; tm, mandibular. General linear models were performed on ranked data. Tests were performed for all teeth combined, subcategories and individual teeth.

Limitations of study design and method

Upon arrival, the animals' previous diets and exact ages were unknown, and their teeth were in various conditions. Because crown height is one of the parameters that influences mesowear score stability within a species (Rivals et al., 2007), it was important to record tooth state before the beginning of the feeding experiment.

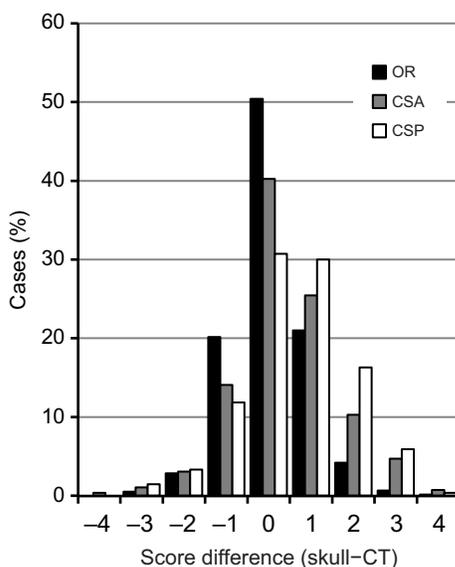


Fig. 3. Difference between the averaged mesowear scores calculated for mesowear variables, taken post mortem on CT images and skulls of goats (*Capra aegagrus hircus*) (n=28) fed four pelleted diets of different abrasiveness for 6 months. Distribution of occlusal relief (OR) differences showed a mild positive skew [95% confidence interval (CI) for skewness 0.008 to 0.400] and positive kurtosis (95% CI kurtosis: 0.870 to 1.654) with a median of 0 and a mean of 0.05. Distribution of anterior cusp shape (CSA) differences showed no skew (95% CI: -0.118 to 0.290) and mild positive kurtosis (95% CI: 0.479 to 1.291) with a median of 0 and a mean of 0.38. Distribution of posterior cusp shape (CSP) differences showed no skew (95% CI: -0.464 to 0.116) and no kurtosis (95% CI: -0.397 to 0.759) with a median of 1 and a mean of 0.59.

To dampen this effect, animals could have been sorted into more homogeneous groups based on age and overall tooth wear state after the initial CT scan. Alternatively, the animals could first have been kept for an extensive period of time (given our results, >6 months) on a common diet, though this was beyond our logistical capacities.

In addition, the experimental diets were provided predominantly in pelleted form. Supplying the animals with solely forage-based diets could have been a better representation of the effect of natural forages. However, the logistics of applying external abrasives consistently to this type of diet over long periods of time was considered impractical, and using different natural forages would automatically have introduced other sources of bias, e.g. differences in digestible energy content between forages as well as individual selectivity or inter-group disparity. The pelleted experimental diets used for the present study were specifically designed to be isocaloric and isonitrogenic, in order to avoid differences in the total amount ingested.

Another consideration is the original statement by Fortelius and Solounias (2000) that the ideal sample size for mesowear scoring should be between 10 and 30 individuals. For a preliminary study, such a large number of animals per diet group would have been excessive and unethical. Furthermore, given our results, it appears doubtful that a higher sample size would have demonstrated a larger effect, but only a more significant one.

Finally, combining the OR and CS scores into a single variable did not provide more insight into diet separation. This may be a convenient procedure for further statistical testing of mesowear in relation to other factors, but should not be considered an improvement of the informative value of the score.

Mesowear methodology

According to the original mesowear technique, the sharper of two molar cusps is often used to provide the final tooth score. In the present study, both cusps were scored and these scores were subjected to statistical testing. Thus, the results of our study on goats support the suggestion by Fortelius and Solounias (2000) that there is generally no difference between the sharpness of the anterior and

Table 2. Significance of differences in mesowear scores of teeth of goats (*Capra aegagrus hircus*) ($n=28$) fed four pelleted diets of different abrasiveness for 6 months, from CT scans taken at the end of the experiment and on macerated skulls representing the same time point, as well as the effect of diet on this difference for occlusal relief (OR), anterior cusp shape (CSA) and posterior cusp shape (CSP)

Tooth	Difference between skull and CT			Effect of diet on the difference					
	OR	CSA	CSP	OR		CSA		CSP	
	<i>P</i>	<i>P</i>	<i>P</i>	<i>P</i>	<i>post hoc</i>	<i>P</i>	<i>post hoc</i>	<i>P</i>	<i>post hoc</i>
All teeth	0.257	<0.001	<0.001	0.241	–	0.049	GR>GRS		
All premolars	0.231	<0.001	–	0.068	–	0.214	–		
All molars	0.649	<0.001	<0.001	0.312	–	0.186	–	0.751	–
Upper premolars	0.842	0.001	–	0.039	–	0.781	–		
Lower premolars	0.312	<0.001	–	0.007	GR>GRS	0.044	GR>GRS		
Upper molars	0.144	<0.001	<0.001	0.537	–	0.025	GR>GRS	0.246	–
Lower molars	0.692	0.008	0.030	0.007	L,G<GRS	0.924	–	0.786	–
txP2	0.783	0.425	–	0.958	–	0.647	–		
txP3	0.864	0.035	–	0.411	–	0.866	–		
txP4	0.847	0.007	–	0.505	–	0.920	–		
txM1	0.810	0.108	0.001	0.494	–	0.211	–	0.721	–
txM2	0.336	0.003	<0.001	0.542	–	0.321	–	0.506	–
txM3	0.258	0.015	<0.001	0.681	–	0.582	–	0.248	–
tmP2	0.864	0.153	–	0.643	–	0.828	–		
tmP3	0.105	<0.001	–	0.701	–	0.504	–		
tmP4	0.449	0.036	–	0.091	–	0.115	–		
tmM1	0.321	0.040	0.060	0.344	–	0.764	–	0.910	–
tmM2	0.621	0.333	0.955	0.056	–	0.856	–	0.394	–
tmM3	0.653	0.102	0.025	0.523	–	0.819	–	0.751	–

Comparisons between skulls and CT scans using Wilcoxon signed-rank tests for paired samples. Effect of diet [L, lucerne ($n=7$); G, grass ($n=7$); GR, grass/rice husks ($n=7$); GRS, grass/rice husks/sand ($n=7$)] was tested using general linear models performed on ranked data. tx, maxillary; tm, mandibular. Tests were performed for all teeth combined, subcategories and individual teeth.

posterior cusps within the same molar, at least for measures performed on the skulls.

Originally, the mesowear technique scored only the upper M2 ‘for simplicity’ (Fortelius and Solounias, 2000). Yet, the second molar could be the ideal tooth to represent the mesowear signal, as the first molar erupts first and endures more wear, while the third molar erupts later on and may not have been fully subjected to wear. Our results indicate that of all individual teeth investigated, the second molar showed changes closest to our expectations at

statistical significance (in four out of six individual tests in Fig. 5), even though several other teeth, in particular the first and third molar, followed the same trend, albeit less often significantly so.

When scoring mesowear on CT scans, the results indicate OR scoring as similar between CT scans and real skulls. Cusp sharpness, however, is reduced in CT imagery as medical CT scanners are restricted by resolution. The very sharp points are therefore lost at lower resolution, though general relief is conserved (Fig. 6). When results are ranked, diet differences between

Table 3. Significance of differences in the final scores of teeth of goats (*Capra aegagrus hircus*) ($n=28$) fed four pelleted diets of different abrasiveness for 6 months, quantified by mesowear occlusal relief and cusp shape of the anterior and posterior cusp in CT scans at the end of the experiment (corresponding in time point to the scores on the skulls tested in Table 1)

Tooth	Occlusal relief final CT		Cusp shape anterior final CT		Cusp shape posterior final CT	
	<i>P</i>	<i>post hoc</i>	<i>P</i>	<i>post hoc</i>	<i>P</i>	<i>post hoc</i>
All teeth	<0.001	L>G,GR,GRS	<0.001	L>G,GR,GRS		
All premolars	<0.001	L>G,GR,GRS	0.001	L>G,GR,GRS		
All molars	<0.001	L>G,GR,GRS	0.003	L>G,GR,GRS	0.045	L>GR
Upper premolars	<0.001	L>G,GR,GRS	0.052	–		
Lower premolars	0.001	L>GR,GRS	0.003	L>GR		
Upper molars	0.103	–	0.034	L>GR	0.502	–
Lower molars	<0.001	L>GR,GRS	0.14	–	0.074	–
txP2	0.035	L>GRS	0.078	–		
txP3	0.021	L>G	0.368	–		
txP4	0.118	–	0.867	–		
txM1	0.292	–	0.199	–	0.675	–
txM2	0.706	–	0.396	–	0.488	–
txM3	0.195	–	0.237	–	0.271	–
tmP2	0.019	L>GRS, L>GR	0.061	–		
tmP3	0.027	L>GRS	0.039	–		
tmP4	0.306	–	0.094	–		
tmM1	0.103	–	0.132	–	0.268	–
tmM2	0.004	L>GR,GRS	0.445	–	0.327	–
tmM3	0.176	–	0.628	–	0.514	–

L, lucerne ($n=7$); G, grass ($n=7$); GR, grass/rice husks ($n=7$); GRS, grass/rice husks/sand ($n=7$); tx, maxillary; tm, mandibular. General linear models were performed on ranked data. Tests were performed for all teeth combined, subcategories and individual teeth.

Table 4. Significance of differences in mesowear scores of teeth of goats (*Capra aegagrus hircus*) ($n=28$) fed four pelleted diets of different abrasiveness for 6 months, from CT scans taken at the beginning and end of the experiment, as well as the effect of diet on this difference for occlusal relief (OR), anterior cusp shape (CSA) and posterior cusp shape (CSP)

Tooth	Difference between initial and final CT			Effect of diet on the difference					
	OR	CSA	CSP	OR		CSA		CSP	
	<i>P</i>	<i>P</i>	<i>P</i>	<i>P</i>	<i>post hoc</i>	<i>P</i>	<i>post hoc</i>	<i>P</i>	<i>post hoc</i>
All teeth	0.231	0.568	0.651	<0.001	L<G,GR,GRS	0.001	L<GR,GRS		
All premolars	0.864	0.121	–	0.004	L<G	0.268	–		
All molars	0.089	0.030	0.651	<0.001	L,G<GR,GRS	<0.001	L<G,GR,GRS	<0.001	L<GR,GRS GR<GRS
Upper premolars	0.644	0.508	–	0.006	L<G	0.139	–		
Lower premolars	0.101	0.023	–	0.395	–	0.490	–		
Upper molars	0.830	0.142	0.654	0.116	–	<0.001	L<GR,GRS	0.010	L<GR
Lower molars	0.445	0.439	0.885	<0.001	L,G<GR,GRS	<0.001	L<GR,GRS	0.001	L<GR
txP2	0.119	0.197	–	0.052	L<G	0.044	–		
txP3	0.252	0.891	–	0.242	–	0.187	–		
txP4	0.858	0.987	–	0.513	–	0.233	–		
txM1	0.626	0.839	0.036	0.420	–	0.181	–	0.044	L<GRS
txM2	0.322	0.027	0.703	0.583	–	0.008	L<GR	0.010	L<GR
txM3	0.176	0.111	0.073	0.162	–	0.038	L<GR	0.921	–
tmP2	0.182	0.008	–	0.258	–	0.019	L>GR		
tmP3	0.364	0.976	–	0.084	–	0.650	–		
tmP4	0.529	0.920	–	0.467	–	0.848	–		
tmM1	0.386	0.508	0.386	0.117	–	0.167	–	0.013	L<GR
tmM2	1.000	0.955	0.396	0.003	L,G<GR	0.003	L<GR	0.343	–
tmM3	0.527	0.136	0.628	0.052	–	0.342	–	0.328	–

Comparisons between initial and final CT scans were made using Wilcoxon signed-rank tests for paired samples. Effect of diet [L, lucerne ($n=7$); G, grass ($n=7$); GR, grass/rice husks ($n=7$); GRS, grass/rice husks/sand ($n=7$)] was tested using general linear models performed on ranked data. tx, maxillary; tm, mandibular. Tests were performed for all teeth combined, subcategories and individual teeth.

individuals are maintained and mesowear scoring remains accurate. It should be noted that 3D renderings do not provide an exact representation of the physical teeth and should therefore only be used to make comparisons within individuals, or within an experimental setup. However, the possibility of applying mesowear scoring to 3D reconstructions of teeth based on medical CT imagery allows expanding the use of mesowear from physical animal skulls to 3D scans of live, sedated animals, and also to observe signal development over time.

Magnitude of mesowear change

The most important finding to emerge from this study is that after spending 6 months on the experimental diet, it was not possible to sort the animals' skulls into correct diet groups based on the mesowear score alone. Even though OR and CS scores showed some significant differences between groups, these differences were of a surprisingly

small magnitude (Fig. 2). It was only after using the differential scores between first and last CT scans that testing whether mesowear development followed the predicted theory became possible.

The current literature is ambiguous on the exact time period a mesowear signal takes to develop, and provides vague estimates at best. To this day, no concrete testing of this duration has been made. Fortelius and Solounias (2000) originally state mesowear as reflecting 'a substantial portion of the individual's life in ecological time', whereas others (Jones and Desantis, 2017; Merceron et al., 2007; Yamada, 2012) describe mesowear as representing an average lifelong diet. Some research has defined mesowear as representing an animal's diet over the last few months or years of its life (Rivals et al., 2007; Ulbricht et al., 2015), with some placing the cap at 1 year (Loffredo and DeSantis, 2014; Louys et al., 2012), or even weeks to years, depending on tooth wear state (Danowitz et al., 2016). According to the present study, mesowear signals resulting from feeding pelleted

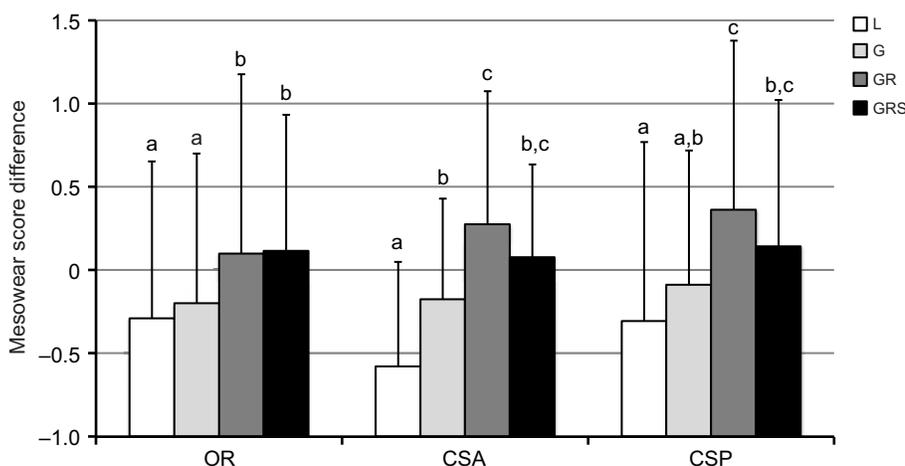


Fig. 4. Averaged score difference between the initial and final CT scan for all molars of goats (*Capra aegagrus hircus*) ($n=28$) fed four pelleted diets of different abrasiveness for 6 months. Each diet is represented according to each of the three scoring parameters: occlusal relief (OR), anterior cusp shape (CSA) and posterior cusp shape (CSP). Letters above s.d. error bars represent significance between diets, within groups. A negative difference indicates that OR became higher, and CS sharper, during the experiment. L, lucerne ($n=7$); G, grass ($n=7$); GR, grass/rice husks ($n=7$); GRS, grass/rice husks/sand ($n=7$).

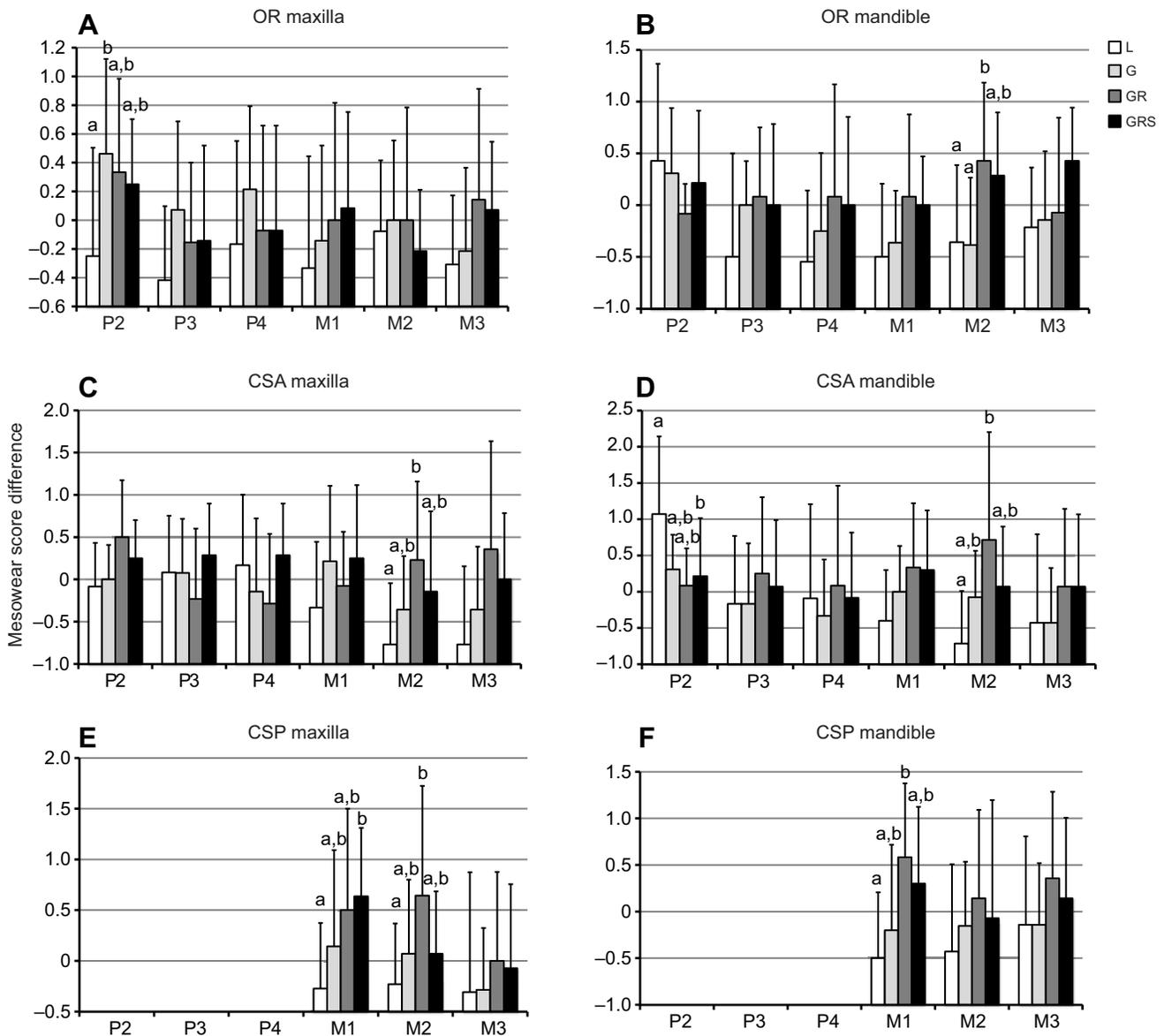


Fig. 5. Averaged score difference between the initial and final CT scan, scored individually for all teeth of goats (*Capra aegagrus hircus*) ($n=28$) fed four pelleted diets of different abrasiveness for 6 months. Letters above s.d. error bars represent significance between diets, within the respective tooth (Table 4). A negative difference indicates that occlusal relief became higher, and cusp shape sharper, during the experiment. (A) Occlusal relief change maxilla. (B) Occlusal relief change mandible. (C) Anterior cusp shape change maxilla. (D) Anterior cusp shape change mandible. (E) Posterior cusp shape change maxilla. (F) Posterior cusp shape change mandible. L, lucerne ($n=7$); G, grass ($n=7$); GR, grass/rice husks ($n=7$); GRS, grass/rice husks/sand ($n=7$).

diets to goats take more than 6 months to develop clearly. Therefore, the assumption that mesowear is of sufficient resolution to track mid-term diet changes such as seasonal diet switches is to be questioned, at least in ruminants. Though most studies tend to use caution when evoking the time frame represented by the mesowear signal, it is often combined with microwear to measure diet seasonality (Kaiser and Schulz, 2006; Muhlbacher and Solounias, 2006; Rivals et al., 2013). Kubo and Yamada (2014) use the standard deviation of the mesowear score for this purpose. If mesowear takes longer than a season to develop, the relationship between mesowear and seasonality will need to be re-evaluated. However, taxon-specific conditions also need to be considered. For example, as mentioned in the Introduction, a mesowear signal in lagomorphs (Fraser and Theodor, 2010; Ulbricht et al., 2015) must develop with 1–2 weeks, given the rate at which cheek-tooth tissue is being replaced.

The change in mesowear score in our study was always less than 1 mesowear unit; more often, this was closer to a change of 0.25. In a very general fashion, one could interpret this as the OR slightly shifting from high towards high–low and the CS shifting from round towards round–round in animals experiencing abrasion. A change of at least 2 mesowear units would be needed to consider animals as having separate diets such as browser or grazer, for example. The small shifts in mesowear signal observed over a period of 6 months brings into consideration the real resolution of the mesowear signal, and offers the possibility of it being more of a lifetime signal in ruminants rather than only representing the last months of an individual's diet. This, in turn, could have an impact on paleontological reconstructions and call for reconsideration of those based on mesowear being a seasonal signal.

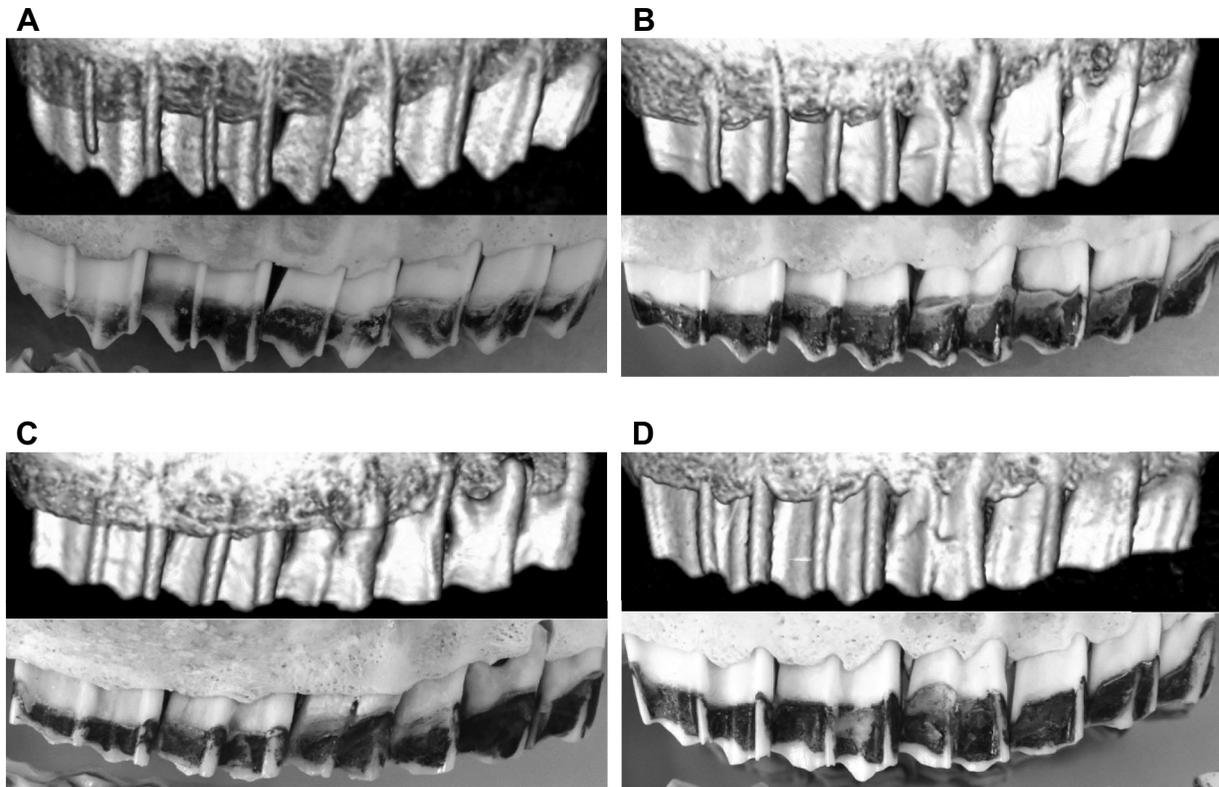


Fig. 6. CT and digital images of the same specimen and position on the upper right tooth row, taken on goats (*Capra aegagrus hircus*) fed four pelleted diets of different abrasiveness for 6 months. (A) Lucerne diet (specimen ID: 13), (B) grass diet (specimen ID: 3), (C) grass/rice husks diet (specimen ID: 27), (D) grass/rice husks/sand diet (specimen ID: 4).

Phytolith effect

The phytolith contents of the experimental diets used in this study are at expected levels based on plant silica content described in Hodson et al. (2005). The hardness of phytoliths and whether they notably affect tooth wear is strongly debated (Baker et al., 1959; Damuth and Janis, 2011; Lucas et al., 2014; Mainland, 2003; Sanson et al., 2007; Xia et al., 2015). In our experimental setting, diets containing more phytoliths, i.e. grass and rice husks versus grass or versus lucerne, caused some abrasive-type wear on ruminant teeth, but the greater change in mesowear (measured as the distance from zero change) was affected by the diet lowest in phytoliths, i.e. lucerne (Fig. 4), suggesting that in our setting, attrition-related sharpening of cusps and deepening valleys was more prominent than abrasion-related blunting of cusps and flattening of valleys. In experiments using the same food with small mammals, tooth wear was not measured by mesowear but by loss of dental tissue via tooth length. In rabbits (Müller et al., 2014) as well as guinea pigs (Müller et al., 2015), when compared within in the same animals (in these animals, repeated measures are logistically possible), the GR diet led to shorter teeth than the G or L diets.

The difference in dietary phytolith content was also hypothesized (though not proven) to be the cause of lower mesowear scores observed in captive giraffe (*Giraffa camelopardalis*) (Clauss et al., 2007), browsing ruminants (Kaiser et al., 2009) and black rhinoceros (*Diceros bicornis*, a browser) (Taylor et al., 2014). These animals putatively receive more grass-related products in captivity than normal for their typically dicot-dominated diets, resulting in abnormally worn teeth. Our observations in the present

study concur with the previous assumptions that phytolith-rich diets induce higher rates of dental tissue loss and lead to rounded, lower mesowear profiles, but more prominently emphasize the putative effect of attrition-related change in dental morphology towards sharper teeth.

Grit (sand) effect

The size of external grit as abrasive dietary particles may be an important factor when considering its effect on mesowear. Large-grained sand, similar to that added to the diets in the present study, tends to cause great amounts of wear in cases such as soil ingestion; this has been documented in agricultural (Healy, 1967; Ludwig et al., 1966) and zoo (Jurado et al., 2008) settings. Another illustration of the effect of external abrasives is the case of the pronghorn antelope (*Antilocapra americana*), an antilocaprid seen as an outlier to the known association between grazing and hypsodonty (Janis and Fortelius, 1988). Both hypsodont teeth, usually related to grazing animals and their highly abrasive diet, and a high/sharp mesowear profile, indicating a browser diet, are present in the pronghorn (Damuth and Janis, 2011; Fortelius and Solounias, 2000; Rivals and Semprebon, 2006; Semprebon and Rivals, 2007). As these animals consume only small amounts of grass, one hypothesis for the high hypsodonty and high mesowear score recorded in this species is that their open environment and close-ground feeding behavior results in the ingestion of large amounts of external abrasives in the form of dust (Damuth and Janis, 2011), therefore mandating high-crowned teeth. However, owing to the lack of deviation from the typical browser mesowear signal in the pronghorn, Kaiser et al. (2013) hypothesized that intrinsic abrasives

(phytoliths) determine facet development (mesowear appearance), whereas external abrasives in the form of small airborne dust particles could cause uniform loss of dental tissue, shortening the teeth while conserving the mesowear pattern. Experimental studies with external abrasives of a variety of size classes are required to further our understanding of the role of grit and dust.

In the present study, when adding large amounts of coarse abrasives (sand) to the experimental diet, the change in mesowear signal between the start and the end of the experiment was similar to, or lower than, that of the same diet without sand, contrary to our expectations. Additionally, in a controlled microwear study on sheep, where mesowear was not determined, Merceron et al. (2016) found a dietary signal difference in dental microwear texture between monocot and dicot roughage, but no relevant additional signal change when dust was added to these diets. In all likelihood, the key to these differences lies in the way the abrasives (phytoliths versus sand or dust) are embedded in the matrix of the food bolus. Ruminants process food repeatedly through the chewing and regurgitation of cud (Gordon, 1968). Whereas phytoliths are embedded in the plant material, added/exogenous grit may possibly be ‘washed off’ and settle in the rumen, resulting in a less abrasive bolus at regurgitation, which would limit the effects of external abrasives on the teeth (Dittmann et al., 2017; Janis et al., 2010). This ‘washing’ mechanism could explain the lack of effect of sand in goats (present study) and of dust in sheep (Merceron et al., 2016), and provide a reason for why low chewing intensity at ingestion, as compared with rumination, might be adaptive in ruminants (Dittmann et al., 2017). Moreover, the observation in the present study that effects on the sand diet appeared even somewhat lower than the same diet without sand could suggest that the goats chewed the sand-containing diet less intensely at ingestion. In humans, reduced chewing intensity owing to the presence of external abrasives has been documented in a chewing gum study (Prinz, 2004). Whether herbivores, ruminant or not, adjust chewing intensity based on the sensory perception of external abrasives in the ingesta, while appearing probable, remains to be investigated.

This hypothesis further suggests that we expect a distinct difference in the effect of external abrasives on tooth wear between ruminant and non-ruminant herbivores. Indeed, results of the same diets used in (non-ruminant) rabbits and guinea pigs (Müller et al., 2014, 2015) indicate a dramatic effect of the external abrasives. The added-sand diet led not only to the relatively shortest cheek teeth and highest wear (and highest growth rates of the hypselodont cheek teeth), but also to abnormal dental morphology. This reflects the fact that these animals are obliged to chew intensively at ingestion, lacking the option of washing off external abrasives. Additionally, in an *in vitro* study (Karme et al., 2016), again using the same experimental diets, horse teeth were subjected to standardized chewing in a mechanical chewing machine. Actual tooth wear was measured by observing volume loss via micro-CT, and results showed that the GRS diet led to the largest amount of tissue loss, mimicking the results of the same diet in non-ruminants. Overall, these results indicate that a diet containing sand affected the teeth of two non-ruminant species but did not have a comparable effect on the teeth of a ruminant. Although we cannot rule out body size effects, these results highlight the different mechanisms adopted throughout species in order to avoid tooth wear.

Conclusions

The results of this preliminary study infer that in goats, 6 months is not enough time for a mesowear signal to develop conclusively.

Accordingly, the lack of a clear distinction between diets after using solely controlled diets for 6 months suggests that in ruminants, the mesowear signal should be considered more as a lifetime or, possibly, depending on future results, an annual rather than a seasonal signal.

The use of CT-based 3D reconstructions for tracing the development of the mesowear signal was an important factor in this study, and further application of this technique could help increase our understanding of mesowear signal development. Similar study designs, including diets with varying sizes and concentrations of grit particles and longer experimental periods, are recommended in order to obtain a solid functional understanding of mesowear-based dietary reconstructions.

Some of the expected effects on mesowear were found, but only once differences between initial and final mesowear scores were observed, a technique difficult to apply in specimens most often subject to mesowear, i.e. fossil or extant-but-deceased specimens. The observed changes indicate, unexpectedly, that diets richer in phytoliths and sand caused less change in mesowear on ruminant teeth than a diet deplete of abrasives (which led to higher/sharper mesowear). Also unexpectedly, the diet with added sand caused mesowear differences similar to those of the same diet without sand, suggesting the possibility that in ruminants, food may be ‘washed’ free of external abrasives when being prepared in the rumen for regurgitation. Thus, the findings also emphasize the relevance of internal abrasives (phytoliths) for the adaptive acquisition of hypselodont molars in ruminants. In the absence of grit effects, internal abrasives may play a more important role in enforcing adaptive responses against tooth wear in ruminants than previously acknowledged.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: T.M.K., D.W.M., P.R.K., M.C., J.-M.H.; Software: P.R.K.; Formal analysis: N.L.A., M.C.; Investigation: N.L.A., D.W.M., J.H., M.C.; Resources: T.M.K., P.R.K., J.H., J.-M.H.; Data curation: M.C.; Writing - original draft: N.L.A., M.C.; Writing - review & editing: N.L.A., D.E.W., E.S., T.M.K., D.W.M., P.R.K., J.H., M.C., J.-M.H.; Visualization: N.L.A., M.C.; Supervision: E.S., T.M.K., P.R.K., M.C., J.-M.H.; Project administration: M.C., J.-M.H.; Funding acquisition: D.W.M., M.C., J.-M.H.

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Data availability

The original data (all mesowear scores) have been deposited in the Dryad digital repository (Ackermans et al., 2018): <https://datadryad.org/resource/doi:10.5061/dryad.658433g/2>.

Supplementary information

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P2

Root growth compensates for molar wear in adult goats (*Capra aegagrus hircus*)

Nicole L. Ackermans, Marcus Clauss, Daniela E. Winkler, Ellen Schulz-Kornas, Thomas M. Kaiser,
Dennis W. H. Müller, Patrick R. Kircher, Jürgen Hummel, Jean-Michel Hatt

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Root growth compensates for molar wear in adult goats (*Capra aegagrus hircus*)

Nicole L. Ackermans¹  | Marcus Clauss¹  | Daniela E. Winkler^{2,4}  |
Ellen Schulz-Kornas^{3,4}  | Thomas M. Kaiser⁴  | Dennis W. H. Müller⁵  |
Patrick R. Kircher⁶  | Jürgen Hummel⁷  | Jean-Michel Hatt¹ 

¹Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

²Institute for Geosciences, Johannes Gutenberg University Mainz, Mainz, Germany

³Max Planck Weizmann Center for Integrative Archaeology and Anthropology, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany

⁴Center of Natural History, University of Hamburg, Hamburg, Germany

⁵Zoologischer Garten Halle GmbH, Halle (Saale), Germany

⁶Division of Diagnostic Imaging, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

⁷Department of Animal Sciences, Ruminant Nutrition, Georg-August-University, Göttingen, Germany

Correspondence

Nicole L. Ackermans, Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Winterthurststrasse 260, 8057 Zurich, Switzerland.
Email: nicole.ackermans@uzh.ch

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Abstract

One reason for the mammalian clade's success is the evolutionary diversity of their teeth. In herbivores, this is represented by high-crowned teeth evolved to compensate for wear caused by dietary abrasives like phytoliths and grit. Exactly how dietary abrasives wear teeth is still not understood completely. We fed four different pelleted diets of increasing abrasiveness (L: Lucerne; G: grass; GR: grass and rice husks; GRS: grass, rice husks, and sand) to four groups of a total of 28 adult goats, all with completely erupted third molars, over a six-month period. Tooth morphology was captured by medical computed tomography scans at the beginning and end of the controlled feeding experiment, and separation lines between the crown and root segments were defined in the upper right second molar (M2), to gauge absolute wear. Using bootstrapping, significant differences in volume loss between diets L/G and GR/GRS were detected. A small but nevertheless consistent volume gain was noted in the roots, and there was a significant, positive correlation between crown volume loss and root volume gain. This growth could possibly be attributed to the well-known process of cementum deposition and its relation with a putative feedback mechanism, in place to attenuate wear caused by abrasive diets.

KEYWORDS

3D imaging, cementum, controlled feeding experiment, dental wear, ruminant teeth, tooth volume

1 | INTRODUCTION

Teeth are a prime representation of evolution, in which the varying dental morphologies of vertebrates have adapted to cope with diverse diets. In this view, the adaptation of mammalian teeth is one of the main reasons for their evolutionary success (Reilly, McBrayer, & White, 2001). Mammalian teeth are mainly composed of dentin, covered by enamel in the crown and by cementum in the roots.

A pulp canal runs through the hollow inside of the tooth, accommodating the nerves and vessels that supply the dentine core (Ungar, 2010). Whether teeth are brachydont (low-crowned) or hypsodont (high-crowned) depends on crown height and tooth width (Van Valen, 1960). This can also be determined by observing the junction between the root and the crown, although determining where the enamel and dentin layers end and where the cementum begins is not always clear, especially for hypsodont teeth. In many

species of rodents or lagomorphs, teeth may be rootless and thus grow throughout the lifetime of the individual; these are referred to as hypsodont teeth (Hillson, 1986; Koenigswald, 2011).

Hypsodonty has evolved and increased in proportion over time (Jernvall & Fortelius, 2002; Samuels & Hopkins, 2017) as a response to high amounts of wear on the dental tissue related to the ingested diet (Damuth & Janis, 2011; Janis & Fortelius, 1988), where the two main factors causing wear are phytoliths and grit (Damuth & Janis, 2011; Toljagić, Voje, Matschiner, Liow, & Hansen, 2018; Williams & Kay, 2001). Grass contains more phytoliths (hard opaline silicates) than browse (Hodson, White, Mead, & Broadley, 2005; Sanson, Kerr, & Gross, 2007). Phytoliths are thought to cause wear shaping the pattern of tooth facet development (Kaiser et al., 2013), which could facilitate discrimination between grazers and browsers based on the tooth wear pattern. Ruminants with a diet mainly composed of grasses often have high crowned teeth (Simpson, 1955; Williams & Kay, 2001). Because of their lower feeding height compared with browsers, grazers also tend to ingest soil abrasives along with plant matter, further increasing tooth wear (Damuth & Janis, 2011; Janis & Fortelius, 1988). However, exactly how much internal or external abrasives contribute to wear, and how long this takes, is still under debate (Baker, Jones, & Wardrop, 1959; Lucas et al., 2013; Mainland, 2003; Sanson et al., 2007; Xia et al., 2015).

Wear caused by both, internal and external abrasives, can be observed at different levels. Microscopic wear (e.g., dental microwear texture (Grine, 1986; Scott et al., 2005; Ungar, Brown, Bergstrom, & Walker, 2003) and 3D surface texture (Calandra & Merceron, 2016)) measures complex topographic features on the tooth's surface, deemed to represent an animal's diet over the last few days (Percher et al., 2018; Schulz et al., 2013; Scott et al., 2005; Teaford & Oyen, 1989b; Ungar, Merceron, & Scott, 2007). On a macroscopic level, wear is described by mesowear (Fortelius & Solounias, 2000), scoring occlusal relief and cusp shape of the tooth ectoloph to reconstruct an animal's diet. Mesowear is thought to represent more of an average lifetime signal (Ackermans et al., 2018; Brent Jones & Desantis, 2017; Merceron, Schulz, Kordos, & Kaiser, 2007; Yamada, 2012).

Precisely quantifying tooth wear by observing loss of dental tissue on a macroscopic scale is difficult. The established method to quantify species- or population-specific volume loss is to measure the crown height of a series of teeth from individuals that died at various ages and, based on this, estimate a wear rate characterizing the population. These estimated rates have been generated for many species (reviewed in Damuth & Janis, 2014), but recently, more modern techniques are able to provide exact information on volume loss in experimental conditions, using 3D imaging techniques (Karme, Rannikko, Kallonen, Clauss, & Fortelius, 2016). Our approach here consists of using 3D volume analysis of computed tomography (CT) image stacks to define the absolute loss of dental tissue over time, and determine the relation to different diets.

Diet-related differences in dental wear have been reported experimentally in a range of species, including goats (*Capra aegagrus hircus*, Linnaeus 1758; Ackermans et al., 2018; Solounias, Tariq, Hou, Danowitz, & Harrison, 2014), rabbits (*Oryctolagus cuniculus* Linnaeus

1758, Müller et al., 2014), guinea pigs (*Cavia porcellus* Linnaeus 1758, Müller et al., 2015) and vervet monkeys (*Chlorocebus pygerythrus* Cuvier 1821; Teaford & Oyen, 1989a). Concerning external abrasives, fine dust has been suggested to create a volume loss effect without affecting the macroscopic mesowear pattern (Kaiser et al., 2013), and in experimental microwear studies on sheep (*Ovis aries* Linnaeus 1758), in which fine dust (<100 µm) was added to forage, Merceron et al. (2016) noted little effect of the extrinsic dust on microwear. Additionally, when adding sand to experimental diets, Hoffman, Fraser, and Clementz (2015) observed microwear pitting for medium grain-sized silica sand (250–425 µm), but no grit effect was evident when smaller fine-grained silica sand (180–250 µm) was added. Large-grained sand originating from ingested soil, on the other hand, creates severe, even pathological tissue loss, often reshaping wear-facets entirely. This was first described in New Zealand sheep where high wear, recorded as incisor volume loss, was related to soil ingestion on very eroded pastures (Barnicoat, 1957; Healy, 1967; Ludwig, Healy, & Cutress, 1966; Madden, 2014), and also seen in zoo animals feeding on sandy soils (Martin Jurado, Clauss, Streich, & Hatt, 2008). In small mammals, as part of the experiments mentioned above (Müller et al., 2014; Müller et al., 2015), diets containing coarse sand resulted in larger amounts of dental tissue loss on incisors and cheek teeth than the same diet without added sand. Using these same diets in an in vitro study on horse molars subjected to mechanical standardized chewing, Karme et al. (2016) found the diet with added sand to cause the most volume loss when observed with a micro-CT.

In a previous publication (Ackermans et al., 2018) we reported mesowear signal development in goats during a controlled feeding experiment over six months. Additional 3D data from this study was used here to report total tooth volume of the upper right second molar (M2) of 26 adult female goats using CT imaging and 3D visualization, to record variations in tooth volume over time in relation to diets of increasing abrasiveness. Tooth crown volume is expected to decrease according to the abrasiveness of the diet; in other words, the group fed the most abrasive diet (GRS > GR > G > L) is expected to show the most absolute volume loss.

2 | MATERIALS AND METHODS

2.1 | Animals

Animal experiments were performed with the approval of the Swiss Cantonal Animal Care and Use Committee Zurich (animal experiment license N°115/2009). Twenty-eight adult female domestic goats (*C. aegagrus hircus*, Linnaeus 1758) of mixed breeds and varying ages (Saanen goats, Chamois colored goats, and Toggenburger goats; average body mass = 60 ± 8 kg, estimated age = 3–10 years, exact ages unknown but all with erupted third molars) were acquired from various sources across Switzerland. For logistic and financial reasons, we were not able to acquire animals of the required age class, and with the obligatory specification of not being pregnant, with reliably registered birth dates. The animals were divided randomly into four

groups, consisting of seven individuals each, and kept in an indoor stable (40 m²/group) covered by industrial carpet, with access to a concreted outdoor compartment (12 m²/group). The feeding experiment was from 182 to 198 days long for 24 of the animals, as euthanasia had to be staggered to facilitate a detailed dissection of each animal for another study, and from 107 to 176 days long for four other animals that were euthanised before the end of the experiment because of reasons unrelated to the study. At the end of the experimental period, the animals were slaughtered and the skulls were skeletonized by enzymatic maceration at the Center of Natural History, University of Hamburg, where they are permanently curated in the Mammalogy Collection.

2.2 | Diets

Experimental diets were designed to contain different levels and types of abrasives between groups, with abrasiveness increasing from lucerne pellets (L), grass pellets (G) to grass pellets with rice husks (GR), and grass pellets with rice husks with an addition of sand (GRS; playground sand, grain size 0–1 mm, REDSUN garden products BV, Heijen, Denmark; mean particle size measured by sieve analysis of 0.233 mm). These diets were of the same batch as those used in experiments with rabbits (Müller et al., 2014), guinea pigs (Müller et al., 2015), and in vitro with horse teeth (Karme et al., 2016). The pelleted diets were designed so the proportion of indigestible silica abrasives in the GRS diet was mimicked by a similar proportion of indigestible, nonabrasive filler (pure lignocellulose, Arbocel, JRS Pharma, Rosenberg, Switzerland) in the other diets, to ensure comparable levels of energy and nutrients per amount of pellets (Müller et al., 2014). Grass hay was provided to all groups except for the lucerne group, which was fed lucerne hay. Each animal received 1,500 g of pelleted food and 100 g of hay daily. It should be noted 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. Water was available for ad libitum consumption at all times. Samples of all diet items and fecal samples of all animals before slaughter were analyzed for acid-detergent insoluble ash as a measure for silica (abrasives) content (Hummel et al., 2011); a nutritional analysis of the pelleted diets and the silica concentrations have been reported previously (Ackermans et al., 2018; Müller et al., 2014).

2.3 | Computer tomography

CT images were acquired from a helical multislice Siemens scanner (Siemens Medical Solutions, Erlangen, Germany) housed at the University of Zurich Veterinary Hospital. Throughout the experiment, parameters were kept constant: tube voltage at 120 kVp, image matrix of 512 × 512 pixels, field of view of 980 × 332 pixels, slice thickness of 0.6 mm, and B60s convolution kernel. At the start of the experiment, before being assigned to the experimental diets, the animals were CT scanned to have a baseline for the tooth condition upon arrival. Another CT scan was performed at the end of

the experiment, post-mortem. As is the general procedure for CT scans, general anesthesia was achieved by administering ketamine, 10 mg/kg (Ketonarkon®, Streuli Pharma AG, Uznach, Switzerland) and xylazine 0.1 mg/kg (Xylazin Streuli, Streuli Pharma AG, Uznach, Switzerland) intramuscularly. Anesthesia was maintained with isoflurane (Attane®, Provet AG, Lyssach, Switzerland) mixed in oxygen with a facemask.

Following data acquisition, the CT datasets were converted to DICOM medical imaging format and rendered into 3D surface models using Amira 5.6 (Mercury Computer Systems/3D Viz group, San Diego, CA) 3D visualization software and using Horos v3.0.1 (Horos Project 2015) for additional visualization.

Using orthographic CT slices at a dorsoventral projection through the upper right M2 and proceeding apically through the image stack, three landmarks at different levels in the tooth were considered as division planes of the crown-root border (Figure 1). Crown-segment 1 was defined as the volume between the tip of the crown and crown point 1 (C1), set at the coronal–most junction of the inner pulp cavity with the dentin (three visible cavities). Crown-segment 2 was from the tip of the crown to crown point 2 (C2), set at the slice before separation of the central pulp cavity (one visible cavity) into two or more root cavities, and crown-segment 3 was set from the tip of the crown to crown point 3 (C3), defined at the slice where distinct roots became visible. For each crown point, the part of the image stack ranging from the defined crown point to the root tips was defined as

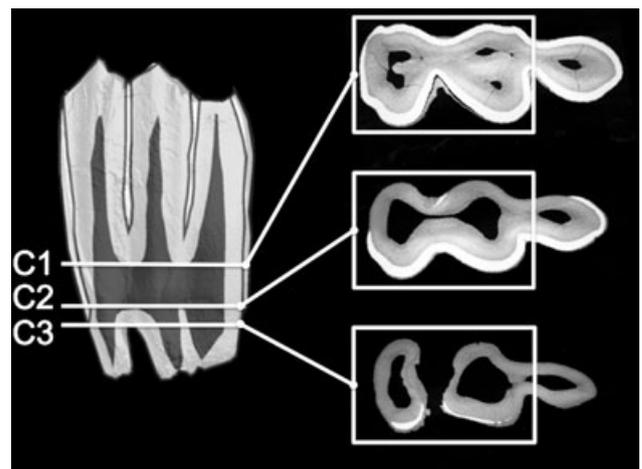


FIGURE 1 Schematic sagittal representation of three possible delimitation lines between molar crown and roots, established to measure crown and root volume in goats (*C. aegagrus hircus*) fed diets of different abrasiveness for six months. Crown segment 1 is defined as the volume between the tip of the crown to crown point 1 (C1), and the volume from C1 to the tip of the roots is defined as root segment 1, idem for crown point 2 (C2) and crown point 3 (C3). White frames represent the transversal tooth-based morphology as seen on X-ray micro-CT slices, used to determine the exact delimitation lines; for C1, one slice further down would show the joint pulpal cavity; for C2, one slice further down would show clearly separated pulp cavities for the two main roots; for C3, one slice further upwards would show the cementum link between the two main roots. The tooth represented is the lower left third molar of animal N°8

root-segment R1, R2, and R3, respectively. Figure 1 was obtained by scanning a lower left m3 (animal N°8; the m3 was used for visualization as it was naturally loosened during maceration, wishing not to damage any other specimens) with an industrial micro-CT scanner (Nikon XT H 225ST) housed at the University of Zurich. Though the m3 has a third cusp, the same method applies.

Manual thresholding of the slice images was performed so as to take the inner pulp cavity of the tooth into account as part of the total volume. From this point on, the upper right M2 was measured in each individual, for each of the two CT scans. The white balance was set at a pixel range of -200 to 2384 pixels and the threshold was set to the full range (-1024 to 3071). The tooth was then delimited manually with the paintbrush tool on multiple slices and interpolated in between for slices with similar morphology. All slices were then checked visually for inconsistencies before the final volume measurement. The total tooth volume was measured, followed by an independent measurement of each section: the tip of the crown to C1, C1 to C2, C2 to C3, and C3 to root tips. The same observer (NLA) performed all of the volume measurements, blinded to the diet groups. Two animals (N°s 11 and 27) were excluded from analysis due to artefacts during the CT process affecting the M2, resulting in a total of 26 animals analyzed. Volume change was calculated by subtraction for all different measures, resulting in data of total volume loss, $\Delta C1$, $\Delta C2$ and so forth for each individual.

2.4 | Statistical analysis

Because all data were not distributed normally, comparisons between treatment groups were performed using ranked data and General Linear Models (GLM) with Tukey's post hoc tests. For all groups, and for each diet group separately, values for C1-3, R1-3 and the total volume were compared between start and end using paired t-tests.

The difference in crown tissue loss and root tissue gain measurements between start and end were compared between groups using analysis of variance (ANOVA) and Sidak's post hoc tests. Because post hoc tests did not always indicate significant differences between groups after a significant ANOVA, the ANOVA was repeated in these cases with bootstrapping (1,000 bootstraps, stratified by diet, 95% confidence intervals of differences), to test whether the confidence interval of the difference between groups included zero. Simple correlations between measures were assessed using Pearson's *R*. The effect of crown tissue loss and diet group on root tissue gain was assessed using GLMs, confirming normal distribution of residuals, with root tissue gain as the dependent variable, crown tissue loss as the independent variable, and diet as cofactor. GLMs were first performed including the crown tissue loss * diet interaction, but this was never significant, and the GLMs were repeated without the interaction. All analyses were performed in SPSS 22.0 (IBM, Armonk, NY), with the significance level set to $P < 0.05$.

3 | RESULTS

The paired t-test of the total M2 volume at the start versus end of the experiment indicated a significant overall decrease in volume (Table 1). This volume loss was also highly significant in t-tests for the crown segments, regardless of the method applied, and a numerical volume gain was visible for all root segments, albeit nonsignificant for R2 (Table 1). When accounting for diet, volume loss was numerically evident, though significant in t-tests only for GR and GRS. Specifically for diet G, $\Delta C1$ is the sole measure showing the numerical loss, though nonsignificant (Table 1). In the roots, the numerical gain in volume is evident overall and significant for the GR and GRS diets (Table 1).

TABLE 1 Mean \pm SD of volume measurements in μm^3 on different crown segment (C) loss and root segment (R) gain, of the upper right second molar (M2) in goats (*C. aegagrus hircus*) fed diets of different abrasiveness for six months

	n	Total volume		C1		C2		C3	
		Start	End	Start	End	Start	End	Start	End
All	26	4,104 \pm 910	4,000 \pm 843**	2,509 \pm 1,080	2,307 \pm 1,043***	2,959 \pm 1,057	2,808 \pm 1,051***	3,535 \pm 1,052	3,392 \pm 959***
L	6	4,121 \pm 1,001	3,982 \pm 870	2,585 \pm 1,136	2,509 \pm 1,238	3,015 \pm 1,118	2,886 \pm 1,171*	3,655 \pm 1,185	3,462 \pm 1,075*
G	6	3,742 \pm 434	3,793 \pm 469	2,352 \pm 466	2,256 \pm 524	2,577 \pm 447	2,652 \pm 580	3,193 \pm 590	3,227 \pm 599
G/R	7	4,033 \pm 1,028	3,884 \pm 952*	2,642 \pm 1,213	2,334 \pm 1,136***	2,938 \pm 1,243	2,682 \pm 1,174**	3,494 \pm 1,251	3,308 \pm 1,105*
G/R/S	7	4,470 \pm 1,052	4,308 \pm 1035*	2,444 \pm 1,441	2,151 \pm 1,289*	3,257 \pm 1,282	3,001 \pm 1,306***	3,766 \pm 1,180	3,559 \pm 1,130**
	n	R1		R2		R3			
		Start	End	Start	End	Start	End	Start	End
All	26	1,595 \pm 764	1,692 \pm 719*	1,145 \pm 408	1,191 \pm 959	569 \pm 379	607 \pm 360*		
L	6	1,536 \pm 227	1,473 \pm 416	1,106 \pm 298	1,095 \pm 342	467 \pm 223	520 \pm 245		
G	6	1,389 \pm 228	1,537 \pm 217	1,164 \pm 223	1,141 \pm 256	549 \pm 221	565 \pm 150		
G/R	7	1,391 \pm 326	1,550 \pm 338*	1,095 \pm 353	1,202 \pm 336*	539 \pm 352	577 \pm 313		
G/R/S	7	2,026 \pm 1,390	2,156 \pm 1,229	1,213 \pm 667	1,306 \pm 735	704 \pm 598	749 \pm 582*		

Note. L: Lucerne; G: grass; G/R: grass/rice husks; G/R/S: grass/rice husks/sand. Means of start and end differ significantly (paired t-test) at *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

TABLE 2 Mean differences \pm SD (95%CI) [bootstrap confidence interval] on different crown segment (C) loss and root segment (R) gain in the upper right second molar (M2) in μm^3 between start and end volume measurements, in goats (*C. aegagrus hircus*, $n = 26$) fed diets of different abrasiveness for six months

Measure	L	G	G/R	G/R/S
Δ Total volume*	139.5 \pm 176.7 (-45.9;325.0) [1.7; 261.9] ^B	-51.0 \pm 108.9 (-165.3; 63.3) [-132.4; 23.9] ^A	148.9 \pm 143.2 (16.5; 281.3) [53.4; 248.7] ^B	162.5 \pm 115.6 (55.5; 269.4) [87.4; 236.0] ^B
Δ C1*	76.2 \pm 176.3 (-108.8;261.2) [(-46.3;207.8)] ^A	96.5 \pm 129.8 (-39.7;232.7) [6.0;187.9] ^A	308.0 \pm 103.6 (212.2;403.8) [245.2;383.9] ^B	292.8 \pm 233.9 (76.5;509.2) [114.9;438.8] ^B
Δ C2*	129.2 \pm 118.8 ^B (4.8;253.9) [34.4; 200.5] ^B	-74.6 \pm 158.3 ^B (-240.6;91.5) [(-215.0;18.7)] ^A	255.6 \pm 105.0 ^B (158.5;352.7) [188.7; 330.8] ^C	256.0 \pm 87.0 ^B (175.6;336.4) [197.7; 310.5] ^C
Δ C3*	192.7 \pm 140.7 (45.1; 340.4) [100.4; 308.1] ^B	-34.7 \pm 96.4 (-135.9; 66.5) [-98.6; 44.9] ^A	186.4 \pm 198.8 (2.5; 370.2) [58.4; 334.3] ^B	207.4 \pm 144.3 (73.9; 340.9) [93.0; 299.4] ^B
Δ R1	-63.3 \pm 285.7 (-363.2; 236.5) [-]	147.5 \pm 149.5 (-9.4; 304.3) [-]	159.1 \pm 156.2 (14.6; 303.5) [-]	130.4 \pm 269.0 (-118.4; 379.2) [-]
Δ R2	-10.3 \pm 245.4 (-267.8; 247.3) [-]	-23.6 \pm 87.7 (-115.6; 68.4) [-]	106.7 \pm 85.4 (27.7; 185.7) [-]	93.5 \pm 119.4 (-16.9; 204.0) [-]
Δ R3	53.2 \pm 98.8 (-50.5; 156.9) [-]	16.3 \pm 134.1 (-124.5; 157.0) [-]	37.4 \pm 92.2 (-47.8; 122.7) [-]	45.0 \pm 45.2 (3.2; 86.7) [-]

Note. L lucerne ($n = 6$), G grass ($n = 6$), G/R grass/rice husks ($n = 7$), G/R/S grass/rice husks/sand ($n = 7$)

*ANOVA $p < 0.05$.

^{a,b}significant differences (Sidak post hoc test) between groups.

^{A,B}bootstrap 95% confidence interval of difference between groups does not include zero.

Comparing the four diet groups by ANOVA (no bootstrapping), significant differences were apparent for the total volume loss as well as Δ C1, Δ C2, and Δ C3, but without subsequent differences at post hoc testing (except for Δ C2, where the G diet was significantly lower than the other ones; Table 2). Bootstrapping also isolated diet G when observing the total volume loss, Δ C2, and Δ C3. In contrast, L and G were significantly different from GR and GRS for Δ C1 and for Δ C2 after bootstrapping (Table 2).

Pearson's correlation coefficient was highest for the Δ C1- Δ R1 relationship at $R = 0.74$. The correlations between Δ C2- Δ R2 and Δ C3- Δ R3 were also significant, but at decreasing R as the separation line approached the root tips (Tables 2 and 3, Figures 1 and 2). This observation was confirmed using GLMs (Table 4). Finally, the diet cofactor only showed a trend ($p = 0.056$) in the GLMs using the C1/R1 approach, but not for the C2/R2 or C3/R3 approaches. The relationship of Δ R1 to Δ C1 was additionally characterized using linear regression statistics, where the resulting equation (Figure 2) included 0 in the 95% confidence interval of the intercept and 1 in the 95% confidence interval of the slope.

4 | DISCUSSION

After six months on experimental diets, an overall volume loss was recorded in the upper right M2 for the four diet groups, predominantly in the crown segment. Most interesting, a slight but consistent volume gain in the root segment that was correlated to crown tissue loss was observed; a finding to our knowledge which has not been reported so far in hypsodont teeth. Diets higher in

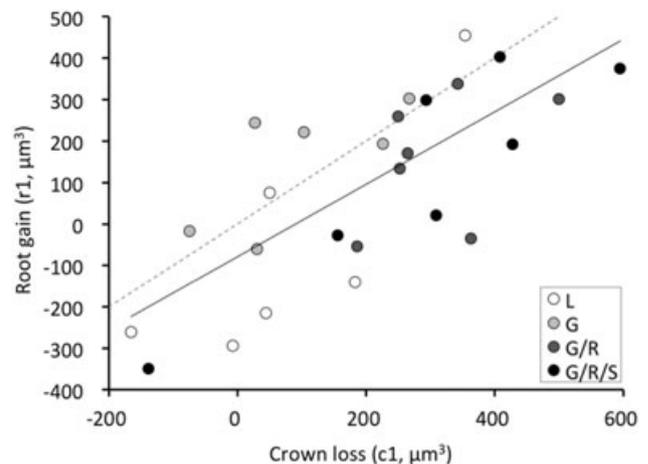


FIGURE 2 The relationship between crown tissue-loss (wear; using method C1) and root tissue-gain (growth, using method R1), in goats (*C. aegagrus hircus*, $n = 26$) fed diets of different abrasiveness for six months. The regression equation, with 95% confidence intervals, was $y = -79 [-172;14] + 0.87 [0.54;1.21] x$ ($R^2 = 0.55$). L lucerne ($n = 6$), G grass ($n = 6$), G/R grass/rice husks ($n = 7$), G/R/S grass/rice husks/sand ($n = 7$). The dotted line denotes $y = x$.

TABLE 3 Pearson's correlation coefficients between different crown segment (C) volume gain and root segment (R) volume loss in the upper right second molar (M2) in μm^3 in goats (*C. aegagrus hircus*) ($n = 26$) fed diets of different abrasiveness for six months

	ΔC2	ΔC3	ΔR1	ΔR2	ΔR3
ΔC1	$R = 0.56$ $P = 0.003$	$R = 0.28$ $p = 0.170$	$R = 0.74$ $p < 0.001$	$R = 0.50$ $p = 0.009$	$R = 0.26$ $p = 0.195$
ΔC2		$R = 0.54$ $p = 0.004$	$R = 0.068$ $p = 0.742$	$R = 0.55$ $p = 0.004$	$R = 0.00$ $p = 0.985$
ΔC3			$R = -0.35$ $p = 0.081$	$R = -0.26$ $p = 0.205$	$R = 0.45$ $p = 0.022$
ΔR1				$R = 0.66$ $p < 0.001$	$R = 0.28$ $p = 0.165$
ΔR2					$R = 0.09$ $p = 0.647$

phytoliths led to more crown loss and root growth; surprisingly, GRS did not cause more wear than the GR.

4.1 | Limitations of study design

The animals used in this experiment were of variable ages and breeds, with varying tooth conditions. A more homogenous sample would have been ideal. But because breeding-animals of the required age class are comparatively expensive, the logistics of keeping such animals until they gave birth, before our experiment, were considered prohibitive, and nonbreeding animals are mostly not kept up to the age we required for the study, we therefore accepted a non-homogenous sample. The requirement of erupted (and in-use) third molars was met by all animals. Additionally, in an effort to simulate "normal" pasture conditions, the amount of added grit in the GRS diet was 5%, as the average amount of soil ingested for sheep ranges from 5% to 9% of daily intake, and up to 33% in extreme cases (reviewed in Damuth & Janis, 2011). Incorporating sand into a pelleted diet is not an accurate representation of natural forage, though consistently adding external abrasives to forage diets would have been impractical over the long term. Evidently, the sand used in

our experiment cannot represent all forms of grit found in nature; nevertheless, it is a valid experimental representation of a highly abrasive substrate. Finally, there can be little doubt that a pelleted diet, while allowing standardized conditions over a long period of time, will evoke different chewing patterns than natural forages, in particular, chewing intensity (chews per dry matter intake) will be lessened on a pelleted diet (Kennedy, 1985). Therefore, it is possible that natural forages of similar nutrient and abrasives content would have a greater effect on tooth wear than pelleted diets.

A further consideration is that the use of a higher resolution CT scanner would have yielded more precise results and higher quality renderings; however, the necessity to scan live, sedated animals only allowed for the use of a medical-grade CT scanner. Further improvements may include the manual segmenting method used in the Amira 3D visualization software, which had a standard error of $\pm 200.57 \mu\text{m}^3$. An automated technique might have served to reduce this error, though detecting the difference between root material and alveolar bone automatically is complicated and furthermore, this technique would not have taken into account the pulp cavity as part of the total volume.

With regard to ANOVA and bootstrapping tests (Table 2), the grass group was often different from the other groups. This could be a chance finding of these particular animals having higher amounts of cementum deposition, as also evident in Figure 2.

TABLE 4 Results of general linear models (incl. adjusted R^2) linking root tissue gain (growth) with crown tissue loss (wear), in goats (*C. aegagrus hircus*) ($n = 26$) fed diets of different abrasiveness for six months

Dependent: Root growth	Model	Independent: Crown wear	Cofactor: Diet
$\Delta\text{R1-}\Delta\text{C1}$	$F_{4,21} = 11.150$ $p < 0.001$ $R^2 = 0.62$	$F_{4,21} = 34.330$ $p < 0.001$	$F_{3,21} = 2.950$ $p = 0.056$
$\Delta\text{R2-}\Delta\text{C2}$	$F_{4,21} = 2.786$ $p = 0.053$ $R^2 = 0.22$	$F_{4,21} = 5.988$ $p = 0.023$	$F_{3,21} = 0.489$ $p = 0.694$
$\Delta\text{R3-}\Delta\text{C3}$	$F_{4,21} = 1.514$ $p = 0.234$ $R^2 = 0.08$	$F_{4,21} = 5.460$ $p = 0.029$	$F_{3,21} = 0.223$ $p = 0.879$

4.2 | Absolute volume methodology

The C1/R1 delineation was selected, as it appeared to be the most efficient at excluding interference with root-growth (Tables 3 and 4, Figure 2). According to the results obtained for the different crown point placements, it seems as if volume gain does not only occur in the root tips but also to a certain extent on the sides of the root walls, which most probably indicates lateral cementum deposition, as for example described for bison (Moffitt, 1998). In future experiments, one could consider creating a permanent mark on the teeth, by drilling a small burr for example, so as to define crown loss and root gain based on this mark.

4.3 | Diet

Significant volume loss appeared reliably in the paired *t*-test between feeding groups on diets with high phytoliths content, as in the GR and GRS diets. Using C1, GR showed the highest volume loss and, contrary to expectations, GRS did not show more loss than GR, though GRS contained additional external abrasives. These results correspond to findings in Ackermans et al. (2018), where mesowear scored on these same goats indicated equal or slightly less wear on GRS when compared with GR, which is also similar to observations by Merceron et al. (2016) when observing dental microwear textures. Indeed, the absence of effect from the GRS diet can be explained by the washing mechanism in place in ruminant digestion (Ackermans et al., 2018; Dittmann, Kreuzer, Runge, & Clauss, 2017; Janis, Constable, Houpt, Streich, & Clauss, 2010; Muhlbacher, Campbell, Ayoub, Chen, & Ghani, 2016) where large sand settles to the bottom of the rumen, resulting in a less abrasive bolus upon regurgitation and therefore dampening the potential wear effect of added sand on the teeth. Whether differences noted in the degree of root growth at similar levels of wear, as for example evident between several animals on diets L and G (with less root growth on diet L), are related to differences in the chewing load or intensity (with a hypothesized lower effort on L compared with G) would have to be corroborated by further experiments.

4.4 | Correlation between crown and root volume change

Hypsodonty is positioned on a scale between brachydonty and euhyposodonty, with a varying balance between the formation of the tooth's crown and roots during the animal's life (Koenigswald, 2011). In the euhyposodont incisors of rodents, the crown and roots are controlled by different genetic pathways, flexible between species (Tummers & Thesleff, 2009) indicating that root and crown growth may happen independently. Knowledge concerning the underlying genetic factors that control euhyposodonty is most often restricted to mice and rats, as they are the most accessible models in a laboratory setting, though this leads to poor comprehension of tooth replacement, being that these species are monophyodont (Juuri et al., 2013). The murine incisor is nevertheless the main model for hypo- and euhyposodont studies. As these continuously growing teeth require persistent stem cell presence at the base of the tooth, a developmental regulatory structure called the cervical loop (Jernvall & Thesleff, 2012; Renvoisé & Michon, 2014) promotes tooth growth there when the crown material is lost to wear by attrition (Harada et al., 1999).

The mouth contains a multitude of proprioceptors working to protect the teeth (Sanson, Kerr, & Read, 2017); periodontal ligaments, for example, contain mechanoreceptors that provide feedback on tooth load (Hughes, 2015), at least in humans. Based on a hypothesis by Janis and Fortelius (1988) that the transmission of occlusal stress from tooth to bone will pass through the root to the area of root secretion, Renvoisé and Michon (2014, p. 8) hypothesize that *“the physical forces of occlusion, dependent on the animal diet, its*

volume, soil grit and/or tooth attrition, might have a mechanical effect on the tooth, and in turn, affects the stem cell niche, through a feedback loop pathway.” The concept of cementum growth compensating for wear has been suggested by Attwell (1980, p. 121): *“as [cementum] layers are laid down throughout life, the process may serve to continue eruption and consequently compensate for the loss of crown height resulting from tooth wear”*, and also by Klevezal (1996, p. 4): *“molars of [...] some ungulates are considered as evergrowing teeth. Their dentin stops growing in length rather early but on the root cementum deposits intensively compensating to some extent [sic] the wear of the crown,”* although neither author cited empirical data as proof for the observation. It would thus not be far-fetched to imagine the existence of a tooth-specific feedback mechanism in ruminants, aimed at compensating wear by inducing root growth by means of cementum deposition, as suggested by our findings. The effects of such a mechanism have been shown experimentally in rabbits (Meredith, Prebble, & Shaw, 2015; Müller et al., 2014; Ness, 1956) and guinea pigs (Müller et al., 2015), with independent growth rates between cheek teeth and incisors, where there was a relation between wear and compensatory growth in the incisors and lower premolar p3.

Camelids, suids or hippopotamids are only some examples of animals showcasing the combination of molars with fixed growth and ever-growing incisors or canines, indicating the possibility of euhyposodont teeth present in hypsodont animals (Koenigswald, 2011). Sequential replacement in the form of delayed molar eruption is an evolutionary adaptation to wear (Janis & Fortelius, 1988), although eruption timing can also be delayed by other external factors such as general malnutrition or specific nutritional deficiencies (calcium and phosphorous), especially in younger animals when the dentition is rapidly developing (Greenfield & Arnold, 2008; Moran & O'Connor, 1994; Popkin, Baker, Worley, Payne, & Hammon, 2012). Historically, tooth eruption schedules for ovicaprids (reviewed by Zeder, 2002) show that molar eruption seems to follow a specific time frame, with only slightly longer time periods in wild or feral animals, most likely due to nutritional differences (Caughley, 1965; Silver, Brothwell, & Higgs, 1969; Taber, 1971; Vigal & Machordom, 1985). Still, to date, no continued growth after the final eruption has been reported (Greenfield & Arnold, 2008). Interestingly, seasonal deposition of cementum layers on the root area of teeth has been recorded and used to age animals for many years (Laws, 1952). It is, however, never described as a type of growth, even though the material is clearly being added to the tooth root structure.

Lieberman (1993) tested the effect of changes in diet on cementum microstructure in a controlled feeding experiment on goats, using diets of different hardness (normal or softened pellets) and with a different protein, mineral (calcium and phosphorus) and vitamin content. The diet hardness, before rumination, impacted cementum growth by affecting the orientation of the Sharpey's fibers, which re-align to counter tensile forces that press teeth in the alveoli during occlusion. This difference in orientation altered visual representation of the incremental cementum lines when viewed under polarized light. The differences in diet mineralization affected the formation rate of the collagen matrix, resulting in darker

cementum bands. This demonstrates that diet hardness and mineral content affect cementogenesis. In any case, growth is happening at the root level, although more accurate equipment is necessary to observe precisely which tissue layer of the roots is gaining volume, whether it is indeed cementum that is the main source of growth in reaction to diet, and how exactly the feedback mechanism works.

5 | CONCLUSION

Volume changes in goat molars show the possible existence of a previously undetected feedback mechanism in ruminants, possibly related to the amount of wear inflicted on the tooth, which reacts and changes even in the absence of euhypsodonty. The observed increase in root volume might be linked to the well-described factor of cementum deposition, already known to react differently depending on various seasonal and physiological forces, though this hypothesis would benefit from further physiological-based research. This mechanism suggests itself to be common to any ruminant, and even to all herbivores in which the investigation of cementum deposits has been described as a method for age determination (Klevezal, 1996). Investigating the spread of this mechanism across species, and for example its link to brachyodont or hypsodonty, might shed further light on evolutionary adaptations to tooth wear. Following studies on differential growth of individual teeth in rodents and rabbits (Ness, 1956; Schour & Medak, 1951), performing an experimental manipulation (such as a graded grinding down) of selected teeth of an adult ruminant and a subsequent detailed investigation of the cementum reaction of treated versus nontreated teeth, might help elucidate the feedback mechanism and outline the scope and limits of compensatory cementum deposition. Such approaches might be useful in estimating the respective costs and constraints of the cementum deposition mechanism as compared to other strategies like euhypsodonty.

Measuring absolute volume allows us to better understand the development of tooth wear over time. Here, it confirmed that diets higher in phytoliths cause more wear, whether it is recorded in the form of volume loss or mesowear, as in our previous article, and that in ruminants, large sand has little effect, probably because it is washed off the ingesta before rumination.

Though a medical grade CT scanner was sufficient to observe slight morphological changes in this study, a detailed approach into the precise mechanics of each tooth layer with a higher-resolution technique should be investigated in future research.

The outcome of this study reveals the need to consider the implications of root growth reacting to (putatively diet-specific) wear from many more points of view. Further research may search to tease apart how much of this signal is due to phenotypic plasticity and how much is genetic. The implications for hypsodonty and euhypsodonty from an evolutionary viewpoint are then also to be questioned. This study is a starting point, but wider use of this technique will provide more information on the matter and serve to create a database of tooth volume changes, of the crown and roots, which we believe will help in understanding dental wear

more than the technique of measuring volume on ontogenetic series of teeth, used up to date.

In any case, teeth should be seen not as a dead structure that is just worn away, but more as a part of the living, growing body.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

MC, TMK, DWHM, PRK, and JMH designed the study, DWHM and MC performed the animal experiment, PRK supervised the CT scanning, JH supervised the nutritional analyses, NLA created the 3D renderings and performed volume measurements, NLA and MC analysed the data and drafted the first version of the manuscript which then received input by all other co-authors.

ORCID

Nicole L. Ackermans  <http://orcid.org/0000-0001-8336-1888>

Marcus Clauss  <http://orcid.org/0000-0003-3841-6207>

Daniela E. Winkler  <http://orcid.org/0000-0001-7501-2506>

Ellen Schulz-Kornas  <http://orcid.org/0000-0003-1657-8256>

Thomas M. Kaiser  <http://orcid.org/0000-0002-8154-1751>

Dennis W. H. Müller  <http://orcid.org/0000-0001-9996-064X>

Patrick R. Kircher  <http://orcid.org/0000-0002-9677-0522>

Jürgen Hummel  <http://orcid.org/0000-0002-8876-7745>

Jean-Michel Hatt  <http://orcid.org/0000-0002-7043-7430>

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P3

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Author contributions: DWHM, MC and JMH designed the original study, DWHM and MC fed the animals from the original study, JBS performed mesowear III measurements under supervision of NLA, JBS and MC analysed the data, and NLA and JBS drafted the first version of the manuscript which then received input by all other co-authors.

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Testing inner-mesowear III on goats (*Capra aegagrus hircus*) fed experimental diets

Julian B. Stauffer¹, Marcus Clauss², Dennis W. H. Müller³, Jean-Michel Hatt² & Nicole L. Ackermans^{2,*}

¹ University of Zurich, CH-8006, Zurich, Switzerland

² Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, CH-8057, Zurich, Switzerland (*corresponding author's e-mail: nicole.ackermans@uzh.ch)

³ Zoologischer Garten Halle GmbH, D-06114, Halle (Saale), Germany

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The wear patterns on ungulate's teeth help to understand dietary habits during an individual's lifetime. Mesowear is a series of techniques used to detect tooth wear patterns at low magnification. While in mesowear I, and its simplified version mesowear II, the outer profile of a herbivore's molar cusps is observed to assign a diet, mesowear III (or inner-mesowear) scores the wear facets on the inner enamel band of the tooth. This approach was developed to detect finer dietary signals for shorter periods than the two former mesowear methods. We tested this method on skulls of 26 goats (*Capra aegagrus hircus*) fed different diets for six months, for which mesowear I and II had already been scored. Our goal was to explore the effects of diets with varying abrasiveness on the dietary signal, comparing signals recorded with both techniques. We found no differences in mesowear III signals among diets, regardless of visible tendency of more abrasive diets leading to higher mesowear scores. We also found no difference in time resolution between mesowear III and I.

Introduction

Mesowear is a series of methods used to assess paleodiets of herbivorous ungulates by analysing different morphological aspects of the animal's molars. Mesowear signals are detectable by the naked eye or at low magnification, and can reflect a considerable fraction of an individual's diet during its lifespan (Fortelius & Solounias 2000). Tooth wear patterns are divided into the wear modes of abrasion and attrition that are the result of food-on-tooth and tooth-on-tooth wear, respectively. By evaluating the relative severity

of attrition as well as abrasion and scoring these wear modes accordingly, mesowear can be used to separate herbivorous ungulates into different diet groups, ranging from grazers to browsers (Fortelius & Solounias 2000). While grazers typically show abrasion-dominated tooth wear, resulting in low, blunt cusps on the molar crowns, browsers cover the other end of the herbivorous dietary spectrum with attrition-dominated tooth wear, showing higher, sharper molar cusps. Though successfully applied in many instances to observational data of extant as well as extinct species (Franz-Odenaal & Kaiser 2003, Clauss

et al. 2007, Rivals *et al.* 2007, Schulz *et al.* 2007, Croft & Weinstein 2008), mesowear has rarely been applied to animals fed experimental diets in controlled conditions (Solounias *et al.* 2014, Kropacheva *et al.* 2017, Ackermans *et al.* 2018), so understanding the extent of the dietary signal assessed by mesowear scoring is therefore still limited.

During the last 20 years, mesowear techniques were constantly developed and adapted to new tasks. Originally developed by Fortelius and Solounias (2000), the mesowear method consisted of two tooth characters: the cusp shape and the cusp relief of ungulate upper molars, scored for one apex per individual. This was further expanded to lower molars for equids (Kaiser & Fortelius 2003) and artiodactyls (Franz-Odenaal & Kaiser 2003). The original technique and its expanded version were termed “mesowear I” by Solounias *et al.* (2014). A new, four-point scoring approach was then introduced by Muhlbachler and Solounias (2006), and was named “mesowear type II” — this is a more simplified method of measuring mesowear I, and the two are considered equivalent in this paper. The mesowear method has since then been further extended, simplified or adapted for specific species (reviewed in Green & Croft 2018). The “mesowear III” technique, also called “inner mesowear”, was established by Solounias *et al.* (2014), and pertains to scoring the wear facets of the inner second enamel band (previous versions scoring the outer third enamel band, visible on the tooth’s profile). This technique was established in order to detect a shorter-term signal with more precision, and was originally used in an experiment lasting for 40 days, a period considered likely too short to record a signal using mesowear I or II. Mesowear III was also used on its own to fossil camelid teeth (Bravo-Cuevas & Jiménez-Hidalgo 2015), in combination with mesowear II on Miocene giraffids (Danowitz *et al.* 2016, Solounias & Danowitz 2016) and extant artiodactyls alongside the experimental goats in Solounias *et al.* (2014), and in combination with mesowear I on fossil bison (Díaz-Sibaja *et al.* 2018) and Pleistocene ungulates (Strani *et al.* 2018b, Strani *et al.* 2018c, Strani *et al.* 2018a). Although combining the methods provides robustness, the methods do not always

produce identical results for each species at different time scales.

As we had already applied the expanded mesowear I technique to goats fed experimental diets of varying abrasiveness for six months (Ackermans *et al.* 2018), in this study we applied mesowear III to the same set of samples to compare the two techniques. As in the previous experiment, mesowear I had not yielded a significant difference among the feeding groups, we expected mesowear III to be able to differentiate among these groups. We therefore anticipated that abrasive-rich diets would result in higher mesowear III scores than less abrasive ones.

Methods

Animals and their diet

The animal experiments were performed with approval of the Swiss Cantonal Animal Care and Use Committee Zurich (animal experiment license no. 115/2009). The skulls analysed in the present study are housed in the mammal collection at the Center of Natural History, University of Hamburg (ID numbers in Table 1).

The animals were all domestic-goat (*Capra aegagrus hircus*) does ($n = 28$) from mixed breeds with unknown feeding history. They were the same individuals as those used in Ackermans *et al.* (2018), as were the diets and feeding regimes, the duration of the feeding experiment, and the skull preparation. In short, prior to the beginning of the experiment, all individuals were fed *ad libitum* lucerne (*Medicago sativa*) hay and lucerne pellets for two weeks. Subsequently, the goats were randomly divided into four groups of seven animals. For about six months, each group was fed one of the following four diets varying in abrasiveness: lucerne (L), no abrasives; grass (G), low in phytoliths; grass and rice husks (GR), high in phytoliths; grass, rice husks and sand (GRS), high in phytoliths and high abrasives. These diets were from the same batch as used in experiments with rabbits (*Oryctolagus cuniculus*) by Müller *et al.* (2014). The experimental diets were provided as pellets, 1500 g per animal per day, in addition to 100 g per animal per day of either lucerne hay for the lucerne group, or grass

hay for the remaining three dietary groups. To make sure that the levels of energy and nutrients in the pelleted diets were comparable, they were formulated to be isocaloric and isonitrogenic (using an indigestible, non-silicacious filler and soybean meal) (cf. Müller *et al.* 2014). Water was available *ad libitum*.

Mesowear III

In the mesowear III method (Solounias *et al.* 2014), scores from 1 to 4 are assigned to the

occlusal view of the inner enamel band of molar teeth. Score 1 reflects a typical browser wear pattern (sharp edge, flat surface), and score 4 a typical grazer pattern (round edge, uniform arched surface). For scoring, the enamel band is divided into three sections which are scored separately: (1) the mesial part, (2) the distal part and (3) the midpoint of these two areas named *j* for junction. As suggested by Solounias *et al.* (2014) and Danowitz *et al.* (2016), we also calculated averages of the mesial and distal scores to obtain a mean score (cf. Tables 1 and 2). In this study, primarily the paracone of the second right-side

Table 1. Mesowear III scores on molars of domesticated goats (*Capra aegagrus hircus*, $n = 26$), fed experimental diets for six months.

Animal ID ^a	ZMH ID ^b	Tooth ^c	Diet ^d	Side	Mesowear III score			Score	
					distal ^e	mesial ^f	<i>j</i> ^g	average	ranked
1	10879	M3	GRS	left	2	2	3	2	3
2	10880	M2	GRS	right	3	4	4	3.5	6
3 ^h	10881	M2	L	right					
4 ^h	10882	M2	GR	right					
5	10883	M3	G	right	4	4	3	4	7
6	10884	M2	GRS	right	3	3	2	3	5
7	10885	M2	GR	right	3	3	2	3	5
8	10886	M2	GR	right	2	3	4	2.5	4
9	10887	M2	G	left	3	3	4	3	5
10	10888	M2	L	right	3	4	4	3.5	6
11	10889	M2	L	left	4	3	3	3.5	6
12	10890	M2	GR	right	2	3	2	2.5	4
13	10891	M2	L	right	2	3	2	2.5	4
14	10892	M2	G	right	3	3	3	3	5
15	10893	M2	L	right	2	3	2	2.5	4
16	10894	M2	GR	right	3	2	3	2.5	4
17	10895	M2	G	right	2	2	2	2	3
18	10896	M2	GRS	right	4	3	3	3.5	6
19	10897	M2	GRS	right	3	4	3	3.5	6
20	10898	M2	GRS	right	3	4	3	3.5	6
21	10899	M2	G	right	2	2	4	2	3
22	10900	M2	GR	right	3	3	3	3	5
23	10901	M2	L	left	1	2	2	1.5	2
24	10902	M2	L	right	2	1	1	1.5	2
25	10903	M2	GR	right	4	3	3	3.5	6
26	10904	M2	GRS	right	3	4	2	3.5	6
27	10905	M2	G	right	2	2	2	2	3
28	10906	M2	G	right	1	1	1	1	1

^a same as in Ackermans *et al.* (2018).

^b ID numbers assigned by the Zoological Museum of Hamburg.

^c M2 = maxillary second molar, M3 = maxillary third molar.

^d L = lucerne, G = grass, GR = grass and rice husks, GRS = grass, rice husks and sand.

^e mesowear III score assigned to the distal facet of the tooth's third enamel band.

^f mesowear III score assigned to the mesial facet of the tooth's third enamel band.

^g mesowear III score assigned to the midpoint of the tooth's third enamel band.

^h teeth too worn to be scored for mesowear III.

mandibular molar was scored. If this tooth was too worn and/or damaged, the same location on the left-side mandibular molar was scored. If this tooth was also worn and/or damaged, the third molar was used for scoring. In all, we scored 22 maxillary right-side molars, of which one was the third molar and the rest second molars; and 4 maxillary left-side molars, of which one was the third molar and three were second molars. Two goat skulls had teeth too worn to be scored for mesowear III. The sample size was thus $n = 26$.

Mesowear I scores of the same teeth were taken from Ackermans *et al.* (2018). In comparisons between mesowear I and III, animals that were not scored in either of the techniques were excluded, resulting in the sample size of $n = 25$.

Statistical analysis

Because the mesowear III scores were not normally distributed, the data were analysed using non-parametric tests. The Kruskal-Wallis test was used to find out whether mesowear III scores differed among the diet groups, and Spearman's rank-order correlation analysis to study the relationships between III scores and between mesowear I and III scores. All analyses were performed in SPSS 22.0 (IBM, Armonk,

NY). Differences were considered significant and correlations existing at $p < 0.05$. Although the data were non-normally distributed, standard deviation and means were plotted to better visualise possible differences (cf. Fig. 1).

Results

We found no statistical differences among diet groups when scoring tooth wear with mesowear III (Kruskal-Wallis test: $p = 0.171$ for the mesial mesowear III score, $p = 0.389$ for the distal mesowear III score, $p = 0.212$ for the mean of the mesial and distal mesowear III score, and $p = 0.700$ for the junction mesowear III score; Fig. 1). GRS, GR and G diets tended to produce higher mean mesowear scores than the L diet for mesial, junction and averaged characters, while GRS and GR produced higher mean mesowear scores than the L and G diets for the distal character. GRS was the diet that resulted in the highest mean mesowear III score for all four mesowear III characters, and the diet with the lowest mean mesowear III score was either G or L. All measured mean mesowear III scores were greater than 2, most being between 2 and 3 (cf. Table 1). Although the four mesowear III scores were correlated (R between 0.43 and 0.90

Table 2. The scoring of mesial part, distal part and midpoint (j) of the tooth's third enamel band, and the score descriptions of mesowear III (following Danowitz *et al.* 2016) used to assess mesowear signals.

Location	Score	Description
mesial/distal	1	The occlusal surface of the enamel band is flat and planar and there are no gouges or indentations on the surface of the enamel.
mesial/distal	2	The surface of the enamel band is nearly flat and contains several gouges that traverse the surface from either edge. The labial and lingual edges of the enamel band are somewhat rounded. Instead of a single surface as in score 1, several facets are discernible.
mesial/distal	3	The enamel surface is similar to score 2, but is more rounded with less defined edges and more gouges.
mesial/distal	4	The enamel surface is rounded. The surface is smooth without gouges, and there are no well-defined edges.
midpoint	1	The mesial and distal surfaces join at a sharp, well-defined junction.
midpoint	2	The midpoint between the mesial and distal surfaces is somewhat sharp, and often contains a gouge.
midpoint	3	The midpoint on the enamel band is rounded, but the mesial and distal sides appear as distinct, separate surfaces.
midpoint	4	The j point lacks a discrete apex, and the mesial and distal sides of the enamel band form one continuous surface.

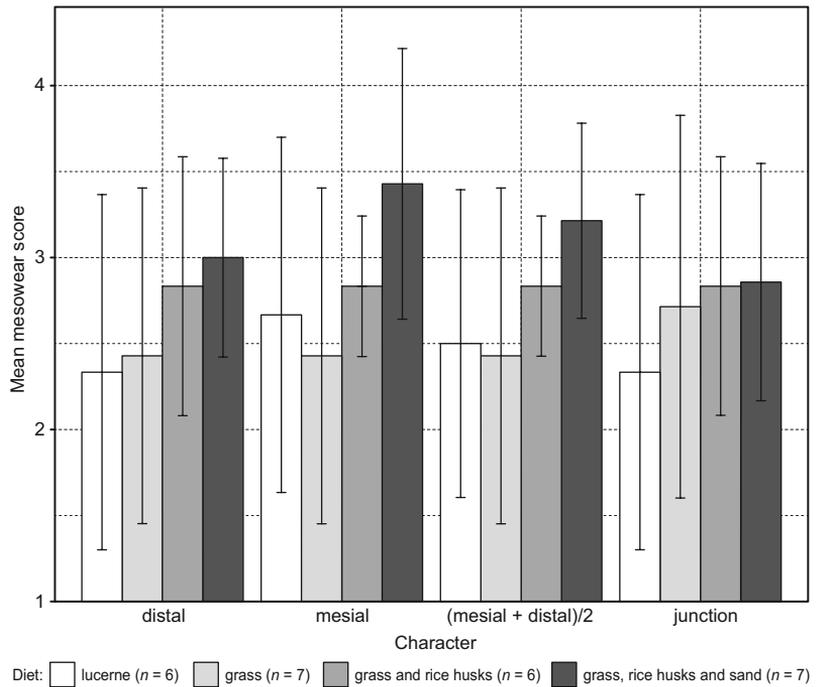


Fig. 1. Mean (\pm SD) mesowear III scores on molars of domesticated goats (*Capra aegagrus hircus*, $n = 26$), fed four experimental diets for six months. The x-axis represents the three separately scored enamel characters (distal, mesial, junction) and the average of two of them [(mesial + distal)/2].

and p between 0.031 and < 0.001), none of the mesowear scores were correlated with the corresponding mesowear I cusp-score on the same tooth ($R = 0.10\text{--}0.19$, $p = 0.377\text{--}0.627$).

Discussion

Diets differing in abrasiveness did not result in differences in maxillary M2 mesowear III scores in goats. Mesowear III scores seemed to increase with the diet abrasiveness (cf. Fig. 1), but this was not supported by the statistical analysis of the data. Similar results were found by Ackermans *et al.* (2018) for mesowear I.

In their original mesowear III study, Solounias *et al.* (2014) fed two groups of four goats browsing and grazing diets. On days 10, 20, 30 and 40 one goat from each group was sacrificed and their skulls and jaws were skeletonised. They found that in the goats that shifted from natural food to browsing, the teeth became incrementally flatter after 30–40 days of browsing, while in the goats that shifted from natural food to grazing, the teeth had become incrementally rounder during the first 30 days of grazing, and did not change anymore during the last 10 days.

However, as Solounias *et al.* (2014) recognized themselves, their experimental setting had several weaknesses: all the goats had a browsing diet before the experiment, and the sample size was small (only one animal per sampling, hence no statistical comparisons were possible). In addition, the original wear state of these animals was based on mesowear III scored on other individuals feeding in the same location. Therefore individual differences in tooth wear among experimental goats at the start of the experiment could not be taken into account in the comparisons. As such, by applying mesowear III scoring in a longer-term experiment with a larger sample size, we hoped to ascertain the applicability of this technique. The lack of significant difference in mesowear scores among the goats fed the different diets in the present study indicates that six months may not be long enough for a stable mesowear I or III signal to develop.

Although the results obtained by Solounias *et al.* (2014) were considered preliminary, several subsequent studies used this technique. For example, Danowitz *et al.* (2016) showed that mesowears II and III are correlated when predicting browser or grazer profiles. The correlations between the two mesowear techniques, however,

were weaker than those between mesowear III scores. As similar results were obtained in other studies as well (Strani *et al.* 2018b, Strani *et al.* 2018a), combined usage of mesowear II and III techniques has been recommended. Bravo-Cuevas and Jiménez-Hidalgo (2015), however, used mesowear III alone to determine a browser or grazer diet. Even if mesowear III scores could distinguish between the two diet types, and could be considered a short-term dietary signal in this case, it should not be used as a stand-alone method to define species-specific, long-term, diets. It would thus be a good practice to systematically record mesowear I or II in conjunction with mesowear III.

The mesowear method is based on more or less subjective observations, and the descriptions of scoring can be interpreted differently depending on the observer, which is why a single observer is often used to reduce inter-observer errors. Another factor that could have affected the scoring in the present study were large age differences among individuals. Age can affect mesowear scores because an old, flat, and round tooth is scored as blunter, due to more abrasion, than a younger, sharper tooth. Additionally, differences in goat breeds and unknown previous diets might have also increased variations in the data set. On the other hand, obtaining a large number of animals with equal wear could be rather challenging. It might also be interesting to study whether goats might be less sensitive to mesowear changes than for example sheep, or whether ruminants as a group are less sensitive than non-ruminants, because ruminants can wash off external abrasives from their diet before rumination (Hatt *et al.* 2019), most likely reducing dental wear. Nevertheless, our finding that after 6 months of controlled feeding no produce differences among the diet groups, indicates that similarly to mesowear I, mesowear III should probably not be considered a short-term dietary signal by default until the signal length can be confirmed in additional experiments.

According to the results of this study, mesowear III could be used in combination with mesowear I or II by adding dietary signal information to a data set, but its relationship to mesowear I or II, as well as its responsiveness to the diet cannot so far be confirmed with certainty.

All in all, mesowear is the most cost-effective method to quickly determine the general diet of a large number of specimens. The time this technique requires to respond to different diets, however, remains to be experimentally determined.

Conclusions

Based on the results of our study we can conclude that, at least when small ruminants and short time intervals are concerned, mesowear III is not a finer tooth-wear measurement method than mesowear I or II. More caution is thus needed when it comes to applying mesowear, especially mesowear III, as the amount of time necessary for the dietary signal to develop, and the strength of the signal are not yet fully quantified.

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P3

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

Jean-Michel Hatt, Daryl Codron, Dennis W. H. Müller, Nicole L. Ackermans, Louise F. Martin,
Patrick R. Kircher, Jürgen Hummel, Marcus Clauss

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Author contributions: DWHM, PRK, MC and JMH designed the study; DWHM and MC performed the animal experiment; PRK supervised the CT scanning; JH supervised the nutritional analyses; JMH and NLA performed the volumetric analysis of CT scans; DC analysed the data, JMH and MC and NLA drafted the first version of the manuscript which then received input by all other co-authors.



Original investigation

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

Jean-Michel Hatt^a, Daryl Codron^b, Dennis W.H. Müller^a, Nicole L. Ackermans^a, Louise F. Martin^a, Patrick R. Kircher^c, Jürgen Hummel^d, Marcus Clauss^{a,*}

^a Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Winterthurerstr. 260, 8057 Zurich, Switzerland

^b Department of Zoology and Entomology, University of the Free State, PO Box 339, 9300 Bloemfontein, South Africa

^c Clinic for Diagnostic Imaging, Vetsuisse Faculty, University of Zurich, Winterthurerstr. 260, 8057 Zurich, Switzerland

^d Ruminant Nutrition, Department of Animal Sciences, University of Goettingen, Kellnerweg 6, 37077 Goettingen, Germany

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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 mastication 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.

* Corresponding author.

E-mail address: mclauss@vetclinics.uzh.ch (M. Clauss).

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 depleted in silica compared to the diet, indicating a washing effect in the rumen.

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 (cm³) were calculated by manually defining regions of interest (ROIs) on every 6th 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

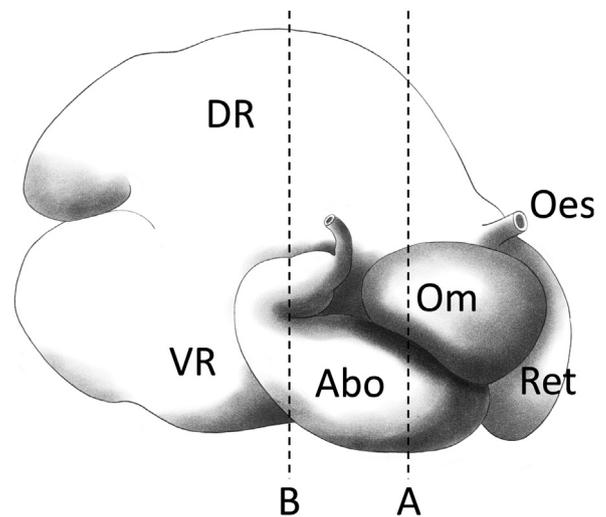


Fig. 1. Schematic representation of the ruminant forestomach, viewed from its right side. The cranial part is to the right, with the oesophagus (Oes), and the caudal part to the left. While the forestomach is positioned next to the left abdominal wall of the animal, on its right side, between the scheme 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 thoracic cavity with the dorsal part of the lungs, not shown here. Drawing modified from Nickel et al. (2004).

(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 (Zeitl 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,

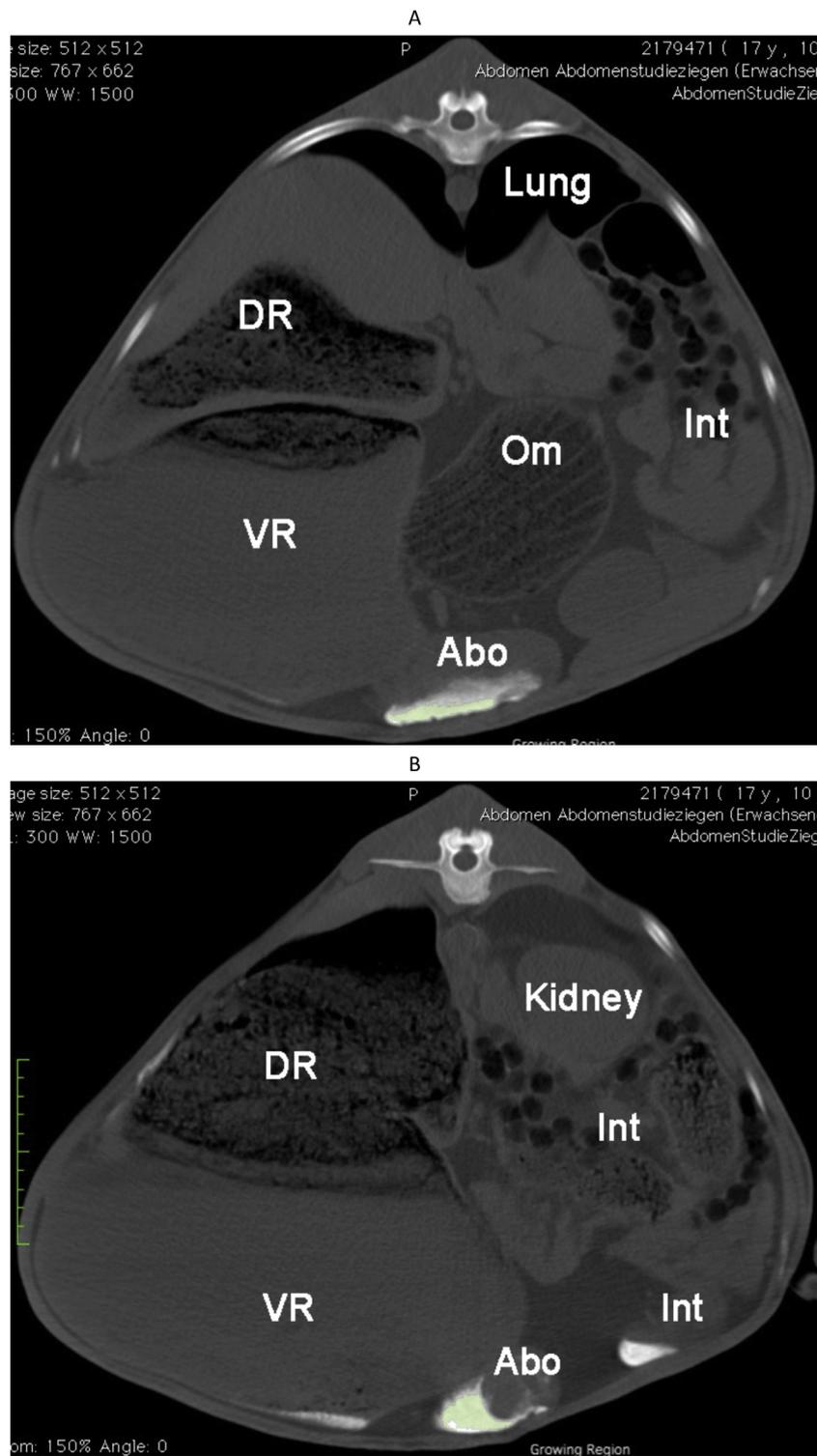


Fig. 2. Computed tomographic scans of a goat (*Capra aegagrus hircus*) fed a pelleted diet of grass and rice husks containing sand (GRS) for six months. Animal in sternal recumbency. Images represent vertical slices through the thorax and abdomen (A) or, more caudally, through the abdomen only (B), as 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, $48.1 \pm (\text{SD}) 32.0 \text{ cm}^3$. Note that this did not cause clinical problems

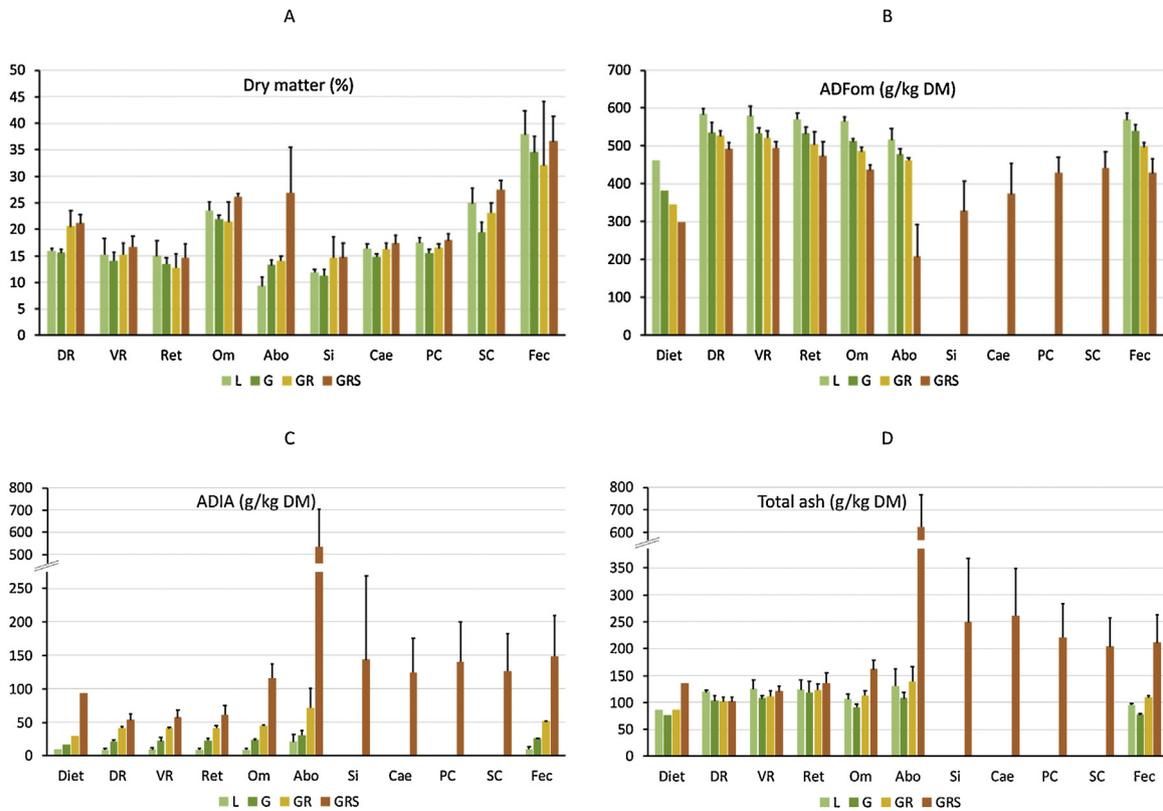


Fig. 3. The (mean +SD) concentration of (A) dry matter (DM), (B) acid detergent fibre (ADFom), (C) acid detergent insoluble ash (ADIA; a measure for silica) and (D) total ash (including insoluble ash as well as acid detergent soluble ash, i.e. minerals) in the diet and digestive tract of goats (*Capra 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.

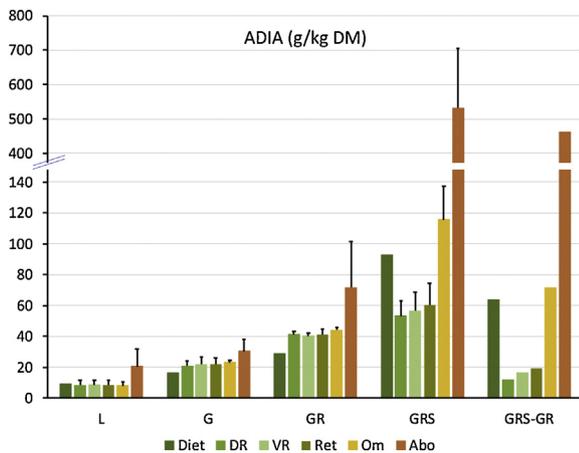


Fig. 4. Concentration of acid-detergent insoluble ash (ADIA, in dry matter; means +SD) in the diet and different sections of the (fore)stomach in goats (*Capra aegagrus hircus*) fed diets of different abrasive content (lucerne L; grass G; grass and rice husks GR; grass, rice husks and sand GRS). DR dorsal 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 $14.2 \pm 12.0 \text{ cm}^3$ 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 diet	3,17	0.115 22.108	0.735 <0.001	GRS > L/G/GR; GR > G all diets: Fec > all
diet/GIT	36,169	31.492	<0.001	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 diet	3,33	0.060 151.613	0.807 <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 diet	3,32	5.527 53.479	0.019 <0.001	GRS > L/G/GR; GR > L
diet/GIT	25,123	21.457	<0.001	GRS: Abo > all
Total ash				
individual diet	3,28	16.051 37.610	<0.001 <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.

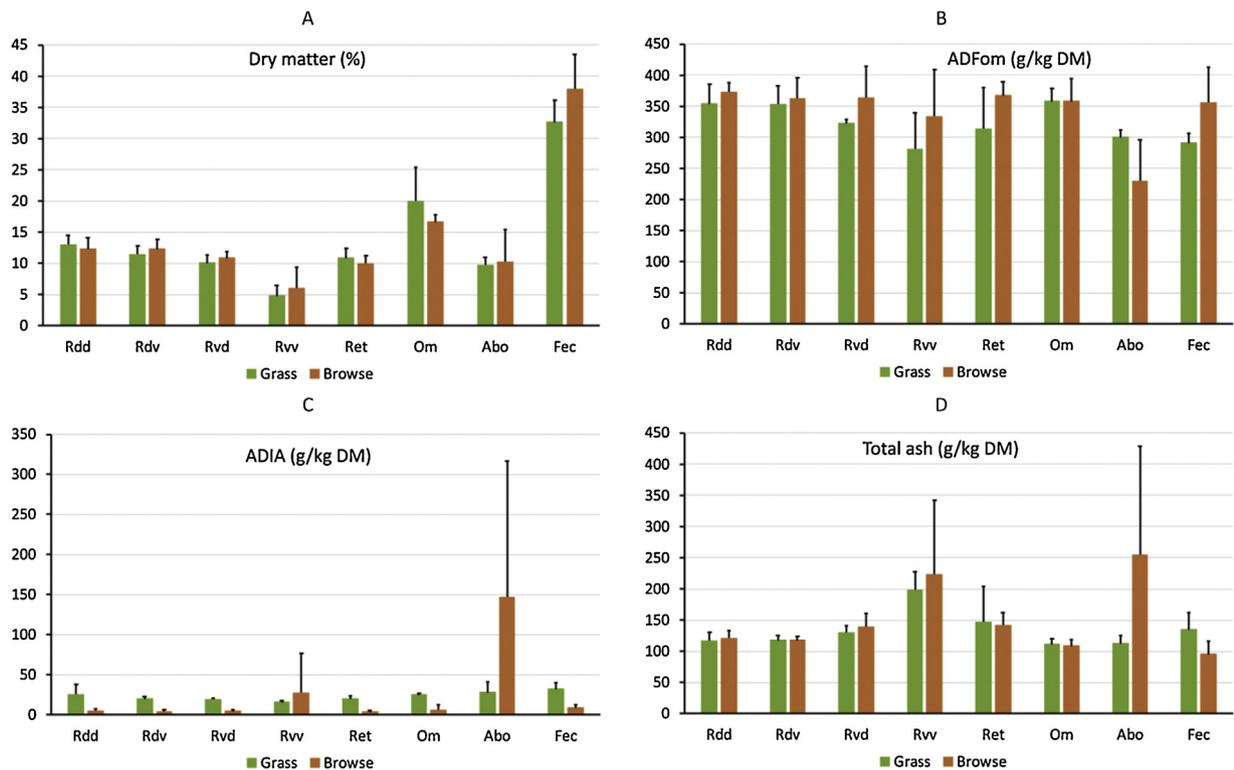


Fig. 5. The (mean + SD) (A) concentration of dry matter (DM), (B) acid detergent fibre (ADFom), (C) acid detergent insoluble ash (ADIA) and (D) total ash in the digestive tract of goats (*Capra aegagrus hircus*) fed either grass hay or dried browse. Rdd dorsal part of dorsal rumen, Rdv ventral part of 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

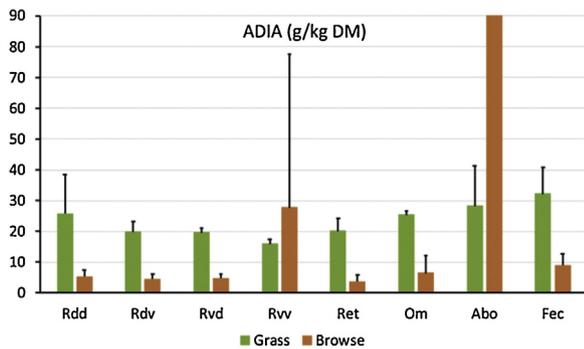


Fig. 6. The (mean + SD) concentration of acid detergent insoluble ash (ADIA) in the digestive tract of goats (*Capra aegagrus hircus*) fed either grass hay or dried browse. Rdd dorsal part of dorsal rumen, Rdv ventral part of dorsal rumen, Rvv ventral part of ventral rumen, Ret reticulum, Om omasum, 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 diet	1,8	12.974	<0.001	
diet/GIT	14,54	80.469	<0.001	both diets: Fec > all B: Om > Rvv/Ret G: Om > all; Rdd/Rdv > Rvv
ADFom				
individual diet	1,8	2.961	0.085	
diet/GIT	14,54	4.875	<0.001	B: Abo < all
ADIA				
individual diet	1,8	0.141	0.707	
diet/GIT	14,55	3.131	<0.001	B: Abo > all
Total ash				
individual diet	1,8	0.352	0.553	
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; Mühlbachler 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 compilations 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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M2

Everything matters: molar surface texture in goats fed diets of different abrasiveness

Ellen Schulz-Kornas, Daniela E. Winkler, Marcus Clauss, Jessica Carlsson, Nicole L. Ackermans,
Louise F. Martin, Jürgen Hummel, Dennis W. H. Müller, Jean-Michel Hat, Thomas M. Kaiser

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Everything matters: molar surface texture in goats fed diets of different abrasiveness

Ellen Schulz-Kornas^{1,2,7}, Daniela E. Winkler^{2,3,7}, Marcus Clauss^{4*}, Jessica Carlsson², Nicole L. Ackermans⁴, Louise F. Martin⁴, Jürgen Hummels⁵, Dennis W. H. Müller^{4,6}, Jean-Michel Hatt⁴, Thomas M. Kaiser²

¹Max Planck Weizmann Center for Integrative Archaeology and Anthropology, Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, 04103, Leipzig, Germany

²Center of Natural History, University of Hamburg, Hamburg, Germany

³Institute for Geosciences, Johannes Gutenberg University Mainz, Mainz, Germany

⁴Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

⁵Department of Animal Sciences, Ruminant Nutrition, Georg-August University, Göttingen, Germany

⁶Zoologischer Garten Halle GmbH, Fasanenstr. 5a, 06114, Halle (Saale), Germany

⁷these authors contributed equally

* to whom correspondence should be addressed at mclauss@vetclinics.uzh.ch

Abstract

Goats were fed diets on increasing phytolith-based abrasiveness (L: lucerne based pellets, very low phytolith abrasion diet, acting as the control. G: grass-based pellets, medium abrasive phytolith diet. GR: grass and rice husk pellets, high abrasion phytolith diet), including a diet with added external abrasives (GRS: the GR diet with addition of large-grained sand as a source of external abrasives), for 6 months. Increasing dietary abrasion in different experimental diets (L<G<GR<GRS) led to gradually increasing roughness and larger volume in surface texture features. Highest void volumes (largest pits) were found in the GRS group. This is consistent with texture results from rabbits fed the same pelleted diets. We conclude that the same diet forms similar surface texture, even in two very different mammal species. It further indicates a general diet-dependent principle of surface texture formation. In the direct comparison of

maxillary and mandibular molars, the sand diet had a significantly more pronounced effect on the maxillary molars, which showed more voluminous valleys and larger texture direction values. This contradicts the concept of gravity making mandibular molars more susceptible to impact from external abrasives, but supports the concept of maxillary and mandibular molars as an inverted pestle-and-mortar system. The results indicate that external abrasives (of the size and concentration of the present experiment) do have a 3D texture effect in goats that could be relevant for dietary and environmental reconstructions based on this method.

Introduction

Wear traces on teeth have been used for the reconstruction for the diets and the habitats of fossil vertebrates for decades. Usually, these wear traces are grouped according to the level of assessment, into macroscopic (mesowear) and



microscopic (2D and 3D microwear) traces (Davis and Pineda-Munoz, 2016b; Calandra et al., 2019).

The research field of tooth wear is the subject of a classic scientific dilemma: on the one hand, substantial calibrating catalogues of extant species exist, which allow the differentiation of feeding types – such as browsers vs. grazers. These diet types can be, in some systems, very reliably differentiated by mesowear (Fortelius and Solounias, 2000), 2D microwear (Walker et al., 1978b), or 3D microwear (dental microwear texture analysis, DMTA) (Scott, 2012a; Calandra and Merceron, 2016b). The patterns that allow the classification are, in many cases, very convincing, and have a strong differentiation power. Additionally, clear differences between habitats of differing dietary regimes have been demonstrated, especially between free-ranging and zoo animals (Kaiser et al., 2013b).

On the other hand, the actual processes that lead to the wear traces have often not been identified, but have been described only by hypothetical explanations. These explanations focus on the presence of abrasives in or on the diet, or the putative proportions of abrasion and attrition, but also include other physical aspects of the diet, teeth, and chewing physiology (Kaiser et al., 2016). This can lead to a situation close to circular argumentation, when a certain wear type is hypothetically explained to be caused by abrasion, with the derived conclusion that the diet clearly linked to this wear type must be abrasive, and this assumption then obtains

factual status without being subjected to experimental testing. In other words, while wear proxies evidently *work*, as can be demonstrated in extant species grouped into diet categories, the hypothetical explanations *why they work* mostly remain speculative, and require testing.

For example, while mesowear has been widely shown to differentiate between browsing and grazing ruminants (Fortelius and Solounias, 2000), or between free-ranging and captive specimens of the same species (Clauss et al., 2007); experimental approaches (that so far, however, did not include the long-term feeding of grass or browse forages), have not been successful in recreating that differentiation statistically (Solounias et al., 2014; Ackermans et al., 2018a; Stauffer et al., 2019). Experimental tests of DMTA measures have yielded unexpected results that contradict traditional explanations, for example suggesting that a more variable signal need not necessarily indicate a more variable diet, but can be triggered by a monotonous diet with certain characteristics (Schulz et al., 2013e). The extent to which species-specific properties influence the wear traces on teeth is another area of debate (DeSantis et al., 2018; Fraser et al., 2018; Clauss, 2019), where it has been difficult to explain patterns across a broad range of species for some measurements (Kaiser et al., 2016; Mihlbachler et al., 2016). Differences between teeth within individuals (*i.e.* between mandibular or maxillary teeth, or between tooth facets (Ramdarshan et al., 2017b; Arman et al., 2019)),



further complicate the functional understanding of wear processes. Finally, the extent to which different wear proxies are functionally related, e.g. whether a certain DMTA pattern is expected to be linked to a certain mesowear pattern, remains to be explored. When combining different wear proxies (such as mesowear and DMTA), or combining wear proxies and analyses of the actual diet as derived from stomach contents or isotopes, it appears that often the results are in discrepancy (Schubert et al., 2006; Merceron et al., 2010b; Louys et al., 2012; Ecker et al., 2013; Tütken et al., 2013; Loffredo and DeSantis, 2014; Rivals et al., 2015; Saarinen et al., 2015; Rivals and Lister, 2016; Sánchez-Hernández et al., 2016; Brent Jones and Desantis, 2017; Mihlbachler et al., 2018; Strani et al., 2018). While this can be explained to some extent by the different time resolution of the respective diet proxies, one would, stochastically, expect these proxies to be more often in accord than not, if they indeed record the same dietary signal.

Perhaps the most prominent scientific debate in the field of dental research, revolves around whether internal abrasives (phytoliths) or external abrasives (dust, grit) are the most important-, or even the sole entities responsible for wear (Sanson et al., 2007a; Lucas et al., 2013c; Rabenold and Pearson, 2014; Sanson et al., 2017). The question has a very high relevance, relating to the selective pressures that led to the evolution of hypsodonty and hypseledonty (Jardine et al., 2012; Tapaltsyian et

al., 2015). This includes general habitat aridification (increasing dust loads) (Eronen et al., 2010a; Eronen et al., 2010b), volcanic activity that affects the characteristics of environmental dust (Madden, 2014a), or a general spread of plants that contain phytoliths (i.e., monocots), amongst others (Semperebon et al., 2019). For ruminants, the current evidence on effects of internal or external abrasives are not conclusive. Data on hypsodonty, diet, and habitat, suggest habitat is an important predictor of the degree of hypsodonty (Damuth and Janis, 2011a), but habitat does not appear related to mesowear (Kaiser et al., 2013b), suggesting different effects of external abrasives on absolute tooth wear in the form of tissue loss and macroscopic tooth shape. Experimental studies on ruminants in which diets were supplemented with external abrasives in a controlled manner, did not detect an effect of these abrasives on absolute crown wear of the maxillary M2 (Ackermans et al., 2019) and mesowear of maxillary and mandibular molars (Ackermans et al., 2018a), or 3D texture of the mandibular molars in a previous experiment (Merceron et al., 2016b), but did cause a distinct microwear signal in the maxillary molars in yet another earlier experiment (Hoffman et al., 2015).

In our view, controlled *in vitro* and *in vivo* experimental approaches remain the standard to test our functional understanding of tooth wear, which is originally derived from the epidemiological approaches used when assessing extant specimens whose diet is only assumed. In

order to assess how DMTA patterns reflect differences in internal- and external abrasive content, and relate to other proxies of wear and tooth position, we applied DMTA to teeth from an experiment in which goats were fed four different diets for half a year (Ackermans et al., 2018a; Ackermans et al., 2019b). In particular, we were interested in whether the addition of sand to a phytolith-rich diet would have an effect on DMTA results, as it had no discernible effects on absolute tissue loss or mesowear.

Methods

The feeding experiment from which samples were analysed was performed with approval of the Swiss Cantonal Animal Care and Use Committee Zurich (animal experiment licence N° 115/2009), and details on absolute tooth wear, mesowear, and diet preference have been reported previously (Ackermans et al., 2018a; Ackermans et al., 2019b; Ackermans et al., 2019d). In brief, 28 adult domestic goats (*Capra aegagrus hircus*) were divided randomly into four groups of seven individuals, and kept for half a year on one of four isocaloric, isonitrogenous pelleted diets designed to contain different levels and types of abrasives. Abrasiveness theoretically increased from lucerne pellets (L), grass pellets (G) to grass pellets with rice husks (GR) and grass pellets with rice husks with an addition of sand (GRS; mean particle size of sand 0.233 mm). For a more detailed description of the diets as well as the concentration of silica in the faeces and digestive

tracts of the goats, refer to previous publications (Müller et al., 2014a; Clauss et al., 2018; Hatt et al., 2019b). At the end of the experimental period the animals were slaughtered and the skulls were prepared by enzymatic maceration at the Center of Natural History, University of Hamburg, where they are permanently curated in the Mammals Collection.

DMTA of the samples was performed following the standard technique in Schulz et al. (2013a). We applied 46 DMT parameters using the ISO 25178 (roughness), motif, furrow, isotropy, ISO 12871 (flatness), and Scale-sensitive fractal analysis (SSFA). Parameters were grouped into the following categories for simplification: area (*Sda, Sha, mea*), complexity (*Sdr, nMotif, Asfc*), density (*Sal, Spd, medf*), direction (*Std, Str, Tr1R, Tr2R, Tr3R, IsT, epLsar*), height (*S10z, S5p, S5v, Sa, Sku, Sp, Sq, Ssk, Sv, Sxp, Sz, meh, madf, metf, FLTt, FLTp, FLTq, FLTv*), peak sharpness (*Spc*), plateau size (*Smc, Smr*), Slope (*Sdq*), and volume (*Sdv, Shv, Vm, Vmp, Vmc, Vv, Vvc, Vvv*) (Table S1).

For each specimen, four sites on the same facet were analysed, when possible: the posterior facet of the anterior cusp on the second enamel band (from buccal) for the right maxillary M2 (Fig. S1), or alternatively the facet on the fourth enamel band when the former was damaged or too worn. In the case that both sites could not be used in a right tooth, the left side was used. The antagonist facet to the M2 for was selected for each specimen on the mandibular m2: the anterior facet of the posterior cusp on the first



enamel band (from buccal) was selected on the m2 when the ideal facet was measured on the M2 (Fig. S1); if it was not intact, or if the fourth enamel band was measured on the M2, the second enamel band was selected on the m2.

Target facets were cleaned with rubbing alcohol and then moulded using putty (Provil® novo vinylpolysiloxane type 3 dental putty). A copper thread was inserted on the mould to indicate direction, and moulds were placed in a microtiter plate (Schulz et al., 2013a). The plate was then fixed to the table of the confocal disc scanning microscope μ surf custom (NanoFocus AG, Oberhausen, Germany) with a blue LED (470 nm) and high-speed progressive-scan digital camera (984x984 pixel), set to a 100x long distance objective (resolution in x, y = 0.16 μ m, step size in z = 0.06 μ m), housed at the Center of Natural History of the University of Hamburg. Four scans were rendered per facet of 160x160 μ m, taking care not to overlap scanning areas. The data was processed with MountainsMap Premium v. 7.4.8803 Software (DigitalSurf, Besançon, France, www.digitalsurf.com). 3D scan images were created using MountainsMap.

The supplementary material contains the descriptive statistics for all measurements (Table S1) and the results of statistical tests (Table S2), as well all data visualisation in boxplots (Fig. S2). Bivariate scatter plots are additionally given for measurements often put into context in DMTA studies: a measure of direction (*epLsar*) vs. a measure of complexity (*Asfc*), and a

measure of furrow density (*medf*) vs. a measure of furrow height (*metf*) (Fig. S3).

Statistical analysis was carried out using the R software (R Core Team, 2017, version 3.3.1) using the packages *xlsx* (Dragulescu, 2014), *rJava* (S, 2016), *doBy* (Højsgaard and Halekoh, 2016), and *R.utils* (Bengtsson, 2016). Significance was tested using a combination of three statistical tests following Calandra et al. (2012) and Schulz et al. (2013a). As DMT data is generally non-normally distributed, we used the procedure of Wilcox (Wilcox, 2012), applying a robust T1 way heteroscedastic Welch-Yuen omnibus test, coupled with a heteroscedastic pairwise “Dunnett’s T3 test”, with significances confirmed using the robust heteroscedastic rank-based test according to Cliff (pairwise comparison with bootstrap). Data were assessed, for each jaw separately, for a difference between the feeding groups, and for each feeding group separately for a difference between the jaws. The significance level was set to 0.05. Boxplots for each measurement, and selected biplots were made for data visualisation. To delineate the expected reference magnitudes, the corresponding measurements for giraffe (*Giraffa camelopardalis*), an exclusive browser, and wildebeest (*Connochaetes taurinus*), a strict ground-level grazer, taken from Schulz et al. (2013a), were included in some of the graphic results.

Results

The visual impression of the reconstructed surface images of the enamel facets, was that of increasing roughness from L<G<GR<GRS, with no evident difference between the maxillary and mandibular molars (Fig. 1).

Nevertheless, both the boxplots and the scatter plots (Fig. S2, Fig. S3) indicate that the maxillary and mandibular molars often deviate in the DMTA pattern. Using Dunnett's T3, many differences between the jaw positions were significant, although this was only once confirmed using Cliff's method (for height parameter Sz , Table S2). In particular, the visual pattern across the diets differed distinctively for some complexity ($nMotif$), density (Spd , $medf$),

and direction (Std , $Tr1R$) parameters (Fig. S2). Specifically focussing on a difference in the effect of diet GRS as compared to GR, the ranking of the diets in the upper vs. the lower jaw appeared mostly similar, but was notably different for direction parameter IsT , height parameters Sa , Spd , Sq , $medf$, peak sharpness parameter Spc , plateau size parameter Smc , and volume parameter Vvc (Fig. S2).

Plotting anisotropy against complexity ($epLsar$ vs. $Asfc$) showed some diet group distinction in the maxillary, but none in the mandibular molar; in particular, diet GR (but not GRS) led to the more complex and anisotropic patterns in the maxillary M2 (Fig. S3).

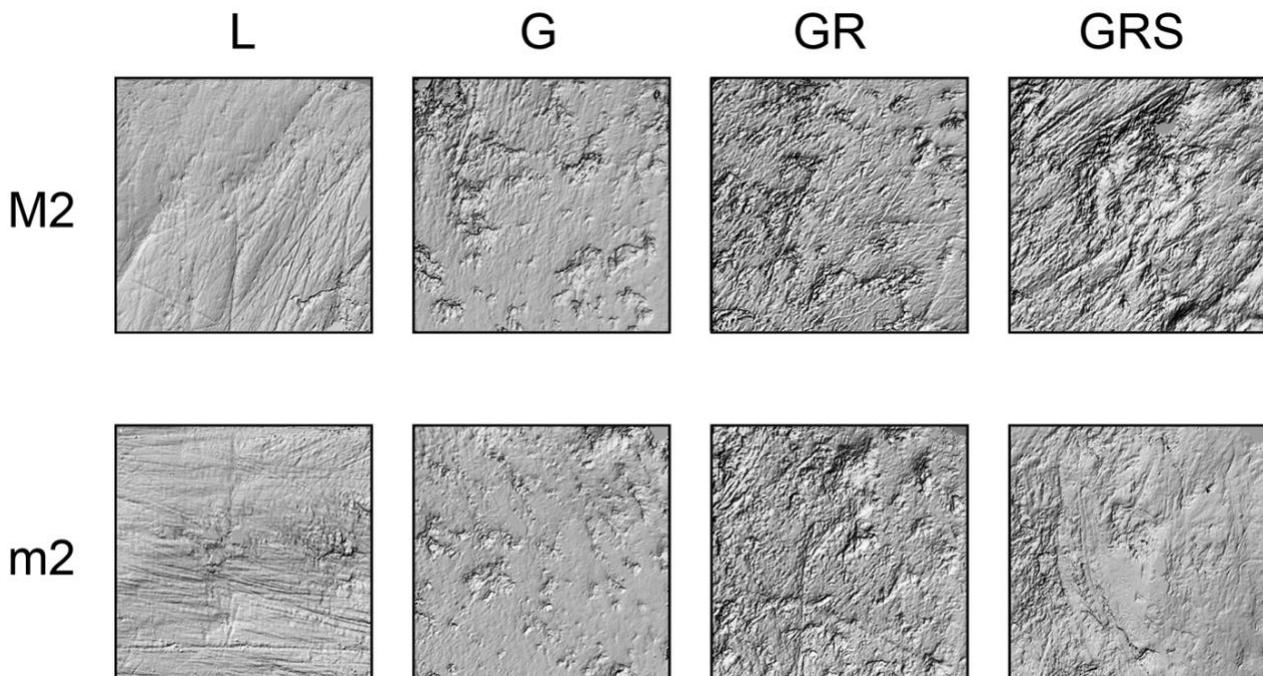


Figure 1 Microwear texture photosimulations of the enamel surface of goats (*Capra hircus*, n=28) fed diets of varying abrasiveness for 6 months. Images created using the MountainsMap software. Specimens imaged here are ZMH1161715 (Lucerne L), ZMH1116806 (Grass G), ZMH1298140 (Grass and rice husks GR), ZMH1421691 (Grass, rice husks and sand GRS), showing a facet of the maxillary M2 and antagonist facet for the mandibular m2. All images are taken with the same orientation with anterior to the top and buccal side to the left of the figure, each image represents a 160 x 160 μm facet.

Plotting density and depth of furrows against each other (*medf* vs. *metf*) showed a more pronounced distinction between diets, and even more pronounced in the lower than in the upper molar, with a decreasing density and increasing depth of furrows from L to G, GR and GRS (Fig. S3), a pattern also evident in the facet surface visualisation (Fig. 1).

Two of three area parameters showed a pattern in relation to diet, with a continuous increase from L<G<GR<GRS (*Sda*, *mea*; Fig. 2A, Fig. S2); the pattern had statistical support once by Cliff's method (*Sda*; Table S2).

Complexity parameters showed either an increase from L<G<GR, with no additional increase or even a decrease in GRS (*Sdr*, *Asfc*; Fig. S2 and S3), or a continuous decline as L>G>GR>GRS (*nMotif*; Fig. 2B). Only the latter decline achieved statistical support by Cliff's method (Table S2).

Density parameters either showed no diet effect (*Sal*, Fig. S2) or a decrease from L>G>GR>GRS in the lower molar with deviating patterns in the upper molar (*Spd*, *medf*; Fig. 2C, S2, S3), but none of these patterns had statistical support by Cliff's method (Table S2).

Direction parameters had several contrasting patterns between the maxillary and mandibular molars (*Std*, *Str*, *Tr2R*, *epLsar*; Fig. S2). Generally, G or GR showed the most

extreme values, and GRS either was similar to, or had lower values than, diet G or GR (Fig. 2D, S2, S3). This latter pattern showed one incidence of statistical support by Cliff's method (Table S2).

Most height parameters showed a similar pattern, with a systematic shift from L to G to GR, and GRS being similar as, or less pronounced than, GR (Fig. 2EF, S2, S3). As mentioned above, there were several parameters for which this sequence of diet ranking differed between the maxillary and mandibular molars, typically with the maxillary molar showing a more pronounced value for GRS. There were many cases in which the sequence of height parameters found statistical support by Cliff's method (Table S2).

Peak sharpness (*Spc*) was lowest on L, and was similar for G, GR, and GRS in the maxillary molar but increased from G to GR in the mandibular molar, with a decrease again for GRS (Fig. 2G); the pattern had statistical support by Cliff's method (Table 2).

Plateau size parameter *Smc* and slope parameter *Sdq* also showed similar patterns, including the difference between the jaws (Fig. 2HI), also with support from Cliff's method (Table S2). By contrast, plateau size parameter *Smr* did not yield any pattern (Fig. S2).

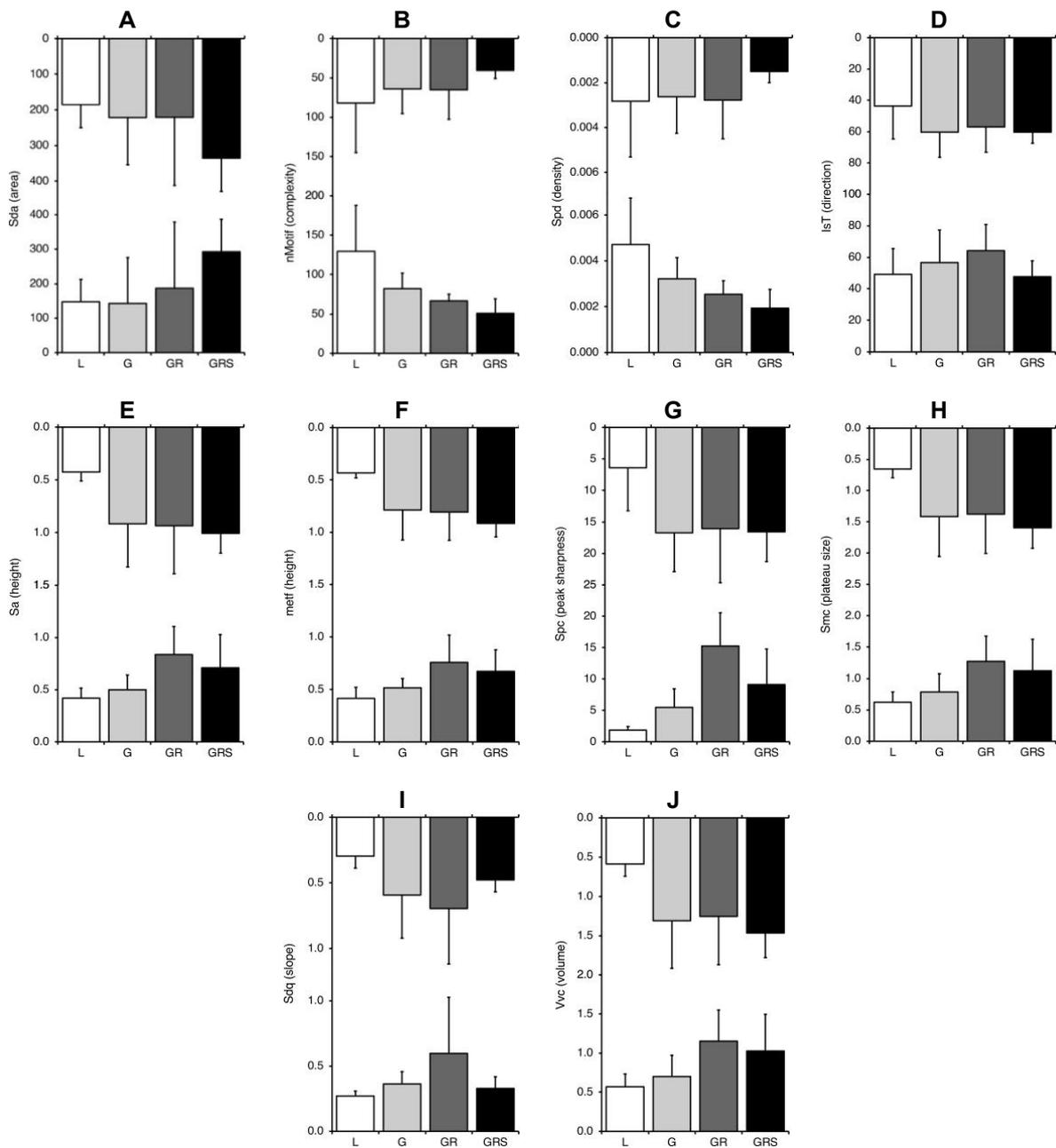


Figure 2 Selected mean (\pm SD) dental microwear texture analysis parameters of the maxillary M2 (upper columns) and the mandibular m2 (lower columns) in goats (*Capra hircus*, n=28) fed four different pelleted diets (L lucerne, G grass, GR grass and rice hulls, GRS grass, rice hulls and sand) for half a year. For statistics, see Table S2.

Volume parameters generally showed patterns similar to the height parameters, with an increase L<G<GR and GRS being similar to, or higher than, GR in the maxillary molar, and less than GR in the mandibular molar (V_m , V_{mp} , V_{mc} , V_v , V_{vc} , V_{vv} ; Fig. 2J, S2). A continuous increase L<G<Gr<GRS was only evident in S_{dv}

(Fig. S2). The patterns had statistical support by Cliff's method in several instances (Table S2).

When compared to previously reported values for giraffe and wildebeest, the current measurements in the goats appeared to span the range from a strict browser to a strict grazer feeding close to the ground (Fig. 3).

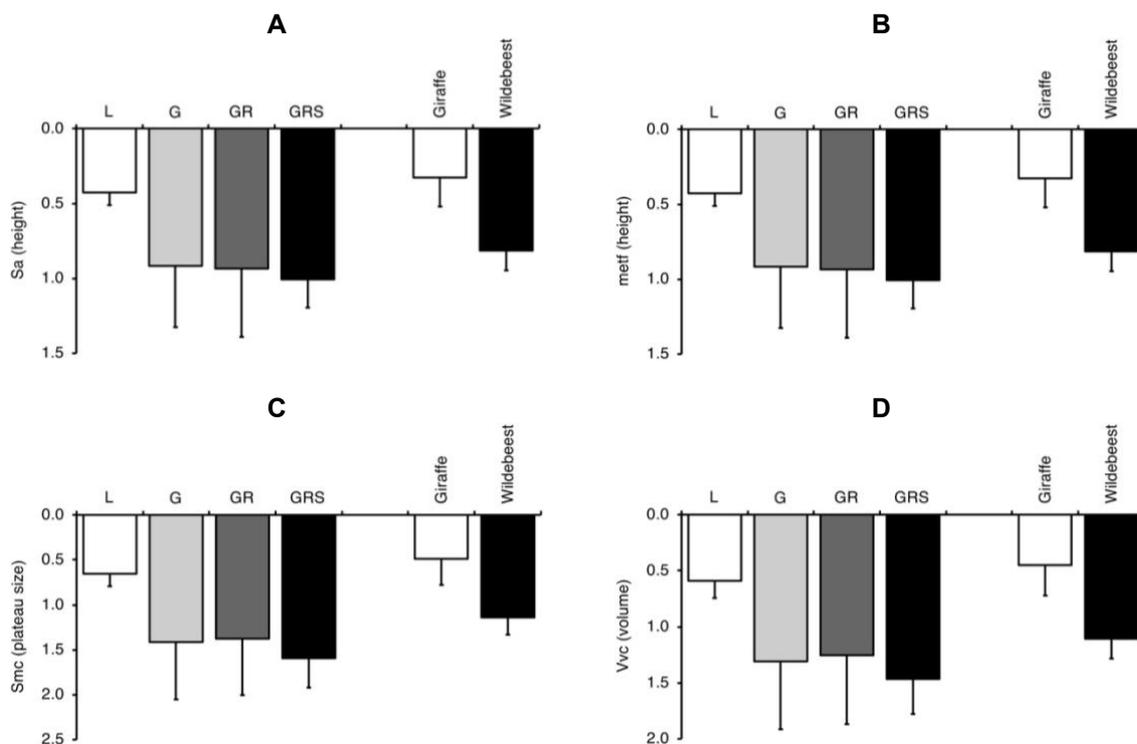


Figure 3 Selected mean (\pm SD) dental microwear texture analysis parameters of the maxillary M2 in goats (*Capra hircus*, n=28) fed four different pelleted diets (L lucerne, G grass, GR grass and rice hulls, GRS grass, rice hulls and sand) for half a year, as compared to corresponding measurements on the maxillary M2 in free-ranging giraffe (*Giraffa camelopardalis*, n=X) and wildebeest (*Connochaetes taurinus*, n=X) from Schulz et al. (2013a).

Discussion

The findings of the present study can most conveniently be summarized, as ‘everything matters’, with respect to the influence of the different experimental diets, in a twist to the title of Merceron et al.’s (2016b) experimental study in sheep that concluded that ‘dust does not matter’. In that study, about 1 % of quartz dust had been added to clover or grass silages fed to sheep for 70

days, with no significant effect of the addition on various DMTA measures (reported for mandibular molars only). In the present study, the addition of an external abrasive was not only at a much higher concentration (5% of the pelleted diet), but also contained larger abrasive particles (classifying as ‘sand’ rather than as ‘dust’). Although for some DMTA parameters, there appeared to be no additional effect of this diet (GRS) when

compared to the diet with the highest concentration of internal abrasives (phytoliths, diet GR). Visually, in some cases even a lesser effect, the GRS diet stood out for some other DMTA parameters. Additionally, a pattern showing an effect of GRS was not consistent for all measures between the maxillary and mandibular teeth, suggesting some functional differences between either jaws in relation to sand. In particular, for some height parameters, sand seemed to leave more distinct traces on the maxillary teeth (though without changing the diet ranking), possibly related to the inverted pestle-and-mortar-function, where food ‘resting’ on the mandibular teeth is moved along the maxillary antagonist tooth, with potentially a higher relative movement of food along the maxillary than the mandibular molar surface.

The GRS diet stood out for some parameters (area, complexity, peak sharpness and plateau size parameters), but less in others, such as direction, height or volume parameters. Currently, there appears to be no simple (or even complex) explanation for the variation in the effect of diets on the different parameters, in particular because of a lack of controlled studies allowing a change of only a single factor. Ideally, *in vitro* chewing systems (Hua et al., 2015a; Karne et al., 2016b) would be used to determine the effect of a different types of abrasives when concentration and chewing pressure is varied, in order to understand how the different DMTA parameters interact. To date, there is no logical method to decide whether, in general, the patterns found indicate an

additional effect of GRS as compared to GR (e.g., in *Sda*, Fig. 2A), or whether the diet with the same concentration of internal abrasives but without the sand had the most pronounced effect (e.g., in *Spc*, Fig. 2G). We are left with the conclusion that both, internal and external abrasives have an effect on DMTA in our system.

One important aspect of our understanding of tooth wear has, to our knowledge, hardly been addressed in the literature: the extent to which signals detected in 2D microwear or DMTA actually translate into macroscopic tooth wear. Macroscopic tooth wear, or quantitative tissue loss, represents a rate measurement where loss of tooth height is measured per time unit. This can be done by comparing dental measurements of animals of different ages within populations (Damuth and Janis, 2014b; Sanson et al., 2017), by measuring changes in the distance between tooth crowns and burr marks (Müller et al., 2014b; Meredith et al., 2015a; Müller et al., 2015b; Ackermans et al., in prep.-b), or by volumetric measurements of 3D-rendered dental CT scans taken from the same tooth at different time points (Karne et al., 2016b; Ackermans et al., 2019a; Ackermans et al., in prep.-b). Alternatively, macroscopic wear can be described qualitatively between species or populations, e.g. comparing dental dimensions of animals on different diets (Martin et al., 2019), or by mesowear (Fortelius and Solounias, 2000). Mesowear can also be translated into a rate when assessing individuals of different ages in a population (Rivals et al., 2007b) or assessing CT scans made in the same

individuals over time (Ackermans et al., 2018a; Ackermans et al., in prep.-a). And in particular when observing mesowear changes in cusp shape or in occlusal relief, tissue loss can be parsimoniously implied. In contrast to all these examples, 2D microwear or DMTA yield information on the traces on the tooth's surface, and if measured repeatedly within individuals, can inform on the rate at which these traces change their pattern (Teaford and Oyen, 1989a; Teaford and Lytle, 1996; Teaford et al., 2017b). However, it is not possible to deduct rates of tissue loss from these measurements so far. Some authors even suggest that some part of the DMTA patterns might be rather due to plastic deformation, rather than to tissue loss (Lucas et al., 2013c). Therefore, comparisons of actual tissue loss rates with the associated DMTA measurements would be highly interesting, and would enable to test whether a relationship between the two wear proxies can be established.

Such a test can be done qualitatively, when comparing the current DMTA data of experimental goats with data of free-ranging ruminants. Given their natural diets (data collection in Dittmann et al., 2015a), giraffe and wildebeest should span the range of diet abrasiveness in ruminants. Giraffe typically ingest phytolith-poor dicot material far from the ground, whereas wildebeest mainly ingest grass, feeding at ground level and hence on material putatively more contaminated with dust and soil. Silica levels measured in faeces of free-ranging giraffe and wildebeest corroborate this pattern (Hummel et al.,

2011a). For several of the DMTA measurements, the range covered by our experimental goats corresponds to that between giraffe and wildebeest (Fig. 3). This correspondence in DMTA is contrasted, however, by the lack of a similar range in mesowear scores achieved after half a year on the experimental diets (Ackermans et al., 2018a). These observations suggest that DMTA and mesowear may not necessarily be linked, at least within the time period of half a year.

A quantitative test for the relationship between DMTA measures and mesowear or absolute dental tissue loss is to compare the DMTA results of the present study, on an individual basis, to the changes in mesowear score and crown volume loss of the same teeth (the maxillary M2). Even if mesowear scores did not develop to a full browser-grazer difference, the directionality of the development of the mesowear score could nevertheless show some relationship with the DMTA signal. Scatter plots of the corresponding data are given in Fig. S4-S6, indicating no discernible correlation between any of the wear proxies. We do not know the extent to which the experimental diets used in the present study really represent diets consumed by animals in the wild. In addition, the lack of a coherent pattern throughout different wear proxies suggests that we cannot apply DMTA to represent mechanisms leading to mesowear and tooth volume changes, at least not without further investigation.

Such investigations may reveal counter-intuitive findings. For example, *in vitro* studies



with human enamel and silica particles found their concentration to be negatively correlated with wear (Borrero-Lopez et al., 2018). If this result is robust, then common conceptions about the dosage effect of abrasives need to be reconsidered. Additionally, animals' behavioural reactions to abrasive diets are rarely explored. For the goats of the present study, results from a preference test suggest the capacity to detect the sand in the GRS diet (Ackermans et al., 2019d). Humans have been shown to reduce chewing intensity when exposed to external abrasives (Prinz, 2004a), and a comparison of faecal particle sizes in chimpanzees (*Pan troglodytes*) between seasons with and without dust contamination, suggest a similar reaction. Therefore, one possible reason for the GRS diet showing an effect that was lower than expected as compared to GR, could be a reduced chewing intensity at ingestion on the part of the goats (cf. Dittmann et al., 2017), coupled with a washing mechanism in their forestomach that removed the sand from the digesta prior to regurgitation for rumination (Hatt et al., 2019b).

A lack of functional understanding of DMTA signals is sometimes evident in studies where DMTA results are compared to parallel diet records. Either from observations in the same population from which dental samples for DMTA were used (Stuhlträger et al., 2019), or from stomach contents of the same individuals whose teeth were submitted to DMT analysis (Merceron et al., 2010b). So far, statistical analyses have mainly focussed on whether categorical differences between groups (either experimental

units, or assumed feeding categories for museum specimens) are reflected in DMTA patterns. These analyses have tried to identify the measurements yielding the highest differentiation power (Francisco et al., 2018), or address issues of sampling such as tooth position, or jaw, for diet discrimination (Ramdarshan et al., 2017b). To our knowledge, there is a lack of studies investigating how precisely diet can be predicted based on a catalogue of existing DMTA data, using individuals for which the diet consumed during the weeks prior to DMTA is known.

In conclusion, the experimental diets of the present study triggered DMTA pattern differences resembling differences between free-ranging ruminants of different feeding types. An expected result based on intuitive assumptions on the effect of external abrasives. However, results also showed DMTA patterns that challenged that same intuition, without any correlation between DMTA patterns and measurements of absolute dental tissue loss or mesowear change. This additionally raised the question about the meaning of DMTA traces for macroscopic wear.

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Feeding selectivity for diet abrasiveness in sheep and goats

Nicole L. Ackermans, Louise F. Martin, Jürgen Hummel, Dennis W. H. Müller, Marcus Clauss,

Jean-Michel Hatt

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Feeding selectivity for diet abrasiveness in sheep and goats

Nicole L. Ackermans^{a,*}, Louise F. Martin^a, Jürgen Hummel^b, Dennis W.H. Müller^c,
Marcus Clauss^a, Jean-Michel Hatt^a

^a Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, CH-8057, Zurich, Switzerland

^b Department of Animal Sciences, Ruminant Nutrition, Georg-August-University, Göttingen, D-37075, Germany

^c Zoologischer Garten Halle GmbH, D-06114, Halle (Saale), Germany



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ABSTRACT

Tooth wear can be a considerable factor in shortening the lifespan of herbivores. It is mainly caused by the ingestion of silica particles, either phytoliths - internal to the ingested plants - or external abrasives, such as dust or grit. The avoidance of these abrasives can therefore be favourable to maintain healthy teeth, though it is not known to what extent herbivores are able to select diets based on the level of abrasive contamination. In order to better understand herbivores' selective capabilities, controlled feeding trials were performed on goats and sheep, offered a choice between high- and low-abrasive pelleted diets. Adding coarse grit resulted in the goats avoiding the diet, whereas the addition of finer dust, yet in high proportion, did not elicit any diet preference response in the sheep. Though preliminary, the experimental results provide an insight into the possible reactions of goats and sheep in response to dietary contamination by external abrasives and suggest that below a certain grain size, abrasives may no longer be detectable by these animals.

1. Introduction

One of the main factors capable of shortening herbivore's natural lifespan is excessive tooth wear (Janis and Fortelius, 1988). The principal culprits contributing to dental wear are silica particles from the herbivore's natural diet that can either be encapsulated in plant matter in the form of opaline silicates called phytoliths, or that can be ingested as external abrasives in the form of dust and grit. Grasses high in phytolith content are the principal component of grazer diets, subjecting these animals to especially high amounts of wear (reviewed by Damuth and Janis, 2014; Healy, 1967; Hummel et al., 2011). Evolutionary adaptations such as hypsodonty suggest a high value in avoiding large amounts of abrasives and therefore point out that the ability to detect abrasiveness may influence ruminant feeding behaviours (Massey et al., 2009) and serve as an intentional control for the reduction of dental wear.

Food choice experiments in voles (*Microtus orchogaster*) demonstrated an avoidance of high silica content grasses (Gali-Muhtasib et al., 1992; Massey and Hartley, 2006). The presence of abrasives was found to be tolerated in guinea pigs (*Cavia porcellus*) (Müller et al., 2015) and rabbits (*Oryctolagus cuniculus*) fed diets of varying abrasiveness (Müller et al., 2014), although other studies on rabbits (Cotterill et al., 2007)

show a reduction of preference for plants with above-average silica rates.

It has also been suggested that ruminants should avoid the ingestion of abrasive feeds (Hindelang and Peterson, 1993), though studies testing this assumption are rare. Shewemaker et al. (1989) determined dietary preference of ewes and lambs for different forage species during different seasons. Grass species preference varied depending on phenological stages, though when silicon was measured no preference was found. In a later study, Massey et al. (2009) assessed the preference of sheep by manipulating silica levels of five grass species. Again, preference was driven by grass species rather than silica levels within species.

In other demonstrations of silicate avoidance, animals have been observed to prefer removing grit by washing their food before eating it. This has been the case with primates (Allritz et al., 2013; Visalberghi and Fragaszy, 1990), suids (Ito et al., 2017; Sommer et al., 2016) and otters (Neunteufel, 2007). Even in humans, chewing intensity was reduced in the presence of external abrasives added to chewing gum (Prinz, 2004), an observation also seen in chimpanzees (*Pan troglodytes verus*) (Schulz-Kornas et al., 2019). A current representation of the detrimental effects of tooth wear is clearly visible in the livestock industry, where excessive tooth wear is at the root of considerable

* Corresponding author at: Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, 8057, Zurich, Switzerland.

E-mail address: nicole.ackermans@uzh.ch (N.L. Ackermans).

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economic loss for sheep and goat farmers. In sandy feeding pastures considered areas of high wear, ewes are often culled from flocks at an early age, being no longer able to graze efficiently due to excessively worn down teeth (Erjavac and Crossley, 2010). This occurrence was first studied by Healy and Ludwig (1965), when New Zealand sheep feeding on highly eroded pastures consumed above-average amounts of soil (up to 400 g/day), which was linked to high amounts of wear (recorded by incisor length). Extreme tooth wear can result in numerous pathologies, such as loss of teeth or premature mortality, reducing the ewes' reproductive lifespan and impacting production in sheep (McGregor, 2011) as well as in goats (McGregor and Butler, 2011). While soil erosion may be caused by high stocking rates, it can also be impacted by climate and vary with the seasons (Madden, 2014). To what extent sheep and goats actively avoid external abrasives, when given a choice, is still unknown.

We conducted a controlled feeding experiment on goats and sheep, by providing a choice of more or less abrasive feeds to observe preference selection. We predicted the animals to avoid the added-abrasives diet, implying that small ruminants are able to influence their own diet when it comes to abrasiveness selection, and thus reduce tooth wear.

2. Material and methods

2.1. Animals and husbandry conditions

The goats partaking in this dietary preference trial were part of a long-term tooth wear experiment (Ackermans et al., 2018) during which a short preference trial was performed. With approval by the Swiss Cantonal Animal Care and Use Committee Zurich (animal experiment license N°115/2009), five female goats (*Capra aegagrus hircus*) of mixed age, weight and breed (four Saanen goats and a Chamois Coloured goat; average body mass: 60 ± 8 kg, estimated age: 3–10 years, exact ages unknown) were subjected to a choice trial during which they were provided with two different pelleted feeds. Prior to the experiment, these animals had been fed a pelleted diet consisting of grass hay, rice hulls and sand (GRS), and a minimum allotment of grass hay for five months. At the time of the food preference trial, each animal was isolated in a concrete-floored pen and provided with access to two feeding boxes, one containing 500 g of the GRS diet, and one containing the same pelleted diet but without added sand, for two minutes. The amount of feed consumed was determined by weighing the boxes before and after the experiment. The experiment was repeated four times over one day for each individual.

A second experiment was performed on sheep (*Ovis aries*); also as part of a long-term tooth wear experiment during which a period was dedicated to preference trials. The animal experiments were performed with approval of the Swiss Cantonal Animal Care and Use Committee Zurich (animal experiment license N° 10/2016). Thirteen ewes of mixed age, weight and breed (average body mass: 75 ± 16 kg, estimated age: 4–10 years; exact ages unknown) were subjected to a feeding trial during which they were provided with a choice of eight different pelleted feeds (Table 1). At the time of the food preference trial, each animal was isolated in a concrete-floored feeding pen (10 m²) allowing nose-to-nose contact with the other sheep through the barrier to reduce stress. Each individual had equal access to seven buckets, containing 500 g of each diet. A timer was set off once the animal started eating and observation ensued for 10 min. The amount of feed consumed was determined by weighing the buckets before and after the experiment. The order of the food was changed between repetitions to avoid any positional preference. The experiment was repeated seven times across four months for each individual.

2.2. Experimental diets

The pellet base of the experimental diet used in the experiment on

goats was composed of grass hay and rice hulls, with or without the addition of “playground sand” (REDSUN garden products B.V., Heijen, Denmark; grain size 0–1 mm, mean particle size of 233 µm measured by sieve analysis). During diet production, the proportion of indigestible abrasives in the GRS diet was designed to be mimicked in the other diet by a similar proportion of indigestible, non-abrasive filler, in order to provide comparable levels of energy per amount of pellets (see nutritional analysis in Müller et al. (2014)). The animals had previously been maintained on the GRS diet for five months.

In the experiment performed with sheep, seven experimental pelleted diets of increasing abrasiveness levels were formulated to be isocaloric and isonitrogenic. The pellets had a base of lucerne meal, which contains no phytoliths. Diets were designed to represent a spectrum of abrasive sizes and concentrations. External abrasives were added in the form of silica dust in three different sizes: small, medium and large abrasives (SCR-Sibelco N.V., Antwerp, Belgium. Small abrasives: SIRCON® M500, mean particle size of 4 µm; medium abrasives: MICROSIL® M4, mean particle size of 50 µm; large abrasives: METTET AF100, mean particle size of 130 µm). For each diet of a different abrasive size, abrasives were added in two concentrations [4%] and [8%], resulting in seven different diets: small abrasives [4%], small abrasives [8%], medium abrasives [4%], medium abrasives [8%], large abrasives [4%], large abrasives [8%] and a control diet with no abrasives (Table 1). The two groups of sheep used in this experiment had been kept for seven months on the “control diet” and the “large abrasives [8%] diet”, respectively.

2.3. Statistical analysis

All analyses were performed in SPSS 22.0 (IBM, Armonk, NY, USA) with the significance level set to $P < 0.05$. For the goats, comparisons between individuals were performed using a paired *t*-test. For the sheep, data were expressed as proportion of the total intake per session for each diet, so as to avoid spurious effects due to differences in the total amount eaten per session, and either used with the data of all repetitions (13 animals from two groups, 7 diets, 4 repetitions = 364 data points) or with an average value per individual per diet (13 animals from two groups, 7 diets = 91 data points). As data were not normally distributed, General Linear Models (GLMs) with ranked data were performed. GLMs included the proportion of intake as the dependent variable, and either group and diet, or group, abrasive size and abrasive concentration as independent factors.

3. Results

For the goats, the paired *t*-test showed a significant difference between diets ($P = 0.017$, $t = 3.955$; $df = 4$), showing a higher consumption for the diet without added abrasives (Fig. 1).

For the sheep experiment, using data for all individual repetition experiments and diets, there was no significant difference between diets ($F = 1.571$, $P = 0.155$) or groups ($F = 0.378$, $P = 0.539$), with no significant diet * group interaction ($F = 1.738$, $P = 0.111$); similarly, there was no significant effect of abrasive size ($F = 1.086$, $P = 0.339$), abrasive concentration ($F = 0.027$, $P = 0.869$), or groups ($F = 0.056$, $P = 0.814$), but the abrasive size * abrasive concentration interaction was significant ($F = 3.931$, $P = 0.020$), indicating that differences between abrasive concentration were not the same for all abrasive sizes. Whereas intake was lower for the “small abrasives [8%]” diet, intake was higher for the “medium abrasives [8%]” and “large abrasives [8%]” diets compared to the respective [4%] diets (Fig. 2).

Using average data from the four repetition experiments for each diet, there was no significant difference between diets ($F = 1.794$, $P = 0.111$) or groups ($F = 0.005$, $P = 0.942$), with no significant diet * group interaction ($F = 1.021$, $P = 0.418$); similarly, there was no significant effect of abrasive size ($F = 0.347$, $P = 0.708$), abrasive concentration ($F = 0.234$, $P = 0.630$), or groups ($F = 0.030$, $P = 0.864$),

Table 1
Composition of different pelleted diets fed to sheep (*Ovis aries*, n = 13) during a controlled dietary choice experiment.

Ingredients	Control	[4%] Small abrasives	[8%] Small abrasives	[4%] Medium abrasives	[8%] Medium abrasives	[4%] Large abrasives	[8%] Large abrasivesAAA
Lucerne flour (%)	71.15	71.15	71.15	71.15	71.15	71.15	71.15
Quartz dust ^a (%)	–	4	8	4	8	4	8
Beetroot molasses (%)	3	3	3	3	3	3	3
Mineral premix ^b (%)	2.8	2.8	2.8	2.8	2.8	2.8	2.8
Soybean oil (%)	2	2	2	2	2	2	2
Binding solution (%)	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Monosodium phosphate (%)	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Sodium bicarbonate (%)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dry matter (% as fed)	89	89	89	89	89	89	89
Nutrient composition (g/kg DM)							
Acid detergent fibre ^c	326	303	270	290	270	293	259
Acid detergent insoluble ash ^d	12	31	52	34	66	58	100
Ash	114	150	187	157	192	156	205

^a Quartz dust was added to the diets at either [4%] or [8%], in different sizes depending on the diet: small, medium and large abrasives (mean particle size of 4 µm, 50 µm, and 130 µm respectively).
^b Cu - controlled for sheep.
^c ash corrected.
^d a measure for abrasives content.

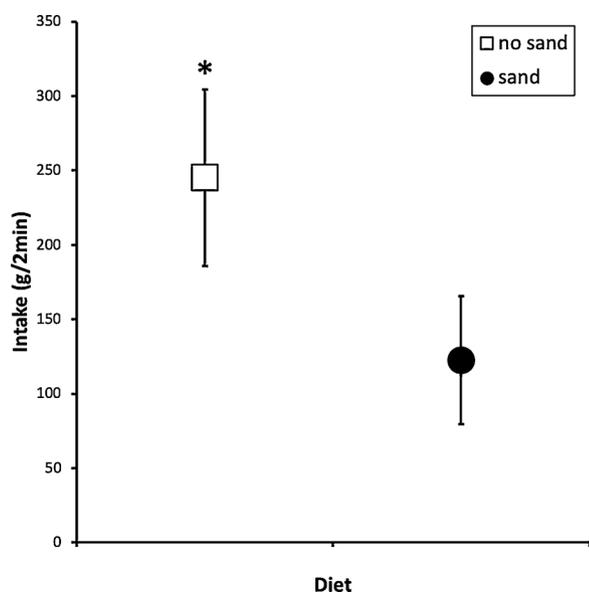


Fig. 1. Average intake of diets with and without added abrasives, fed to goats (*Capra aegagrus hircus*, n = 5) during a controlled dietary choice experiment. The animals were offered a diet containing added abrasives in the form of sand (mean size 233 µm) and a diet without added abrasives for two minutes. * Indicates significance ($P < 0.005$).

but the abrasive size * abrasive concentration interaction was again significant ($F = 5.259, P = 0.007$).

4. Discussion

Results show that the experimental goats favoured the diet without added sand. Although the goats had been accustomed to the GRS diet for months before the experiment, it is likely that the coarse, large-grained sand added to their diet created a perceptible sensation while chewing that the animals chose to avoid this diet. Anecdotally, a grinding noise was audible emanating from the masticating of the goats feeding on the GRS diet during the experiment. It should be noted that in studies where the same diets were fed to rabbits and guinea pigs, the addition of sand did not affect diet acceptance (Müller et al., 2014, 2015).

In the dietary experiment performed on sheep, results suggest no

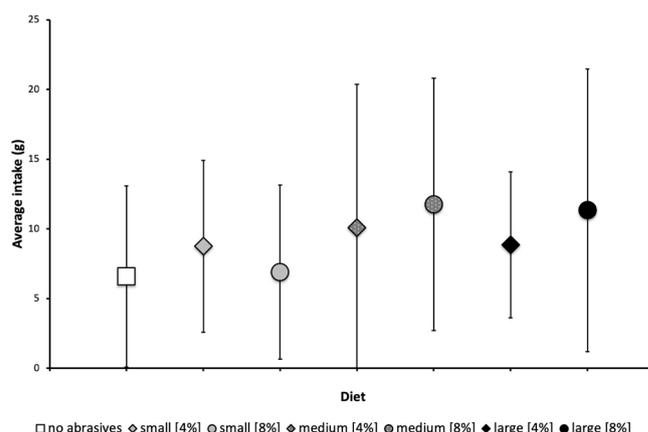


Fig. 2. Average intake of diets of varying abrasiveness, fed to sheep (*Ovis aries*, n = 13) during a controlled dietary choice experiment for ten minutes. Small, medium or large indicate the grain size of the abrasive added to the experimental diet in the form of silica dust (mean sizes: small 4 µm, medium 50 µm, and large 130 µm) and 8% or 4% indicate the concentration of added abrasives.

particular preference for any of the experimental diets. The silica particles used in this experiment were much smaller than those in the goat diets, suggesting that sheep may not be able to perceive contamination by dust during food intake. A study on sheep fed grass with manipulated silica rates (Massey et al., 2009) also showed no preference, the abrasives in question being phytoliths. The sheep studied by Healy and Ludwig (1965) had no other choice but to feed on highly eroded pastures, thereupon sustaining high amounts of tooth wear. Though ruminants display varying feeding strategies, one may question whether goats consciously avoiding grit is indicative of similar behaviour in other ruminant species and if so, to what extent ruminants can consciously avoid tooth wear when given the possibility.

Limiting factors to these experiments include the relatively small number of animals (goats n = 5, sheep n = 13) and the difference in experimental setup between studies. Although sheep and goats are not equivalent and do not represent all herbivores, we believe that the experimental results, which precluded an effect of novelty in sheep due to the repetitions the individuals were exposed to, provide insight into the different mechanisms of dietary selectiveness in regards to tooth wear. Furthermore, controlled feeding experiments are rare and an important baseline for better understanding animals' eating habits. A

better representation of natural diets could have been achieved through forage-based diets, though this would have been impractical logistically and could have introduced other sources of bias. Using pelleted diets for the present study allowed the avoidance of differences in the total amount ingested, as the pellets were designed to be isocaloric and isonitrogenic.

In these diet preference trials, abrasive size averaged 233 μm in the goat diet and ranged from 4 to 150 μm in the sheep diet. In literature, abrasive sizes vary depending on location, (Kaiser et al., 2013; Strömberg, 2006) and can range from 5 to 250 μm and higher; specifically, grasslands show a range of 5–60 μm (mean diameter of 25 μm). Dust related to the Harmattan dust storm on the West African sub-continent blows particles from the Sahara Desert into the Gulf of Guinea and further. There, fine deposits were recorded as ranging between 20 and 40 μm with particles that travel long distances being smaller than 5 μm , while coarser dust raised by local disturbances in the same location ranged between 50 and 70 μm (McTainsh et al., 1997). If one were to generalise, abrasive particles could be separated into sand (62.5–2000 μm) and dust (< 20–70 μm), the latter more readily suspended by wind (Kok et al., 2012). Concerning plant-based abrasives, grass phytoliths - like those in the experimental goat diets - range from 10 to 50 μm , while lucerne contains no phytoliths, which is why it was used in the sheep diets. In the literature however, phytoliths cover a broad spectrum of sizes and can range from 5 to 250 μm (mean diameter of 25 μm for grasslands) (Strömberg, 2004). These are only some of the examples indicating the variability of abrasive sizes depending on context and location, and the near impossibility of categorising phytoliths, dust and grit sizes from every location in the world.

At precisely what size and/or concentration can animals start to detect abrasives, and what effect on tooth wear do these different sizes have? It has been suggested that various abrasives cause drastically different types of wear, with dust causing uniform wear without affecting the mesowear signal and large grit creating pathological wear at the other extreme (Kaiser et al., 2013), though this has yet to be tested experimentally. Volcanic ash is yet another example, as it is especially abrasive and is often reported to lead to excessive tooth wear in livestock (Flueck, 2016; Wilson et al., 2011). If dust is indeed imperceptible, evolutionary adaptations distinct from behaviour may be important in limiting the effects of its abrasion.

High-crowned, or hypsodont, teeth observed in grazers have evolved as a response to increasing wear related to the ingested material (dietary or otherwise), with morphological (Damuth and Janis, 2011; Janis and Fortelius, 1988; Mendoza and Palmqvist, 2008; Williams and Kay, 2001) and possible physiological (Dittmann et al., 2017) adaptations countering the detrimental effect of these abrasives. One physiological adaptation for wear compensation was recorded in goats and African buffaloes (*Syncerus caffer*), where increasing molar wear was correlated to small amounts of increasing root growth in the tooth cementum (Ackermans et al., 2019; Sanson et al., 2017). A further example of wear avoidance is the ruminant washing mechanism, a digestive function occurring when large grit ingested with plant matter is washed off by the liquid in the rumen before the material is regurgitated for rumination, thus reducing tooth wear. A more in-depth exploration of how different categories of external abrasives affect dietary preferences, as well as determining the point at which animals may no longer make a selection based on these abrasives, are interesting prospects that still require further investigation.

5. Conclusion

In this pilot study, goats were shown to be capable of avoiding coarse grit in their diet, while sheep showed no preference for diets with no- or varying levels of finer abrasiveness. Though preliminary, these results provide insight on how these small ruminants detect external abrasives. These animals may select food motivated by the avoidance of tooth wear, but seemingly only up to a certain point at

which abrasives are no longer detectable. Undetectable contamination could prompt more detrimental tooth wear when small abrasives are present in large quantities and either go unnoticed, or cannot be avoided.

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Competing statement

The authors declare no conflicts of interest.

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**Mesowear resembles a lifelong signal in sheep (*Ovis aries*) within a
long-term feeding experiment**

Nicole L. Ackermans, Louise F. Martin, Daryl Codron, Jürgen Hummel, Patrick R. Kircher, Henning
Richter, Marcus Clauss, Jean-Michel Hatt

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Author contributions: JMH and MC designed the study, NLA, LFM, MC and JMH performed the experiment, HR and PRK supervised the CT scanning, JH supervised the nutritional analyses, TMK supervised the maceration of skulls and dental cast production, NLA performed the mesowear scoring, DC analysed the data, NLA and MC wrote the first draft of the manuscript, which then received input from all co-authors.

Mesowear resembles a lifelong signal in sheep (*Ovis aries*) within a long-term feeding experiment

Nicole L. Ackermans^{a*}, Louise F. Martina^a, Daryl Codron^b, Jürgen Hummel^c, Patrick R. Kircher^a, Henning Richter^d, Thomas M. Kaiser^e, Marcus Clauss^a, Jean-Michel Hatt^a

^a Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, CH-8057 Zurich, Switzerland

^b Department of Zoology and Entomology, University of the Free State, PO Box 339, 9300 Bloemfontein, South Africa

^c Department of Animal Sciences, Ruminant Nutrition, Georg-August University, 37073 Göttingen, Germany

^d Division of Diagnostic Imaging, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

^e Center of Natural History, University of Hamburg, Hamburg, Germany

*Corresponding author.

Email: nicole.ackermans@uzh.ch

Address: Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, 8057 Zurich

Abstract

A herbivore's diet can affect its teeth by causing different types of wear. Browsers typically have sharper, higher cusps, while grazers show lower, blunter cusps, presumably due to the more abrasive nature of their diet. On the macroscopic scale, this allows the reconstruction of herbivore diets based on the shape of the tooth's profile, using a method called mesowear. However, the duration of the dietary signal represented by mesowear has not been precisely defined in the literature. To obtain a more precise delimitation of this timeframe, sheep (*Ovis aries*, n=39) were fed pelleted diets containing external abrasives of different sizes (\varnothing 4 μ m, \varnothing 50 μ m, and \varnothing 130 μ m), and concentrations (4%, or 8%) for 17 months. Mesowear was scored on the skulls at the end of the experiment, as well as on computer-tomographic scans and on resin casts of the teeth taken at different timepoints along the experiment. These datasets were compared, and changes in score were calculated using the CT and cast data from the beginning and end of the

experiment. Overall, even though a visual trend appeared of more wear on higher abrasion diets, it was of an extremely small magnitude, and the dietary effect on the mesowear scores or the change of the mesowear scores in these animals was never significant. This leads us to conclude that, at least in small ruminants, mesowear is more of a general signal than a seasonal one, and needs to be considered as such for tooth wear-based palaeodietary reconstructions. Experiments with natural forages are required to corroborate this conclusion.

Keywords: dietary signal, ruminant, palaeodiet, abrasives, tooth wear



1. Introduction

Methods calibrated using data from extant species can be used to recreate diets of the past, and thus paleoenvironments. Dental wear has long been of interest in zoology and palaeontology to inform on a specimen's diet and ecology (Brent Jones and Desantis, 2017; Damuth and Janis, 2011; Fortelius, 1985; Janis and Fortelius, 1988; Kovalevsky, 1874). Wear patterns can be used to estimate age (Grant, 1982; Spinage, 1976), while a tooth's general shape can inform on a specimen's place in the evolutionary tree. Only since the mesowear technique was established (Fortelius and Solounias, 2000) has it been possible to rapidly and affordably define a herbivore's diet on the macroscopic scale. Today, mesowear is a series of techniques used to measure tooth wear with the naked eye, based on the profile of a herbivore's molar teeth (reviewed in Ackermans, in prep.). Using this technique, high and sharp molar cusps along with high occlusal reliefs are indicative of a browser diet, while low, blunt cusps and low reliefs are more often related to grazer-type diets.

In the literature, the exact dietary timescale represented by mesowear is unclear, and studies have claimed that it is either a weekly to monthly signal (Danowitz et al., 2016), a seasonal dietary variation (Kaiser and Schulz, 2006; Marom et al., 2018; Schulz and Kaiser, 2013), a yearly signal (Louys et al., 2012; Rivals et al., 2007; Ulbricht et al., 2015), or a general lifetime signal (Brent Jones and Desantis, 2017; Fortelius and Solounias, 2000; Merceron et al.,

2007; Yamada, 2012), though these claims were not based on experimental observations. Even within an individual's lifespan the mesowear signal itself is thought to be unstable; whereas it changed continuously with age in a brachyodont fossil (*Leptomeryx sp.*), the signal seemed to be stable in the intermediate stages of adulthood in reindeer (*Rangifer tarandus*) and fossil bison (*Bison priscus*), and these wear signals were also closely related to initial crown height (Rivals et al., 2007).

Feeding experiments cannot perfectly reproduce nature, but they remain the gold standard for assessing the robustness of proxies we use for paleodiet reconstruction. In a previous study, we performed a pilot experiment, feeding experimental diets of varying internal and external abrasiveness to goats (*Capra aegagrus hircus*) for six months (Ackermans et al., 2018), to obtain an indication of the length of the dietary signal represented by mesowear. That study was the first long-term experimental test of mesowear, and it found no significant dietary effect on tooth wear after half a year, suggesting that mesowear recorded a rather long-term signal in goats. As a follow-up to the pilot experiment, the present study presents a much larger feeding experiment, testing the effect of external abrasives of varying sizes and concentrations over 17 months on 39 sheep. Our goal was to better understand how external abrasives affect diet and how the dietary signal is created in the long term, informing the place of external abrasives in the evolution of hypsodonty.



2. Methods

2.1. Experimental animals

The animal experiments were performed with approval of the Swiss Cantonal Animal Care and Use Committee Zurich (animal experiment license N° 10/2016). Forty-nine sheep of mixed breeds (average body mass = 75 ± 16 kg) confirmed to be older than 3 years due to erupted third molar took part in this study (refer to supplemental material for additional information on breed, age and weight). The animals were kept for 17 months and group housed in partially covered outdoor enclosures on approx. 15 m² (10m² covered area with soil or sawdust) bedding and 5 m² concreted flooring) exceeding the minimum legal requirement of 12 m² for groups of 8 sheep (≥ 90 kg bodyweight) set by the Swiss Animal Welfare Ordinance. The animals were also provided with enrichment and salt licks.

All animals were subjected to a general health examination before arrival at the premises. The animals' overall health was documented daily during the experiment, and they underwent monthly weighing, hoof trimming, veterinarian examinations and were shorn in spring and autumn. During the course of the study, nine animals were euthanized due to health complications unrelated to the experiment. One animal was euthanised after showing clinical symptoms of copper overdose traced to an error in one of the experimental diets (exchanged immediately after), leaving a total of n=39 animals for the final evaluation.

2.2. Experimental diets

The composition and nutritional analysis of the experimental pelleted diets is described in detail in Ackermans et al. (2019). To design diets of increasing abrasiveness, external abrasives were added to the pelleted diets in the form of quartz silica abrasives of three different sizes (SCR-Sibelco N.V., Antwerp, Belgium). The small abrasives (SIRCON® M500) had a mean particle size of \varnothing 4 μ m, the medium abrasives (MICROSIL® M4) had a mean particle size of \varnothing 50 μ m, and the large abrasives (METTET AF100) had a mean particle size of \varnothing 130 μ m. All diets were formulated to be isocaloric and isonitrogenic, using an indigestible non-sillacious filler and soybean meal (cf. Müller et al., 2014). For each size, abrasives were added in two concentrations: [4%], and [8%], resulting in seven different diets: small abrasives (4%*s* and 8%*s*), medium abrasives (4%*m* and 8%*m*), large abrasives (4%*l* and 8%*l*), and a control diet with no abrasives (C).

Upon arrival, the animals were submitted to a two-week regimen of grass hay *ad libitum* as an adaptation period and were switched gradually to a low abrasive lucerne hay regimen (*Medicago sativa*) in the days before their first CT scan. Consequently, the herd was divided into seven groups with similar weight distribution. During a second adaptation phase, all animals were fed the C diet and lucerne hay (low phytoliths) for one week. Then, each group was assigned a pelleted diet of different



abrasiveness, fed for the remaining 17 months of the study. The daily feeding regimen was as follows: 200 g of lucerne hay per individual, distributed within hay nets; 1.2 kg (up to 2 kg during colder or stressful periods) of pellets per individual, distributed into feeding troughs, and *ad libitum* fresh water access. The enclosures, including food troughs, were cleaned daily.

Diet samples for all groups including the lucerne hay and faecal samples for all individuals were taken periodically over the course of the experiment, and samples were analysed for acid detergent insoluble ash as a measure for silica (abrasives) content (Hummel et al., 2011).

2.3. Dental imprints

Dental imprints were taken under general anaesthesia before CT scans (December 2016, April 2017, and June 2018) and additionally in October 2017. Anaesthesia was induced Ketamine 10 mg/kg bodyweight (Ketonarkon®, Streuli Pharma AG, Uznach, Switzerland) and Xylazine 0.1 mg/kg bodyweight (Xylazin Streuli, Streuli Pharma AG, Uznach, Switzerland) intramuscularly. Teeth were then gently cleaned with a toothbrush and flushed with water until visibly free of debris while. Then, two layers of dental putty (Provil® novo vinylpolysiloxane type 0 and type 3) was injected into four custom moulds, one for each molar row, and held in place on the animal's teeth for approximately 3 minutes, then labelled and left to dry. The imprints were later cast in epoxy resin (Injektionsharz EP, Reckli-Chemiewerkstoff,

Herne, Germany) at the University of Hamburg and once hardened, they were used to score mesowear, herein referred to as “dental casts”.

2.4. Computer tomography imaging

The computer tomography was performed as described by Ackermans et al. (2018). Images were acquired from a helical multislice Siemens scanner (Siemens Medical Solutions, Erlangen, Germany) housed at University of Zurich Tierspital. Throughout the experiment, parameters were kept constant: tube voltage at 120 kVp, image matrix of 512 x 512 pixels, field of view of 980 x 332 pixels, slice thickness of 0.6 mm, and B60s convolution kernel. To minimize movements artefacts anaesthesia depth was increased with Isoflurane (Attane®, Provet AG, Lyssach, Switzerland) administered in oxygen using a facemask during scanning. To reverse Xylazine, Tolazoline 2mg/kg bodyweight was slowly administered intravenously (after minimum anaesthesia time of 45minutes) and the animals closely supervised until fully recovered.

The first CT scan was used as baseline for the tooth condition at the start of the experiment, as the animals were in groups but not yet on experimental diets, and the final CT scan was performed post-mortem. The CT datasets were converted to DICOM medical imaging format and rendered into 3D surface models using Amira 5.6 (Mercury Computer Systems/3D Viz group, San Diego, CA, USA) as well as Horos v3.0.1 (Horos Project 2015) for additional visualisation.

2.5. Skull preparation

At the end of the experiment the animals were anesthetized with 0.5 mg/kg body weight xylazine (*xylazinum*, Xylazin Streuli ad us vet; Streuli Pharma AG, Uznach, Switzerland), and 5 mg/kg body weight ketamine (Ketanarkon 100 ad us vet; Streuli Pharma AG) intra-muscularly and euthanized with pentobarbital (*pentobarbitalum natricum*, Esconarkon ad us vet; Streuli Pharma AG; 100–150 mg/kg body weight) dosed to effect intravenously until cessation of the heartbeat. The skulls were scanned in CT and prepared by enzymatic maceration at the Center of Natural History, University of Hamburg, where they are permanently curated in the Mammals Collection.

2.6. Mesowear

Mesowear was scored as in Ackermans et al. (2018), on all molar and premolar teeth, using the extended method according to Taylor et al. (2016), adapted from the original (Fortelius and Solounias, 2000), and extended (Winkler and Kaiser, 2011) mesowear methods. Scoring was performed physically on the skulls and dental casts with aid of a x12 magnifying lens, as well as digitally on 3D reconstructions of the sheep's skulls from the CT data. To allow for mesowear scoring of the 3D renderings in Amira, dynamic models of the data were rendered by defining a fixed iso-surface threshold to achieve the highest bone resolution while avoiding artefacts, and view mode was set to orthographic.

All teeth and cusps were scored unless they were chipped, cracked, or otherwise damaged. Resin casts of dental moulds and 3D renderings were scored for four and three different timepoints respectively (beginning, middle, and end of the experiment), and the change in mesowear scores was calculated by subtracting the scores of the final timepoint from those of the initial timepoint. In the chosen scoring system, cusp shape (CS) was measured for all cusps, and scored as sharp (highest score), round-sharp, round, round-round, or blunt (lowest score). Occlusal relief (OR) was scored based on the height and width of the valley between the two molar cusps (or a fictitious mirrored second cusp if it was a premolar). Occlusal relief was scored as high-high (highest score), high, high-low, low, or flat-negative (lowest score). We investigated results from these separate scores and also combined CS and OR into ordinal scores for statistical analysis (using score values inversed from the continuous system in Winkler and Kaiser (2011), with 0 being the bluntest score and 17 being the sharpest). This scoring system is not species-specific and is applicable to ruminants.

Mesowear scoring was performed blinded to diet groups, and the scoring of the skulls was separate from that of the CT scans and that of the resin casts. All scoring was performed by the same investigator (N.L.A.), and the descriptive scores (e.g. high, sharp etc.) were transferred into numerical values as described above. The mesowear score changes on skulls,

CT scans or resin casts were not used to adjust scoring, but were part of this investigation. Due to technical reasons, mainly misshapen casts, not all scoring methods were applied to all cusps and reliefs.

2.7. Statistical analysis

Comparisons of mesowear scores between diet groups were made with Generalized Linear Mixed Effects Models (GLMMs), using the "glmer" function of lme4 (Bates et al., 2014), and assuming Poisson distributions for ordinal data. Jaw (mandible or maxilla) and side (left or right) were random effects. Because of the unbalanced design, fixed effects were hierarchically nested in the sequence method/date/tooth/abrasives_size/abrasives_concentration, with either OR, CSA, CSP, or the ordinal mesowear score as dependent variable. For the first set of models, patterns in CT and cast (MLD) data were evaluated separately, and correspondingly "method" was excluded as a term. In the second set of models, the change in mesowear score

from start to finish was evaluated, correspondingly "date" was excluded as a term. Similarly, models evaluating patterns from skull (SK) data did not have a "method" or "date" term. Significance (alpha-level 0.05) of fixed effects and nested terms was determined based on Likelihood ratios which approximate a chi-square distribution (package "car", Fox and Weisberg, 2018; Luke, 2017), and multiple comparisons were made with Tukey's HSD post hoc test.

3. Results

After feeding sheep experimental diets of increasing abrasiveness for 17 months, few significant differences between diets were detected by mesowear scoring, and those that were significant were generally of a very low magnitude. The results of the statistical analyses are summarised in Table 1 (for the change in mesowear score between the first and last timepoint), and Table 2 (for the last timepoint of the experiment).



Table 1. Statistical analysis of mesowear scores at the end of an experiment where sheep (*Ovis aries*, n=39) were fed experimental diets for 18 months. Mesowear was applied to the teeth, casts of the teeth, and 3D reconstructions of the teeth based off of CT scans.

Occlusal Relief - Computed Tomography							
<i>n</i>	<i>K</i>	Effect	<i>df</i>	<i>F</i>	<i>X</i> ₂	<i>p</i>	<i>post hoc</i>
2631	128	date	2	8.310	16.621	0.0002	1,2 vs. 4
		date/tooth	15	19.803	297.052	0.0000	many tooth combinations
		date/tooth/size	54	1.322	71.372	0.0568	M2 and M3 all sizes vs. c
		date/tooth/size/amount	54	1.066	57.567	0.3446	M1: 8%L vs. 4%L
Cusp Shape Anterior - Computed Tomography							
<i>n</i>	<i>K</i>	Effect	<i>df</i>	<i>F</i>	<i>X</i> ₂	<i>p</i>	<i>post hoc</i>
1352	65	date	2	1.687	3.374	0.1851	1,2 vs. 4
		date/tooth	6	16.492	98.950	0.0000	many tooth combinations
		date/tooth/size	27	1.273	34.365	0.1557	n.s.
		date/tooth/size/amount	27	0.660	17.828	0.9087	n.s.
Cusp Shape Posterior - Computed Tomography							
<i>n</i>	<i>K</i>	Effect	<i>df</i>	<i>F</i>	<i>X</i> ₂	<i>p</i>	<i>post hoc</i>
1352	65	date	2	1.687	3.374	0.1851	1 vs. 4
		date/tooth	6	16.492	98.950	0.0000	many tooth combinations
		date/tooth/size	27	1.273	34.365	0.1557	M2: m vs. c
		date/tooth/size/amount	27	0.660	17.828	0.9087	n.s.
Ordinal Score - Computed Tomography							
<i>n</i>	<i>K</i>	Effect	<i>df</i>	<i>F</i>	<i>X</i> ₂	<i>p</i>	<i>post hoc</i>
2618	128	date	2	26.644	53.285	0.0000	1,2 vs. 4
		date/tooth	15	81.914	1228.750	0.0000	many tooth combinations
		date/tooth/size	54	5.710	308.365	0.0000	p2: m vs. c
		date/tooth/size/amount	54	6.253	337.710	0.0000	n.s.
Occlusal Relief - Casts							
<i>n</i>	<i>K</i>	Effect	<i>df</i>	<i>F</i>	<i>X</i> ₂	<i>p</i>	<i>post hoc</i>
3007	170	date	3	9.077	27.111	0.0000	1,2 vs. 3, 1,2 vs. 4
		date/tooth	20	9.009	180.030	0.0000	many tooth combinations
		date/tooth/size	72	0.699	50.340	0.9755	n.s.
		date/tooth/size/amount	72	0.659	47.438	0.9888	n.s.
Cusp Shape Anterior - Casts							
<i>n</i>	<i>K</i>	Effect	<i>df</i>	<i>F</i>	<i>X</i> ₂	<i>p</i>	<i>post hoc</i>
2946	170	date	3	0.904	2.703	0.4398	n.s.
		date/tooth	20	4.533	90.644	0.0000	many tooth combinations
		date/tooth/size	72	0.976	70.255	0.5362	n.s.
		date/tooth/size/amount	72	0.751	54.081	0.9432	n.s.
Cusp Shape Posterior - Casts							
<i>n</i>	<i>K</i>	Effect	<i>df</i>	<i>F</i>	<i>X</i> ₂	<i>p</i>	<i>post hoc</i>
1475	86	date	3	0.964	2.851	0.4152	n.s.
		date/tooth	8	7.268	57.918	0.0000	M1 vs. M2, M3
		date/tooth/size	36	1.340	48.239	0.0835	M2: m vs. c
		date/tooth/size/amount	36	0.885	31.849	0.6664	n.s.

Ordinal Score - Casts							
<i>n</i>	<i>K</i>	Effect	<i>df</i>	<i>F</i>	<i>X</i> ₂	<i>p</i>	<i>post hoc</i>
2946	170	date	3	13.633	40.719	0.0000	1,2,3 vs. 4
		date/tooth	20	34.408	687.615	0.0000	many tooth combinations
		date/tooth/size	72	4.435	319.290	0.0000	M2: m vs. c
		date/tooth/size/amount	72	4.345	312.832	0.0000	n.s.

Occlusal Relief - Skull							
<i>n</i>	<i>K</i>	Effect	<i>df</i>	<i>F</i>	<i>X</i> ₂	<i>p</i>	<i>post hoc</i>
879	44	tooth	5	34.415	172.104	0.0000	many tooth combinations
		tooth/size	18	2.427	43.699	0.0006	p2: s vs. c; M2: m,l vs. c; M3: m,l vs. c, s vs. l
		tooth/size/amount	18	0.982	17.678	0.4771	n.s.

Cusp Shape Anterior - Skull							
<i>n</i>	<i>K</i>	Effect	<i>df</i>	<i>F</i>	<i>X</i> ₂	<i>p</i>	<i>post hoc</i>
877	44	tooth	5	23.169	115.884	0.0000	many tooth combinations
		tooth/size	18	1.265	22.777	0.1993	n.s.
		tooth/size/amount	18	1.034	18.624	0.4153	n.s.

Cusp Shape Posterior - Skull							
<i>n</i>	<i>K</i>	Effect	<i>df</i>	<i>F</i>	<i>X</i> ₂	<i>p</i>	<i>post hoc</i>
454	23	tooth	2	39.225	78.507	0.0000	M1 vs M2,M3; M3 vs. M2
		tooth/size	9	1.886	16.985	0.0490	M2: s,m,l vs. c
		tooth/size/amount	9	0.338	3.045	0.9625	n.s.

Ordinal Score - Skull							
<i>n</i>	<i>K</i>	Effect	<i>df</i>	<i>F</i>	<i>X</i> ₂	<i>p</i>	<i>post hoc</i>
873	44	tooth	5	152.815	764.253	0.0000	many tooth combinations
		tooth/size	18	7.703	138.675	0.0000	M2: m vs. c
		tooth/size/amount	18	7.471	134.501	0.0000	n.s.

Table 2. Statistical analysis of the change in mesowear scores on sheep teeth (*Ovis aries*, n=39) between the end and the beginning of an experiment where the animals were fed an experimental diet for 18 months. Mesowear was applied to the teeth, casts of the teeth, and 3D reconstructions of the teeth based off of CT scans.

Occlusal Relief - Computed Tomography score change							
<i>n</i>	<i>K</i>	Effect	<i>df</i>	<i>F</i>	χ^2	<i>p</i>	<i>post hoc</i>
858	44	tooth	5	0.485	2.423	0.7880	n.s.
		tooth/size	18	1.918	34.527	0.0108	n.s.
		tooth/size/amount	18	1.361	24.490	0.1396	n.s.

Cusp Shape Anterior - Computed Tomography score change							
<i>n</i>	<i>K</i>	Effect	<i>df</i>	<i>F</i>	χ^2	<i>p</i>	<i>post hoc</i>
854	44	tooth	5	3.507	17.533	0.0036	p2,p3,p4 vs. M3
		tooth/size	18	1.393	25.078	0.1228	n.s.
		tooth/size/amount	18	1.477	26.588	0.0871	M2: 8% <i>m</i> vs. 4% <i>m</i>

Cusp Shape Posterior - Computed Tomography score change							
<i>n</i>	<i>K</i>	Effect	<i>df</i>	<i>F</i>	χ^2	<i>p</i>	<i>post hoc</i>
450	23	tooth	2	0.195	0.392	0.8222	n.s.
		tooth/size	9	1.975	17.793	0.0377	n.s.
		tooth/size/amount	9	0.940	8.468	0.4878	n.s.

Ordinal Score - Computed Tomography score change							
<i>n</i>	<i>K</i>	Effect	<i>df</i>	<i>F</i>	χ^2	<i>p</i>	<i>post hoc</i>
856	44	tooth	5	7.086	35.432	0.0000	p2,p3,p4 vs. M3
		tooth/size	18	6.627	119.283	0.0000	p3: s vs. l
		tooth/size/amount	18	2.487	44.772	0.0004	n.s.

Occlusal Relief - Cast score change							
<i>n</i>	<i>K</i>	Effect	<i>df</i>	<i>F</i>	χ^2	<i>p</i>	<i>post hoc</i>
602	44	tooth	5	1.007	5.035	0.4116	n.s.
		tooth/size	18	0.676	12.165	0.8386	n.s.
		tooth/size/amount	18	1.294	23.300	0.1793	n.s.

Cusp Shape Anterior - Cast score change							
<i>n</i>	<i>K</i>	Effect	<i>df</i>	<i>F</i>	χ^2	<i>p</i>	<i>post hoc</i>
570	44	tooth	5	5.289	26.447	0.0001	p2 vs. M1,M2,M3; p3 vs. M3; p2 vs. P4
		tooth/size	18	1.409	25.355	0.1154	n.s.
		tooth/size/amount	18	0.763	13.727	0.7467	n.s.

Cusp Shape Posterior - Cast score change							
<i>n</i>	<i>K</i>	Effect	<i>df</i>	<i>F</i>	χ^2	<i>p</i>	<i>post hoc</i>
290	23	tooth	2	1.355	2.711	0.2579	n.s.
		tooth/size	9	1.985	17.868	0.0367	M2: s,l vs. m
		tooth/size/amount	9	1.034	9.305	0.4096	n.s.

Ordinal Score - Cast score change							
<i>n</i>	<i>K</i>	Effect	<i>df</i>	<i>F</i>	χ^2	<i>p</i>	<i>post hoc</i>
576	44	tooth	5	5.060	25.298	0.0001	n.s.
		tooth/size	18	4.076	73.369	0.0000	n.s.
		tooth/size/amount	18	2.264	40.747	0.0016	n.s.

Dietary silica content

As intended, the diets with larger abrasive sizes and with higher abrasive content increased in silica content (Fig. 1A). The faecal ADIA levels indicated a similar pattern as the dietary samples, albeit, due to the disappearance of digestible components, at generally higher levels (Fig. 1B).

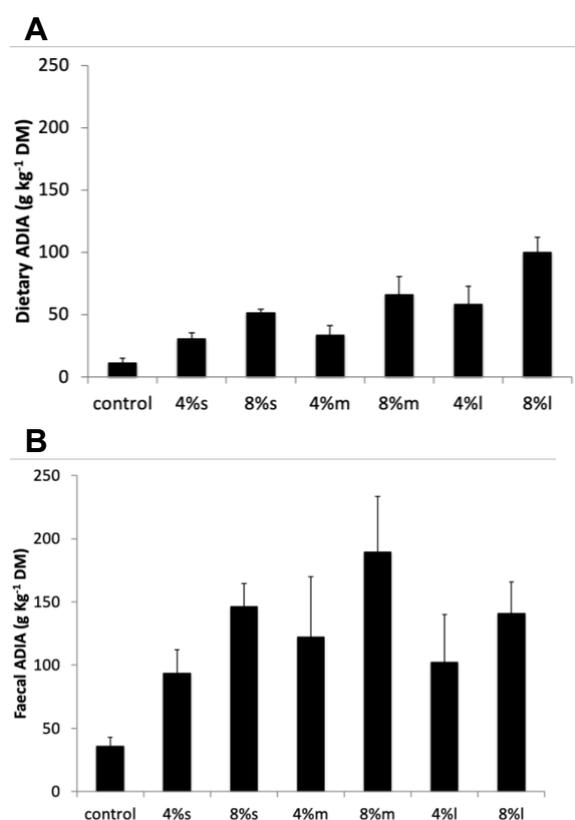


Figure 1: Silica content of the different diets (A) and faecal samples (B), measured by Acid Detergent Insoluble Ash (ADIA), for sheep (*Ovis aries*, n=39) fed diets of varying abrasiveness for 17 months.

Means \pm s.d. The experimental diets include a control diet without added abrasives, two diets containing small abrasives (\varnothing 4 μ m) at two different concentrations 4%_s and 8%_s, and the same with both the medium and large abrasives (\varnothing 50 μ m, and 130 μ m).

3.2. Absolute mesowear scores

Mesowear scored on CT scans indicated a significant difference of the final vs. initial

scans (Table 2). At the initial CT scan, no systematic difference between the groups was evident (Fig. 2). The scores from the final timepoint appear to indicate an effect on OR, with a trend of wear being caused mostly by concentration and not size of abrasives – though it is a non-significant, visual pattern (Fig. 3). There was a significant effect on cusp shape for both the anterior and posterior cusps (CSA and CSP), but without any specific diet differences at post hoc testing (Table 1). When OR and CS were combined into an ordinal score, effects again emerged for size and concentrations of abrasives, though there were no significant differences in any post hoc test. For OR, CS, and the ordinal score, the only significant effects that appeared in post hoc testing were differences between individual tooth positions (Table 1).

The mesowear data from the dental casts also indicated a significant difference of the final vs. the initial measurements (Table 1). Using the casts, no diet effects on OR, CSA or CSP were detected (Table 1). For the ordinal score, both size and concentration of abrasives showed significant effects, but the only significant difference at post hoc testing was in the M2 between the medium-sized abrasives and the control (Table 1). Similarly to the CT data described above, most significant post hoc effects were only seen between individual tooth positions (Table 1).

The average scores for OR and CS at the end of the experiment, as read directly from the

skulls, suggested more wear on the silica-containing diets than on the control diet, with an effect of abrasive concentration but less so of abrasive size (Fig. 3). However, the wear difference seen on the skulls was of a magnitude of 0.5 for OR (corresponding to “low-low” vs. slightly higher) and 0.25 for CS (corresponding to “round” vs. slightly sharper). Statistical analyses revealed a significant effect of abrasive size on OR and CSP (but not CSA). Post hoc differences were generally between the control and the treatment diets (Table 1). For the ordinal score, both size and concentration of abrasives showed significant

effects, but the only significant difference at post hoc testing was again in the M2 between the medium-sized abrasives and the control (Table 1).

When comparing those scores for which all three supports (skull, CT, cast) had been available for the same structure, it was evident that for OR, CT scores were more similar to skull-based scores than scores made from casts (Fig. 4A). By contrast, cusp sharpness was more similar between skull-based and cast-based scores than CT scores for the sharp-sharp and the round categories (Fig. 4BC).

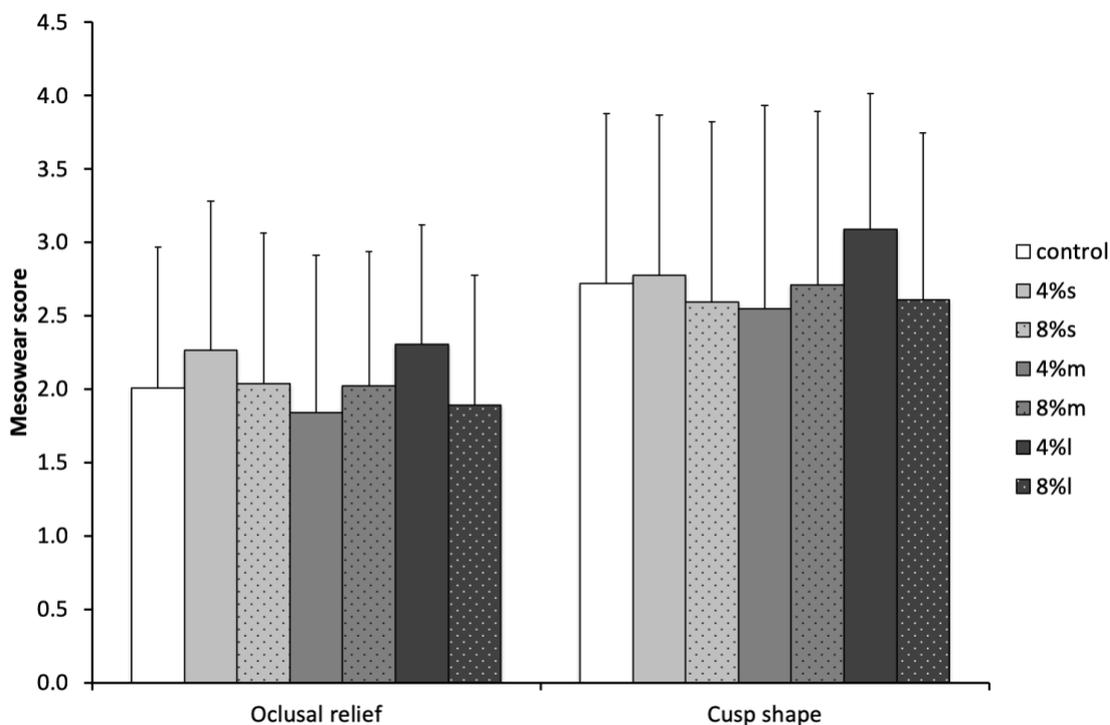


Figure 2. Initial tooth wear state of experimental sheep (*Ovis aries* n=39) scored with mesowear on CT scans before the beginning of a 17-month feeding experiment. A higher score indicates less tooth wear. The experimental diets include a control diet without added abrasives, two diets containing small abrasives (\varnothing 4 μ m) at two different concentrations 4%s and 8%s, and the same with both the medium and large abrasives (\varnothing 50 μ m, and 130 μ m).

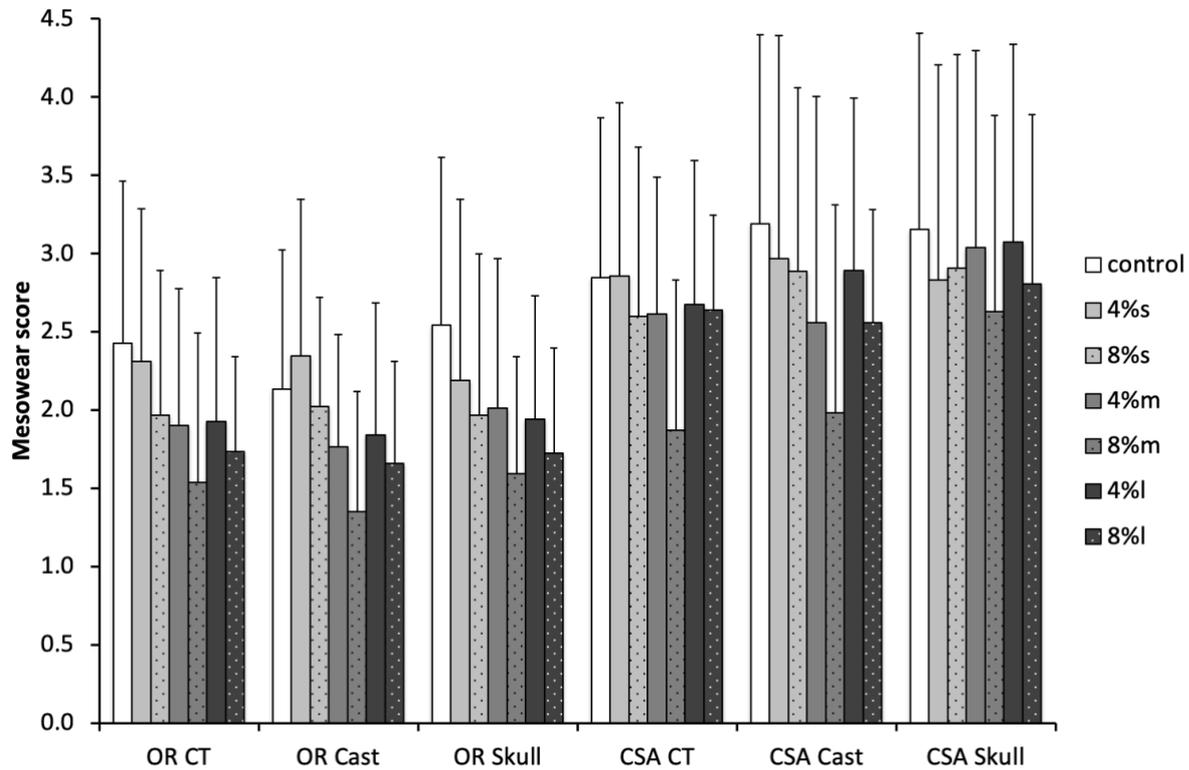


Figure 3. Mesowear scored on molar teeth of sheep (*Ovis aries*, n=39) fed diets of varying abrasiveness for 17 months, represented by three different supports: CT scans, resin casts and the physical skulls. OR represents the occlusal relief of the mesowear score, CS represents cusp shape. A higher score indicates less tooth wear. The experimental diets include a control diet without added abrasives, two diets containing small abrasives (\varnothing 4 μ m) at two different concentrations 4%s and 8%s, and the same with both the medium and large abrasives (\varnothing 50 μ m, and 130 μ m).

3.3. Mesowear score change over the course of the experiment

The magnitude of the change in mesowear score during the experiment was obtained for CT as well as cast data. CT scan data showed a gradient of low abrasion diets with lower changes in scores, representing low abrasion, and more changes for the high-abrasion diets, (except for the 4%l diet) (Fig. 5). The average change in OR and CS scores were of a magnitude of -0.1 in OR and -0.2 in CS for the control diet, suggesting a minute increase in

relief height and sharpness, and up to 0.95 and 0.7 for the abrasive diets, suggesting a decrease in relief and sharpness by less than one mesowear score step. Significant effects for the size of abrasives were indicated for the CT for changes in OR and in CSP, as well as in the ordinal score. Significant effects for the concentration of abrasives were only found for the change in ordinal score. Post hoc testes, however, showed no systematic differences (Table 2). The situation was similar for cast data (Table 2).

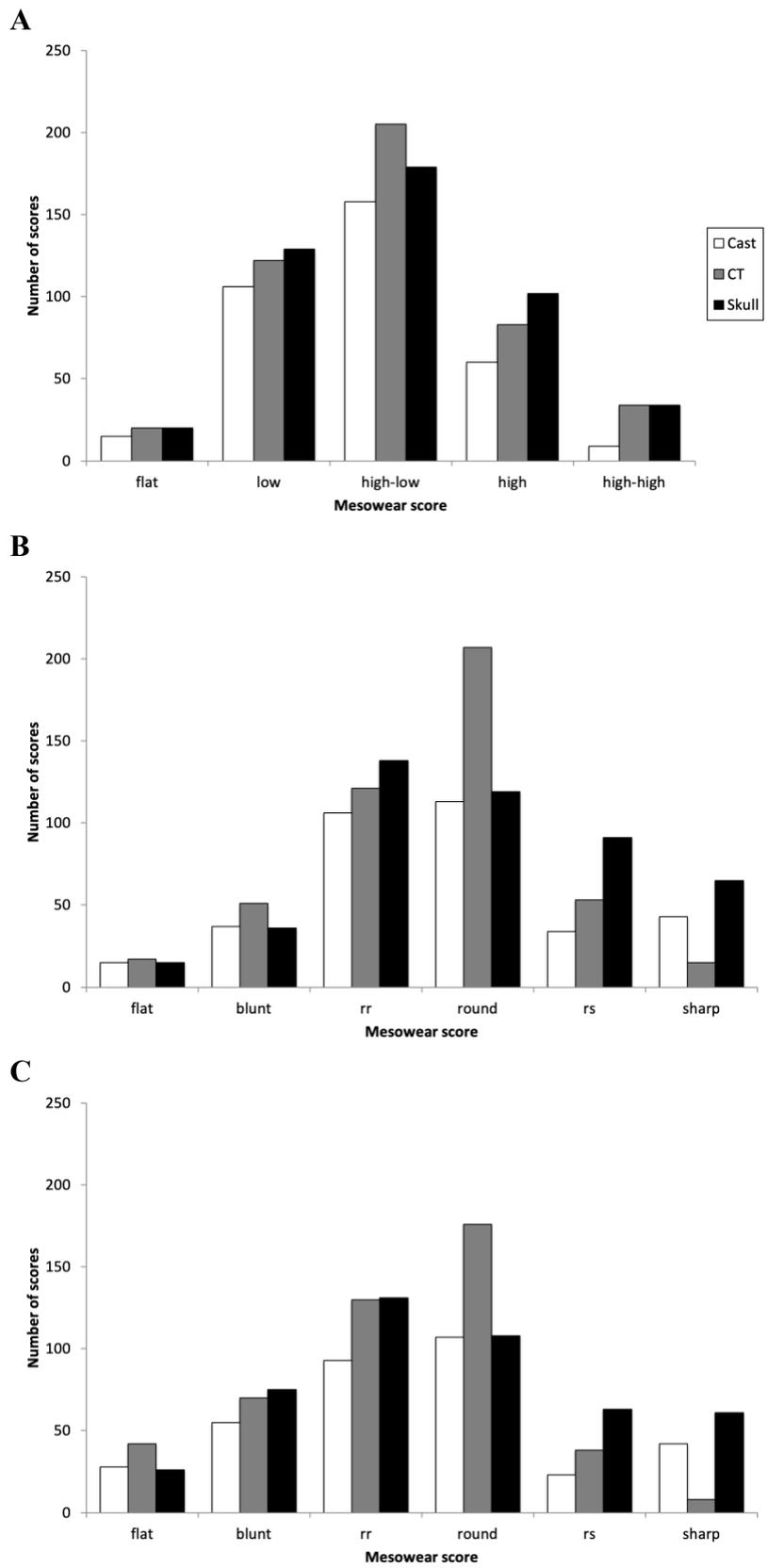


Figure 4. Distribution of mesowear scores from different supports, scored on molar teeth of sheep (*Ovis aries*, n=39) fed diets of varying abrasiveness for 17 months. The supports scored were resin casts of the teeth, computed tomographic scans of the teeth (CT), and the teeth themselves within the skull, for (A) the occlusal relief of the mesowear score, (B) the cusp shape of the anterior molar cusp, and (C) the cusp shape of the posterior molar cusp

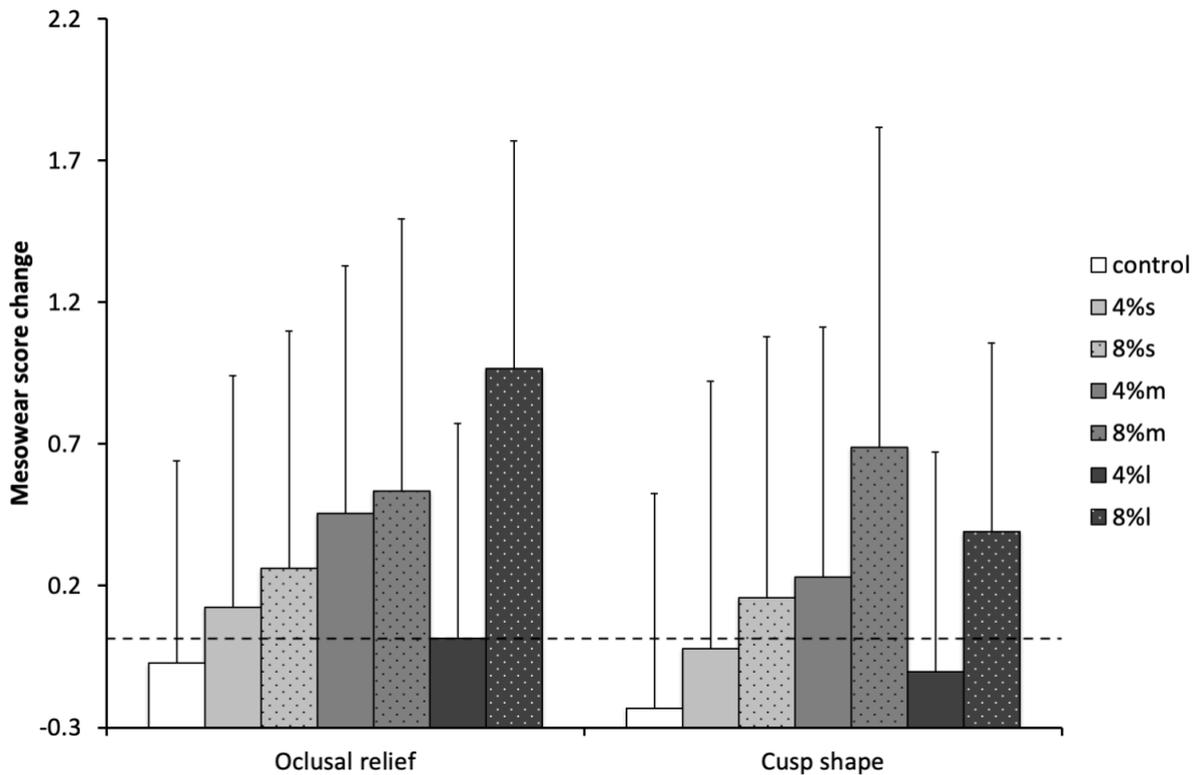


Figure 5. Average change in occlusal relief and cusp shape scores taken on molar teeth, between the start to the end of the experiment determined, from CT scans of sheep (*Ovis aries*, n=39) fed diets of varying abrasiveness for 17 months. A higher change in score indicates more tooth wear, dotted line represents the zero mark. The experimental diets include a control diet without added abrasives, two diets containing small abrasives (\varnothing 4 μ m) at two different concentrations 4%s and 8%s, and the same with both the medium and large abrasives (\varnothing 50 μ m, and 130 μ m).

4. Discussion

Overall, the change in mesowear score over time was statistically significant, though not to the point that it allowed to distinguish between diets. When there were statistically significant differences, most appeared between teeth within a single individual, and the general concept of more and easily detectable abrasion on diets with higher abrasiveness in terms of both concentration and size of silica particles was not supported in our sample. Given the long feeding period of the present sheep experiment, these findings not only corroborate the conclusions derived from the goat pilot study – that the mesowear changes very slowly under our experimental conditions – but extend this

conclusion substantially from an experimental period of half a year, to one three times as long. The sheep did show more change in mesowear score than the goats, though it was minute (a magnitude of 0.5 for the goats, up to 1 for the sheep, which would represent a shift from “sharp” to “round-sharp”, for example).

4.1. Limitations of the study

Pelleted diets were used in this experiment as a means to efficiently convey the added abrasives in each diet. Using natural forages would have been a more realistic approach, but the logistics involved of this type of feeding over the long-term would have introduced additional variation to the study and

substantially increased the daily workload during the experiment. The present study is not directly comparable with its pilot because of the difference in diets used as well as the difference in model species; nevertheless, both studies show the same overall pattern. Note that the main reason for using different species was a logistical one, as it was not possible to acquire the same number of goats of the required sex, age and reproductive status (non-pregnant) in Switzerland at the time of the present experiment.

The high variability present in our results and the lack of statistical significance are most likely related to variation between individuals, as not all specimens were of the same age, weight, or breed (Fig. 6). It would have been ideal to achieve a homogenous study population in this respect. Given agricultural practices in Switzerland, this would only have been feasible by acquiring lambs of the same season from a single breeder and raising them to the age where all permanent teeth are considered erupted. Though desirable, this was beyond our means. Another limitation may be the number of individuals within the study. Though our sample was large by experimental standards, Fortelius and Solounias (2000) recommend a minimum of $n=10$ individuals per group for a sound mesowear signal. Such a large number of individuals, however, was considered logistically unfeasible for our study.

4.2. Mesowear considerations

Intraspecific mesowear variation remains largely unexplored in its quantitative aspect. In Fig. 6, data reported from the literature shows mesowear score distribution in caprids to be relatively variable within a study, including in the present study, but also in those from the literature where more than a single value per species is given. Although the plots for the pilot goat study (Ackermans et al., 2018, Fig. 6A) and the present sheep study (Fig. 6B) show, with respect to the distribution of sharp and round cusps, a slight staggering of percentages as predicted by study design, the general pattern of distribution of blunt, round and sharp cusps rather indicates stability across diets (especially given the long experimentation period). *Capra ibex*, *Ovis canadensis* (Fortelius and Solounias, 2000, Fig. 6C); Fig. 6C), or *Ovis ammon antiqua* (Rivals et al., 2008, Fig. 6D) differed distinctively from the experimental goats and sheep, either due to a much higher proportion, or much lower proportion to absence of blunt cusps. By contrast, the score distribution of four experimental grazing goats (Solounias et al., 2014, Fig. 6E) or archaeological caprids (Marom et al., 2018, Fig. 6F location 2) indicate a similar pattern as that of our experimental animals. The findings of our experimental studies make it difficult to ascribe the difference between these literature datasets with certainty to differences in diets between the populations. A broader dataset would be required to further investigate



intraspecific variation in mesowear scores beyond the influence of age (Rivals et al., 2007).

The mesowear signal is not as straightforward as one could wish. The signal was found not to be stable over an entire animal's lifetime (Rivals et al., 2007) and can give different results for a juvenile and senile specimen, depending on the species' initial crown height. When excluding these overly young and old individuals, the change in mesowear score observed over time is less than an order of magnitude (from approx. 0.3 to 0.6) for a large assemblage of wild reindeer from the same study, suggesting stability in this age group. If one were to take a wild adult browser from its natural habitat and provide it with highly abrasive food in captivity, the logical expectation would be for its teeth to become progressively blunter. Though the present study intended to replicate this scenario, the results do not fulfil this logical expectation. Studies comparing the dentition of wild ruminants to their captive conspecifics do indeed find strong differences in mesowear between the two settings (Clauss et al., 2007; Kaiser et al., 2009), possibly related to non-natural feeding practices in captivity. This indicates that within a species, macroscopic tooth shape has the possibility to change from sharp to blunt or vice versa during a lifetime. However, such a large change in mesowear has yet to be observed in an experimental setting. This puts into question whether the experimental diets used in the present study were abrasive enough to cause the

expected amount of wear, or whether mesowear changes can be triggered by artificial diets to the same extent as natural diets, at least during a reasonable time period. We also question whether factors other than the addition of abrasives are more relevant for mesowear, such as internal abrasives that cannot be washed off and will have an effect during rumination (Hatt et al., 2019), or the physical structure of forages. This would correspond to predictions made by Kaiser et al. (2013), that external abrasives (as connotated by habitat) have a lesser effect on mesowear than internal abrasives, in an interspecific comparison. However, in our goat pilot study, the effect of diets with increased internal abrasives was similarly small (Ackermans et al. 2018).

Another question arising from the present study is whether any mesowear effects would be stronger at an earlier stage in life. With respect to a certain age during which mesodont or hypsodont teeth are more susceptible to a certain mesowear signal, it would be interesting to follow a similar feeding study where the development of tooth wear could be measured from eruption to several years of wear by repeated CT imaging.

4.3. Method comparisons

In graphical representations (Fig. 3), scoring mesowear on the skull, casts or CT data does not appear to differ in terms of average mesowear score (for the last timepoint only), only a slight blunting of cusp shape is visible



when scoring on the CT scans. Among the different methods, the casts show overall less significance than CTs, which themselves have a blunting effect due to the resolution of the medical CT scanner, an effect also noted in the pilot study (Ackermans et al., 2018). The CTs in turn show less significance in comparison to the skull scores, visible when the distribution of mesowear scores is compared between techniques (Fig. 4). The lower accuracy of the casts is probably due to dental casts being formed for the most part on live animals with a limited mouth gape and while under general anaesthesia

still performed chewing movements due to strong stimulus from oral manipulation, resulting in imperfect moulding. Though resin casts may be a more cost-effective alternative to CT scanning, they present their own abundance of logistical difficulties. Technique aside, it is important to have a baseline of tooth state before beginning a feeding experiment, as individual tooth morphologies can affect the final results (Rivals et al., 2007). Given our experiences, the CT method recommends itself for future long-term studies.

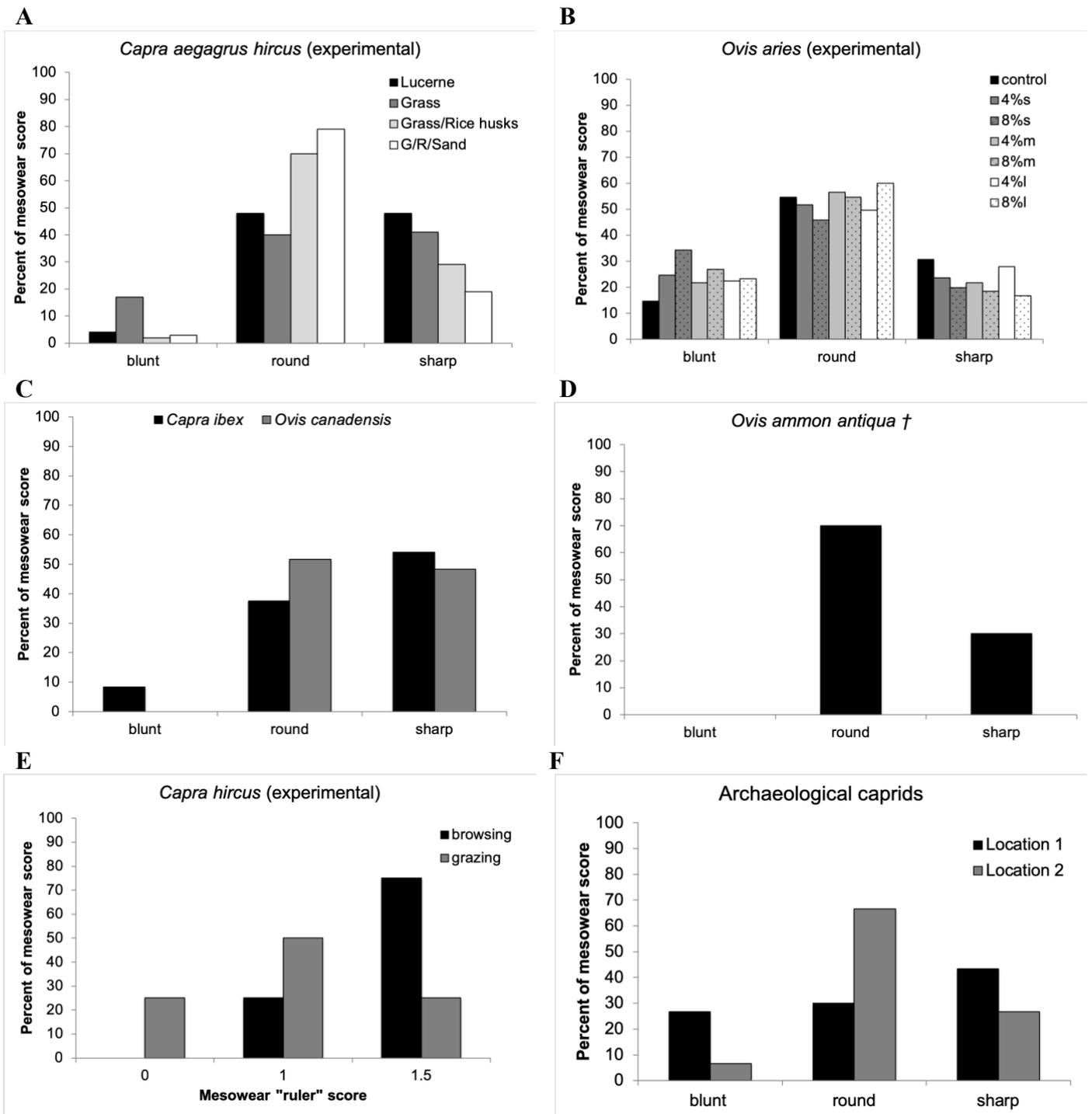


Figure 6. Distribution of mesowear scores in various caprid mesowear studies.

A and B were converted to conservative mesowear scoring for visualisation. A: Ackermans et al. (2018b) (7 per group, total n=28); B: Present study (control n=7, 4%*s* n=4, 8%*s* n=5, 4%*m* n=7, 8%*m* n=5, 4%*l* n=6, 8%*l* n=5, total n=39); C: Fortelius and Solounias (2000) (*C. ibex* n=24, *O. canadensis* n=34); D: (Rivals et al., 2008) (n=17); E: Solounias et al. (2014) (browsing n=4, grazing n=4), scored with the mesowear "ruler" technique (Mihlbachler et al., 2011); F: Marom et al. (2018) (Location 1 n teeth=30, location 2 n teeth=15).

4.4. Dietary effects

Changes in mesowear appear in the expected direction, towards a blunting of the teeth; however, this trend was not significant. Occlusal relief and cusp shape scored on the skulls showed lower mesowear scores (more wear) than the control diet (Fig. 2), though the main concern is that for all measurements, the order of magnitude is very small. This was also the case in the pilot experiment (Ackermans et al., 2018), though the teeth were overall more worn in the sheep (approximate score change of one order of magnitude over 17 months) than in the goats (approximate score change of half an order of magnitude over 6 months). One would expect mesowear score changes in cusp shape of around two orders of magnitude, when related to a long-term ingestion of abrasives, representing a change from sharp to round cusps.

Given the lack of significant differences in the present study, some remarks on the visual patterns are given here, under the assumption that they are meaningful, and that we would have been able to achieve statistical significance with a larger number of animals. These considerations should be considered hypothetical, due to the absence of statistical corroboration. In the present study, we remarked in the case of the very small abrasives that they are either washed off more intensely than larger abrasives and therefore affect the teeth less, or that the abrasive particles are so small that they do not affect the teeth very much, which has been previously hypothesised, in that dust maintains the

mesowear profile while wearing down the teeth uniformly (Kaiser et al., 2013). Based on our parallel study of the ruminant washing mechanism (Hatt et al., 2019), it appears more likely that small particles are those that adhere best to the ingesta when submitted the ruminant's washing mechanism, and thus the more likely conclusion is that they do not affect mesowear as much as larger abrasives.

The yearly intake of external abrasives per sheep ranged from approximately 0 to 44 kg/year during our experiment. In our pilot study, goats ingested approximately 52 kg/year on a diet enriched with sand, which had no additional effect on mesowear. In a study on wild buffalo, Sanson et al. (2017) estimated an external abrasive load of 10-28 kg/year (in addition to a phytolith intake of 300-400 kg/year), without any notable differences in wear between regions differing in both components, and concluded that silica abrasives intake may not affect tooth wear as importantly as previously thought. Our results appear to support this conclusion for smaller ruminants, which would make new explanations necessary for the development of absolute tooth wear and mesowear in ruminants. Structural properties of the diets, and the corresponding chewing movements and forces, appear logical next candidates. Again, this calls for experimental approaches using whole forages.

In contrast, rabbits and guinea pigs consuming the same diet as the goats, (Müller et al., 2014; Müller et al., 2015), showed a clear impact of both internal and external abrasives on



tooth length, and in an in vitro system, horse teeth lost more volume on the diets with more abrasives (Karme et al., 2016). Most likely, ruminants and non-ruminants experience dental wear differently, with external abrasives being washed off the ingesta in ruminants prior to the most important chewing process, i.e. rumination (Hatt et al., Submitted). However, this would not explain the lack of a clear effect of internal abrasives, as also suggested by (Sanson et al., 2017). Very recently, an in vitro study with human enamel yielded the counter-intuitive result that higher concentrations of abrasive particles led to less rather than more enamel wear (Borrero-Lopez et al., 2018). It appears that the experimental investigation of dietary properties other than abrasive content on tooth wear is a very important next step in the understanding of tooth wear processes.

5. Conclusion

In sum, experimental diets did not create significantly different mesowear signals between groups of sheep consuming small, medium or large abrasive diets at low or high concentrations, after 17 months. Even though a pattern of higher abrasion causing more wear appears visible on graphical presentations, the magnitude is extremely small, and no statistical differences were found between diets. If one were to make a crude projection based on the present study and its pilot, it would take around eight years of abrasive consumption to see the expected change in mesowear score of two orders of magnitude in

these animals, if abrasives are indeed the main determinant of mesowear. Based on these experiments, the mesowear signal either appears to take a substantial amount of time to form, and may well represent more of a general- or lifetime signal, at least for small ruminants; or mesowear is not affected by abrasive content, and different properties of the diets that remain to be identified are responsible for mesowear formation. These findings need to be taken into account when reconstructing palaeodiets based on tooth wear, especially for herbivores of a similar size.

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Conflict of interest

The authors declare no conflict of interest.

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M4

**Absolute wear and digital measurements sheep (*Ovis aries*)
teeth within a controlled long-term feeding experiment**

Nicole L. Ackermans, Louise F. Martin, Patrick R. Kircher, Daryl Codron, Marcus Clauss,

Jean-Michel Hatt

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Author contributions: JMH and MC designed the study, NLA, LFM, MC and JMH performed the experiment, PRK supervised the CT scanning, NLA performed the volumetric and calliper measurements, DC analysed the data, NLA and MC wrote the first draft of the manuscript, which then received input from all co-authors.



Absolute wear and digital measurements on sheep teeth (*Ovis aries*) within a controlled, long-term feeding experiment

Nicole L. Ackermans^{a*}, Louise F. Martina^a, Daryl Codron^b, Partick R. Kircher^c, Henning Richter^c, Marcus Claussa, Jean-Michel Hatt^a

^a Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland.

^b Department of Zoology and Entomology, University of the Free State, PO Box 339, 9300 Bloemfontein, South Africa

^c Division of Diagnostic Imaging, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland.

*Corresponding author.

Email: nicole.ackermans@uzh.ch

Address: Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, 8057 Zurich

Abstract

Diet can affect many factors of an animal's anatomy, but teeth are a specific focus of dietary research, as their durability lends them to record information on a large variety of scales. Abrasive diets like those of grazing herbivores are known to wear down teeth, but how that wear affects tooth growth and the relations between its different morphological components, are rarely investigated. Eight pelleted diets varying in abrasive size and concentration were fed over a 17-month period to 49 sheep (*Ovis aries*), of which n=39 qualified for morphology measurements. Using computed tomography, scans of the skulls were made over the course of the experiment, and the impact of diet-related wear was observed on tooth volume and morphology, including the position of dental burr marks, over time. Digital calliper measurements were applied to 3D renderings of the teeth, and the crown and root volumes were rendered and investigated separately. Most burr marks wore off with the crown

material over time, reducing the number of observations. Crown volume loss was correlated to root volume gain, in the range of a similar pilot experiment on goats. Height and burr mark measurements indicated a much higher experimental tooth wear than that previously reported for free-ranging animals, the reason for which is unclear. There was no relationship between tooth height and dentin basin depth. In general, for all parameters, there was no effect of diet; hence, while the measurements corroborate general understanding of tooth wear and compensatory processes, these methods appear not suitable to assess subtle differences between feeding regimes.

Key words: dietary signal, ruminant, tooth volume, 3D imaging, tooth wear



Introduction

Mammalian teeth are mainly composed of layers of mineralised tissue. The inner pulp provides innervation and vascularisation (Ungar, 2010), and is covered by a thick layer of dentin, itself covered by a hard enamel surface in the crown and by cementum in the roots. Cementum is an avascular mineralised tissue belonging to the periodontium, or dental attachment apparatus, and mainly serves to anchor the periodontal ligament to the root surface (Gonçalves et al., 2005). Cementum layers are deposited continuously throughout life, and have been used to determine an animal's age based on the number of cementum rings on the roots of its teeth (Klevezal, 1996), as they are not subject to reabsorption. Klevezal (1996) also notes that the volume of these cemental layers are determined by the amount of mechanic stress to which the tooth is subjected, as some of the thickest layers are seen, for example, in beaver (*Castor fiber* and *C. Canadensis*). In an experiment on goats (*Capra aegagrus hircus*), harder foods impacted cementum production, microscopically translated by a reorganisation of the Sharpey's fibres in the cementum microstructure, as a response to increasing tensile forces (Lieberman, 1993). This is indicative of a feedback mechanism compensating for tooth wear, which has been discussed in the literature but has yet to be explored in detail. Cementum growth compensating for tooth wear has been

hypothesised (Attwell, 1980; Klevezal, 1996; Renvoisé and Michon, 2014), and shown experimentally in our pilot study on goats (Ackermans et al., 2019b). During this pilot study, volumetric measurements of molar teeth showed a small but consistent gain in root volume when the crown was worn down over time, and the highest wear, caused by high-abrasion diets, was correlated to the highest amount of root growth.

As a follow-up of the goat pilot study, the present study consists in a larger feeding experiment, lasting three times as long, in which sheep (*Ovis aries*) were fed pelleted diets of different abrasive concentration and size. The aim of the present study was to test whether a similar signal of root growth compensating for wear could be detected in the sheep, and if this mechanism would be affected by a variety of abrasives. In addition to the methods used in the goat pilot study, we compared digital calliper measurements on 3D renderings of the teeth from the beginning and end of the experiment. We also evaluated the use of burr marks applied to the sheep's molars as a tool to measure changes in the wear and growth of the teeth. Burr marks have been used successfully in determining wear and growth rates in hypselodont teeth (Müller et al., 2014a; Meredith et al., 2015b; Müller et al., 2015a), but to our knowledge, have never been applied in non-hypselodont molars.



Material and methods

Experimental animals and diets

The animal experiment on which the present study is based is the same as in (Ackermans et al., 2019a; Ackermans et al., in prep.-a). The experiment was performed with approval of the Swiss Cantonal Animal Care and Use Committee Zurich (animal experiment license N° 10/2016). Forty-eight ewes and one wether (*Ovis aries*, n=49, wether: ZMH109537) of mixed breeds (Braunköpfiges Fleischschaf, Dorper, Ile-de-France Suisse, Oxford, Schwarzbraunes Bergschaf, Skudde, Spiegelschaf, Texel, and Weissen Alpenschaf; average body mass = 75 ± 16 kg) and varying ages (estimated age = 3-10y, exact ages unknown - all had an erupted third molar) were part of this study. The animals were divided into seven groups and fed pelleted diets of varying abrasiveness. Two concentrations (4% and 8%) and three sizes (s: \varnothing 4 μ m, m: \varnothing 50 μ m, and l: \varnothing 130 μ m) of quartz abrasives were added to a lucerne based pellet diet to create a total of eight diets, including a control with no abrasives (C, 4%s, 8%s, 4%m, 8%m, 4%l, 8%l). All diets were formulated to be isocaloric and isonitrogenic, using an indigestible non-silicaceous filler; a complete description of the diets is reported in Ackermans et al. (2019a). Ten animals were euthanized due to health complications unrelated to the experiment goal, leaving a

total of n=39 animals for the final evaluation. The animals were kept for 17 months and group-housed on soil or sawdust bedding with concreted outdoor space (total surface of ca. 15 m² per group of eight) (the minimum requirement for eight sheep \geq 90 kg is 12 m²). The animals were also provided with enrichment and salt blocks as a supplement.

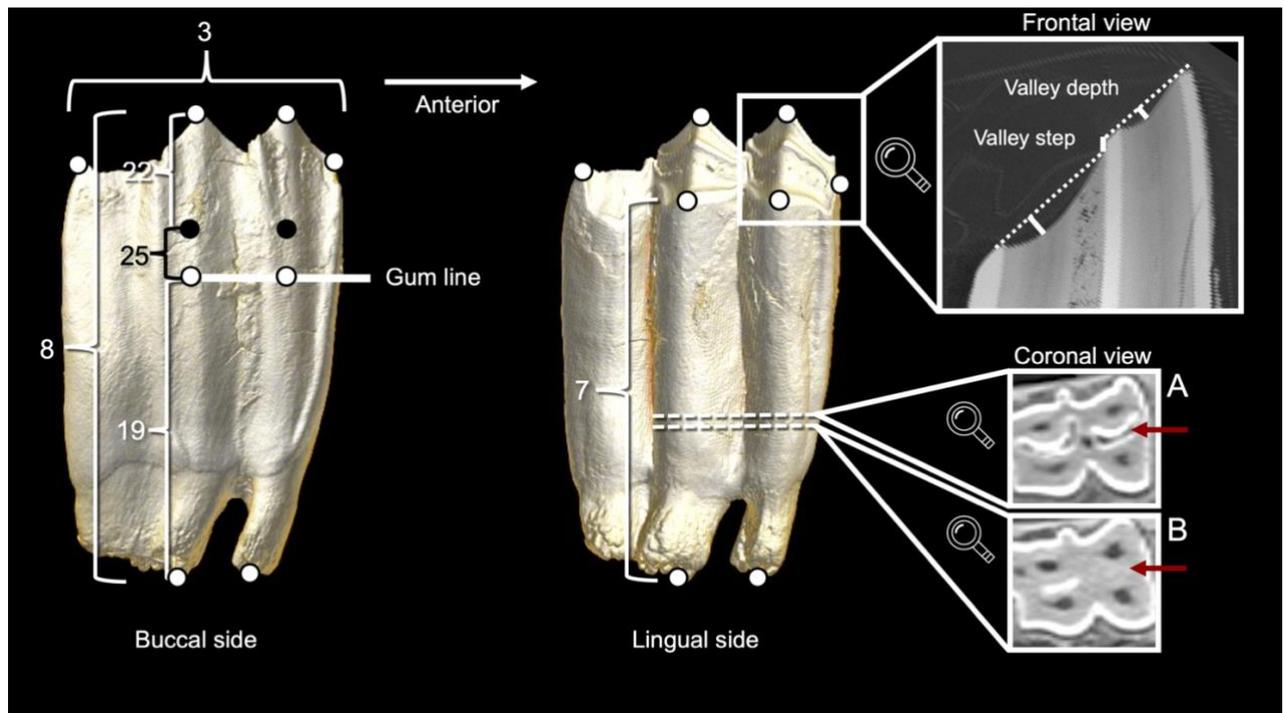
Computed tomographic (CT) scans were carried out under general anaesthesia at two timepoints during the experiment (T1: December 2016, T2: April 2017) and on the skull of the animals after the experiment (T3: May 2018). Then the skulls were rendered in 3D for volumetric calculations and digital calliper measurements (Fig. 1). At the time of the first CT, while animals were under anaesthesia, burr marks were made on the buccal side of the maxillary second molar teeth using a dental drill, taking care not to drill through the enamel completely. When these marks were visible on the CT (Fig. 2), they were used to track tooth wear by measuring this point in relation to the crown tip, alveolar socket, and root tips (Table 1, Fig. 1). Because several of these burr marks had worn off by the time of the second CT, evaluations regarding burr marks could only be done on a limited number of animals, and only between T1 and T2.



Table 1. Digital calliper and volumetric measurements applied to the teeth of experimental sheep (*Ovis aries*, n=31). All measurements were made in millimetres with either a linear measurement tool (L) or a traced, with a non-linear measurement tool (T), volume was measured in mm³ (V) by semi-automatic segmentation.

#	measurement	description	tool
1	crown volume	volume of the crown from tip until crown/root delimitation	V
2	root volume	volume of the roots from the tip to the crown/root delimitation	V
3	tooth surface length	craniocaudal length of the tooth surface	L
4	height buccal ant	tooth height from the tip of the crown to the tip of the roots on the buccal side of the anterior cusp	T
5	height buccal post	tooth height from the tip of the crown to the tip of the roots on the buccal side of the posterior cusp	T
6	height lingual ant	tooth height from the tip of the crown to the tip of the roots on the lingual side of the anterior cusp	T
7	height lingual post	tooth height from the tip of the crown to the tip of the roots on the lingual side of the posterior cusp	T
8	mean tooth height	mean height calculated from the 4 height measurements per tooth	
9	valley buccal ant	depth of the crown surface valley on the buccal side of the anterior cusp	L
10	valley lingual ant	depth of the crown surface valley on the lingual side of the anterior cusp	L
11	valley buccal post	depth of the crown surface valley on the buccal side of the posterior cusp	L
12	valley lingual post	depth of the crown surface valley on the lingual side of the posterior cusp	L
13	mean valley depth	mean valley depth calculated from the 4 valley measurements per tooth	
14	valley step ant	depth of the step between both valleys on the anterior cusp	L
15	valley step post	depth of the step between both valleys on the posterior cusp	L
16	mean step height	mean valley step height calculated from the 2 valley step measurements	
17	burr to roots ant	measurement from burr mark to tip of roots for the anterior cusp	T
18	burr to roots post	measurement from burr mark to tip of roots for the posterior cusp	T
19	mean burr-root distance	mean burr-root distance calculated from the 2 burr-root measurements	
20	burr to crown ant	measurement from burr mark to tip of crown for the anterior cusp	T
21	burr to crown post	measurement from burr mark to tip of crown for the posterior cusp	T
22	mean burr-crown distance	mean burr-crown distance calculated from the 2 burr-crown measurements	
23	burr to gum ant	measurement from burr mark to alveolar margin for the anterior cusp	T
24	burr to gum post	measurement from burr mark to alveolar margin for the posterior cusp	T
25	mean burr-gum distance	mean burr-alveolar margin distance calculated from the 2 burr-alveolar margin measurements	

Figure 1. Landmarks for digital calliper measurements applied to the teeth of experimental sheep (*Ovis aries*, n=31). Tooth represented here is the left mandibular M3 of a goat (ZMH10881), though the measurements were performed on a right mandibular M2. White points indicate points between which digital calliper measurements were made, listed in Table 1, black points indicate burr marks. A and B indicate CT sections visualising the landmark (red arrow) used to section the tooth into crown and roots. A represents the section closest to the crown, while B represents the section closest to the roots, where the infundibula disappear. Micro-CT image courtesy of the Institute of Anthropology at the University of Zurich.



At the end of the experiment the animals were anesthetized with 0.5 mg/kg body weight of xylazine (*xylazinum*, Xylazin Streuli ad us vet; Streuli Pharma AG, Uznach, Switzerland), and 5 mg/kg body weight of ketamine (Ketanarkon 100 ad us vet; Streuli Pharma AG) intra-muscularly and euthanized with 100 to 150 mg/kg body weight of pentobarbital (*pentobarbitalum natricum*, Esconarkon ad us vet; Streuli Pharma AG; 100–150 mg/kg body weight) intravenously, and the death of the animals was confirmed by heartbeat reversal. The skulls were prepared by enzymatic maceration at the Center of

Natural History, University of Hamburg, where they are permanently curated in the Mammals Collection.

Computed tomography imaging

The computed tomography methodology is as in (Ackermans et al., 2018b) and (Ackermans et al., in prep.-a). Images were acquired from a helical multislice Siemens scanner (Siemens Medical Solutions, Erlangen, Germany) housed at University of Zurich Tierspital. Throughout the experiment, parameters were kept constant: tube voltage at 120 kVp, image matrix of 512 x 512 pixels, field of view of

980 x 332 pixels, slice thickness of 0.6 mm and B60s convolution kernel. During scans, the animals were placed under general anaesthesia with Ketamin 10 mg/kg (Ketonarkon®, Streuli Pharma AG, Uznach, Switzerland) and Xylazin 0.1 mg/kg (Xylazin Streuli, Streuli Pharma AG, Uznach, Switzerland) intramuscularly. Anaesthesia was maintained with isoflurane (Attane®, Provet AG, Lyssach, Switzerland) administered in oxygen using a facemask. The first CT scan was used as baseline for the tooth condition at the start of the experiment, as the animals were in groups but not yet on experimental diets, and the final CT scan was performed post-mortem. The CT datasets were converted to DICOM medical imaging format and rendered into 3D surface models using Amira 5.6 (Mercury Computer Systems/3D Viz group, San Diego, CA, USA) as well as Horos v3.0.1 (Horos Project 2015) for additional visualisation.

To allow for mesowear scoring of the 3D renderings in Amira, dynamic models of the data were rendered by defining a fixed iso-surface threshold to achieve the highest bone resolution while avoiding artefacts, and view mode was set to orthographic. Excluding missing or otherwise damaged teeth, as well as CT artefacts, we were left with a group of n=31 individuals for volume and n=38 for some digital calliper measurements; with respect to the visibility of the original burr

marks at T2, this number was even more reduced to n=18 individuals.

To allow for digital calliper measurements to be taken on the CT scans in Horos, 3D MPR mode was selected. The slices were focused on the right maxillary M2 and the following measurements were taken using the “length” tool for linear measurements and the “opened polygon” tool for traced measurements. Digital calliper measurements involving burr marks were made for T1 and T2. Crown and root volume measurements were made for T1 and T3, based on a modified version of the method established in the pilot experiment (Ackermans et al., 2019b), i.e. when moving from the crown to the roots through the image stack, the disappearance of the bottom margin of the anterior infundibulum, and hence also the disappearance of the central structure separating the two infundibula, was used as a landmark to establish a horizontal plane sectioning the tooth into a crown and a root segment (Fig 1A and B). Afterwards, semi-automatic sectioning was applied to segment each volume.

On each M2, tooth surface length, tooth height, depth of dentine valleys, height of the step between valleys, and the distance of the burr mark to the crown, gumline (defined as the margin of the alveolar socket on the CT), and roots were measured, to account for tooth movement within the alveolar socket and wear over the course of the experiment (Table 1,

Fig. 1). Individual calliper measurements were made for each tooth side and cusp on 3D renderings of the CT scans from T1, T2, and T3, when applicable. The measurements were averaged for comparisons in the case of the four “height” measurements (crown tip to root tip on the buccal and lingual sides of the anterior and posterior cusps), the four “valley” measurements (the depth of the dentine valley on the buccal and lingual sides of the anterior and posterior cusps), and “step” measurements (the step created by the infundibulum between the buccal and lingual dentine valley on the anterior and posterior cusps).

Statistical analysis

In the first step of data evaluation, measurements taken at the first and last time points for the same parameter and were plotted against each other to assess whether data showed a general directionality. In particular, we predicted some measurements to become smaller over time (crown volume, tooth height, burr-crown distance), some measurements to become larger over time (root volume, burr-root distance, burr-gumline distance), and some measurements to show no systematic change (tooth length, valley depth, step height). These predictions were tested by paired tests (either t-test or Wilcoxon test), depending on whether or not data were normally distributed, as assessed by Kolmogorov-Smirnov test.

Secondly, correlations were assessed by correlation analysis, using Pearson’s or Spearman’s test, depending on data normality. Tests occurred between crown-volume loss and root-volume gain, between crown-wear and root-growth as measured by burr marks, and between the tooth-height or step-height and the valley-depth. Due to the generally low sample size per diet, and the lack of an evident pattern in the graphical representations, we did not test for an effect of diet. Analyses were performed using SPSS 25.0 (IBM, Armonk, New York, USA), with the significance level set to 0.05.

Results

When results were averaged across all specimens, there was a systematic decrease in crown volume, tooth height, and distance between burr mark and crown over time (Table 2, Fig. 3A, C, G). By contrast, root volume increased significantly over the course of the experiment (Table 2, Fig. 3B). For measures of tooth length, valley depth, step height, and the distances between the burr mark and the alveolar margin or the root, no significant change over time appeared (Table 2, Fig. 5A, B, C). Crown-volume loss and root-volume gain were significantly correlated (Fig. 4A, Table 3), although the relationship was not as consistent as previously documented in goats (Fig. 4B). However, when the crown-height loss and root-height gain was calculated from burr

mark measurements, they did not show a systematic relationship (Fig. 3, Table 3). The calliper measurements generally indicated that higher teeth had higher steps between the basin valleys (Fig. 5A, Table 3), but they did not show a correlation to valley depth (Fig. 5B, Table 3). Nevertheless, teeth with higher steps had deeper valleys (Fig. 5C, Table 3).

Table 2. Mean (\pm SD) measurements taken over different time periods and results of statistical pair-wise comparisons measured on teeth of sheep fed diets of varying abrasiveness for 17 months. Comparisons made by paired t-test or by Wicoxon signed rank test (if data were not normally distributed). See Table 1 and Fig. 1 for a definition of measurements.

Measurement	N	Start	End	Time period (months)	Material loss/gain rate (/year)	P
Crown volume (μm^3)	31	2613 \pm 1331	1926 \pm 892	17	520.06 \pm 415.29	< 0.001
Root volume (μm^3)	31	1507 \pm 477	1685 \pm 517	17	138.23 \pm 119.20	< 0.001
Tooth length (mm)	39	15.9 \pm 1.8	15.7 \pm 1.7	17	0.22 \pm 1.00	0.224
Tooth height (mm)	39	32.1 \pm 5.6	29.3 \pm 4.1	17	1.29 \pm 4.02	< 0.001
Valley depth (mm)	38	1.08 \pm 0.35	1.18 \pm 0.30	17	0.08 \pm 0.24	0.096 _a
Step height (mm)	38	0.69 \pm 0.53	0.74 \pm 0.62	17	0.17 \pm 0.45	0.397 _a
Burr-crown (mm)	18	3.9 \pm 1.8	3.3 \pm 1.3	4	1.78 \pm 3.65	0.002 _a
Burr-gumline (mm)	18	7.6 \pm 1.9	8.0 \pm 2.3	4	0.51 \pm 7.14	0.157 _a
Burr-root (mm)	18	25.1 \pm 6.1	27.3 \pm 6.7	4	3.27 \pm 19.28	0.102 _a

_a Wicoxon signed rank test

Table 3. Correlations between measurements on teeth of sheep fed diets of varying abrasiveness for 17 months. Correlations based on Pearson's test or Spearman's test (if data were not normally distributed). see Table 1 and Fig. 1 for a definition of measurements.

y - x	N	R	P
Root volume gain – Crown volume loss	31	0.49	0.005
Burr mark growth – Burr mark wear	18	-0.23	0.345 _a
Step height – Tooth height	38	0.47	0.003
Valley depth – Tooth height	38	0.26	0.119
Valley depth – Step height	38	0.44	0.006 _a

_a Spearman's test

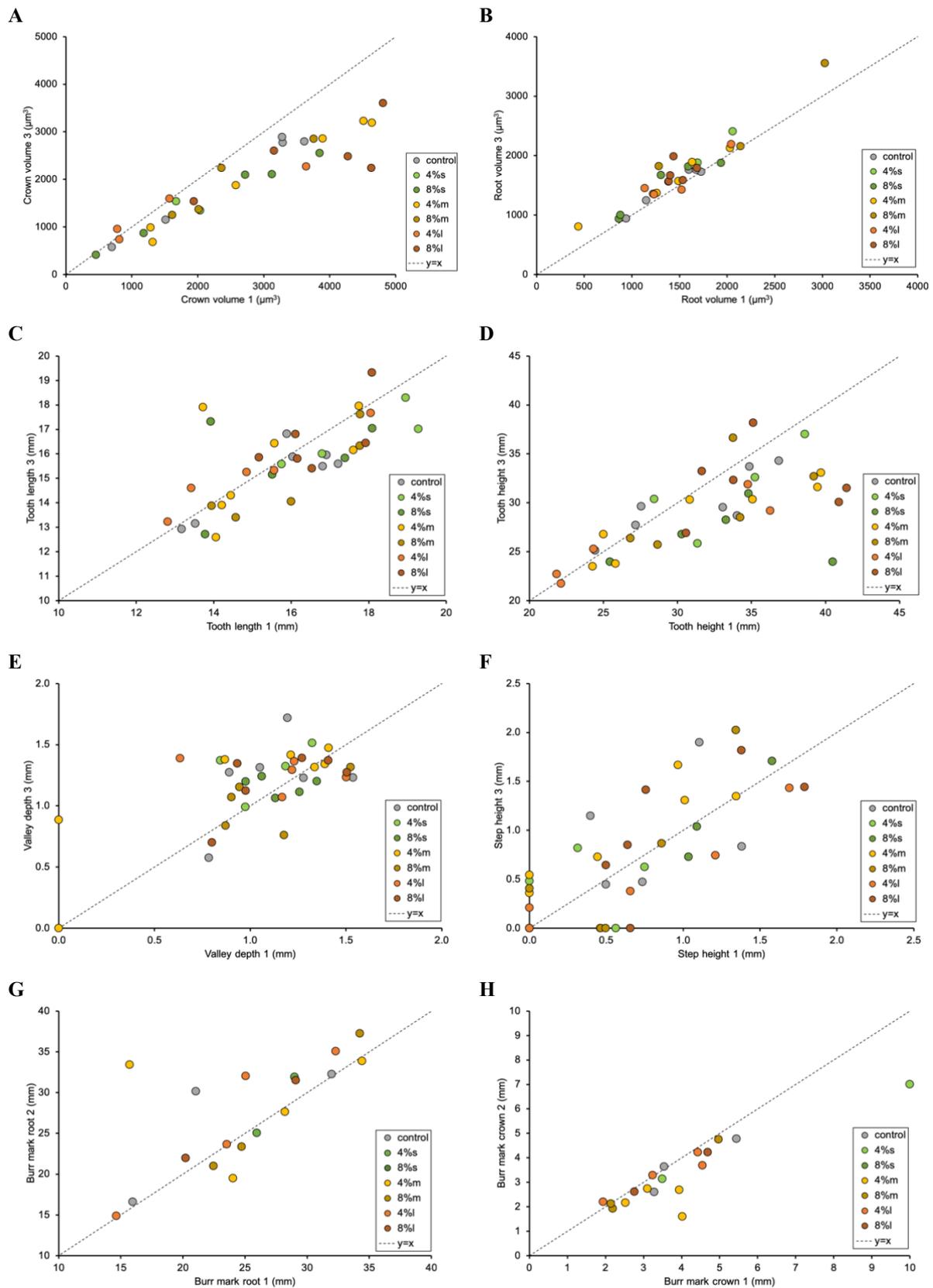


Figure 3. Comparisons of measurements made at the beginning (x-axis) and end of the observation period (y-axis), measured on teeth of sheep fed diets of varying abrasiveness for 17 months. The experimental diets include a control diet without added abrasives, two diets containing small abrasives at two different concentrations (4%_s, 8%_s), and the same with both the medium (4%_m, 8%_m) and large abrasives (4%_l 8%_l). For statistics, see Table 3.

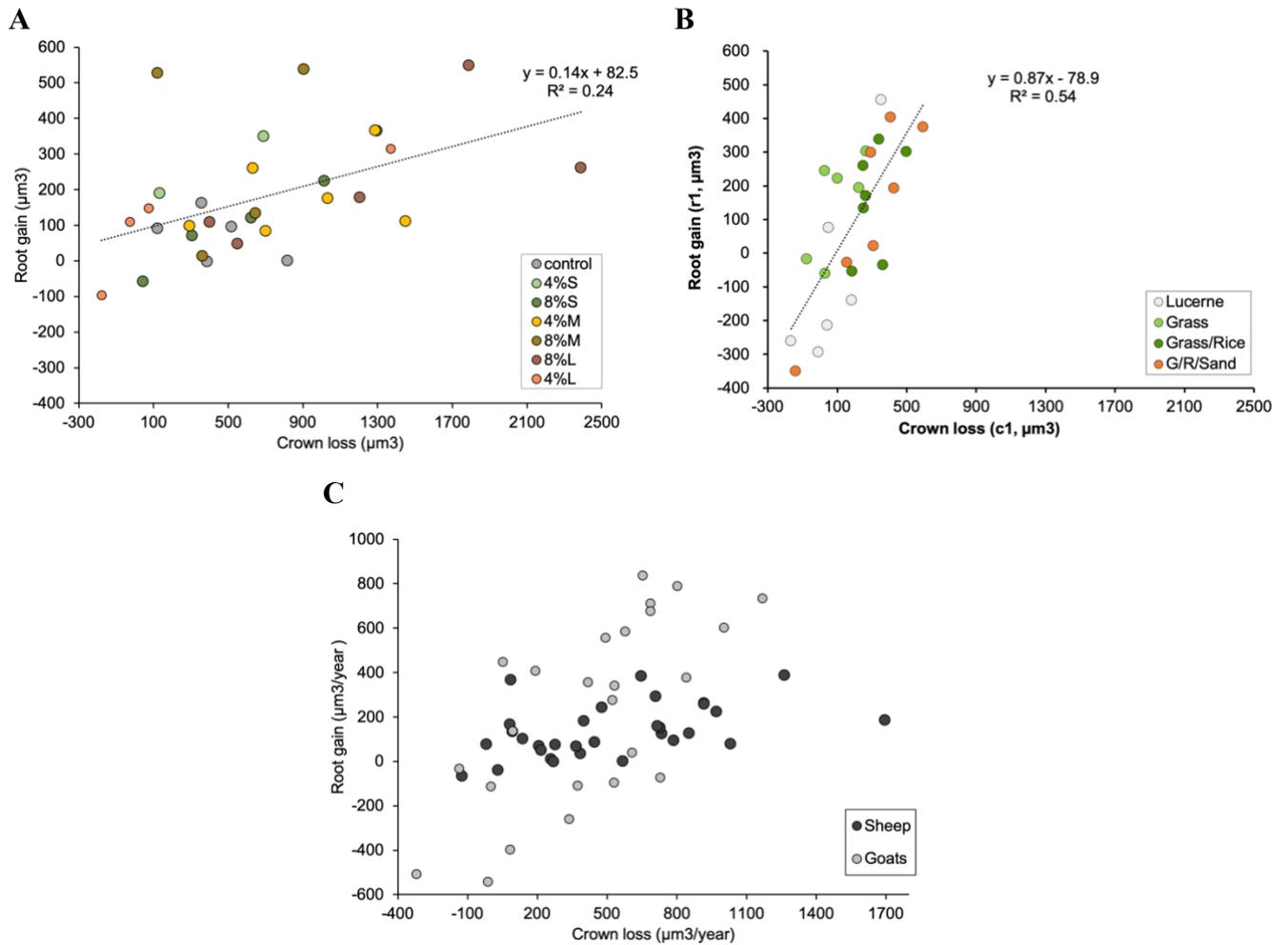


Figure 4. Correlation of crown loss and root gain on the right maxillary M2 for sheep (A, *Ovis aries*, n=31) and goats (B, *Capra aegagrus hircus*, n=26, adapted from Ackermans et al. (2019b)) fed diets of varying abrasiveness for 17 and 6 months respectively. The experimental diets (A) include a control diet without added abrasives, two diets containing small abrasives at two different concentrations (4%*s*, 8%*s*), and the same with both the medium (4%*m*, 8%*m*) and large abrasives (4%*l* 8%*l*). C and represents the yearly wear rate in sheep and goats plotted together.

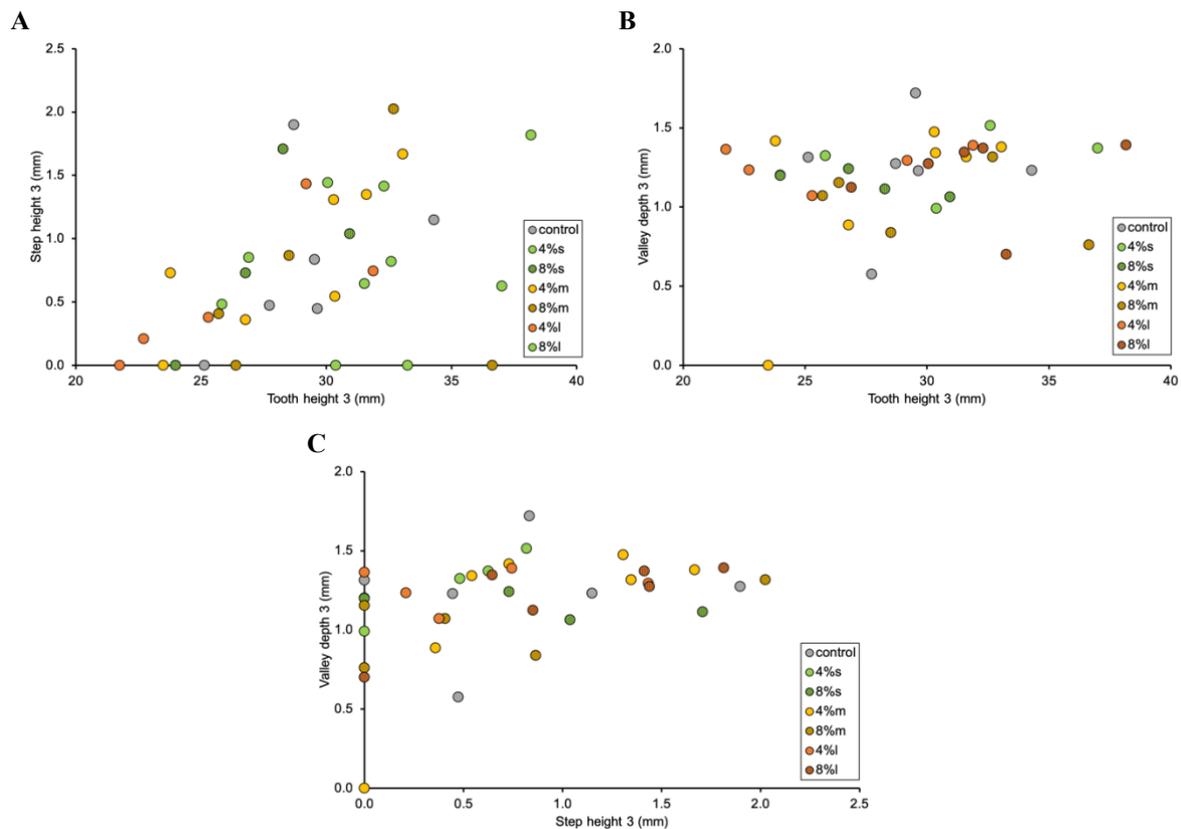


Figure 5. Correlations between tooth height, step height, and valley depth, measured on teeth of sheep fed diets of varying abrasiveness for 17 months. The experimental diets include a control diet without added abrasives, two diets containing small abrasives at two different concentrations (4%_s, 8%_s), and the same with both the medium (4%_m, 8%_m) and large abrasives (4%_l, 8%_l).

Discussion

It has been previously suggested that in herbivores with hypsodont (but not hypselodont) teeth, crown wear is partially compensated by the process of ‘continuous eruption’ in the absence of enamel or dentine growth (Witzel et al., 2018). This is facilitated by the accretion of cementum tissue directly on the root surface, in acellular and cellular layers, which are then attached to the alveolar bone via periodontal ligaments (Berkovitz and Shellis, 2018). By microscopically measuring cementum thickness in wild buffalo (*Syncerus caffer*), Sanson et al. (2017) recorded a

significant negative correlation between crown height and cementum thickness, also corroborating the compensatory mechanism. Seemingly, hypsodont teeth continuously adapt during an animal’s lifetime, controlling their height as a reaction to crown wear, as demonstrated by Sanson et al. (2017), our pilot experiment on goats (Ackermans et al., 2019b), and the present study.

A comparison of cellular mechanisms involved in the compensatory reaction of hypsodont and hypselodont teeth appears a promising area of future research. In hypselodont teeth, stem cells basal to the open

root differentiate into odontoblasts and ameloblasts, producing enamel and dentin (Hu et al., 2014; Thesleff, 2018), whereas in hypsodont teeth, basal cells differentiate into cementoblasts and secrete cementum (Berkovitz and Shellis, 2018). In spite of this difference, the feedback mechanisms that control the system should be of a similar- or the same nature, and must be linked to mechano-proprioception that reacts to pressures, or lack of pressures, induced by contact with an antagonist, or lack thereof.

After feeding sheep experimental diets of varying abrasiveness for 17 months, crown-volume loss was significantly correlated to root-volume gain, though there was visually no dietary distinction. Compared to the pilot experiment on goats (Fig. 4B), sheep appeared to have a higher crown volume loss and less root volume gain than the goats (Fig. 4A), which is best explained by the substantially longer experimental period. When calculating the measurement as rates (*e.g.*, on a yearly basis), there was substantial overlap between the two species, although the relationship between crown loss and root gain did not appear as stringent and steep in the sheep compared to the goats (Fig. 4C). The differences in root growth could be related to the experimental abrasives being much larger in the goats (mean \varnothing 230 μm in the GRS diet, vs. 4-130 μm in the sheep), and to three of the four goat diets containing phytoliths, which may have created a larger effect of

proprioception triggering an increase in cementogenesis. Cementum growth was recorded to be influenced by sex, higher rates having been recorded in female buffalo (Sanson et al., 2017), though sex cannot explain the differences here, as all animals were female (except one wether).

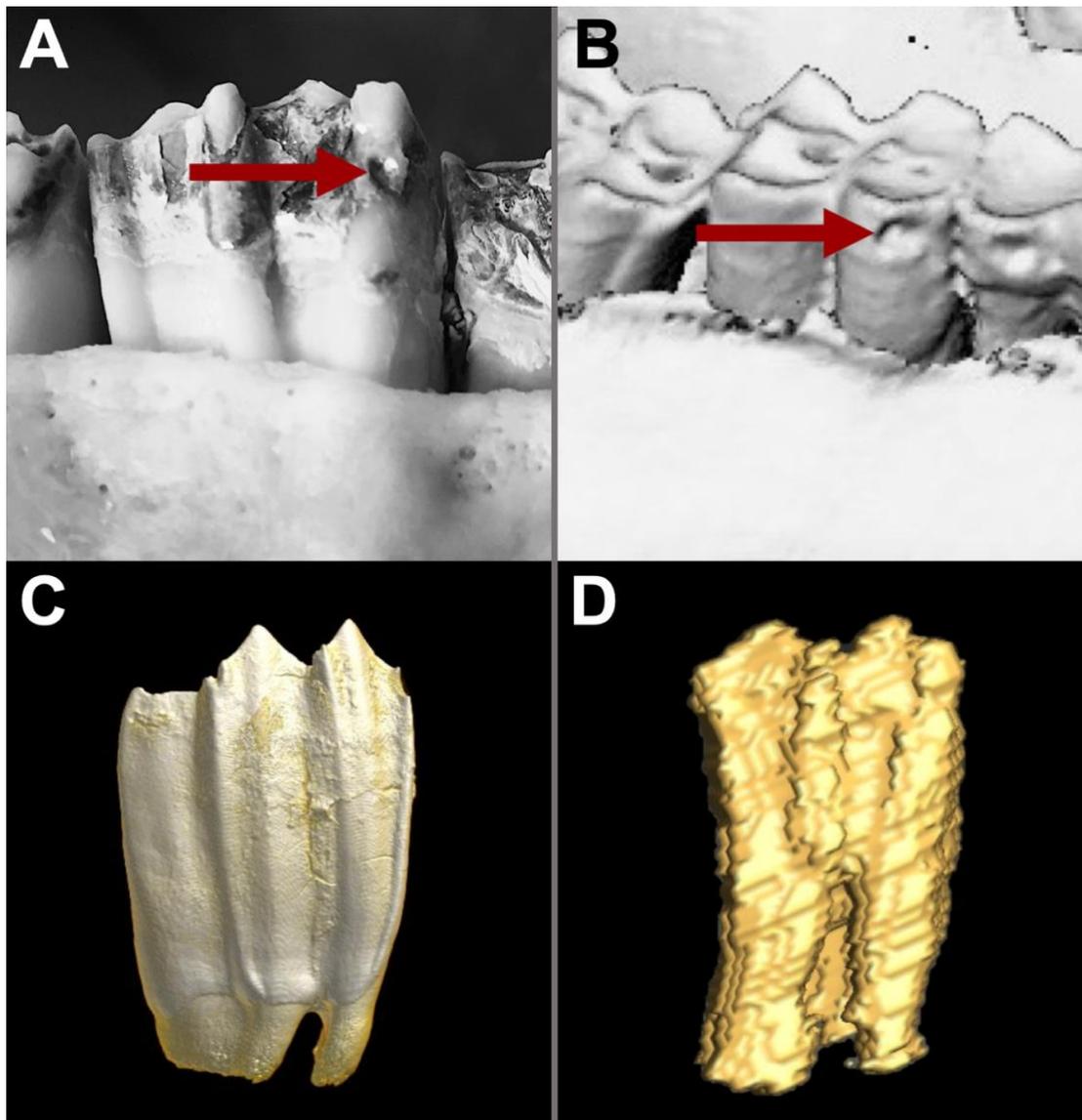
Tooth height loss in the sheep was *ca.* 1.78 mm/year as measured from burr mark to crown, but 1.29 mm/year when total tooth height was measured (Table 2), with the difference being most likely due to of the root gain. Thus, the two measures seem to roughly corroborate each other. In comparison, a review of wear rates in mammals (p8, of dissertation) indicates distinctively less molar wear in other studies, at 0.24-0.30 mm/year in ibex (*Capra pyrenaica*) (Fandos et al., 1993), and 0.83 mm/year of incisor wear in domestic sheep (Healy and Ludwig, 1965). Why our experimental sheep (and, by extrapolation from Fig. 4C, also the goats) experienced up to seven times more wear than that recorded in these studies remains unclear. Notably, this apparently high wear rate was not matched by distinct changes in mesowear scores for either group (Ackermans et al., 2018b) (Ackermans et al., in prep.-a). Compared to the faecal silica content of other wild herbivores (Hummel et al., 2011b), neither the faeces of the goats (Ackermans et al., 2018b) nor of the sheep (Ackermans et al., in prep.-a) showed particularly high silica levels, suggesting that the levels of experimental abrasives were not

excessive compared to natural situations. Awaiting further corroboration, these enormous differences caution against a simplistic ascription of species-specific dental wear rates.

In principle, absolute wear measurements, either volume reconstruction, or digital calliper without or with using burr marks, record tooth height loss, as expected. The expectations for a gain in root volume was

met, but root length measurements using the burr mark (measuring either the distance to the alveolar margin or the root), were not significant, although they showed a numerical increase (Table 1). The resulting impression is that these measurements have potential for investigating general principles of tooth wear, but are comparatively crude and are not suited to detect more subtle dietary differences.

Figure 2. Visualisation of sheep teeth with different media. A and B respectively represent an image and 3D rendering from a medical CT scan of a second molar. Burr marks are indicated by arrows. C is a micro-CT scan of a third molar and D is a second molar rendered by segmentation from medical CT images.



The sensitivity of volumetric measurements is tightly related to CT scan quality (Fig. 2). Micro-CT scans would have been ideal for these types of absolute wear volume measurements (comparing Fig. 2 C and D), but to date it is not yet possible to perform micro-CT scans on live animals. Secondly, though marking the teeth with burrs is certainly durable, tooth wear, chipping, and CT visualisation mitigate their persistence (Fig. 2 A and B). The low number of specimens with measurable burr marks was most likely an important factor in some of the discrepancies between measurements related to this parameter.

Digital measurements revealed some correlations between different anatomical features within the tooth. Namely, higher teeth generally also had higher steps at the infundibulum, suggesting that in the process of wear, the molar surface is generally flattened. Nevertheless, some teeth did not have a measurable step, even though they were not among the most worn (Fig. 5A). There was no change in the dentin basin, or valley depth over time, matching a similar observation of Sanson et al. (2017) on wild buffalo. Martin et al. (in press) suggested that in animals with hypsodont (as opposed to ever-growing, hypselodont) teeth, there is a rather constant equilibrium, where dentine erosion exposes the enamel and hence contributes to enamel wear, with a constant enamel-dentine ratio. This is also suggested in

our results, by the consistency of the valley depth measurement (Fig. 5B).

Conclusion

The correlation between crown-volume loss and root-volume gain observed first in the pilot study, and a secondly in the present experimental study, anchors the existence of a feedback mechanism triggering root growth as a form of compensation for crown wear. As the understanding of this mechanism is rudimentary at best, much work remains in exploring its extent across the dentition of hypsodont animals and their various diets. For example, the effect abrasive diets create for root growth remains to be investigated in non-ruminant ungulates. And furthermore, if it is indeed the cemental layer that causes the recorded growth. Though we expect cementum to be the main factor, we cannot say for certain that this is the case without more precise imaging techniques. The mechanism connecting dietary properties such as abrasion and hardness, to cell proliferation - and thus root growth - remains poorly understood. Further experiments are required to understand the basic workings of this mechanism, including the participation of each of the dental layers, whether a precise loading point triggers the tooth's proprioceptors, and how this mechanism reacts to external versus internal abrasives from herbivore diets.

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Conflict of interest

The authors declare no conflict of interest

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M5

Differences in the rumen washing mechanism in sheep with concentration and size of abrasives

Jean-Michel Hatt, Daryl Cordon, Nicole L Ackermans, Louise F Martin, Henning Richter,
Patrick R Kircher, Christian Gerspach, Jürgen Hummel, Marcus Clauss

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Differences in the rumen washing mechanism in sheep fed diets with abrasives of various concentrations and sizes

Jean-Michel Hatt^a, Daryl Codron^b, Nicole L. Ackermans^a, Louise F. Martina^a, Henning Richter^c, Patrick R. Kircher^c, Christian Gerspach^d, Jürgen Hummel^e and Marcus Clauss^{a,1}

^a Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Winterthurerstr. 260, 8057 Zurich, Switzerland

^b Department of Zoology and Entomology, University of the Free State, PO Box 339, 9300 Bloemfontein, South Africa

^c Clinic for Diagnostic Imaging, Vetsuisse Faculty, University of Zurich, Winterthurerstr. 258c, 8057 Zurich, Switzerland

^d Clinic for Ruminants, Food Animal Department, Vetsuisse Faculty, University of Zurich, Winterthurerstr. 260, 8057 Zurich, Switzerland

^e Ruminant Nutrition, Department of Animal Sciences, University of Goettingen, Kellnerweg 6, 37077 Goettingen, Germany

* Corresponding author at: Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Winterthurerstr. 260, 8057 Zurich, Switzerland

E-mail address: mclauss@vetclinics.uzh.ch (M. Clauss)

Abstract

The processes during the digestion in the ruminant organism lead, possibly inadvertently, to a washing of material before it is regurgitated for rumination chewing. Therefore, ruminants might experience less wear from external abrasives such as dust and grit, which can be washed off, compared to nonruminant herbivores. Details of the washing mechanism, including its efficiency in relation to abrasive size, have not been explored so far. Here, we describe the location and quantity of external abrasives in the gastrointestinal tract of sheep fed seven diets containing external silica abrasives varying in concentration (0, 4, and 8 %) and size (4, 50, and 130 μm). As typical for ruminants, external abrasives accumulated mainly in the fourth stomach section, the abomasum. Compared to the diet, the dorsal rumen contents – from where material for regurgitation and rumination is recruited – was

depleted in external abrasives for the 130 μm silicates, and for the 8% concentration of 50 μm silicates, but not for the 5 μm silicates or the 4% concentration of 50 μm silicates. These results suggest that the rumen washing mechanism is probably more protective against abrasion from grit and sand, and potentially less efficient against fine dust.

Keywords: ruminant, rumen, tooth wear, chewing, rumination, washing

Introduction

Traditionally, the main function of the ruminant stomach complex has been considered to lie in the microbial digestion of plant material (Van Soest, 1994). At the same time, the microbes that grow during this process are passed on into the lower digestive tract and are also digested by the ruminant host (Van Soest, 1994). An important peculiarity that sets the ruminant forestomach apart from

the forestomach of nonruminant foregut fermenters is a sorting mechanism that ensures a differential treatment of particles that are well-comminuted and digested, and larger particles that require further chewing activity (Dittmann et al., 2015b). The sorting mechanism utilizes the floating and sedimenting properties of the digesta in a liquid medium (Lechner-Doll et al., 1991). During all these processes, the digesta in the first and second section of the ruminant stomach complex, the rumen and the reticulum, are subjected to peristaltic movements as well as soaking by, and mixing with, rumen fluid.

One side-effect of these processes is that digesta is washed by the rumen fluid, and one can expect dense material to sediment in such an environment. Theoretically, this should lead to a situation where the majority of particle-size reducing chewing, i.e. rumination, is performed on material that is not only softer but also contains fewer external abrasives than the ingested diet. In return, it may contain, due to the expedient digestion of easily digestible substances and the concomitant concentration of less digestible plant parts, potentially higher concentrations of internal abrasives (phytoliths). This assumption was used to explain differences in the anatomy, physiology and behaviour associated with the chewing process between ruminants and nonruminants (Fletcher et al., 2010; Janis et al., 2010;

Williams et al., 2011; Dittmann et al., 2017; Zhou et al., 2019), as well as differences in tooth wear patterns (Mihlbachler et al., 2016). The actual depletion of external abrasives of the dorsal rumen contents (from where digesta is regurgitated for rumination) was shown in a fistulated sheep by Baker et al. (1961), and more comprehensively in goats fed an experimental diet that contained sand of a grain size of 230 μm (Hatt et al., 2019b).

The ruminant digestive tract, therefore, treats grit or sand differently than other parts of the digesta. In particular, external abrasives first accumulate somewhat in the ventral rumen, but are passed on via the reticulum and omasum (3rd section of the ruminant stomach complex) to the abomasum (the 4th and glandular section of the ruminant stomach complex). Here, the dense material accumulates but is also passed on continuously into the lower digestive tract, usually without evident problems. This has been reported not only in experimental goats, but also in free-ranging and captive wild ruminants (Fig. 1).

As an expansion of our study in goats (Hatt et al., 2019b), the aim of the present study was to evaluate the digesta washing effect in relation to the size and concentration of the external abrasives. We hypothesized that the washing effect would be more distinct at larger particle sizes and higher concentrations of abrasives in the diet.



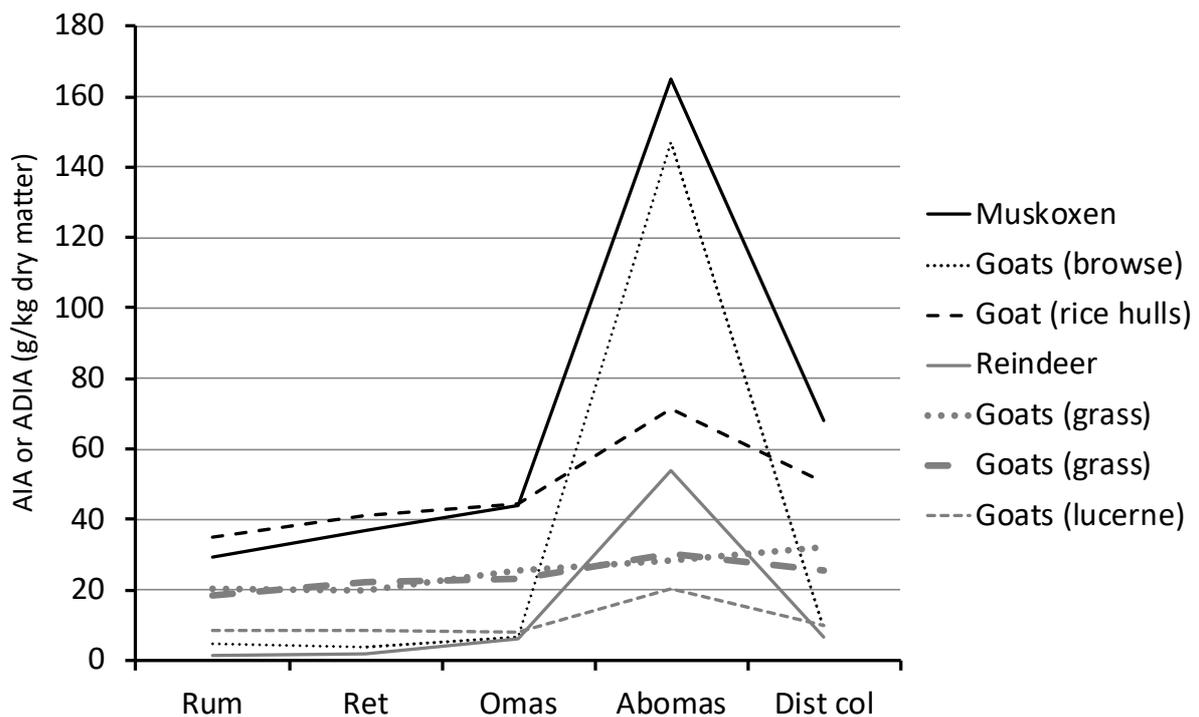


Figure 1 Reported levels of silica (measured either as acid insoluble ash [AIA] or acid-detergent insoluble ash [ADIA] in dry matter) in the digestive tract of goats (*Capra aegagrus hircus*) fed different diets (Hatt et al., 2019b), captive reindeer (*Rangifer tarandus*) fed lichen (Staaland et al., 1986; read from graph), and free-ranging muskoxen (*Ovisbos moschatus*) (Staaland and Thing, 1991; read from graph). Data displayed for the four sections of the ruminant stomach complex rumen (Rum), reticulum (Ret), omasum (Om), abomasum (Abomas) as well as the distal colon (Dist col, equivalent to faeces). Note the accumulation of silica in the abomasum above levels at any other section in many cases, indicating contamination of the ingested material with external abrasives.

Methods

Experiments were performed with approval of the Swiss Cantonal Animal Care and Use Committee Zurich (animal experiment licence 10/2016). 42 mature, female, non-reproducing sheep were divided into seven groups that received, for 17 months, one of seven pelleted diets varying in the concentration and size of external abrasives (Ackermans et al., 2019d). The pellets had a base of lucerne meal, which contains no phytoliths. External abrasives were added in the form of silica in three different sizes: small, medium and large abrasives

(SCR-Sibelco N.V., Antwerp, Belgium. Small abrasives: SIRCON® M500, mean particle size of 4 µm; medium abrasives: MICROSIL® M4, mean particle size of 50 µm; large abrasives: METTET AF100, mean particle size of 130 µm). For each diet of a different abrasive size, abrasives were added in two target concentrations (4% and 8%), resulting in seven different diets: small abrasives (4%S), small abrasives (8%S), medium abrasives (4%M), medium abrasives (8%), large abrasives (4%L), large abrasives (8%L) and a control diet with no added abrasives. To ensure

all pelleted diets were isocaloric and hence ingested in similar amounts, they were designed so that the proportion of indigestible silica abrasives was matched by a similar proportion of indigestible, non-abrasive filler (pure lignocellulose, Arbocel, JRS Pharma, Rosenberg, Switzerland) in diets of lesser silica content. The measured concentrations of acid detergent insoluble ash (a proxy for silica) was 12 g/kg dry matter (DM) in the control diet, 31 and 52 g/kg DM in the small abrasives diets, 34 and 66 g/kg DM in the medium abrasives diet, and 58 and 100 g/kg DM in the large abrasives diets respectively (Ackermans et al., 2019d). Lucerne hay was provided to all groups. Each animal received 1400 g of pelleted food and 230 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 4 months, CT images were acquired using a helical multislice Siemens scanner (Siemens Medical Solutions, Erlangen, Germany) to image the abdomen of the sheep (tube voltage at 120 kVp, image matrix of 512 x 512 pixels, field of view of 1329 x 762 pixels, slice thickness of 0.5 mm, B30s 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 and dosed to effect. CT data sets were converted to DICOM medical imaging format and evaluated in Horos v3.0.1 (Horos Project 2015). Radiodense silica volumes (cm³) were calculated by manually defining regions of interest (ROIs) on every 6th 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. 2.

After 17 months, the sheep were euthanised (using the same anaesthetic protocol as for CT followed by intravenous pentobarbital administration until cessation of heartbeat) within two hours after their last feeding, and the gastrointestinal tract (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.

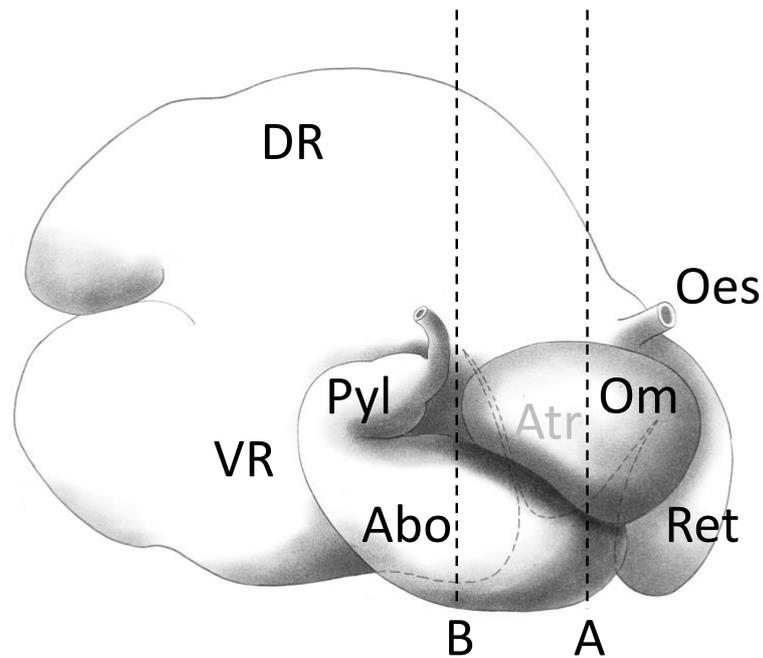


Figure 2 Schematic representation of the ruminant forestomach, viewed from its right side. The cranial part is to the right, with the oesophagus (Oes), and the caudal part to the left. While the forestomach is positioned next to the left abdominal wall of the animal, the intestines are placed on its right side in the abdominal cavity, between the scheme and the viewer, and are not shown here. DR dorsal rumen (from where contents are regurgitated for rumination), VR ventral rumen (partly overlaid by the abomasum and omasum), Ret reticulum (partially overlaid by the omasum), Om omasum, Abo abomasum (fundus), Pyl abomasum (pylorus), and ATR atrium (overlaid in this view by the omasum). The stipled lines (A,B) indicate the planes of the CT images shown in Fig. 3A and 3B. 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). Note that the pylorus is drawn as pointing dorsally, but in subsequent CT images, rather points to right side of the body (towards the viewer).

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 acid detergent insoluble ash (ADIA) (Hummel et al., 2011a).

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, ADIA, ash) was tested in

models nesting GIT section within diet, which consisted in itself of size (small, medium, large) nested within dose (0, 4, 8%), with Tukey's HSD post hoc test for multiple comparisons (significance level at 0.05). For the assessment of CT measurements, this approach was applied separately to the ventral rumen, the abomasum, and the whole stomach complex.

Results

Inspection of the CT scans indicated accumulation of radiodense material in the ventral rumen, the *Atrium ruminis*, and the abomasum (Fig. 3 and 4). In the abomasum, two different locations were evident – in the fundic region, where a partitioning of the radiodense material by the folds typical for the fundic mucosa of the abomasum could be observed (Fig. 3B, Fig. 4A), and at the pylorus (the exit of the abomasum towards the small intestine) (Fig. 4BC). Radiodense material was not visible in the dorsal rumen, the reticulum, the omasum or the intestines. The measured dimensions of accumulated radiodense material in the ventral rumen and abomasum showed a clear pattern with the feeding groups, with higher values linked to higher concentrations, and larger particle size (Fig. 5, Table 1). Note that, as previously reported for goats (Hatt et al., 2019b), this accumulation did not cause clinical problems (and the sheep lived for another 12 months on the same diets after the CT on the same diets).

The complete findings for DM and ADIA are displayed in Fig. 6. DM followed the typical ruminant pattern, with drier contents in the dorsal than the ventral rumen, very dry contents in the omasum, higher moisture content in the abomasum and small intestine, and then a continuously increasing DM content along the large intestine (Fig. 6A). In the present study, the only exception was the DM content in the abomasum that was particularly

high on the high concentrations of medium and large external abrasives, indicating massive silica accumulation. ADIA concentrations were higher in the ventral than in the dorsal rumen, increased towards the omasum (Fig. 6B, Fig. 7) and were extremely high in the abomasum (Fig. 6B). From the small intestine to large intestine, ADIA concentration again increased and then remained constant.

The visual interpretations were confirmed in the statistical analyses (Table 2). The highest concentration of silica led to generally higher DM values, and differences between sites of the gastrointestinal tract were as expected (with many sites differing from the distal colon, spiral colon and omasum), with the evident exception of the abomasum that differed from all other sites. For total ash and ADIA, values increased with concentrations of abrasives in the diet, with no statistical effect of abrasives size. The main significant difference was between the abomasum and all other sites.



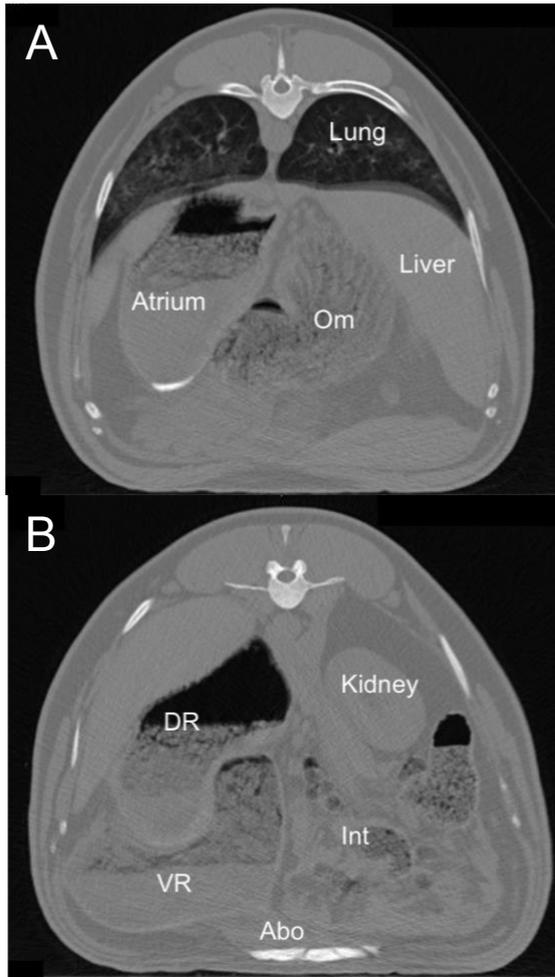


Figure 3 Computed tomographic scans of a sheep (*Ovis aries*) fed diet of lucerne meal pellets containing external abrasives (medium 4%) for four months. Animal in sternal recumbency. Images represent vertical slices through the thorax and abdomen (A) or, more caudally, through the abdomen only (B), 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. Silica accumulation is visible as hyperattenuating (white) agglomerations in the atrium and abomasum (Abo) Note the partitioning of the sand in the abomasum, which is caused by the fundic mucosal folds. Other radiodense structures correspond to vertebrae and ribs. Sand is not visible at other locations. The typical stratification of rumen contents are visible, 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)

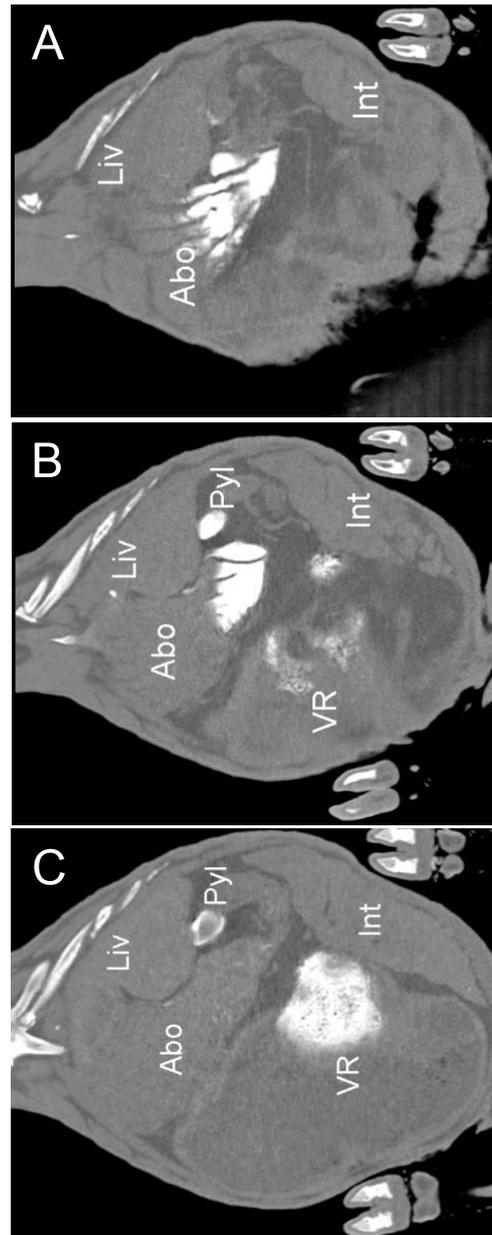


Figure 4 Computed tomographic scans of a sheep (*Ovis aries*) fed diet of lucerne meal pellets containing external abrasives (small 4%) for four months. Animal in sternal recumbency. Images represent horizontal-oblique slices through the thorax and abdomen at a more dorsal (A), intermediate (B) or ventral (C) position. The hind feet are visible on the lower part of the images. Silica accumulation is visible as hyperattenuating (white) agglomerations in the fundic region of the abomasum (Abo), partitioned by the abomasal fundic mucosal folds (A), in the aboral fundic region of the abomasum as well as in the pylorus (Pyl) of the abomasum, and, in outlines, in the ventral rumen (VR) (B), and more ventrally only in the pylorus and the ventral rumen (C). Note the outline of the abomasum in (c). Other radiodense structures correspond to vertebrae and ribs. Sand is not visible at other locations. Liv liver, Int intestines.

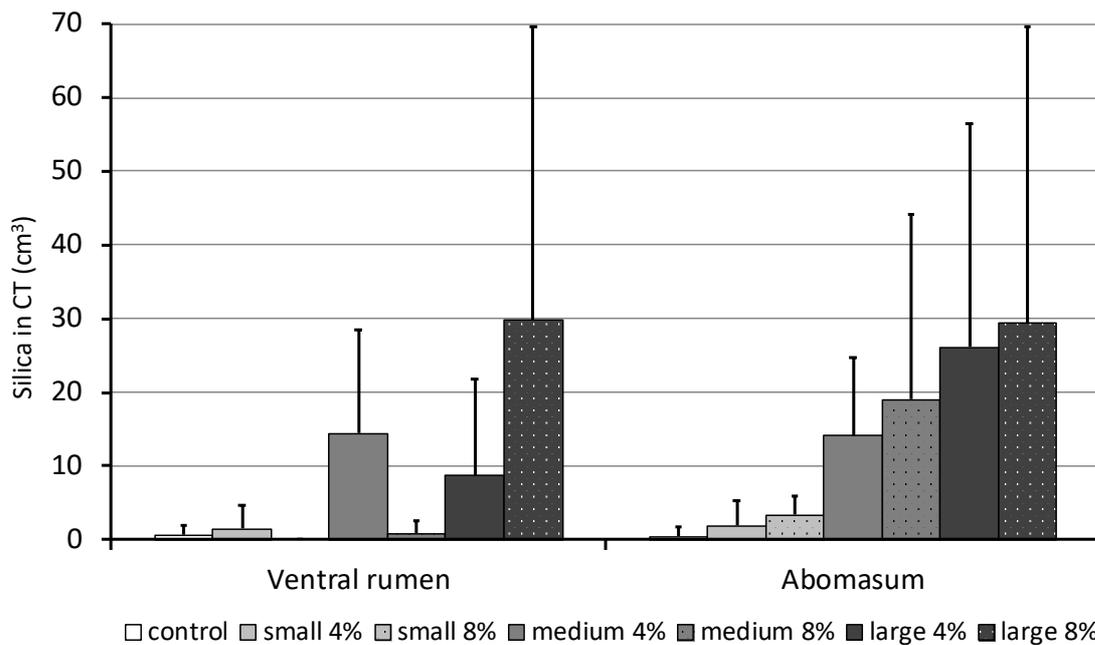


Figure 5 Average accumulation of radiodense material, as quantified on CT images, in the ventral rumen and abomasum in sheep (*Ovis aries*) fed diets of different abrasive concentrations (small abrasives [4%], small abrasives [8%], medium abrasives [4%], medium abrasives [8%], large abrasives [4%], large abrasives [8%] and a control diet with no added abrasives).

When calculating the difference in ADIA concentration between the diet and the different stomach sections, it was evident that dorsal rumen contents were relatively depleted in ADIA for large abrasives, and for 8%M, but not for 4%M or the small abrasives (Fig. 8A). The same was evident for the reticulum. Omasal contents were either of similar ADIA concentration as, or higher ADIA concentration than, the diet, and the abomasum showed an enrichment of ADIA compared to the diet for all groups (Fig. 8B). When comparing the dorsal and the ventral rumen, the ventral rumen contents had higher ADIA concentrations again for large abrasives, and for 8%M, but not for 4%M or the small abrasives. Actually, on 8%S, it even seemed

that the dorsal rumen contents did not release the abrasives as readily as for the other groups (Fig. 8C). In statistical assessments of differences in DM, total ash or ADIA between the dorsal and ventral rumen (nested in abrasives concentration and size), there was a general effect of the organ site (Table 3), although there were no diet-specific (i.e., nested) differences for DM or ADIA. For ash and ADIA, the silica concentration in the diet but not the grain size had an effect on the concentrations measured at the sites (Table 3). By contrast, the difference between dorsal and ventral rumen total ash content was significant for the two diets with the largest grain size (Table 3).

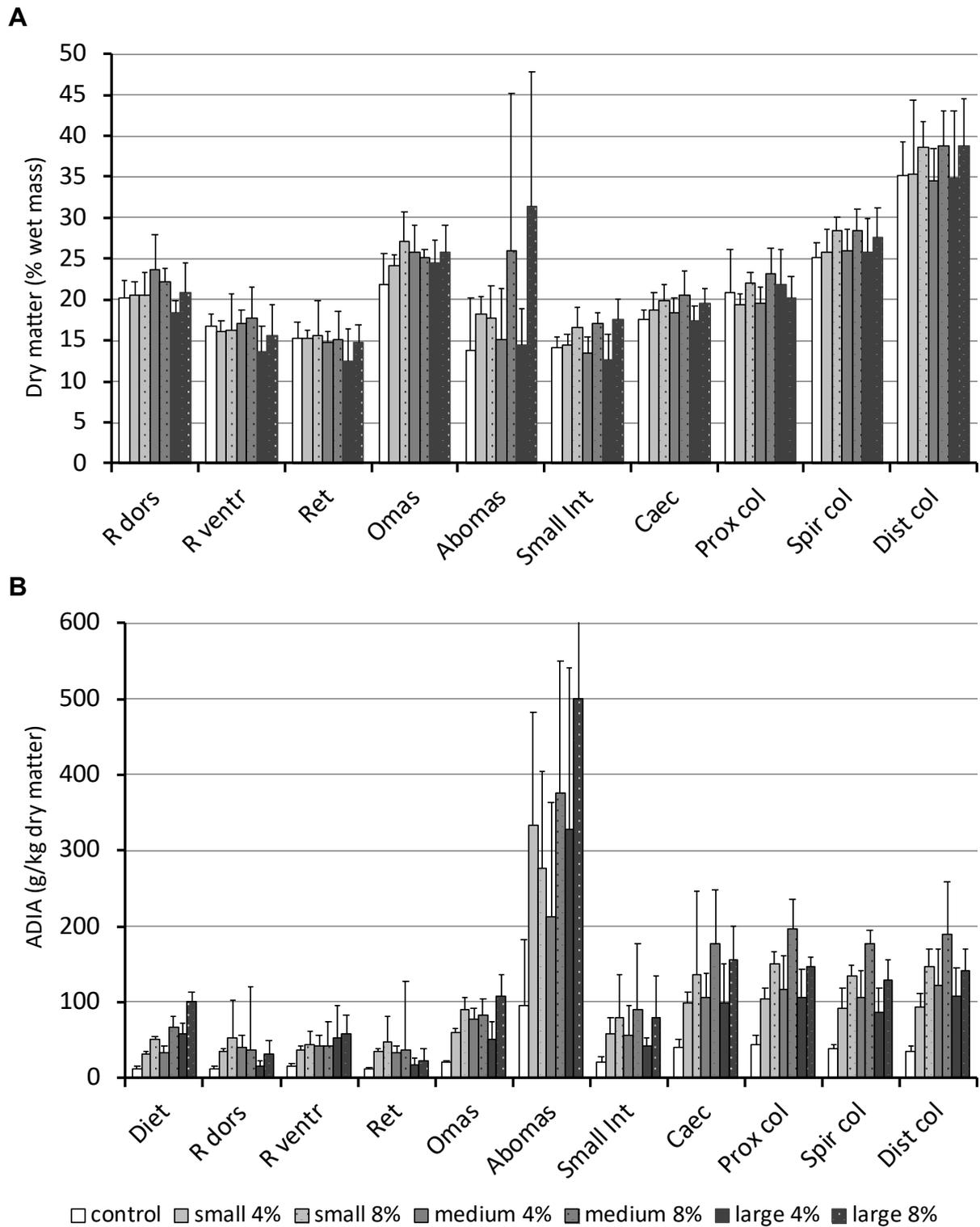


Figure 6 The (mean +SD) concentration of (A) dry matter (DM), (B) acid detergent insoluble ash (ADIA; a measure for silica) in the diet and digestive tract of sheep (*Ovis aries*) fed diets of different abrasive concentrations and sizes. R dors dorsal rumen (from where contents are regurgitated for rumination), R ventr ventral rumen, Ret reticulum, Om omasum, Abo abomasum, Small int small intestine, Caec caecum, Prox col proximal colon, Spir col spiral colon, Dist col distal colon/faeces. For a more detailed look at (B) see Fig. 7.

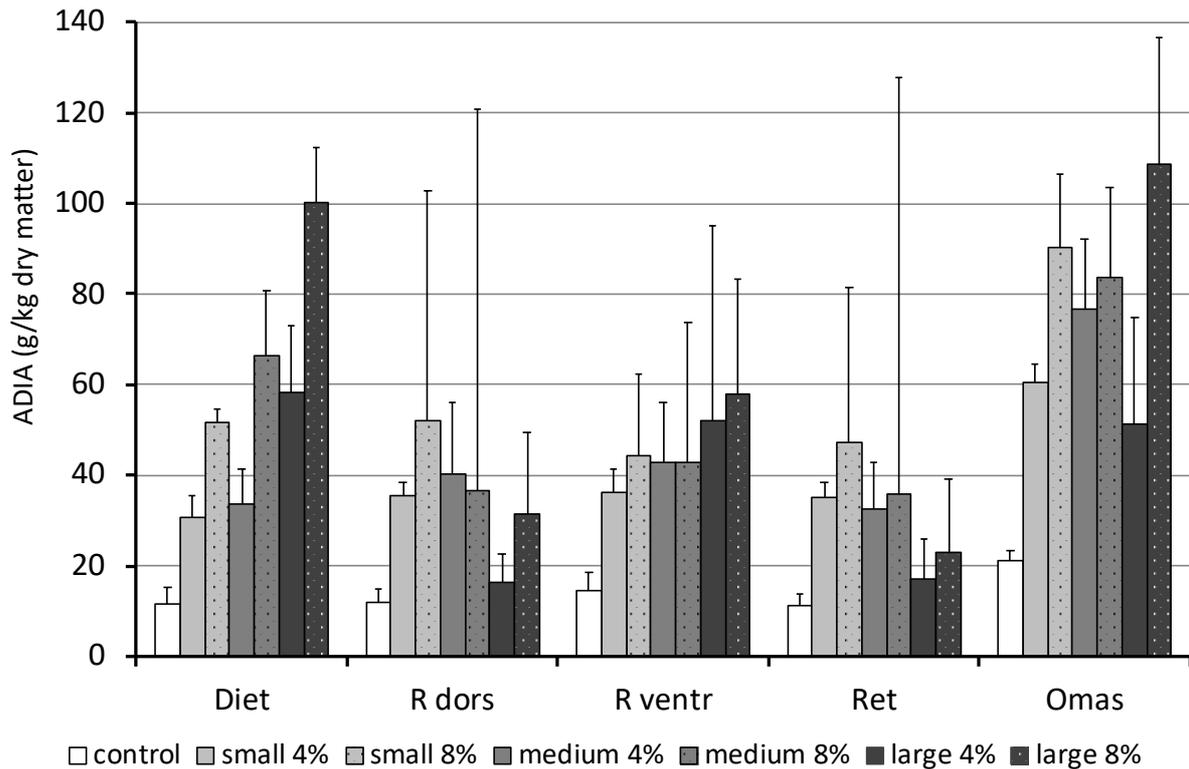


Figure 7 Concentration of acid-detergent insoluble ash (ADIA, in dry matter; means +SD) in the diet and different sections of the forestomach in sheep (*Ovis aries*) fed diets of different abrasive concentrations and sizes. R dors dorsal rumen (from where contents are regurgitated for rumination), R ventr ventral rumen, Ret reticulum, Om omasum.

Table 1 Results of statistical analyses for differences between diets in the accumulation of radiodense material in the ventral rumen, the abomasum and the whole stomach of sheep (*Ovis aries*) fed diets of different doses and sizes of abrasives (small abrasives [4%], small abrasives [8%], medium abrasives [4%], medium abrasives [8%], large abrasives [4%], large abrasives [8%] and a control diet with no added abrasives) for four months

Effect	df	effect <i>F</i>	effect <i>P</i>	post hoc
Radiodense volume (cm³) ventral rumen				
dose	2	2.183	0.128	n.s.
dose/size	4	3.440	0.019	n.s.
Radiodense volume (cm³) abomasum				
dose	2	3.712	0.036	c<4; c<8 at <i>P</i> = 0.084
dose/size	4	1.214	0.324	n.s.
Radiodense volume (cm³) whole stomach				
dose	2	5.817	0.007	c<4/8
dose/size	4	3.148	0.027	n.s.

Table 2 Results of statistical analyses for differences between in dry matter, total ash, and acid detergent-insoluble ash along the gastrointestinal tract of sheep (*Ovis aries*) fed diets of different doses and sizes of abrasives (small abrasives [4%], small abrasives [8%], medium abrasives [4%], medium abrasives [8%], large abrasives [4%], large abrasives [8%] and a control diet c with no added abrasives) for 1.5 years

Effect	df1	df2	effect F	effect P	post hoc
Dry matter concentration (% fresh matter)					
dose	2	315	27.410	<0.001	c/4 < 8
dose/size	4	315	6.447	<0.001	n.s.
dose/size/organ	63	285	13.572	<0.001	mainly differences to abomasum, distal colon, spiral colon, omasum
individual	1		0.370	0.543	
Total ash (% dry matter)					
dose	2	317	43.181	<0.001	c < 4 < 8
dose/size	4	317	5.583	<0.001	n.s.
dose/size/organ	63	286	8.687	<0.001	only differences of other organs to abomasum
individual	1		0.134	0.715	
Acid detergent insoluble ash (% dry matter)					
dose	2	315	59.721	<0.001	c < 4 < 8
dose/size	4	315	10.972	<0.001	n.s.
dose/size/organ	63	286	9.849	<0.001	only differences of other organs to abomasum
individual	1		0.997	0.318	

Individual was a random effect

Table 3 Results of statistical analyses for differences between in dry matter, total ash, and acid detergent-insoluble ash between the dorsal and the ventral rument of sheep (*Ovis aries*) fed diets of different abrasive concentrations (small abrasives [4%], small abrasives [8%], medium abrasives [4%], medium abrasives [8%], large abrasives [4%], large abrasives [8%] and a control diet with no added abrasives) for 1.5 years

Effect	df1	df2	effect F	effect P	post hoc
Dry matter concentration (% fresh matter)					
dose	2	55	1.117	0.335	n.s.
dose/size	4	55	2.946	0.028	(4): medium > large
dose/size/organ	7	32	13.474	<0.001	n.s.
individual	1		5.625	0.018	
Total ash (% dry matter)					
dose	2	52	0.954	0.392	c /4 < 8
dose/size	4	52	3.149	0.022	n.s.
dose/size/organ	7	32	12.468	<0.001	(4/8(large)): dorsal < ventral
individual	1		8.379	0.004	
Acid detergent insoluble ash (% dry matter)					
dose	2	58	1.904	0.158	c < 4 < 8
dose/size	4	58	2.556	0.048	n.s.
dose/size/organ	7	32	4.004	0.003	n.s.
individual	1		3.359	0.067	

Individual was a random effect

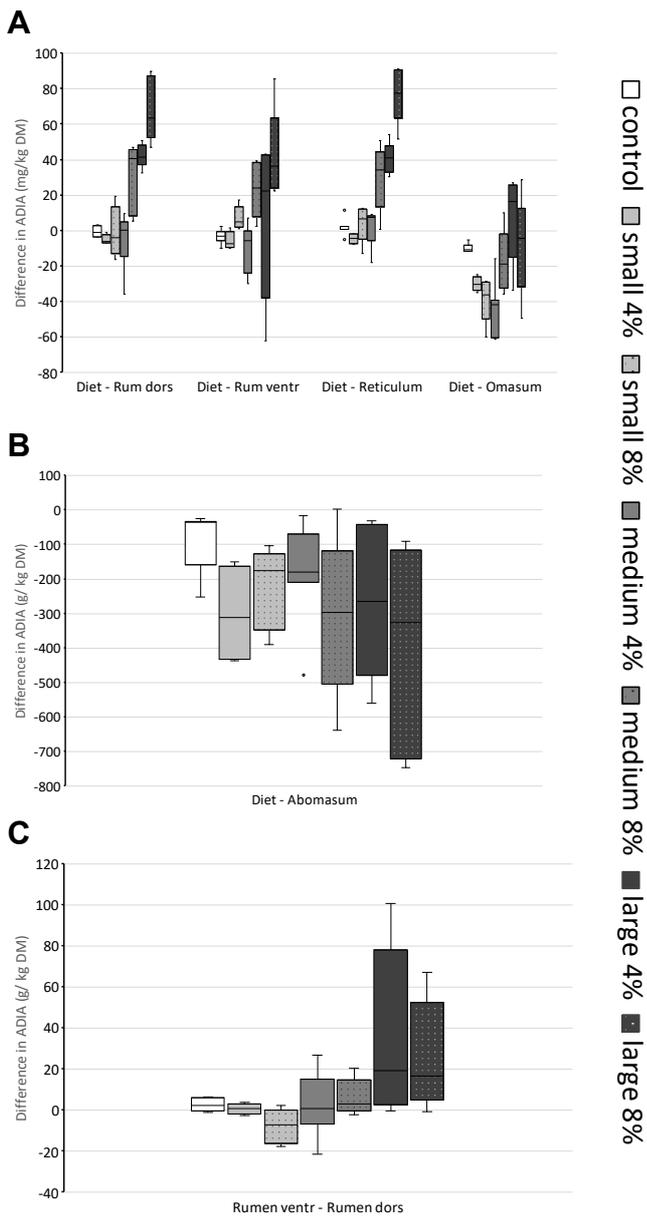


Figure 8 Differences in the concentration of acid detergent insoluble ash (ADIA in g/kg dry matter) between (A) the diet and digesta in different sections of the ruminant stomach compartments (Rdors dorsal rumen, Rventr ventral rumen, Ret reticulum, Omas omasum), (B) the diet and digesta in the abomasum, and (C) the digesta in the ventral and the dorsal rumen.

For the dorsal rumen, there was a decrease, within each concentration level, of ADIA concentrations with abrasive size (Fig. 8A), suggesting that in this study, larger abrasives were more easily washed out of the digesta at this location than smaller abrasives. For the abomasum, the opposite pattern was evident (Fig. 8B); in other words, larger abrasives were more concentrated in the abomasum than the smaller ones.

Discussion

The present study corroborates the recently described general concept of a washing mechanism in the ruminant forestomach complex, which depletes the digesta of external abrasives in certain compartments, and concentrates them in the glandular stomach (Hatt et al., 2019b). Apparently, the principle is easily replicated. Here, we expand this concept by assessing differences in the mechanism for different concentrations and sizes of abrasives. The results suggest that in particular very small abrasives – of a size that would be considered ‘dust’ (<20 to 70 μm) – are less efficiently washed off the digesta, and are less selectively retained in the abomasum, than larger abrasives of a size that is closer to ‘grit’ (>70 μm).

Whether this difference is a real effect operating in nature, and the extent to which it is linked to the production of the artificial diets used in the present study, is difficult to judge. One could argue that smaller silica particles,

with a higher surface:volume ratio and hence also likely higher surface interactions with other particles, (i) may combine more tightly with the general food matrix during the compression of the pelleting process, (ii) may be more retained by surface tension than larger particles, and (iii) may be still contained within swallowed food fractions that are not completely moistened by saliva and require some time to dissolve. Larger particles are less likely to be contained within pieces of food matrix and may therefore be washed out of the ingested material at a faster rate. Should this reasoning be correct, we would expect a less distinct size-related effect in ruminants consuming natural foods, where all external abrasives are on the outside of the plant material and unlikely to be partially trapped in pieces of food matrix. Investigating the presence and size of abrasives along the gastrointestinal tract of free-ranging ruminants, with a sampling regime that represents the stratification, and hence the difference between dorsal and ventral rumen contents (Sauer et al., 2017), would be required to further investigate these questions.

In comparison with the previous study in goats (Hatt et al., 2019b), the main accumulation sites of the silica were again in the abomasum, and to a lesser extent in the ventral rumen. For the abomasum, the major accumulation site was between the fundic laminae, but also partially in the pylorus. The fact that these animals lived on these diets for

1.5 years, and that variation in dietary abrasives levels between the groups corresponded to differences in levels in the faeces, indicates that the accumulation is only temporary, and that an equilibrium develops. In other words, the inflow of abrasives into the abomasum must, at least when integrated over longer time periods, match the outflow from this organ. Even though the abomasal surface does not consist of a keratinized epithelium like the preceding forestomach sections, but rather consists of a soft, glandular epithelium, it is most likely protected by the mucous produced by stomach glands, which also protects the mucosa against the hydrochloric acid produced by the deeper parietal cells (Goff, 2015). The findings in wild and domestic ruminants, summarized in Fig. 1, additionally suggest that the accumulation of dense material at this site can be considered a normal process. African buffalo (*Syncerus caffer*) were estimated to ingest up to 28 kg of external silicates per year (Sanson et al., 2018), bison (*Bison bison*) ingesting natural forages can apparently withstand daily intake rates of soil of 6 % in dry matter (Beyer et al., 1994), and cattle on ranges or pasture have been estimated to ingest up to one kilogram of soil per day (Healy, 1968; Mayland et al., 1975; Mayland et al., 1977), without accompanying reports of clinical problems.

In domestic ruminants, cases and case series of abomasal sand impaction have been reported in the literature. However, the

condition seems to be uncommon. Gravel can occasionally be palpated in the abomasum during exploratory laparotomy, but without any signs of obstruction (Cebra et al., 1996). In general, sand impaction of the abomasum in cattle is caused by sand contaminated feed (Hunter, 1975; Erickson and Hendrick, 2011) or water (Simsek et al., 2015), or because of pica (ingestion of non-nutritive substances) apparently triggered by an acidogenic diet (Melendez et al., 2007).

The amount of sand accumulating in the abomasum depends on the amount of sand ingested, but also on gastrointestinal motility (Dirksen, 2002). According to this author, healthy cows fed 10 kg sand daily over 35 days with their diet did not show any clinical signs of obstruction, similar to the animals of the present study. Dirksen (2002) suggests that underlying diseases affecting the motility and mucosa of the abomasum, as well as feed quality, are important factors for developing sand impaction in domestic cattle. The clinical signs in cattle with sand impaction are rather unspecific and attributable to forestomach dysfunction or intraluminal intestinal obstruction. In those cases reported in the literature, sand impaction has been diagnosed by exploratory laparotomy or necropsy.

A result of the CT analyses in the present study that had not been reported previously is the notable accumulation of radiodense material in the *Atrium ruminis*. During dissections, we had not considered it feasible

to sample this material separately, due to the large connection between the preceding rumen chambers and this part. The atrium links the dorsal and ventral rumen chambers to the reticulum, and dense material that collects in the ventral rumen should next be passed on to the Atrium, followed by the reticulum (Wyburn, 1980). Apparently, more intense contractions in the reticulum, as compared to the atrium, prevent a similar accretion of radiodense material at the former site. Why accumulations in the atrium had not been visible in the goats, with the coarsest silica particles, could be related to species-specific differences in motility, or in rumen fluid throughput (Clauss et al., 2006). Differences in the degree of fluid throughput between ruminant species that cannot be explained as adaptations to the natural diet could, in theory, also represent adaptations to the degree to which food is contaminated with dust or grit in the natural habitat (Przybyło et al., 2019), but this hypothesis still awaits testing.

Hatt et al. (2019b) summarized findings in comparative herbivore dental anatomy and dental wear, as well as chewing physiology, that are affected by the existence of a washing mechanism in the forestomach of ruminants. These include the general difference in hypsodonty between equids and ruminants even when ingesting similar natural diets (Damuth and Janis, 2011a), differences in wear traces between hindgut fermenting and ruminant ungulates on putatively similar diets

(Mihlbachler et al., 2016), distinct differences in feeding experiments between the wear effect of the same diets on live rabbits and *in vitro* assays versus live ruminants (Müller et al., 2014b; Karne et al., 2016b; Ackermans et al., 2018; Ackermans et al., 2019b), the catching conclusion that for ruminant tooth wear as measured by microtexture, “dust does not matter” (Merceron et al., 2016b), differences in mandibular anatomy (Fletcher et al., 2010; Zhou et al., 2019) and chewing forces (Williams et al., 2011) as well as chewing patterns (Dittmann et al., 2017). Whether the presence of a washing mechanism influences the comparative feeding selectivity, with nonruminants avoiding grit-contaminated food to a higher degree than ruminants, remains to be investigated.

Various reasons may exist why the same abrasives might cause different wear patterns in different species, ranging from dental anatomy, differences in enamel characteristics, to different chewing patterns and forces. For ruminants and – by extrapolation, potentially also camelids – the forestomach washing mechanism is just another one. Ideally, addressing the use of tooth wear as a habitat or climate proxy should not cause trench lines between supporters of taxon-specific and taxon-free approaches (DeSantis et al., 2018; Fraser et al., 2018; Clauss, 2019), but will simply benefit by prudent interpretations and attempts to ensure constancy among the species used. In our opinion, categorically

rejecting taxon-free approaches, even if a relative constancy of species distributions has been achieved, may be as unproductive as categorically rejecting the use of methods that account for differences between taxa, such as statistics accounting for the phylogenetic structure of the data. The mechanisms of herbivore tooth wear most likely still hold much to discover.

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M6

**A long last supper: response of dental microwear texture
in sheep (*Ovis aries*) fed various concentrations and sizes of
quartz abrasives for 17 months**

Nicole L. Ackermans, Daniela E. Winkler, Louise F. Martin, Thomas M. Kaiser, Marcus
Clauss, Jean-Michel Hatt

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A long last supper: response of dental microwear texture in sheep (*Ovis aries*) fed various concentrations and sizes of quartz abrasives for 17 months

Nicole L. Ackermans^{a,*}, Daniela E. Winkler^{b,c}, Thomas M. Kaiser^c, Marcus Clauss^a, Jean-Michel Hatt^a

^a*Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, CH-8057 Zurich, Switzerland*

^b*Applied and Analytical Paleontology, Institute for Geosciences, Johannes Gutenberg University Mainz, 55099 Mainz, Germany.*

^c*Center of Natural History, University of Hamburg, 20146 Hamburg, Germany.*

*Corresponding author.

Email: nicole.ackermans@uzh.ch

Address: Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, 8057 Zurich

ABSTRACT

External abrasives ingested along with the herbivore diet are considered main contributors to dental wear, though how different abrasive sizes and concentrations influence wear remains unclear. Dental microwear texture analysis (DMTA) is an established method for dietary reconstruction which describes surface topography on a micrometre scale. In the present study, a long-term feeding experiment was performed on sheep (*Ovis aries*), fed seven diets of different abrasiveness. Our aim was to discern the individual effects of abrasive size and concentration on dental wear, applying DMTA to four tooth positions where mesowear had already been measured. Microwear textures differed between individual teeth, but showed no gradient along the molar tooth row, and the strongest differentiation of experimental groups was achieved when combining data of all maxillary molars. Overall, a pattern of

increasing height, volume and complexity appeared with increasing abrasive size, and when compared to the control, the small abrasive diets showed a polishing effect. Results indicate abrasive size as more important for DMTA traces than abrasive concentration. And when compared, mesowear and DMTA do not seem to record the same physiological process, though further exploration is required to understand how microscopic traces created by abrasives translate quantitatively to absolute wear.

Keywords: microtexture, feeding experiment, ruminant, diet, herbivore, abrasives



Supplemental information:

Table S1: Microtexture parameter description and grouping

Table S2: Descriptive statistics of all parameters on all individual teeth.

Table S3: Descriptive statistics of all parameters for all diets on individual maxillary molars

Table S4: Descriptive statistics of parameters for all diets on antagonists M2 and m2

Table S5: P values and standard deviation for all parameters on combined maxillary molars

Table S6: P values and standard deviation for all parameters on M1

Table S7: P values and standard deviation for all parameters on M2

Table S8: P values and standard deviation for all parameters on M3

Table S9: P values and standard deviation for all parameters on m2

Figure S1: Boxplots for all parameters on all individual teeth

Figure S2: Boxplots for select parameters on all diets for the maxillary molar gradients

Figure S3: Scatterplots for select parameters for all diets for the maxillary molars

Supplemental information – PCA

Introduction

Tooth wear is an important factor for dietary reconstruction, and herbivores have been well documented to show contrasting dental wear for different diets. A diet of browse is thought to induce more attrition, resulting in teeth with sharp molar cusps (Fortelius and Solounias, 2000), and on a microscopic level, an enamel surface with wear facets dominated by pits and a microtexture with low roughness (Schulz et al., 2013b; Schulz et al., 2013d; Winkler et al., 2019a). A diet of grass, on the other hand, is

thought to include internal and often adherent external abrasives that wear down the tooth material, resulting in blunted molar cusps, scratch-dominated microwear facets, and a microtexture with high roughness, anisotropy, and low complexity (Scott, 2012b; Schulz et al., 2013b). It is still under debate whether phytoliths – the hard opaline silicates internal to plants – or external abrasives, like dust, are the main cause of tooth wear in herbivores (reviewed in Winkler et al., 2019a), though the general consensus is that they both contribute at least to some extent.

Baker et al. (1959) were the first to observe microscopic pitting on sheep teeth caused by phytoliths, in what is thought to be the first application of the 2D microwear technique, before (Walker et al., 1978a) introduced 2D microwear as a systematic method to deduct herbivore diets. Microwear is used to analyse microscopic wear on a tooth's surface, quantifying small scratches and pits in order to ascertain the diet of a specimen. As the turnover for these surface marks is a matter of days or weeks (Teaford and Oyen, 1989c), the short timeframe of this proxy has earned it the moniker of “the last supper” effect (Grine, 1986). Adaptations of industrial systems have led to less use of 2D microwear in favour of 3D techniques using scanning-electron, and confocal-disc scanning microscopes (reviewed in Scott et al., 2006; and Fig. 1 in Calandra et al., 2019). Today, the two leading techniques in dental microwear texture analysis (DMTA) are

scale-sensitive fractal analysis (SSFA), and 3D surface texture analysis (3DST). Both SSFA and 3DST quantify the texture of the whole surface as a unit (Clementz, 2012) and use profilometry at sub-micron resolution to represent 3D geometry and distribution of topographic features on the tooth's enamel surface (Scott et al., 2006; Schulz et al., 2010). SSFA uses mainly four parameters (*Asfc*, *epLsar*, *HAsfc*, and *Tfv*) to describe various surface features that vary with scale of observation. With SSFA, grazers tend to exhibit high anisotropy and either systematically low (Ungar et al., 2007; Scott, 2012b; Merceron et al., 2014) or high (Schulz et al., 2013b; Schulz et al., 2013d; Winkler et al., 2019a) complexity values, while browsers show the opposing tendency. This is most likely due to inter-microscope differences, and is a technical issue that requires further calibration and research. 3DST characterises wear features using over 40 parameters subdivided into six categories of analysis (direction, furrow, isotropy, ISO 12781, ISO 25175, and motif). Using 3DST, grazers generally show high surface roughness, high peaks in great quantity, deep dales and a general pattern that is low in variability. Browsers, on the other hand, generally show flatter surfaces with lower peaks. Overall, both SSFA and 3DST are used to determine diet based on its inherent mechanical properties, though the exact connection between these properties and the wear traces they leave is so far mainly derived from logical reflections

rather than empirical relationships (Kaiser et al., 2015). This is mainly due to the lack of rigorous diet characterisation linked to DMTA effects. Consequently, controlled feeding experiments are the only means by which to corroborate the interaction between microtexture formation and dietary properties.

In palaeontology, microwear and DMTA serve to reconstruct the diet of a specimen around its time of death, though studies comparing them to stomach content (Merceron et al., 2010a; Purnell et al., 2012; Kubo et al., 2019) rarely find correspondence between the measures, indicating that microscopic wear is more of an average signal of at least a few days. Combining microwear/DMTA with other dietary proxies such as mesowear and isotopes (Ecker et al., 2013; Rivals et al., 2015; Brent Jones and Desantis, 2017) can reveal a wide scale of information concerning paleoenvironmental reconstruction, though again, the different techniques do not always correspond to each other in terms of dietary reconstruction. This is generally explained by the assumption that they represent different scales of time resolution, with mesowear and isotopes representing long-term dietary signals of months, and microwear and DMTA representing short-term dietary signals of days or weeks. Yet, how diet translates through different proxies at different scales remains poorly understood.

Microwear and DMTA have been measured on several non-mammalian taxa,

including reptiles (Winkler et al., 2019b), and fish (Purnell et al., 2012), but mostly on mammals (Ungar et al., 2007; Schubert et al., 2010; Merceron et al., 2014; Brent Jones and Desantis, 2017; Aiba et al., 2019), including extinct humans (Pérez-Pérez et al., 2003). Experimental studies have also been performed on a wide variety of mammalian taxa, such as possums (Kay and Covert, 1983), primates (Teaford and Oyen, 1989b; Teaford and Oyen, 1989c; Teaford et al., 2017a), including humans (Romero et al., 2012), rabbits (Schulz et al., 2013d), sheep (Hoffman et al., 2015; Merceron et al., 2016a; Ramdarshan et al., 2017a), guinea pigs (Winkler et al., 2019a), voles (Kropacheva et al., 2019), rats (Mihlbachler et al., 2019), and *in vitro* with various chewing machines (Hua et al., 2015b; Daegling et al., 2016; Karme et al., 2016a).

A long-term feeding experiment was performed by Ackermans et al. (2019a), (**Manuscript M3**) to track the development of tooth wear over time between groups of sheep (*Ovis aries*) fed experimental diets of varying abrasiveness. The use of experimental diets in this setting presents an opportunity to complete a growing dataset for the investigation of DMTA on multiple diet groups and tooth positions, complementing measurements of mesowear I and II (Ackermans et al., in prep.-a) (**Manuscript M3**), absolute wear (Ackermans et al., in prep.-b) (**Manuscript M4**), and investigation of the rumen washing mechanism (Hatt et al., Submitted) (**Manu-**

script M5); thus, providing a complete overview of 17 months of a controlled dental wear experiment.

In the present study we used DMTA to investigate microscopic tooth wear on various tooth positions. Microtexture was measured on the maxillary M1-M2-M3 molar gradient, along with the maxillary M2's antagonist, the mandibular m2; and the results were analysed in these groups, and individually. By feeding sheep with seven diets of increasing abrasiveness, we aimed to measure the effect of silica abrasive size and concentration on various teeth and compare several proxies to observe this pattern on multiple scales.

We expected a pattern of increasing wear with increasing abrasive size and concentration, as well as a decreasing pattern of wear on the gradient from the maxillary M1 towards the M3, and higher wear on the mandibular m2 in comparison to its maxillary counterpart.

Material and methods

Samples

The teeth analysed in this study belonged to the specimen collection related to the feeding experiment described in (Ackermans et al., in prep.-a) (**Manuscript M3**). Forty-eight ewes and one wether (*Ovis aries*, n=49) were fed experimental diets during 17 months in a controlled experimental setting. The animals were divided into seven groups based on weight, and fed pelleted diets of varying abrasiveness. Using a lucerne based pellet,

three sizes (\varnothing 4 μm , \varnothing 50 μm , and \varnothing 130 μm) and two concentrations (4% and 8%) of quartz abrasives were added to create the different diets, leading to a total of seven diets: a control diet with no added abrasives (C), small abrasives (4%*s* and 8%*s*), medium abrasives (4%*m* and 8%*m*), and large abrasives (4%*l* and 8%*l*). A complete description of the diets is reported in Ackermans et al. (2019a). At the end of the experiment the skulls were skeletonised and housed in the mammal collection at the Center of Natural History of the University of Hamburg.

Microwear

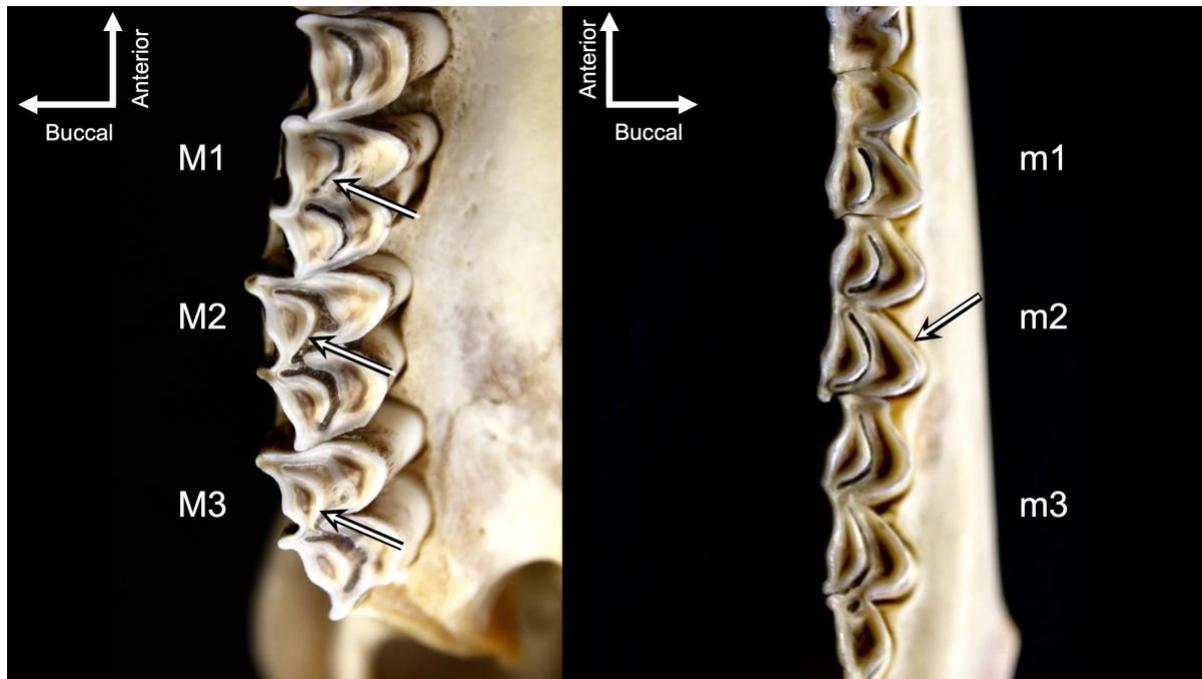
DMTA of the samples was performed following the standard technique in Schulz et al. (2013b). We applied 46 DMT parameters using the ISO 25178 (roughness), motif, furrow, isotropy, ISO 12871 (flatness), and Scale-sensitive fractal analysis (SSFA). Parameters were grouped into the following categories for simplification: area (*Sda*, *Sha*, *mea*), complexity (*Sdr*, *nMotif*, *Asfc*), density (*Sal*, *Spd*, *medf*), direction (*Std*, *Str*, *Tr1R*, *Tr2R*, *Tr3R*, *IsT*, *epLsar*), height (*S10z*, *S5p*, *S5v*, *Sa*, *Sku*, *Sp*, *Sq*, *Ssk*, *Sv*, *Sxp*, *Sz*, *meh*, *madf*, *metf*, *FLTt*, *FLTp*, *FLTq*, *FLTv*), peak sharpness

(*Spc*), plateau size (*Smc*, *Smr*), Slope (*Sdq*), and volume (*Sdv*, *Shv*, *Vm*, *Vmp*, *Vmc*, *Vv*, *Vvc*, *Vvv*) (description in Table S1). Excluding the animals that died early during the feeding experiment and those unsuitable for microtexture analysis due to cracked or chipped cusps, we were left with a sample size of $n=37$.

For each specimen, four sites on the same facet were analysed, when possible: the posterior facet of the anterior cusp on the second enamel band (from the buccal side) for the right maxillary M1, M2, and M3 (Fig. 1), using the same facet on the fourth enamel band when the previous was damaged or too worn. In the case where both sites were not measurable in a right tooth, the left side was used for all tooth positions. The antagonist facet to the M2 for was selected for each specimen on the mandibular m2: the anterior facet of the posterior cusp on the fourth enamel band (from the buccal side) was selected on the m2 when the ideal facet was measured on the M2 (Fig. 1), however when the fourth enamel band was measured on the M2, the second enamel band was selected on the m2. In preparation for measurements, the target facets were cleaned with rubbing alcohol and then moulded using putty (Provil® novo type 3 dental putty).

Figure 1. Wear facet measured using dental microtexture analysis on teeth of sheep (*Ovis aries*).

Arrows indicate wear facets where microtexture was sampled and scanned. The left image represents the right maxillary molar row and the right image represents the right mandibular molar row.



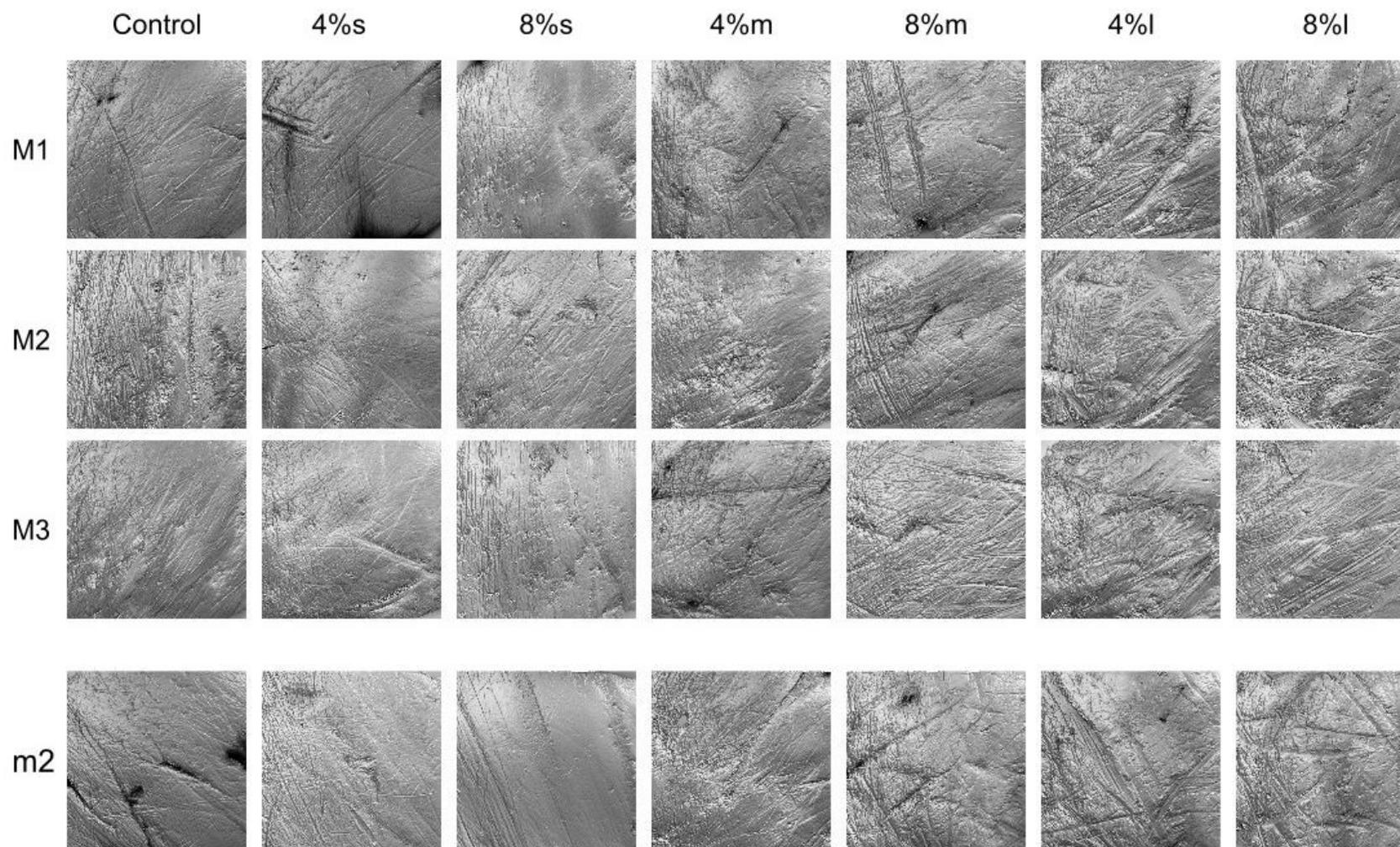
The plate was then fixed to the table of the confocal disc scanning microscope μ surf custom (NanoFocus AG, Oberhausen, Germany) with a blue LED (470 nm) and high-speed progressive-scan digital camera (984x984 pixel), set to a 100x long distance objective (resolution in x, y = 0.16 μ m, step size in z = 0.06 μ m), housed at the Center of Natural History of the University of Hamburg. Four scans were rendered per facet of 160x160 μ m, taking care not to overlap scanning areas. The data was then separated by tooth or grouped into upper molars (M1+M2+M3) or antagonist teeth (M2+m2) and then and processed with MountainsMap Premium v. 7.4.8803 Software (DigitalSurf, Besançon, France,

www.digital surf.com). 3D scan images were created using MountainsMap (Fig. 2

Statistical analysis

Statistical analysis was carried out using the R software (R Core Team, 2017, version 3.3.1) using the packages xlsx (Dragulescu, 2014), rJava (S, 2016), doBy (Højsgaard and Halekoh, 2016), and R.utils (Bengtsson, 2016). Analysis were performed on the following groupings: all teeth, maxillary teeth (M1, M2, and M3), antagonist group (M2, and m2), as well as each individual tooth; M1, M2, M3, and m2.

Figure 2. Microwear texture photosimulations of the enamel surface, on experimental sheep fed diets of varying abrasiveness for 17 months. Images created using the MountainsMap software. Specimens imaged here are ZMH10941 (Control), ZMH10942 (4%*s*), ZMH10922 (8%*s*), ZMH10910 (4%*m*), ZMH10956 (8%*m*), ZMH10919 (4%*l*), and ZMH10913 (8%*l*). The same facet was imaged for the maxillary M1, M2 and M3, and the antagonist facet of M2 was imaged for the mandibular m2. Images are all taken with the same orientation with anterior to the top and buccal side to the left of the figure, each image represents a 160 x 160 μm facet.



Significance was tested using a combination of three statistical tests. As DMT data is generally non-normally distributed, we used the procedure of Wilcox (Wilcox, 2012), applying a robust T1 way heteroscedastic Welch-Yuen omnibus test, coupled with a heteroscedastic pairwise “Dunnett’s T3 test”, with significances confirmed using the robust heteroscedastic rank-based test according to Cliff (pairwise comparison with bootstrap) – methodology following that of Calandra et al. (2012) and Schulz et al. (2013b). The significance level was set to 0.05. Boxplots and selected biplots were created for each grouping, with different letters indicating significant differences at $P < 0.001$, and significance indicated with * if $P < 0.05$, ** if $P < 0.01$, and *** if $P < 0.001$). The principal component analysis (PCA) was created in R v3.3.1 using the built-in function *prcomp()* with singular value decomposition (SVD) and *ggbiplot* (Vu, 2011) for visualisation. Predicting variables were z-transformed and the PCA based on correlations in order to ignore different scale of variables. A Kaiser-Meyer-Olkin measure of sampling adequacy (value > 0.5) using the function “*paf*” of the R package *rela* (Chajewski, 2009) and

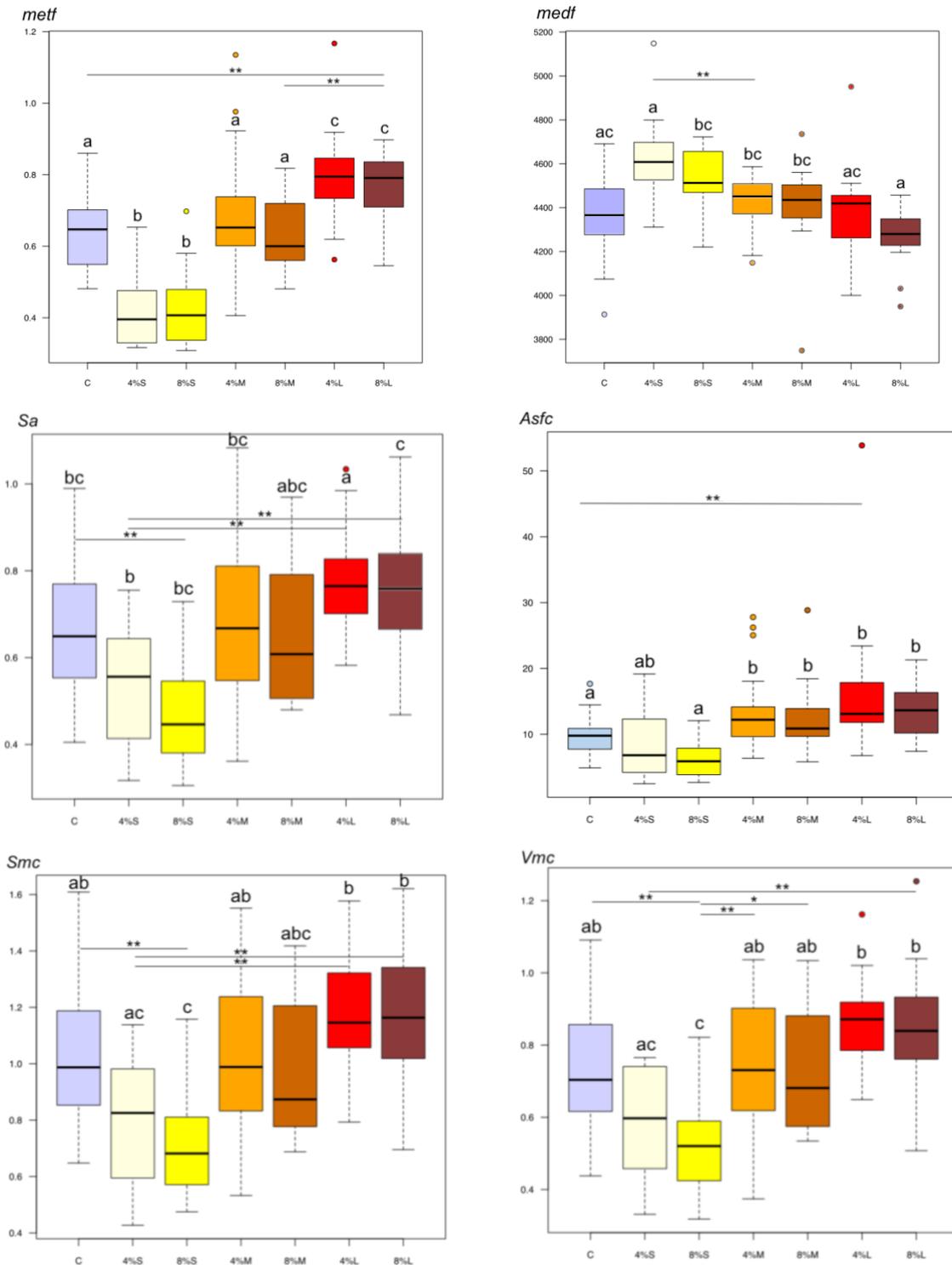
Bartlett’s Test indicate that the formal requirements for conducting a PCA are met by our data. Detailed results for the PCA are given in the supplements (Supplemental Information - PCA). In order to facilitate different statistical approaches to data analysis by other researchers, the original data is provided as an electronic supplement.

Results

Boxplots and descriptive statistics for all individual measurements are provided in the supplemental information (Tables S1-9, Figure S1). Figure 2 represents sample images of the surface texture of the enamel facet for all teeth from a single individual from each diet group. Visually, texture directionality is more or less consistent between maxillary molars and shows an opposing directionality on the mandibular molar. A clear polishing effect is visible across teeth for the 8%*s* diet and the 4%*s* diet to a lesser extent, and surface roughness appears to increase with dietary abrasive size, with the largest abrasives creating large, deep scratches. Boxplots of select parameters for all maxillary molars combined are provided in Figure 3.

Figure 3. Boxplots of combined molars (M1+M2+M3)

Different letters indicate a significant difference of <math><0.001</math>, same letters indicate no differences. Significant differences of metf and *medf* represent depth and density of furrows respectively, *Sa* represents mean height and surface roughness, *Asfc* represents complexity, *Smc* represents plateau size, and *Vmc* represents volume.



Biplots and principal component analysis

Plotting complexity against anisotropy (*Asfc* vs. *epLsar*) showed no clear diet group distinction for all maxillary molars combined (Fig. 4A), though the small abrasive dietary groups separated somewhat from the other groups. Using complexity (*Asfc*) on its own provided better differentiation between diet groups (Fig. 3 *Asfc*). Plotting depth and density of furrows against each other (*metf* vs *medf*) for all maxillary molars combined showed a lot of scatter in the control and medium abrasives group, though both small abrasive groups were well separated by high density and shallow furrows (*medf* and *metf*). The large abrasive groups showed the opposite distribution of low *medf* and high *metf* (Fig 4 B).

This parameter combination showed a similar resolution for the dietary groups as compared to a PCA with the 12 best separating parameters. On PC1, groups were mainly separated by height and volume parameters, with the large abrasive diets showing larger parameter values. On PC2 the smallest abrasives were mainly separated by density of furrows (*medf*) (Fig. 4 C).

Maxillary molar wear gradient

Overall, there were very few significant inter-tooth differences (Table S3) and there was no significant indication or visual pattern of a gradient along the upper molar row (Fig. S2).

Antagonist tooth (M2 and m2) wear patterns

An obvious pattern was seen for directional parameters for the small- and medium- abrasive diets when comparing the two second molars (Table S4, Fig. S1). The texture direction (*Tr1R*) of the mandibular m2 showed wear features predominantly aligned at higher angles (predominantly buccally oriented) and distributed more randomly (Fig. S1, *Tr1R*), while the maxillary M2 showed strong features oriented predominantly at lower angles (Fig. S1, *Tr1R*: 50°, *Tr2R*: 40-60°).

Diet differences for all teeth

Area parameters showed no clear pattern in relation to diet for combined or individual teeth, though the values in mandibular m2 were slightly lower when compared to other teeth (Tables S2-4, Fig. S1 Area).

Complexity parameters showed significant differences between diets (Table S2). When upper molars were combined, they displayed an increasing trend in complexity (Fig. 3, *Asfc*), and the same was true for individual teeth, with M1 and m2 showing increasing complexity (*Sdr*, *Asfc*) with increasing particle size, and smaller particles showing lower complexity as compared to the control group. However, that pattern was not visible in the M2 and M3, except for in the 8% diet, which showed lower complexity (*Sdr*, *Asfc*) (Fig. S1 Complexity).

Density parameters showed decreasing density of furrows (*medf*) with increasing particle size when all maxillary molars were combined (Fig.

3, *medf*), and when considering individual teeth, the same pattern was seen for all but the M2. There was also a tendency for increasing density of peaks (*Spd*) with increasing particle size in the M1 and lower m2 (Fig. S1 Density). In general, the diets with larger particles created fewer features (*medf*) but also more peaks (*Spd*).

For direction parameters, when upper molars were combined, they showed some variation but the direction of wear features (*TrIR*) was predominantly between 50-60°. For individual teeth, *TrIR* was the same in M1 and M2 (around 50°), and more variable in the M3, while it was much higher in m2 (80-100°). Small abrasive diets showed lower isotropy (*IsT*) in the M1 and M3, and M3 has higher anisotropy (*epLsar*) for the larger abrasives (Fig. S1 Direction).

Most height parameters indicated that when combining the upper molars, the smallest abrasive diets of either concentration created lower height and less overall roughness (Fig. 3, *metf*, *Sa*), a pattern that was also visible for individual teeth. In the mandibular m2, a tendency of increasing height (*meh*) and roughness (*Sq*) with increasing abrasive size appeared; this trend, however, was not as pronounced in the individual maxillary teeth. The most important finding is that depth of furrows (*metf*) was consistently lowest in the small abrasive diets and increased with particle size for all teeth. Only in the maxillary M3 did the 4% diet diverge from the general pattern (Tables S2-4, Fig. S1 Height).

Combined molars showed plateau size increasing significantly with abrasive size (Fig. 3, *Smc*), and the same trend was visible for individual teeth (Fig. S1 Plateau size).

The slope parameter indicated only a slight tendency for increasing slope with increasing particle size in combined molars, and this trend was much more pronounced for M1, M2, and m2 (Fig. S1, *Sdq*).

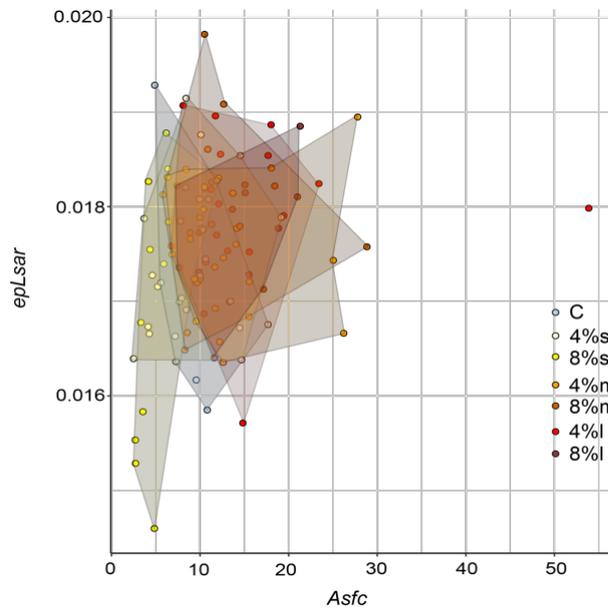
Finally, volume parameters, similarly to height parameters, indicated significant increases in topography with abrasive size for combined molars (Fig. 3, *Vmc*) as well as individual molars (Fig. S1 Volume).

Discussion

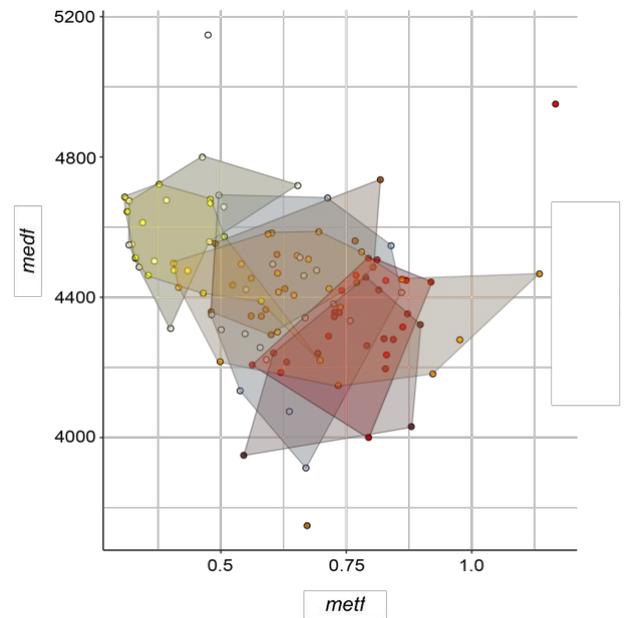
After sheep were fed experimental diets of increasing abrasive size and concentration for 17 months, applying DMTA to the sheep's teeth revealed no molar gradient between the maxillary M1-M3, and few differences were observed between the second molar and its mandibular antagonist. Individual tooth differences appeared, and combining all scans provided the best overall dietary distinction. In terms of diet, the small-abrasive diets created a polishing effect on the enamel surface, and diets with increasing abrasive size created increasing enamel surface roughness, while abrasive concentration showed little effect. Finally, these results do not perfectly correspond to those resulting from the mesowear analysis on the same teeth.

Figure 4. Biplots and PCA of combined maxillary molars (M1+M2+M3). Diets are the following: C for control diet, small (s), medium (m) and large (l) abrasives at 4% and 8% concentration in the diet. A: Biplot of *Asfc* (complexity) and *epLsar* (anisotropy); B: Biplot of *medf* and *metf* (mean density and depth of furrows, respectively); C: PCA of 12 strongest parameters

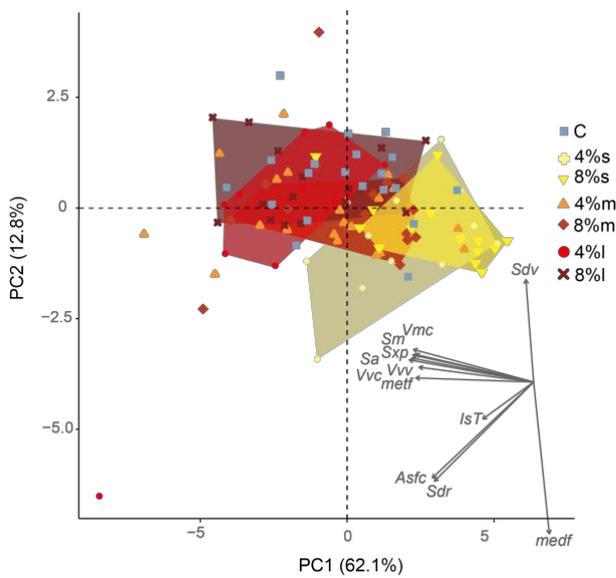
A



B



C



Individual and grouped tooth differences

Contrary to our expectations, there was no indication of a wear gradient measured by DMTA along the maxillary molar row. When recording DMT or 2D microwear in mammalian herbivores, the maxillary M2 is most often used as the tooth of reference. This is because based on the sequence in which the molars erupt (Monson and Hlusko, 2018), and hence wear macroscopically, we expect the M1 to show more wear on the occlusal surface, the M2 to show intermediate wear, and the M3 to show the least amount of wear. This macroscopic wear gradient has been recorded in literature for many species, including cercopithecine monkeys (Gantt, 1979), ibex (Fandos et al., 1993), giraffes (Clauss et al., 2007), rhinoceroses (Taylor et al., 2013), and between premolars and molars in wild equids (Taylor et al., 2016). Whereas macroscopic wear may be related to the age of the tooth, this is not necessarily the case for DMTA, as this pattern of increasing wear does not appear in our results. Here, the M2 had the least discrimination between dietary groups, when number of significant parameters is taken as a measure, out of the three maxillary molars evaluated. In other microtexture studies, molar measurements are often combined into a single dataset regardless of position to maximise sample size (Ungar et al., 2007; Merceron et al., 2010a; Schulz et al., 2013b; Merceron et al., 2014). Tooth position and gradient effects have been investigated using DMTA in ungulates. Schulz et al. (2010) observed a molar gradient

for density (*Sal*), and texture direction (*Std*) parameters in wildebeest, which they interpreted to be linked to rumination and the high curvature of the tooth row in wildebeest as compared to zebra. Ramdarshan et al. (2017a) found no such gradient along the tooth row in a controlled feeding trial with experimental sheep. Antagonist teeth were also investigated by Ramdarshan et al. (2017a), and when applying SSFA parameters, no differences were observed. This is similar to our own results, where the only significant differences between M2 and m2 were related to texture direction (*TrIR*). These differences in texture direction may be caused by the mortar and pestle function of the maxillary and mandibular antagonist (Kaiser and Fortelius, 2003). Our results also showed M3 having the highest number of significant parameters, while M1 showed the best differentiation between groups, and that the dietary differentiation for the mandibular m2 was comparable to that of M1. With individual teeth each showing differing trends in our results, it is important to reflect these differences in the interpretation, by considering to measure the complete molar tooth row, rather than focusing on the M2, which may not capture a complete signal. For differentiating between dietary groups, the best discrimination was achieved by combining all maxillary molars, showing that regardless of tooth position, more scans across different teeth may be preferable for parameter significance.

Dietary differences

All parameters considered, a general trend of larger abrasives resulting in larger and higher microtexture features is evident for all teeth. Different abrasive concentrations generally only had negligible effect on the creation of microtexture features. The only exception was the 8%*s* diet, which often created a much stronger polishing effect than the 4%*s* diet, as visible in Figure 2. The base pellets used for the control diet already created a fair amount of surface roughness. The addition of small abrasives to the pellets allowed the diet to “polish-off” that roughness on the whole surface texture, while the other abrasive sizes did not show a polishing effect. Specifically, decreasing density of peaks (*Spd*) with particle size indicates that smaller particles abrade the peaks. This also explains the lower number of significant parameters for the medium-sand diets, as their range of wear was often overlapping with that of the control diet (Fig. 3). A similar “polishing effect” has been previously discussed by Sanson et al. (2017), in that a large particle can cause a scratch, and smaller particles could then wear away the edges of this scratch, resulting in the appearance of less roughness. In an experiment testing the effect of external abrasives on sheep using DMTA, Merceron et al. (2016a) saw no effect from the addition of less than 0.8% of dust ($\varnothing > 100 \mu\text{m}$, comparable in size to our medium-sand diet, though in lower concentrations) to a browse- or graze-based diet for 70 days. Therefore, abrasives in the range of

100 microns may not create a distinctive microwear texture on small ruminant teeth, a result emulated in the present study.

In our results, the height and volume parameters show the best dietary discrimination (e.g. *Sa*, *metf*, *Vmc*), and height, volume, and as well as complexity parameters relatively consistently increase with abrasive size for all teeth. *Medf* and *metf* (mean density and depth of furrows, respectively) were particularly strong parameters in the present study, which also was the case for guinea pigs and lepidosaurs (Winkler et al., 2019a; Winkler et al., 2019b), and additionally, Schulz et al. (2010) also noted high *medf* values specifically in ruminants compared to non-ruminants of similar diets. In the present study, *Asfc* (complexity) on its own showed strong discrimination between dietary groups, with large abrasives resulting in high complexity (Fig. 3). As expected, direction parameters rarely differed between teeth or diet groups, as they reflect chewing direction, which should be consistent within a species. Pathologies and age might induce the variation visible when teeth are combined for other parameters (Fig. 3).

Mesowear scored on the same dataset (Ackermans et al., in prep.-a) (**Manuscript M3**, Fig. 5) only showed a visual pattern of increasing macroscopic wear with abrasive size and content when the difference in score was calculated, and even then, there were no significant differences between diets after 17 months. The present results corroborate these

findings to an extent. Height, volume, and complexity parameters did indeed show strong dietary separation, and were especially high for the large-abrasive diets. However, abrasive concentration did not show as much as an effect for DMTA, and in this case, contrary to mesowear measurements, the control diet was not the least abrasive and indeed not distinguished from the medium-abrasive diets. It seems dietary abrasiveness does not necessarily create the same effect in DMTA and mesowear. Potentially, more abrasive diets lead to a blunt tooth profile in mesowear but more peaks with larger volume in DMTA. On the other hand, less abrasive diets lead to sharper molar cusps in mesowear, but less peaks, fewer furrows and overall “flatter” surfaces in DMTA (Schulz et al., 2013b). Seemingly, a more abrasive diet (i.e. with larger abrasives) leads to more wear on all scales, with more overall tissue loss and deeper microscopic furrows, and higher concentrations of abrasives might not necessarily aggravate this trend. Whether deeper furrows and higher roughness translate to more tissue loss is still uncertain, and microscopic wear may in some cases be the result of tissue deformation rather than tissue loss, where a scratch represents merely a plastic deformation of the enamel (Lucas et al., 2013b). In particular, DMTA cannot quantify the frequency by which the microwear traces have occurred on a tooth; in theory, deeper furrows, while detectable, might be created at a much lower frequency than small furrows, and hence not represent a difference in

the rate of tissue loss. There is as of yet no simple translation of diet abrasiveness to larger furrows, higher tissue loss, more mesowear, and more absolute wear. DMTA might therefore rather be considered as a measure of microscopic traces left by a diet, instead of actual wear in the sense of quantitative tissue removal, until a better understanding of wear acquisition and development is reached on both the microscopic and macroscopic scales.

An important note is that ruminants such as sheep, may be less sensitive to macroscopic tooth wear than non-ruminants, owing to the capacity to wash off external abrasives in their rumen (Hatt et al., 2019a); a mechanism also applicable to pelleted diets. Schulz et al. (2010) suggest cud to be less abrasive than the initial ingesta, due to washing. These authors suggested that that a meso-distal wear gradient may still appear in ruminants, because the abrasives are washed out of the food bolus by saliva during the ingestive processing from the front to the rear of the oral cavity – a hypothesis that is difficult to test. Additionally, Merceron et al. (2016a) state that most microscopic abrasion is caused while ruminating cud, though it may be lessened, as digestion softens plant fibres, creating less of an abrasive effect. The washing mechanism may be less sensitive to small particles that are less prone to being washed and can continue to abrade the teeth during rumination, though this remains to be further explored. Both washing and pre-digestion are wear-reduction strategies that may result in

ruminant teeth losing tissue at a slower rate than other ungulates, an issue to take into account when applying dental wear proxies on both macroscopic (Ackermans et al., 2018) and microscopic scales in these animals. Therefore, wear traces of the same diets could be expected to be more distinct in non-ruminant animals.

Conclusion

When analysing the molar teeth of sheep fed experimental diets with DMTA, there was no indication of an increase in microscopic traces along the molar row. However, DMT parameters indicated individual tooth differences, questioning the use of M2 as the ideal tooth of reference as it is currently being used. Regardless of tooth position, combining scans allowed for the strongest dietary differentiation, emphasizing that a higher number of measurements is recommendable. Experimental diets with increasing abrasive size were distinguished by an increasing complexity, height and volume of the enamel surface textures, and when compared to the control diet, the smallest abrasives created a polishing effect.

Mesowear and DMT analysis applied to teeth from the same dataset provided some similar patterns, of increasing in wear and roughness, notably for the 4% diets. However, there were also several discrepancies, and the two proxies did not follow a corresponding pattern for all diets. This may indicate that these proxies may not only be recording abrasion at different scales, but may be recording different

process altogether. Whether microscopic traces on the enamel surface translate to a tooth's absolute wear is unknown. Further research is necessary to better understand if indeed wear is created by processes that can be differentiated microscopically, and how it translates to the macroscopic scale.

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3 | Conclusion and outlook

The aim of this body of work was to establish an experimental precedent, following the development of tooth wear over time, while obtaining more precision on the duration of the dietary signal under different abrasive conditions. In this, multiple dietary proxies with increasing timescales were applied (microwear, absolute wear, mesowear III, and mesowear I) to create an overview of the mechanics of diet-related wear, reflected by each technique and its respective scale. We also explored the theoretical basis surrounding tooth wear; including the evolution of hypsodonty, the reaction of dental tissue to wear, the effect of different types of abrasives, and how they all pertain to the wear process.

Our findings resulting from both the goat (**Publications P1-5, Manuscript M2**) (Ackermans et al., 2018; Ackermans et al., 2019a; Ackermans et al., 2019b; Hatt et al., 2019; Stauffer et al., 2019; Schulz-Kornas et al., in prep.) and sheep experiments (**Manuscripts M3-6, Publication P5**) (Ackermans et al., 2019a; Ackermans et al., in prep.-b; Ackermans et al., in prep.-c; Ackermans et al., in prep.-d; Hatt et al., Submitted) created an experimental basis for long-term tooth wear analysis, specifically concerning small ruminants. We first established the possibility of scoring mesowear on 3D images, allowing us to track its development over time (**Publication P1**) (Ackermans et al., 2018), and as a result, both experiments showed mesowear as representing a more general signal than previously expected (**Publication P1, Manuscript M3**) (Ackermans et al., 2018; Ackermans et al., in prep.-b). Experimental diets enriched with either internal or external abrasives had no significant effect on mesowear, though they did show minute visual patterns of increasing wear with abrasive size and concentration. The goat teeth were also measured with mesowear III (**Publication P3**) (Stauffer et al., 2019), but this method did not offer a more precise signal than the original mesowear measurements, and requires additional testing to determine its viability.

Tooth wear was additionally investigated on the microscopic scale, using DMTA (**Manuscript M2 and M6**) (Ackermans et al., in prep.-d; Schulz-Kornas et al., in prep.), showing surface roughness to increase when animals were fed diets with larger abrasive sizes. Surprisingly, higher concentrations of abrasives showed little effect. The diets with the smallest abrasives created a polishing effect on the enamel surface when compared to the control diet. Comparing these DMT results to the mesowear analysis on the same teeth reveals a somewhat different trend: the highest amount of mesowear in the sheep experiment was caused by higher concentrations of abrasives rather than their size. This was not completely surprising, considering microscopic wear and mesowear rarely correspond when applied together (Davis and Pineda-Munoz, 2016), even though they represent different timescales. The interaction between these techniques is not yet fully understood. Still, contrasting results from both

proxies, applied to a same controlled experiment, contradicts the hypothesis that mesowear and microscopic wear measure the same process on different scales (Fig. 5). We believe that rather, microscopic wear and mesowear measure different processes on different scales, and the signals recorded with DMTA are rather microscopic traces than proxies for quantitative tissue loss. In particular, DMTA does not provide information on the rate of trace formation, which may well differ between diets. For example, diets leading to more distinct abrasive DMTA signals may also be subject to more restrained ingestive chewing cycles, where the trace pattern is overwritten by new traces much more slowly than that of less abrasive diets. Also, abrasion on the microscopic scale possibly creates more of a plastic deformation, rather than actual enamel wear *per se*. Sharp cusps on the mesowear scale show “flatter” microscopic surfaces in DMTA, while more DMT roughness is not necessarily translated to macroscopic wear of the cusp shape. How microscopic traces are linked to the absolute wear of enamel and thus macroscopic wear, is a process that remains to be investigated.

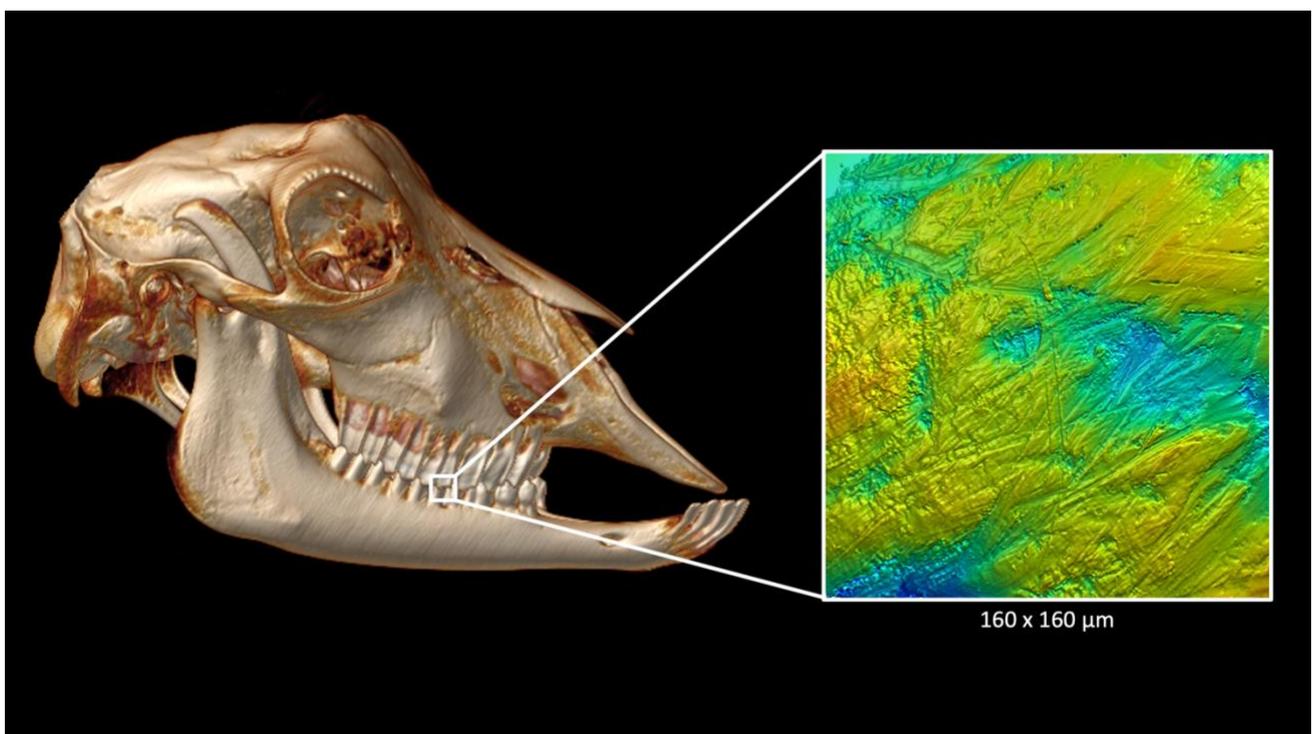


Figure 5. 3D rendering of a sheep skull and a dental microwear texture photosimulation of the enamel surface of the right maxillary M1 at the end of a 17-month feeding experiment.

The experiments also informed upon the previously untested question of how tooth roots react to wear, by measuring the absolute volume of the crown and roots separately in both absolute wear studies (**Publication P2, Manuscript M4**) (Ackermans et al., 2019b; Ackermans et al., in prep.-c). In the goats, crown-wear caused by the increasingly abrasive diets was correlated to a matching increase in root-volume. This pattern, however, was not as clearly visible for the sheep, though they showed

higher crown-volume loss, most likely related to the longer duration of the experiment (**Manuscript M4**, fig. 2) (Ackermans et al., in prep.-c). It is possible the small size of the quartz abrasives added to the sheep's experimental diets were not able to cause an increase in tensile force strong enough to trigger cell proliferation in the roots (Lieberman, 1993). Interestingly, in both experiments, the wear rate was extremely high when compared to other studies on domestic and wild caprids, though the cause is unclear. Digital calliper measurements taken on the 3D renderings of the sheep teeth (**Manuscript M4**, fig 1) (Ackermans et al., in prep.-c) informed on the relatedness of different aspects of tooth morphology and how they wear in relation to each other. In particular the dentin basins on each cusp seem to wear at a similar rate to the cusps themselves. Implementing this simple measurement technique in future studies is a simple way to learn more about tooth morphology, even though it is not strong enough to record diet.

The combination of all of the aforementioned results can be used as an image to explain some aspects of the evolution of hypsodonty. As observed in both absolute wear experiments (**Publication P2 and Manuscript M4**) (Ackermans et al., 2019b; Ackermans et al., in prep.-c), abrasive particles, whether they be phytoliths or external abrasives, do not only leave traces on the tooth surface, they also cause physical wear and loss of dental tissue. We still do not understand how exactly this abrasion happens, but preference trials have shown that ruminants can choose to avoid abrasives, though they may only be able to detect them above a certain size (**Publication P5**) (Ackermans et al., 2019a). Dietary selection, rumination, and hypsodonty have all evolved as protective mechanisms in response to tooth wear inflicted by dietary abrasives. This makes abrasive diets a key factor in the evolution and diversification of herbivores. Further exploring these evolutionary processes in extant species through controlled experiments will continue to expand our knowledge about their extinct ancestors and inform on past environments.

Though this extensive experimental dataset has led us to many answers, as most often is the case in science, it has raised even more questions. As of yet, the tooth wear model of small ruminants as an experimental representation for large mammals, and rabbits as a model for small mammals, are well established. However, if large or small abrasives do not seem to affect the teeth of goats and sheep in a significant manner, and neither do phytoliths on a macroscopic scale, then one might question whether this is also true for non-ruminant herbivores, or if the signal varies between species in general. Moreover, if mesowear represents a more general signal, as this body of work seems to indicate, its scale has yet to be precisely defined. Additional long-term experiments involving a wider variety of species should be considered to explore this line of questioning. Furthermore, the true influence of external abrasives, as well as experimental wear patterns related to browsing vs. grazing diets remains

to be seen. This gap of knowledge exists both on the microscopic and macroscopic scale, and more still needs to be elucidated concerning the relation between these wear proxies.

The discrepancies between mesowear and microwear may have become commonplace in natural settings, but finding them in an experimental case has raised questions about the robustness of these methods. Possibly, an experiment following the development of tooth wear macroscopically and microscopically in young sheep, raised on different phytolith- and silica-based abrasive diets over the long term, could provide further insight on these questions of wear and create a stronger foundation for their application to extinct species. Additionally, an *in vitro* experiment using a chewing machine equipped with various types of teeth could be used to test the effects of various parameters such as abrasive concentration and size, effect of experimental diets, the effect of phytoliths versus grit, etc., in a controlled manner and at gradual scales.

If teeth do indeed show different physiological reactions to increasing levels of abrasion, it remains to be seen which tooth layer causes the increase in volume. Though we suspect the cementum to be the main dental layer causing root proliferation, this again requires experimental confirmation. One solution involves applying imaging techniques with higher resolution capacities than those of medical CT scanners, to animals before and after consumption of heavy-wear diets, perhaps mandated by tooth extraction, as micro-CT is not applicable to live specimens as of yet. This would not only answer our questions related to absolute wear, but also inform on general tooth morphology, and how its different components react in relation to each-other when subject to heavy wear.

The many proxies that exist to inform on a specimen's diet (**Manuscript M1, Publication P3, Fig. 4**) (Stauffer et al., 2019; Ackermans, in prep. -a) are an indication of the plethora of information that can be recovered from a single tooth. Cementum lines and crown wear can help infer an animal's age, while the cusp shape can provide information on a herbivore's dietary group. With microwear, one can glean a general idea of the last few suppers a specimen consumed before its death; and even if all other traces are not measurable, stable isotope analysis can predict a general herbivore diet of C3 or C4 plants. Fortunately, teeth are the most abundant remains of the mammalian fossil record, and thus our main source of insight into prehistoric life. For extant species, dental wear can be coupled with bio-sample and behavioural data to inform a wide manner of biological and ecological questions. In both extinct and extant research, the rapid advancement of imaging techniques and related technologies constantly elicit new lines of thought, and continue to shine light into the mouth of the beast.

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