Dental microwear texture gradients in guinea pigs reveal that material properties of the diet affect chewing behaviour

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Summary statement

Dental wear analyses are often used for diet reconstruction. We show how different wear gradients develop along the cheek tooth row in guinea pigs depending on the diet they received.

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Abstract

Dental microwear texture analysis (DMTA) is widely used for diet inferences in extant and extinct vertebrates. Often, a reference tooth position is analysed in extant specimens, while isolated teeth are lumped together in fossil datasets. It is therefore important to test whether DMT is tooth position specific, and if so, what the causes for wear differences are. Here we present results from controlled feeding experiments with 72 guinea pigs, which either received fresh or dried natural plant diets of different phytolith content (lucerne, grass, bamboo), or pelleted diets with and without mineral abrasives (frequently encountered by herbivorous mammals in natural habitats). We test for gradients in dental microwear texture along the upper cheek tooth row. Regardless of abrasive content, pelleted diets display an increase in surface roughness along the tooth row, indicating that posterior tooth positions experience more wear compared to anterior teeth. Guinea pigs feedings on plants of low phytolith content and low abrasiveness (fresh and dry lucerne, fresh grass) show almost no DMT differences between tooth positions, while individuals feeding on more abrasive plants (dry grass, fresh and dry bamboo) show a gradient of decreasing surface roughness along the tooth row. We suggest that plant feeding involves continuous intake and comminution by grinding, resulting in posterior tooth positions mainly processing food already partly comminuted and moistened. Pelleted diets require crushing, which exerts higher loads, especially on posterior tooth positions, where bite forces are highest. These differences in chewing behaviour result in opposing wear gradients for plant versus pelleted diets.

1. Introduction

Ingesta processing during chewing generates considerable mechanical stress on teeth and thus modifies the occlusal surface during an individual's life. Food material properties are one important factor amongst many others to consider when trying to understand the dental wear processes that affect the occlusal surface (for a review see Schulz-Kornas et al., 2020). During one chewing

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sequence, the food particles break down and a bolus is formed (Stokes at al., 2013). It is known that external bolus properties as well as food material properties change gradually during that sequence (Foster et al., 2006; Woda et al., 2006; Witt and Stokes, 2015). This in turn could lead to gradual changes of the dental wear along the tooth row. Additionally, in many large mammals (i.e., ungulates), the macroscopic morphology of the molars differs systematically with position in the tooth row (Winkler and Kaiser, 2015a, b) and could further influence gradual changes depending on the size and shape of the molar occlusal area and the force of the power stroke, which both vary between teeth (Pérez-Barberia and Gordon, 1998, 1999). Macroscopic wear gradients with the first molar showing the most and the third molar the least wear have been reported for several ungulates (Fandos et al., 1993; Clauss et al., 2007; Taylor et al., 2016) and for primates (Teaford, 1982, 1983). However, only a few studies have focused on microscopic tooth wear analysis along the tooth row (Schulz et al., 2010; Ramdarshan et al., 2017; Arman et al., 2019; Ackermans et al., 2020).

More often, microscopic wear analyses are applied to successfully differentiate general feeding habits. A common dental wear analysis for diet inferences of extant and fossil mammals, including hominins (for a review see Ungar et al., 2019), is dental microwear texture analysis (DMTA) (Ungar et al., 2003; Scott et al., 2005, 2006; Schulz et al., 2010, 2013a). It has advantages compared to (stereomicroscopic SEM and low-magnification) microwear analysis (DeSantis, 2016), because it is less observer-biased and captures the whole 3D topography of an enamel surface (Scott et al., 2005; DeSantis et al., 2013; Kubo et al., 2017), not only relying on singular wear features (i.e., pits and scratches).

DMTA has been applied to herbivorous ungulates (Ungar et al., 2007; Merceron et al., 2010; Schulz et al., 2010, 2013a; Scott, 2012; Winkler et al., 2013), primates (Calandra et al., 2012; Schulz-Kornas et al., 2019), carnivores with varying levels of bone-consumption (Ungar et al., 2010; DeSantis et al., 2012, 2013), but also to infer diet in non-mammalian taxa such as fish (Purnell and Darras, 2016) and reptiles (Winkler et al., 2019a; Bestwick et al., 2019, 2020).

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Beyond the application of DMTA to detect dietary differences between species or populations, it is of importance to understand how the microscopic wear features form, and what diet properties cause which kind of microwear texture. For example, high surface complexity in hominins like *Paranthropus robustus* was ascribed to with hard object feeding (Scott et al., 2005), but such hypotheses need to be validated in extant taxa, preferably under controlled experimental conditions. However, results from in-vitro indentation experiments recently questioned this correlation (van Casteren et al., 2020), as they could not produce high surface complexity on enamel through contacts with hard foods such as seeds. Feeding experiments with large and small mammals have shed light on the long-lasting debate about which factors contribute more to dental (micro)wear formation - internal (i.e., phytoliths) or external abrasives (i.e., mineral dust) (see Winkler et al. 2019b; 2020a and references within). For sheep, there is evidence that under low grit contamination (~1%) the effect of quartz abrasives ingested with clover or grass silage is negligible, and textural differences are predominantly due to mechanical properties of the plants, and thus most likely their phytolith content (Merceron et al., 2016). On the other hand, 4-8% of fine silt-sized (~4 µm) and fine sand-sized (~150 µm) quartz abrasives added to pelleted diets resulted in either distinctly flatter or rougher microwear textures compared to an abrasive-free control diet (Ackermans et al., 2020). For small mammals, there is abundant evidence that both internal and external abrasives contribute substantially to dental wear (Schulz et al., 2013b; Müller et al., 2014, 2015; Martin et al., 2019), but in different ways. Notably, external abrasives result in microwear textures with significantly higher roughness, deeper, and more complex wear features compared to mineral abrasive-free plant feeds of high phytolith content (Winkler et al., 2019b, 2020a).

If differences in (microscopic) wear pattern (microwear texture) along the tooth row were just an effect of the tooth eruption sequence and time of their exposure to wear, systematic variation in DMTA would not be expected, as the same patterns would emerge on teeth as they erupt, soon reaching an equilibrium. Experiments with small mammals have shown that the turnover time of microwear texture is between 2-3 weeks (Winkler et al., 2020b). If, however, specifics of dental

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morphology or chewing behaviour correspond to position in the tooth row, then systematic variation along the row would be predicted. Schulz et al. (2010) report no significant microwear texture inter-tooth differences in wild zebra (*Equus grevyi*) and wildebeest (*Connochaetes taurinus*); however, they detected specific gradients along the maxillary tooth row. In *E. grevyi*: distal teeth showed lower textural fill volume (a measure of lesions on the enamel surface), likely attributed to distal teeth encountering reduced abrasive particle sizes. In *C. taurinus*, such an effect was absent, which may be attributed to differences in digestive strategy of ruminants versus hindgut fermenters like the zebra. In the former there is a “rumen washing mechanism” (Hatt et al., 2019), leading to less abrasive cud chewed during rumination and possibly levelling texture gradients caused on first ingestion. Studies of other ruminants, i.e. sheep, in controlled feeding experiments support this assertion: Ramdarshan et al. (2017) found no significant wear differences between mandibular teeth, and Ackermans et al. (2020) came to a similar conclusion for the maxillary dentition, although they also highlighted that the upper second molar (M2), which is often employed as the reference tooth position in large herbivorous mammals, had the lowest discriminatory power between experimental diets of varying abrasiveness. Ackermans et al. (2020) further found the strongest differentiation between diet groups when lumping measurements of all upper molars together. This is likely due to the increased number of measurements per feeding group. In macropods, tooth position influenced surface complexity (*Asfc*), with the highest inter-tooth variation being seen between the fourth molar and all other teeth (Arman et al., 2019). In summary, there might be inter-tooth differences in dental wear patterns of large mammals, or even gradients, but evidence is scarce.

For small mammals, inter-tooth differences in microwear texture have not yet been reported. Amongst several other rodents, guinea pigs (*Cavia porcellus*) possess dentitions with similar morphology and occlusal area for each tooth. Their occlusal surface is characterized by a rasp-facet, in which the functional enamel crests form a grinding surface (von Koenigswald, 2016). The permanent dentition already erupts in-utero (Teaford and Walker, 1983) and is comprised of one premolar and three molars (Berkovitz, 1972), all rootless and ever-growing (euhypsodont).

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Therefore, unlike in ungulates (von Koenigswald, 2016), their molar teeth are always in the same wear stage and show no macroscopic wear differences, and do not require considerations of eruption sequence. Guinea pigs are hindgut fermenters and have a unidirectional food passage, so that chewing of less abrasive cud like in ruminants will not affect any potential surface texture gradients along the tooth row. Therefore, guinea pigs are an ideal model organism to assess the effect of tooth position on dental microwear texture formation. Moreover, finite element analysis predicted increasing bite forces for guinea pigs from the fourth premolar (P4) to the third molar (M3), with bite forces at M3 exceeding those of P4 by a factor of 1.8 (Cox et al., 2012). We expect that bite force differences are a key masticatory parameter influencing dental microwear texture formation along the tooth row (Kaiser et al., 2016).

To test for a gradient in microwear texture formation in guinea pigs, we analysed all teeth of one side of the upper cheek dentition in 72 individuals. The animals received either a plant diet (lucerne, grass, bamboo) free of adherent mineral abrasives (Winkler et al., 2019b), or a pelleted diet with admixed mineral abrasives of different sizes, shapes, and quantities (Winkler et al., 2020a) for 3-5 weeks. Plant diets were chosen to represent different phytolith contents (lucerne < grass < bamboo) and fed fresh or as hay. We put forward the following hypotheses:

(1) A microwear texture gradient along the tooth row in guinea pigs is detectable, showing increasing surface roughness from the fourth premolar (P4) towards the third molar (M3), due to the increase of chewing forces in the distal direction.

(2) Abrasive-free natural plant feeds are expected to leave a less pronounced wear gradient compared to diets with external mineral abrasives.

If gradients are indeed detectable, using random tooth positions for dietary inference is expected to reduce the resolution of dietary differences. Moreover, such a result would mean that the common practice of lumping isolated teeth of different or unknown tooth positions from fossil species into a single dataset for diet analysis might be problematic. However, in fossil samples, often only isolated teeth are preserved, and different tooth positions lumped together in order to increase sample sizes.

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To test which DMTA parameters still have adequate dietary resolution using randomised tooth positions, we tested correct dietary categorisation when either a random subset of 25% or 75% of all teeth is subjected to the analysis.

2. Material and Methods

2.1 Animal housing and diets

The feeding experiment was performed during September and October 2017 at the Vetsuisse Faculty, University of Zurich, with approval of the Swiss Cantonal Animal Care and Use Committee Zurich (animal experiment licence N° ZH135/16). All animals in this experiment were adult female Dunkin Hartley (HsdDhl:DH) Guinea pigs (3-4 weeks old at the start of the experiment). Guinea pigs belong to the family Caviidae and likely descended from wild *Cavia tschudii* Fitzinger, 1867 (Spotorno et al., 2004), that are adapted to feeding on grasses in their natural habitat of grasslands in South America (Dunnum, 2015; Lacher, 2016). Animal housing conditions and diets are described in detail in Winkler et al. (2019b, 2020a). Animals received their designated diets for a duration between three and five weeks, except for the IsoL group, which was kept on the diet for 8 weeks (Table 1). This time span has been found to be sufficient to form dietary informative wear (Winkler et al., 2020b). Guinea pigs that received plant diets (initial body mass, 263 g ± 14 g) were housed in six groups of six individuals (total: 36) in sheltered outdoor stables (2.25 m^2 each), each protected from direct sunlight and rain, equipped with an infrared lamp, a ground covered in a thick layer of sawdust, two large wooden shelters, and two plastic tubes, two open dishes, and two nipple drinkers. Stables were sheltered to the sides by canvas to prevent airborne dust from contaminating the forages, as were stable floors below the sawdust. Guinea pigs kept on pelleted diets (initial body mass: 249.4 g ± 12.5 g SD) were housed in groups of six or three (six individuals per diet, total: 36) in inside stables (0.58 m^2 each), equipped with an infrared lamp, a ground covered in a thick layer of sawdust, a large shelter and two plastic tubes, one open dish for water and food and one nipple drinker. All groups were provided with water (supplemented with vitamin C at 200 mg/L) and food for ad libitum consumption and received no extra gnawing material.

2.2 Dental microwear texture analysis

After the feeding periods, animals were sacrificed, and after maceration of the skulls in an enzyme bath, their teeth were moulded for surface texture measurements. These were acquired using the high-resolution confocal disc-scanning surface measuring system µsurf Custom (NanoFocus AG, Oberhausen, Germany) with a blue LED (470 nm) and high-speed progressive-scan digital camera (984 x 984 pixel), set to a 100x long distance objective (resolution in x, y = 0.16 µm, step size in z = 0.06 µm, numerical aperture = 0.8), and processed with MountainsMap Premium v. 7.4.9175 Software (DigitalSurf, Besançon, France, www.digitalsurf.com). Following Winkler et al. (2019a, 2020a), we measured preferably the anterior enamel band on all cheek teeth of one maxillary tooth row, obtaining 3-4 non-overlapping scans per tooth (see Fig. 1 for exemplary 3D images for each dietary group). If the anterior enamel band was damaged on both sides, we switched to the third enamel band (99 out of 1033 scans). Forty-six surface texture parameters were quantified using the ISO (International Organization for Standardization) 25178 (roughness), motif, furrow, isotropy, ISO 12871 (flatness) and scale-sensitive fractal analysis (SSFA) implemented in MountainsMap Premium (Table S1). Parameters were grouped according to their main characterising feature in the following categories: area, complexity, density, direction, height, peak sharpness, plateau size, slope, and volume. Processing followed the protocol described by Schulz et al. (2010, 2013) and applied in Winkler et al. (2019b, 2020a), except for the flatness parameters *FLTp*, *FTLq*, *FLTt*, *FLTv* (ISO 12871), where we adjusted the S-filter (λs) and used a Gaussian filter of 0.0025 mm instead of the default value of 0.025 mm, because the default filter resulted in NA-values for several individual surfaces. Results for these parameters therefore deviate from those published in Winkler et al. (2019, 2020a).

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2.2.1 Dental Microwear Texture parameters

The vast majority of the DMTA parameters are field parameters (Blateyron, 2013) using every data point of the tooth surface (field or area) measured, i.e. characterising surface heights, slopes, complexity, and wavelength content. In addition, there are feature parameters that consider specific points, lines, or areas of the measured surface. In the ISO 25178, parameters are grouped according to five main algorithm groups used for the calculation (Table S1). For example, it applies to the group of height parameters using the statistical distribution of the height along the z-axis; the spatial parameters using the spatial periodicity of the surface; the hybrid parameters using the spatial shape; function and related parameters calculated from the material ratio curve; feature parameters derived from segmentation of the surface into motifs (dales and hills) according to the watershed method. In addition to 30 ISO 25178 parameters, we used 3 motif, 3 furrow, 4 isotropy, 4 ISO 12871 (flatness) and 2 scale-sensitive fractal (SSFA) parameters. In sum, this results in 46 parameters analysed. For clarity reasons we sorted these parameters in nine parameter groups consisting of similar parameters as follows:

Area: Parameters describing overall surface area, or area of specific features (hills, dales) are summarised here. Diets of higher abrasiveness generally result in larger values of the area parameters.

Complexity: Surface complexity refers to distribution of wear features, with dental microwear textures characterised by differently sized features, which overlap each other, having high complexity. Hard-object feeding and more abrasive diets generally result in larger complexity, except for the parameter *nMotif* (number of motifs).

Density: Features of the surface such as peaks or furrows can be characterised according to their mean density. Peak density is often larger in soft diets, while furrow density is higher with presence of higher loads of external abrasives.

Direction: Both, parameters indicating periodic motifs of the surface in X and Y direction, and dominant direction of wear features are included in this category. The directionality can be in one or

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several directions (e.g., having a predominant texture direction, "lay") for anisotropic surfaces or being without predominant texture direction (isotropic). Anisotropy is larger in individuals feeding on diets with high quantities of internal and/or external abrasives (grazers).

Height: All parameters that refer to either overall (mean) height differences, or height measures between specific features of the surface (e.g., depth of furrows, maximum peak height) are summarized in this category. Diets of higher abrasiveness generally result in larger height differences on the dental surface topography.

Peak sharpness: There is only one parameter referring to form of peaks, being either sharper or rounded.

Plateau size: Bearing ratio parameters *Smr and Smc* are aimed at characterising functional behaviour of the surface, such as wear and lubrication. The areal material ratio *Smr* is used to set the amount of bearing area (plateau) remaining after a certain depth of material is removed. The inverse areal material ratio *Smc* can be used to describe an optimum crevice volume necessary for a sealing surface to allow for some lubrication entrapment to flush out wear particles.

Slope: Completely flat surfaces have no slope, thus less abrasive diets resulting in flat surface texture profiles have lower slope values.

Volume: Parameters referring to volume measures of the complete surface, or particular features, are summarised in this category. Since large volumes measures could be achieved by deepening of surface features there is a strong dependency to height parameters, thus, abrasive diets lead to overall larger volume parameters.

2.3 Statistics

For each of the 46 DMT variables, the effects of diet and tooth position was evaluated using linear models with the variables *Diet* (6 levels) and *Tooth* (4 levels), as well as the interaction between them, as fixed effects. Tukey's HSD *post hoc* test for multiple pairwise comparisons was applied where necessary (α-level = 0.05 for all tests, Table S2; S3.). To account for non-independence incurred by

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multiple measurements of the same set of individuals, we included Individual as a random factor (Kristensen and Hansen, 2004). Normality and heteroscedasticity of residuals was tested using Shapiro-Wilk's and Levene's tests, respectively. If these assumptions were not met, data were ln-transformed and, if normality was not achieved by data transformation, ranked data were used. Analyses were carried out in the software R v 3.5.2 (R Core Team 2018), with the package lmerTest used for mixed effects linear models (Kuznetsova et al., 2017). Data from natural and pelleted diets were analysed separately.

If wear responses do differ across tooth positions, the common practice in palaeoecological research of lumping isolated teeth to improve sample size is likely to inflate especially Type II errors, i.e., reducing the probability of detecting significant diet differences across specimens. To test for this effect, we used a randomized numerical experiment that simulated the 'lumping' approach, in order to determine the likelihood that any set of isolated teeth would reflect the overall expected trend. Here we ranked the six diet treatments per experiment from 1 to 6 according to the means for each DMT variable, and then evaluated correlations (using Spearman's Rank correlations) between overall rankings with rankings obtained from random subsets of data, i.e. subsets containing a randomized assortment of tooth positions. Randomized subsets included first 25% and then 75% of the total data, sampled without replacement. Sub-sampling was repeated over 10^4 iterations, but correlations were only performed when all six diets were represented in the sub-sample. The mean and standard deviation (SD) of the R_s coefficient were determined, as well as the proportion of significant ($p <$ 0.05) correlations, for each variable. These simulations were carried out in the software R v. 3.5.2, again repeating the analyses for natural and pelleted diets separately (Table S4; S5).

Principal Component Analysis (PCA) including 10 DMTA parameters (employing median parameter values per specimen) was conducted in JMP Pro (v.14.2.0) using varimax rotation and covariances rather than correlations, separately for the natural and pelleted diets (Fig. 2). Selection of parameters was based on the PCA included in Winkler et al. (2020a).

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2.4 Terminology

When referring to "chewing behaviour", we incorporate several aspects in this term. We refer to how much forage is taken into the oral cavity and loaded on parts of the dentition, where the processing is happening (i.e., anteriorly, or posteriorly), jaw motion and how much force is exerted during mastication. Note that most inferences on chewing behaviour of our experimental animals are drawn indirectly from the observed dental microwear texture pattern, with the exception of the interpretation of the supplementary movie.

When using the term "abrasion", we are referring to mechanical processes occurring during food-tooth contacts described in dental literature (Mair, 1992; Eisenburger and Addy, 2002a, b; Grippo et al., 2004) as friction between a tooth and exogenous agents, i.e. food itself, or ingested (mineral) particles such as grit and dust. It results in material deformation (plastically deformed enamel) and/or displacement, which may result in visible wear features (furrows, dales, peaks) and/or tissue loss.

3. Results

3.1 Natural diets

Guinea pigs feeding on natural plant diets (Table 1) were observed to continuously ingest plant matter (Supplementary movie) in what we describe as a "conveyor belt" feeding strategy.

In DMTA, strong gradients of increasing or decreasing parameter values along the tooth row were mostly seen in the diet groups Gd (grass dry), Bf (bamboo fresh), and Bd (bamboo dry) but not on other diets (Figs. 1; 3; S1). Therefore, the interaction diet x tooth was often significant (Table S2).

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Area:

All area parameters showed decreasing values along the tooth row towards the posterior end for all diets. Both diet and tooth position significantly affected area parameter values, but only for *Sda* a significant interaction between diet x tooth was found (Table S2). The P4 had significantly larger values than other tooth positions, but for the Bf group also more variance was found (Table S2, Figs. 3; S1).

Complexity:

Asfc and *Sdr* increased along the tooth row for Ld (Lucerne dry), Gf (grass fresh), and Bd (bamboo dry) (Fig. S1). Both bamboo diets and Gf showed significantly larger values compared to both lucerne diets for both parameters. *nMotif*, on the contrary, was smaller in both bamboo diets compared to the lucerne and grass diets, and a significant interaction for diet x tooth was found (Table S2). *nMotif* also increased from P4-M3 for Lf, Gd, Bf, and Bd, while for Ld and Gf the parameter values were more variable, and no clear trend was visible. The P4 generally showed significantly lower complexity values compared to all molar teeth.

Density:

Spd and *medf* increased along the tooth row, regardless of diet. This trend was less expressed in Ld and Gf due to higher variability especially in M2 and M3 (Fig. S1). Overall, both bamboo diets showed lower *Spd* and *medf* than all other diets. For *Sal*, a decreasing trend was seen in Gd and Bd; the other diets were more variable. Here a significant interaction between diet x tooth was found (Table S2).

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Direction:

There was no clear pattern in parameters relating to the main texture direction (*Str*, *Sdr*, *Tr1*, *Tr2*, *Tr3*). Concerning the overall orientation of features, *IsT* decreased along the tooth row in the grass and lucerne diets, while it increased in Bd. In contrast, *epLsar* showed the opposite pattern, with an increase (or no gradient) in the lucerne and grass diets, and a decrease in Bd. Bf showed high variability for P4 and no clear gradient (Fig. S1). The interaction diet x tooth was significant for *Str* and *IsT* (Table S2).

Height:

16 out of 18 height parameters (with the exception of *madf* and *FLTv*) showed a significant interaction between diet x tooth (Table S2). There were two distinct patterns, with the diets Lf, Ld and Gf showing no or only a minor gradient between tooth positions, while Gd, Bf and Bd showed a significant decrease in parameter value posteriorly along the tooth row (Figs. 3; S1).

Peak sharpness:

No significant effect of tooth position was found for *Spc*, but the interaction between diet x tooth was significant (Table S2). Lf had significantly smaller values than the other diets. For Ld, Gf and Bd, P4 had smaller values than the other tooth positions, while for Bf P4 was larger (Fig. S1).

Plateau size:

There was a significant interaction between diet x tooth for *Smc* and *Smr* (Table S2). The lucerne diets showed no gradient along the tooth row, for Gf values slightly increased (*Smc*) or decreased (*Smr*), while for the bamboo diets P4 had significantly larger (*Smc*) or smaller values compared to the other molar tooth positions (Fig. S1).

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Slope:

Ld, Gf and Bd showed a gradient of increasing *Sdq* along the tooth row (Fig. S1). For Lf, Gd and Bf, no clear trend was visible. Generally, P4 showed significantly lower values compared to M1 and M3, except for Bf.

Volume:

Volume parameter indicated two distinct patterns, with the diets Lf, Ld, Gf showing no distinct change along the tooth row, while for Gd, Bf and Bd values significantly decreased from P4 to M1, and in several instances along the complete tooth row (Figs. 3; S1). All volume parameters except for *Shv* and *Vvv* showed a significant interaction between diet x tooth (Table S2).

3.2 Pelleted diets

During the experiment, guinea pigs feeding on pelleted diets were not captured on video while feeding. All pelleted (Table 1) diets caused strong gradients in microwear texture along the tooth row (Fig. 1) of either increasing or decreasing parameter values (Figs. 3; S1). Therefore, almost no significant interaction between diet x tooth was found (Table S3).

Area:

Diet and tooth individually had significant effects on all area parameters (Table S3). Parameter values increased along the tooth row, with a less expressed gradient in 4sS and 4lVA (Figs. 3; S1).

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Complexity:

Diet and tooth individually had significant effects on all complexity parameters (Table S3). *Asfc* and *Sdr* increased along the tooth row for all diets. Only for 4lVA, M1 did not follow that pattern and showed lower values compared to P4, M2 and M3. The diets IsoL and 4sS showed less pronounced gradients (Fig. S1). For *nMotif*, there was a significant decrease in parameter values from P4 to M1, and a tendency for decreasing parameter values along the whole tooth row.

Density:

All density parameters showed a significant effect of diet and tooth individually (Table S3). *Sal* increased along the tooth row for all diets, but the M3s for 4sS and 4lVA did not follow the general pattern and had lower values compared to M2s. *Spd* and *medf* decreased from P4-M2, with similar or higher values for M3 compared to M2 (Fig. S1).

Direction:

There was no clear pattern and high variability in parameters relating to the main texture direction (*Str*, *Sdr*, *Tr1*, *Tr2*, *Tr3*) and orientation of wear features (*IsT*, *epLsar*) (Fig. S1). Diet had a significant effect on *IsT* and *epLsar*, with 4sS and 8sS showing significantly higher *epLsar* than C, 4lS and 4lVA, and 4sS and 8sS showing significantly smaller *IsT* compared to C and 4lS (Table S3).

Height:

All height parameters (except *Sku*) displayed a distinct, significant gradient of increasing parameter values along the tooth row for almost all diets (Figs. 3; S1). Only for 4sS, M1-M3 are very similar, while P4 still was distinctly smaller.

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Peak sharpness:

Peak sharpness (*Spc*) displayed a significant diet x tooth interaction (Table S3). Values generally increased along the tooth row for IsoL, 8sS, 4lS and 4lVA, slightly increased for 4sS, and were similar between tooth positions for C (Fig. S1).

Plateau size:

Both plateau size parameters showed a significant effect of diet and tooth individually (Table S3). *Smr* showed distinct gradients for decreasing parameter values along the tooth row, while *Smc* increased (Fig. S1). For IsoL and 4sS, the gradient was less expressed, but still P4 was significantly different to the other teeth.

Slope:

Sdq either increased along the tooth row, or was significantly smaller in P4 compared to M2 and M3 (Table S3, Fig. S1).

Volume:

All volume parameters showed a significant effect of diet and tooth individually (Table S3). Volume parameters displayed significant gradients of increasing parameter values along the complete tooth row, or from P4-M2 (Figs. 3; S1). For the diets IsoL, C, 4sS and 4lVA, M3 values were in several parameters lower compared to M2, but still higher than M1.

3.3 Principal Component Analysis

Principal components 1 and 2 accounted for more than 75% of variation observed for Guinea pigs receiving either natural or pelleted diets when all teeth were included in the analysis (Fig. 2). PC1 mainly reflects increasing surface height and volume, while surface complexity increases along PC2. The dietary spaces of Guinea pigs receiving natural diets is separated between diets of lower

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abrasiveness (Lf, Ld, Gf) and diets of higher abrasiveness (Bf, Bd) mainly along PC1, with Gd lying in between. For Lf, Ld and Gf, all tooth positions cluster together, while for Bf and Bd, the P4 is separated along PC1.

Dietary spaces of Guinea pigs receiving pelleted diets hugely overlap. Within each diet group, a separation of P4/M1 and M2/M3 can be observed along PC1, and for the 4lVA group also along PC2.

3.4 Randomized tooth sample (simulated fossil assemblage)

For natural diets, correlations between diet group rankings based on observed versus randomly subsampled data were significant on < 70% of occasions for all but 16 parameters (*Sal*, *medf*, *Sda*, *mea*, *nMotif*, *S5v*, *S10z*, *Sq, Sv*, *Sxp*, *Sz*, *meh*, *FLTq*, *Sdv*, *Vmc*, and *Vvv*; Table S4). This result was consistent for both 25% and 75% subsets. The best correlation between diet and DMTA parameter was found for *Sdv* (mean $R_s > 0.91$), followed by *S10z* (mean $R_s > 0.89$) and *FLTq* (mean $R_s > 0.88$). For pelleted diets, only *mea* (mean $R_s > 0.85$) and *Sdv* (mean $R_s > 0.84$) resulted in significant correlations between observed and randomized subsets with > 70% frequency significantly ranked diets for draws comprising either 25% or 75% of all teeth (Table S5).

4. Discussion

Dental microwear textures from guinea pigs feeding on pelleted diets generally exceeded those of animals on natural plant diets in enamel surface roughness. They showed larger wear features (dales, furrows, plateaus) as compared to those feeding on plant diets. The least abrasion was observed in both lucerne (Lf, Ld) and the fresh grass group (Gf), while dry grass (Gd) and both bamboo groups (Bf, Bd) displayed DMT patterns with overall larger roughness, indicating that these diets were more abrasive (Winkler et al., 2019b). Amongst the pelleted diets, those containing 4% fine sand-sized quartz (4lS) and volcanic ash (4lVA) stood out with the greatest height (for 4lS) and complexity (for 4lVA) values. Large mineral abrasives have been found to result in rougher surfaces, signified by larger height and volume parameters, while smaller mineral abrasives display a surface

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polishing effect (Winkler et al., 2020a). Furthermore, large angular mineral particles resulted in more complex DMTA patterns (Winkler et al., 2020a). For a more detailed discussion of implications from these results, see Winkler et al. (2020a). In the following discussion, we will focus solely on the different dental wear gradients observed between pelleted and natural diets; the details of how material properties of plants or mineral abrasives affect dental microwear texture formation are beyond the scope of the present study and discussed in Winkler et al. (2020a).

4.1 Different chewing behaviour on pellets and plants

We observed a strong gradient of increasing surface roughness along the maxillary tooth row in the guinea pigs fed pelleted diets (Figs. 1; 3). The gradient was similar regardless of whether guinea pigs received an abrasive-free pelleted diet or a pelleted diet containing different concentrations and sizes of external mineral abrasives. For individuals receiving fine silt-sized quartz abrasives in 4% or 8% (mean grain size 4.4 µm, 4sS and 8sS) concentration or 4 % volcanic ash (mean grain size 96 µm, 4lVA), the gradient was often restricted to P4-M2, with M3 showing values closer to M1. This increased roughness was most pronounced in area, height, and volume parameters, indicating larger wear features on the posterior tooth positions (Fig. 3). In former studies, we found that in ungulates from the wild (Schulz et al., 2013a) as well as guinea pigs feeding on natural diets (Winkler et al. 2019b), large area, height, and volume parameter values are characteristic of diets of high abrasiveness. However, due to the experimental setting in our current study, the increasing parameter values along the tooth row in five of the six pelleted diets indicate that in addition to dietary abrasiveness, other masticatory parameters (i.e., bite force, distribution of food within the oral cavity, mechanical food properties) influenced the wear pattern. This pattern of increasing surface roughness along the tooth row was consistent, which is also evident from the mostly not significant diet x tooth interaction for pelleted diets (Table S3).

Surprisingly, the guinea pigs feeding on natural plant diets showed not only a less pronounced wear gradient, but often an opposed trend to the guinea pigs receiving pelleted diets (Figs. 1; 3). While the low abrasive lucerne diets Lf, Ld and fresh grass diet Gf showed almost no differences in DMTA between tooth positions, the more abrasive dry grass diet Gd and bamboo diets Bf and Bd exhibited distinct decreases in surface roughness along the tooth row. Therefore, chewing behaviour of guinea pigs feeding on pelleted diets and guinea pigs feeding on plant diets seem to be different, which is likely due to differences in food ingestion and distribution within the oral cavity.

Guinea pigs have a grinding dentition with serial enamel ridges forming an occlusal surface of rasp-facets (Berkovitz and Shellis, 2018), that is adapted to feeding on grasses, which consist of slender leaves with blades and stems. Cylindrical compressed pelleted diets have a very different geometry compared to leafy grass blades, and we propose that pelleted diets require to be fractured by crushing rather than reduced by grinding. Typically, crushing dentitions differ in morphology compared to grinding dentitions. Teeth adapted for crushing typically are more mortar and pestle shaped (Ungar, 2015), bunodont, and covered by a thick enamel cap. The thin enamel ridges and narrow teeth of guinea pigs are not well suited for crushing and might even be susceptible for damage if used in this manner. Cox et al. (2012) calculated that bite forces increase along the tooth row in guinea pigs, with forces being 1.8x higher on the M3 as compared to the P4. In combination with an unusual chewing mode, the processing of pelleted diets is apparently prone to wear posterior teeth more in comparison to anterior teeth. In contrast to natural diets, pellets also seem not be reduced initially by gnawing using the incisors. When feeding guinea pigs with pellets and pellet-sized pieces of carrot, Byrd (1981) only recorded gnawing cycles for carrot feeding. He further recorded longer activity of the deep masseter, superficial masseter, and medial pterygoid when guinea pigs chewed pellets compared to carrots. These muscles are most important during mandibular elevation, which indicates that pellets were chewed with greater force. We therefore hypothesise that pelleted diets are either processed by loading all teeth simultaneously with a new "haul" of food (implying that with increasing chewing force, posterior teeth experience higher loads and thus more wear). Alternatively, pelleted diets are processed to a higher degree at the rear dentition, in order to exert higher forces for easier fracture of the diet. Both scenarios would explain

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how higher forces at the rear of the dentition result in higher abrasion, expressed in higher surface roughness.

When feeding on plants, guinea pigs continuously ingest leaves in a “conveyer belt” feeding strategy, where material is gnawed with the incisors and pushed backwards to the cheek teeth (Supplementary movie). The plant matter is successively reduced in size while it is shifted further back into the oral cavity. Therefore, the actual processing of plant matter on the posterior part of the dentition is less as compared to the front, as material arriving at the M3 has already been comminuted to some extent by the P3-M2, and hence, despite the different forces, the M3 is more likely to meet comminuted material. We also suggest that the already size reduced plant parts are more easily immersed in saliva, which additionally softens and hydrates them. The processed plant matter thus probably quickly becomes softer and less abrasive. Most abrasion by ingested plant matter can therefore be expected at the anterior part of the dentition, when leaves are still larger in size and drier. This would explain why we observe decreasing surface roughness along the tooth row for the more abrasive plant feeds (Figs. 3; S1).

On the contrary, pellets are not easily drenched in saliva, they need longer to fracture in small parts that can be soaked and become softer. This results in pelleted diets being more abrasive and leading to more tooth wear, which in combination with possibly higher bite forces leads to increased wear at the rear of the dentition. We therefore partly accept hypotheses 1 and 2, but note that plant feeds not only show a less expressed wear gradient along the dentition, but have a different wear pattern from pelleted diets, which is likely attributed to different chewing behaviour on pelleted and natural plant feeds.

4.2 Randomised tooth sample

The analysis of the randomized tooth sample (simulating a fossil assemblage) indicated that with more expressed DMT gradients along the tooth row, and with a less distinct DMTA dichotomy in the possible diets, correct ranking of diets using random tooth positions becomes less correct. For

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pelleted diets, only *Sdv* and *mea* had a high frequency (> 70%) of correct diet rankings regardless tooth composition of the sample (Table S5). Guinea pigs feeding on natural plant diets, on the other hand, had a less pronounced gradient along the tooth row for the low abrasive diets (Lf, Ld, Gf), and only a distinct gradient for the higher abrasive diets (Gd, Bf, Bd) (Figs. 3; S1), and generally showed a dichotomy of three low-abrasion and three high-abrasion diets irrespective of tooth position. This led to a greater degree of variation across diets which accounts for the more significant diet rankings overall (Table S4), and to a higher number of DMTA parameters (16 out of 46) that discriminated well between diet extremes in the randomized tooth sample (*Sal*, *medf*, *Sda*, *mea*, *nMotif*, *S5v*, *S10z*, *Sq,* *Sv*, *Sxp*, *Sz*, *meh*, *FLTq*, *Sdv*, *Vmc*, *Vvv*). Most DMTA measures significantly reflect diet rankings, but the postulated differences in chewing behaviour mean that signals were not consistent for all teeth, or at least signals for each tooth differed in intensity.

In general, these results imply that differences in most DMT variables across individuals are strongly affected by the specific teeth (representing different tooth positions) in the sample, and that "lumping" approaches should therefore proceed with caution. Therefore, we note that accounting for tooth position is necessary when evaluating DMT within and across samples, either by keeping specific teeth separate, or including this as a factor in statistical analyses. That said, DMT parameters like *Sdv* and *mea* appear to retain good resolution of diet-related wear differences across the different natural and pelleted diets and could still be used as focal measures for samples that, by necessity, comprise teeth from multiple positions. Overall, height, volume, area, and density parameters performed best for dietary classification for all diets tested in this study, which suggests that these are the most stable parameters that most reliably record dietary differences.

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5. Conclusions

Guinea pigs fed with natural plant foods show different dental microwear texture gradients along the tooth row than guinea pigs fed with pelleted diets. We attribute this observation to combined effects from different ingestion and food processing behaviour together with bite force gradients along the tooth row. While plant diets are continuously comminuted employing the whole length of the dentition, pelleted diets are likely crushed using the posterior molars, where highest bite forces are exerted. The guinea pig dentition is not adapted for such a chewing behaviour, which likely results in more dental wear observed on posterior molars when chewing pellets. Wear gradients can therefore affect diet reconstruction based on DMTA when only isolated teeth are available. Even though correct diet ranking in a randomized tooth sample was possible for natural plant diets, we advise for only comparing the same tooth positions to each other, if possible. In diets of naturally low inherent abrasiveness (browse), and without contamination of external mineral abrasives, inter-tooth differences might be negligible. Otherwise, the parameters *Sdv* and *mea* displayed the best dietary discrimination and could be employed for reliable dietary discrimination in samples that are composed of different tooth positions, for example in fossil assemblages.

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Author contributions

DEW, MC and TT designed the study and feeding experiments, DEW and MC performed the animal experiments and DEW and MR the texture measurements, DC performed statistical analyses, DEW, ESK and DC analysed the data, TMK facilitated dental microwear texture analysis, DEW, MC and TT wrote the first draft of the manuscript that then received input from all co-authors.

Data availability

All original, unfiltered surface texture scans used in this study are stored at the database of the public natural history museum (University of Hamburg, Center of Natural History Hamburg, mammal collection) and can be accessed via the open-source data repository of the University of Hamburg. The supplementary Figure S1 is also available under the same DOI (doi: 10.25592/uhhfdm.9163).

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Figures

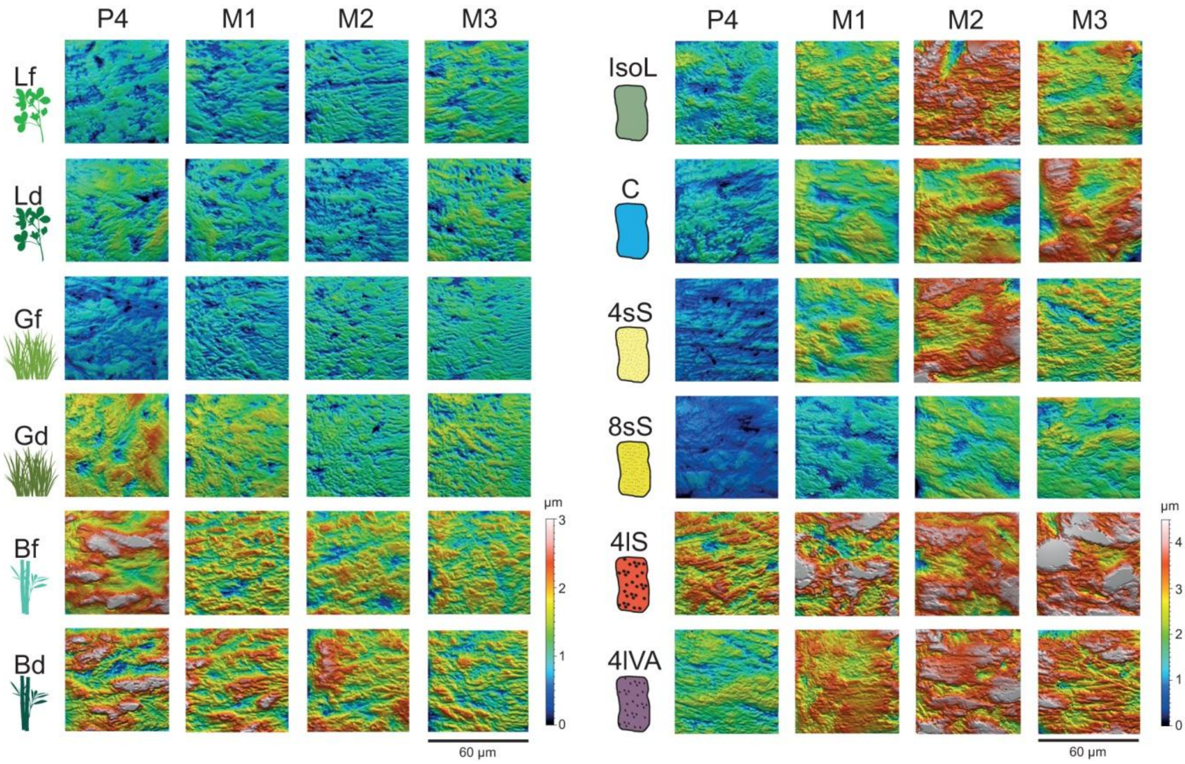

Figure 1. Exemplary 3D images of dental microwear textures for each tooth position (P4 to M3) from one individual per diet group. Left plate depicts natural diets, right plate pelleted diets. Note that all scans are scaled to the same vertical scale given within each plate. Blue and green colours refer to low topographic profile height, while yellow and red colours relate to larger height. White areas represent the largest elevation of microwear texture profiles. Note that profile height is similar between tooth positions, or slightly decreasing along the tooth row, for natural diets (left), while height increases along the tooth row for all pelleted diets (right). **Lf** = lucerne fresh, **Ld** = lucerne dry, **Gf** = grass fresh, **Gd** = grass dry, **Bf** = bamboo fresh, **Bd** = bamboo dry, **IsoL** = lucerne-meal based pellet type 1, **C** = lucerne-meal based pellet type 2, **4sS** = 4 % small quartz (~4.4 µm), **8sS** = 8 % small quartz (~4.4 µm), **4lS** = 4 % large quartz (~166.0 µm), **4lVA** = 4 % large volcanic ash (~96.4 µm).

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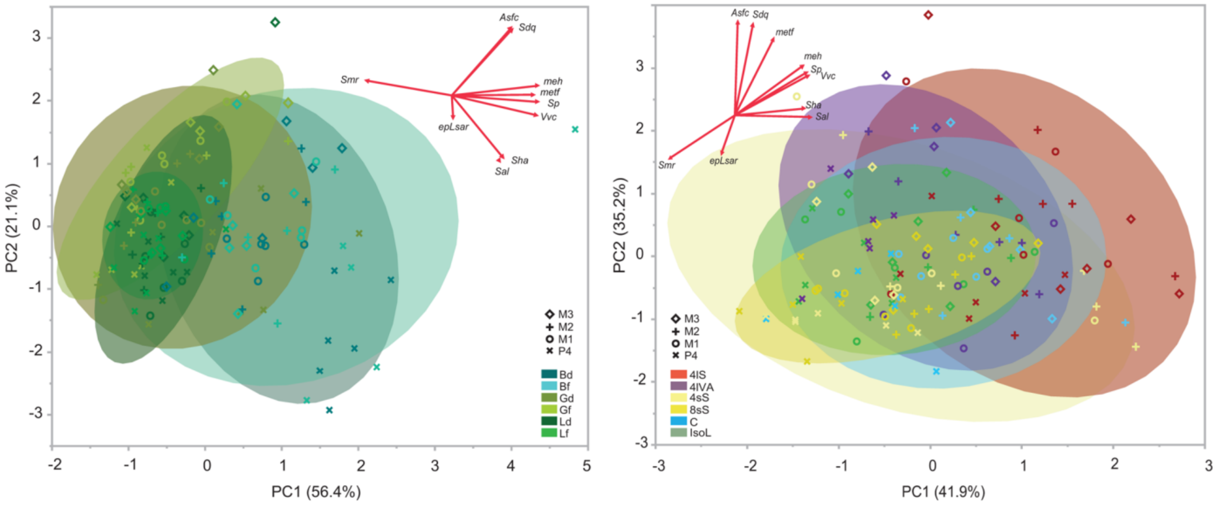

Figure 2. **Principal Component Analysis for all Guinea pigs fed natural (left) and pelleted diets (right).** Ellipses account for 90% of variation and depict dietary space. Tooth positions are indicated using different marker symbols. Note that overlap of dietary space is largest in Guinea pigs receiving pelleted diets.

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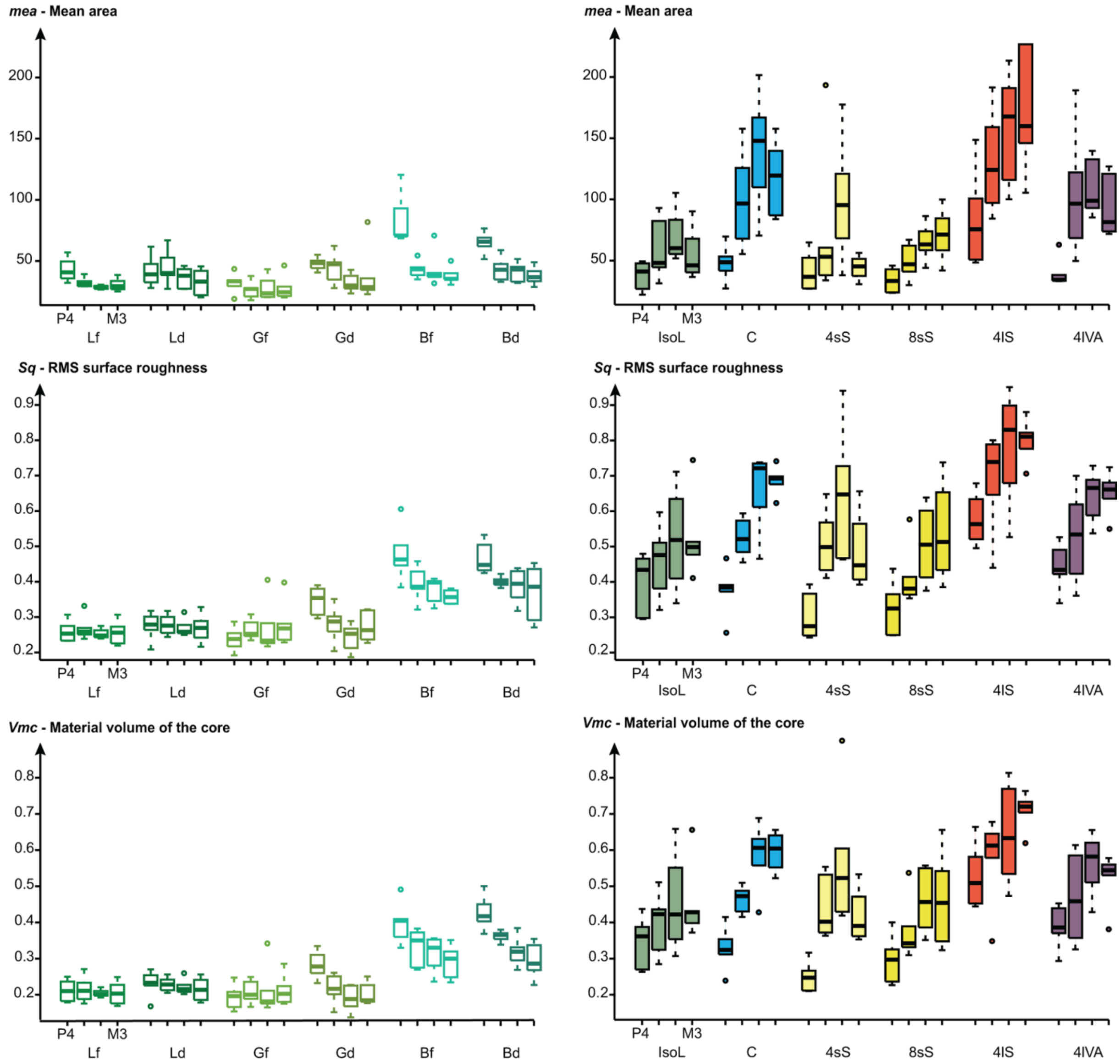

Figure 3. Representative DMTA parameters from area, height, and volume category. Left column shows natural plant diets, right column pelleted diets, with P4-M3 for each group (n=6). Note the higher parameter values for pelleted diets compared to plant diets as well as stronger gradients for all parameters in pelleted diets.

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Table(s)

Table 1. Overview of characteristics of natural and pelleted diets fed to guinea pigs, with experiment duration (in days). Data from [1]Winkler et al. (2019b), [2]Winkler et al. (2020a).

	Diet	Abbreviation	Experiment duration	ADIA content[1] (% DM)	Mean particle size[2] ± SD (µm)
Natural diets	Lucerne fresh	Lf	21 d	0.47	-
	Lucerne dry	Ld	21 d	0.39	-
	Grass fresh	Gf	21 d	0.58	-
	Grass dry	Gd	21 d	0.67	-
	Bamboo fresh	Bf	21 d	3.21	-
	Bamboo dry	Bd	21 d	3.25	-
Pelleted diets	Lucerne pellet	IsoL	59 d	-	-
	Control	C	35 d	-	-
	4% Small quartz	4sS	35 d	-	4.41 ± 1.39
	8% Small quartz	8sS	35 d	-	4.41 ± 1.39
	4% Large quartz	4lS	35 d	-	166.01 ± 15.47
	4% Large volcanic ash	4lVA	35 d	-	96.44 ± 9.03

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Table S1. Dental microwear texture parameter descriptions. Standard and units according to ISO 25178, motif, furrow, texture direction, texture isotropy, and flatness (ISO 12781) analysis and scale-sensitive fractal analysis (SSFA). Functional group has been assigned by the authors for easier reference to similar parameters.

Parameter	Description (condition)	Standard	Functional group	Unit
Sda	Closed dale area	ISO 25178	Area	µm ^{2}
Sha	Closed hill area	ISO 25178	Area	µm ^{2}
mea	Mean area	Motif	Area	µm ^{2}
Sdr	Developed interfacial area ratio	ISO 25178	Complexity	%
nMotif	Number of motifs	Motif	Complexity	no unit
Asfc	Area-scale functional complexity	SSFA	Complexity	
Sal	Auto-correlation length (*s* = 0.2)	ISO 25178	Density	µm
Spd	Density of peaks	ISO 25178	Density	1/ µm ^{2}
medf	Mean density of furrows	Furrow	Density	cm/cm^2
Std	Texture direction	ISO 25178	Direction	–
Str	Texture aspect ratio (*s* = 0.2)	ISO 25178	Direction	no unit
Tr1R	First direction	Direction	Direction	–
Tr2R	Second direction	Direction	Direction	–
Tr3R	Third direction	Direction	Direction	–
IsT	Texture isotropy	Isotropy	Direction	%
epLsar	Anisotropy	SSFA	Direction	
S10z	Ten-point height	ISO 25178	Height	µm
S5p	Five-point peak height	ISO 25178	Height	µm
*S5*v	Five-point valley height	ISO 25178	Height	µm
Sa	Arithmetic mean height or mean surface roughness	ISO 25178	Height	µm
Sku	Kurtosis of the height distribution	ISO 25178	Height	no unit
Sp	Maximum peak height, height between highest peak and mean plane	ISO 25178	Height	µm
Sq	Standard deviation of the height distribution, or RMS surface roughness	ISO 25178	Height	µm
Ssk	Skewness of the height distribution	ISO 25178	Height	no unit
Sv	Maximum pit height, depth between the mean plane and the deepest valley	ISO 25178	Height	µm
Sxp	Peak extreme height difference between *p* = 50% and *q* = 97.5%	ISO 25178	Height	µm
Sz	Maximum height, height between the highest peak and the deepest valley	ISO 25178	Height	µm
meh	Mean height	Motif	Height	µm
madf	Maximum depth of furrows	Furrow	Height	µm
metf	Mean depth of furrows	Furrow	Height	µm
FLTt	Peak to valley flatness deviation of the surface (Gaussian Filter, 0.025 mm)	ISO 12781	Height	µm
FLTp	Peak to reference flatness deviation (Gaussian Filter, 0.025 mm)	ISO 12781	Height	µm
FLTv	Reference to valley flatness deviation (Gaussian Filter, 0.025 mm)	ISO 12781	Height	µm
FLTq	Root mean square flatness deviation (Gaussian Filter, 0.025 mm)	ISO 12781	Height	µm
Spc	Arithmetic mean peak curvature	ISO 25178	Peak sharpness	1/µm
Smc	Inverse areal material ratio (*p* = 10%)	ISO 25178	Plateau size	µm
Smr	Areal material ration, bearing area at given height (c= 1 µm under the highest peak)	ISO 25178	Plateau size	µm
Sdq	Root mean square gradient	ISO 25178	Slope	no unit
Sdv	Closed dale volume	ISO 25178	Volume	µm ^{3}
Shv	Closed hill volume	ISO 25178	Volume	µm ^{3}
Vm	Material volume at a given material ratio (*p* = 10%)	ISO 25178	Volume	µm ^{3}/µm ^{2}
Vmp	Material volume of the peaks	ISO 25178	Volume	µm ^{3}/µm ^{2}
Vmc	Material volume of the core at given material ratio (*p* = 10%, *q* = 80%)	ISO 25178	Volume	µm ^{3}/µm ^{2}
Vv	Void volume at a given material ratio (*p* = 10%)	ISO 25178	Volume	µm ^{3}/µm ^{2}
Vvc	Void volume of the core (*p* = 10%, *q* = 80%)	ISO 25178	Volume	µm ^{3}/µm ^{2}
Vvv	Void volume of the valley at a given material ratio (*p* = 80%)	ISO 25178	Volume	µm ^{3}/µm ^{2}

Table S2. General linear models for natural diets with the variables Diet and Tooth, as well as the interaction between them, as fixed effects. Lf = lucerne fresh, Ld = lucerne dry, Gf = grass fresh, Gd = grass dry, Bf = bamboo fresh, Bd = bamboo dry. *ranked data, °log-transformed data. Please see separate supplementary excel file.

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Table S3. **General linear models for pelleted diets with the variables Diet and Tooth, as well as the interaction between them, as fixed effects.** IsoL = lucerne pellet, C = abrasive-free control pellet, 4sS 4% small quartz, 8sS 8% small quartz, 4lS 4% large quartz, 4lVA 4% large volcanic ash. *ranked data, °log-transformed data. Please see separate supplementary excel file.

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Table S4. Random draws comprising either 25% or 75% of all teeth from natural diet groups. [a]Number of random draws differs from 10000 because random samples that did not cover all 6 diets were discarded. [b]Spearman's rank correlation coefficient of the diet ranking based on 100% of available teeth compared to the diet ranking based on a subsample. [c]Proportion of comparisons of the subsample diet ranking with the 100% diet ranking that produced significant correlations (at $P < 0.05$). Parameters with a proportion of ≥0.70 set in **bold**. Please see separate supplementary excel file.

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Table S5. Random draws comprising either 25% or 75% of all teeth from pelleted diet groups. [a]Number of random draws differs from 10000 because random samples that did not cover all 6 diets were discarded. [b]Spearman's rank correlation coefficient of the diet ranking based on 100% of available teeth compared to the diet ranking based on a subsample. [c]Proportion of comparisons of the subsample diet ranking with the 100% diet ranking that produced significant correlations (at $P < 0.05$). Parameters with a proportion of ≥0.70 set in **bold**. Please see separate supplementary excel file.

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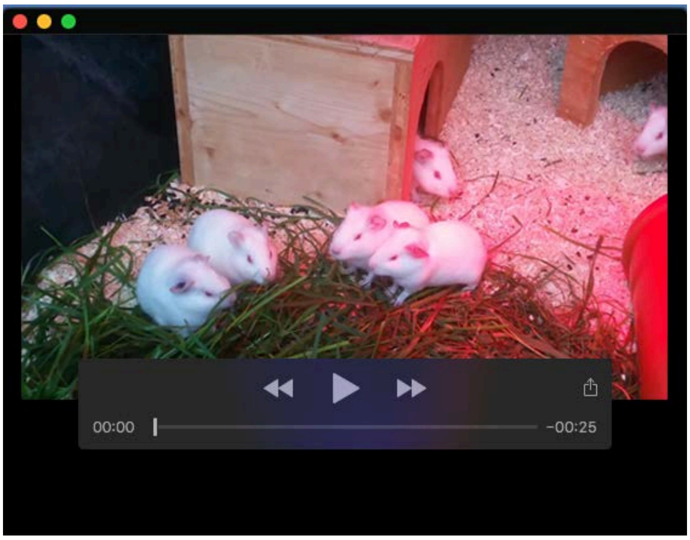

Movie 1. Boxplots for all dental microwear texture parameters for the upper post-canine dentition (P4-M3). The thick horizontal bar represents the median; the box encloses the first (25%) and third (75%) quartiles; the whiskers extend to the full interquartile range; the unfilled dots represent outliers. Upper row shows natural plant diets: Lf = lucerne fresh, Ld = lucerne dry, Gf = grass fresh, Gd = grass dry, Bf = bamboo fresh, Bd = bamboo dry. Lower row shows pelleted diets, partly with addition of mineral abrasives: IsoL = lucerne pellet, C = abrasive-free control pellet, 4sS 4% small quartz, 8sS 8% small quartz, 4lS 4% large quartz, 4lVA 4% large volcanic ash. For parameter descriptions, see Table S1. **This figure is deposited on the open-source data repository of the University of Hamburg and can be accessed under the DOI: 10.25592/ uhhfdm.9163**