Received: 7 July 2021 | Revised: 27 October 2021 | Accepted: 30 October 2021

DOI: 10.1002/jez.b.23104

RESEARCH ARTICLE

JEZ-B MOLECULAR AND DEVELOPMENTAL EVOLUTION WILEY

Macrowear effects of external quartz abrasives of different size and concentration in rabbits (*Oryctolagus cuniculus*)

Louise F. Martin[1] | Nicole L. Ackermans[1] | Henning Richter[2] | Patrick Kircher[2] | Jürgen Hummel[3] | Daryl Codron[4] | Marcus Clauss[1] | Jean-Michel Hatt[1]

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

[2]Clinic for Diagnostic Imaging, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

[3]Divison of Ruminant Nutrition, Department of Animal Sciences, University of Goettingen, Goettingen, Germany

[4]Department of Zoology and Entomology, University of the Free State, Bloemfontein, South Africa

Correspondence

Louise F. Martin, Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, 8057 Zurich, Switzerland.
Email: lfmartin@vetclinics.uzh.ch

Present address

Nicole L. Ackermans, Nash Family Department of Neuroscience, Friedman Brain Institute, Center for Anatomy and Functional Morphology, Icahn School of Medicine at Mount Sinai, New York, New York, USA.

Funding information

National Research Foundation of South Africa, Grant/Award Number: 129172; Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung, Grant/Award Number: 31003A_163300/1; Candoc Forschungskredit of the University of Zurich, Grant/Award Number: FK-16-052

Abstract

External quartz abrasives are one of the driving forces of macrowear in herbivorous animals. We tested to what extent different sizes and concentrations influence their effect on tooth wear. We fed seven pelleted diets varying only in quartz concentration (0%, 4%, and 8%) and size (fine silt: ~4 µm, coarse silt: ~50 µm, fine sand: ~130 µm) to rabbits (*Oryctolagus cuniculus*, $n = 16$) for 2 weeks each in a randomized serial experiment. Measurements to quantify wear and growth of incisors and the mandibular first cheek tooth, as well as heights of all other cheek teeth, were performed using calipers, endoscopic examination, and computed tomography scans before and after each feeding period. Tooth growth showed a compensatory correlation with wear. Absolute tooth height (ATH) and relative tooth height (RTH); relative to the 0% quartz "control" diet) was generally lower on the higher concentration and the larger size of abrasives. The effect was more pronounced on the maxillary teeth, on specific tooth positions and the right jaw side. When offered the choice between different sizes of abrasives, the rabbits favored the silt diets over the control and the fine sand diet; in a second choice experiment with different diets, they selected a pelleted diet with coarse-grained sand, however. This study confirms the dose- and size-dependent wear effects of external abrasives, and that hypselodont teeth show compensatory growth. The avoidance of wear did not seem a priority for animals with hypselodont teeth, since the rabbits did not avoid diets inducing a certain degree of wear.

KEYWORDS

external abrasives, hypsodonty, macrowear, preference, quartz, rabbit

1 | INTRODUCTION

Continuously functioning teeth are a prerequisite for longevity in herbivorous mammals, which is, among other factors, limited by constant exposure to abrasion and attrition during the interaction of feed and teeth (Janis & Fortelius, 1988). Strategies of plasticity for continuing dental function have evolved to respond to dental wear, as has been documented in diverse domesticated populations of several mammalian species (Sánchez-Villagra, 2021).

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2021 The Authors. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* published by Wiley Periodicals LLC.

Apart from direct tooth-to-tooth contact during mastication, major causative agents for macroscopic tooth wear are the silicates inside plant material (endogenous phytoliths) (Martin et al., 2019; Müller et al., 2014) as well as plant-external quartz silica in the form of dust or grit trapped on plant leaves due to wind and rain (Fannin et al., 2021; Madden, 2015; Sanson et al., 2017). The abrasive potential of internal and external abrasives is dependent on several physical characteristics such as size, shape, and hardness (Lucas et al., 2013) in comparison with the affected dental tissues (Kaiser et al., 2018) as well as their overall concentration and distribution in the diet. Large external abrasives are hypothesized to cause detrimental pits and cracks on the tooth surface (Kaiser et al., 2016), but smaller external abrasives may cause more uniform abrasion with a polishing effect (Ackermans, Martin et al., 2020; Winkler et al., 2020). Quartz-based external abrasives exceed the hardness of enamel manifold and are generally accepted to contribute to dental wear (Jardine et al., 2012; Lucas et al., 2013; Müller et al., 2014). Although the softer phytoliths have caused macroscopic dental wear in experimental settings (Müller et al., 2014), their true effect is still discussed controversially (van Casteren et al., 2020; Kaiser et al., 2018; Lucas et al., 2014; Martin et al., 2019; Rabenold & Pearson, 2014; Sanson et al., 2017).

Many animals cannot completely avoid the ingestion of soil or grit with their diet, as shown by the presence of silica in feces or stomach contents (Adams et al., 2020; Beyer et al., 1994; Hummel et al., 2011; Sanson et al., 2017). Herbivores have developed different strategies to minimize the uptake or the impact of abrasive feed: Sand gazelles (*Gazella marica*) were observed to switch from grass to browse during the dry season, which was interpreted as a way to avoid external abrasives (dust) (Schulz et al., 2013). Several species have been reported to wash the abrasives off their diet before ingestion (Allritz et al., 2013; Ito et al., 2017), whereas humans (Prinz, 2004) and other primates (Schulz-Kornas et al., 2019) change their chewing pattern when confronted with a certain amount or size of abrasive particles. Ruminants change their chewing pattern between first ingestion and rumination (Dittmann et al., 2017), possibly to avoid detrimental chewing on abrasives before they are washed out of the diet at the first passage in the rumen (Hatt et al., 2020, 2019). Nevertheless, in an experimental setting, goats (*Capra aegagrus hircus*)—small ruminants—preferred a diet with no added abrasives over one with added large size quartz particles (Ackermans et al., 2019).

Since avoiding tooth wear is impossible, high-crowned, open-rooted (hypsodont) teeth have evolved as a key adaptive response to an abrasive herbivore diet. When the root eventually closes, wear resistance is finite (Ungar, 2015). By retaining the regenerative potential in stem cell niches along the roots, lagomorphs, and rodents perform lifelong dental tissue replacement regulated by complex pathways (Hu et al., 2014; Tummers & Thesleff, 2003). In rabbits and guinea pigs, a flexible tooth growth rate has been demonstrated experimentally, suggesting that their growth rate is adjusted to wear (Meredith et al., 2015; Müller et al., 2014, 2015; Wolf & Kamphues, 1996). The potential for dental regeneration still has some drawbacks: If the teeth are worn at an extreme rate, the compensatory mechanism can be lacking or lag behind (Martin et al., 2019). In addition, regrowth obviously requires allocation of resources and availability of minerals such as calcium (Jekl & Redrobe, 2013), even though the dental tissue lost during wear will be swallowed and digested. Finally, the delicate balance between wear and growth is an Achilles heel prone to malfunction, particularly in the case of malocclusion, as is evident from the high number of dental pathologies seen in pet rabbits (Harcourt-Brown, 2007).

Rabbits are known to regularly ingest relevant amounts of grit or soil with their natural diet (Arthur & Gates, 1988; Rödel, 2005), and have been established as suitable model animals to study wear of hypselodont teeth (Martin et al., 2020; Meredith et al., 2015; Müller et al., 2014). In this study, we investigate the wear effect of seven different pelleted diets, containing external quartz abrasives of various sizes and at various concentrations, on the teeth of rabbits at a macroscopic scale. The purpose of this study was twofold: (1) testing the animals' preference for different concentrations of external abrasives in their diet (2) quantifying the wear effect and corresponding regrowth when rabbits feed on these diets for 14 days each.

2 | MATERIALS AND METHODS

2.1 | Animals

The short-term preference and serial diet switch experiments were approved by the Cantonal Veterinary Office Zurich (license number ZH010/16) and conducted according to the Swiss Animal Welfare Act. Commercially bred tricolored Swiss rabbits were floor-housed individually (0.75 m^2) on woodchip bedding with a raised platform on one end of the enclosure and with no gnawing opportunity other than their diet. Female and male rabbits were kept in two separate rooms with a 12h light/dark cycle. Visual and olfactory contact with neighboring conspecifics was provided through three small windows placed at different heights in the separating walls. Water was provided fresh daily in one open bowl and a nipple drinker. Following arrival, the rabbits were slowly switched from the breeder's diet of hay and pellets to only the pelleted control diet over 7 days. During the experiment, all animals underwent a thorough clinical examination weekly including body weight measurements and dental checks.

2.2 | Main experimental diets

The experimental pelleted diet was lucerne hay-based and contained either no abrasives (control), 4%, or 8% of added quartz abrasives of different size: fine silt (mean particle size: 4 µm, SIRCON® M500; SCR-Sibelco N.V.), coarse silt (mean particle size: 50 µm, MICROSIL® M4; SCR-Sibelco N.V.), or fine sand (mean particle size: 130 µm, METTET AF100; SCR-Sibelco N.V.). The diets were designed to be isocaloric and isonitrogenic by mimicking the indigestible abrasives in the other diets by a similar proportion of the indigestible, nonabrasive filler pure lignocellulose (Arbocel; JRS Pharma), to provide comparable levels of energy per amount of diet. Details on diet composition and hardness analysis (Pharma Test Type PTB 301) can be found in Table 1. The

Ingredients	Control	Fine	Silt	Coarse	Silt	Fine	Sand
Quartz dust size (µm)	–	4	4	50	50	130	130
Quartz dust concentration (%)	–	4	8	4	8	4	8
Cellulose (%)	8	4	–	4	–	4	–
Lucerne meal (%)	71.15	71.15	71.15	71.15	71.15	71.15	71.15
Beetroot molasses (%)	3	3	3	3	3	3	3
Mineral premix (%)	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)							
Crude protein	16.1	16.1	16.1	16.1	16.1	16.1	16.1
Acid detergent fibre[a]	326	303	270	290	270	293	259
Acid detergent insoluble ash[b]	12	31	52	34	66	58	100
Ash	114	150	187	157	192	156	205
Physical characteristics							
Mean pellet hardness (*N*)	85.2	91.7	104.0	97.3	81.6	73.7	91.2

[a]Ash corrected.

[b]A proxy for abrasive content.

TABLE 1 Composition of different pelleted diets fed to rabbits (*Oryctolagus cuniculus*) for in a serial dental wear experiment for 14 days per diet

pelleted diet was fed for ad libitum consumption and the leftover feed was weighed daily, discarded, and replaced by fresh feed.

2.3 | Preference experiment

Fourteen rabbits ($n = 7$ males, $n = 7$ females; 6–18 months old, 3.24 ± 0.94 kg) were presented with the control diet as well as the 8% added abrasive diets (8% fine silt, 8% coarse silt, 8% fine sand) in four feeding dishes placed along a feeding rack for 7 days (stage 1). Dish order was randomized daily using a freeware random number generator to avoid location preference based on an individual dish. Each individual dish contained an amount exceeding the animals' *ad libitum* intake level. The intake of each diet was measured by subtracting the weight of leftovers from the amount offered. The following week (stage 2), the same rabbits were presented with a grass hay-based control pellet, alongside the same pellet containing additionally endogenous plant abrasives in the form of rice hulls, and the grass-rice hull-mixture with the addition of 5% quartz sand (mean particle size: 233 µm; REDSUN garden products B.V.; see Müller et al. (2014) for detailed diet composition).

2.4 | Quantification of macrowear

For the serial feeding experiment, a different group of 16 six-month-old rabbits ($n = 8$ males, $n = 8$ females; 3.0 ± 0.15 kg) were randomly assigned, again using a freeware random number generator, to one of the seven experimental diets for 14 days. After this first feeding period, dental measurements were performed and the rabbits were randomly allocated the next diet so that all rabbits had received all diets by the end of the experiment. During the first feeding period, one male rabbit had to be euthanized due to a severe septicemia unrelated to the experiment. Another rabbit died during recovery from anesthesia after unsuccessful resuscitation. Necropsy could not identify the cause of death in this animal and its respective data were included for complete feeding periods only.

Before the first and after each feeding period, the rabbits were placed under general anesthesia with isoflurane (Attane®; Provet AG) administered to effect in oxygen via a facemask after sedation with 0.2 mg/kg midazolam (Dormicum®; Roche AG) intramuscularly. For nomenclature, mandibular cheek teeth were labeled with lower case letters (p3 to m3) and maxillary cheek teeth with capital letters (P2 to M3). Dental measurements were performed first using a manual caliper (Technocraft®; Allchemet AG, precision 0.01 mm) for the clinical crown of the incisors and the mandibular p3. Additionally, a complete head computed tomography (CT) scan using helical multislice Siemens scanner (Siemens Medical Solutions) at 120 kV tube voltage, 150 mAs was performed in a cranio-caudal direction with a slice thickness of 600 µm to assess the remaining teeth. The CT scans were converted to DICOM imaging files and analyzed in Horos v3.3.3 (Horos Project 2015). Tooth height was recorded as ATH according to the protocol in Martin et al. (2020) and consequently converted to

TABLE 2 Statistical analysis of feeding preference of rabbits (*Oryctolagus cuniculus*; $n = 14$) fed for *ad libitum* consumption for 7 days per stage on diets of different size abrasives

	Diet characteristics			Results	Statistical analysis	
	Conc.	Abrasives	ADIA	% DMI		
Stage 1	0%	Control	12 g/kg DM	17 ± 17%	$F_{3,56} = 8.660$	Control, fine sand < fine silt, coarse silt
(Lucerne)	8%	Fine silt	52 g/kg DM	36 ± 18%	$p < 0.001$	
	8%	Coarse silt	66 g/kg DM	35 ± 17%		
	8%	Fine sand	100 g/kg DM	12 ± 10%		
Stage 2	0%	Control	16 g/kg DM	4 ± 3%	$F_{2,42} = 42.895$	Control < phytoliths < phytoliths + coarse sand
(Grass)	0%	Phytoliths	24 g/kg DM	37 ± 19%	$p < 0.001$	
	5%	Phytoliths + coarse sand	77 g/kg DM	59 ± 20%		

Note: Preference is reported in the mean percentage of dry matter intake ±SD. Data were analyzed with a linear mixed-effects model with subsequent least-square means comparison.

Abbreviations: ADIA, acid-detergent insoluble ash; Conc., concentration of added abrasives; DM, dry matter; DMI, dry matter intake.

Stage 1: Detailed diet description in Table S1.

Stage 2: Detailed diet description in Müller et al. (2014).

relative tooth height (RTH), with tooth height on the pelleted diet without quartz (control) set to 100%.

2.5 | Quantification of tooth wear and growth using burr marks

To quantify wear and growth on the hypselodont teeth, burr marks were placed once the animals were anesthetized before CT scanning. Due to the small mouth gape, only the labial side of each incisor and the cranial aspect of the mandibular third premolar could be marked using a diamond-tipped burr (Henry Schein AG), taking care to only remove a minor portion of the enamel layer when placing a mark approximately 1 mm in diameter. Changes of these marks relative to the tooth tip (to assess wear) or the gingival line (to assess growth) were measured either with a caliper (for incisors) or on CT images (for incisors and mandibular p3). For the p3, additional visual recordings were performed using a portable complete endoscopy unit (TELE PACK VET X LED; Karl Storz GmbH) and a rigid endoscope (170°, 23 cm # 2.0 mm; Richard Wolf GmbH). Images were consequently converted to DICOM images, and the parameters assessed identically to the manual measurements. The constraint of general anesthesia allowed for CT images to be taken only at the beginning and the end of each feeding period, but manual measurements of the incisors were taken weekly, and endoscopy of p3 was additionally performed on day 3 and 7, as the animals readily accepted this procedure.

2.6 | Statistics

For the preference experiments, the data were analyzed using mixed-effects linear models with data for stages 1 and 2 analyzed separately and individuals as a random effect (i.e., a repeated-measurement analysis of variance). The normality of model residuals was confirmed by Shapiro–Wilk test, and diets were compared by their least square means.

The dental measurements were analyzed using mixed-effects linear models with data for mandibles and maxilla analyzed separately and individuals as a random effect. The general model structure was diet concentration/abrasive size (nesting size within concentration) plus side (right or left) plus the interaction of the nested term and side. To account for the effect of body mass, the end body mass (end of each feeding period) was included as a covariate. Allometric scaling was performed separately using nonlinear regression, fitting the power function $y = a\ \mathrm{BM}^b$ for each tooth (Table S3). To analyze laterality, side and CT at the very beginning and end of the experiment were set as factors with individuals as a random effect and each tooth on maxilla and mandible was analyzed individually (Table S4). For the wear/growth measurements, additional covariates were included for specific parameters (Tables S5–S7). If parametric assumptions were not met, dependent variables were replaced with either ln-transformed or, if that also did not meet parametric assumptions, ranks. Where applicable, post-hoc testing was performed to distinguish specific effects. Those analyses were carried out in R 4.0.2 (R Core Team, 2020), with the significance level set to $p < 0.05$.

3 | RESULTS

3.1 | Preference

In stage 1 of the preference experiment, the rabbits preferred the diets with added silt (fine and coarse) compared to the nonabrasive diet and the fine sand diet ($p < 0.001$; Table 2 and Figure 1a). The silt diets amounted to 71% of total dry matter (DM) intake after 7 days of the experiment (Table 2 and Figure 1a). In stage 2, the rabbits opted

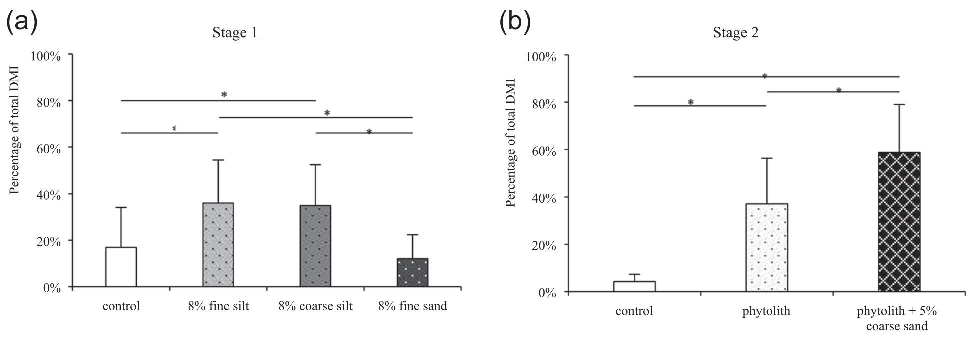

FIGURE 1 Feeding preference of rabbits (*Oryctolagus cuniculus*, *n* = 14) fed for *ad libitum* consumption for 7 days per stage on diets of different-sized abrasives (**(a)** small: 4 µm, medium: 50 µm, large: 130 µm, **(b)** rice phytoliths, rice phytoliths, and coarse sand: 230 µm) (see also Table 2 for statistical analysis). DMI, dry matter intake

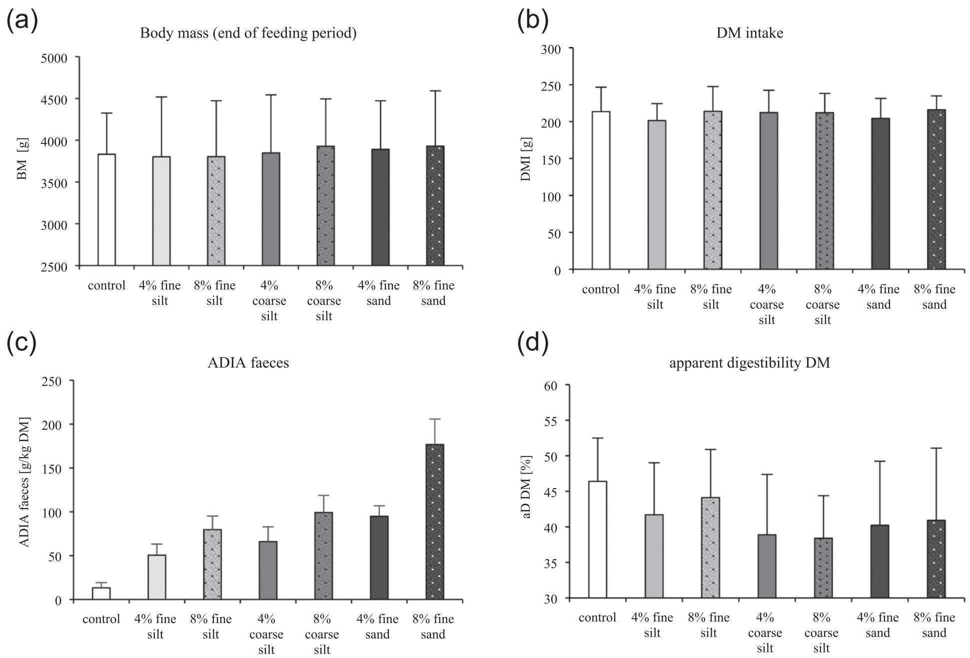

FIGURE 2 **(a)** Body mass at the end of the feeding period, **(b)** daily dry matter intake (DMI) and **(c)** fecal acid detergent insoluble ash (as proxy for abrasiveness), and **(d)** apparent digestibility of dry matter in rabbits (*Oryctolagus cuniculus*, *n* = 16) fed lucerne based pelleted control diet or the same with added 4% or 8% of fine silt, coarse silt or fine sand for 14 days each in a serial feeding experiment (see Table S2 for statistical analysis). ADIA, acid-detergent insoluble ash; DM, dry matter

for the diet with the added coarse sand and phytoliths over the control or the phytoliths-only diet ($p < 0.001$; Table 2 and Figure 1b).

3.2 | Main experiment

Body mass at the start and end of each treatment did not differ significantly ($p = 0.971$ and $p = 0.978$ respectively; Table S1 and Figure 2a), and neither did body mass gain ($p = 0.984$; Table S1) nor daily DM intake ($p = 0.771$; Table S1 and Figure 2b), among the different diets. Acid-detergent insoluble ash can be used as a proxy for the abrasiveness of the diet, and in accordance with the experimental design, it increased in the diets with higher concentration and/or larger size of abrasives (Table 1), as well as in the fecal samples from the corresponding feeding periods ($p < 0.001$; Table S1 and Figure 2c). DM digestibility was highest in the control diet (Table S1 and Figure 2d).

3.2.1 | Tooth height

The rabbits were still growing for the duration of the main experiment (BM before first CT: 2485 ± 372 g; BM at last CT 4521 ± 355 g), which had a significant effect on tooth height in incisors and cheek teeth (Tables 3 and S2) as confirmed by allometric analysis for both measurements (Table S3). Therefore, body

TABLE 3 Mixed effect linear model analyses of absolute and relative tooth height for the cheek teeth of rabbits (*Oryctolagus cuniculus*, *n = 16*) fed lucerne based pelleted control diet or the same with added 4% or 8% of fine silt, coarse silt, or fine sand for 14 days each in a serial feeding experiment

Effects	*F*	*p*	Post-hoc
Absolute tooth height			
Mandibular cheek teeth[a]			
Individual	844.9832	<0.001	
Body mass end	1216.4356	<0.001	
Side	21.1716	<0.001	Left > right
Tooth	53.7008	<0.001	(p3, p4, m1) < m2; *p3 < m1*
Side: tooth	3.4453	0.016	*l*: (p3, p4) < m2 *r*: (p3, p4, m1) < m2
Concentration	4.5657	0.011	n.s.
Conc: side[b]	2.3452	0.097	–
Conc: size[c]	4.7166	0.001	n.s.
Maxillary cheek teeth[a]			
Individual	677.0550	<0.001	
Body mass end	1184.6819	<0.001	
Side	0.3340	0.563	
Tooth	525.0632	<0.001	P2, P3, M2 < M1 < P4 P2 < P3
Side: tooth	1.9677	0.097	
Concentration	103.4128	<0.001	0% > 4% > 8%
Conc: size[c]	677.0550	<0.001	8%: Fine silt > coarse silt > fine sand
Conc: tooth[d]	1.8884	0.059	0%, 4%, 8%: (P2, P3, M2) < M1 < P4
Relative tooth height			
Mandibular cheek teeth[a]			
Individual	480.8120	<0.001	
Body mass end	902.4502	<0.001	
Side	4.8163	0.029	Left < right
Tooth	1.1845	0.315	
Concentration	16.1307	<0.001	*0% > 8%*
Conc: size	8.5837	<0.001	n.s.
Maxillary cheek teeth[a]			
Individual	461.7347	<0.001	
Body mass end	992.4458	<0.001	
Side	0.0051	0.943	
Tooth	5.2203	<0.001	*(P2, M2) > P3*; (P2, M2) > P4
Concentration	94.4755	<0.001	0% > (4%, 8%)
Conc: size[c]	23.8249	<0.001	8%: Fine silt > (coarse silt, fine sand)

(Continues)

TABLE 3 (Continued)

Note: log-transformed data; *italics in post-hoc*: 0.05 < *p* < 0.06.
[a]Ranked data.
[b]Interaction concentration and side.
[c]Size nested in concentration.
[d]Interaction concentration and tooth position.

mass was included as a covariable in all statistical assessments of tooth height. Note that due to the experimental setup, the average body mass did not differ between treatments (Figure 2a).

Neither the absolute (ATH) nor the RTH of the incisors differed between the three concentrations or sizes of abrasives (Table S2 and Figure 3a,b). For the maxillary incisors, size nested in concentration was significant for ATH ($p = 0.022$; Table S2 and Figure 3a), but post-hoc testing did not confirm any differences among the diet groups.

The concentration and size of the abrasives had an inconclusive effect on the ATH in the mandibular cheek teeth, since there was a significant model effect, although with no significant post-hoc result despite numerically different group means, suggesting low statistical power (Table 3 and Figure 3c). The 8% abrasive diets shortened the maxillary cheek teeth more than the 4% diet or the control diet ($p < 0.001$; Table 3 and Figure 3e), and on the higher concentration the abrasive size was linked to a significant difference as well ($p < 0.001$; Table 3 and Figure 3e). P4 had the highest ATH followed by M1 and the other teeth ($p < 0.001$; Table 3 and Figure 3e). The RTH differed significantly among diets in the model for the mandibular cheek teeth (post-hoc not significant; Table 3 and Figure 3d), and more distinctively for the maxillary cheek teeth (concentration $p < 0.001$, 0% < [4%, 8%]; concentration:size $p < 0.001$; Table 3 and Figure 3f). The coarse silt and fine sand caused significant shorter RTH on the high concentration diet than the fine silt. The maxillary cheek teeth on the outermost positions of the tooth row (P2, M2) had a higher RTH than P3 and P4, respectively (Table 3 and Figure 3f).

Over the whole study period, incisors and maxillary cheek teeth showed no difference between the left and right side on ATH and RTH, but the mandibular right cheek teeth were overall shorter than their counterparts on the left side ($p < 0.001$; Table S4 and Figure S1). The laterality was also consistent on the maxillary M1 ($p = 0.029$;

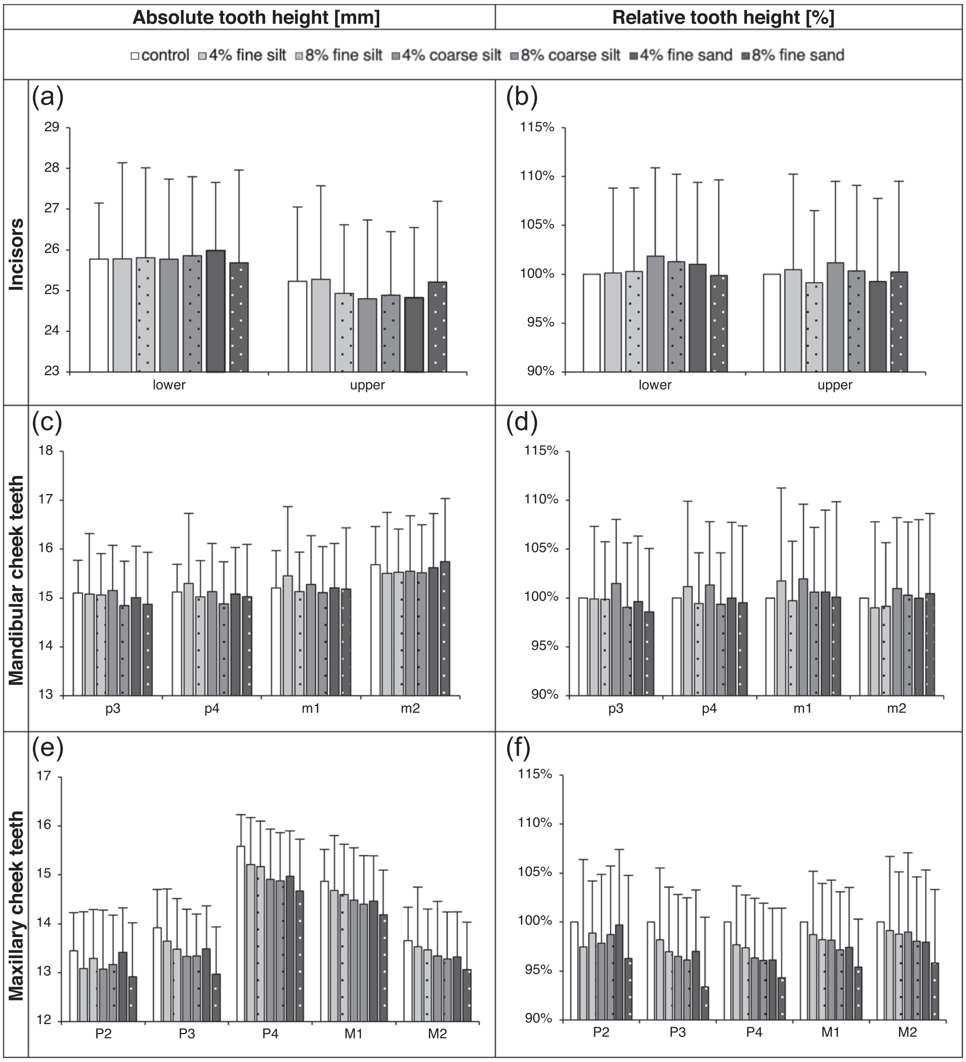

FIGURE 3 Absolute **(a,c,e)** and relative **(b,d,f)** tooth height (with control diet as 100%) for incisors **(a,b)**, mandibular **(c,d)** and maxillary **(e,f)** cheek teeth of rabbits (*Oryctolagus cuniculus*, *n = 16*) fed lucerne based pelleted control diet or the same with added 4% or 8% of fine silt, coarse silt, or fine sand for 14 days each in a serial feeding experiment (see also Table 3)

Table S4 and Figure S1) and M2 ($p = 0.059$; Table S4 and Figure S1). In the maxillary tooth row, laterality was more pronounced at the end of the experiment (Figure S1).

3.2.2 | Wear and growth

The manual wear and growth measurements showed no effect of the concentrations or abrasive sizes on the incisors or the mandibular third premolar (Table S5 and Figure 4a–c). Wear and growth differed between left and right mandibular premolar (left < right; $p < 0.01$; Tables S5 and S6; Figure 4d). Wear and growth of the incisors correlated well ($p < 0.001$; Table S6). Integrating wear in the first week as a covariable, first-week wear only showed an effect on second-week growth in the maxillary incisors while there was no effect on the mandibular incisors (Table S6). The different methods for quantification of wear on the mandibular p3 all showed a significant correlation between wear and growth (Table S7).

4 | DISCUSSION

The results of this study confirm the abrasive effect of fine silt-, coarse silt- and fine sand-sized quartz on the cheek teeth of rabbits when added at a 4% and especially an 8% concentration to a pelleted diet for 14 days of feeding. The effect differed depending on tooth position, mandibular or maxillary tooth row, and jaw side. The wear on the incisors and the mandibular third premolar was compensated with the corresponding regrowth without a measurable difference between abrasive concentration or size.

4.1 | Feeding preferences

During the preference experiment, the rabbits favored diets with added abrasive elements, in particular the silt diets. Wild rabbits spend roughly 15 h a day foraging (Lehmann, 1990) to feed mainly on

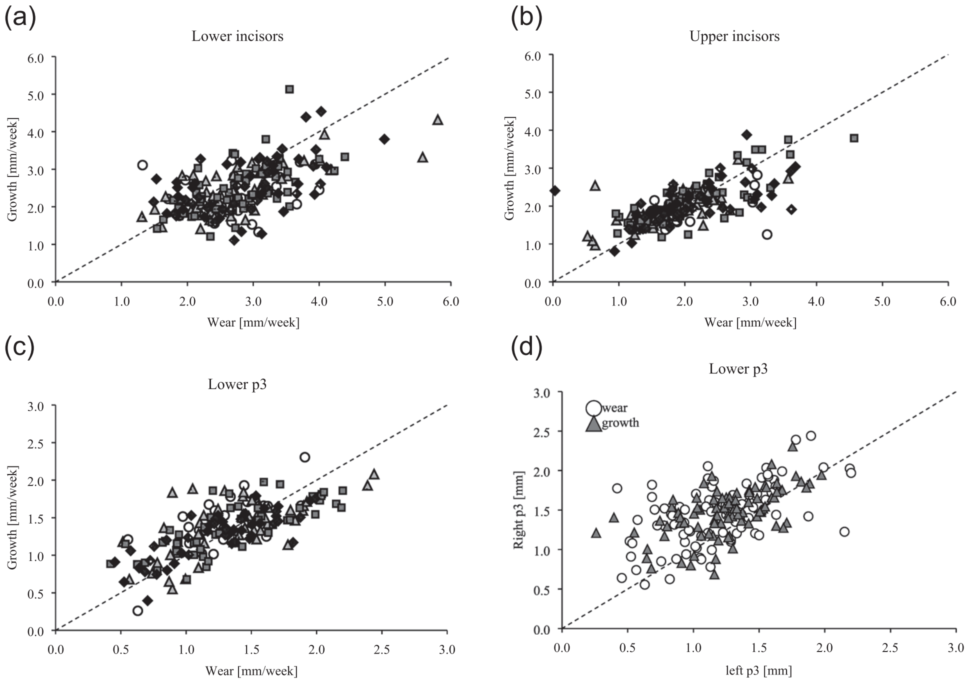

FIGURE 4 Wear and growth of the incisors **(a, b)** and the mandibular third premolar **(c)** in rabbits (*Oryctolagus cuniculus*, $n = 16$) fed lucerne based pelleted control diet or the same with added 4% or 8% of fine silt, coarse silt or fine sand for 14 days each in a serial feeding experiment measured with a manual caliper and the help of burr marks. The dotted line denotes $y = x$ (see also Table S5 for statistical analysis). **(d)** Wear and growth (manual measurements only) for the mandibular left and right third premolar

soft, energy-dense grass (Williams et al., 1974), yet consume—accidentally or on purpose—around 6.3% of soil in their daily feed uptake (recorded for black-tailed jackrabbits (*Lepus californicus*) by Arthur and Gates (1988), with reported fecal sand concentrations of 8% DM (Rödel, 2005). The study results clearly show that the animals must have been able to differentiate between the control diets and the diets containing external abrasives. The threshold for the interocclusal tactile sensitivity in humans is on average 15 µm (Utz, 1986), and coarseness perception is significantly influenced by the hardness and shape of the individual particles (Engelen et al., 2005). Quartz sand is heterogenous in shape and harder than tooth enamel (Erickson, 2014), suggesting its presence must be detectable by the animals. Thus, it seems that the rabbits were able to perceive differences among the diets and purposefully chose to eat the abrasive diets. By this active selection, the rabbits also influence a morphophysiological measure—dental wear and (responding) growth rate (see below); thus giving an example of behavior influencing a morphological outcome (Diogo, 2017). In other words, a certain plasticity in behavior is followed by plasticity in development, as demonstrated by the compensatory growth in the ever-growing teeth of rabbits that are selected for a more abrasive diet.

The hypselodont dentition of rabbits is a specific adaptation to tooth wear, and dental malformation can occur on either very low-abrasive (Meredith et al., 2015) or very high-abrasive diets (Müller et al., 2014). Avoidance of low-abrasive diets are shown clearly in both preference experiments (Figure 1). In stage 2, the rabbits significantly preferred the diet with 5% coarse sand, which was previously shown to cause clinical tooth abnormalities (such as tooth spurs, step formation, and more) after feeding for 14 days (Müller et al., 2014). Feeding preference, therefore, does seem to be guided both by size and concentration of abrasive elements, without resulting in any changes in DM intake during the 14 day feeding periods of the main experiment (Figure 2b). A limitation intrinsic to all short-term preference experiments is the unattainability of stable patterns, which would allow for more robust results. Even though the diets were designed to be isocaloric and isonitrogenic and of similar hardness (see Table 1), a difference in taste quality cannot be ruled out, and the diet containing no abrasives showed the highest DM digestibility. The results let us hypothesize that rabbits prefer a certain amount of abrasive stimulation in their diet.

4.2 | Tooth wear

The experimental diets in this study were designed to be low in phytoliths but supplemented with either 4% or 8% added external quartz abrasives—so they were either below or above the percentages of soil intake reported for wild rabbits (Arthur & Gates, 1988). An in vitro experiment showed that a watery sludge with 1% abrasive particles had a higher microwear effect on human enamel than with 5%, potentially due to a broader distribution of forces over a larger area in the higher concentration slurry (Borrero-Lopez & Lawn, 2018). By contrast, the 8% diet caused significantly more macrowear than the 4% diet (even though the microwear signal remains to be explored in the future). Small size abrasives are thought to cause a polishing effect (Ackermans, Winkler et al., 2020; Winkler et al., 2020) without siginificantly wearing down teeth, while large size abrasives have the potential to cause cracks and pits on the enamel surface initiating further macrowear. Even though tooth wear was lower on the fine silt diet compared to the coarse sand, the silt still caused significant tooth height differences compared to the control diet (Table 3), suggesting that the polishing effect also includes dental tissue removal. In a natural environment, fine silt can be transported by wind onto plants in the dry season (Fannin et al., 2021; Kok et al., 2012), while larger particles only end up on leaves when they are propelled from the ground upwards by the splash of rain drops (Sanson et al., 2017) during the rainy season. Shorter hypselodont teeth could thus reflect a sandy environment with low grass coverage during the rainy season, rather than a windy dry season. These types of interpretations add to the difficulties in paleontological diet and habitat reconstructions.

The same diets fed to the rabbits here were used in a 17-month tooth wear study involving sheep (with hypsodont dentition). The sheep showed no macrowear difference among diets (Ackermans, Martin et al., 2020). The difference between the two animal models might lie in the hardness of the enamel (Martin et al., 2020), with hypselodont teeth made of a softer enamel that may be affected by abrasives after even a short amount of time, whereas the more durable enamel of ungulate's permanent teeth may make tissue loss much more difficult to detect experimentally. Additionally, in ruminants such as sheep, external abrasives are washed off the digesta in the forestomach before rumination mastication (Hatt et al., 2020, 2019). Hence, differences in both, physical properties of enamel as well as the physiology of mastication, may contribute to species-specific wear signals.

4.3 | Tooth wear patterns/gradients

Abrasive diets affect mandibular and maxillary tooth rows or even individual tooth positions differently (Figure S1), following patterns previously established in rabbits and guinea pigs (Martin et al., 2020; Müller et al., 2014, 2015): abrasive diets caused more wear in maxillary than in mandibular check teeth, on one side of the mandibular cheek than the other, and on teeth in the middle of (especially the maxillary) tooth row compared to those on either end. These patterns were consistent regardless of abrasive concentration or size. Therefore, the rabbits did not change their chewing movements in response to the large abrasives or the higher concentration, nor did they decrease their overall intake in response to the extreme diets (Figure 2b). The disproportionate wear of the maxillary cheek teeth compared to the mandibular ones has previously been used to invoke an "inverted pestle-and-mortar" system, where food is placed on the mandibular cheek teeth and moved long the occluding surface of the maxillary ones (Müller et al., 2014). The fact that teeth in the middle of the tooth row are more affected than those at its ends leads to the hypothesis that the diet load is highest in the middle, whereas at the ends, food may more easily move off towards the respective front or back during mastication.

Laterality of motor function in general, and masticatory handedness in particular, have been researched in humans and some other mammal species (Ginot et al., 2018; Llorente et al., 2011; Martinez-Gomis et al., 2009) but little is known about this topic in rabbits (Pares-Casanova & Cabello, 2020). In the present study, rabbits had shorter teeth on the right mandible compared to the left (except for m2), and some differences were also evident on the maxilla already at the start of the experiment. At the very end of the experiment, laterality had increased on p4, m1, and m2, while the pattern was reversed on the maxilla P2, P3, P4, and M2 (see also Figure S1), which would indicate more intense tooth wear on the left side of the "inverted pestle-and-mortar" system. Whether these findings indicate more intense chewing on the left side (with more wear on the left maxillary cheek teeth) or more intense chewing on the right side (with more wear on the right mandibular cheek teeth) remains to be investigated in the future.

4.4 | Tooth wear and growth

Pelleted diet formulations reduce tooth wear on the incisors compared to natural diet items such as hay, because the pellets are manipulated only shortly until first breakage. Nevertheless, pellets with added coarse sand had a shortening effect on rabbit incisor teeth in a previous study (Müller et al., 2014). In the present study, incisor wear was compensated by corresponding growth irrespective of diet, resulting in no difference in absolute or RTH (Figure 3a,b). Incisor wear/growth rate corresponds to the results from a tooth wear experiment in young growing rabbits (Meredith et al., 2015) but is higher than previously recorded (see literature review in Müller et al. (2014)). As regrowth matches wear, the different growth rates could be explained by the considerably different abrasive potential of the diets offered across these studies as well as the different age groups. The enamel hardness—as one factor influencing wear—may be lower in younger animals (Shakila et al., 2015) than in older rabbits (Hakki et al., 2015). But since the maximum unimpeded growth rate for adult rabbits incisors was shown to be around 13.3 mm/2 weeks (Moxham & Berkovitz, 1974), it follows that even the 8% fine sand diet could not significantly impact the height of the incisors in these growing animals significantly (Figure 3a) due to the flexibility of the hypselodont teeth to regrow in a compensatory fashion.

Hypselodont teeth represent a comparatively novel morphophysiological unit in mammalian evolution (Tapaltsyan et al., 2015). The fact that this unit comprises a functional feedback whereby dental wear triggers compensatory growth on the basis of individual teeth has been known for a long time, and the changes in occlusal pressure when teeth are "worn out of ideal conclusion" have long been suspected as the triggering physiological signal (An et al., 2018; Mittag, 1932; Ness, 1956; Risnes et al., 1995; Wetzel, 1927). The growth-triggering effect of a lack of occlusal pressure has been confirmed both in experimental approaches that shorten a hypselodont tooth out of occlusion, inducing particularly fast growth (Wetzel, 1927), and in approaches that prevent tooth wear or apply controlled pressure and hence reduce growth (Proffit & Sellers, 1986; Steigman et al., 1981; Weinreb et al., 1969). The stem cells as the basis of hypselodont teeth and many of their signaling pathways have recently been identified (Yu & Klein, 2020), but the direct link between the pressure signal and these pathways remains to be detected.

In the present study, variables were recorded as the change in distance of the burr mark to the crown tip via manual caliper and CT measurements at the end of each 2-week feeding period. The mandibular p3 was the only cheek tooth where burr marks could be placed due to the small mouth gape in rabbits. After 14 days feeding, many burr marks had been worn away, introducing a potential error in the manual and CT measurements. The additional endoscopic examinations took place on days 3 and 7 during each feeding period and therefore should in theory be more sensitive to effects of abrasive size and concentration than CT readings, as corroborated in the present experiment (Table S5). For future experiments, we recommend further increasing the frequency of endoscopic examination.

5 | CONCLUSIONS

Macrowear and tooth regrowth in rabbits with hypselodont dentition remain challenging to quantify, but tooth dimensions on CT scans can differentiate between diets with various sizes and concentrations of abrasive quartz particles, and may be more sensitive than manual readings of wear using burr marks. Especially, the 8% fine sand diet caused significant wear along previously described tooth wear gradients with some additional laterality. The rabbits did not change their chewing pattern or food intake in response to the high abrasive diet, and showed a preference for abrasives diets rather than of a low-abrasive control diet. As compared to results in sheep on the same diet, it appears that the rabbit model is more sensitive in detecting differences in dental tissue loss due to abrasion.

ACKNOWLEDGMENTS

This study was part of project 31003A_163300/1 funded by the Swiss National Science Foundation. The work of Louise F. Martin was funded by the Candoc Forschungskredit of the University of Zurich. Daryl Codron was funded by the National Research Foundation of South Africa (Grant ID 129172). Open access funding provided by Universitat Zurich.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Marcus Clauss and Jean-Michel Hatt designed the study. Louise F. Martin, Nicole L. Ackermans, and Marcus Clauss performed the animal experiment. Patrick R. Kircher and Henning Richter supervised the computed tomography scanning, and Jürgen Hummel the nutritional and fecal analysis. Daryl Codron and Marcus Clauss analyzed the data. Louise F. Martin and Marcus Clauss wrote the first draft of the manuscript which then received input by all co-authors.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU and Swiss standards for the protection of animals used for scientific purposes.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the Supporting Information Material of this article.

ORCID

Louise F. Martin http://orcid.org/0000-0003-4326-0788
Nicole L. Ackermans http://orcid.org/0000-0001-8336-1888
Henning Richter http://orcid.org/0000-0003-3696-6993
Patrick Kircher http://orcid.org/0000-0002-9677-0522
Jürgen Hummel http://orcid.org/0000-0002-8876-7745
Daryl Codron http://orcid.org/0000-0001-5223-9513
Marcus Clauss http://orcid.org/0000-0003-3841-6207
Jean-Michel Hatt http://orcid.org/0000-0002-7043-7430

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/jez.b.23104.

REFERENCES

Ackermans, N. L., Martin, L. F., Codron, D., Kircher, P. R., Richter, H., Clauss, M., & Hatt, J.-M. (2020). Confirmation of a wear-compensation mechanism in dental roots of ruminants. *Anatomical Record*, *304*, 425–436. https://doi.org/10.1002/ar.24402

Ackermans, N. L., Martin, L. F., Hummel, J., Müller, D. W. H., Clauss, M., & Hatt, J.-M. (2019). Feeding selectivity for diet abrasiveness in sheep and goats. *Small Ruminant Research*, *175*, 160–164. https://doi.org/10.1016/j.smallrumres.2019.05.002

Ackermans, N. L., Winkler, D. E., Martin, L. F., Kaiser, T. M., Clauss, M., & Hatt, J.-M. (2020). Dust and grit matter: Abrasives of different size lead to opposing dental microwear textures in experimentally fed sheep (*Ovis aries*). *Journal of Experimental Biology*, *223*(Pt 3): jeb220442. https://doi.org/10.1242/jeb.220442

Adams, N. F., Gray, T., & Purnell, M. A. (2020). Dietary signals in dental microwear of predatory small mammals appear unaffected by extremes in environmental abrasive load. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *558*, 109929. https://doi.org/10.1016/j.palaeo.2020.109929

Allritz, M., Tennie, C., & Call, J. (2013). Food washing and placer mining in captive great apes. *Primates*, *54*(4), 361–370. https://doi.org/10.1007/s10329-013-0355-5

An, Z., Sabalic, M., Bloomquist, R. F., Fowler, T. E., Streelman, T., & Sharpe, P. T. (2018). A quiescent cell population replenishes mesenchymal stem cells to drive accelerated growth in mouse incisors. *Nature Communications*, *9*(1), 378. https://doi.org/10.1038/s41467-017-02785-6

Arthur, W. J., & Gates, R. J. (1988). Trace element intake via soil ingestion in pronghorns and in black-tailed jackrabbits. *Journal of Range Management*, *41*(2), 162–166. https://doi.org/10.2307/3898955

Beyer, W. N., Connor, E. E., & Gerould, S. (1994). Estimates of soil ingestion by wildlife. *The Journal of Wildlife Management*, *58*(2), 375. https://doi.org/10.2307/3809405

Borrero-Lopez, O., & Lawn, B. R. (2018). Role of particulate concentration in tooth wear. *Journal of the Mechanical Behavior of Biomedical Materials*, *80*, 77–80. https://doi.org/10.1016/j.jmbbm.2018.01.024

van Casteren, A., Strait, D. S., Swain, M. V., Michael, S., Thai, L. A., Philip, S. M., Saji, S., Al-Fadhalah, K., Almusallam, A. S., Shekeban, A., McGraw, W. S., Kane, E. E., Wright, B. W., & Lucas, P. W. (2020). Hard plant tissues do not contribute meaningfully to dental microwear: evolutionary implications. *Scientific Reports*, *10*(1), 582. https://doi.org/10.1038/s41598-019-57403-w

Diogo, R. (2017). Evolution driven by organismal behavior. Springer Publishing.

Dittmann, M. T., Kreuzer, M., Runge, U., & Clauss, M. (2017). Ingestive mastication in horses resembles rumination but not ingestive mastication in cattle and camels. *Journal of Experimental Zoology. Part A, Ecological and Integrative Physiology*, *327*(2-3), 98–109. https://doi.org/10.1002/jez.2075

Engelen, L., Van Der Bilt, A., Schipper, M., & Bosman, F. (2005). Oral size perception of particles: effect of size, type, viscosity and method. *Journal of Texture Studies*, *36*(4), 373–386. https://doi.org/10.1111/j.1745-4603.2005.00022.x

Erickson, K. L. (2014). Prairie grass phytolith hardness and the evolution of ungulate hypsodonty. *Historical Biology*, *26*(6), 737–744. https://doi.org/10.1080/08912963.2013.841155

Fannin, L. D., Singels, E., Esler, K. J., & Dominy, N. J. (2021). Grit and consequence. *Evolutionary Anthropology*. https://doi.org/10.1002/evan.21927

Ginot, S., Agret, S., & Claude, J. (2018). Bite force performance, fluctuating asymmetry and antisymmetry in the mandible of inbred and outbred wild-derived strains of mice *(Mus musculus domesticus)*. *Evolutionary Biology*, *45*(3), 287–302. https://doi.org/10.1007/s11692-018-9450-2

Hakki, S. S., Siddikmalkoc, S., Dundar, N., Kayis, S. A., Hakki, E. E., Hamurcu, M., Baspinar, N., Basoglu, A., Nielsen, F. H., & Götz, W. (2015). Dietary boron does not affect tooth strength, micro-hardness, and density, but affects tooth mineral composition and alveolar bone mineral density in rabbits fed a high-energy diet. *Journal of Trace Elements in Medicine and Biology*, *29*, 208–215. https://doi.org/10.1016/j.jtemb.2014.10.007

Harcourt-Brown, F. M. (2007). The progressive syndrome of acquired dental disease in rabbits. *Journal of Exotic Pet Medicine*, *16*(3), 146–157. https://doi.org/10.1053/j.jepm.2007.06.003

Hatt, J.-M., Codron, D., Ackermans, N. L., Martin, L. F., Richter, H., Kircher, P. R., Gerspach, C., Hummel, J., & Clauss, M. (2020). The effect of the rumen washing mechanism in sheep differs with concentration and size of abrasive particles. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *550*, 109728. https://doi.org/10.1016/j.palaeo.2020.109728

Hatt, J.-M., Codron, D., Müller, D. W. H., Ackermans, N. L., Martin, L. F., Kircher, P. R., Hummel, J., & Clauss, M. (2019). The rumen washes off abrasives before heavy-duty chewing in ruminants. *Mammalian Biology*, *97*, 104–111. https://doi.org/10.1016/j.mambio.2019.06.001

Hu, J. K. H., Mushegyan, V., & Klein, O. D. (2014). On the cutting edge of organ renewal: Identification, regulation, and evolution of incisor stem cells. *Genesis*, *52*(2), 79–92. https://doi.org/10.1002/dvg.22732

Hummel, J., Findeisen, E., Südekum, K.-H., Ruf, I., Kaiser, T. M., Bucher, M., Clauss, M., & Codron, D. (2011). Another one bites the dust: Faecal silica levels in large herbivores correlate with high-crowned teeth. *Proceedings of the Royal Society B-Biological Sciences*, *278*(1712), 1742–1747. https://doi.org/10.1098/rspb.2010.1939

Ito, M., Macdonald, A. A., Leus, K., Atmaja, I. D. G. A., & Balik, I. W. (2017). Food preparation behaviour of Babirusa (*Babyrousa celebensis*). *Journal of Zoo and Aquarium Research*, *5*(2), 97–103. https://doi.org/10.19227/jzar.v5i2.274

Janis, C. M., & Fortelius, M. (1988). On the means whereby mammals achieve increased functional durability of their dentitions, with special reference to limiting factors. *Biological Reviews*, *63*(2), 197–230. https://doi.org/10.1111/j.1469-185X.1988.tb00630.x

Jardine, P. E., Janis, C. M., Sahney, S., & Benton, M. J. (2012). Grit not grass: Concordant patterns of early origin of hypsodonty in Great Plains ungulates and Glires. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *365*, 1–10. https://doi.org/10.1016/j.palaeo.2012.09.001

Jekl, V., & Redrobe, S. (2013). Rabbit dental disease and calcium metabolism—the science behind divided opinions. *Journal of Small Animal Practice*, *54*(9), 481–490. https://doi.org/10.1111/jsap.12124

Kaiser, T. M., Braune, C., Kalinka, G., & Schulz-Kornas, E. (2018). Nano-indentation of native phytoliths and dental tissues: Implications for herbivore-plant combat and dental wear proxies. *Evolutionary Systematics*, *2*(1), 55–63. https://doi.org/10.3897/evolsyst.2.22678

Kaiser, T. M., Clauss, M., & Schulz-Kornas, E. (2016). A set of hypotheses on tribology of mammalian herbivore teeth. *Surface Topography-Metrology and Properties*, *4*(1), 014003. https://doi.org/10.1088/2051-672x/4/1/014003

Kok, J. F., Parteli, E. J., Michaels, T. I., & Karam, D. B. (2012). The physics of wind-blown sand and dust. *Reports on Progress in Physics*, *75*(10), 106901. https://doi.org/10.1088/0034-4885/75/10/106901

Lehmann, M. (1990). Beschaeftigungsbeduerfnis junger Hauskaninchen: Rohfaseraufnahme und Tiergerechtheit. *Schweizer Archiv Tierheilkunde*, *132*, 375–381. https://doi.org/10.5169/seals-592612

Llorente, M., Riba, D., Palou, L., Carrasco, L., Mosquera, M., Colell, M., & Feliu, O. (2011). Population-level right-handedness for a coordinated bimanual task in naturalistic housed chimpanzees: Replication and extension in 114 animals from Zambia and Spain. *American Journal of Primatology*, *73*(3), 281–290. https://doi.org/10.1002/ajp.20895

Lucas, P. W., Casteren, A., Al-Fadhalah, K., Almusallam, A. S., Henry, A. G., Michael, S., Watzke, J., Reed, D. A., Diekwisch, T. G. H., Strait, D. S., & Atkins, A. G. (2014). The role of dust, grit and phytoliths in tooth wear. *Annales Zoologici Fennici*, *51*(1–2), 143–152. https://doi.org/10.5735/086.051.0215

Lucas, P. W., Omar, R., Al-Fadhalah, K., Almusallam, A. S., Henry, A. G., Michael, S., Thai, L. A., Watzke, J., Strait, D. S., & Atkins, A. G. (2013). Mechanisms and causes of wear in tooth enamel: implications for hominin diets. *Journal of the Royal Society Interface*, *10*(80), 9. https://doi.org/10.1098/rsif.2012.0923

Madden, R. (2015). Hypsodonty in mammals: Evolution, geomorphology, and the role of earth surface processes. Cambridge University Press.

Martin, L. F., Krause, L., Ulbricht, A., Winkler, D. E., Codron, D., Kaiser, T. M., Müller, J., Hummel, J., Clauss, M., Hatt, J. M., & Schulz-Kornas, E. (2020). Dental wear at macro- and microscopic scale in rabbits fed diets of different abrasiveness: A pilot investigation. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *556*, 109886. https://doi.org/10.1016/j.palaeo.2020.109886

Martin, L. F., Winkler, D. E., Tütken, T., Codron, D., De Cuyper, A., Hatt, J.-M., & Clauss, M. (2019). The way wear goes: Phytolith-based wear on the dentine-enamel system in guinea pigs (*Cavia porcellus*). *Proceedings of the Royal Society B-Biological Sciences*, *286*(1912), 20191921. https://doi.org/10.1098/rspb.2019.1921

Martinez-Gomis, J., Lujan-Climent, M., Palau, S., Bizar, J., Salsench, J., & Peraire, M. (2009). Relationship between chewing side preference and handedness and lateral asymmetry of peripheral factors. *Archives of Oral Biology*, *54*(2), 101–107. https://doi.org/10.1016/j.archoralbio.2008.09.006

Meredith, A. L., Prebble, J. L., & Shaw, D. J. (2015). Impact of diet on incisor growth and attrition and the development of dental disease in pet rabbits. *Journal of Small Animal Practice*, *56*(6), 377–382. https://doi.org/10.1111/jsap.12346

Mittag, W. (1932). Über das Wachstum der Kaninchenschneidezähne unter verschiedenen Funktionsbedingungen und ihr teleologisches Verhalten. *Wilhelm Roux' Archiv für Entwicklungsmechanik der Organismen*, *126*(1), 104–122. https://doi.org/10.1007/BF00586377

Moxham, B. J., & Berkovitz, B. K. (1974). The effects of root transection on the unimpeded eruption rate of the rabbit mandibular incisor. *Archives of Oral Biology*, *19*(10), 903–909. https://doi.org/10.1016/0003-9969(74)90053-3

Müller, J., Clauss, M., Codron, D., Schulz, E., Hummel, J., Fortelius, M., Kircher, P., & Hatt, J. M. (2014). Growth and wear of incisor and cheek teeth in domestic rabbits (*Oryctolagus cuniculus*) fed diets of different abrasiveness. *Journal of Experimental Zoology*, *321*(5), 283–298. https://doi.org/10.1002/jez.1864

Müller, J., Clauss, M., Codron, D., Schulz, E., Hummel, J., Kircher, P., & Hatt, J.-M. (2015). Tooth length and incisal wear and growth in guinea pigs (*Cavia porcellus*) fed diets of different abrasiveness. *Journal of Animal Physiology and Animal Nutrition*, *99*(3), 591–604. https://doi.org/10.1111/jpn.12226

Ness, A. R. (1956). The response of the rabbit mandibular incisor to experimental shortening and to the prevention of its eruption. *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character*, *146*(922), 129–154. https://doi.org/10.1098/rspb.1956.0077

Pares-Casanova, P. M., & Cabello, M. (2020). Patterns of mandibular asymmetries in two types of companion rabbits. *Anatomia, Histologia, Embryologia*, *49*(2), 227–232. https://doi.org/10.1111/ahe.12517

Prinz, J. F. (2004). Abrasives in foods and their effect on intra-oral processing: A two-colour chewing gum study. *Journal of Oral Rehabilitation*, *31*(10), 968–971. https://doi.org/10.1111/j.1365-2842.2004.01328.x

Proffit, W. R., & Sellers, K. T. (1986). The effect of intermittent forces on eruption of the rabbit incisor. *Journal of Dental Research*, *65*(2), 118–122. https://doi.org/10.1177/00220345860650020401

Rabenold, D., & Pearson, O. M. (2014). Scratching the surface: A critique of Lucas et al. (2013)'s conclusion that phytoliths do not abrade enamel. *Journal of Human Evolution*, *74*, 130–133. https://doi.org/10.1016/j.jhevol.2014.02.001

Risnes, S., Septier, D., & Goldberg, M. (1995). Accelerated eruption of rat lower incisor. Relationship between impeded and unimpeded eruption rates, rate of attrition, tooth length, and production of dentin and enamel. *Connective Tissue Research*, *32*(1–4), 183–189. https://doi.org/10.3109/03008209509013722

Rödel, H. G. (2005). Winter feeding behaviour of European rabbits in a temperate zone habitat. *Mammalian Biology*, *70*(5), 300–306. https://doi.org/10.1016/j.mambio.2005.03.001

Sánchez-Villagra, M. R. (2021). *The process of domestication*. Princeton University Press.

Sanson, G. D., Kerr, S., & Read, J. (2017). Dietary exogenous and endogenous abrasives and tooth wear in African buffalo. *Biosurface and Biotribology*, *3*(4), 211–223. https://doi.org/10.1016/j.bsbt.2017.12.006

Schulz, E., Fraas, S., Kaiser, T. M., Cunningham, P. L., Ismail, K., & Wronski, T. (2013). Food preferences and tooth wear in the sand gazelle (*Gazella marica*). *Mammalian Biology*, *78*(1), 55–62. https://doi.org/10.1016/j.mambio.2012.04.006

Schulz-Kornas, E., Stuhltrager, J., Clauss, M., Wittig, R. M., & Kupczik, K. (2019). Dust affects chewing efficiency and tooth wear in forest dwelling Western chimpanzees (*Pan troglodytes verus*). *American Journal of Physical Anthropology*, *169*(1), 66–77. https://doi.org/10.1002/ajpa.23808

Shakila, N., Ali, A., & Zaidi, S. (2015). Micro hardness of dental tissues influenced by administration of aspirin during pregnancy. *International Journal of Morphology*, *33*, 586–593.

Steigman, S., Michaeli, Y., & Zajicek, G. (1981). The influence of calibrated loads upon the rate of eruption of mandibular rat incisors. *Archives of Oral Biology*, *26*(4), 327–331. https://doi.org/10.1016/0003-9969(81)90055-8

Tapaltsyan, V., Eronen, J. T., Lawing, A. M., Sharir, A., Janis, C., Jernvall, J., & Klein, O. D. (2015). Continuously growing rodent molars result from a predictable quantitative evolutionary change over 50 million years. *Cell Reports*, *11*(5), 673–680. https://doi.org/10.1016/j.celrep.2015.03.064

Tummers, M., & Thesleff, I. (2003). Root or crown: A developmental choice orchestrated by the differential regulation of the epithelial stem cell niche in the tooth of two rodent species. *Development*, *130*(6), 1049–1057. https://doi.org/10.1242/dev.00332

Ungar, P. S. (2015). Mammalian dental function and wear: A review. *Biosurface and Biotribology*, *1*(1), 25–41. https://doi.org/10.1016/j.bsbt.2014.12.001

Utz, K. H. (1986). The interocclusal tactile fine sensitivity of the natural teeth as studied by aluminum oxide particles. *Deutsche zahnärztliche Zeitschrift*, *41*(3), 313–315.

Weinreb, M. M., Michaeli, Y., & Silberman, G. (1969). Role of attrition and occlusal contact in the physiology of the rat incisor. IV. Prevention of attrition in the articulating incisor. *Journal of Dental Research*, *48*(1), 120–130. https://doi.org/10.1177/00220345690480010401

Wetzel, G. (1927). Die regulationen der nagetierschneidezähne. *Wilhelm Roux' Archiv für Entwicklungsmechanik der Organismen*, *112*, 455–479.

Williams, O. B., Wells, T. C. E., & Wells, D. A. (1974). Grazing management of Woodwalton Fen: Seasonal changes in the diet of cattle and rabbits. *The Journal of Applied Ecology*, *11*(2), 499. https://doi.org/10.2307/2402204

Winkler, D. E., Tütken, T., Schulz-Kornas, E., Kaiser, T. M., Müller, J., Leichliter, J., Weber, K., Hatt, J. M., & Clauss, M. (2020). Shape, size, and quantity of ingested external abrasives influence dental microwear texture formation in guinea pigs. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(36), 22264–22273. https://doi.org/10.1073/pnas.2008149117

Wolf, P., & Kamphues, J. (1996). Untersuchungen zu Fütterungseinflüssen auf die Entwicklung der Incisivi bei Kaninchen, Chinchilla und Ratte. *Kleintierpraxis*, *10*, 723–732.

Yu, T., & Klein, O. D. (2020). Molecular and cellular mechanisms of tooth development, homeostasis and repair. *Development*, *147*(2): dev184754. https://doi.org/10.1242/dev.184754

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Martin, L. F., Ackermans, N. L., Richter, H., Kircher, P., Hummel, J., Codron, D., Clauss, M., & Hatt, J.-M. (2022). Macrowear effects of external quartz abrasives of different size and concentration in rabbits (*Oryctolagus cuniculus*). *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, 338, 586–597. https://doi.org/10.1002/jez.b.23104