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

Louise F. Martin1 | Nicole L. Ackermans1 | Henning Richter2 | Patrick Kircher2 | Jürgen Hummel3 | Daryl Codron4 | Marcus Clauss1 | Jean-Michel Hatt1

1Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
2Clinic for Diagnostic Imaging, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
3Division of Ruminant Nutrition, Department of Animal Sciences, University of Goettingen, Goettingen, Germany
4Department 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.

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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).
Apart from direct tooth-to-tooth contact during mastication, major causative agents for macroscopic tooth wear are the silicates inside plant material (endoogenous 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 m²) 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, MICROSL® 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
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 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).

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

| 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 8 8       |           |
| Cellulose (%)                        | 8 4 4   | 4 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 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 2   |             |           |
| Binding solution (%)                 | 1.5 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     | 0.6       |
| Sodium bicarbonate (%)               | 0.5 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 | 89 89 89 |           |
| Nutrient composition (g/kg DM)      |         |           |             |           |
| Crude protein                        | 16.1 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       |           |

\(^a\)Ash corrected.
\(^b\)A proxy for abrasive content.
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 (TELEPACK 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 comparison.

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 $y = a \cdot BM^b$ 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 for...
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

**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
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). The laterality was also consistent on the maxillary M1 ($p = 0.029$; Table S4 and Figure S1).

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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 |
| Tooth | 53.7008 | <0.001 |
| Side: tooth | 3.4453 | 0.016 |
| Concentration | 4.5657 | 0.011 |
| Conc: side$^b$ | 2.3452 | 0.097 |
| Conc: size$^c$ | 4.7166 | 0.001 |
| 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 |
| Side: tooth | 1.9677 | 0.097 |
| Concentration | 103.4128 | <0.001 |
| Conc: size$^c$ | 677.0550 | <0.001 |
| Conc: tooth$^d$ | 1.8884 | 0.059 |
| Relative tooth height |
| Mandibular cheek teeth$^a$ | | | |
| Individual | 480.8120 | <0.001 |
| Body mass end | 902.4502 | <0.001 |
| Side | 4.8163 | 0.029 |
| Tooth | 1.1845 | 0.315 |
| Concentration | 16.1307 | <0.001 |
| Conc: size | 8.5837 | <0.001 |
| Maxillary cheek teeth$^a$ | | | |

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.

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 3c), 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). The laterality was also consistent on the maxillary M1 ($p = 0.029$; Table S4 and Figure S1).
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
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 inter-
occlusal 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 dif-
fferences among the diets and purposefully chose to eat the abrasive
diets. By this active selection, the rabbits also influence a morpho-
physiological measure—dental wear and (responding) growth rate
(see below); thus giving an example of behavior influencing a mor-
phological 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 in-
trinsic 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 re-
mains to be explored in the future). Small size abrasives are thought
species enamel as well as the physiology of mastication, may contribute to differences in both, physical properties of durable enamel of ungulate’s permanent teeth may make tissue loss abrasives after even a short amount of time, whereas the more hypsodont teeth made of a softer enamel that may be affected by 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 hypsodont 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 disproportional 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 move more easily off towards the respective front or back during mastication.

Laterality of motor function in general, and masticatory hard- edness in particular, have been researched in humans and some other mammal species (Ginot et al., 2018; Llorente et al., 2011; Martínez-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 (Mosham & 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.

Hypsodont teeth represent a comparatively novel morphophysiological unit in mammalian evolution (Tapalsyan 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
hypsodont 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 hypsodont 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 hypsodont 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.

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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

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